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Original Contribution

Histological subtypes in triple negative breast cancer are associated with specific information on survival

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ABSTRACT

Much research has focused on finding novel prognostic biomarkers for triple negative breast cancer (TNBC), whereas only scattered information about the relation between histopathological features and survival in TNBC is available. This study aims to explore the prognostic value of histological subtypes in TNBC.

A multicenter retrospective TNBC cohort was established from five Dutch hospitals. All non-neoadjuvantly treated, stage I-III patients with estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 negative breast cancer diagnosed between 2006 and 2014 were included. Clinical and follow-up data (overall survival; OS, relapse free survival; RFS) were retrieved and a central histopathological review was performed.

Of 597 patients included (median follow up 62.8 months, median age at diagnosis 56.0 years), 19.4% developed a recurrence. The most prevalent histological subtypes were carcinoma of no special type (NST) (88.4%), metaplastic carcinoma (4.4%) and lobular carcinoma (3.4%). Collectively, tumors of special type were associated with a worse RFS and OS compared to carcinoma NST (RFS HR 1.89; 95% CI 1.18–3.03; $p = 0.008$; OS HR 1.94; 95% CI 1.28–2.92; $p = 0.002$). Substantial differences in survival, however, were present between the different histological subtypes.

In the presented TNBC cohort, special histological subtype was in general associated with less favorable survival. However, within the group of tumors of special type there were differences in survival between the different subtypes. Accurate histological examination can provide specific prognostic information that may potentially enable more personalized treatment and surveillance regimes for TNBC patients.

1. Introduction

All breast tumors are tested for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). These biomarkers have been shown to yield both prognostic and predictive information and are therefore widely used to determine further patient management. About 15–20% [1] of all breast cancers test negative for all three receptors and are referred to as triple negative breast cancer (TNBC). In addition, every invasive tumor is assigned a WHO class of histological subtype [2]. Invasive carcinoma of no special type (NST) is the most common malignant tumor type and accounts for up to 75% of all invasive epithelial breast tumors [2,3]. As many as 47 more subtypes are recognized, which are collectively referred to as 'special subtypes'. These subtypes range from relatively common (e.g. invasive lobular carcinoma) to very rare (e.g. glycogen-rich clear cell carcinoma). It is known that special histological breast cancer subtypes are associated with lack of ER, PR expression and HER2 overexpression [4-9].

TNBC is known to display more aggressive behavior than hormone receptor positive breast cancer. About one third of the patients with TNBC will be faced with a distant recurrence within the first 8 years after diagnosis [10,11]. In the first three years after diagnosis the incidence of recurrences displays a sharp peak, after which the risk of recurrence levels off to that of the average breast cancer population. Reported median survival time for metastasized TNBC is only 9.0 to 13.0 months [11,12], whereas patients with metastasized hormone

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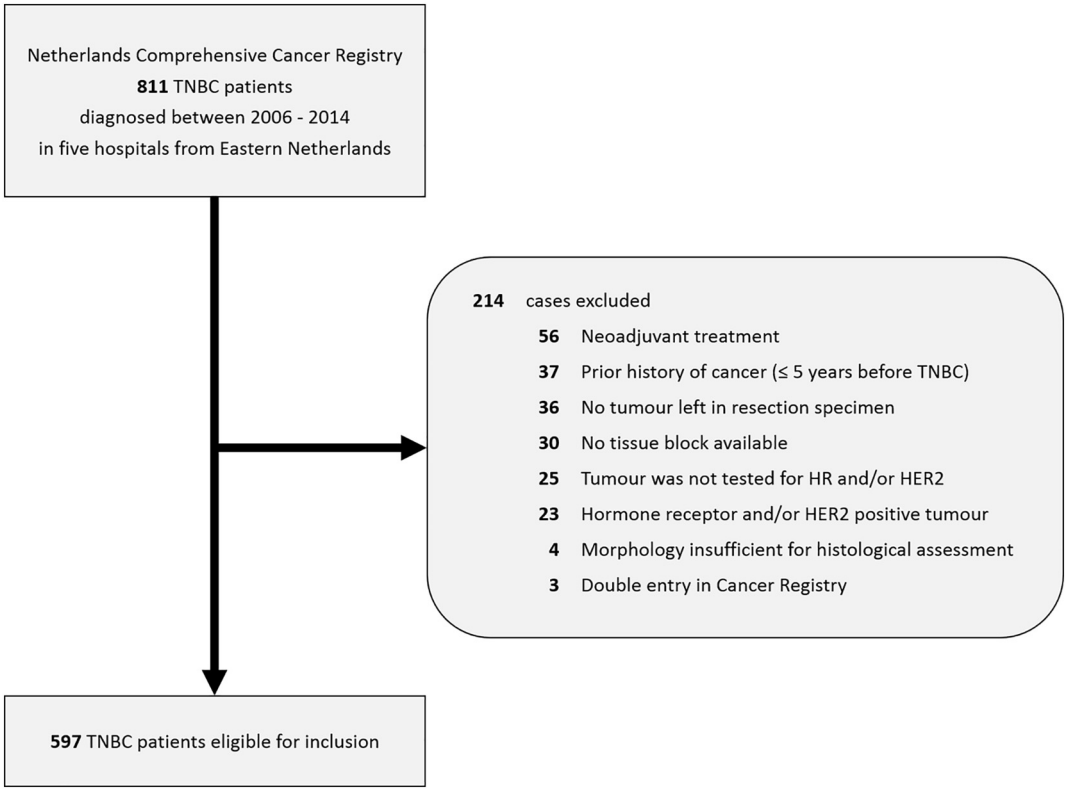

