Pathological, molecular pathological features and prognosis of early-onset breast cancer

PhD Thesis Outlines

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INTRODUCTION

Breast cancer (BC) is the most common malignancy of women affecting 1 million patients yearly all over the world. Altough BC is mainly a disease of postmenopausal women, in 5,5% of the cases the women affected are younger then 40 years of age and 2% of breast cancer patients are younger than 35 years. It is the most common malignancy related cause of death in these young age groups. EUSOMA (European Society of Breast Cancer Specialists) defines breast cancer of young women as that of affecting women ≤ 40 years, while that affecting women ≤ 35 years, as breast cancer of very young women.

Several data suggest that early-onset BC has unfavourable biology and prognosis compared to BC of older women. Genetic predisposition is always suspected in early-onset breast cancer and about 5% of these cases are indeed related to mutation of high penetrance genes with an autosomal dominant inheritance, most commonly germline mutation of BRCA1 or BRCA2.

Early-onset breast cancer in 10 % of the cases is pregnancy associated: either presenting during pregnancy or in the postpartum period.

The majority of breast malignancies are of epithelial origin, the most common form being invasive (ductal) carcinoma, while invasive lobular carcinomas are somewhat less common (both types may also be associated with in situ carcinomas).

Gene expression studies have subdivided breast cancer into 6 main groups: Luminal A, Luminal B, Her2-enriched, basal, molecular apocrin and caludin-low. These subgroups differ considerably in occurrence, prognosis and their response to therapy. ER+ and ER- tumors may also be subdivided by immunohistochemistry predicting treatment modalities: Luminal A and Luminal B proliferating (Her2-) groups are separated by Ki67 labeling index and/or PgR expression, while the Luminal B Her2+ subgroup is characterized by ER positivity and HER2 amplification/protein expression. ER negative breast cancer is further subdivided into HER2 enriched (ER-,PgR-, Her2+) and triple negative (ER-, PgR-, Her2-) subtypes.

Early-onset BC has unfavourable biology: these tumours are more often hormone receptor negative, Her2+, high grade carcinomas with increased proliferative activity. Several studies have confirmed the unfavourable prognosis of early-onset BC; compared to older women, these young patients more commonly suffer from loco-regional and systemic relapses, and BC related death is also more common in this age group. Early-onset BC is usually detected in a more

advanced stage, probably also explained by the fact that these young women are from the non-screened population (≤ 45 years). BC related family anamnesis is often positive, genetic predisposition is always suspected in this young age group of patients.

Young women with breast cancer usually encounter some special problems related to their age; they should face treatment induced eventual fertility loss, thus for those who are nulliparous or planning to have more children, fertility preservation strategies should be offered. In 0,6% of cases BC is pregnancy related (Pregnancy Associated BC-PABC); either manifests during pregnancy, lactation or in the postpartum period, within a year after delivery.

Pregnancy has dual influence on BC; it is protective on the long term, but it is also associated with increased risk of BC development after delivery. The time frame of this increased BC risk is 2-15 years or even more in older primipara.

The incidence of PABC in western countries is 1/10000-1/3000 pregnancies. Since there is a tendency of postponing childbearing to a later age, and with age, cancer risk is also increasing, the incidence of PABC is expected to rise.

AIMS

Clinicopathological characteristics, outcome and family history data of early-onset breast cancer

We have sought to investigate differences in T and N stage, IHC-based subtypes and prognosis of BC cases of very young and older women. We have searched for differences in prognosis of young women with BC, based on family history (breast and ovarian cancer or other malignancies); comparing those cases with a positive family history (FH) for breast and ovarian cancer, or any other malignancy with those of a negative FH. We have further investigated the association of treatment modalities and prognosis.

Morphological and immunophenotypical heterogeneity in BC of young and elderly women

In this study, we sought to investigate morphological and immunophenotypical differences of early-onset BC and that affecting older women. We looked for intratumoural heterogeneity within BC samples of these age groups.

