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RESEARCH

Significance of Histomorphology of Early Triple-Negative Breast Cancer

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Abstract Triple-negative breast cancer (TNBC) is a heterogeneous disease. Possibly genetic characterisation provides the most appropriate information on tumour biology and prognosis, but it is only limitedly available in clinical practice. The aim of this investigation was to explore what additional prognostic information could be gained from detailed histomorphologic report. Patients and method: patients were selected retrospectively operated from 2005 to 2009 in one institution and charts were revised. Beyond age, tumour and nodal status, histologic grade and therapy, the additional pathologic characteristics were also involved in analysis: necrosis, lymphocytic infiltration, peritumoural vascular invasion (PVI), perineural invasion, DCIS extent and grade, perinodal spread, mitotic activity index (MAI). Results: 295 early TNBC were involved. In univariate survival analysis with a mean follow-up of 3.57 years the tumour size, the nodal status, type of operation (conservation or mastectomy), irradiation, PVI and perinodal spread proved to be significantly connected with both disease free survival (DFS) and breast cancer specific overall survival (BSOS), and necrosis and chemotherapy with BSOS. Necrosis analysed together with lymphocytic infiltrate showed greater predicting power. In multivariate analysis nodal metastasis, necrosis positive/lymphacytic infiltration negative status and lack of irradiation has significant negative impact on DFS (p=<0.0001 HR:1.98 [1.4–2.77], p=<0.017 HR:2.1 [1.1–3.8], p=<0.001 HR:0.25 [0.11–0.57], respectively) and BSOS (p=<0.0001 HR:2.47 [1.8–3.4], p=<0.017 HR:3.7 [1.6–8.2], p=<0.0017 HR:0.24 [0.1–0.58], respectively). For DFS perivascular invasion also showed significant effect (p=<0.042 HR:2.5 [1.0–6.0]). Nodal status was the strongest prognostic parameter but other histomorphologic parameters can be used for prognosis prediction.

Keywords Triple-negative breast cancer · Vascular invasion · Necrosis · Irradiation

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Introduction

Triple-negative breast cancers (TNBC) even in early setting has poor prognosis and it has no accepted targeted therapy so far [1]. The triple-negative subgroup of breast cancers is heterogeneous. In early gene expression cluster analysis five subgroups were established in breast cancer: luminal A and B, Her 2, basal-like and normal breast like [2]. Two of these five subtypes, the basal-like and normal-like show considerable overlap with TNBC tumours. In more recent gene analysis investigators distinguished more, at least five or six subtypes in TNBC with characteristic "driver" signalling pathways and their unique promising targeted therapies [3–5]. Perhaps the genomic characterisation could give the best biologic classification of tumours (i.e. basal-like genotype, different prognostic and predictive platforms) but the cost of genetic testing and the need for validation of tests hinders its use in daily practice.

While gene analysis is not available for most patients great effort has been taken by investigators to distinguish patients with different prognosis even with conventional pathology methods like H&E or immunohistochemistry. It is generally accepted that tumour size and lymph node status have strong prognostic value in this subgroup of disease [1]. On the other hand it is also known from conventional pathology studies that 10 % of TNBCs, which have otherwise unfavourable clinical course, have a much better

outcome. Similarly some rare, special histological type have better (i.e. medullary cancer) or unanimously good prognosis (i.e. adenoid cystic or secretory carcinoma) [6]. Certain immunohistochemical characteristics have been investigated, too with much (basal markers) or less (androgen receptor, BCL-2, claudins, p53, insulin-like growth factor receptor) acceptance as prognostic factor, although their role in prognosis is not completely clear.

