University of Latvia

Faculty of Medicine

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PATHOGENIC ROLE OF BRCA1 GENE MUTATIONS AND HEREDITARY FACTORS IN PATIENTS WITH BREAST AND OVARIAN CANCER

SUMMARY OF ACADEMIC DISSERTATION

Specialty – oncology

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Background information and rationale of the study

Breast cancer comprises 23% of all newly discovered cancer cases in women and 10% of total number of malignant diseases. Every year approximately 1.1 million women are diagnosed with breast cancer (UICC, 2006). Besides, it is the main reason of death among malignant diseases in active age (35 to 64 years) women in EU states (Bray, Sankila et al., 2002).

Breast cancer is the most common oncologic disease in Latvia with about 1000 newly discovered cases per year. During the last 10 years the incidence has increased for 15%. Regretfully, the mortality rate is still high (about 450 cases per year) and the main reason for that is delayed diagnostics that affects the total survival and 5-year survival as well (Latvian Cancer Registry, 2003,2004).

This figure hasn't substantially changed during the last 20 years despite of further progress in diagnostics and medical technologies. And thus every attempt to improve early diagnostics both by mammographic screening and risk group selection should be encouraged. Positive family history and RBCA1 gene mutations are of special importance as women bearing these risk factors do develop their cancers at an earlier age.

Every year about 300 new cases of ovarian cancer are diagnosed in Latvia. This is the third or forth most common cause of cancer mortality among women (Latvian Cancer Registry, 2003, 2004). Ovarian cancer diagnosis often is delayed and also the treatment options are quite restricted. At the same time many women with this particular disease do get their cancer at untypical earlier age or do have positive family history that may point to some probable inheritance, including proven BRCA1/2 gene mutations (Boyd, Rubin, et al., 1997). Therefore selection of these women with probably inherited predisposition to development of ovarian cancer could be of great importance.

It has been noticed that breast cancer more often affects women whose close relatives have had this particular disease at a reproductive age or had bilateral disease (Adami, Hansen et al., 1980; Satin, Rubin et al., 1985; McPherson, Vessey et al., 1987; Mettlin et al., 1990;; Byrne, Brinton et al., 1991;; Andriev, Duffy et al., 1995; Pharoah, Nicholas et al., 1997 Kampova-Polevaja and Cistjakovs 2006). A similar tendency has been observed also among patients with ovarian cancer. Positive ovarian cancer family history increases the risk of the proband to develop the same cancer later (Greggi, Ponder et al., 1991; Amos, Shaw et al., 1992; Nguyen, Averette et al., 1994).

Nowadays there are recognized several mutations of different genes that may lead to development of breast, ovarian and other cancers. The most studied are mutations of two big genes – BRCA1 and BRCA2 that may affect different fragments and loci of the same gene. At the same time there is evidence that certain ethnic communities and regions do bear specific mutations of a certain gene fragment, the so-called founder mutations that facilitates gene testing.

The possibility of getting breast or ovarian cancer among patients with proven BRCA1 gene mutations ranges between 23% to 85% and 16% to 50% respectively (Ford, Easton et al., 1998; Risch, McLaughlin et al., 2001; King, Marks et al., 2003; Domchek and Weber, 2006; Narod, 2006). At the same time one should keep in mind other independent risk factors like gene modificators, reproductive factors etc, that may additionally facilitate the development of the cancer.

It has been established at the Biomedical Center of the University of Latvia, that there are two mutations (5382insC and 4154delA) that are found in up to 80% of Latvia's female population. So far there have been recognized seven clinically significant BRCA1 gene mutations (5382insC, 4154delA, 300A>G, 3650delT, 185delAG, 962del4, 4476+1G>A), the presence of which does correlate with higher incidence of breast, ovarian and other cancers (Csokay, Tihomirova et al., 1999; Tikhomirova, Sinicka et al., 2005; Sinicka, Tihomirova et al., 2005).

The knowledge of gene and environmental interaction facilitate treatment planning for patients with proven BRCA1 gene mutations. Chemoprophylaxis with antiestrogens and regular check-ups may substantially lower the risk of getting and delaying the disease. While removal of ovaries can reduce the risk of ovarian cancer for more than 90% and breast cancer for about 50%.

Our future goal is to individualize the personal risk detection according not only to patients' age, reproductive status, personal and family history but also according to certain gene mutations. This would help to provide the optimal treatment schedule in each individual case as well as to select the most appropriate prophylactic approach – medical or surgical in order to improve early diagnostics or even to prevent the disease.

AIM OF THE STUDY

To study the impact of BRCA1 gene mutations and positive family or personal cancer history on the development and clinical course of breast or ovarian cancer.

OBJECTIVES OF RESEARCH:

1. To compare the clinical course and morphological features of breast and ovarian cancer in patients with or without BRCA1 gene mutations.
2. To detect the impact of positive family history on development of breast or ovarian cancer.
3. To analyze the incidence of BRCA1 gene mutation between breast and ovarian cancer patients with positive previous cancer history.
4. To determine the spectrum of clinically significant BRCA1 gene mutations among patients with proven breast or ovarian cancer.
5. To assess the course of the disease between breast and ovarian cancer patients according to the status of BRCA1 gene mutations.
6. To develop practical recommendations for BRCA1 gene testing and prophylactic measures for patients with breast or ovular cancer.

THESES FOR DEFENSE

The detection of BRCA1 gene mutations and hereditary factors analysis provides us with additional significant information about disease prognosis, clinical course and morphological characteristics as well as necessity for prophylactic and medical procedures.

NOVELTY OF THE THESIS

It was for the first time in Latvia that the role of BRCA1 gene mutations has been evaluated according to the spectrum of clinically significant BRCA1 gene mutations, course of the disease, morphological features and positive personal or family history.

We have developed recommendations for BRCA1 gene testing and recommendations for tailoring treatment plan according for patients with proven BRCA1 gene mutations and patients with increased cancer risk.

MATERIAL AND METHODS

This case-control study was performed with the permission of the Latvian Central Ethics Committee (30.08.1999.Nr.10) and Ethics Committee of the Medical Academy of Latvia (30.11.2000).

Patients

A total of 317 patients - 209 with breast and 108 with ovarian cancer have been studied at the Oncology Center of Latvia between 1998 and 2004. The principal inclusion criteria were morphologically proven breast or ovarian cancer and written permission for the usage of tissue material for genetic testing. The age of breast cancer patients ranged from 19 to 71 years and the age of ovarian cancer patients ranged from 25 to 72 years. More detailed information about the course of disease, its morphological characteristics, and tumor markers, steroid receptors state and other indicators were acquired from case-records, ambulatory cards and The Registry of Latvian Cancer Patients.

Pedigrees

All 317 patients filled questionnaires about cancer cases among their 1^{st}, 2^{nd} and 3^{rd} degree relatives, including information about the type of tumor and age of onset. This information served for creating pedigrees for altogether 316 patients.

Detection of mutations

Mutations were detected at the Laboratory of Biomedical Research and Study Centre University of Latvia by using standard methodology and equipment for DNA isolation and mutation testing. At the beginning the testing of the whole BRCA1 gene was performed in 74 patients with breast and 24 patients with ovarian cancer. Thus we detected the spectrum of BRCA1 gene mutations for breast and ovarian cancer. In order to simplify genetic testing we performed detection of the most common and clinically significant mutations. Thus we tested 134 breast and 84 ovarian cancer patients.

Statistics

Statistic analysis was performed with the aid of software programs: SPSS for Windows 10.0 (by SPSS, Ldc., USA), Microsoft Excel 8.0, EpiInfo 2001 software “StatCalc” and software (CIA) for analyzing confidence intervals created by D.Altman (Altman, 1991; Altman, 2000). Generally accepted statistic methods were used for group description (Altman, 1991; Altman, 2000; Teibe and Berķis, 2001). We used SPSS software for life expectancy analysis using the Kaplan-Mayer method within various risk groups and between them.