Predicting BRCA mutation status with BRCAPRO

We sought to investigate whether BRCA mutation status can be predicted, based on detailed family history and BC immunophenotype of very young women, by applying the BRCAPRO model.

BRCA mutation related and claudin-low BC: stepsisters or blood relatives?

We have investigated whether BRCA mutation associated and claudin-low BC are identical subtypes.

Clinicopathological features and outcome of Pregnancy Associated Breast Cancer (PABC)

In a retrospective matched case control study we have assessed differences of PABC and non-PABC cases, based on clinicopathological features, immunohistochemistry, IHC-based subtypes, treatment modalities and outcome.

METHODS

Clinicopathological characteristics, outcome and family history data of early-onset breast cancer

Patients. We have assessed 107 BC cases of very young women, diagnosed in the 2nd Department of Pathology, Semmelweis University, Budapest, Hungary between 01/01/1999 and 31/12/2009. Clinicopathological features of these cases-group 1- were compared to that of 55 women (36-45 years) –group 2, 214 women (46-65 years) –group 3, 110 women (66-75 years) –group 4. and 58 women (older than 75 years) –group 5. *Pathological features, immunohistochemistry.* We have assessed tumor type, nuclear grade, ER, PgR, Her2 expression and KI67 labeling index according to standard guidelines. *IHC-based subtypes.* Tumour samples were considered Luminal A if ER+, PgR+/- and Ki67 labeling index lower than 20%, Luminal B if ER+, PgR+/- and Ki67 index more than 20% or Her2+. ER-, PgR- and Her2-tumour samples were assessed as triple negative (TNBC), while ER-, PgR- and Her2+ tumours

as Her2+. *Family history.* Patients ≤35years and their families were contacted, personal disease history and family history investigated. *Statistics.* Clinicopathological and prognostic data were compared by chi^2 test and Kruskal-Wallis test. Disease free survival was assessed by Kaplan-Meyer method. We set $p<0.05$ as significance level. Statistical analysis was performed with Statistica 9.0 software (StatSoft, Tulsa, OK, USA) and SPSS 17 (SPSS Inc., Chicago, IL, USA).

Morphological and immunophenotypical heterogeneity in BC of young and elderly women

Patients. We have compared BC tumour samples of 41 young (≤ 35 years) and 33 elderly (> 65 years) women. *Microscopic morphology.* HE stained tumour samples were divided into morphologically heterogeneous areas and digitalized (Mirax MIDI, 3DHistech Ltd, Budapest). *Immunohistochemistry (IHC) and Fluorescent In Situ Hybridization (FISH).* IHC expressions were evaluated according to intensity (0-3) and ratio (0-100), then combined together into a score of 0-300 (Histoscore). FISH results were interpreted according to available guidelines. *Statistics.* Analysis was performed by using the SPSS 15.0 Family Pack softver (SPSS Inc., Chicago, IL, USA). Results were evaluated by chi-square test, two-sided tests were used..

Predicting BRCA mutation status with BRCAPRO

Patients. We have analyzed BC cases of very young (≤ 35 years) women, diagnosed in the 2nd Department of Pathology, Semmelweis University Budapest, Hungary between 01/01/2000 and 31/12/2007. According to available guidelines, these young patients should be referred for genetic testing of BRCA. *Family history.* Patients and their families were contacted and asked to complete a questionnaire. *Pathological features, Immunohistochemistry (IHC) and Fluorescent In Situ Hybridization (FISH).* BC samples were assessed, histological type, grade, ER, PgR, CK5/6 expression, Her2 status recorded. *BRCAPRO model.* We have applied the BRCAPRO genetic risk prediction model (CancerGene, BayesMendel, Dallas, TX, USA). It estimates the probability that the patient carries a deleterious mutation of BRCA1 or BRCA2 as well as her risk of developing cancer. If the assessed risk is high (10% or more), the patient is advised for genetic counseling. *Genetic testing.* Results of BRCA genetic testing were provided by Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary.

BRCA mutation related and claudin-low BC: stepsisters or blood relatives?

