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PRECLINICAL STUDY

The impact of distinct triple-negative breast cancer subtypes on misdiagnosis and diagnostic delay

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Abstract

Background Triple-negative breast cancer (TNBC) includes mostly aggressive types of breast cancer with poor prognosis. Due to its growth pattern, misinterpretation in clinical imaging is more frequent than in non-TNBC. As the group of TNBC contains heterogeneous types of tumors, marker expression-based subtypes have recently been established. We analyzed clinical features and false-negative imaging findings that could potentially lead to diagnostic delay within the subtypes.

Methods An exploratory analysis compared the imaging features across the a priori defined subtypes and related these findings to molecular subtype, disease stage, potential diagnostic delay, and patient outcome.

Results TNBC cases were categorized into basal-like (BL; 38.6%), mesenchymal-like (ML; 19.9%), luminal androgen receptor (LAR; 28.3%), and immunomodulatory (IM; 13.3%) subtype. In almost every third patient, malignant classification was missed in at least one imaging method. Misclassification in mammogram was more frequent in ML, while benign ultrasound features were reported more often in the BL subtype. Diagnostic delay due to misclassification in imaging led to tumor growth and/or upgrading of the tumor stage in 8.9% of BL tumors, which had the lowest overall survivals. Despite misclassification rate was higher in the ML subtype it showed better outcomes. Misdiagnosis of axillary lymph node metastasis was higher in LAR; however, this subtype showed a higher percentage of affected axillary lymph nodes.

Conclusion TNBC subtypes have different clinical features, benign appearances, and diagnostic delay, which can lead to tumor stage upgrade. Future clinical studies on TNBC outcomes might consider the confounder of clinical delay in the subtypes.

Keywords Triple-negative breast cancer · TNBC subtypes · Imaging features · Diagnostic delay · Breast ultrasound

Introduction

Triple-negative breast cancer (TNBC) includes a heterogeneous group of carcinomas characterized by the lack of both estrogen receptor (ER) and progesterone receptor (PR) expression, and the lack of overexpression and/or amplification of human epidermal growth factor receptor 2 (HER2) [1]. They account for 15–20% of breast cancers and have been associated with young age, BRCA1 germline mutation, and poor prognosis [2]. Pathologic complete response (pCR) to chemotherapy is high in TNBC patients [3]. However, overall survival (OS) and pCR differ clearly within the group of TNBC, which indicates the heterogeneity of this disease [4]. Previous studies have been focused on developing further molecular TNBC subtypes which would potentially correlate with clinical impact [5–7]. Initially, Lehmann et al. identified subtypes of TNBC by gene expression and biological function which are a common basis for TNBC classification today [5]. Further molecular subtyping with overlapping profiles was established by Turner&Reis-Fihlo, with three main molecular/gene expression profiles: basal-like cancers (BL), mesenchymal-like cancers (ML), and luminal androgen receptor cancers (LAR) [8]. A further

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subtype of immunomodulatory cancers (IM) is defined by a considerable lymphocytic infiltration (Table 1) [8, 9]. Despite some promising clinical studies, gene expression and molecular analysis to define TNBC subtypes are not yet routinely used in clinical practice [10].

Earlier studies emphasized the characteristic features of TNBC in clinical imaging, such as more circumscribed, lobulated masses, that may be more frequently misinterpreted as a benign tumor than in non-TNBC [11, 12]. According to the Breast Imaging Reporting and Data System (BI-RADS), the category of "probably benign" (BI-RADS 3) is recommended to have a follow-up interval of 4–6 month [13]. Depending on the patient's compliance, follow-up can be additionally delayed by several months [14]. TNBC tumors are often larger in size at initial diagnosis, due to their mostly aggressive growth pattern [2]. Early detection of these tumors is therefore of utmost importance to improve clinical outcomes. PCR is more likely to be achieved in earlier tumor states, and the rate of radical therapeutic procedures can be reduced [15]. As TNBC subtypes have different prognoses and therapy responses, the current study focuses on their different clinical features, and whether diagnostic misinterpretation leads to delayed treatment initiation [4, 16]. To our knowledge, it is the first study to evaluate imaging features of TNBC and correlate it with molecular subtypes, diagnostic rate, and clinical outcome. Understanding the clinical aspects of the heterogeneous group of TNBC is crucial for future clinical trials and targeted therapy.

