Original Article

Histo- and clinico-pathological analysis of a large series of triple-negative breast cancer in a single center in China: Evidences on necessity of histological subtyping and grading

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Abstract

Objective: To investigate histo-pathological distribution and clinico-pathological significance in a large Chinese triple-negative breast cancer (TNBC) patients serials based on the latest understanding of its clinico-pathological diversity, and to provide more information to clinicians to improve precision of individualized treatment of TNBC.

Methods: A retrospective analysis was performed on patients with TNBC at Breast Disease Center, Peking University First Hospital between January 2010 and December 2019. Histo- and clinico-pathological characteristics were analyzed by Chi-square test and Student's *t*-test, and prognoses were calculated using Kaplan-Meier method and a Cox proportionate hazards model. Bonferroni correction was used to correct for multiple comparison.

Results: Conventional type of TNBC (cTNBC) were identified in 73.7% of 582 TNBC, while special type of TNBC (sTNBC) were 26.3%, including 71 apocrine carcinoma, 20 medullary carcinoma, 31 metaplastic carcinoma, 18 invasive lobular carcinoma, 7 invasive micropapillary carcinoma, 5 adenoid cystic carcinoma and 1 acinic cell carcinoma. Compared to sTNBC, cTNBC was associated with high histologic grade (P<0.001) and lower androgen receptor (AR) expression (P<0.001). TNM stage of low-grade cTNBC was significantly lower than that of high-grade cTNBC (P=0.002). Although no significant difference, there was a trend that the rate of 5-year disease-free survival (DFS) and 5-year overall survival (OS) were longer in high-grade cTNBC than in high-grade sTNBC (P=0.091 and 0.518), and were longer in low-grade sTNBC than in high-grade sTNBC (P=0.051 and 0.350). Metaplastic carcinomas showed larger tumor size (P=0.008) and higher proliferative Ki67 index (P=0.004) than cTNBCs.

Conclusions: Results from our cohort imply that sub-categorization or subtyping and histological grading could be meaningful in pathological evaluation of TNBC, and need to be clarified in more large collections of TNBC.

Keywords: Histological type; prognosis; triple-negative breast cancer; tumor grading

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Introduction

Triple-negative breast cancer (TNBC) is a molecularly and histologically highly diverse group of breast cancer characterized by negative biomarkers estrogen receptor (ER), progesterone receptor (PR), and human epidermal

growth factor receptor 2 (HER2). It represents 10%–20% of breast cancers and in general is associated with more aggressive clinical features, including early onset of metastatic disease, visceral metastases, rapidly progressive disease, short response duration to available therapies and inferior outcomes (1). TNBCs have been challenging due to a considerable disease heterogeneity concerning age of diagnosis, prognosis and response to treatment.

In contrast to hormone receptor positive- and HER2-positive (HER2+) breast cancers for which effective targeted therapies are available, chemotherapy remains the standard of care for TNBC. With rapid advances in molecular studies, the understanding of heterogeneity of TNBC is evolving, which has shed light on its treatment. TNBC can be clustered into at least six subtypes on the basis of gene ontology and expression: basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor (LAR) subtype, and corresponding targets have been explored (2). Meanwhile, *BRCA* and related homologous recombination genes involved in DNA repair have been found to alter in some TNBCs. Owing to these discoveries, numerous ongoing clinical studies are investigating a wide range of potential targets in TNBC including poly-ADP ribose polymerase (PARP) inhibitors, immune check point inhibitors, androgen receptor (AR) targeting agents, and antibody-drug conjugates targeting the AKT pathway. It is conceivable that these novel therapeutic approaches will result in a paradigm shift in TNBC treatment and improve patients' outcome in the future (3).

TNBCs are not only heterogeneous in their molecular characteristics, but also diverse in histo-pathological features. Most of TNBCs are invasive ductal carcinomas with a high-grade histology, i.e. high nuclear grade, brisk mitotic activity, and solid growth pattern with minimal glandular formation. However, some special histological types can belong to TNBC. These types include metaplastic carcinomas, both with high-grade and low-grade features, medullary carcinoma, and apocrine carcinoma. Moreover, TNBCs may present themselves as adenoid cystic carcinoma, secretory carcinoma and acinic cell carcinoma, which belong to the group of salivary gland-like tumors of breast. Although all of these subtypes of tumors account for a relative low percentage in TNBCs group, the diversity of histo-pathological changes in TNBCs suggests that they are associated with more complicated molecular features. Recently, knowledge on histologic spectrum of TNBCs has evolved (4,5). TNBCs could be clarified as conventional type of TNBC (cTNBC) and special type of TNBC (sTNBC), mainly reflecting their deriving cell lineage. And for each of the subtypes, high or low two-tier histological grade is introduced. Both of histological grouping and grading might reflect their clinical behavior or prognosis. Meanwhile, progression from low grade to high grade has been established in some histological subtypes of TNBC.

Based on the latest understanding of clinico-pathological diversity of TNBCs, here we reviewed medical records of TNBC patients in Breast Disease Center, Peking University First Hospital over 10 years, with the goal to investigate the clinico-pathological features of TNBC in a large Chinese cohort, and to provide more information to clinicians to improve precision of individualized treatment of TNBCs.

Materials and methods

Patient information

Breast cancer files of Breast Disease Center, Peking University First Hospital from January 1, 2010 to December 31, 2019 were reviewed. In the consecutive series of 4,748 patients of primary invasive breast cancer diagnosed, 582 (12.3%) TNBC patients were identified and the inclusion criteria were: 1) ER-negative (ER–)/PR-negative (PR–)/HER2-negative (HER2–); 2) tumor size larger than 1 mm; and 3) clinico-pathological data were available. Among 582 TNBC patients, 426 patients had surgical excision specimens in addition to core needle biopsy samples, while other 156 patients had only core needle biopsy specimens for diagnosis in our archives and were transferred to other hospitals for treatment.

This study was a retrospective study, which had been approved by the Ethics Committee of Peking University First Hospital to exempt patient's informed consent. Clinico-pathological data of TNBC patients, including histological subtype, histological grade and score, tumor size, ductal carcinoma *in situ* (DCIS), lymphocytes in tumor stromal, nerve infiltration, tumor emboli, invasion of epidermis, expression of AR, proliferative index Ki67, age, TNM stage and prognosis stage, option and effect of neoadjuvant therapy, were collected. All patients included had been followed up regularly every other year since breast cancer diagnosis. The median follow-up time was 41 months. Overall survival (OS) was defined as the time from the date of initial diagnosis to the date of death from any

cause. Disease-free survival (DFS) was defined as the duration from the date of initial diagnosis to the first detection of breast cancer recurrence or distant metastasis.

Specimen preparation and hematoxylin/eosin (HE) slicing

The protocol of tissue handling was standardized according to recommendations of the 2007 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines (6). The cold ischemia time from tissue acquisition to fixation was as short as possible, limited within one hour. The breast tissue samples were fixed in 10% neutral buffered formalin from at least 6 h to no more than 48 h. The samples were sliced at 5 mm intervals and placed in sufficient volume of neutral buffered formalin. After appropriate gross inspection and sampling, materials were placed in processing cassettes, dehydrated through a serial alcohol gradient, and embedded in paraffin wax blocks. And then sections were stained with HE. Histological subtype and grading were reviewed and distinguished by two experienced pathologists according the World Health Organization (WHO) classification of breast cancer and Nottingham grading (7). Although there was controversy about the histological grade criteria of metaplasitc carcinomas, subtyping of fibromatosis-like and low-grade adenosquamous carcinoma was straightly considered as low histological grade and may have a better clincinal outcome than other types of metaplastic breast cancer (7). Miller-Payne system was used to assess the effect of neoadjuvant therapy (8).