Patients- In silico analysis. Sixteen patients with BRCA mutation (BRCAmut) (12 GSE50567, 2 GSE18864 and 2 GSE 3744), and 17 patients with BRCA wild type tumours (BRCAwt) –as controls- (10 GSE18864 and 7 GSE50567) were involved in this study. Gene expression data were retrieved from 3 public databases. Case control study was performed. We have downloaded unprocessed .CEL files which were normalized using MAS5 R statistical environment (R, version 2.10.1, Vienna, Austria). *Patients-IHC analysis.* A separate cohort of 22 BRCA mutation carrier women with available BC tissue was selected at Sheba Medical Center (CSMC), Tel Hashomer, Israel and 19 women- as a control cohort- with available BC FFPE samples were selected from the files of the 2nd Department of Pathology, Semmelweis University Budapest, Hungary. BRCA mutation analysis of these patients was performed in the 1st Department of Pathology, Semmelweis University, Budapest, Hungary. *Tissue microarrays (TMA).* TMAs were composed using 2 cores of 2mm per case, which were placed consecutively in 2 blocks with a TMA builder instrument (MTA-1, Beecher Instruments, Inc. Sun Prairie, WI, USA). *Immunohistochemistry (IHC) and Fluorescent In Situ Hybridization (FISH).* Antibodies used according to standard protocols: AR, CD24, CD44, CK5/6, claudin-1, claudin-3, claudin-4, claudin-7, E-Cadherin, EGFR, ER, EZH2, HER2, Ki67, P53, PgR, vimentin. The IHC slides were scanned by Pannoramic 250beta (3DHistech Ltd., Budapest, Hungary). The evaluation of examined proteins was based on the combined assessment os staining intensity (0-3) and frequency (0-5) of positive tumour cells in the core regions. The raw data were then added resulting in an Allred-like number (0, 2-8) Ki67 was evaluated on a scale from 0 to 100%. Evaluation of ER, PgR and Her 2 was done according to current guidelines. For other reactions a value <3 was considered negative, 3-4 was low, and 4< was considered high. *Statistics.* The subtypes of tumors in the datasets were assigned in R statistical environment using the PAM50 predictor described previously. Chi-square and Kruskall-Wallis test was performed to test relation of nominal and numeric ordinal variables. Numeric scale variables were compared with student's t-test using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Two-sided tests were used and we set a significance level of *.05*.

Clinicopathological features and outcome of Pregnancy Associated Breast Cancer (PABC)

Patients. PABC and control patients were selected from breast cancer cases of women ≤45 years, diagnosed in the 2nd Department of Pathology, Semmelweis University, Hungary between 01/01/1998 and 01/11/2012. *Pathological features, immunohistochemistry (IHC),*

FISH. Histopathology information on tumor type, grade, size, T, N, Lympho-vascular invasion (LVI), Nottingham Prognostic Index (NPI), associated in situ lesions and IHC characteristics: ER, PgR, HER2, Ki67, p53 were recorded, IHC and FISH assessment was performed according to current guidelines. *IHC-based subtype.* Tumour samples were considered Luminal A if ER+, PgR+/- and Ki67 labeling index lower than 14%, Luminal B proliferating if ER+, PgR+/- and Ki67 index more than 14% or Luminal B Her2+ if ER+, PgR+/- and Her2+. ER-, PgR- and Her2- tumour samples were assessed as triple negative (TNBC), while ER-, PgR- and Her2+ tumours as Her2 positive. Clinical, management and outcome information was analysed. *PABC cases.* Those patients were selected as cases whose BC had developed during pregnancy or within a year after delivery. *Control cases.* Each PABC patient was matched by age (± 1 year) and year of first BC diagnosis to a control patient with non-PABC. *Statistics*. Asymmetrical numeric data were analyzed by matched Wilcoxon-test. Categorical data were compared using Chi-square and Fisher's exact test. Overall survival analyses were done using the Kaplan-Meier method. The comparison between survival functions for different strata was assessed with the log-rank statistic. Multivariate analysis of prognostic factors was done using Cox's regression model. Differences were considered significant when $p < 0.05$. All statistical analyses were performed using Statistica 9.0 (StatSoft Inc. Tulsa, OK) software.