RESULTS

Breast cancer

The mean age of the disease onset in the observed population was 45.8±9.9 years.

The distribution of patients by various stages of disease did not differ substantially among patients without reference from discovery of BRCA1 gene mutations and personal or family anamnesis of breast or ovarian cancer. Medullar carcinomas were observed more frequently (13.8%) among mutation carriers than the examined population in whole. During our research these patients BRCA1 gene mutations (OR=5.6, 95%CI 1.16-26.33; RR=3.56; 95%CI 1.57-8.05) had been discovered credibly more often (p=0.0067).

BMI (body mass index) in the breast cancer patient group ranged from 17 to 48 kg/m2, (mean BMI was 27.9±5.6 kg/m2). There was an overall tendency of being overweight among our study population.

The expression of estrogen receptors (ER+) was found in 40.7% of patients, while the progesterone receptor (PR+) in 43.1% of cases respectively. Patients with BRCA1 gene mutations had significantly more often ER-negative (p=0.003; χ^2=11.52) and PR-negative (p=0.018; χ^2=8.07) disease (see Table1).

Table1.

Expression of ER and PR in tumor tissue according to BRCA1 gene mutations.

		BRCA1 gene mutation				**Total**	
		Present		**Not proven**			
Estrogen receptors		**N**	**%**	**N**	**%**	**N**	**%**
	Unknown	16	8.9	2	6.9	18	8.6
	Positive	81	45.0	4	13.8	85	40.7
	Negative	83	46.1	23	79.3	106	50.7
	Total	180	100.0	29	100.0	209	100.0
Progesterone receptor	Unknown	16	8.9	2	6.9	18	8.6
	Positive	84	46.7	6	20.7	90	43.1
	Negative	80	44.4	21	72.4	101	48.3
	Total	180	100.0	29	100.0	209	100.0

Clinically significant BRCA1 gene mutations we recognized in altogether 29 patients out of 209(13.9%). At the same time out of the seven so far registered in Latvia clinically significant BRCA1 gene mutations we founded only five (see Table 2.). The most common were mutations 5382insC (16 out of 20 or 55.2%) and 4154delA (9 out of 29 or 31%).

Table2.

Spectrum of BRCA1 gene mutations in study population

BRCA1 gene mutations	**Number**	**(%)**
5382insC	16/ 209	7.7
4154delA	9/ 209	4.3
300T>G	2/ 209	1.0
185delAG	1/ 209	0.5
962del4	1/ 209	0.5
Not proven	180/ 209	86.1

During the first stage of the study we analyzed the whole BRCA1 gene for altogether 74 patients. In this group that was carefully selected by including patients with positive family history and early disease onset (less than 40 years of age) BRCA1 gene mutations were found in every fifth woman (in altogether 15 patients out of 74).

In the next phase we screened our study population for the most common types of so far registered mutation in Latvia (5382insC, 4154delA, 300T>G, 185delAG, 962del4). This was done in altogether 134 breast cancer patients. BRCA1 gene mutations were founded in 14 out of 134 patients (10.4%). Later these particular BRCA1 gene mutations were found also in 11 relatives of our study patients: one patient's mother with breast cancer, five sisters of patients, including one with breast cancer and one twin sister, and five patient's daughters. BRCA1 gene mutations were found only in one case – in a healthy daughter of the patients from C330 family.

The age of the onset of the disease did not differ significantly among patients with proven BRCA1 gene mutations. The difference was more evident when comparing patients with and without BRCA1 gene mutations (see Table 3).

Table3.

The age of the onset of the disease according to BRCA1 gene mutations.

Patients	**N**	**Range (years)**	**Median age (years)**	**SD**
Without any mutations	180	19- 71	46.64	9.71
With proven BRCA1 mutations	29	22- 62	40.17	10.86
Mutation 5382insC	16	33- 60	41.13	10.31
Mutation 4154delA	9	29- 62	40.77	11.24

We compared the overall survival among patients with and without BRCA1 gene mutations as well as among patients with different BRCA1 gene mutations. The median survival tended to be longer in patients without BRCA1 gene mutations and in patients with 4154delA mutation. The overall survival was shorter in patients with 5382inC mutation when compared to 4154delA mutation carriers although this difference was not statistically significant (Table 4 and Picture 1).

Table4.

Median survival according to BRCA1 gene mutation status

Patients groups	**Survival (months)**	**95%CI**	**M**	**SED**
Patients without BRCA1 mutations (N=180)	183	143- 223	138	20
Patients with proven BRCA1 mutations (N=29)	148	103- 193	122	23
Patients with 5382insC mutation (N=16)	147	91- 203	122	29
Patients with 4154delA mutation (N=9)	178	109- 247		35

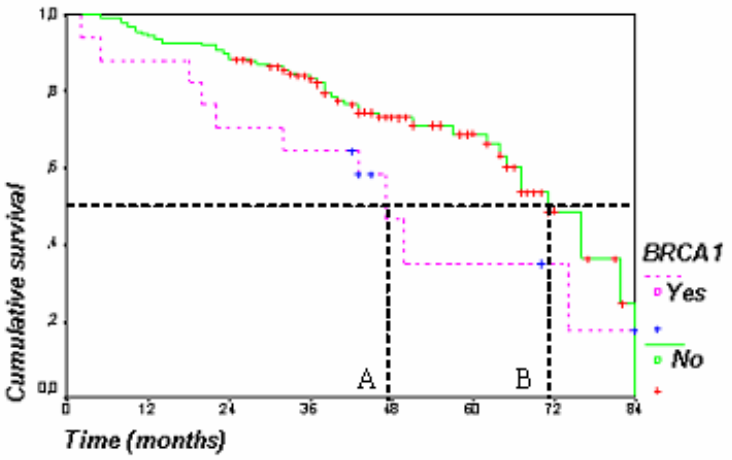

Figure1. Survival curves in patients with and without BRCA1 gene mutations

Positive family history (different malignancies) has been registered in altogether 137 patients out of 209 (65.6%). In 35 cases malignancy has affected patients father (16.7%) and in 69 cases (33%) - patients mother. The most common malignancy among diseased mothers was breast cancer (31 of 69). Breast and/or ovarian cancer among the first and/or second degree relatives was found in altogether 66 cases out of 209 patients (see Table 5). BRCA gene mutations were considerably more often found in patients with a positive family history.

Table5.

Breast and/or ovarian cancer among 1st and 2nd degree relatives according to patients BRCA1 gene mutation status

1st, 2nd degree relatives with proven breast and/or ovarian cancer	**BRCA1 gene mutation**				**Total**	
	Unproven		**Proven**			
	N	**%**	**N**	**%**	**N**	**%**
Not known	129	71.7	14	48.3	143	68.4
Known	51	28.3	15	51.7	66	31.6
Total	180	100.0	29	100.0	209	100.0

When assessing the possibility to getting breast cancer in BRCA1 gene mutation carrier according to breast and/or ovarian cancer history in patients' mother, sister or aunt, we discovered that mother's breast and/or ovarian cancer history had the greatest impact (see Table 6).

Table6.

Breast and/or ovarian cancer in patient mothers and BRCA1 gene mutations in patients

Mother with breast and/or ovarian cancer	BRCA1 gene mutation				Total	
	Unproven		Proven			
	N	%	N	%	N	%
Not known	154	85.6	17	58.6	171	81.8
Known	26	14.4	12	41.4	38	18.2
Total	180	100.0	29	100.0	209	100.0

When estimating the cross-reference (OR) between mutation carriers and relatives with breast and/or ovarian cancer we discovered that the probability of cancer was greater if patients mother has had a breast or ovarian cancer (see Table 7)

Table7.

