A single-centre chart review exploring the adjusted association between breast cancer phenotype and prognosis

C. Hamm MD,† M. El-Masri RN PhD,*† G. Poliquin MD,‡ V. Poliquin MD,‡ J. Mathews MD,* S. Kanjeekal MD,* Y. Alam MD,* S. Kulkarni MD,* and T. Elfiki MD**

ABSTRACT

Purpose and Methods

Using a retrospective chart review, we investigated the differences in survival and prognostic factors between patients with triple-negative breast cancer (TNBC) and those with non-TNBC. The review included 1018 breast cancer patients who were diagnosed between 2000 and 2005 in Essex, Kent, and Lambton counties in Ontario, Canada.

Results

Our findings indicate that, although the unadjusted results suggested that patients with TNBC were more likely than patients with non-TNBC to die [hazard ratio (HR): 2.29; 95% confidence interval (CI): 1.33 to 2.93], an adjusted survival analysis revealed no significant difference in overall survival between the groups (HR: 1.22; 95% CI: 0.63 to 2.39). The significant predictors of survival in the adjusted analysis were age, stage of cancer, and size of cancer.

Conclusions

Our findings support those of earlier reports, which suggest that presenting tumour size is the most important prognostic factor in TNBC. Investigations into unique screening methods to identify these tumours at an earlier stage and to prevent advanced-stage cancer in this patient subpopulation are necessary.

KEY WORDS

Triple-negative breast cancer, survival, tumour size, cancer-related death, age

1. INTRODUCTION

Increasingly, breast cancer is being recognized as a heterogeneous disease. A patient's treatment and prognosis are determined by a multitude of factors that include, but are not limited to, age; stage of cancer; tumour characteristics such as estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (HER2/*neu*) status; and histologic grade. Gene expression profiling has revealed new classifications of breast cancer: two are ER-positive (luminal A and B), and two are ER-negative (HER2-positive and basal-like) [1]. Basal-like tumours usually demonstrate low levels of estrogen and HER2 gene expression, strongly correlating with triple-negative breast cancer (TNBC), which is ER-, PR-, and HER2-negative. Unfortunately, gene profiling is not clinically available at this time, and many investigators allocate patients to groups that can be identified using immunohistochemistry.

Triple-negative breast cancer has been associated with younger age groups and patients presenting with a higher stage of disease [2], and it is thought to have a worse prognosis [3]. More recent studies [4,5] have reported that the tumour size at presentation is the most important predictor of survival in TNBC. It is important that the differences in characteristics between patients with TNBC and with other breast cancers be further investigated. The purpose of the present study was therefore to compare survival rates and prognostic factors between patients with TNBC and those with other breast cancers.

2. METHODS

2.1 Study Design

We conducted a retrospective chart review for all breast cancer patients seen between 2000 and 2005 at a referral facility with a catchment area of approximately 500,000 people. The patients were referred from 4 different hospitals, 3 of which perform independent pathology reviews. A central pathology review of the patients was held at the referral centre.

The ER and PR analyses used Novocastra antibodies (Leica Microsystems, Concord, ON), with cutoff levels for receptor positivity of more than 10%. Although

ER and PR positivity are considered at more than 1% in current clinical practice, 10% was the accepted level during the reported time period. In many charts, results were reported only as positive or negative; details of percentage positivity were omitted. Positivity for overexpression of HER2 was evaluated using the Novocastra CB11 monoclonal antibody (Leica Microsystems). Results were reported as 1+ to 3+ density of staining. A result of 3+ was reported as positive, 2+ was reported as indeterminate, and 1+ was reported as negative. Tumours reported as 2+ were then tested for gene overexpression using fluorescence *in situ* hybridization (FISH). Threshold for a positive HER2 FISH result was a HER2-to-CEP17 ratio exceeding 2.2.