Fig. 1. Flow diagram for the formation of the TNBC cohort.
Flow diagram showing the initial number of 811 triple negative breast cancer patients as retrieved from the Dutch Cancer Registry. After histopathological review and during obtaining archival tissue blocks, 214 cases were removed from the study. Numbers of excluded patients are listed per factor in descending order.

responsive tumors show a median survival of up to 44.0 months [12]. Because of the high risk for recurrence, stage I-III TNBC patients often undergo aggressive (loco)regional and systemic treatment. However, to date it remains unclear which of these TNBC patients will progress to metastatic disease and no biomarkers are available to assess the individual risk for recurrence. Much current research is focused on unravelling molecular features of TNBC to obtain prognostic and predictive starting points for optimized patient care [13-15]. Surprisingly little research on phenotypical heterogeneity as a prognostic marker has been performed by thorough examination of histological morphology.

In the present study, we aim to assess the prognostic value of routinely assessed clinical and histopathological characteristics of TNBC. In addition, we will evaluate the distinct survival characteristics of individual special histological subtypes. For this, a large retrospective Dutch TNBC cohort was set up of which the conventional clinicopathological features will be presented.

2. Material and methods

2.1. Patient selection

Using the Netherlands Comprehensive Cancer Registry (IKNL) (a nationwide registry in which all malignancies in the Netherlands are registered), we set up a multicenter, retrospective cohort study from one academic hospital (Radboudumc, Nijmegen) and four general hospitals (Canisius-Wilhelmina Hospital, Nijmegen; Jeroen Bosch Hospital, 's-Hertogenbosch; Bernhoven Hospital, Uden and Hospital Pantein, Boxmeer) from Eastern Netherlands. The inclusion criteria were as follows. Patients from these hospitals diagnosed with TNBC during the years 2006 until the end of 2014 and who were surgically treated were selected. TNBC was defined as estrogen and progesterone receptor < 10% positive and HER2 negative. HER2 was defined as either a negative in situ hybridization result (no amplification), or negative immunohistochemistry (score 0 or 1+). Cases with a 2+ result on HER2 immunohistochemistry with negative in situ hybridization reflex test were considered HER2 negative [16-18]. In the Netherlands Comprehensive Cancer Registry, every tumor is labelled with a systematic international nomenclature code [19]. This allowed selection of tumors of epithelial origin, which is the focus of the present study. Mesenchymal tumors of the breast (e.g. phyllodes tumors) were not included in this study. Invasive tumors with pathological T-stages 1–4 (i.e. having any size in the excision specimen) were included [20]. All possible pathological regional lymph node stages were included, also cases in which the regional lymph nodes were not assessed (pNx). Patients with distant metastases (stage M1) at time of initial presentation were excluded. Also, patients who were treated with neoadjuvant therapy and patients who were diagnosed with an invasive carcinoma (excluding non-melanoma skin cancers) during the 5 years before they were diagnosed with TNBC, were excluded. Follow up data were retrieved from the Netherlands Comprehensive Cancer Registry (overall survival; OS) and from local patient files (relapse free survival; RFS). The requirement for ethical approval was waived by the institutional review board (case number 2015–1711) of the Radboud University Medical Center (Radboudumc). All patient material and data were treated according to the Code of Conduct for the Use of Data in Health Research [21] and the Code of Conduct for dealing responsibly with human tissue in the context of health research [22].