Immunohistochemistry (IHC)

Biomarkers of ER, PR, HER2, proliferative index Ki67 and AR were evaluated in all TNBC. The sections were cut in 4-μm-thick and stained with immunohistochemical stainer Ventana, Bench-mark XT for HER2 and Dako, Autostainer Link48 for other markers. All sections were dewaxed, antigen repaired, incubated at 37 ℃ for 24 min, separated with antibodies ER (1D5, Dako, at 1:50 dilution), PR (636, Dako, at 1:200 dilution), HER2 (4B5, Ventana), Ki67 (MIB1, Dako, at 1:100 dilution), or AR (EP120, GBI) together with the OptiView DAB IHC detection kit, then hematoxylin stained.

Result interpretation: 1) ER and PR: The proportion and intensity of positive staining of tumor cell nuclei were recorded. According the 2010 ASCO/CAP guideline (9), ER or PR was considered positive if ≥1% of tumor cells nuclei were immunoreactive. And then the 2019 update to this guideline had maintained this threshold. In addition, 1%−10% of ER or PR immunoreactive tumor cells should be reported as "Low Positive" (10). And ER or PR was always considered negative if <1% of tumor cell nuclei were immunoreactive in the presence of evidence that the sample can express ER or PR (internal control cells presented and stained as expected). 2) HER2: Appropriate positive and negative controls were used for each run. The normal breast epithelial cells were used as the internal negative control, and tissue microarray of the negative, uncertain and positive cases was used as the external control for HER2. Testing algorithms of HER2 was described with a score of 0 or 1+ interpreted as HER2−, a score of 3+ interpreted as HER2+, and a score of 2+ interpreted as equivocal. Cases between 2010 and 2013 were evaluated according to the 2007 ASCO/CAP guideline (6): 3+, uniform intense membrane staining of <30% of invasive tumor cells; 2+, complete membrane staining either non-uniform or weak in intensity of more than 10% of tumor cells, or rarely intense, complete membrane staining of 30% or fewer tumor cells; 0 or 1+, no staining or weak, incomplete membrane staining in any proportion of tumor cells. While cases between 2014 and 2019 were scored according to the 2013 updated ASCO/CAP guideline recommendation (11). And a positive 3+ HER2/neu reflects a homogeneous, contiguous population and within >10% of the invasive tumor cells. All cases with equivocal for HER2 protein expression were suggested test with fluorescence *in situ* hybridization (FISH) for *HER2* gene amplification. 3) Ki67 was visually scored for percentage of tumor cell nuclei with positive immunostaining above the background level. According to the 2011 St. Gallen International Expert Consensus, IHC surrogates were adopted for molecular classification, and the criteria are as follows: luminal A [ER-positive (ER+), PR-positive (PR+), HER2− and Ki67<14%]; luminal B (ER+, PR+, HER2+/− and Ki67≥15%); HER2-overexpressed (ER−, PR−, HER2+); TNBC (ER−, PR−, HER2−) (12). 4) AR: Expression of AR was described by proportion and intensity of positive staining of tumor cell nuclei.

FISH

Sections were baked overnight at 60 °C. Before hybridization, tissue sections were deparaffinized in xylene and hydrated. The slides were then immersed in 1 mol/L NaSCN at 80 °C for 30 min for pretreatment wash.

Following protease treatment of the slides (0.1 g/L protease solution at 37 °C for 3 min), they were dehydrated in 100% ethanol and air-dried. The HER2 LSI DNA probes, with Spectrum Orange, were combined with Spectrum Green labeled centromeric probe, CEP 17 (Vysis, Abbott). The slides were co-denatured for 5 min at 85 °C and hybridized for 18 h at 37 °C on a ThermoBrite Slide Oven (Abbott). The slides were 4',6-diamidino-2-phenylindole (DAPI) counterstained before being viewed by fluorescent microscope (Axio Imager M2, Zeiss). At least 20 cells were counted by two pathologists for each case. All cases were scored according to the 2018 ASCO/CAP guidelines (13). Group 1 = HER2/CEP17 ratio ≥2.0, ≥4.0 HER2 signals/cell; Group 2 = HER2/CEP17 ratio ≥2.0, <4.0 HER2 signals/cell; Group 3 = HER2/CEP17 ratio <2.0, ≥6.0 HER2 signals/cell; Group 4 = HER2/CEP17 ratio <2.0, ≥4.0 and <6.0 HER2 signals/cell; Group 5 = HER2/CEP17 ratio <2.0, <4.0 HER2 signals/cell. Almost IHC equivocal cases were tested by FISH in our series, so Group 2, 4 and 5 were classified as negative, Group 1 and 3 were classified as positive.

Some other FISH tests were needed for diagnosis of sTNBC. In this series, *MYB/NFIB* translocation was identified for adenoid cystic adenocarcinoma using *MYB* separate probe (Anbiping, Guangzhou, China). FISH signals were analyzed by two pathologists for break-apart signals (orange and green signals for telomeric and centromeric ends of *MYB* gene, respectively). Cells were counted as rearranged when at least one set of orange and green signals were two or more signal diameters apart, or when there was a single orange signal without a corresponding green signal in addition to fused and/or broken-apart signals. Whole section of the tumor was screened initially for signals. At least 100 non-overlapping interphase tumor cell nuclei per case were counted initially. A case was considered positive only if >15 of 100 cells rearranged (14).

Statistical analysis

For clinico-pathological features, continuous variables were compared by Student's *t*-test, and categorical variables were compared using Chi-squared test or Fisher's exact test. The treatment outcomes were measured by OS and DFS using by Kaplan-Meier method, and log-rank test was used to compare difference between two groups. Cox regression models were used to estimate prognostic risk factors. All P-values involved in this study were two-sided. P<0.05 was considered significant, and Bonferroni correction was used to correct P value for multiple comparisons (P=0.05/n). All statistical analyses were performed with the IBM SPSS Statistics (Version 20.0; IBM Corp., New York, USA).

Results

General information

Age distribution of 582 patients with TNBC, whose median age was 54 (range: 24–93) years is shown in *Figure 1*, and all patients were female. Taxane- and anthracycline-based combination chemotherapy was administered to 426 patients as the standard treatment approach, and 190 of them received treatment in a neoadjuvant setting.

Histo-pathological subtypes

Distribution of different histo-pathological subtypes is shown in *Figure 2*. In total, 429 (73.7%) of 582 patients were re-evaluated as cTNBC (namely, invasive breast carcinoma of no special type), and the remaining 153 (26.3%) patients were sTNBC, which included apocrine carcinoma, medullary carcinoma, metaplastic carcinomas, invasive lobular carcinoma, invasive micropapillary carcinoma, adenoid cystic carcinoma and acinic cell carcinoma. In particular, metaplastic carcinoma constituted a group of histo-pathologically distinct tumors, including fibromatosis-like metaplastic carcinoma, spindle cell carcinoma, squamous cell carcinoma, metaplastic carcinoma with heterologous mesenchymal differentiation and mixed metaplastic carcinoma (*Figure 2*).

In the group of cTNBC, 344 (80.2%) patients were high Nottingham histologic grade, 84 cases were of grade 2 and one case was of grade 1. Representative cases were shown in *Figure 3*. The uncommon case of grade 1 cTNBC was initially diagnosed in core needle biopsy. After treatment of neoadjuvant chemotherapy, the tumor almost achieved pathological complete remission evaluated in mastectomy. The histological grade may be underestimated because there were focally intermediate-grade ductal carcinoma *in situ* (DCIS) and few invasive carcinoma in the tumor bed. However, to date, 57 months after the diagnosis, the patient is well with no evidence of this disease.