Risk of breast cancer in patients with BRCA1 gene mutations according to family cancer history

Breast and/or ovarian cancer in close relatives	OR	95%CI	RR	95%CI	P value
Mother	4.18	1.65<OR<10.58	3.18	1.66<RR<6.08	0.0005
Sister	1.77	0.36<OR<7.54	1.61	0.55<RR<4.66	0.39
Aunt	2.67	0.84<OR<8.28	2.22	1.01<RR<4.85	0.054
Any 1^{st} /2^{nd} degree relative	2.71	1.14<OR<6.46	2.32	1.19<RR<4.52	0.012

As one can see the risk of breast cancer in our population (209 patients) was higher, if the patient herself carries BRCA1 gene mutation and has a positive breast and/or ovarian cancer family history. This was especially evident if breast and/or ovarian cancer have been diagnosed in patients mother or 1^{st} / 2^{nd} degree relative.

Previous personal cancer history has been documented in altogether 41 patients (19.6%). In 23 cases breast cancer has been diagnosed as a secondary malignancy. In 9 patients ovarian cancer has been diagnosed as a second or third malignancy. Positive breast and/or ovarian cancer family history was noted in 19 cases out of 41 (46.3%). Personal previous cancer history according to BRCA1 gene mutation findings are shown in Table 8.

Table8.

BRCA1 gene mutation status according to personal cancer history.

Multiple malignancies	**BRCA1 gene mutation**				**Total**	
	Unproven		**Proven**			
	N	**%**	**N**	**%**	**N**	**%**
Not registered	152	84.4	16	55.2	168	80.4
Known	28	15.6	13	44.8	41	19.6
Total	180	100.0	29	100.0	209	100.0

When assessing the coincidence of BRCA1 gene mutations and presence of multiple malignancies it came out that the OR was 4.41 (95%CI 1.77-11.01) and RR - 3.3 (95%CI 1.74-6.36). This correlation was also statistically significant (p=0.00023). Only in one case out of 13 BRCA1 gene mutation carriers the secondary malignancy was not breast or ovarian cancer, but renal carcinoma.

Patients with multiple personal malignancies did get their first disease at an earlier age (median 42.8 years) comparing to patients with solely breast cancer (at an average age of 46.5 years; p=0.031).

The overall survival in patients with or without personal other cancer history did not differ significantly and was about 167 months for patients with only breast cancer and 173 months for patients with breast and other malignancies.

Ovarian cancer

The mean age of the disease onset in the observed population was 48.4±9.9 years.

The distribution of the different stages of the disease was equal independently on BRCA1 gene mutations and positive personal or family cancer history.

Cistadenocarcinoma was the dominating morphologic type of ovarian cancer. We founded it in altogether 80.6% of cases. Patients without BRCA1 gene mutations more often had other morphological subtypes (19.3%) in comparison with BRCA1 gene mutation carriers (8%) although this difference was not statistically significant (p=0.09).

The median BMI among ovarian cancer study patients was 27.8±6.1 kg/m2 ranging from 17 to 47 kg/m2. Also the ovarian cancer patients seemed to be overweight as well as our breast cancer patients.

Prior to cancer therapy the level of serological marker CA125 was within normal (0-21 U/ml) limits in altogether 15.7% of patients. In 13.9% of patients the level was slightly elevated (up to 65 U/ml) and in 47.3% it was considerably increased (above 65 U/ml). The level of CA125 according to BRCA1 gene mutation status is depicted in Table 9.

Table9.

Level of CA125 in ovarian cancer patients according to BRCA1 gene mutation status

Ca125	**BRCA1 gene mutation**				**Total**	
	Unproven		**Proven**			
	N	**%**	**N**	**%**	**N**	**%**
Not applicable	22	26.5	3	12.0	25	23.1
Normal (0- 21)	15	18.0	2	8.0	17	15.7
Under 65 U/ml	12	14.5	3	12.0	15	13.9
Over 65 U/ml	34	41.0	17	68.0	51	47.3
Total	83	100.0	25	100.0	108	100.0

Patients with BRCA1 gene mutation had significantly higher basal CA125 levels in comparison with patients without mutations (p=0.017; χ^2=5.63). We got an evidence that increased basal CA125 indicate a higher probability of BRCA1 gene mutation- OR=3.06 (95%CI 1.09-8.8) and RR=2.38 (95%CI 1.12-5.03). Thus it could help to select patients for whom genetic testing would be desirable.

BRCA1 gene mutation was found in altogether 25 patients out of 108 (23.1% - see Table 10). In our material we found only three mutations out of altogether seven so far in Latvia recognized clinically significant BRCA1 gene mutations.

Table10.

The spectrum of BRCA1 gene mutations in study population with ovarian cancer

BRCA1 gene mutations	**N**	**%**
5382insC	14	13.0
4154delA	10	9.3
300T>G	1	0.9
Total	25	23.1
Unproven	83	76.9
Total	108	100.0

During the first stage of study we analyzed the whole BRCA1 gene in altogether 24 ovarian cancer patients. Mutations were found in six patients (25%). In the next stage of research we screened for most common mutations ever recognized in Latvia in altogether 84 patients. Mutations were found in 19 cases (22.6%).

Patients with ovarian cancer and BRCA1 gene mutation 5382insC fell ill earlier than 4154delA mutation carriers. But neither this nor the difference between any other mutation carriers and non-carriers was statistically significant (see Table 11).

Table11.

Age of onset of ovarian cancer according to BRCA1 gene mutation status

Patients groups	**N**	**Range (years)**	**Median age (years)**	**SD**
Without any mutations	83	25- 72	48.69	10.62
With proven BRCA1 mutations	25	34- 61	47.60	7.08
Mutation 5382insC	14	34- 60	46.14	6.15
Mutation 4154delA	10	40- 61	48.70	8.04

During the study we analyzed the median survival of patients with ovarian cancer according to BRCA1 gene mutation status. These results are shown in Table 12. We didn't separate the only patient with a rare type of mutation and included it in the group of mutation carriers. It turned out that the median survival was longer for patients with BRCA1 gene mutations and mutation 4154delA although this difference was not statistically significant.

Table12.

Median survival of ovarian cancer patients according to BRCA1 gene mutation status

Patients groups	**Survival (mo)**	**95%CI**
Patients without BRCA1 mutations (N=83→80)	44 ± 4	37- 51
Patients with BRCA1 mutations (N=25)	67 ± 7	54- 80
Patients with 5382insC mutation (N=14)	61 ± 7	47- 75
Patients with 4154delA mutation (N=10)	64 ± 11	43- 86

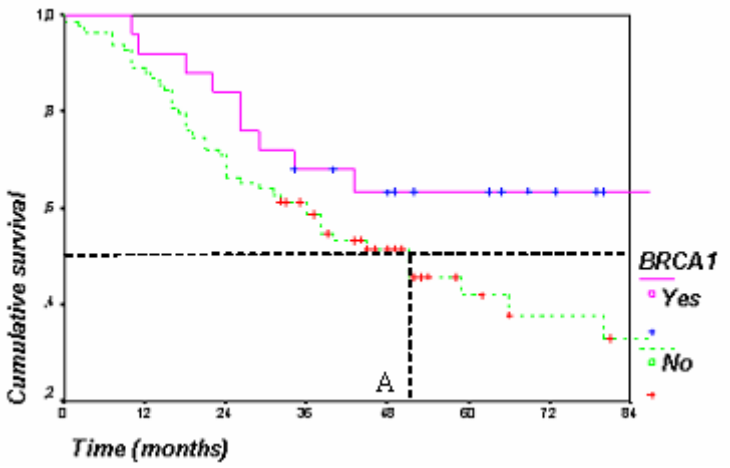

Figure2. Survival curves of ovarian cancer patients according to BRCA1 gene mutation status

When comparing the survival curves of patients with and without BRCA1 gene mutations the divergence seems to be more obvious (see Figure2).

Positive family history (different malignancies of various locations) was found in altogether 81 patients out of the 108 (75%) studied patients. Cancer in patient's father was noted in 25 cases (23. 1%). Mother has been affected in 34 (31.5%) cases.