Materials and methods

Patients

This observational study includes 166 patients diagnosed with TNBC between 2002 and 2016 in the Breast-Center Zurich, Switzerland. The patients' medical records were reviewed for personal data, clinical symptoms, diagnostic process, and treatment. The median follow-up was 60 months (95% CI 50 to 72 months, inverse Kaplan–Meier method).

Clinical imaging

Diagnostic process and treatment were evaluated retrospectively. All patients were examined by specialized physicians of the Breast-Center Zurich with at least 5 years of experience. Mammography was performed in two standard imaging planes (mediolateral oblique and craniocaudal). For ultrasound performance, the radiologists used 5–12 MHz transducers. Breast density was classified into four categories according to the American College of Radiology (ACR). Sonographic and mammographic masses were categorized by shape, margin, orientation, echo pattern, lesion boundary, posterior features, and vascularity (BI-RADS classification) [17]. Standard two-view mammography was performed and evaluated by two specialized radiologists.

Histological diagnosis

Surgical specimens were processed as formalin-fixed, paraffin-embedded tumor tissues according to a standardized protocol of the Department of Pathology and Molecular Pathology, University Hospital Zurich, Switzerland. TNBC was defined as lacking the expressions of ER and PR (< 1% by immunohistochemistry) and for Her2 (assessed by immunohistochemistry and/or by fluorescence in situ hybridization). In cases of pCR ($n = 8$), initial biopsy specimen was submitted for subtyping. Immunostains were performed in the Laboratory of Special Technics in the Department of Pathology and Molecular Pathology, University Hospital Zurich, using standardized procedures, ready-to-use antibodies, and the automatic Benchmark staining machines.

TNBC subtyping

Molecular subtyping was carried out using a panel of antibodies based on the recommendations of Lehmann et al. and Turner et al. (Table 1) [5, 8]. Subtypes were identified as follows: basal-like: preferential expression of basal cytokeratins (CK5/6); immunomodulatory: presence of abundant intratumoral lymphocytes; mesenchymal-like: preferential

Table 1 TNBC subtypes adapted to Turner et al. [8]

TNBC-subtype	Molecular features
Basal-like (BL)	Basal keratin expression
	DNA damage repair pathway
	Cell cycle-related genes
Immunomodulatory (IM)	Encoding immune antigens, cytokine, and core immune signal transduction pathways as a consequence of lymphocytic infiltration
Mesenchymal-like (ML)	Genes involved in epithelial–mesenchymal-transition and growth factor pathways
Luminal androgen receptor (LAR)	Luminal gene expression and androgen receptor

expression of PIC3kinase; luminal androgen receptor: predominant expression of androgen receptors. Subtyping was done immunohistochemically using a semi-quantitative scoring system from 0, +, ++, and +++.

Statistics

This was an exploratory analysis on observational data collected in routine clinical practice. The descriptive analysis used mean (standard deviation) or median [IQR] for continuous variables and number (percentage) for categorical variables. To enhance interpretation, stacked bar plots were used to visualize some categorical variables. The χ^2 test and Fisher's exact test were used to test for differences in proportions across subtypes. Differences in center were analyzed using the Kruskal–Wallis rank-sum test. Benign/malignant classification rates by mammogram and ultrasound were compared using odds ratios and further summarized by *p*-values from McNemar's test for matched data. Overall survival and disease-free survival were visualized with Kaplan–Meier plots and differences across groups tested with the log-rank test. All analyses were performed in the R programming language.