As part of general histology report several additional parameters are also described such as the vascular invasion and proliferation activity, although their significance in prognosis is not completely elucidated. In prior studies the prognostic value of peritumoural vascular invasion (PVI) has been detected in node-negative patient population [7, 8] or among early breast cancer patients without adjuvant chemotherapy [9]. It is debated whether there is significant prognostic or predicting role of PVI in the subgroup of triple-negative breast cancer, although recently has been published an analysis of 108 TNBC cases where PVI was independent prognostic factor for survival in node-positive and node-negative patients [10]. The histologic grade and also most of genetic testing for prognostication are strongly connected to proliferation. In spite of this the additional prognostic value of proliferation markers (Ki67, mitotic index, etc.) over commonly used prognostic indices is still unclear [31] and mostly observed in node negative breast cancer patients [11]. Their role in TNBC is waiting for further clarification. In a study with limited cases central fibrosis and lymphocyte infiltration had also prognostic value which was not reproduced in subsequent analysis [3]. The aim of this study was to explore clinical utility in prognostication of histomorphologic characteristics based on retrospective analysis of data from early breast cancer patients.

Patients and Method

In this study consecutive cases have been selected with triple-negative breast cancer (TNBC) operated in the National Institute of Oncology (Budapest) between 01 Jan 2005 and 31 Dec 2009. Only data of patients with early breast cancer with proper resection was accepted. Cases with operation for another breast cancer in the previous 10 years and also cases with relapse between 60 days after primary operation were excluded from analysis (supposing synchron metastasis). Triple-negativity was defined as complete absence of staining for ER and PR and staining null or 1+ for Her2, or 2+ but negative by FISH. Patients' charts have been evaluated retrospectively for clinical history and tumour characteristics in a uniform fashion. Information on therapy, local, regional and distant recurrence has been gained. All pathologic reports were reviewed for T and N status, histologic grade, age, mitotic count per 10 high-power field /MAI/, peritumoural vascular invasion (PVI), necrosis, lymphocytic infiltration, perineural invasion, presence and grade of DCIS, perinodal spread in node positive tumours. The Nottingham Prognostic Index (NPI) has been calculated and used distinguishing 3 subgroups as had been described previously [12]. PVI was defined positive if either blood vessel or lymphovascular invasion was present. Adjuvant chemotherapy and radiotherapy was also incorporated in analysis because it may alter patient outcome and thus prognosis [1, 13]. Disease free survival (DFS) and breast specific overall survival (BSOS) have been investigated in connection with the above mentioned factors. DFS was calculated in months from the date of operation (or date of diagnosis if primer systemic chemotherapy was given) until local or distant recurrence or the last contact of a disease free patient if patient was lost to follow-up. The appearance of operable contralateral breast cancer was not counted for survival analysis since in the 7 cases of contralateral breast cancer in follow-up period survival data was not altered and they were counted as a second primary. BSOS was calculated from the date of operation (or date of diagnosis if primer systemic chemotherapy was given) until documented death caused by breast cancer. The database has been closed on 20 Jan 2011.

The pathology evaluation was in a uniform fashion. The MAI was evaluated as described before [11]. PVI was assessed on H&E stained section and in uncertain cases CD31 (DAKO) staining was used. Necrosis, lymphocytic infiltrate, perineural invasion, perinodal spread in node positive tumours and proportion of DCIS were assessed on H&E stained sections. In pathology reports some parameters were evaluated semi quantitatively by the pathologist. Cases were distinguished where a certain marker was not just present but markedly present. According to it for the first step we retain this distinction using three categories (absent, present, markedly present) in classifying the degree of necrosis, lymphocytic infiltration, PVI and perineural invasion.

Statistical Analysis

Statistical analysis was performed using BMDP Statistical Software (LA, USA,1990). We analyzed the association of different prognostic markers with disease free and overall survival by univariate Kaplan-Meier survival function, multivariate stepwise Cox proportional-hazards regression test and also stepwise discriminate test. Patients lost to follow-up with a follow-up period shorter than 12 month and no progression were excluded discriminant analysis. Correlation of different features was examined by χ^2 test. Estimates were considered statistically significant for two tailed values

of P 0.05. The study was approved by the Institutional Ethics Committee.