2.2. Tissue selection

Using the data from the Netherlands Comprehensive Cancer Registry, archival slides were retrieved from each resection specimen from the respective hospitals. All available slides were microscopically inspected by the principal investigator (MCAB) after which one slide per case was selected under the supervision of a pathologist with expertise in breast cancer (PB, CAPW, WV or SJM). This slide was selected

Table 1
Patient and tumor characteristics of the triple negative breast cancer cohort.

	n	%
Sex		
Female	597	100.0
Age, years		
≥50 (50–96)	397	66.5
< 50 (25–49)	200	33.5
Hospital of care		
Radboud University Medical Centre, Nijmegen	97	16.2
Canisius Wilhelmina Hospital, Nijmegen	173	29.0
Jeroen Bosch Hospital, 's-Hertogenbosch	171	28.6
Bernhoven Hospital, Uden	123	20.6
Hospital Pantein, Boxmeer	33	5.5
Primary tumor stage[a]		
T1	316	52.9
T2	257	43.0
T3	19	3.2
T4	5	0.8
Regional lymph node stage[a]		
N0 (including isolated tumor cells)	399	66.8
N1	134	22.4
N2	28	4.7
N3	17	2.8
Nx (regional lymph nodes cannot be assessed)	19	3.2
TNM stage[a]		
I	255	42.7
II	289	48.4
III	53	8.9
Histological type		
Invasive carcinoma of no special type	528	88.4
Special histological subtypes	71	11.6
Histological grade [24]		
1	1	0.2
2	64	10.7
3	532	89.1
Primary treatment		
Mastectomy	219	36.7
Breast conserving surgery	378	63.3
Adjuvant treatment (any)		
No	102	17.1
Yes	495	82.9
Adjuvant radiation therapy		
No	213	35.7
Yes	384	64.3
Adjuvant chemotherapy		
TAC regime	204	34.2
FEC regime	138	23.1
Other regimes	17	2.8
None	238	39.9
Development of recurrence[b]		
No	481	80.6
Yes	117	19.4
Site of initial recurrence		
Brain	20	17.1
Hepatic	15	12.8
Locoregional[c]	31	26.5
Lymphatic	2	1.7
Osseous	11	9.4
Other	4	3.4
Pulmonary	30	25.6
Skin	4	3.4
Deceased (overall)		
No	447	74.9
Yes	150	25.1
Cause of death		
TNBC	71	47.3
Other	31	20.7
Unknown	41	32.0

Abbreviations: TAC: taxotere, adriamycin and cyclophosphamide; FEC: 5-fluorouracil, epirubicin and cyclophosphamide.

[a] Primary tumor stage, regional lymph node stage and TNM stage are classified according to TNM 6th edition [26] for the years 2006 until 2009 and TNM 7th edition [27] was in use from 2010. However, no changes considering the classification of the pathological T-stage and N-stage were made in the TNM 7th edition [27], resulting in comparable stages between the 6th and 7th TNM edition.

[b] The presence of a recurrence was confirmed either clinically (imaging studies) or with additional pathological examination.

[c] Locoregional recurrence: presence of triple negative breast cancer in ipsilateral breast, chest wall, axilla, infraclavicular, supraclavicular or parasternal lymph node region [26,28].

based on the presence of the tumor burden and the presence of a transition from tumor to normal breast tissue (the border of the tumor, often referred to as “invasive margin”) [23]. From each corresponding archival tissue block, a new hematoxylin and eosin (H&E) stained slide was produced and labelled with a study number. All tissue blocks were centrally cut and stained in batches in the laboratory of the Radboudumc. Using the new H&E slides, all tumors were centrally reviewed in the Radboudumc by the principal investigator (MCAB) under the supervision of a specialist consultant in breast pathology (PB) who examined all cases together. Both observers were blinded for any clinical or pathological information. Histological type was defined by using the 4th edition of the World Health Organization's classification system [4] and histological grade by the Nottingham grading system [24]. No immunohistochemical stainings were used to assess histological type or grade.

2.3. Statistical analysis

RFS was defined as the interval between the date of diagnosis of TNBC via core needle biopsy or fine needle aspiration and the date of clinically and/or pathologically detected (loco)regional or distant recurrence of invasive TNBC [25]. The occurrence of hormone receptor and/or HER2 positive breast cancer was considered as a new primary tumor and not as a recurrence. If no recurrence occurred, patients were censured at the date of last follow up. For OS, the interval between date of diagnosis of TNBC and date of death or the date of last follow up was used. To visualize uncorrected associations between survival and histological subtype, tumor stage and lymph node stage, Kaplan-Meier curves with the log rank test were used to compare survival distributions. Univariable Cox regression models were used to calculate hazard ratios over time. For all regression analyses, the proportional hazard assumption was tested and valid. To remain close to breast cancer research practice, we chose to dichotomize age in two classes (under 50 years versus 50 years and older). To allow comparison of hazard ratios of adjacent classes, T2 was set as reference in primary tumor stage and N1 in lymph node stage.