Results

Patient and tumor characteristics

Among our study cohort of 166 patients, mean age was 57 years (14 years), and a majority of patients were postmenopausal (65.7%, $n = 109$) (Table 2). Sixty-four cases were classified as BL subtype (38.6%), 33 (19.9%) as ML, 47 (28.3%) as LAR, and 22 (13.3%) as IM. For patients primarily treated with surgery or with sentinel lymph node excision prior to chemotherapy ($n = 129$), LAR subtype tumors had the highest rate of histopathologic affected lymph nodes (18/37, 48.6%) versus 15/52 (28.8%), 2/12 (16.7%), and 6/28 (21.4%) in BL, IM, and ML, respectively (p value = 0.065), despite these tumors more often appeared well differentiated (14/47, 29.8%, p-value < 0.012). Overall, the HE morphology was of no special type (NST or ductal according to the latest 2012 WHO classification of breast tumors, 126/166, 75.9%), except the lymphogenic morphology of IM (22/22, 100%). The mesenchymal subtype presented metaplastic morphology in 10/33 patients (30.3%) and no special type otherwise. Most patients (132/166, 79.5%) were treated with a standard taxane and anthracycline-based adjuvant or neoadjuvant chemotherapy. In 43 patients who were treated with neoadjuvant chemotherapy, eight patients (18.6%) showed pathologic complete remission (pCR).

Clinical imaging and BI-RADS classification

For comparison of diagnostic classification by imaging methods, eight of the total 166 patients were excluded because one imaging technique was not used prior to the histopathologic diagnosis, and three patients were excluded because they declined a mammogram. To exclude the confounder of very small tumors, four patients with tumors less than 1 cm were also excluded. Observations from 151 patients were used to compare the diagnostic imaging features. At initial diagnosis, median tumor size of TNBC in clinical imaging was 20 mm with no evidence of significant differences across the subtypes. The percentage of images determined to contain suspicious lymph nodes varied from 30.0% in IM to 45.2% and 45.5% in ML and LAR, but these differences were statistically insignificant (Table 3). Breast density was heterogeneously distributed in all subtype groups. In 44 women (29.1% out of 151), at least one of the diagnostic methods missed the malignant diagnosis (Table 3 and Fig. 1): in 24 patients (15.9%), mammogram resulted in a benign classification, whereas ultrasound criteria were malignant, in 16 patients (10.6%), both mammogram and ultrasound classifications were benign, and in four cases (2.6%), mammogram was classified as suspicious, whereas ultrasound was classified as benign. Figure 1 shows the largest percentage for each subtype accounts for joint malignant classification by the methods. Per subtype, ultrasound catches 10.0% (IM) to 19.4% (ML) of cases missed by mammogram, while mammogram only detects between 0% (IM, ML) and 5.4% (BL) of cases missed by ultrasound. Odds ratio point estimates indicate that being classified as malignant is less likely with mammogram than with ultrasound, but the confidence intervals indicate that there is not enough evidence to call the difference significant. This is also reflected by McNemar test results summarized by *p*-values (Table 4).

Diagnostic delay and outcome

Delay in diagnosis was reported in 13 cases in total (8.6%), mainly in cases with simultaneous misclassification in both diagnostic methods ($n = 7$) and distributed to 1 of 20 IM cases (5%), 3 of 44 in LAR (6.8%), 5 of 56 in BL (8.9%), and 4 of 31 in ML (12.9%). Delayed diagnosis caused a tumor stage upgrade in 8 cases. The mean diagnostic delay due to misclassification in ultrasound was 2.2 months (SD 3.9), and due to misclassification in mammogram 1.4 months (SD 3.1) (Table 5). Overall, local recurrence was only experienced by 9 of the 166 patients (5.4%) of patients and metastases by 42 patients (25.3%). Lung and CNS locations were the most common locations of metastases (Table 2). Figures 2 and 3 show the Kaplan–Meier plot for overall survival, and