RESULTS

Clinicopathological characteristics, outcome and family history data of early-onset breast cancer

Median age at the time of diagnosis in the early-onset BC group was 31.64 years. Histology showed a high incidence of grade III tumors in this group of patients (70 cases, 67.96%), while only four cases (3.88%) were considered grade I. In 29 cases (28.16%) grade II cancer was diagnosed. According to immunophenotypical results, 22 tumors were enrolled in LumA (22.22%), 35 in LumB (35.35%), 9 in HER2+ (9.09%) and 33 in TNBC (33.33%) subgroups.

The control groups had a mean age at time of diagnosis as follows, 36-45 years: 41.95, 46-65 years: 54.53, 66-75 years: 70.09 and over 76 years: 80.66. We have compared tumor characteristics of the women included in the study. Significant difference was detected in the comparison of the subtypes and grades between the early-onset breast tumors and the other age

groups. Pathological "T" status also differed but tumor size measured in mm was similar in the population. Pathological "N" status did not show significant difference.

To assess the prognosis of all the age groups, we have completed Kaplan-Meier analysis. The early-onset group displayed the worse prognosis when compared to others. The detailed questionnaire related to family history was completed and received in 49 cases (45.79%). Analysis of these data revealed positive family history of breast or ovarian carcinoma reported in first and second degree relatives in 25 cases (51.02%). In 13 cases (26.53%) no family history of breast or ovarian carcinoma was mentioned, whereas in 11 cases (22.44%) other types of tumors were mentioned in first and second degree relatives. An increased proportion (52%) of TNBC was observed among younger women with a family history of the disease whereas in the group of patients without family history of breast and ovarian carcinoma the LumA tumours were found in higher percentage (46.15%). *Survival.* Within the early-onset BC group 25 (23.36%) women died. We did not find significant association between OS and negative family history versus a family history of breast or ovarian carcinoma in first and second degree relatives (p = 0.975). No statistically significant differences were observed when comparing OS in patients with negative family history and patients having a family history of other types of tumors (p = 0.188). No significant differences in survival (p=0.188) were detectable between the applied therapies (neoadjuvant chemotherapy or adjuvant chemotherapy). When analysing the subtype of tumors in the group of patients who died (25 patents) we found that the highest number of deaths was observed in the LumB subgroup (36%), followed by the TNBC subtypes (32%), LumA types (20%) and HER2+ cases (12%).

Morphological and immunophenotypical heterogeneity in BC of young and elderly women

Morphologically, relatively homogeneous areas were noticed in 19 cases out of 41 early-onset BC cases (46.3%): the tumor was considered as having only one morphological component. Seventeen (41,5%) tumors showed 2 morphologically different areas. Two tumor samples had 4 regions (4,8%). One tumor sample had 3, another 5 and another 6 components (2.4-2.4-2.4%) based on histomorphology pattern. Twenty of elderly women's BC samples (60,6%) had one, 10 (30,3%) two, and 3 (9,1%) three morpholigally different components. Mean tumor regions of young women was higher then that of elderly women (1.82 vs. 1.48 areas/tumour), and showed increased intratumoral heterogeneity (53,6% vs. 39,4%, p = 0.353).

We have assessed IHC reactions without taking into consideration morphological heterogeneity, then we have compared morphological and IHC regions. In all but one early-onset breast cancer cases morphological and immunophenotypical regions overlapped. (p<0.001). In the latter case same morphological regions have demonstrated IHC heterogeneity of ER; one region was ER negative but another area with the same morphology has shown ER expression of Histoscore 60.