Results

Clinical and pathology data of 295 female early breast cancer patients were involved in the analysis. The mean age of patients at the time of diagnosis was 56.3 year, the mean tumour size was 27.4 mm and in 57.5 % of cases no lymph node metastasis was detected. The tumours were predominantly ductal carcinomas (84 %) and other types were diagnosed in much less proportion (lobular:4.1 %, papillary:1.7, medullary:2.4, apocrin:3.4, metaplastic:3.1, adenoid cystic:0.7, neuroendocrin and acinic cell:1-1 %). The basic characteristics of patients, tumours and therapy are shown in Table 1. From these traditional parameters in univariate survival analysis with a mean follow-up of 3.57 years the tumour size, the nodal status (consequently the stage and NPI value), type of operation (conservation or mastectomy) and irradiation proved to be significantly connected with both DFS and BSOS, and chemotherapy with BSOS (Fig. 1). Interestingly age, histologic group and histologic grade showed no significant correlation with prognosis although on the score of age and histology group a tendency could be observed. In the younger age group more recurrences occurred than in older patients (recurrence rate was 31.6 % in 19 patients <35 year and 22.1 % in 72 patients >60 year, $p=0.59$). In the 11 tumours with more favourable histology (group1) there were fewer (only one) recurrences than in group 2 (9 % and 25 %, respectively).

The investigated additional histomorphologic features are summarized in Table 2. In univariate survival analysis PVI and perinodal spread (among patients with lymph node metastasis) had significant impact on both DFS and BSOS, necrosis on BSOS (Fig. 1).

The MAI value characteristically is high in TN tumours. In our cases its mean value was 29.9 and most of the cases fell in high range (84 % of cases >10). Similarly to histologic grade the MAI also did not influenced significantly survival data in our analysis. With different subdivision according to MAI values did not changed this finding (0–9/10–19/≥20 or WHO sorting 0–5/6–10/>10) and even in the lowest range of MAI values the progression rate remained high (3 progression in 6 patients with MAI<3).

PVI was present in 48 % of cases. We defined three subgroups of tumours according to PVI. On the base of pathology report we distinguished cases where PVI invasion was absent, present or extensively present. The presence and extent of vascular invasion were strongly correlated with survival ($p<0001$). With this analysis more extensive vascular invasion correlated with shorter disease free and overall survival (Fig. 1). In correlation analyses vascular invasion was strongly correlated with tumour size ($p=0.0009$) and lymph node status ($p<0.0001$) using χ^2-test.

It is noteworthy that in lymph node positive diseases the perinodal spread had significant prognostic value. The extent of spread seemed to have no prognostic effect.

Surprisingly the more extended operation (ablation vs. conserving surgery) and chemotherapy was accompanied with shorter survival. However both ablation and chemotherapy was correlated with higher stage and chemotherapy with younger age which may explain this phenomenon.

The site of recurrence was in connection with BSOS. The locoregional relapses as first recurrence were mostly operated and connected with better survival but it did not reach the level of significance ($p=0.09$, Fig. 1).

We searched which grouping of pathologic characteristics gives the most in prognostication. According to univariate analysis using three values for necrosis as ‘absent’, ‘present’ and ‘markedly present’ the test gave nearly significant result for BSOS ($p=0.08$). The survival data of patients with necrosis ‘present’ and ‘markedly present’ were very similar, thus a new analysis was performed grouping together the tumours where any necrosis was described (negative vs. positive) . In this second analysis the presence of necrosis gained significant impact on overall survival ($p=0.025$) but not for DFS ($p=0.18$). Similar investigation was performed based on other additional pathologic parameters (lymphocytic infiltration, DCIS, perivascular invasion, perineural invasion) resulting in dichotomic subgrouping. Lymphocytic infiltration (absent/present vs. markedly present) and perineural invasion (absent vs. present) remained insignificant on survival, but DCIS (<10 % vs. ≥10 %) showed significant effect on DFS ($p=0.034$ for DFS, $p=0.079$ for BSOS). There was no correlation between DCIS group and place of recurrence ($p=0.96$). Dichotomic grouping for PVI (absent or present) also demonstrate superior prediction over the three value classification. The dichotomic subgroupig has been used in multivariate analysis in these pathologic characteristics.