Table 2 Patient and tumor characteristics

	Overall	BL	IM	ML	LAR	*p*-value
n	166	64	22	33	47	
Age [year; mean (SD)]	57.3 (14.0)	55.6 (15.0)	51.5 (15.8)	57.2 (12.0)	62.5 (11.8)	0.014
Menopausal status (%)						0.003
Premenopausal	57 (34.3)	27 (42.2)	12 (54.5)	11 (33.3)	7 (14.9)	
Postmenopausal	109 (65.7)	37 (57.8)	10 (45.5)	22 (66.7)	40 (85.1)	
Reason for first consultation (%)						
Self-detected palpable mass	98 (59.0)	44 (68.8)	15 (68.2)	20 (60.6)	19 (40.4)	0.18
Screening	50 (30.1)	13 (20.3)	6 (27.3)	12 (36.4)	19 40.4)	
Symptoms (discharge/pain)	6 (3.6)	2 (3.1)	0	1 (3.0)	3 (6.4)	
Other	12 (7.2)	5 (7.8)	1 (4.5)	0	6 (12.8)	
Family history of breast cancer (%)						
Yes	30 (18.1)	11 (17.2)	5 (22.7)	6 (18.2)	8 (17.0)	0.92
No	133 (80.1)	53 (82.8)	17 (77.3)	27 (81.8)	36 (76.6)	
Unknown	3 (1.8)	0	0	0	3 (6.4)	
Histopathologic tumor size (%)	43	14	10	8	11	
ypT0	8 (18.6)	4 (28.6)	1 (10.0)	1 (12.5)	2 (18.2)	0.95
ypT1	23 (53.5)	7 (50.0)	6 (60.0)	4 (50.0)	6 (54.5)	
ypT2–4	12 (27.9)	3 (21.4)	3 (30.0)	3 (37.5)	3 (27.3)	
	122	49	12	25	36	0.27
pT1	55 (45.1)	21 (42.9)	8 (66.7)	13 (52.0)	13 (36.1)	
pT2–4	67 (54.9)	28 (57.1)	4 (33.3)	12 (48.0)	23 (63.9)	
Histopathologic axillary lymph node (%)	37	12	10	5	10	0.43
ypN0	16 (43.2)	6 (50.0)	6 (60.0)	1 (20.0)	3 (30.0)	
ypN1–3	21 (56.8)	6 (50.0)	4 (40.0)	4 (80.0)	7 (70.0)	
	129	52	12	28	37	0.065
pN0	88 (68.2)	37 (71.2)	10 (83.3)	22 (78.6)	19 (51.4)	
pN1–3	41 (31.8)	15 (28.8)	2 (16.7)	6 (21.4)	18 (48.6)	
HE morphology (%)						
Any special type	40 (24.1)	4 (6.2)	22 (100)	10 (30.3)	4 (8.5)	< 0.001
NST	126 (75.9)	60 (93.8)	0	23 (69.7)	43 (91.5)	
Tumor grading (%)						
G1–2	26 (15.7)	5 (7.8)	4 (18.2)	3 (9.1)	14 (29.8)	0.012
G3	140 (84.3)	59 (92.2)	18 (81.8)	30 (90.9)	33 (70.2)	
Local recurrence (%)						
Yes	9 (5.4)	4 (6.2)	1 (4.5)	1 (3.0)	3 (6.4)	0.93
No	156 (94)	59 (92.2)	21 (95.5)	32 (97)	44 (93.6)	
Distant metastasis (%)						
Overall	42 (25,3)	16 (25.0)	7 (31.8)	6 (18.2)	13 (27.7)	0.68
Lung	17 (10.2)	10 (15.6)	3 (13.6)	1 (3.0)	3 (6.4)	
Liver	11 (6.6)	5 (7.8)	2 (9.1)	1 (3.0)	3 (6.4)	
Other visceral	9 (5.4)	3 (4.5)	1 (4.5)	3 (9.1)	2 (4.3)	
CNS	15 (9.0)	5 (7.8)	2 (9.1)	3 (9.1)	5 (10.6)	
Distant lymph nodes	2 (1.2)	1 (1.6)	0	0	1 (2.1)	
Bone	1 (0.6)	0	0	0	1 (2.1)	

for disease-free survival by TNBC subtype. At 5 years follow-up, most patients were still living and disease-free and most patients were still living at 10 years of follow-up. No median disease-free or overall survival could be computed for these follow-up periods. Survival for patients with ML subtype trended better than others, but no statistically significant differences in OS or DFS could be detected across TNBC subtypes (log-rank tests, $p = 0.48$

Table 3 Clinical imaging and diagnostic delay for patients undergoing ultrasound and mammography without previous histopathologic diagnosis and tumor size ≥ 1 cm