In the group of sTNBC, apocrine carcinoma presented as large cells with abundant eosinophilic granular cytoplasm and enlarged nuclei with prominent nucleoli (*Figure 4A*). Medullary carcinoma showed high histological grade, pushing margins, syncytial architecture and

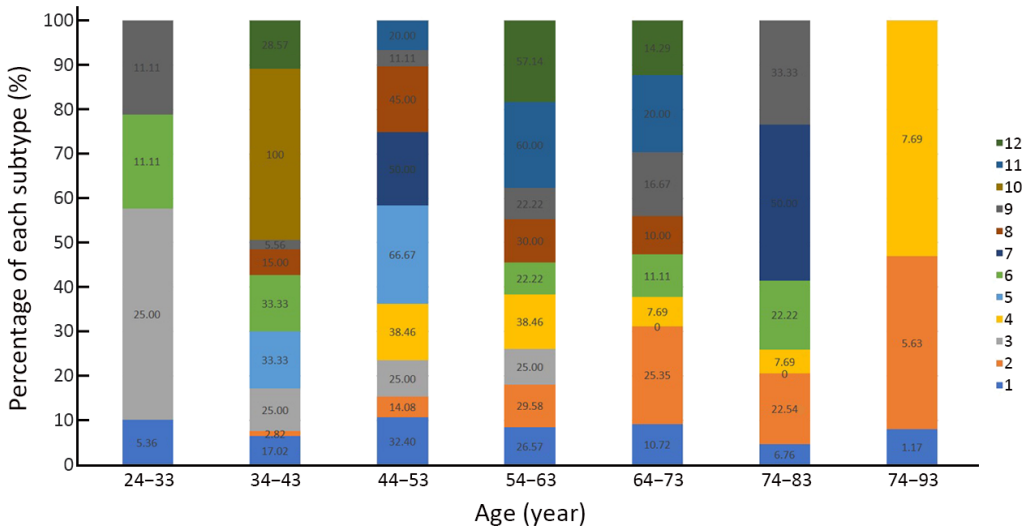

Figure 1 Age distribution of variant subtypes of 582 TNBC patients. 1, cTNBC; 2, apocrine carcinoma; 3, mixed metaplastic carcinoma; 4, squamous cell carcinoma; 5, metaplastic carcinoma with heterologous mesenchymal differentiation; 6, spindle carcinoma; 7, fibromatosis-like metaplastic carcinoma; 8, medullary carcinoma; 9, invasive lobular carcinoma; 10, acinic cell carcinoma; 11, adenoid cystic carcinoma; 12, invasive micropapillary carcinoma. TNBC, triple-negative breast cancer; cTNBC, conventional type of triple-negative breast cancer.

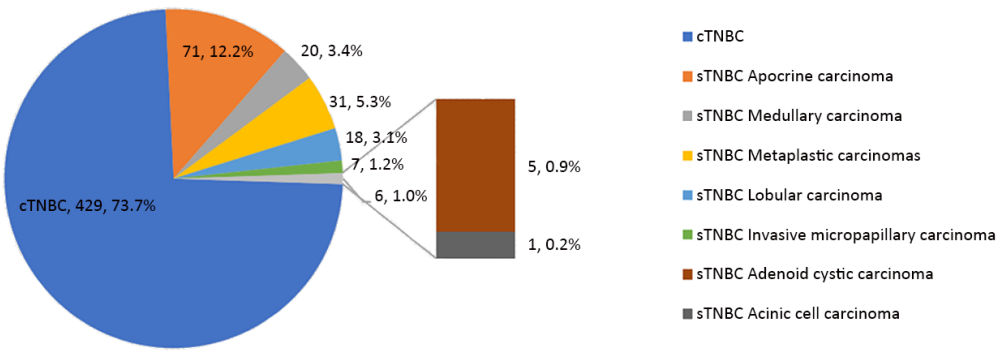

Figure 2 Distribution of different histo-pathological subtypes of TNBC in study series. TNBC, triple-negative breast cancer; cTNBC, conventional type of triple-negative breast cancer; sTNBC, special type of triple-negative breast cancer.

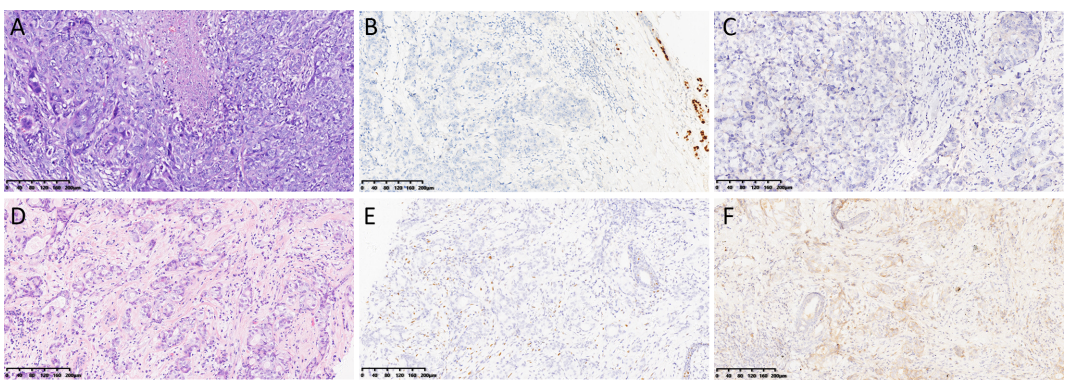

Figure 3 Morphological features and immunohistochemical phenotype of cTNBC. (A) High-grade cTNBC, without ER (B) and HER2 (C) expression; (D) Low-grade cTNBC, without ER expression (E) and with low HER2 expression (F). cTNBC, conventional type of triple-negative breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

prominent tumor infiltrating lymphocytes (*Figure 4B*). Metaplastic carcinomas were heterogeneous, with differentiation of neoplastic epithelium towards squamous cells, spindle cells, fibromatosis-like or heterologous mesenchymal elements (*Figure 4C–F*). In pleomorphic lobular carcinoma, cells were markedly pleomorphic, more than 4 times the size of lymphocytes, and lacked cell-to-cell cohesion (*Figure 4G*). Invasive micropapillary carcinoma cells were surrounded by clear spaces with an inside-out growth pattern (*Figure 4H*). Adenoid cystic carcinomas were composed of epithelial and myoepithelial neoplastic cells arranged in tubular, cribriform, and solid patterns, with basophilic matrix (*Figure 4I*).

Clinico-pathological features

Clinico-pathological features of 582 patients with TNBCs are summarized in *Table 1*. Among them, 23 (4.0%) patients are of grade 1, 149 (25.6%) patients grade 2, and 410 (70.4%) patients grade 3. And 399 patients of TNBC had available data of TNM stage with median tumor size of 1.80 (range: 0.15–14.00) cm. The prevalence rates of TNM stage (1–4) of TNBC patients were 36.8% (147/399), 38.6% (154/399), 5.5% (22/399) and 19.0% (76/399), respectively. Clinico-pathological features of different subtypes of TNBC were also summarized and compared as follows.

Clinico-pathological features of 429 patients with cTNBC and 153 patients with sTNBC were analyzed. Patients with cTNBC were associated with younger age (P<0.001), higher histological grade (P<0.001), more high-grade DCIS (P=0.002) and lower expression of AR (P<0.001). More patients with cTNBC (38.9%, 167 cases) accepted neoadjuvant therapy compared with patients with sTNBC (15.0%, 23 cases) (P<0.001), and cTNBC seemed to have a better response assessed by Miller-Payne System however it did not reach statistical significance (P=0.057).