 When assessing IHC; in BC samples of young women ER expression has demonstrated areas with different protein expression in 6 cases (14,6%), with PgR also in 6 cases (14,6%), with Her2 8 (19,5%), and Ki67 14 cases (34,1%). HER2 FISH has demonstrated polysomy in a region of 2 cases. TOP2A gene copy number in all but two cases showed correlation with that of HER2. In BC samples of elderly women ER expression has demonstrated areas with different protein expression in 6 cases (18,2%), with PgR 3 (9,1%), with Her2 0, and with Ki67 7 cases (21,2%). In a HER2 positive case with 2 morphological components one of the regions did not show amplification. In this same case TOP2A gene copy number was normal. Comparing young and elderly women's BC samples HER2 IHC heterogeneity was significantly different (p=0,007). In case of ER- (p=0,681), PgR (p=0,468), p53 (p=0,552) and Ki67 (0,220) tendencies were detected only..

Predicting BRCA mutation status with BRCAPRO

By the end of our study 13 out of 73 patients died of their BC. Our questionnaire was answered by 32 patients, in their families 73 malignancies occurred in first and second degree relatives (median: 2,28 malignancies/family). Eleven patients were assessed as of high risk for BRCA1 mutation, and 2 patients for BRCA2 mutation. According to the archives of the Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary, BRCA genetic testing was performed only in 11 cases out of the 73 patients involved in our study. Amongst the 32 patients who had answered the survey only 5 were tested genetically for BRCA mutation; 3 had BRCA1 mutation and 2 patients tested negative.

BRCA mutation related and claudin-low BC: stepsisters or blood relatives?

In silico analysis. Based on published gene expression data, we have investigated the expression of CLDN1 (ROC=0.785,p=2.6E-4), CDH1 (ROC=0.785,p=2.2E-4), CLDN7 (ROC=0.723,p=8.8E-3), CLDN3 (ROC=0.696,p=0.020) and CLDN4 (ROC=0.685,p=0.027) for the purpose of distinguishing between the BRCAmut vs. BRCAwt BC. When comparing the expression of claudins between the groups it was noted that CLDNs 1, 3, 4, 7, 10 are expressed at higher mRNA levels in the BRCAmut than in the BRCAwt tumors (p=0.339,p=0.062,p=0.126,p=0.082,p=0.784, respectively). We noted that ESR1, ERBB2 were lower (p=0.770,p=0.477, respectively), while EGFR, TP53, VIM and MKI67 showed a tendency towards higher expression (p=0.179,p=0.064,p=0.002,p=0.347, respectively) in BRCAmut vs. BRCAwt cases.

The "PAM50" predictor was applied (modelled in silico) to classify the tumors according to gene expression derived intrinsic subtype, which did not always reflect the subtype of samples previously assigned: 4 of the BRCAmut BLBCs turned out to be Luminal B's, while 1 of the BRCAwt BLBC was Her2-E and 1 of the BRCAwt Luminal case was Basal-like according to our re-classification.

The genes as positive and negative predictors, published as the 9CLCLP previously, were identified in the tumors of the downloaded dataset. Out of the 437 positive predictor and 370 negative predictor genes, 161 and 354 were available for analysis, respectively. The centroid±SD of the positive predictors showed a higher expression tendency in the BRCAwt tumors (2258.73±716.39) than in the BRCAmut cancers (1904.40±544.27), p=0.114; while the negative genes were more likely to be expressed at lower levels in the BRCAwt tumors (1553.60±490.40) than in the BRCAmut cancers (1621.82±360.62), p=0.647. We have found that the group of BRCAmut tumors does not display the claudin-low phenotype, and that BRCAmut tumors do not display a uniform group as compared to BRCAwt tumors based on either the positive or negative predictor genes (Figure S3 and S4), and they do not form a uniform cluster.

Immunohistochemical analysis. In the patient cohort with FFPE samples we have performed immunophenotypical characterization. One Her2+ tumor was found both among BRCA1 and

BRCA2 mutation carriers. All other BRCA2 tumors were Luminal, and majority of BRCA1 tumors were TNBC.

Claudin-1, -3 and -7 were barely expressed in any BRCAmut tumors, while claudin-4 was expressed in 45.5% (10/22) of the tumors as well as E-cadherin with 68.5% (15/22) positive cases (Table 4, Figure 5). None of these markers differed significantly across BRCA1 and BRCA2 tumors (p=1.000,p=1.000,p=0.344,p=0.200,p=0.501, respectively).