	Overall	BL	IM	ML	LAR	*p*-value
n	151	56	20	31	44	
Tumor size in ultrasound in mm (median [IQR])	20 [13,28]	18 [12,28]	20 [12,23]	20 [17,27]	24 [15,28]	0.62
Tumor size in mammogram in mm (median [IQR])	20 [12,25]	18 [10,25]	17 [11,21]	20 [11,27]	25 [14,27]	0.35
Unifocal tumor (%)						
Yes	126 (83.4)	49 (87.5)	19 (95.0)	25 (80.6)	33 (75.0)	0.19
No	25 (16.6)	7 (12.5)	1 (5.0)	6 (19.4)	11 (25.0)	
BI-RADS mammography						
Benign features (1–3)	40 (26.5)	15 (26.8)	5 (25.0)	10 (32.3)	10 (22.7)	0.84
Malignant features (4–5)	111 (73.5)	41 (73.2)	15 (75.0)	21 (67.7)	34 (77.3)	
Breast density						
Low (ACR a and b)	77 (51.0)	33 (58.9)	10 (50.0)	10 (32.3)	24 (54.5)	0.11
High (ACR c and d)	74 (49.0)	23 (41.1)	10 (50.0)	21 (67.7)	20 (45.5)	
BI-RADS ultrasound						
Benign features (1–3)	20(13.2)	9 (16.1)	3 (15.0)	4 (12.9)	4 (9.1)	0.79
Malignant features (4–5)	131 (86.8)	47 (83.9)	17 (85.0)	27 (87.1)	40 (90.9)	
Sonographic suspicious lymph nodes						
Yes	59 (39.1)	19 (33.9)	6 (30.0)	14 (45.2)	20 (45.5)	0.47
No	92 (60.9)	37 (66.1)	14 (70.0)	17 (54.8)	24 (54.5)	
Patients with benign features in ultrasound (*n*)	20	9	3	4	4	0.36
Diagnostic delay (month) (mean [SD])	2.15 [3.9]	0.67 [1.1]	0.67 [1.2]	3.5 [3.5]	5.3 [7.5]	
Patients with benign features in mammogram (*n*)	40	15	5	10	10	0.55
Diagnostic delay (month) (mean [SD])	1.4 [3.1]	0.5 [1.2]	0.4 [0.9]	2.0 [3.0]	2.6 [5.1]	
Tumor growth during delay (%)						
< 1 cm	5 (3.3)	2 (3.6)	1 (5.0)	1 (3.2)	1 (2.3)	0.85
≥ 1 cm	4 (2.6)	2 (3.6)	0	2 (6.5)	0	
≥ 1 cm and newly diagnosed lymph node metastasis	4 (2.6)	1 (1.8)	0	1 (3.2)	2 (4.50)	

Breast tissue density was classified by ACR [17] and split into low (ACR a and b) and high (ACR c and d)

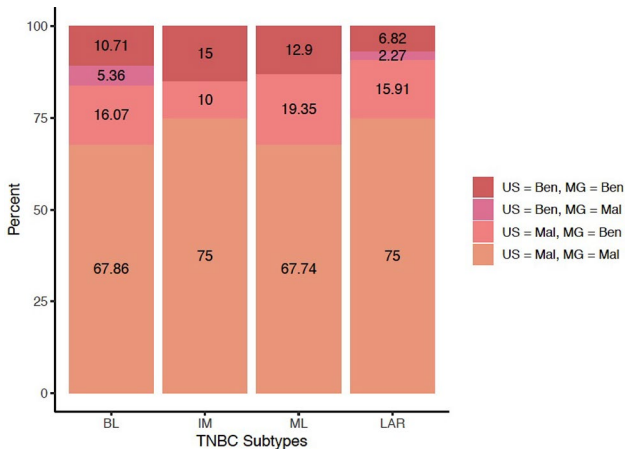

Fig. 1 stacked bar plots displaying BI-RADS classification (benign and malignant) for ultrasound (US) and mammogram (MG) per subtype. Percent is displayed in the bars

Table 4 Odds ratio estimates with confidence intervals and *p*-values from McNemar's test for matched ultrasound/mammogram data per subtype

	Benign features (MG)	Malignant features (MG)	Sum
Benign features (US)	16	4	20
Malignant features (US)	24	107	131
Sum	40	111	151

and $p = 0.47$). This procedure was repeated, including only patients treated with standard chemotherapy ($n = 132$), with no change in results.