The necrosis and lymphocitic infiltration were also evaluated together. The necrosis positive and lympocytitic infiltration negative/scarce (necrosis/lymphocyte positive) vs. necrosis negative and lymphocytic infiltration abundant (necrosis/lymphocyte negative) vs. intermediate group was compared. The results seemed more pronounced than the effect of necrosis ($p=0.12$ for DFS and $p=0.01$ for BSOS) and this grouping was also considered afterwards.

Previously it was observed that the ratio of positive and removed lymph nodes has prognostic value. We made three subgroups from lymph node positive cases (≤0,2 vs. 0,21 to 0,65 vs. >0,65) as it was described before [14] and we analysed its role in lymph node positive patients. In this analysis the lymph node ratio has no significant effect on survival in lymph node positive cases (DFS $p=0.15$, BSOS $p=0.09$).

Table 1 Conventional characteristics of patients and tumours used in prognosis prediction and their relationship with survival in univariate Kaplan-Meier analysis. Histology group 1: ductal, lobular, papillary, apocrin or metaplastic carcinoma. Histology group 2: medullary, adenoid cystic, acinic cell or low-grade neuroendocrin carcinoma

Feature	Proportion (%)	*p* value for DFS (Mantel-Cox)	*p* value for BSOS (Mantel-Cox)
Age (*n*=295)		0.78	0.58
<35y	5.4		
35–55	42.9		
≥55y	51.7		
Histology (*n*=295)		0.23	0.49
Group 1	96.2		
Group 2	3.8		
T status (*n*=261)		<0.0001	0.0002
T1	40.3		
T2	45.9		
T3	9		
T4	4.8		
Nodal status (*n*=286)		<0.0001	<0.0001
N0	57.5		
N1	20.7		
N2	13		
N3	8.8		
Stage (*n*=281)		<0.0001	<0.0001
St1	29		
St2	48.6		
St3	22.4		
Histologic grade (*N*=273)		0.72	0.24
1	1.5		
2	14.3		
3	84.2		
NPI (*n*=244)		<0.0001	<0.0001
<3,4	11.5		
3,4–5,4	70.8		
>5,4	17.7		
Type of operation (*n*=293)		0.0002	0.0002
consevation	32		
mastectomy	68		
Chemotherapy (*n*=290)		0.15	0.008
no	13.8		
yes	86.2		
Irradiation (*n*=248)		0.004	0.0015
no	11.7		
yes	88.3		

Investigating surgical margin a tendency could be observed according to the width of intact surgical margin (<2 mm vs. 2 mm to <10 mm vs. ≥10 mm) but it has not reached the significant level nor in local recurrence rate (p=0.5) nor in distant metastasis occurrence (p=0.66).

Multivariate Cox regression analysis was performed on the 160 cases when all the researched characteristics were available. It gave the result that nodal metastasis, necrosis/lymphacytic infiltration positive status and lack of irradiation has negative impact on DFS and BSOS. For DFS perivascular invasion also showed significant effect (Table 3). Multivariate discriminant analysis has been also performed. This method showed, too that nodal status is the prominent determining parameter for DFS and BSOS, but with less importance PVI and irradiation for DFS and necrosis and iradiation for BSOS had significant effect. Nevertheless these parameters could predict recurrence and