There were 71 apocrine carcinomas in sTNBC group. Compared with cTNBC, patients of this type (mean age: 64.80 years old) were significantly older (P<0.001), and with lower histological grade (P<0.001), more intermediate grade of DCIS (P=0.001) and high expression of AR (P<0.001). There was no significantly difference in term of TNM stage between apocrine carcinomas and cTNBC.

There were 31 metaplastic carcinomas, which as a group showed larger tumor size (P=0.008), higher Ki67 proliferation index (P=0.004), and higher prognostic stage (P=0.003) compared with cTNBC.

There were 20 medullary carcinomas, whose TNM stage was significantly lower than that of cTNBC (P=0.009), mainly because of lower N stage (P=0.001).

Clinico-pathological features of 172 low-grade (grade 1

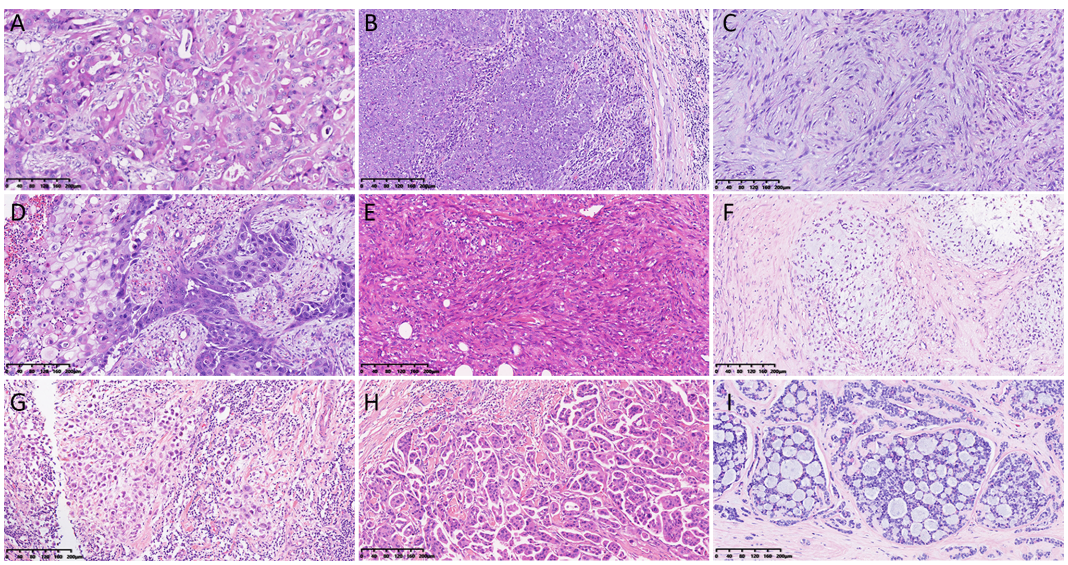

Figure 4 Spectrum of sTNBCs. (A) Breast carcinoma with apocrine differentiation; (B) Breast carcinoma with medullary feature; (C) Fibromatosis-like metaplastic carcinoma; (D) Metaplastic carcinoma-squamous cell carcinoma; (E) Metaplastic carcinoma-spindle cell carcinoma; (F) Metaplastic carcinoma with heterologous mesenchymal differentiation; (G) Pleomorphic lobular carcinoma; (H) Invasive micropapillary carcinoma; (I) Adenoid cystic carcinoma. TNBC, triple-negative breast cancer. sTNBC, special type of triple-negative breast cancers.

Table 1 Comparison of clinico-pathological features of 582 patients with TNBC

Variables	cTNBC (n=429) [n (%)]	sTNBC (n=153) [n (%)]	P	Apocrine carcinoma (n=71) [n (%)]	cTNBC *vs.* apocrine carcinoma	Metaplastic carcinomas (n=31) [n (%)]	cTNBC *vs.* metaplastic carcinomas	Medullary carcinoma (n=20) [n (%)]	cTNBC *vs.* medullary carcinoma
Age (year) *	53 (24–93)	60 (25–88)	<0.001	65 (39–88)	<0.001	55 (27–86)	0.539	52 (39–68)	0.451
DCIS									
Low	10 (6.5)	13 (17.3)	0.002	7 (13.7)	0.001	0 (0)	0.809**	0 (0)	0.318**
Middle	78 (50.3)	45 (60.0)		36 (70.6)		3 (60.0)		2 (28.6)	
High	67 (43.2)	17 (22.7)		8 (15.7)		2 (40.0)		5 (71.4)	
TILs									
Little	59 (28.8)	10 (20.8)	0.330	5 (27.8)	0.062	4 (40.0)	0.307**	0 (0)	0.033**
Middle	35 (17.1)	12 (25.0)		7 (38.9)		3 (30.0)		1 (8.3)	
Large	111 (54.1)	26 (54.2)		6 (33.3)		3 (30.0)		11 (91.7)	
HER2									
0	204 (47.8)	62 (40.8)	0.200	16 (22.9)	<0.001	22 (71.0)	0.028**	14 (70.0)	0.128**
1+	154 (36.1)	57 (37.5)		32 (45.7)		8 (25.8)		5 (25.0)	
2+	69 (16.2)	33 (21.7)		22 (31.4)		1 (3.2)		1 (5.0)	
AR (%)*	10 (0–90)	40 (0–90)	<0.001	63(0–90)	<0.001	12 (0–90)	0.673	19 (0–90)	0.297
Neoadjuvant therapy	167 (38.9)	23 (15.0)	<0.001	7 (10.0)	<0.001	8 (25.8)	0.128	3 (15.0)	0.032**
Tumor regression score									
G1	27 (16.3)	5 (23.8)	0.057	2 (40.0)	0.422	2 (25.0)	0.051**	0 (0)	0.684**
G2	18 (10.8)	3 (14.3)		0 (0)		1 (12.5)		1 (33.3)	
G3	49 (29.5)	5 (23.8)		2 (40.0)		1 (12.5)		1 (33.3)	
G4	24 (14.5)	7 (33.3)		1 (20.0)		4 (50.0)		0 (0)	
G5	48 (28.9)	1 (4.8)		0 (0)		0 (0)		1 (33.3)	
Tumor size (cm)*	1.80 (0.15–14.00)	2.37 (0.20–12.00)	0.491	2.00 (0.33–5.80)	0.157	3.25 (0.20–12.00)	0.008	1.91 (0.70–3.30)	0.107
Grade									
1	1 (0.2)	22 (14.6)	<0.001	13 (18.3)	<0.001	0 (0)	0.726	0 (0)	0.593**
2	80 (18.8)	63 (41.7)		44 (62.0)		4 (13.3)		2 (10.0)	
3	344 (80.9)	66 (43.7)		14 (19.7)		26 (86.7)		18 (90.0)	
Score*	8 (5–9)	7 (3–9)	<0.001	6 (3–9)	<0.001	8 (7–9)	0.118	8 (7–9)	0.338
Duct formation									
1	0 (0)	3 (2.0)	<0.001	3 (4.3)	<0.001	0 (0)	>0.999**	0 (0)	>0.999**
2	12 (2.9)	18 (12.0)		14 (20.0)		0 (0)		0 (0)	
3	407 (97.1)	129 (86.0)		53 (75.7)		30 (100.0)		20 (100.0)	

Table 1 (*continued*)

Table 1 (*continued*)