Breast and/or ovarian cancer cases in probands family are summarized in Table 13. It is evident that positive family history correlates with more often finding of BRCA1 gene mutation.

Table13.

Breast and/or ovarian cancer in 1st and 2nd degree relatives according to patients BRCA1 gene mutation status.

1st,2nd degree relatives with proven breast and/or ovarian cancer	**BRCA1 gene mutation**				**Total**	
	Unproven		**Proven**			
	N	**%**	**N**	**%**	**N**	**%**
Not known	60	72.3	8	32.0	68	63.0
Known	23	27.7	17	68.0	40	37.0
Total	83	100.0	25	100.0	108	100.0

When evaluating the probability of getting ovarian cancer in BRCA1 gene mutation carrier according to cancer cases among probands mother, sisters or aunts, it turned out that the strongest impact on the risk has mothers' breast and/or ovarian cancer history (see Table14).

Table14.

Breast and/or ovarian cancer in patients mother according to patients BRCA1 gene mutation status

Mother with proven breast and/or ovarian cancer	**BRCA1 gene mutation**				**Total**	
	Unproven		**Proven**			
	N	**%**	**N**	**%**	**N**	**%**
Not known	71	85.5	13	52.0	84	77.8
Known	12	14.5	12	48.0	24	22.2
Total	83	100.0	25	100.0	108	100.0

When establishing the cross reference (OR) between mutation carriers and breast and/or ovarian cancer cases among close relatives, it appeared that the highest probability of having ovarian cancer was found in patients whose mothers or any other close 1^{st} or 2^{nd} degree relative have had breast and/or ovarian cancer (see Table 15).

Table15.

Risk of ovarian cancer in BRCA1 gene mutation carriers according to family history

Breast and/or ovarian cancer in close relatives	**OR**	**95%CI**	**RR**	**95%CI**	**P value**
Mother	5.46	1.82<OR<16.71	3.23	1.70<RR<6.13	0.0004
Sister	2.97	0.60<OR<14.40	2.10	0.92<RR<4.77	0.11
Aunt	2.06	0.53<OR<7.79	1.68	0.75<RR<3.75	0.23
Any 1^{st} /2^{nd} degree relative	6.27	2.15<OR<18.78	3.91	1.86<RR<8.22	0.00008

When analyzing the onset of cancer among patients close relatives it became apparent that in average both patients and their relatives got their cancer at approximately the same age (48.4± 9.9 years for relatives at 50.8 ± 11.2 years for patients respectively). We made a pair comparison using the t-test and discovered that the difference was not statistically significant. But, using the method of linear regression we found a correlation ($r=0.451$; $p=0.004$) between the morbidity age of probands and their relatives – the later the cancer was discovered in patients relatives, the later the disease affected the proband.

Positive personal cancer history was found in 11 ovarian cancer patients (10.2%). The second most common cancer among ovarian cancer patients was breast cancer – 6 cases out of 11.

We found 2 cases of multiple malignancies among 25 ovarian cancer patients with BRCA1 gene mutations. There were 9 cases of multiple malignancies among altogether 83 ovarian cancer patients without proven BRCA1 gene mutations. Because of the small number of cases we did not calculate OR and RR for other cancer risk in patients with proven ovarian cancer.

According to our data patients with ovarian and other cancers did fell ill at an earlier age (44.6±8.6 years) in comparison to patients with only ovarian cancer (48.9±10 years) although this difference was not statistically significant ($p=0.18$).

The overall survival of patients with multiple malignancies including ovarian cancer was slightly smaller than for patients with only one – ovarian cancer, although this difference was not statistically significant.

Pedigrees

A pedigree analysis was performed for every patient of our study. Creation of a family tree has a long history and is currently used nowadays, especially in cases of congenial or hereditary diseases. Various software programs have been developed not only to draw a family tree but also to provide us with a chance to process and analyze the data. To illustrate this we provide an insight in some pedigrees of our study patients.

In family (C330) with four cases of breast cancer they all were mother-sided (see Figure 3). BRCA1 gene mutation was found in proband II:2 who had bilateral breast cancer. BRCA1 gene mutation was found in one out of her three daughters (III:1). The second daughter did not have BRCA1 gene mutation, but the third daughter was still a teenager and the gene testing was not performed. Theoretically all the three daughters could be attributed to a high risk group and could be involved in breast cancer screening program from age 25. In the same time the oldest daughter (III:1) could be recommended to perform prophylactic oophorectomy after child bearing, thus minimizing the risk of development of breast or ovarian cancer.

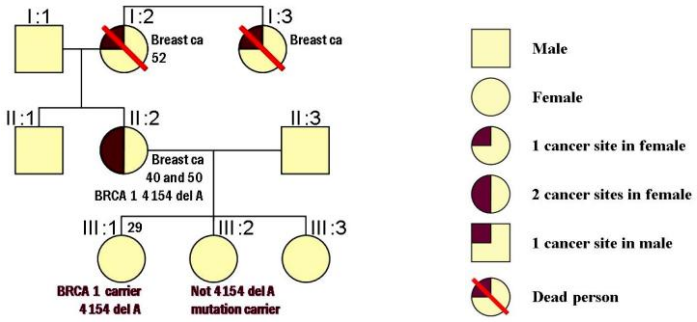

Figure3. Pedigree of the family C330 with four cases of breast cancer and proven BRCA1 gene mutations in proband and her daughter.

In another family (C121) breast cancer had affected four family members (see Figure 4). BRCA1 gene mutations were not detected neither in proband (II:4), nor in her sister (II:3). Despite these negative results, this family also bears special attention and should be recommended to start early screening.

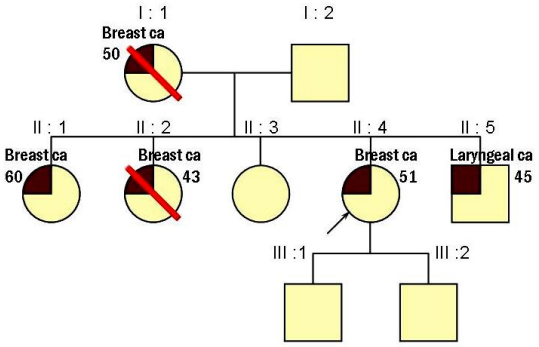

Figure4. Pedigree of the family C121 with four cases of breast cancer and unproven BRCA1 gene mutations

There were three known cases of ovarian cancer in the family C470 (see Figure 5) although BRCA1 gene mutation in the 56 years old probande (II:2) with metastatic ovarian cancer was not found. Because of known positive family history the patient has undergone prophylactic oophorectomy although this did not prevent further development of metastatic peritoneal carcinoma. This family also should be attributed to high risk group and should follow early screening recommendations, including patients daughter (III: 4) and sister's daughter (III:1).

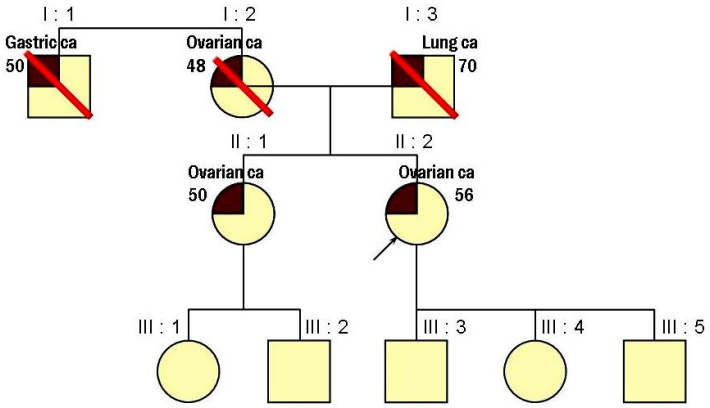

Figure5. Pedigree of the family C470 with three cases of ovarian cancer and unproven BRCA1 gene mutations in the proband

Three cases of breast and ovarian cancer have been established in the family C451 (see Figure 6) following both maternal and paternal lines. Clinically significant BRCA1 gene mutation 5382insC was detected in the proband (II:4). Genetic testing would be highly

recommended for patient's sister and daughters after reaching adulthood. Irrespectively of the further testing results all the family female relatives should be recommended to join cancer screening program starting at the age of 25.