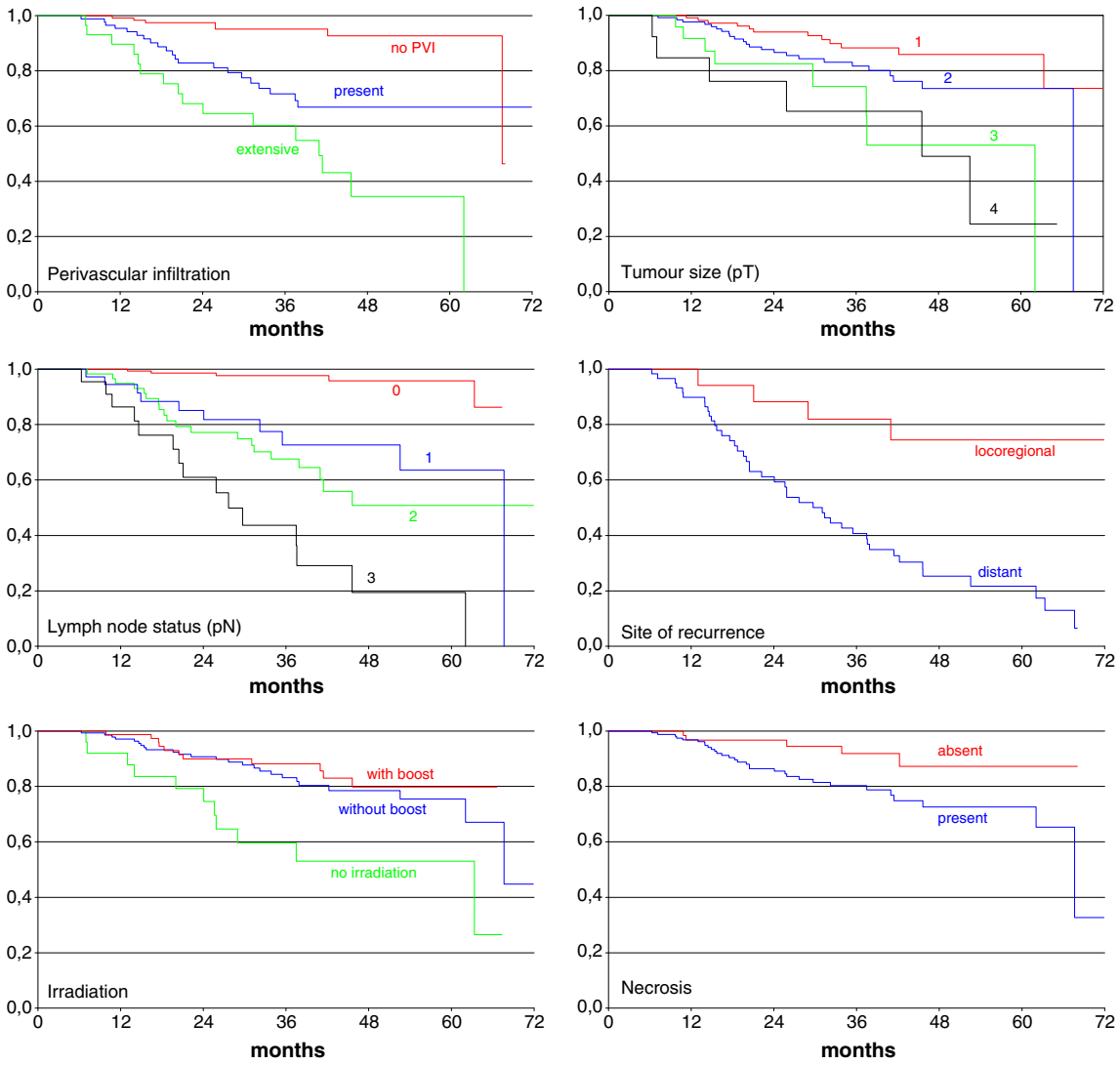

Fig. 1 Kaplan-Meier curves according to BSOS

survival with low accuracy (sensitivity 53 % and specificity 85.5 % for DFS, sensitivity 47 % and specificity 93.8 % for BSOS). However, the disease outcome was not predictable with acceptable accuracy, there are subgroups where good prognosis may be awaited, like tumours with "good hystologic type" (n= 11, only one distant metastasis), low grade (grade 1) tumours (n=4, no relapse) and tumours with abundant lymphocyte infiltration but without necrosis (necrosis/lymphocyte negative, n= 11, one local relapse). On the other hand there are characteristics which may predict outstandingly worse prognosis like extensive lymph node involvment (>9 lymph node) expecially when perinodal spread (recurrance rate 82.4 %, n=17) or necrosis/lymphocytic infiltration positive status (recurrence rate 65 %, n=23) is also present.

The irradiation, the proportion of DCIS and surgical margin were expected to have impact on site of recurrence (local or distant) but it was not the case in this cohort with correlation analysis.

Discussion

In this investigation we searched whether there is a pathology feature based on H&E sections in TNBC that can be used for prognostication besides tumour size and lymph node status. Additionally we searched for a subgroup in TNBC where the prognosis is good and least intensive adjuvant chemotherapy could be given or on the contrary a subgroup where potent adjuvant chemotherapy is highly important.

Investigating optimal characterisation we found that dichotomic grouping give better tool for prognostication in case of irradiation (yes or no), necrosis (yes or no), vascular invasion (yes or no), DCIS (<10 % or ≥10 %) and lymphocytic infiltration (extensive or no/scarce). Connecting necrosis status with lymphocytic infiltrate may ameliorate prognostic effect: necrosis with no or scarce lymphocytic infiltration predict worse outcome than the lack of necrosis accompanied with abundant lymphocytic infiltration.