Variables	cTNBC (n=429) [n (%)]	sTNBC (n=153) [n (%)]	P	Apocrine carcinoma (n=71) [n (%)]	cTNBC *vs.* apocrine carcinoma	Metaplastic carcinomas (n=31) [n (%)]	cTNBC *vs.* metaplastic carcinomas	Medullary carcinoma (n=20) [n (%)]	cTNBC *vs.* medullary carcinoma
Nuclear grade									
1	1 (0.2)	8 (5.3)	<0.001	2 (2.9)	<0.001	0 (0)	0.123**	0 (0)	0.625**
2	233 (55.6)	96 (64.0)		57 (81.4)		11 (36.7)		9 (45.0)	
3	185 (44.2)	46 (30.7)		11 (15.7)		19 (63.3)		11 (55.0)	
Mitotic activity									
1	29 (6.9)	59 (39.3)	<0.001	38 (54.3)	<0.001	1 (3.3)	0.623**	1 (5.0)	0.944**
2	64 (15.3)	35 (23.3)		22 (31.4)		6 (20.0)		3 (15.0)	
3	326 (77.8)	56 (37.3)		10 (14.3)		23 (76.7)		16 (80.0)	
Mitotic count*	25 (0–100)	13 (0–63)	<0.001	7 (0–39)	<0.001	22 (2–63)	0.336	23 (5–38)	0.588
Ki67 (%)*	68 (0–95)	42 (2–95)	<0.001	28 (3–90)	<0.001	56 (15–90)	0.004	72 (20–95)	0.510
TNM									
1	105 (37.8)	42 (34.7)	0.020	25 (42.4)	0.099	6 (23.1)	0.353**	9 (52.9)	0.009**
2	107 (38.5)	47 (38.8)		23 (39.0)		11 (42.3)		3 (17.6)	
3	9 (3.2)	13 (10.7)		5 (8.5)		2 (7.7)		3 (17.6)	
4	57 (20.5)	19 (15.7)		6 (10.2)		7 (26.9)		2 (11.8)	
Prognosis stage									
1	0 (0)	1 (0.8)	0.318	0 (0)	0.120	1 (3.8)	0.003**	0 (0)	0.441**
2	106 (38.3)	48 (39.7)		29 (49.2)		5 (19.2)		9 (52.9)	
3	114 (41.2)	53 (43.8)		24 (40.7)		13 (50.0)		6 (35.3)	
4	57 (20.6)	19 (15.7)		6 (10.2)		7 (26.9)		2 (11.8)	
T									
1	158 (57.0)	61 (50.4)	0.647	36 (61.0)	0.764	8 (30.8)	0.010**	11 (64.7)	0.721**
2	100 (36.1)	50 (41.3)		21 (35.6)		12 (46.2)		6 (35.3)	
3	12 (4.3)	7 (5.8)		1 (1.7)		4 (15.4)		0 (0)	
4	7 (2.5)	3 (2.3)		1 (1.7)		2 (7.7)		0 (0)	
N									
0	176 (64.2)	79 (65.3)	0.429	36 (61.0)	0.825	20 (76.9)	0.489**	12 (70.6)	0.001**
1	83 (30.3)	31 (25.6)		18 (30.5)		6 (23.1)		2 (11.8)	
2	5 (1.8)	5 (4.1)		2 (3.4)		0 (0)		3 (17.6)	
3	10 (3.6)	6 (5.0)		3 (5.1)		0 (0)		0 (0)	
M									
0	279 (79.7)	104 (77.6)	0.431	55 (82.1)	0.161	19 (65.4)	0.626**	17 (89.5)	0.381**
1	71 (20.3)	30 (22.4)		12 (17.9)		9 (34.6)		2 (10.5)	

TNBC, triple negative breast cancer; cTNBC, conventional type of triple negative breast cancer; sTNBC, special type of triple negative breast cancer; DCIS, ductal carcinoma *in situ*; TIL, tumor infiltrating lymphocyte; HER2, human epidermal growth factor receptor; AR, androgen receptor; *, median (range); **, categorical variables were analyzed by Chi-squared test or Fisher's exact test and continuous data were analyzed using the Student's *t* test.

or grade 2) patients and 410 high-grade (grade 3) patients were compared (*Table 2*). Patients with low-grade TNBCs showed significant higher AR expression and more frequent low-grade DCIS compared to patients with high-grade TNBC (P<0.001 and P<0.001, respectively). Higher proportion of high-grade TNBC patients underwent neoadjuvant chemotherapy. Within the cTNBC group, 85 low-grade (grade 1 or grade 2) and 344 high-grade (grade

Table 2 Comparison of clinico-pathological data between different histological grades of TNBC

Variables	n (%)		P	n (%)		P
	LG-cTNBC (n=85)	HG-cTNBC (n=344)		LG-TNBC (n=172)	HG-TNBC (n=410)	
Age (year) [median (range)]	55 (24–93)	53 (24–92)	0.096	59 (24–93)	53 (24–92)	<0.001
DCIS						
Low	5 (14.3)	5 (4.2)	<0.001	18 (20.0)	5 (3.6)	<0.001
Middle	24 (68.6)	54 (45.0)		59 (65.6)	64 (45.7)	
High	6 (17.1)	61 (50.8)		13 (14.4)	71 (50.7)	
TILs						
Little	9 (32.1)	50 (28.2)	0.656	13 (26.5)	56 (27.5)	0.264
Middle	6 (21.4)	29 (16.4)		13 (26.5)	34 (16.7)	
Large	13 (46.4)	98 (55.4)		23 (46.9)	114 (55.9)	
HER2						
0	35 (41.7)	169 (49.3)	0.366	59 (34.7)	207 (50.6)	<0.001
1+	32 (38.1)	122 (35.6)		67 (39.4)	144 (35.2)	
2+	17 (20.2)	52 (15.2)		44 (25.9)	58 (14.2)	
AR (%) [median (range)]	18 (0–90)	8 (0–90)	0.011	36 (0–90)	10 (0–90)	<0.001
Neoadjuvant therapy	26 (31.7)	141 (42.7)	0.079	36 (21.8)	154 (38.9)	<0.001
Tumor regression score						
G1	1 (3.7)	26 (18.7)	0.158*	4 (11.1)	28 (18.5)	0.164*
G2	1 (3.7)	17 (12.2)		1 (2.8)	20 (13.2)	
G3	11 (40.7)	38 (27.3)		13 (36.1)	41 (27.2)	
G4	5 (18.5)	19 (13.7)		9 (25.0)	22 (14.6)	
G5	9 (33.3)	39 (28.1)		9 (25.0)	40 (26.5)	
Tumor size (cm) [median (range)]	1.90 (0.15–8.00)	2.32 (0.20–14.00)	0.133	1.96 (0.15–8.00)	2.42 (0.20–14.00)	0.005
Grade						
1	1 (1.2)	0 (0)	<0.001*	23 (13.9)	0 (0)	<0.001*
2	80 (98.8)	0 (0)		143 (86.1)	0 (0)	
3	0 (0)	344 (100)		0 (0)	410 (100)	
Score [median (range)]	7 (5–7)	8 (8–9)	<0.001	6 (3–7)	8 (8–9)	<0.001
Duct formation						
1	0 (0)	0 (0)	<0.001*	3 (1.8)	0 (0)	<0.001*
2	12 (15.4)	0 (0)		30 (18.4)	0 (0)	
3	66 (84.6)	341 (100)		130 (79.8)	406 (100)	
Nuclear grade						
1	1 (1.3)	0 (0)	<0.001*	9 (5.5)	0 (0)	<0.001*
2	71 (91.0)	162 (47.5)		141 (86.5)	188 (46.3)	
3	6 (7.7)	179 (52.5)		13 (8.0)	218 (53.7)	

Table 2 (*continued*)

Table 2 (*continued*)