The chart review identified a total of 1114 patients, of whom 1018 were deemed eligible for study inclusion. A patient was deemed eligible if her chart contained enough information to declare with certainty that her disease was TNBC or non-TNBC. Patients whose charts lacked phenotype data or had data on only 1 or 2 of the receptors were excluded. Demographic information (age at diagnosis, time between diagnosis and first visit to an oncologist, menopausal status, family history, county of origin, need for a translator, employment status) were collected for the included patients, as were clinicopathologic features of the cancer (tumour size, tumour pathologic subtype, grade, TNM staging, ER status, PR status, and HER2 status). Data were also collected concerning the type of surgical intervention, type of chemotherapy, hormonal treatment, and radiotherapy.

2.2 Data Analysis

Data were analyzed using the PASW statistical software package (version 18.0: SPSS, Chicago, IL, U.S.A.). Before the actual analyses, the data were explored for accuracy of entries, missing data, and statistical assumptions. Data analysis procedures included basic descriptive statistics, bivariate analyses (life tables, chi-square tests, and the Student *t*-test), and Cox proportional hazards modelling. Chi-square and *t*-tests were used to compare demographic and prognostic factors between women with TNBC and non-TNBC. Life tables were used for time-to-event (for example, death from cancer) comparisons between women with TNBC and non-TNBC, in 12-month intervals. The decision to use life tables instead of Kaplan–Meier analysis was based on the fact that the survival curve did not reach 0.5 on the cumulative survival axis, which made the median for 5-year survival incalculable. Cox regression analysis was used to determine the crude and adjusted hazard ratios (HRs) for factors associated with death from cancer. A 95% confidence interval (95% CI) was set as the criterion to establish statistical significance. Factors were considered for inclusion in the adjusted Cox regression based on a p value of 0.25 or less in the bivariate analysis [6].

3. RESULTS

3.1 Sample Characteristics

Of the 1018 patients in the study sample, 99 (9.7%) were identified as having TNBC; the remaining patients (90.3%) were identified as having breast cancer with at least 1 positive marker (that is, non-TNBC). Estrogen receptor status was available for all patients (83.7% positive, 16.3% negative). With regard to PR status, 68.1% of patients were positive, and 31.6% were negative; 0.3% were not known. With regard to HER2 status, 11.5% were positive, and 44.2% were negative; 44.3% were unknown. No patients were ER-negative, PR-negative, and HER2 unknown.

Table I presents the demographic characteristics of the sample and compares those characteristics for the TNBC and non-TNBC groups. The mean age (with standard deviation) for the entire sample was 60.2 ± 13.3 years; the mean age for the TNBC group was statistically lower than that for the non-TNBC group (53.2 years and 60.9 years respectively; $t = 5.56$, $p < 0.001$). The frequency of self-detection was higher among patients with a TNBC tumour than among patients with a non-TNBC tumour (76.8% and 56.4% respectively; $p < 0.001$). Patients with a family history of cancer (any first- or second-degree relative with breast cancer) constituted 44.7% ($n = 455$) of the sample. However, family history was not statistically different between the groups ($\chi^2 = 0.071$; $p = 0.791$).

Although only 27% of the sample ($n = 277$) had a grade III breast cancer, the frequency of grade III cancer was higher in the TNBC group than in the non-TNBC group (67.7% vs. 22.9%; $p < 0.001$). Stage I was the most frequent cancer stage in the study sample ($n = 466$, 45.8%), followed by stage II ($n = 437$, 42.9%). The most frequent cancer size was 1–2 cm ($n = 433$, 42.5%), followed by cancers that ranged from more than 2 cm to 5 cm ($n = 404$, 39.7%).

The data revealed that the two groups were statistically different with regard to stage ($\chi^2 = 24.68$, $p < 0.001$) and size of cancer ($\chi^2 = 46.57$, $p < 0.001$). The groups were also different with regard to the frequency of chemotherapy. Of the TNBC patients, 85% had received some form of chemotherapy; only 36% of the non-TNBC patients had received chemotherapy ($\chi^2 = 98.51$, $p < 0.001$). Among the non-TNBC patients, 682 (74.2%) had received some kind of hormonal therapy; among TNBC patients, only 2 had received hormonal therapy ($\chi^2 = 211.1$, $p < 0.001$).