Table 2 Univariate analysis, additional potential prognostic factors in pathology report (H&E section). The DCIS grade was described only in cases where DCIS was present. Similarly perinodal spread and lymph node ratio (metastatic lymph node/all resected axillar lymph node) could be judged only in lymph node positive cases

Feature	Proportion (%)	*P* value for DFS (Mantel-Cox)	*P* value for BSOS (Mantel-Cox)
Mitotic index (MAI) (*n*=261)		0.15	0.85
0–9	11.6		
10–19 *mitosis/10 NNP*	37		
≥20	51.4		
Necrosis (*n*=233)		0.19	0.025
absent	28		
present	72		
Lymph. Infiltration (*n*=231)		0.34	0.27
absent	18		
present	55		
markedly present	27		
Perivascular invasion (*n*=257)		<0.0001	<0.0001
absent	52		
present	35.5		
markedly present	12.5		
Perineural invasion (*n*=292)		0.07	0.28
absent	85		
present	14		
markedly present	1		
DCIS (*n*=249)		0.13	0.35
absent	49.2		
<10 %	25.8		
10–25 %	16.5		
>25 %	8.5		
DCIS grade (*n*=128)		0.67	0.36
low-grade	26.5		
high-grade	73.5		
Perinodal spread in lymph node positives (*n*=107)		0.01	0.0033
absent	56.6		
present	36.8		
markedly present	6.6		
Lymph node ratio (*n*=100)		0.11	0.09
≤0,2	49		
>0,2 and ≤0,65	27		
>0,65	24		
Surgical margin (*n*=251)		0.9	0.78
<2 mm	23.5		
≥2 mm and <10 mm	56.5		
≥10 mm	20		

In univariate analysis tumour size, nodal status, stage, NPI value, type of operation (conservation or mastectomy), irradiation, vascular invasion and perinodal spread were significantly connected with DFS and the same plus chemotherapy and necrosis (alone or combined with lymphocytic infiltration) for BSOS. In multivariate Cox regression analysis only nodal status, necrosis/lymphocytic infiltration and irradiation has significant effect on DFS and BSOS, although for DFS perivascular infiltration also proved to be important. In multivariate discriminant analysis besides nodal status with less importance PVI and irradiation for DFS and necrosis and irradiation for BSOS showed significant effect.