For all analyses, confidence intervals were set at the 95% level and a minimal p value of < 0.05 was considered statistically significant. Analyses were carried out in IBM SPSS version 24.0, Chicago, USA.

3. Results

3.1. Patient demographics and tumor characteristics

From the Netherlands Comprehensive Cancer Registry, a total cohort of 811 patients who underwent surgery for primary breast cancer between 2006 and 2014 in the 5 participating hospitals from Eastern Netherlands was available. After applying the exclusion criteria and after the retrieval of archival tissue blocks, 597 patients were eligible for inclusion (Fig. 1). Characteristics of the included patients and tumors are presented in Table 1. The median age in the cohort was 56.0 years (range 25–96 years, SD 15.2 years). The majority of tumors were grade 3 (89.1%) whereas only one grade 1 tumor (0.2%) was present in this cohort. Fourteen different histological subtypes were present in the cohort, with invasive carcinoma NST (88.4%) being the most prevalent histological subtype. Several rare histological subtypes, such as lipid rich carcinoma and secretory carcinoma were present in small numbers. Fig. 2 shows examples of images of some histological subtypes in the cohort. One hundred and twelve (18.8%) and 135

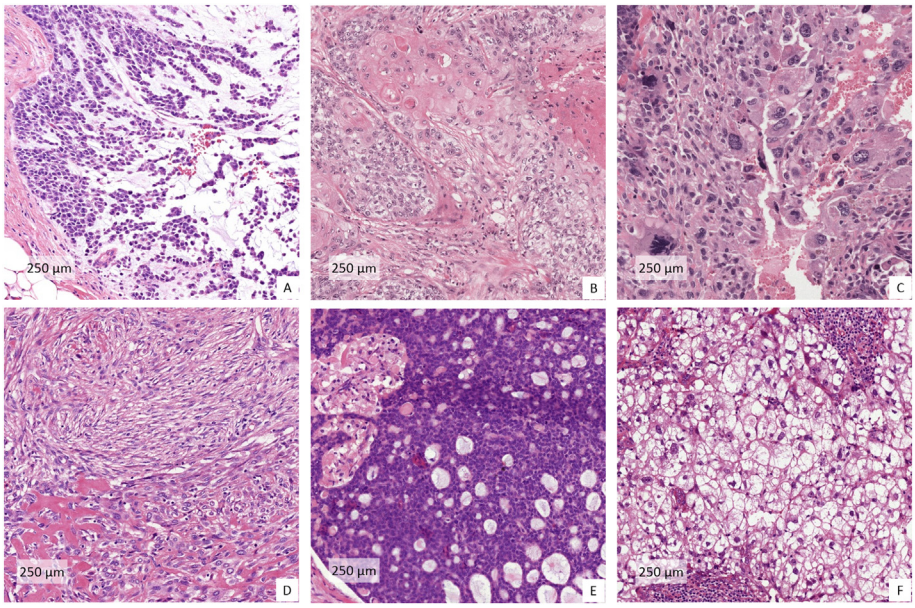

Fig. 2. Examples of different histological subtypes in the TNBC cohort.
A: Metaplastic carcinoma, matrix producing type. The tumor has a circumscribed border with a high density of tumor cells in the periphery. The tumor cells are surrounded by a cartilaginous like matrix. B: Metaplastic carcinoma, squamous type. This tumor showed extended areas of squamous differentiation with accompanying keratinization. Because of the conversion of adenocarcinoma to squamous cell differentiation (elsewhere in the lesion, not shown in image), this tumor was not classified as a pure squamous cell carcinoma. C: Metaplastic carcinoma, mixed type. Image shows a metaplastic carcinoma with choriocarcinomatous like morphology; high grade giant cells with bizarre nuclei are present with haemorrhagic foci in between them. This tumor also showed areas of squamous cell differentiation and of chondrosarcomatous differentiation (not shown in this image). D: Metaplastic carcinoma, spindle cell type. The spindle cells have a storiform appearance. E: Adenoid cystic carcinoma. The tumor shows the characteristic mixture of proliferating epithelial and myoepithelial cells which produce mucinous and basement membrane substance, respectively. F: Glycogen-rich clear cell carcinoma. The tumor grows in sheets with areas of lymphocytic infiltration and focal necrosis in between. The tumor cells have a typical polygonal appearance with clear cytoplasm and distinct cell borders.