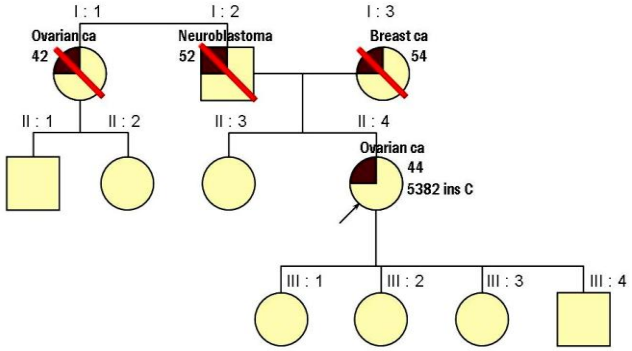

Figure6. Pedigree of the family C451 with two cases of ovarian and one case of breast cancer and proven BRCA1 gene mutations in the proband

In another family (C9) a total of six cases of breast and ovarian cancer have been established (see Figure 7). A BRCA1 gene mutation was detected in the 41 year old proband following both maternal and paternal lines. The BRCA1 gene mutation for this patient was proven during her treatment. As the prophylactic ovarian removal was not performed a second malignancy in ovaries developed later. It would be advisable to examine probands sister (III:3), daughter (IV:4) and daughter of stepsister (IV:6) for gene mutations. All three should be included in screening programs irrespective of genetic testing results.

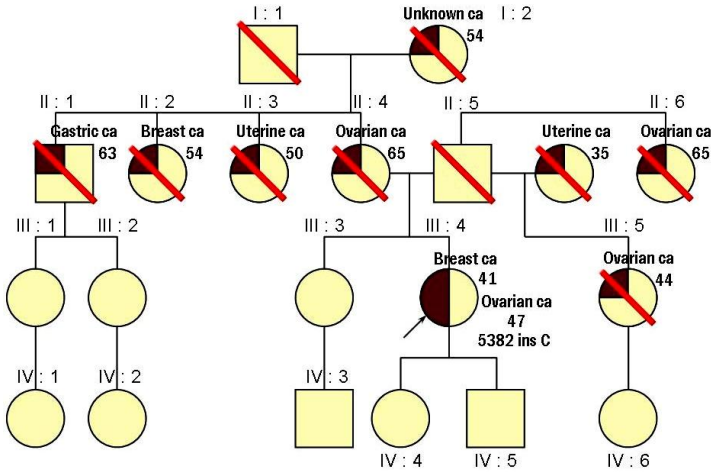

Figure7. Pedigree of the family C9 with six cases of breast and ovarian cancer and proven BRCA1 gene mutations in the proband

Discussion

Age of the disease onset

Our study patients got their cancer at a younger age (45.8±9.9 years for breast cancer and 48.4±9.9 years for ovarian cancer) in comparison with the general population. The peek incidence of breast and ovarian cancer in Latvia is between 60-74 years (Latvian Cancer Registry, 2001-2004). This coincides with the observations of other authors who note that women with hereditary predisposition for breast or ovarian cancer (positive family anamnesis, proven BRCA1 gene mutations) develop cancer 5-15 years earlier than it is observed in sporadic cases (Claus, Risch et al., 1994; Struewing, Hartage et al., 1997).

It is interesting that our study patients in breast cancer group developed their disease 2.6 years earlier than patients in the ovarian cancer group. Similarly there was a difference in the proportion of breast (72.7%) and ovarian cancer (56%) which developed their disease before age of 50. When comparing the age of onset of the disease among patients with and without BRCA1 gene mutations, it came out that patients with proven BRCA1 gene mutations get their breast cancer earlier (40.1±10.9 years) than patients without mutations (46.6±9.7 years of age). Due to wide age distribution (19-71) this difference was not statistically significant. We did not noticed similar trend among ovarian cancer patients. The age of cancer onset in mutation carriers (47.6±7.6 years) was close to age of patients (48.7±10.6) without BRCA1 gene mutations.

The fact that hypothetically predisposed ovarian cancer patients get their disease a few years later than breast cancer allows us to plan prophylactic measures. Young women before 50 years of age and positive family history should be proposed to provide prophylactic removal of ovaries irrespectively of their breast tumor receptor status. The most commonly nowadays used method of medical castration could be ineffective since the target organ for further probable cancer development was left intact.

Body mass index

Overweight was noticed in both our study groups. The average body mass index for breast cancer patients was 27.9±5.6 kg/m^2 and 27.8±6.1 kg/m^2 for ovarian cancer patients. There was a tendency of increasing BMI with increasing age. It has been estimated that at least 25% of breast cancer could be attributed to overweight no matter of it's genesis (IARC Handbook`s of Cancer Prevention, 2002). It has been observed that the risk of getting breast cancer correlates positively with BMI (McTiernan, 2003). The relative risk (RR) of

developing breast cancer for patients with BMI from 25 to 29.9 kg/m2 was 1.34 in comparison with normal weighted counterparts, while for patients with BMI ranging from 30 to 34.9 kg/m2 the RR was already 1.63. For patients with BMI that exceeds 40 kg/m2 the RR was 2.12.

Similar conclusions can be drawn from observations among ovarian cancer patients. BMI exceeding 25 kg/m2 significantly increases the risk of developing ovarian cancer (OR=1.95; 95%CI 1.44-2.64) (Pan, Johnson et al., 2004).

Stage of the disease

We did not find remarkable differences of stage distribution among patients with and without positive family history. It looks like a little attention is paid in these families for early prophylaxis (more frequent check-ups and targeted examinations). There are references suggesting that earlier finding of cancers in families with affected members the families themselves and society in general do pay more attention to cancer alertness (Gomes, Gulmaraes et al., 1995; Pharoah, Nicholas et al., 1997). Close relatives keeping in mind their increased risk start screening earlier than general population. Thus, even if a tumor starts developing it is discovered at an early stage. Therefore the society need to be enlightened and informed that some cancers could be inherited.

Morphological subtypes of cancer

Medullar carcinoma was found more often (13.8%) in mutation carriers than in patients without BRCA1 gene mutation (2.8%). Similar findings are described by other authors recommending genetic testing for patients with this type of breast cancer (Eisinger, Jacquemier et al., 1998; Lakhani, Jacquemier et al., 1998). There was none patient with *in situ* carcinoma in our study population. There is evidence that BRCA1 gene mutations are rarely found in patients with *in situ* carcinoma (Breast Cancer Linkage Consortium 1997).

Ovarian cystadencarcinoma was the most common morphological type of ovarian cancer patients irrespective of BRCA1 gene mutation finding (92% in mutation carriers and 77.1% in patients without mutations). The proportion of other histological types was different but this difference wasn't statistically significant ($p>0.05$) neither in mutation carrier group (8%) nor in the group without mutations (19.3%). Other authors share our observations. According to their data patients with BRCA1 gene mutations have cystadenocarcinoma in 94% of all cases while the

percentage of this type in case of sporadic ovarian cancer is only about 60% of all cases (Berchuk, Heron et al., 1998).

Serological tumor markers

The majority of serologic tumor markers help follow-up the course of the disease and seldom are used for detecting malignant growth. Because of its low specificity and sensitivity CA125 serves mainly for disease monitoring. There is evidence that elevated serum CA125 levels above 65U/ml indicate higher possibility for ovarian cancer development in premenopausal women and in women in early menopause (Eltabbakh, Belinson et al., 1997). This was also in accordance with our observation that the highest basal CA125 level was observed among patients with early onset of the disease and proven BRCA1 gene mutations.