In previous studies several definitions of triple-negativity were used. The ER threshold for being positive varied from 1–10 % in publications, and the 1 % threshold has been

Table 3 Characteristics with significant impact on survival in multivariate Cox proportional-hazards regression analysis (n=160)

Feature	DFS	BSOS
Nodal status	<0.0001 (HR:1.98 [1.4–2.77])	<0.0001 (HR:2.47 [1.8–3.4])
Necrosis/lymphocytic infiltration	<0.017 (HR:2.1 [1.1–3.8])	<0.017 (HR:3.7 [1.6–8.2])
Irradiation	<0.001 (HR:0.25 [0.11–0.57])	<0.0017 (HR:0.24 [0.1–0.58])
Perivascular invasion	<0.042 (HR:2.5 [1.0–6.0])	

recently recommended [32], however the Her2 status was worldwide clarified according to ASCO/CAP guideline [15]. However, it is shown that using 10 % cut-off value for identifying ER-negative tumours only a tiny proportion of the cases has luminal-B characteristics and this may have no significant effect on prognosis [16]. We used the 1 % threshold for identification of TN diseases, but result from studies using 10 % threshold may have relevant data on TN phenotype, too.

Most of publications studying the connection between TNBC and prognosis searched the significance of triple-negative characteristic in contrast to non-TNBC [13, 17–22]. Most of these publication found that TN characteristic has significant negative effect on prognosis.

The pathologic characterisation of TNBC was quite unanimous in these descriptions. These tumours are more likely ductal carcinomas (NOS), larger primary tumours with more lymph node metastasis and show poorly differentiated histology than the average, although not significantly different from other subtypes with poor prognosis (high grade luminal and Her2 positive tumours).

The characteristics of cases in our cohort were in line with previous results, where TNBCs' dominant histology type is intraductal carcinoma (83–92 %, in this study 84 %), the mean tumour size was 22-31 mm (in this study 27.4 mm) and 30–51 % cases showed lymph node positivity (in this study 42.5 %). The rate of poorly differentiated cancer falls between mostly 76–86 % (in this study 84.2 %). DCIS of 19 % was reported in only one paper (in this study 50.8 %) [3], but chemotherapy and radiotherapy was counted in six previous trials with the rate of 48–92 % and 17–88 %, respectively (in this study 86.2 % and 88.3 %, respectively) [1, 3, 13, 18, 20, 23]. Marked lymphocytic infiltration was present in prior studies in 2–12 % (in this study 27 %), and perivascular invasion in 11–41 % of cases (in this study 48 %) [3, 10, 22–24].

These previous investigations stated that the strongest prognostic factors are the tumour size and lymph node status, although additional variable with significant effect on prognosis can not be established. Only two trials found the grade or mitotic index [17, 21], one the chemotherapy [13] and one the lymphocytic infiltration and central fibrosis [3] as independent prognostic factor. Surely the results of these investigations were influenced by which characteristics were involved in multivariate analysis.

Lin et al. reported on 167 TNBC patients that grade had significant prognostic effect but was maintained only in lymph node positive and not in lymph node negative tumors [17]. Rakha et al. specifically scrutinized the data of 282 triple-negative patients with 1 % cut-off value. They showed that in multivariate analysis involving age, tumour size, lymph node status, androgen receptor and basal phenotype only tumour size and lymph node status keeps its independent prognostic significance in TNBC cases, but grade did not [1]. In our analysis the histological grade and mitotic index also did not have significant effect on survival. Presumably the cause why grade could not be used is that only few patients has low grade tumour in triple-negative subgroup. It must be noted about our cohort that in the four low grade tumours no relapse occurred.