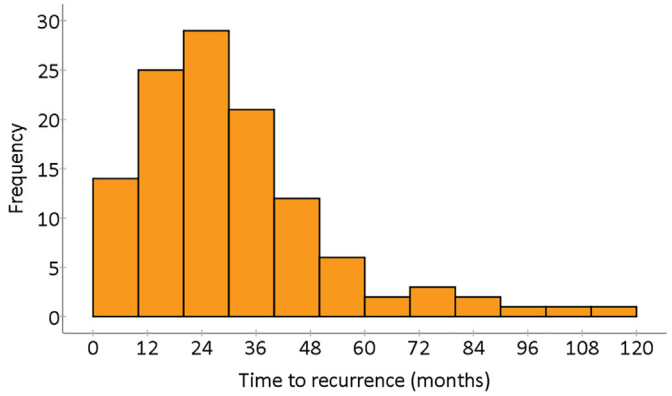

Fig. 3. Time to recurrence for TNBC patients.
Number of TNBC patients who were confronted with a recurrence, clustered per time period after initial diagnosis.

patients (22.6%) received only adjuvant chemotherapy or only radiotherapy, respectively. A group of 102 patients (17.1%) did not receive any form of adjuvant treatment. One hundred and sixteen patients (19.4%) were confronted with a recurrence of TNBC after a median duration of 25.8 months after initial diagnosis (Fig. 3), of which (loco) regional (26.5%) and pulmonary (25.6%) were the most prevalent sites of initial recurrence. Of the patients with a recurrence, 71 patients (60.8%) died from metastatic disease. In total, 150 of the 597 patients (25.1%) of the cohort deceased (age range 32–96 years, median age 66 years, SD 16.1 years) during the follow up period. Median overall follow up duration of the total cohort was 62.8 months (range 1–132 months, SD 34.0 months).

3.2. Associations of clinicopathological features with survival

Baseline univariable analyses showed that a higher tumor stage and lymph node stage were associated with RFS and OS (Fig. 4A–D, Table 2). In addition, higher age was associated with a worse OS (HR 1.74; CI 1.19–2.53; p = 0.004). Histological grade did not show an association with survival. Adjuvant treatment, both (loco)regional radiotherapy and chemotherapy, as well as the combination of both were associated with beneficial hazard ratios for RFS and OS in comparison to no adjuvant treatment after primary surgery. Hazard ratios and confidence intervals of primary tumor stage T4 and histological grade 1 could not be assessed because of too small numbers present in the cohort.

3.3. Survival characteristics of special histological subtypes

Univariable analyses and Kaplan-Meier curves of histological subtypes showed a worse RFS and OS for the group of special histological subtypes as compared to invasive carcinoma NST (Table 2, Fig. 4E–F). Of the 597 patients in the cohort, 71 (11.6%) were assigned to a special histological subtype. Table 3 summarizes survival characteristics for the individual subtypes. In the group of special subtypes, there were in total 22 patients with a TNBC recurrence (31%), whereas in the group of patients with invasive carcinoma NST 95 (19%) developed a recurrence. Of the 20 invasive lobular carcinomas, 8 patients developed a recurrence within a median time to recurrence of 23.7 months. A relative high recurrence rate was noted for patients with metaplastic carcinomas, in particular metaplastic carcinomas with mixed components and matrix producing types.

4. Discussion

In this study, we assessed the prognostic value of clinicopathological features of TNBC. We set up a retrospective, multicenter TNBC cohort and performed univariable survival analysis for the available clinicopathological parameters. In addition, we presented for every histological subtypes in our cohort the survival characteristics. We showed that patients with special histological subtype carcinomas as a group had a higher risk of developing a recurrence. However, within this group of special histological subtypes there were marked differences in survival.

In the most recent (2012) edition of the WHO classification of tumors of the breast [2], the nomenclature of the most prevalent breast cancer type was altered from ‘invasive ductal carcinoma, not otherwise specified (NOS)’ into invasive carcinoma of no special type (NST). Invasive carcinoma NST comprises a morphologically heterogeneous group of tumors, in contrast to all other histological types which show distinctive morphological features by which they are grouped. The