Estrogen and progesterone receptors

It is a well-known fact that steroid receptor expression in tumor tissue correlates with a better overall prognosis and it has a positive predictive value concerning hormonal treatment efficacy. Our data on receptor negativity in BRCA1 gene mutation carriers correlates to observations of other authors (Lakhani, Van DE Vijver et al., 2002; Lakhani, Reis-Filho et al., 2005). Both steroid receptor negative and BRCA gene mutation induced tumors are often tumors with poor prognosis. Thus steroid receptor negativity and BRCA1 gene mutations could be assumed as independent prognostic markers. Probably the combination of both has different pathogenic mechanisms. Although mutations were found in only 29 our study breast cancer patients, the difference of receptor expression among patients with and without BRCA1 gene mutations was statistically significant (ER $p=0.003$; PR $p=0.018$) (See Table 1).

Treatment

All the patients, including our study population did receive standard treatment recommended by the physicians' council. Unfortunately in no case the treatment plan has been changed after obtaining the results of genetic testing. Breast cancer patients were mostly (72% of all cases) treated surgically, followed by chemotherapy and irradiation. Ovarian cancer treatment was usually an operation followed by chemotherapy.

17 of 317 patients included in this research had both breast and ovarian cancer and BRCA1 gene mutations were discovered in 7 cases. Considering the recommendations for high-risk women a prophylactic ovarian ablation should have been advised for five patients after detecting breast cancer and proven BRCA1 gene mutation. According to literature it should decrease the risk of developing further ovarian cancer by 90% (Rebbeck, Levin et al., 1999; Kauff, Satagopan et al., 2002; Eisen, Lubinski et al., 2005; Narod, 2006; Domchek and Weber, 2006). It means that all of these five women thus could have escaped later ovarian cancer. Other two mutation carriers with first ovarian and later breast cancer should have been offered chemoprevention with Tamoxifene, bilateral mastectomy or inclusion tight screening program. According to literature it could either prevent getting breast cancer or to facilitate its earlier detection (Domchek and Weber, 2006; Narod, 2006). In our case the treatment plan has not been corrected. In our study altogether 21 out of 209 breast cancer patients had bilateral breast cancer and BRCA1 gene mutations were found in 7 of them. Three women had simultaneous breast cancers in both breasts at the time of discovery and two of them had proven BRCA1 gene mutations. According to literature they should have been advised a preventive ovarian ablation that wasn't done (Domchek and Weber, 2006; Narod, 2006). Three mutation carriers with breast cancer get their disease at the end of 80s and the beginning of 90s when there were no gene testing possibilities. All these examples show that adjustment of treatment according to BRCA1 gene testing results for high risk patients is essential and that educational work should be continued amongst the strategic decision makers. The prevention strategy and treatment options for patients with proven BRCA1 gene mutations should be included in treatment standards for cancer patients.

Cancer family history

Most of the patients included in our research – 137 (65.6%) in breast cancer and 81 (75%) of ovarian cancer group – had a positive family history. The most commonly affected relatives were patients' mothers - 33% in breast and 31.5% in ovarian cancer group. Certainly we should keep in mind that it was always much easily to get family history about mother than other close relatives. Similarly one should bear in mind that the families in Latvia are small and that makes it more difficult to interpret the data. We also had problems with discovering diseases in 1^{st} and 2^{nd} level relatives because we usually had oral evidence that could be incorrect.

Out of all examined breast cancer patients some cancer affected first or second degree relatives have had breast or ovarian cancer in 66 (31.6%) cases. In 15 cases BRCA1 gene mutations were found either in proband or one of examined family members. Altogether 40 patients with ovarian cancer (37%) have had positive family history with cases of breast and ovarian cases and in 17 cases BRCA1 gene mutation was found either in proband or some close relative. It should be stressed that genetic testing in patients with known positive family history is of utmost importance and should be included in complex examination program. It would allow selecting patients who will need personalized treatment planning.

The proportion of families with more than two breast and/or ovarian cancer cases wasn't high – 24 (11.5%) in breast cancer and 17(15.7%) in ovarian cancer group. It could be explained by small number of relatives in the family. But this stresses the need for genetic testing in these families and to provide them further tailored treatment and prevention planning. BRCA1 gene mutations were found in 5 families out of 24 (20.8%) in breast cancer group and in 8 families out of 17 (47.0%) in the ovarian cancer group. This our finding correlates with literature about more frequent discovery of BRCA1 gene mutations in high-risk families (Struewing, Tarone et al., 1996; Struewing, Hartage et al., 1997).

Our study comprises more families with moderate risk (one case of breast and/or ovarian cancer). There were 42 (20.1%) such cases in breast and 23 (21.3%) cases in ovarian cancer group respectively. The need for genetic testing in moderate-risk families is still discussed (Struewing, Tarone et al., 1996; Malone, Daling et al., 1998). We discovered BRCA1 gene mutations in 10 breast cancer patients (23.8%) and in 9 ovarian cancer patients (39.1%) from moderate-risk families. Small families are the main confusing factors that may underestimate the need genetic testing and interpretation of the results. We suggest genetic testing also for moderate-risk families.

It was interesting, that we found pretty many BRCA1 gene mutations among patients without positive family history. Here we could speak about sporadic cases that may be additionally blurred by the small number of members in the observed families. Usually BRCA1 gene testing is not recommended in sporadic cancer cases. The main inclusion criterion of our study was patients' written consent to use her tissue material for further genetic testing. Therefore the number of patients without positive family history was so high. However there were a considerable proportion of cases with proven BRCA1 gene mutations. Mutations were found in 14 breast cancer patients out of 143 (9.8%) and 8 ovarian cancer patients out of 68 (11.8%) respectively. It has been shown in literature that the proportion of mutations among sporadic

breast cancer cases ranges from 3.5% in Great Britain (Peto, Collins et al., 1999; Ellis, Greenman et al., 2000) to 13% in Israel (Fitzgerald, MacDonald et al., 1996) and 13.5% in Poland (Menkiszak, Gronwald et al., 2003). The higher number of proven mutations in sporadic cases in our material we explain first, with incomplete information about the real situation of cancer cases among relatives. Secondly it is possible that women concerned about their personal cancer risk or women with known positive family history were eager to participate in this study. Finally we are reluctant to state that BRCA1 gene mutations exceed the number of similar mutations elsewhere in Europe. Our material was too small to extrapolate these data to the whole population.

According to published data, ovarian cancer among close relatives is much stronger predictive factor for the development of breast/ovarian cancer than breast cancer (Greggi, Ponder et al., 1991; Antoniou, Pharoah et al., 2003). We observed that the most significant risk factor for breast cancer development was breast cancer in a close relative. In our material breast cancer among close relatives was five times more often than ovarian or ovarian and breast cancer. We did not recognize such strong correlation among our breast cancer patients. Our breast cancer patients had equally often breast and ovarian cancer cases among their close relatives.

In case of hereditary cancers the following generations usually develop cancers earlier than their ancestry (Greggi, Ponder et al., 1991; Pharoah, Nicholas et al., 1997). We too tried to analyze how the age of disease onset in family history affects the age of cancer onset in proband. On average patients developed breast cancer for approximately eight years earlier than their diseases relatives, while ovarian cancer developed about two years earlier, although this difference was not statistically significant.

Patients' cancer history

According to observations of numerous authors patients with BRCA gene mutations more often suffered from different malignancies (Risch, McLaughlin et al., 2001; Antoniou, Pharoah et al., 2003). Similar observations have been stated about positive family history (Narod, 2002). In our study there were patients with BRCA1 gene mutation and at least one other malignancy before or after breast or ovarian cancer. This correlation was evident for breast cancer (in 41 patient out of 209 (19.6%)) rather than for patients with ovarian cancer (11 out of 108 (10.2%)). In the majority of cases the second cancer was other breast or ovarian cancer. Altogether 8 different tumors were recognized as secondary malignancies in our study population.