The vascular invasion has been studied previously and it was shown that in TNBC patients the neo-vascularisation is more active with higher level of VEGF and higher microvessel densitiy which may be associated with shorter survival time [25, 26]. In papers focusing on peritumoral vascular invasion it may also have prognostic value [10, 24, 27]. In our analysis the presence and intensity of vascular invasion had strong prognostic effect both on DFS and BSOS in univariate analysis but it was highly correlated with tumour size and lymph node involvement which could be the explanation why it lost its significance on multivariate analyses. Sabatier et al. also report correlation of vascular invasion and lymph node status. In their work age, tumour size and PVI were significant in multivariate analysis for metastasis free survival (MFS) but not the lymph node status. Of note contrary to our study they used threshold of 10 % for ER, applied CD31 and podoplanin staining for every section and have longer follow-up (>5 years) which may cause why PVI had stronger effect on disease outcome than nodal status in their study. The generalized CD31/podoplanin staining has improved PVI detection especially in lymph node negative cases. They reported that lymph node negative/PVI positive patients have worse prognosis than lymph node positive patients (51 % and 63 % 5 year MFS, respectively). On the contrary in our study DFS was in the same groups 75 % and 54.5 %, MFS was 87.5 % and 61 %, respectively after a mean 3.57 years follow-up. In our study vascular invasion in TNBC had limited additional prognostic value.

In retrospective trials lymph node ratio (LNR) also proved to be significant regarding survival [14, 28, 29]

although specifically the TNBC subgroup has not been investigated in this regard. We made a classification for LNR identical as previous studies. In this cohort LNR has no significant effect on survival in the lymph node positive tumours. On the other hand in lymph node positive tumours the perinodal spread was significant again both for DFS and BSOS, but lost its effect in multivariate analysis. However, in node positive cases its presence may predict an especially unfavourable prognosis.

In a much smaller study only large amount of lymphocytic infiltrate and absence of central fibrosis reached statistical significance in multivariate analysis for survival in TNBC cases [3]. In this study we used necrosis which had been described in pathology report substituting central fibrosis. Grouping according to the absence and presence of necrosis it has significant effect only on BSOS in univariate testing which was the same when it was combined with lymphocytic infiltration status. However, in multivariate analysis the necrosis/lymphocytic infiltration became independent prognostic factor in our cohort, too.

Interestingly besides nodal status and necrosis/lymphocytic infiltration status the irradiation was independent predictor of survival. Interestingly it was not connected with site of recurrence (local or distant). Considering that such effect of irradiation in this patient population was not observed previously further exploration is needed to explain this phenomenon.

However, the disease outcome was not predictable with acceptable accuracy, there are subgroups where good prognosis may be awaited, like tumours with "good hystologic type" (n=11), low histologic grade (grade 1) (n=4) or abundant lymphocyte infiltration but without necrosis (necrosis/lymphocyte negative, n=11).

It should be stated that this analysis has shortcomings. It was a retrospective analysis, more pathologist were involved in section analysis without central reviewing which can cause important bias [30]. The fact that the same pathology department worked up the samples with the same platforms, antibodies and standards may attenuate this effect. The follow-up (mean 3.56 years) was rather short comparing with other retrospective trials, although it may be relevant considering TNBC tend to recur in the first three years. Special immunohistochemical staining was not goal of this study and later research in this aspect could give more detailed information on the same patient population.

In conclusion this trial is one of the biggest retrospective analyses on TNBC with detailed assessment of pathologic characteristics. It showed that patient data and H&E stained sections could be used in prognostication even in this patient population. Evaluating new pathologic characteristics (like necrosis/lymphocytic infiltration) may lead us to new independent prognostic factors, but it seemes that further characteristic is yet needed to ameliorate prognostication in TNBC. Hopefully genetic evaluation, grouping on the base of molecular results will explore new prognostic and predicting factors which can be captured in phenotype, too and bring us closer distinguishing tumours which need aggressive chemotherapy, targeted therapy (like PARP-inhibitor or anti-EGFR therapy) or rare cases where we could omit adjuvant therapy.

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