3.2 Survival Time Analysis

The data show that, during the follow-up period of 8 years, 75 of the 919 non-TNBC patients (8.2%) died as a result of their breast cancer; 16 of the 99 (16.2%) TNBC patients died as a result of their breast cancer.

Table II sets out the life table results, which suggest that the median survival time was statistically

TABLE I Comparison of prognostic factors and demographic characteristics between triple-negative and other breast cancers

Variable	*Group*			t	χ^2	p
	Triple-negative	*Others*	*Overall*			*Value*[a]
Mean age (years)	53.2±12.7	60.9±13.2	60.2±13.3	5.56		<0.001
Family history of breast cancer [*n* (%)]						
No	43 (43.4)	412 (44.8)	455 (44.7)		0.071	0.791
Yes	56 (56.6)	507 (55.2)	563 (55.3)			
Grade [*n* (%)]						
1	12 (12.1)	286 (31.1)	298 (29.3)		90.72	<0.001
2	20 (20.2)	423 (46.0)	443 (43.5)			
3	67 (67.7)	210 (22.9)	277 (27.2)			
Stage [*n* (%)]						
I	27 (27.3)	439 (47.8)	466 (45.8)		24.68	<0.001
II	54 (54.5)	383 (41.7)	437 (42.9)			
III	16 (16.2)	58 (6.3)	74 (7.3)			
IV	2 (2.0)	39 (4.2)	41 (4.0)			
Size						
<1 cm	3 (3.0)	117 (12.7)	120 (11.8)		46.57	<0.001
1–2 cm	22 (22.2)	411 (44.7)	433 (42.5)			
>2–5 cm	58 (58.6)	346 (37.6)	404 (39.7)			
>5 cm	16 (16.2)	45 (4.9)	61 (6.0)			
Chemotherapy [*n* (%)]						
None	15 (15.2)	586 (63.8)	601 (59.0)		98.51	<0.001
Anthracycline (A)	48 (48.5)	184 (20.0)	232 (22.8)			
Taxane (T)	23 (23.2)	61 (6.6)	84 (8.3)			
Neither A nor T	13 (13.1)	88 (9.6)	101 (9.9)			
Hormone therapy [*n* (%)]						
No	97 (98.0)	237 (25.8)	334 (32.8)		211.1	<0.001
Yes	2 (2.0)	682 (74.2)	684 (67.2)			

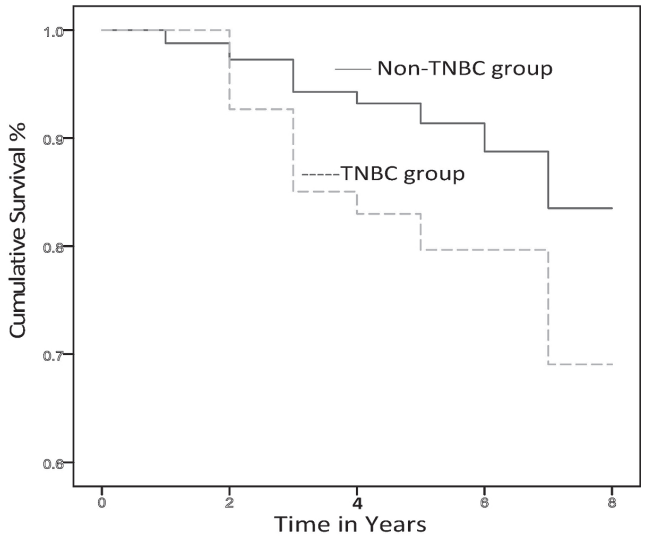
[a] Significant two-tailed *p* value at an alpha of 0.05.