Table 5 In 166 patients with TNBC, there is no evidence of a correlation between age and diagnostic delay, nor between reason for initial presentation and diagnostic delay

	No delay	Delay	*p*-value
n (166)	114	52	
Age at first presentation [year; mean (SD)]	58.7 (13.9)	54.4 (13.7)	0.072
Reason for first presentation (%)			0.22
Self-detected palpable lump	69 (60.5)	29 (55.8)	
Screening	31 (27.2)	19 (36.5)	
Symptoms (discharge/pain)	6 (5.3)	0	
Follow-up cancer care	3 (2.6)	3 (5.8)	
Incidental finding during diagnostic imaging for other reasons	5 (4.4)	1 (1.9)	

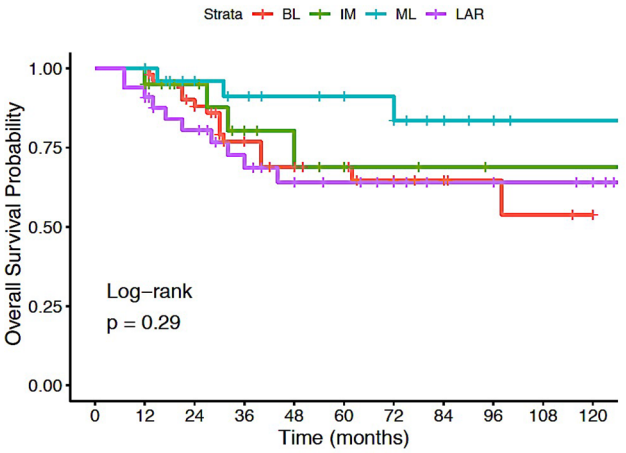

Fig. 2 Kaplan–Meier plot for overall survival (until 10 years) by TNBC subtype

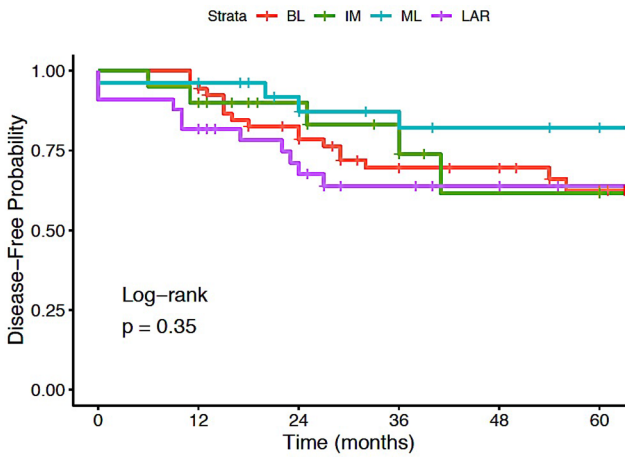

Fig. 3 Kaplan–Meier plot for disease-free survival (until 5 years) by TNBC subtype

Discussion

TNBC subtypes

The complex and heterogeneous group of TNBC can be classified into subtypes based on gene expression profiles, which correlate to different therapeutic responses [4]. Imaging features of TNBC frequently show benign criteria, such as circumscribed, lobulated masses in the ultrasound, and oval masses less associated with calcifications in mammography [11, 12]. As these features are similar to benign tumors such as fibroadenomas, misinterpretation is more frequent than in non-TNBC. In a recent study of Zeng et al. TNBC was divided into two subtypes (basal-like, BL, defined as CK5/6 and/or EGFR positive, and normal-like) to compare imaging features [18]. BL presented with larger tumor mass, and more often microlobulated or spiculated margins in ultrasound than normal-like TNBC. The categorization of TNBC used in the current study overlaps with the standard histologic definitions of BL breast cancer, but the gene expression pattern also includes additional proliferation genes and DNA damage response that are more specific for the BL-TNBC subtype [5]. In the presented study, we found a difference in clinical detection varied across TNBC subtypes, although without statistical significance.