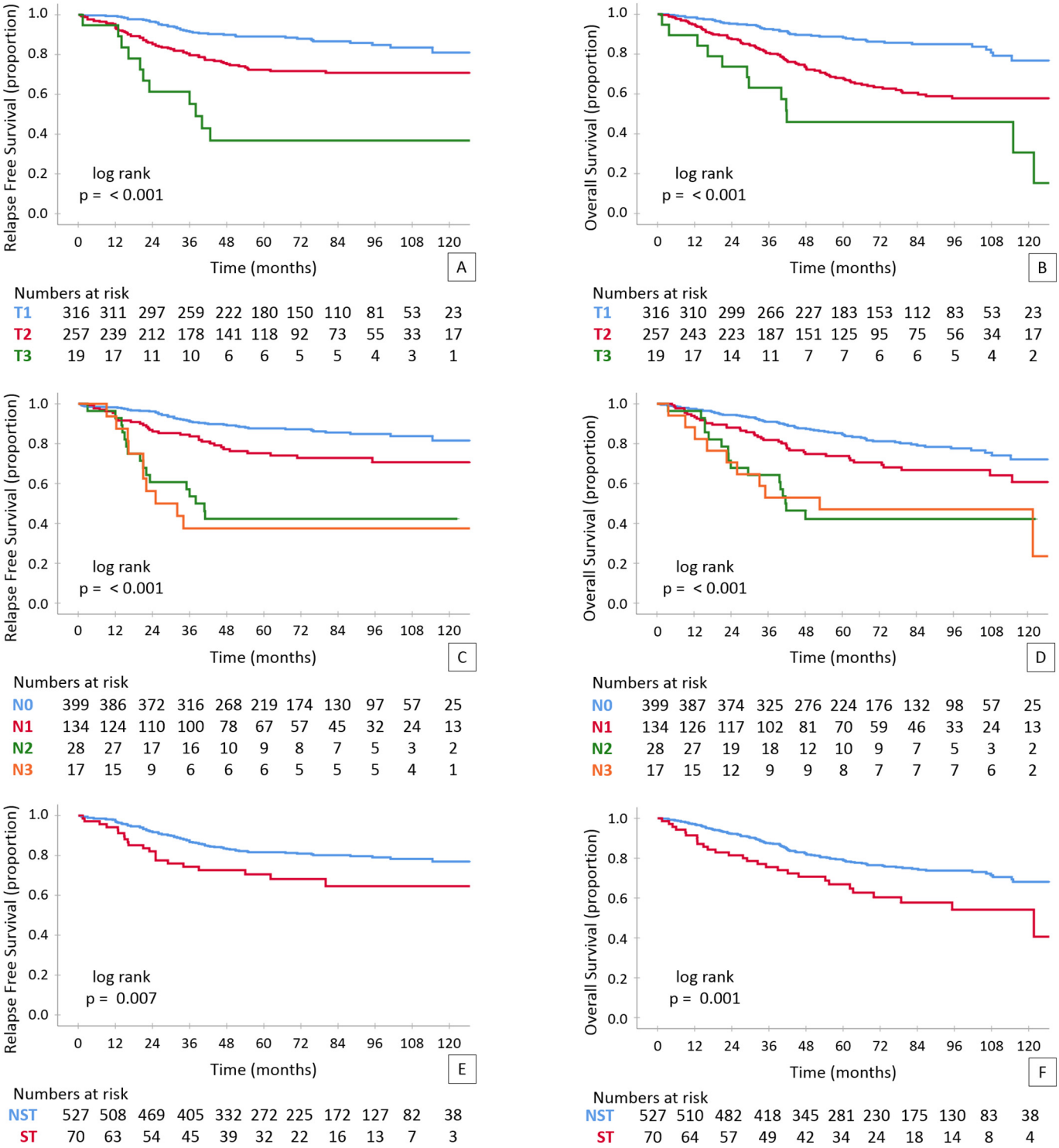

Fig. 4. Kaplan-Meier curves for relapse free survival and overall survival.
Kaplan-Meier curves for relapse free survival and overall survival stratified for pathological primary tumor stage (A, B), lymph node stage (C, D) and for histologic subtypes (E, F). The numbers below the survival curves indicate the numbers at risk with intervals of 12 months.

present study is limited by a confined number of tumors with special histological features, as a result of the low prevalence of these subtypes. This unfortunately hampers more detailed analyses of the prognostic potential of the individual histological subtypes. Although displaying diverse patterns of characteristic histological features, all special histological types were grouped for our univariable analysis, fitting the nomenclature of the dichotomous classification of the prevailing WHO classification [2]. Our results reveal that tumors with a special histological pattern were associated with a worse RFS and OS compared to no special type tumors. However, our results showed marked survival differences exist between the different special histological subtypes.

Only limited research about the relationship between histological subtypes and prognosis in TNBC is available and results are contradictory. In 2013, Montagna et al. [28] published a prospectively collected, single center cohort study in which nearly 800 TNBC were analyzed for recurrence patterns based on histological subtypes. They

Table 2
Univariable analyses for relapse free survival and overall survival for the triple negative breast cancer cohort.