TABLE II Life-table comparisons of median survival times for patients having triple-negative and non-triple-negative breast cancer

Variable	*Mean ± standard error*	*Median*	*Wilcoxon–Gehan test*	p *Value*
Triple-negative	6.01±0.23	7.0	10.1	0.002
Non-triple-negative	7.40±0.07	8.0		

different in the two groups, with median years of survival being less in the TNBC group (median: 7 years TNBC and 8 years non-TNBC; Wilcoxon–Gehan test: 10.1; $p = 0.002$). Figure 1 shows the unadjusted survival curves for the two groups in 12-month intervals. Throughout the first 6 years of follow-up, patients in the TNBC group tended to have a survival rate lower than that for patients with non-TNBC.

Table III shows the unadjusted and adjusted HRs from the Cox regression analyses. In the unadjusted model, the HR for death in the TNBC group, compared



Tumour type	*Patients (*n*)*								
Triple-negative									
Remaining	919	882	796	615	436	367	182	48	1
Censored	26	73	159	173	61	177	127	47	1
Non-triple-negative									
Remaining	99	99	85	55	26	23	11	3	0
Censored	0	7	24	28	2	12	7	3	0

FIGURE 1 Life table survival curves for the study groups.

with the non-TNBC group, was 2.29 (95% CI: 1.33 to 3.93). However, the HR became statistically insignificant after adjustments for the additional variables listed in Table III (HR: 1.24; 95% CI: 0.64 to 2.42). Figure 2 demonstrates that, in the adjusted Cox regression model, the survival curves of the TNBC and non-TNBC groups are not significantly different.

The adjusted model in Table III further demonstrates that "grade of cancer" and "type of chemotherapy" were not independent predictors of survival; however, stage of cancer was an independent predictor of survival, bearing a hazard for death among patients with stage IV cancer that was 10 times the hazard among patients with stage I disease (HR: 10.01; 95% CI: 4.54 to 22.06). The HR was not different between patients with stage II or III disease and those with stage I. Hormonal therapy was an independent predictor of survival, with women who received hormonal therapy being less likely to die from their breast cancer (HR: 0.48; 95% CI: 0.29 to 0.81).

4. DISCUSSION

The treatment of breast cancer has progressed significantly since the late 1980s. Perhaps the most important advances were the identification of the HER2/*neu* gene and the development of targeted therapy for the group of patients with HER2 overexpression [7,8]. Subsequently, it has been possible to further divide the breast cancer population into molecular subtypes (that is, the luminal A and B and basal subtypes of breast cancer) whose recognition helps in treatment and prognostication. Definition of the TNBC subgroup has evolved over the last few years to delineate a high-risk group of women.

The incidence of TNBC in our study was 9.7% of the presenting breast cancer population, which was somewhat lower than the incidence of 11.2%–26% reported in the literature [2,5,8–10]. That result may depend on differences in the definition of TNBC between various studies. The higher incidence in the literature is believed to be race related, with African American and Korean women having higher reported incidences [9,10]. We also found that patients with TNBC were younger at presentation than their non-TNBC counterparts. That finding accords with previous reports. Specifically, in our study, the median age was 53.2 years for the TNBC group compared with 60.9 years for the non-TNBC group ($p < 0.001$), which was similar in trend to the age range of 48.3–53 years for TNBC patients and 49.1–57.7 years for the non-TNBC patients reported in the literature [2,9]. That result is somewhat concerning because, in Canada, mammographic screening starts at the age of 50 years, which may suggest that many TNBC patients would not, at the point of presentation, have been seen in a screening mammography program. However, the study by Dent and colleagues [2] found that, even in the mammographically screened population, women with TNBC were found to develop interval cancers.

Currently, anyone with more than 1% ER staining would be classified as ER-positive, a level different from that reported in our study. However, our use of the 10% cut-off for ER positivity is consistent with most of the reported studies that use immunohistochemical staining to categorize patients into TNBC phenotypes [2,4,5].

In our study, patients with TNBC were more likely than those with non-TNBC to have a more advanced stage of disease ($p < 0.001$). The findings specifically showed that, although nearly half of the non-TNBC population (47.8%) presented with stage I disease, only 27.3% of the TNBC population presented with this earlier stage. By contrast, stage II was more prevalent in patients in the TNBC group than in the non-TNBC group (54.5% vs. 41.7%), as was stage III (16.2% vs. 6.3%). Those results accord with findings in previous studies and suggest that TNBC patients are more likely to present with more advanced cancer [2,5,11]. This more advanced disease is most likely a reflection of the higher-grade, more aggressive tumours identified in the TNBC population.