BRCA1 gene mutations were found in altogether 13 cases out of 29 breast cancer patients that constitute 44.8% of all mutation carriers. Thus the probability of bearing BRCA1 gene mutation in a case of multiple malignancies in personal history was pretty high (OR=4.41; RR=3.33; p=0.00023).

BRCA1 gene mutations were found in 2 cases out of 25 ovarian cancer patients with multiple other malignancies in personal history that constitute 8% of all mutation carriers. We did not observed convincing evidence of higher ovarian cancer risk among patients with proven other previous malignancies (OR=0.71; RR=0.77; p=0.68). It should be noted that there were few such patients in our study group.

We observed that patients with ovarian or breast cancer and other malignancies did get their disease at an earlier age in comparison with patients affected with only one cancer. Thus our breast cancer patients developed their disease at an average 3.7 years earlier than patients with only one – breast cancer and this difference was statistically significant (p=0.031). Ovarian cancer patients with other malignancies in their personal history did get their first cancer for about 4.2 years earlier, but this difference wasn't statistically significant (p=0.18). Thus we may conclude that the earlier patient gets breast or ovarian cancer, the greater is the possibility to develop other malignancies during the following years.

BRCA1 gene mutations in study patients

Chronologically this is the first study in Latvia in collaboration with the Biomedical Research Center that investigates the incidence and spectrum of BRCA1 gene mutations in patients with breast and ovarian cancer. In the beginning of our study we tested the whole BRCA1 gene for possible mutations. Afterwards we constricted our research for only the most common clinically significant mutations ever recognized in Latvia (Tikhomirova, Sinicka et al., 2005). Altogether clinically significant BRCA1 gene mutations were found in 54 cases out of 317 patients with breast and ovarian cancer. We found five of seven ever recognized in Latvia clinically significant BRCA1 gene mutations in both our study groups (see Table 16) (Sinicka, Tihomirova et al., 2005).

Table16.

BRCA1 gene mutations in study patients

BRCA1 gene mutations	Breast cancer patients		Ovarian cancer patients		Total	
	N	%	N	%	N	%
5382insC	16	55.2	14	56.0	30	55.5
4154delA	9	31.0	10	40.0	19	35.2
300T>G	2	6.9	1	4.0	3	5.5
185delAG	1	3.4			1	1.9
962del4	1	3.4			1	1.9
Total	29	100.0	25	100.0	54	100.0

The most common mutation we found was 5382insC that is also widely distributed in Russia, especially Siberia and whose incidence slowly decreases towards Europe. This mutation is also very common in many Eastern and Central European countries (Gorski, Byrski et al., 2000; Van Der Looij, Szabo et al. 2000; Konstantopoulou, Kroupis et al. 2000; Meindl 2002; Tereschenko, Basham et al., 2002, Loginova, Pospekhova et al., 2003; Menkiszak, Gronwald et al., 2003; Foretova, Machackova et al., 2004; Sinicka, Stengrevics et al., 2004; Tikhomirova, Sinicka et al., 2005; Kampova-Polevaja and Cistjakovs 2006). It is assumed that this mutation has reached us already through population migration during the Middle Ages (Szabo and King, 1997). BRCA1 gene mutation 5382insC is also the commonest amongst Askenazy Hebrews (the so called ancestor mutation) (Tonin, Weber et al., 1996). We found this particular mutation in more than 50% of cases of both breast and ovarian cancer. We found breast or ovarian cancer as a second localization in altogether eight cases out of 30 (26.7%; 95%CI 14.2-44.4) patients with BRCA1 gene mutation 5382insC (two cases of patients with primary breast and two – with primary ovarian cancer). This means that in case of 5382insC mutation the development of breast or ovarian cancer as a primary or secondary malignancy is equally possible. The same coincidence has been observed in Russia and Poland (Gayther, Harrington et al., 1997; Gorski, Byrski et al., 2000). Usually the mutation carriers get breast cancer first (at about the age of 41) and then develop ovarian cancer (at about the age of 46). This is a prompt argument for

prophylactic ovarian ablation in breast cancer patients with this particular mutation irrespective of receptor status.

The second most common mutation in Latvia is 4154delA (Sinicka, Stengrevics et al., 2004; Tikhomirova, Sinicka et al., 2005). We found it in altogether 19 patients with breast and/or ovarian cancer. The proportion was greater amongst patients with ovarian cancer (in 40% of all cases). BRCA1 gene mutation 4154delA is also common in other Eastern European countries – Poland (Gorski, Byrski et al., 2000), Russia (Gayther, Harrington et. Al 1997) and Belarus (Oszurek, Gorski et al., 2000). This particular mutation is frequently observed also in Finland (Sarantaus, Vahteristo et al., 2001), Canada (BIC) and USA (BIC). Breast cancer as a second malignancy was found in altogether 19 mutation 4154delA carriers. Patients with BRCA1 mutation 5382insC bear similar tendency – they usually develop breast or ovarian cancer as a first and one of the already mentioned cancers as a second malignancy. Positive family history has been documented in altogether 13 patients out of 19 (68.4%) carrying mutation 4154delA and in 16 patients out of 30 (53.3%) carrying mutation 5382insC. Both mutations were detected in either patient groups (breast or ovarian cancer).

The third most common BRCA1 gene mutation we founded was 300T>G. We detected this particular mutation in 3 cases for both breast and ovarian cancer patients. It is less common in Latvia than in Eastern Europe. This mutation (300T>G) is a frequent finding among breast and/or ovarian cancer patients in Poland, Hungary, Czech Republic and Germany (Gorski, Byrsku et al., 2000; Van Der Looij, Szano et al., 2000; Meindl 2002; Foretova, Machackova et al., 2004). It is interesting that this mutation we found in the youngest patient of our study – in a 22 year old breast cancer patient. We also found this mutation in a patient with breast cancer and kidney cancer as a second localization. The other two cases were patients with breast cancer and secondary malignancies that were neither breast, nor ovarian cancer. Further research is needed to explore the real incidence and its clinical significance in Latvia.

BRCA1 gene mutation 185delAG we founded in only one case. This patient after ovarian cancer later developed breast cancer. This is a mutation typical for Ashkenazi Hebrews and is thought to be known since already Middle Ages (Tonin, Weber et al., 1996).

One of our breast cancer carried BRCA1 gene mutation 962del4. This patient later developed second ovarian cancer. This particular mutation is prevalent in Austria (Wagner, Moslinger et al., 1998), Germany (Meindl 2002), USA (Janezic, Ziogas et al., 1999).

BRCA1 gene mutations more often were found amongst ovarian cancer patients than among breast cancer patients – 25 of 108 (23.1%) and 29 of 209 (13.9%) respectively. Certainly

our results stress that further investigations with larger patient number is needed to make any decisive conclusions. Ideally if all breast cancer patients below the age of 50 and all the ovarian cancer patients below 60 will undergo genetic testing. About 75% of these patients do concentrate at the Oncology Center of Latvia and therefore this particular Hospital could be the principal center for these studies.