Imaging features

Sonographic benign morphology was reported more often in BL subtype, which resulted in BI-RADS 2 to 3 classification (Fig. 1). These findings can be based on the typical and very fast growth pattern of BL-TNBC. The BL subtype is associated with poorer overall survival [19], and delayed diagnosis due to misinterpretation of clinical imaging may worsen the prognosis [15]. In cases of false-negative radiologic assessment (BI-RADS 1–3), diagnostic delay was a consequence in five patients with BL- TNBC (8.9%). Consistent with earlier investigations, BL-TNBC trends to worse OS, but no significant difference could be observed. The highest percentage of diagnostic delay was found in ML (12.9%, $n=4$),

mainly due to simultaneous misclassification in both diagnostic methods. Misclassification in at least one diagnostic method was most frequent in this subtype (32.3%, $n=10$). Tumor growth during diagnostic delay that led to a higher tumor stage was reported in eight cases overall (5.4%, $n=3$ in BL, $n=0$ in IM, 9.7%, $n=3$ in ML, and 4.5%, $n=2$ in LAR). Interestingly, OS was slightly better in ML than in the other subtypes, although differences were not significant. A better prognosis of ML subtype may be based on a less aggressive behavior; however, delayed diagnosis causing tumor stage upgrade can worsen the outcome.

Mammography had a high rate of missed malignant diagnoses in all TNBC subtypes, with the highest rate in ML (32.3%, $n=10$), and the lowest rate in LAR (22.7%, $n=10$). These findings are in accordance with previous studies and implicate the limitation of mammography for TNBC detection [12, 18]. As in the presented study, almost all patients (98.2%, $n=163$) were examined by mammography and ultrasound. Mammograms presenting benign criteria alone had a lower impact on diagnostic delay than in combination with benign sonographic features. Ultrasound caught up to 19.4% (in ML subtype) of cases which mammogram missed. Most of the TNBC with benign imaging features were diagnosed by immediate ultrasound-guided biopsy. Misclassification was seen in at least one method in 48 cases. However, a delayed biopsy during follow-up was initiated only in 13 cases (27.1%), leading to a delayed diagnosis.

Sonographic suspicious axillary lymph nodes did not differ significantly across the subtypes, although the final histopathologic rate of lymph node metastasis was significant more frequent in LAR (48.6%, $n=18$), and the lowest in IM (16.7%, $n=2$). The axillary ultrasonography is an important marker for lymph nodes metastasis and treatment planning especially in TNBC [20]. Missed axillary metastasis detection leads to inadequate regional surgical or radiological treatment and influenced outcomes [21]. The presented study is the first describing a higher number of missed imaging detection of lymph node metastasis in one subtype (LAR). This phenomenon might be related to a different metastatic growth pattern and further investigations are warranted on this topic.

Diagnostic delay

Diagnostic delay leads to a prolonged interval to therapy initiation and can therefore worsen the patient's prognosis [15, 22, 23]. Reasons for diagnostic delay can be seen on several levels: Firstly, the patient delay from the notice of a symptom, such as a palpable lump, to the contact with a health care provider. Secondly, delay of referral from the health care provider to a specialized center. Thirdly, the diagnostic delay caused by misinterpretation of a specialized doctor [24]. Even in many developed countries, quality and accessibility of the health system are dependent from the patient's insurance status, socioeconomic status, and ethnic/racial background [25, 26]. Our study cohort is from the largest Breast Cancer Center in Switzerland, a country with a high socioeconomic status and a public health system that provides a direct access to specialized centers. Therefore, diagnostic delay due to poor health care resources or lack of insurance can be neglected. The aim of the study was to differentiate the quote of imaging misinterpretation in TNBC subtypes as a potential source of diagnostic delay in a specialized center. False benign classification of clinical images was unexpectedly high in all subtypes, but as in all cases at least two methods of imaging (ultrasound and mammogram) were performed, the number of clinically relevant diagnostic delay was relatively low (8.9%). Furthermore, in a specialized center, the core needle biopsy might be more generously indicated even in lesions with a low likelihood of malignancy (BI-RADS 3). However, we observed a clear difference to previous studies with unselected cohorts of all breast cancer types, in which diagnostic delay was lower than 2% [24, 27].