	Relapse free survival HR (95% CI)	p value	Overall survival HR (95% CI)	p value
Age, years				
< 50	1 (ref)		1 (ref)	
≥50	1.07 (0.73–1.58)	0.721	1.74 (1.19–2.53)	0.004
Primary tumor stage				
T1	0.43 (0.29–0.64)	< 0.001	0.36 (0.25–0.51)	< 0.001
T2	1 (ref)		1 (ref)	
T3	2.82 (1.49–5.33)	0.002	2.09 (1.15–3.83)	0.016
T4	NA		NA	
Regional lymph node stage				
N0	0.48 (0.31–0.75)	0.001	0.58 (0.39–0.84)	0.005
N1	1 (ref)		1 (ref)	
N2	2.88 (1.58–5.23)	0.001	2.30 (1.29–4.10)	0.005
N3	3.27 (1.61–6.64)	0.001	2.12 (1.06–4.23)	0.034
Nx	1.76 (0.78–3.99)	0.174	2.24 (1.12–4.50)	0.023
Histological type				
Invasive carcinoma NST	1 (ref)		1 (ref)	
Special histological subtypes	1.89 (1.18–3.03)	0.008	1.94 (1.28–2.92)	0.002
Histological grade				
1	NA		NA	
2	1 (ref)		1 (ref)	
3	1.25 (0.66–2.40)	0.494	0.80 (0.49–1.29)	0.356
Primary treatment				
Mastectomy	1 (ref)		1 (ref)	
Breast conserving surgery	0.52 (0.36–0.75)	< 0.001	0.441 (0.32–0.61)	< 0.001
Adjuvant treatment				
None	1 (ref)		1 (ref)	
Radiotherapy	0.41 (0.23–0.72)	0.002	0.33 (0.22–0.51)	< 0.001
Chemotherapy	0.49 (0.28–0.84)	0.010	0.24 (0.15–0.39)	< 0.001
Radiotherapy and chemotherapy	0.45 (0.28–0.71)	0.001	0.18 (0.12–0.28)	< 0.001

Abbreviations: CI, confidence interval; HR: hazard ratio; NA: not applicable; NST: no special type.

showed that triple negative invasive lobular carcinoma and metaplastic carcinoma were associated with a relative worse RFS and OS compared to invasive carcinoma NST. Two analyses of the Surveillance, Epidemiology and End Results database showed comparable results [29,30]. A recent publication of Leon-Ferre et al. [31] in which a retrospective, single center cohort of over 600 TNBC tumors was studied for clinicopathological features and their impact on prognosis, showed that histological subtype was not associated with survival outcome in TNBC. However, they did not use the dichotomous classification as in our study. In addition, the histological subgroups that were analyzed in this study (invasive carcinoma with apocrine differentiation, invasive carcinoma with medullary features), would be classified as invasive carcinoma NST according to the 4th edition of the WHO edition. TNBC tumors from special histological subtypes were reported to exhibit a favorable prognosis compared to invasive carcinoma NST in the recent study of Urru et al. [32], which explored clinicopathological features of a large retrospective TNBC cohort from Italy.

Some studies suggest that specific histologic TNBC subtypes such as adenoid cystic carcinoma and secretory carcinoma are outliers of the triple negative spectrum because they are associated with a more favorable disease outcome than 'conventional' triple negative breast tumors [31,33,34]. In our opinion, this phenomenon actually reflects the heterogeneity of disease type and disease course, which is one of the most important hallmarks of TNBC. This was demonstrated in our cohort, in which one of the five patients with adenoid cystic carcinoma developed distant metastases. In general, TNBC has a poorer prognosis as compared to hormone receptor positive breast cancer. However, the strong divergence in outcomes, ranging from very poor (i.e. aggressive metastatic disease for every 1 in 4 patients) to very good poses large clinical challenges. Only small studies for rare triple negative histological subtypes are conducted and conflicting results about their prognostic value are reported [7,8,36,37]. Therefore, in contrast to other studies [31,35], in the present study we included all triple negative breast tumors, regardless of histological subtype.

Our results show that within our cohort the administration of adjuvant treatment (even if limited to administering (loco)regional radiotherapy without adjuvant chemotherapy), was associated with an improved RFS and OS. The positive association of radiotherapy on both RFS and OS for breast cancer patients was previously presented in 2014 in a meta-analysis of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG). They found that radiotherapy reduced the risk for recurrence and mortality in breast cancer patients with positive lymph nodes, even when systemic therapy was given [38].