Variables	n (%)		P	n (%)		P
	LG-cTNBC (n=85)	HG-cTNBC (n=344)		LG-TNBC (n=172)	HG-TNBC (n=410)	
Mitotic activity						
1	29 (37.2)	0 (0)	<0.001*	88 (54.0)	0 (0)	<0.001*
2	44 (56.4)	20 (5.9)		69 (42.3)	30 (7.4)	
3	5 (6.4)	321 (94.1)		6 (3.7)	376 (92.6)	
Mitotic count [median (range)]	9 (0–60)	28 (2–100)	<0.001	7 (0–60)	27 (2–100)	<0.001
Ki67 (%) [median (range)]	47 (8–95)	74(0–95)	<0.001	36 (2–95)	72 (0–95)	<0.001
Sentinel lymph node metastasis	12 (30.8)	40 (21.5)	0.216	24 (27.3)	46 (20.8)	0.231
Lymph node metastasis	20 (52.6)	72 (48.3)	0.717	39 (53.4)	86 (48.6)	0.578
TNM						
1	19 (36.5)	86 (38.1)	0.002*	46 (38.3)	101 (36.2)	0.025
2	20 (38.5)	87 (38.5)		49 (40.8)	105 (37.6)	
3	6 (11.5)	3 (1.3)		11 (9.2)	11 (3.9)	
4	7 (13.5)	50 (22.1)		14 (11.7)	62 (22.2)	
Prognosis stage						
1	0 (0)	0 (0)	0.341*	1 (0.8)	0 (0)	0.038*
2	19 (36.5)	87 (38.7)		52 (43.3)	102 (36.7)	
3	26 (50.0)	88 (39.1)		53 (44.2)	114 (41.0)	
4	7 (13.5)	50 (22.2)		14 (11.7)	62 (22.3)	
T						
1	34 (65.4)	124 (55.1)	0.304	73 (60.8)	146 (52.5)	0.255*
2	15 (28.8)	85 (37.8)		42 (35.0)	108 (38.8)	
3	3 (5.8)	9 (4.0)		4 (3.3)	15 (5.4)	
4	0 (0)	7 (3.1)		1 (0.8)	9 (3.2)	
N						
0	27 (52.9)	149 (66.8)	0.153*	73 (61.3)	182 (65.9)	0.806*
1	19 (37.3)	64 (28.7)		37 (31.1)	77 (27.9)	
2	1 (2.0)	4 (1.8)		3 (2.5)	7 (2.5)	
3	4 (7.8)	6 (2.7)		6 (5.0)	10 (3.6)	
M						
0	46 (86.8)	176 (75.5)	0.099	106 (86.2)	217 (75.6)	0.018
1	7 (13.2)	57 (24.5)		17 (13.8)	70 (24.4)	

TNBC, triple negative breast cancer; cTNBC, conventional type of triple negative breast cancer; LG, low grade; HG, high grade; DCIS, ductal carcinoma *in situ*; TIL, tumor infiltrating lymphocytes; *, categorical variables were analyzed by Chi-squared test or Fisher's exact test, and continuous data were analyzed using the Student's *t*-test.

3) patients were also compared (*Table 2*). Low-grade cTNBC patients were associated with lower TNM stage (P=0.002) and more frequent low-grade DCIS (P<0.001).

Prognostic analysis

A total of 485 patients including 426 patients of cTNBC and 59 patients of sTNBC had follow-up information with a median time of 36 (range: 1–165) months. And 97 patients were lost to follow-up and handled as censored data. The 5-year OS of all 485 patients was 82.3%, and 5-year DFS was 73.8%. The follow-up information of the subgroups is listed in *Table 3*. Although there were no significant differences in both DFS and OS between patients with low-grade cTNBC and high-grade cTNBC

Table 3 Prognosis comparison among groups of TNBCs

Variablels	DFS [average (range)] (month)	Metastasis [n/N (%)]	5-year DFS (%)	OS [average (range)] (month)	Death [n/N (%)]	5-year OS (%)
All TNBCs	38.32 (0–164)	101/484 (20.9)	73.8	40.79 (1–165)	59/485 (12.2)	82.3
cTNBC	38.51 (0–106)	71/350 (20.3)	75.0	40.72 (1–106)	41/351 (11.7)	83.9
sTNBC	37.81 (0–164)	30/134 (22.4)	70.5	40.97 (1–165)	18/134 (13.4)	76.9
LG-TNBC	40.09 (0–164)	26/141 (18.4)	75.5	42.70 (1–165)	13/141 (9.2)	84.9
HG-TNBC	37.59 (0–106)	75/343 (21.9)	72.9	40.01 (1–106)	46/344 (13.4)	81.0
LG-cTNBC	36.71 (0–97)	12/65 (18.5)	74.4	39.69 (1–97)	4/65 (6.2)	90.9
HG-cTNBC	38.92 (0–106)	59/285 (20.7)	75.0	40.95 (1–106)	37/286 (12.9)	82.3
LG-sTNBC	42.99 (1–164)	14/76 (18.4)	76.7	45.26 (1–165)	9/76 (11.8)	80.0
HG-sTNBC	31.03 (0–82)	16/58 (27.6)	62.2	35.34 (1–86)	9/58 (15.5)	72.4
Apocrine carcinoma	41.87 (1–164)	12/67 (17.9)	72.0	43.99 (1–165)	9/67 (13.4)	76.4
Medullary carcinoma	38.11 (5–73)	2/19 (10.5)	87.5	39.74 (5–73)	0/19 (0)	100.0
Lobular carcinoma	35.56 (0–93)	5/16 (31.3)	64.6	39.81 (3–93)	3/16 (18.8)	70.7
Metaplastic carcinomas	25.31 (0–64)	9/26 (34.6)	54.3	31.81 (1–86)	6/26 (23.1)	55.0

TNBC, triple negative breast cancer; cTNBC, conventional type of triple negative breast cancer; sTNBC, special type of triple negative breast cancer; LG, low grade; HG, high grade; DFS, disease-free survival; OS, overall survival.

(P=0.897, P=0.193, respectively, *Figure 5A*,*B*), the rates of 5-year DFS and 5-year OS were both better in low-grade group than in high-grade group. Similar results were seen in patients with low-grade sTNBC compared with those with high-grade sTNBC (P=0.051, P=0.350, respectively, *Figure 5C*,*D*), and were seen in patients with high-grade cTNBC compared with those with high-grade sTNBC (P=0.091, P=0.518, respectively, *Figure 5E*,*F*). While there was no difference in DFS between patients with AR-positive (AR+) cTNBC and AR-negative (AR−) cTNBC (P=0.814, *Figure 5G*). OS of patients with AR+ cTNBC were both longer than that of patients with AR− cTNBC, regardless of whether ≥1% or ≥10% was considered positive, however there was no statistical significance after Bonferroni correction (P=0.046, *Figure 5H*). DFS and OS of patients with metaplastic carcinoma were shorter than that of patients with cTNBC, while there was no statistical significance after Bonferroni correction (P=0.032 and P=0.072, respectively *Figure 5I*,*J*). There was no significant difference in OS or DFS between patients with medullary carcinoma and those with cTNBC (P=0.124 and P=0.319). The number of adenoid cystic carcinoma and invasive micropapillary carcinoma was too small for comparison. DFS of cTNBC after neoadjuvant chemotherapy was better than that of sTNBC (P=0.031, *Figure 5K*), although no significant difference after Bonferroni correction. There was no difference after neoadjuvant chemotherapy in OS between cTNBC and sTNBC (P=0.495, *Figure 5L*).

In Cox regression models, variables included were age, histological grade, TNM stage, tumor emboli, AR expression and special type of metaplastic carcinoma. Multivariate analysis indicated that neither AR expression nor metaplastic carcinoma was associated with DFS or OS (DFS: P=0.420, P=0.586, and OS: P=0.149, P=0.684, *Supplementary Table S1*).