Risk assessment for breast and ovarian cancer

Besides genetic testing we tried to assess the risk of developing breast and/or ovarian cancer according to mutation status, family and personal history and other clinical data. The risk of getting breast or ovarian cancer was statistically significant (p=0.012 for breast cancer patients; p=0.00008 for ovarian cancer patients) in a case of breast/or ovarian cancer among 1st or 2nd degree relatives and proven BRCA1 gene mutation in patient. The OR was 2.71 (95% CI 1.14-6.46) for breast cancer patients and RR was 2.32 (95%CI 1.86-8.22). The OR was 6.27 (95%CI 2.15-18.78) and RR was 3.91 (95%CI 1.86-8.22) among patients with ovarian cancer. In both groups the most significant risk factor apart from existence of a BRCA1 gene mutation was breast and/or ovarian cancer registered in patients' mother. For breast and ovarian cancer patients with proven BRCA1 gene mutation and mother suffering from breast or ovarian cancer the risk of developing breast and/or ovarian cancer was significantly higher OR was 4.18 (95%CI 1.65-10.58) and RR was 3.18 (95%CI 1.66-6.08) in breast cancer patients group and OR was 5.46 (95%CI 1.82-16.71) and RR was 3.23 (95%CI 1.70-6.13) in ovarian patients group. But the family anamnesis is incomplete in too many cases- relatives of patients have been scattered in the territory of former Soviet Union or some other place (like during World War II). Because of all the mentioned facts- assessment of breast and ovarian cancer that is based on family anamnesis is somewhat biased. It could be possible to create a database or registry of high risk persons, using the framework of Latvian Cancer Registry patient database or independently. There would be included no only the data about patients with breast or ovarian cancer risk, but also people with hereditary risk of colon cancer and other tumor with genetically predisposition risk. It would ease the checking and clarification the information about family cancer history.

Life expectancy

During our study we tried to establish correlations with BRCA1 gene mutations, personal and family history, proven multiple malignancies and survival.

The median survival among breast cancer patients with proven BRCA1 gene mutations was 148 months (95%CI 103-193). Survival for patients without BRCA1 gene mutations was longer - 183 months (95%CI 143-223). We used only the existence or absence of BRCA1 gene mutation as a foundation for our life expectancy calculations. We didn't take in mind the stage of disease or TNM classification. This agrees with data from other authors (Marcus, Page et al., 1997; Stoppa-Lyonnet, Ansquer et al., 2000; Phillips 2000; Moller, Borg et al., 2001). Thus it is obvious that the existence of BRCA1 gene mutation is a negative prognostic marker of life expectancy for the patient herself. When we compared median survival among patients with the most common BRCA1 gene mutations we found that it was 147 months (95%CI 91-203) among 5382insC mutation carriers and 178 months (95%CI 109-247) among 4154delA mutation carriers. Thus the 4154delA mutation could be assumed to be a bit more advantageous. This mutation might be the cause of breast cancer but it doesn't affect the prognosis of disease.

The situation with ovarian cancer patients was an opposite one. Patients without BRCA1 gene mutations had worse survival time (average 44 months; 95%CI 37-51) than patients with proven mutations (average 67 months; 95%CI 54-84). Thus in this case the mutation could be viewed as a cause of the disease but not as poor prognostic indicator. This has been observed also by other authors (Rubin, Benjamin et al. 1996). When we compared the survival times among different mutation carriers we noticed that it was almost the same 61 months (95%CI; 47-75) for 5382insC and 64 months (95%CI; 43-86) for 4154delA mutations. In ovarian cancer patient group we cannot say that one of the most common mutations is worse than the other. We made our prognosis using only the existence or absence of mutation in this group.

Comparing the average life expectancy prognosis of breast and ovarian cancer patients we can see that they are significantly different (3-4 times longer for breast cancer patients). Breast cancer is much more often discovered in early stages of disease than ovarian cancer and thus the chance of successful treatment is more likely, as well as it is more advantageous prognostic by itself. Tumors of both localizations determine the life expectancy of the patient by the stage of disease when they are discovered. The earlier it is discovered the better the life expectancy of the patient.

We analyzed the average life expectancy of patients with one and multiple malignant tumors. In both patient groups the average life expectancy was longer for patients with one malignant cancer localization (173 months in breast cancer group, 95%CI 134-212; 50 months in ovarian cancer group, 95%CI 44-56). The life expectancy for patients with multiple localizations of malignant tumors was significantly worse – 18 months in ovarian cancer group (95%CI; 13-24) and 167 months in breast cancer group (95%CI; 134-200). Thus repeatedly proving that ovarian cancer is prognostic less advantageous both alone and as one of several localizations of malignant tumors.

Conclusions

1. In patients with proven BRCA1 gene mutations:
   1.1 and breast cancer, comparing to breast cancer patients without BRCA1 gene mutations, the tumor itself more frequently was medullar carcinoma and receptor negative (ER- and/or PR-) one.
   1.2 and ovarian cancer, comparing to ovarian cancer patients without BRCA1 gene mutations the basic level of CA125 was significantly higher although the there were no significant differences in morphological subtypes.
   1.3 Elevated BMI was observed in both groups and thus could be attributed to independent cancer risk factor.
2. In patients with positive family history:
   2.1 and breast cancer the overall incidence of BRCA1 gene mutations was significantly higher. The most common cancer among proband's mothers was breast cancer. The risk of breast cancer development was significantly higher in patients whose mothers or any other 1^{st} or 2^{nd} degree relatives have had this particular cancer.
   2.2 And ovarian cancer the overall incidence of BRCA1 gene mutations was significantly higher. The risk of ovarian cancer development was significantly higher in patients whose mothers or any other 1^{st} or 2^{nd} degree relatives have had this particular cancer.
3. In patients with multiple malignancies:
   3.1. and breast cancer, the incidence of BRCA1 gene mutations was significantly higher than in patients without any other cancer; significantly more frequently were found BRCA1 gene mutations; the most common secondary malignancy after primary breast cancer was contralateral breast cancer; the onset of the first malignancy occurred significantly earlier than in patients without any other cancer; the overall survival did not differ significantly.
   3.2. and ovarian cancer; BRCA1 gene mutations were found only in two cases out of 25; the most common secondary malignancy after ovarian cancer was breast cancer; the age at onset of the first malignancy did not differ significantly among patients with or without personal cancer history; the overall survival did not differ significantly.
4. Out of altogether seven clinically significant BRCA1 gene mutations recognized in Latvia we founded:

4.1. Five in breast cancer patients (5382insC, 4154delA, 300T>G, 185delAG and 962del14); the most common was 538insC (16 cases out of 29);

4.2. Three in ovarian cancer patients (538insC, 4154delA and 300T>G); the most common was 538insC (14 cases out of 25).

5. In patients with proven BRCA1 gene mutations:

5.1. and breast cancer: the median age of disease onset was insignificantly younger (40.4±10.9 years) than in patients without mutations (46.6±9.7 years); the overall survival was insignificantly shorter (148±23 months) than inpatients without mutations (183±20 months); the overall survival of patients with 5382insC mutation was insignificantly shorter (147±29 months) than in patients with 415delA mutation (178±35 months).

5.2. and ovarian cancer: the median age of disease onset did not differ among patients with and without BRCA1 gene mutations; the overall survival was significantly longer in patients with BRCA1 gene mutations (67±7 months) in comparison with patients without mutations (44±4 months); there was no significant survival difference among patients with two most common BRCA1 gene mutations.

6. Practical recommendations:

6.1. BRCA1 gene testing should be recommended in patients with breast/ovarian cancer and positive family history (breast and/or ovarian cancer diagnosed in patients mother or any other 1^{st} /2^{nd} degree relative) and patients with already proven breast or ovarian cancer in personal history.

6.2. Patients with breast cancer and proven BRCA1 gene mutations should undergo surgical ovarian ablation and contralateral mastectomy irrespective of steroid receptor status.

6.3. Patients with ovarian cancer and proven clinically significant BRCA1 gene mutations should been included in chemopreventive trials with tamoxifen and breast cancer screening program.

6.4. Clinically healthy patients with proven significant BRCA1 gene mutations should be highly recommended to perform ovarian ablation after childbearing as well as be included in breast cancer screening program at an earlier age.

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Abbreviations

Abbreviation	Explanation
BC	breast cancer
BRCA1	breast cancer gene 1
BRCA2	breast cancer gene 2
CA 125	cancer antigen CA125
ER	estrogen receptors
BMI	body mass index
M	mean
N	number of patients
OC	ovarian cancer
OR	odds ratio
p	p value
PR	progesterone receptors
RR	relative risk
r	Pearson correlation
SED	standard error mean
SD	standard deviation
t	t value
χ^2	Chi-squere value

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