Majority of BRCAmut cases did not express CD24 (81.8%, 18/22) with high expression of CD44 (77.3%, 17/22). EZH2 was negative in 5 (22.7%), low in 8 (36.4%) and positive in 9 (40.9%) cases, respectively. Vimentin was mostly negative (10, 45.5%) or low (5, 22.7%) and positive in 7 cases (31.8%). EGFR was negative in 8 (36.4%), low in 5 (22.7%) and positive in 7 (31.8%) cases. Additionally, p53 nuclear positivity was found in 76.2% (16/21) of tumors. Regarding the paternal and maternal alleles, ER and PgR were both lower in the paternally inherited BRCA mutant tumors, than in the maternal ones (p=0.038,p=0.050, respectively).

Upon comparing BRCAmut and BRCAwt tissue samples, we have found that BRCAmut tumors display a lower expression of ER, PgR, and a higher level of EGFR, Her2 and p53. Also, CD24 and CD44 were lower, while EZH2 and vimentin were expressed at a higher level in BRCAmut cases, the ratio of CD24/CD44 was characteristically low/high for these tumors as compared to high/high in BRCAwt. Regarding tight junction molecules and adhesion molecule, claudin-1, -4, -7 and E-cadherin expression was lower and claudin-3 was higher in BRCAmut samples. All markers but claudin-3 were expressed in both wild type and mutant tumors: claudin-3 was not detected in BRCAwt carcinomas.

Correlations. Significant positive and negative correlations were detected between the markers with modest coefficients: strongest positive correlation was noted for CD24 with CD44 and claudin-1; claudin-1 with claudin-4 and Ki67; negatives for E-cadherin with vimentin; and EGFR with ER.

Clinicopathological features and outcome of Pregnancy Associated Breast Cancer (PABC)

Clinical data analysis. Thirty-one BC cases were found to be pregnancy related, 10 manifesting during pregnancy and 21 during lactation or the post-partum period, within 1 year after delivery. Median age at diagnosis was 34 years for the PABC and also for the control group. *Treatment modalities during pregnancy.* Among pregnant patients 3 were diagnosed with breast cancer during the first trimester, 6 during the second trimester and 1 in the third trimester of her pregnancy. Six patients underwent surgery (4 Breast Conserving Surgery-BCS- and 2 Mastectomy, all with axillary lymph node dissection-ALND) while pregnant, followed by adjuvant therapy after delivery in 4 cases. One patient had received neoadjuvant chemotherapy (2xFAC) followed by surgery (Mastectomy with ALND) during her pregnancy and adjuvant therapy after delivery. One patient had surgery (BCS with ALND) followed by adjuvant chemotherapy (6xFEC) while pregnant. *Pregnancy outcome.* One patient underwent induced abortion at 23rd week of gestation, 5 patients elective cesarean section (between the 29th and 34th week of gestation) and 4 patients delivered her baby spontaneously (one preterm delivery). *Treatment modality comparison of PABC and control cases.* There was no significant difference in the modality of surgery (Breast Conserving Surgery versus Mastectomy) between cases and controls. Axillary lymph node dissection (ALND) was the main therapy of choice in both groups. There was no significant difference in neoadjuvant, adjuvant chemo- and radiotherapy frequency or regimen between the PABC and Control group. Adjuvant endocrine therapy was however twice as commonly administered to controls. (p=0,004) *Relapse and survival comparison of PABC and control patients.* Relapse was significantly more common in the PABC group (p=0,003), fourteen (45,2%) patients had systemic relapse against three (9,7%) control patients (p=0,0003). Systemic relapse was the most common in (HR positive, Her2 negative, highly proliferating) LumB prol (6 patients out of 10) and triple negative cases (5 patients out of 15), all these patients died of their disease. Disease free and Overall survival was significantly worse in PABC (p= 0,0004 and p= 0,0007) When Pregnant and post-partum patients' survival data were separately assessed Post-partum patient's group showed significantly worse Disease free (p=0,001) and Overall survival (p=0,00008) compared to controls. Pregnant patients' Disease free survival was inferior compared to controls' (p= 0,007), but Overall survival was not significantly worse. The outcome of PABC was inferior, since thirteen PABC patients (41,9%) died of the disease, 11 patients of them had post-partum breast cancer, while 2 patients (6,5%) died in the control group (p=0,005). *Family history.* There was no difference between the PABC and non-PABC group when Overall and Breast cancer related family anamnesis was evaluated. *Pathological data analysis.* The most common tumor type was high grade invasive ductal carcinoma (IDC) in both groups. There was no significant