Also of interest is the finding that cancer-related deaths were not difference for stage I, II, and III cancers when adjusted for other variables, including

TABLE III Unadjusted and adjusted hazard ratios for cancer-related death associated with each of the study variables

Variable	*Unadjusted*		*Adjusted*	
	HR (95% CI)	p *Value*	*HR (95% CI)*	p *Value*
Triple-negative group	2.29 (1.33 to 3.93)	0.003	1.24 (0.64 to 2.42)	0.522
Age	1.02 (1.0 to 1.03)	0.064	1.02 (1.00 to 1.04)	0.024
Family history	1.10 (0.73 to 1.66)	0.653	Not included in the model	
Grade				
1 (reference)	—	—	—	—
2	1.12 (0.64 to 1.98)	0.684	1.32 (0.73 to 2.36)	0.358
3	2.15 (1.24 to 3.73)	0.006	1.73 (0.91 to 3.28)	0.093
Stage				
I (reference)	—	—	—	—
II	1.46 (0.84 to 2.53)	0.177	0.70 (0.33 to 1.46)	0.340
III	3.69 (1.83 to 7.46)	<0.001	1.08 (0.37 to 3.18)	0.883
IV	23.47 (13.21- 41.69)	<0.001	10.01 (4.54 to 22.06)	<0.001
Size				
<1 cm (reference)	—	—	—	—
1–2 cm	1.62 (0.48 to 5.51)	0.437	2.05 (0.60 to 7.08)	0.255
>2–5 cm	6.04 (1.89 to 19.33)	0.002	6.51 (1.77 to 23.97)	0.005
>5 cm	11.05 (3.22 to 37.91)	<0.001	7.66 (1.70 to 34.54)	0.008
Hormonal therapy	0.61 (0.41 to 0.93)	0.021	0.48 (0.29 to 0.81)	0.006
Chemotherapy				
None (reference)	—	—	—	—
Anthracycline (A)	1.34 (0.82 to 2.19)	0.240	0.95 (0.49 to 1.86)	0.890
Taxane (T)	2.23 (1.11 to 4.48)	0.024	1.00 (0.43 to 2.36)	0.989
Neither A nor T	1.29 (0.68 to 2.44)	0.438	1.18 (0.57 to 2.41)	0.660

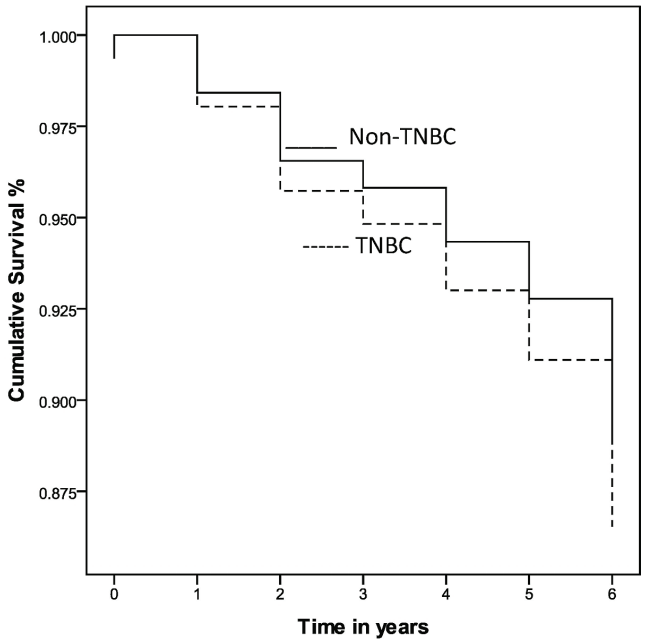


FIGURE 2 Adjusted survival curves for patients with triple-negative and non-triple-negative breast cancer.

age, family history, grade, size, hormonal therapy, and chemotherapy. Possible explanations might be the more aggressive therapy that patients at higher stages received, the relatively small study sizes, or the relatively short median follow-up (3 years).