Discussion

TNBCs show a remarkable diversity of histological patterns and subtypes. The majority of TNBCs (95%) are described as invasive ductal carcinomas of no specific type, but there is a multitude of rare histological special types of breast cancer that are consistently of triple-negative phenotype. For example, medullary carcinoma, various subtypes of metaplastic carcinomas, and adenoid cystic carcinoma are TNBCs. Some TNBCs with distinct pathological features are characterized by low histological grade and an indolent behavior. In a previous analysis of 426 TNBCs for histology, 82% were found to be ductal, 5% lobular, 4% metaplastic, 2.3% medullary, 1.6% apocrine, 0.9% neuroendocrine, 0.5% cribriform and 0.5% mucinous. The 5-year OS rate for ductal TNBC was 62%, and was better for patients with apocrine (100%), medullary (100%) and neuroendocrine (100%) histological types, while worse for patients with papillary (50%) and lobular (68%) histological types (3).

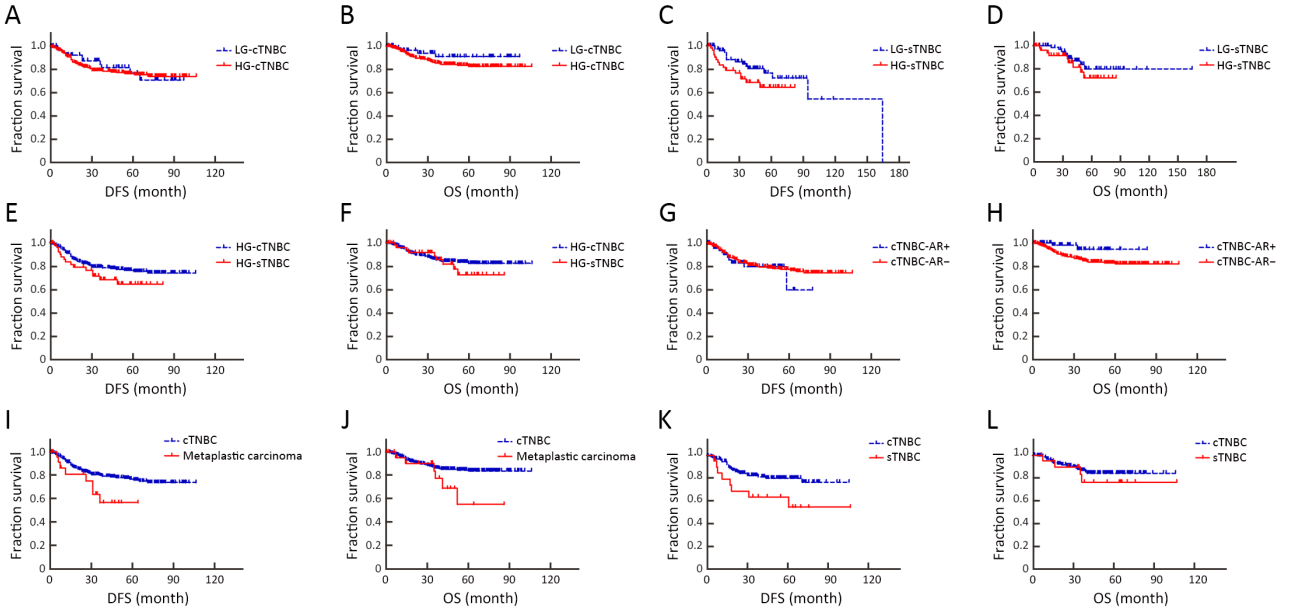

Figure 5 Comparison of DFS and OS between various groups. (A,B) Low-grade and high-grade cTNBC (DFS, log-rank P=0.897; OS, log-rank P=0.193); (C,D) Low-grade and high-grade sTNBC (DFS, log-rank P=0.051; OS, log-rank P=0.350); (E,F) high-grade cTNBC and sTNBC (DFS, log-rank P=0.091; OS, log-rank P=0.518), (G,H) AR+ and AR− cTNBC (DFS, log-rank P=0.814; OS, log-rank P=0.046); (I,J) Metaplastic carcinoma and cTNBC (DFS, log-rank P=0.032; OS, log-rank P=0.072); (K,L) cTNBC and sTNBC patients after neoadjuvant chemotherapy (DFS, log-rank P=0.031; OS, log-rank P=0.495). DFS, disease-free survival; OS, overall survival; cTNBC, conventional type of triple-negative breast cancers; sTNBC, special type of triple-negative breast cancers; AR, androgen receptor.

In total, 4,748 invasive breast carcinomas were identified in our investigation, and 582 (12.3%) were classified as TNBCs, which consisted of 429 (73.7%) patients with cTNBC and 153 patients (26.3%) with sTNBC. Among sTNBC patients, there were 71 (12.2%) apocrine carcinomas, 31 (5.3%) metaplastic carcinomas, 20 (3.4%) medullary carcinomas, 18 (3.1%) lobular carcinomas, 7 (1.2%) invasive micropapillary carcinomas, 5 (0.9%) adenoid cystic carcinomas and 1 (0.2%) acinic cell carcinomas.

The 5-year OS rate for cTNBC and sTNBC was 83.9% and 76.9%, respectively. It was the best for patients with medullary carcinoma (100%), but less favorable for patients with apocrine carcinoma (76.4%), and the worst for patients with metaplastic carcinoma (55.0%). The differences might be associated with cancer staging or treatment.

Among various clinico-pathological factors, histological grade is an important factor in the evaluation of breast cancers. It has been identified as a prognostic factor regardless of tumor size and number of positive lymph nodes (15) and was integrated into tumor staging (16) as one of important biomarkers relevant to prognosis of breast cancer. In this investigation of TNBCs, the histological grade seemed to be associated with prognosis in groups of either cTNBC or sTNBC. It is known that cTNBCs are predominantly of high histological grade, but up to 10% in one study of triple-negative tumors had been shown to be of grade 1 (17). In our data, 344 (80.19%) patients were high histological grade and 85 (19.81%) patients were low grade, including 84 patients of grade 2 and 1 patient of grade 1, and low-grade patients were histologically associated with low-grade DCIS.

There is a multitude of rare histological special types of breast cancer that are consistently of triple-negative phenotype, and this investigation also suggested the importance of subtyping of TNBCs.

Medullary carcinoma is characterized by infiltrating carcinomas with circumscribed pushing borders, high histological grade, syncytial architecture with no glandular structures, a prominent tumor-infiltrating lymphocyte, and a better outcome than other stage-matched high-grade cancers. Most of medullary carcinomas are often triple-negative tumors, however weak hormone receptor expression also occurs in less than 10% of medullary carcinomas (18). In this study, there were 20 (3.4%)

medullary carcinomas, which showed good outcome, and no death happened and the metastasis rate (2/19, 10.5%) was the lowest in the collection of TNBCs.

Metaplastic carcinomas encompass a spectrum of invasive breast carcinomas characterized by differentiation of neoplastic epithelium towards squamous cell and/or mesenchymal-looking components. Histo-pathologically, metaplastic carcinomas constitute a group of distinct patterns, including low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, spindle cell carcinoma, squamous cell carcinoma, and carcinoma with heterologous mesenchymal differentiation. The majority (>90%) of tumors lack expression of ER, PR, and HER2 (19). Although as a group, they display an aggressive clinical behavior, a subset of these cancers are characterized by a low histological grade and a less aggressive clinical course or indolent behavior. In our collection of 31 patients of metaplastic carcinomas, 13 patients of squamous cell carcinoma, 9 patients of spindle carcinoma, 3 patients of metaplastic carcinoma with heterologous mesenchymal differentiation and 4 patients of mixed metaplastic carcinoma were high-grade metaplastic carcinomas, while 2 patients of fibromatosis-like metaplastic carcinoma were considered as low-grade metaplastic carcinoma. As a whole group subtype of sTNBC, DFS after neoadjuvant chemotherapy appeared shorter than that of cTNBC, while OS showed no difference. That result may imply that exploration of genomically characteristics and new target therapies on metaplastic carcinoma could need more attention. However, information offered by small number of cases was limited and additional studies are necessary for further investigation.