difference in tumor T or N stage. When assessing median size of the invasive tumor, if patients were treated with neoadjuvant therapy, tumor size before treatment was considered. Median size of PABC was 24 mm and 22 mm that of controls' (p= 0,13). Notthingham Prognostic Index (NPI) median value for PABC was 6 and 4,65 for the Controls. This finding chategorized PABC as of poor prognosis, while the non-PABC group as of intermediate prognosis (p=0,03). Patients who had complete or partial pathological response after neoadjuvant chemotherapy were excluded from this analysis. Lympho-vascular invasion was common in both groups (no significant difference).

PABC and control cases were ER negative in 58 and 38,8% respectively, the difference in PgR expression was significant: 87,1% of PABC cases while 61,3% of controls did not express PgR (p=0,04). No significant difference was noticed in Her2 status. By Ki67 labeling index, all PABC cases were highly proliferating (p=0,01). There was no difference in p53 positivity. When analyzing IHC-based subtypes, triple negative tumors predominated in PABC (48,4%), followed by LumBprol tumors (32,3%). There were no LumA tumors in this group.

Associated in situ lesions showed significant difference in cases and controls. In 45,2% of the PABC cases, the invasive tumor was associated with high grade Extensive Intraductal Carcinoma Component (also demonstrating comedo necrosis) against 9,7% in controls. Forty-five percent of controls did not have in situ carcinoma associated with the invasive tumor. The predominant in situ lesion of controls was high grade DCIS (22,6%).

On Multivariate Cox proportional model analysis pregnancy related status was associated with survival.

CONCLUSIONS

1. BC affecting very young women (≤ 35 years) has a worse prognosis (23,36% of these patients died of their BC).

2. Biology of early-onset BC is unfavourable (TNBC and LumB IHC-based subtypes predominate).

3. Young women with BC more often have a positive family history of breast and ovarian cancer.

4. Breast cancer of young women with a positive family history is often of the TNBC subtype.

5. To predict BRCA mutation status, a risk-assessment model based on family history and tumor immunophenotype may be used.

6. In the cohort of very young patients involved in our studies the large majority of these women haven't undergone genetic testing for BRCA mutation; this is not in conformity with the current guidelines.

7. BRCA mutation status has a transcription effect on gene expression profiles. (TOP 100 genes' expression profile distinguishes BRCAmut and BRCAwt tumours).

8. BRCA mutation related BC has a special claudin expression profile.

9. BRCA mutation related BC does not show the claudin-low phenotype.

10. The PAM50 single sample predictor-based subtype may differ from that of IHC-based subtype of the same BC sample.

11. Pregnancy associated breast cancer (PABC) has a worse prognosis then non-PABC, especially if manifesting in the postpartum period.

12. The biology of PABC is unfavourable (frequent TNBC and LumBprol IHC-based subtype).

13. Extensive, high nuclear grade DCIS with comedo type necrosis is significantly more frequently found in PABC then in non-PABC.

THESIS RELATED PUBLICATIONS

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1. <u>Madaras L</u>, Kovacs KA, Szasz AM, Tokes AM, Szekely B, Baranyak Z, Kiss O, Kenessey I, Dank M, Kulka J Clinicopathological features and prognosis of pregnancy associated breast cancer: A matched case control study VIRCHOWS ARCHIV 463:(2) p. 248. (2013)

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