Our study has several strengths. Our cohort was set up of cases from 5 different hospitals, including both academic and general hospitals and all tumors underwent central histopathological review. With a total of almost 600 patients, this cohort is one of the largest and most well defined multicenter TNBC cohorts. This study is limited by the constraints of a retrospective analysis, but a conscientious effort was made to obtain high quality and complete follow up data. Because our study focused on patients who were primarily treated by surgery, patients with higher T and higher N stages might be underrepresented in this study, as these patients are usually candidates for neoadjuvant systemic therapy. The central histopathological review on one H&E section might underestimate intratumoral heterogeneity. During our central review, no immunohistochemical stainings or in situ hybridization assays were used to confirm the triple negative status or to support a diagnosis of specific histological subtypes. In the Netherlands, tumors of which < 10% of the tumor cells stain positive for ER and PR are considered negative. We acknowledge that this is not in line with the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines [39] of 1% for ER and PR positivity. However, a large retrospective study of Yi et al. [40] showed that only 2.6% of the cases in their cohort of 9639 patients from MD

Table 3
Survival characteristics of special histological subtypes in the triple negative breast cancer cohort.

Histological subtype [4]	n total	n recurrence (%)	Median age (range)	Median time to recurrence, months
Invasive lobular carcinoma	20	8 (40%)	64.5 (40–88)	23.7
Metaplastic carcinoma, squamous differentiation	9	2 (22%)	71.0 (47–94)	23
Glycogen rich clear cell carcinoma	7	1 (14%)	56.0 (30–67)	16
Metaplastic carcinoma, spindle cell differentiation	7	2 (29%)	74.0 (63–85)	9.9
Adenoid cystic carcinoma	5	1 (20%)	61.0 (52–65)	2
Malignant adenomyoepithelioma	4	1 (25%)	64.5 (38–82)	12.8
Metaplastic carcinoma, matrix producing type	4	3 (75%)	67.0 (42–84)	34
Metaplastic carcinoma, mixed	4	2 (50%)	58.5 (37–84)	26.9
Medullary carcinoma	3	0 (−)	48.0 (47–70)	–
Metaplastic carcinoma, chondroid differentiation	2	0 (−)	52.0 (36–68)	–
Mixed carcinoma (ductal with other type)	2	1 (50%)	56.5 (44–69)	12
Lipid rich carcinoma	1	0 (−)	53.0 (−)	–
Metaplastic carcinoma, choriocarcinomatous differentiation	1	0 (−)	58.0 (−)	–
Secretory carcinoma	1	0 (−)	52.0 (−)	–
Invasive carcinoma with osteoclast like giant cells	1	1 (100%)	81.0 (−)	1.5
All special subtypes	71	22 (31%)	62.6 (30–94)	24.4 (19.6)
Invasive carcinoma NST	526	95 (19%)	56.0 (25–96)	31.4 (21.8)

Anderson Cancer Center showed 1%–9% estrogen positivity. These so-called borderline estrogen positive patients had a comparable outcome with the estrogen negative patients. In the recent publication of van Maaren et al. [41] on patterns and rates of recurrence for breast cancer subtypes in the Netherlands, patients were classified according to the 10% cut off value of ER and PR. The TNBC patient group showed comparable recurrence rates as in cohorts in which the 1% cut off value is used. Although we underline the importance of applying uniform criteria to classify patients, based on the aforementioned studies we expect that the higher cut off values for ER and PR would not change our results. Despite these limitations, our study is one of the largest and most extensive in-depth exploration of the association between special histological subtypes in TNBC and patient outcome, including the influence of conventional clinicopathological variables on this association.

In this large retrospective cohort study on TNBC, we were able to show that special histological subtypes as a group is associated with a worse RFS and OS, compared to invasive carcinoma NST. However, substantial differences in survival are present between the various special histological subtypes. Accurate histological assessment of tumor type therefore may be important for tailored management of TNBC patients.

Author contributions

MCAB, JAWMvdL and PB designed the study. MCAB performed data acquisition under the supervision of PB, CAPW, WV and SJJM. MCAB and PB performed the histological revision of all tumors. MCAB and JAWMvdL performed the statistical analyses. MCAB and JAWMvdL had full access to all study data and take responsibility for the integrity of the data, the accuracy of the data analysis, and interpretation of data. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

Compliance with ethical standards

The requirement for ethical approval was waived by the institutional review board (case number 2015–1711) of the Radboud University Medical Center (Radboudumc). All patient material and data were treated according to the Code of Conduct for the Use of Data in Health Research [21] and the Code of Conduct for dealing responsibly with human tissue in the context of health research [22].

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Declaration of competing interest

JAWMvdL is member of the scientific advisory boards of Philips, the Netherlands and ContextVision, Sweden and receives remuneration for product development for Philips, the Netherlands. The other authors have no conflicts of interest to disclose.

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