A possible weakness of the study is the fact that HER2 status was not known in 44% of our population. That being said, outcomes would not be much different than those in other reported studies, because adjuvant trastuzumab would not have been used regardless of the test results. Voduc *et al.* [12] reported that this HER2-positive group had worse outcomes than the rest of the non-TNBC population. However, the outcomes in our cohort would have been expected to be the same as in other studies [3–5,9,10] because that subpopulation was not reported separately. We also acknowledge that the staging system from the American Joint Committee on Cancer was changed between 2002 and 2003. Unfortunately, that change in staging information was not captured in our review.

Our crude analysis suggested that the hazard for death in the TNBC group was 2.29 times that in the non-TNBC group; however, our results demonstrated

that the survival rates in the two groups were not statistically different once adjustments were made for age, family history, presenting stage and cancer size, and use of hormonal therapy and chemotherapy. In fact, the results of the adjusted analysis showed that the only significant predictors of survival for the TNBC population were age, stage of cancer, and size of cancer. That finding is very different from those of other studies, which reported that even after adjustment for those factors, the TNBC population still carried a higher risk for death than did their non-TNBC counterparts [2,12].

5. CONCLUSIONS

Patients with TNBC present with larger tumours and at more advanced stages, and they present at a younger age. A woman presenting with TNBC may expect the same survival as a patient presenting with non-TNBC after adjustment for variables such as age, stage, grade, size of tumour, and therapy. Despite current screening strategies, TNBC presents at a more advanced stage, and that fact alone adversely affects outcomes. Current screening strategies are inadequate for this small and unique group of women. Novel strategies are needed to allow for earlier detection, and therefore improvements in survival, in this population.

6. CONFLICT OF INTEREST DISCLOSURES

The present study was an unsponsored and unfunded research project.

7. REFERENCES

1. Sørlie T, Perou CM, Tibshirani R, *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98:10869–74.
2. Dent R, Trudeau M, Pritchard KI, *et al.* Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007;13:4429–34.
3. Anders C, Carey LA. Understanding and treating triple-negative breast cancer. *Oncology (Williston Park)* 2008;22:1233–9.
4. Lee JA, Kim KI, Bae JW, *et al.* Triple negative breast cancer in Korea—distinct biology with different impact of prognostic factors on survival. *Breast Cancer Res Treat* 2010;123:177–87.
5. Iwase H, Kurebayashi J, Tsuda H, *et al.* Clinicopathological analyses of triple negative breast cancer using surveillance data from the Registration Committee of the Japanese Breast Cancer Society. *Breast Cancer* 2010;17:118–24.
6. Hosmer DW, Lemshow S. *Applied Logistic Regression.* 2nd ed. New York: John Wiley and Sons; 2000.
7. Perez EA, Baweja M. HER2-positive breast cancer: current treatment strategies. *Cancer Invest* 2008;26:545–52.
8. Smith I, Procter M, Gelber RD, *et al.* 2-Year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet* 2007;369:29–36.
9. Lee K, Lim HT, Park SM. Factors associated with use of breast cancer screening services by women aged >or= 40 years in Korea: the third Korea National Health and Nutrition Examination Survey 2005 (KNHANES III). *BMC Cancer* 2010;10:144.
10. Carey LA, Perou CM, Livasy CA, *et al.* Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 2006;295:2492–502.
11. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. *Cancer* 2007;109:25–32.
12. Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H. Breast cancer subtypes and the risk of local and regional relapse. *J Clin Oncol* 2010;28:1684–91.

Correspondence to: Caroline Hamm, 2220 Kildare Road, Windsor, Ontario N8W 2X3.
E-mail: caroline_hamm@wrh.on.ca

* University of Western Ontario, London, ON.
† University of Windsor, Windsor, ON.
‡ University of Manitoba, Winnipeg, ON.