Apocrine carcinoma is a rare subtype of invasive carcinoma accounting for about 1% of all breast carcinoma, and it is characterized by large cells with abundant eosinophilic granular cytoplasm and enlarged nuclei with prominent nucleoli, resembling features of apocrine sweat glands. AR is consistently expressed in apocrine carcinoma, which always has a characteristic steroid receptor profile that is ER−, PR−, and AR+. Although studies had reported that AR activation was associated with HER2 overexpression, 40%−70% of cases shown HER2− (20). Some studies identified better OS and breast cancer-specific survival for patients with AR+ triple-negative apocrine carcinoma than with other triple-negative tumors (21). In our study, compared with cTNBCs, there were 71/582 (12.2%) patients of apocrine carcinoma with an older age distribution, and no significant difference in prognosis was found. Most (81.7%) of these patients were histological grade 2 or 3, but with the highest (63.0%) AR expression. The data were similar with reports of other series.

Invasive lobular carcinoma constitutes a histologically and genomically distinct subgroup of breast carcinoma, with loss of functional E-cadherin and the characteristic lack of cohensiveness of tumor cells. The vast majority (80%−95%) of invasive lobular carcinoma express ER and PR, but lack HER2 overexpression, however some may occasionally lack hormone receptor expression and HER2 overexpression. In our study, there were 18 (3.1%) patients of invasive lobular carcinoma of triple-negative phenotype, in which 14 (77.8%) cases were histologically grade 2 or 3, including 5 cases of pleomorphic lobular carcinoma. And the small cohort represented less favorable outcome, just as the observation that triple-negative phenotype predicts the worse prognosis for lobular carcinoma (22).

Moreover, in our study there were 7 cases of invasive micropapillary carcinoma, which showed the highest metastasis rate (50%). However, although this type of tumor usually presents worse local events or node metastasis, their OS is not different from cTNBC or even conventional invasive duct carcinomas.

Although salivary gland-type tumors of the breast described below are known to have a triple-negative phenotype, their inclusion in this group is arguable, given their special differentiation and distinct biological features. Even though rarely occurring, the breast can develop all types of tumors encountered in salivary glands, which share morphological features and even molecular alterations found in their salivary gland counterparts, but their clinical behavior is different and most show low aggressive potential, although high-grade transformation has been described in this group of tumors.

Adenoid cystic carcinoma, a well-characterized salivary gland-type tumors of the breast always with a triple-negative phenotype, accounts for less than 0.1% of breast carcinomas, and typically shows a good prognosis, in contrast to poor long-term outcomes of its head and neck counterparts (23). Five patients of adenoid cystic carcinomas were included in this collection, and patients were with a slightly older age range than cTNBC and a good outcome with no high-grade transformation observed. FISH analysis for *MYB-NFIB* fusions, characteristic molecular alterations in this tumor, was performed in 4 cases and all showed high portions of *MYB* rearranged cancer cells.

Acinic cell carcinoma is a rare subtype of salivary gland-type invasive breast carcinoma always with a triple-negative phenotype, and less than 50 cases have been described in the literature. Although knowledge about prognosis of this tumor is still limited, available data indicate that it is a moderately aggressive tumor with a potential to transform and progress to high-grade TNBCs. In our study, there was only 1 case of acinic cell carcinoma, which did not show associated microglandular adenosis/atypical microglandular adenosis nearby or high-grade transformation that has been described in the literature.

TNBCs constitute one of the most challenging groups of breast cancers in genetic level. As a group, TNBCs have been shown to be characterized by high levels of genetic instability and complex patterns of copy number alterations and structural rearrangements. Unlike other forms of breast cancer, where several genes have been found to be mutated in 10% of cases, the only known cancer genes targeted by somatic mutations in ≥10% of TNBCs are *TP53* and *PIK3CA*. Importantly, however, TNBCs display a great variation in mutational content and complex patterns (24,25). A study of targeted ultra-deep (3,000×) sequencing of 104 TNBCs revealed the conclusions with highly clonal *TP53* mutations present in over 80% of samples and more sub-clonal mutations in PI3K pathway (29.8%, mainly *PIK3CA* mutations), mitogen-activated protein kinase (MAPK) signaling pathway (8.7%) and cell-cycle regulators (14.4%).

In contrast to conventional TNBCs, some low-grade salivary gland-type tumors, for example adenoid cystic carcinoma and secretory carcinoma, always lack recurrent *TP53* aberrations, and display quiet genomes and few copy number alterations, but harbor specific/pathognomonic genetic alterations, underpinned by *MYB-NFIB* and *ETV6-NTRK3* fusion genes, respectively. However, when they transform to the high-grade diseases, progression occurred via acquisition of additional genetic events of complex patterns.

Interestingly, acinic cell carcinoma, another salivary gland-type triple-negative tumor, and some high-grade special histological types of TNBCs, for example apocrine carcinoma and some subtypes of metaplastic carcinoma, display a DNA copy-number and mutation landscape similar to that of TNBCs with conventional histological subtype. Therefore, as other cancers, spectrum of TNBCs represents not only genetic heterogeneity, but also diverse cellular lineages, both determine their subtype and grading.

Therefore, histological subtyping of TNBCs is not a mere academic exercise, given that they fundamentally differ from each other although as a group of TNBCs, and actually imply potential clinical implications.

Conclusions

TNBCs are not only clinically heterogeneous, but also quite histo-pathologically diverse. The present investigation reveals that sub-classification of TNBC into cTNBC and sTNBC categories and their histological grading in parallel suggest potential roles in prediction of clinical outcome. Adenoid cystic carcinomas generally present low grade in histology and have a favorable outcome, while metaplastic carcinoma and invasive lobular carcinomas seem less favorable. AR+ is generally a favorable indicator. Results from our cohort imply that sub-categorization or subtyping and histological grading could be meaningful in pathological evaluation of TNBC, and need to be clarified further in more large collections of TNBC.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Table S1 Multivariate analysis of patients' prognosis with TNBCs

Variables	DFS				OS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)
Age (year)	0.106	1.012 (0.997–1.027)	0.738	1.004 (0.980–1.029)	0.333	1.009 (0.990–1.029)	0.567	1.009 (0.979–1.040)
High histological grade 3	0.227	1.264 (0.864–1.847)	0.229	1.948 (0.658–5.774)	0.196	1.501 (0.811–2.778)	0.175	2.770 (0.635–12.082)
High TNM stage 3–4	<0.001	5.385 (4.072–7.120)	<0.001	52.330 (20.242–135.282)	<0.001	21.229 (9.537–47.255)	<0.001	26.657 (7.689–92.424)
Tumor emboli	<0.001	3.643 (2.227–5.960)	0.986	1.006 (0.513–1.974)	<0.001	5.471 (2.826–10.592)	0.007	4.178 (1.479–11.808)
AR expression	0.566	0.998 (0.992–1.004)	0.420	0.994 (0.981–1.008)	0.138	0.993 (0.984–1.002)	0.149	0.980 (0.954–1.007)
Metaplastic carcinoma	0.037	2.098 (1.046–4.211)	0.586	0.727 (0.231–2.290)	0.080	2.153 (0.913–5.078)	0.684	1.366 (0.304–6.145)

TNBC, triple negative breast cancer; AR, androgen receptor; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.