Our data are in line with earlier publications, showing a higher percentage of benign criteria in clinical imaging of TNBC [28]. To the best of our knowledge, it is the first study considering diagnostic delay as a consequence of imaging misinterpretation in TNBC, and even more differentiates the subtypes of the heterogeneous group of TNBC. As TNBC is known to present benign criteria in ultrasound and mammogram [28], the aim of our study was to observe whether benign features in clinical imaging (BI-RADS I–III) could differ within the TNBC subtypes. The clinical relevance of this misinterpretation would be obvious if leading to a significant diagnostic delay and a higher tumor stage.

In a review by Ramirez et al. [29], delayed diagnosis after presentation to a health care provider was correlated to younger age, and other initial symptoms than palpable lump. However, the cited review could not provide information about diagnostic misinterpretation caused by benign imaging features of the breast cancer. The clinical assessment of a presenting patient depends on several factors such as experience of the referred doctor, internal guidelines, and available diagnostic methods. Our study includes a selected group of patients with TNBC, presented in a single highly specialized center, and ultrasound and mammogram were performed in all patients as a standard. Potential bias of younger age, and other symptoms than palpable lump could be excluded in our data analysis, as it was equally distributed in the study group (Tables 3, 6).

In real clinical settings, confounders such as indication for the clinical examination, operator skills, patient age, and medical history must be considered. The current study analyzed the diagnostic and clinical process regarding the clinical routine. Ultrasound was performed in knowledge of

Table 6 Benign imaging features that may lead to a misinterpretation are not correlated to the reason for patient's initial presentation

	Benign features	Malignant features	*p*-value
n (151)	40	111	
Reason for first presentation (%)			0.22
Self-detected palpable lump	24 (60.0)	64 (57.7)	
Screening	14 (35.0)	33 (29.7)	
Symptoms (discharge/pain)	0	6 (5.4)	
Follow-up cancer care	2 (5.0)	4 (3.6)	
Incidental finding during diagnostic imaging for other reasons	0	4 (3.6)	

the mammographic pictures in most cases (75.9%). In almost one-third of patients (32.2%), at least one of the imaging methods resulted in benign classifications, but the combination of methods minimizes the risk of delay. Furthermore, a broad biopsy indication for BI-RADS three lesions leads to a high rate of early diagnosis.

Strengths and weaknesses

The following strengths and weaknesses must be considered in the interpretation of our data: All patients have been diagnosed by specialized physicians of one center, and mammogram and ultrasound were performed during standard procedure. Tumor sizes were homogeneous in all subtype groups at diagnosis, as well as the patient profiles for the confounders age and breast density. The study is exploratory in nature and may be underpowered to detect differences across the four TNBC subtypes. Although 151 patients were included in the analysis of diagnostic delay, the total number of delays is too low and the data granularity too coarse to verify impact on the overall or disease-free survival. The study was not designed to compare delayed diagnosis in patients with TNBC versus non-TNBC.

Due to genetic heterogeneity, the remaining therapy-resistant subclones could be at different histopathologic subtypes after systemic treatment, and TNBC classification in the presented study was made in 35 cases (21%) after neoadjuvant chemotherapy.

Conclusion

Benign mammographic criteria are more often found in the ML-TNBC subtype, and benign sonographic criteria are slightly more frequent in the BL subtype of TNBC. Delay of diagnosis might lead to a higher tumor grade at therapy initiation, which was mostly observed in ML. Familiarity with TNBC subtypes' morphologies in clinical imaging and its correlation to clinical outcomes is important to improve early detection. Future clinical studies on TNBC outcomes should consider the confounder of possible delayed diagnosis in TNBC subtypes.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Cantonal Ethics Committee of Zurich, Switzerland (BASEC-No. 2017-00219), and in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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