Clinical Feasibility and Reproducibility of Tumor Infiltrating Lymphocytes Reporting for Early-Stage Triple Negative Breast Cancer Patients at the University of Washington

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Introduction

- Tumor Infiltrating Lymphocytes (TILs) have prognostic and predictive value in triple negative breast cancer (TNBC)
- Higher TILs correlate with improved rates of pathologic complete response (pCR) following neoadjuvant chemotherapy and lower risk of recurrence and death
- TILs are not routinely clinically reported; Incorporating TILs into pathology reports for patients with early-stage TNBC has the potential to impact patient care and support ongoing research efforts.
- We report our institutional experience standardizing TIL reporting to generate feasible, reproducible TIL evaluation for diagnostic breast biopsies for early-stage TNBC.

Methods

- Stage I-III TNBC cases were identified by breast pathologists at the University of Washington between 2021 and 2023 (IRB #RG1122393)
- Clinical characteristics and survival data was collected by chart review.
- TIL status was independently assessed by two pathologists according to the International TILs Working Group Guidelines: a complete assessment was done using at least a 4-5 um section x200-400 per patient and TILs (including all mononucelear cells) were reported for the stromal component within the border of the invasive tumor (TILs outside the tumor border and those in areas of necrosis/crush artifact were excluded).
- TIL status was categorized as A (low 0%-10%), B (intermediate, 11-59%), or C (high, 60-100%).
- For cases where readers disagreed on the category, the TIL percentage was reassessed by both readers together.
- Read time was collected to assess impact on pathologist workload.

Summary and Discussion

- Using the International TIL Working Group Guidelines, we found TIL reporting to be **highly reproducible between pathologists with 80% concordance.**
- TIL reporting was feasible and the impact on workload was manageable with an average added time of <1 minute per case; however, there are concerns about growing demands on pathologists
- There was no significant difference in pCR rates based on TIL category (likely due to small sample size and how study was powered)
- There was no significant difference between TILs and recurrence free survival (likely due to short median follow-up of 1.8 years and small sample size with only 5 recurrences)
- Standardization of reporting is crucial for ongoing research efforts and valuable for patient care.

Results

Table 1: Patient Characteristics (N=56)

Variable	No. (%) or Median (Range)
Age, years	52 (27 - 88)
<40 years	11 (20%)
40-49 years	15 (27%)
50-64 years	17 (30%)
≥65 years	13 (23%)
Menopausal status	
Premenopausal	23 (41%)
Postmenopausal	32 (58%)
Unknown	1 (1%)
Histologic type	
IDC	51 (91%)
ILC	3 (5%)
Other*	2 (4%)
Grade	
1	2 (4%)
2	6 (11%)
3	48 (86%)
Ki67	52 (5 - 95)
<30%	5 (9%)
30-49%	10 (18%)
50-69%	12 (21%)
≥70%	16 (29%)
Unknown	13 (23%)
Clinical stage	
I	15 (27%)
II	27 (48%)
III	11 (20%)
Unknown	3 (5%)
Systemic therapy	
Neoadjuvant	33 (63%)
Adjuvant	14 (27%)
None	5 (10%)
pCR (if NAC therapy)	
Yes	19 (63%)
No	11 (37%)
Surgery type	
Lumpectomy	26 (48%)
Mastectomy	22 (41%)
Chest wall WLE	1 (2%)
None	5 (9%)
Radiation therapy	
Yes	41 (76%)
No	13 (24%)

Table 1: Selected patient and tumor characteristics. Includes 56 patients with evaluable disease for analysis. For residual disease at surgery, 30 patients received neoadjuvant chemotherapy and were evaluable.

Figure 1: TILs percentages by two readers

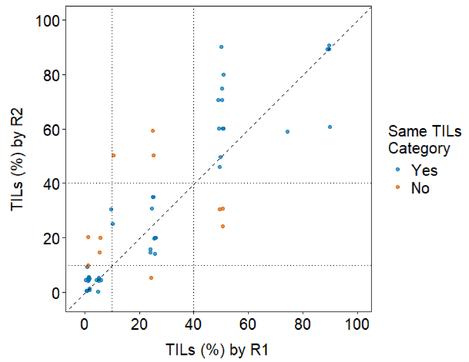


Figure 1: TIL percentage assessed by two readers. A small amount of jitter (<1%) was added to each datapoint to reduce overlap. The dashed lines indicate the cutoffs between categories A and B (10%) and between B and C (40%).

Figure 2: TIL read time by category (N = 56).

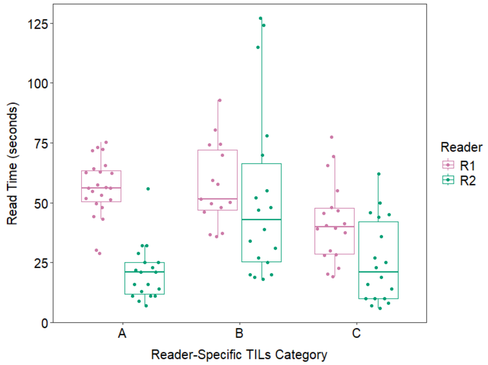


Figure 2: TIL read time by Category. Both reader's read time varied significantly (p = 0.007 for R1 and p = 0.001 for R2) by what their resulting TILs category (based on the TILs percentage). For R1, the read time was similar between groups A and B (p = 0.95) but dropped significantly from groups B to C (p = 0.015). For R2, the read time for group B was significantly higher than for group A (p < 0.001) and group C (p = 0.006). Groups A and C had similar read times (p = 0.76).

Table 2. TILs assessed by two readers (N = 56).

	Readers			
Variable	Reader 1	Reader 2	P-value*	Consensus†
TILs, %			0.060	
Mean ± SD	26 ± 27	30 ± 28		29 ± 27
Median (Range)	25 (1 - 90)	20 (1 - 90)		20 (1 - 90)
TILs category			0.39	
A (<10%)	23 (41%)	20 (36%)		20 (36%)
B (10-39%)	15 (27%)	18 (32%)		14 (25%)
C (≥40%)	18 (32%)	18 (32%)		21 (38%)
Time to review, seconds	51 (19 - 93)	25 (6 - 127)	<0.001	45 (14 - 235)

Values are no. (%), mean ± SD, or median (range) unless otherwise specified;
*Readers were compared using the Wilcoxon signed-rank test.
†For cases where readers disagreed on the category, the TILs percentage was reassessed by both readers together; for other cases, the TILs percentage and time to reviewer were averaged between the two readers.

Table 2: Percent agreement in TIL category was 45/56 (80%, 95% CI: 68-90%). Cohen's kappa was good at 0.70 (95% CI: 0.55-0.86). There wasn't a significant difference in review times between cases where both readers agreed on the TILs category (n = 45) compared to when they disagreed (n = 11) (median time : 40 vs. 41 seconds, mean time: 41 vs. 49 seconds, p = 0.17, Wilcoxon rank-sum test). Times are the average from the two readers

Figure 3: pCR rate by TILs consensus category (N=30)

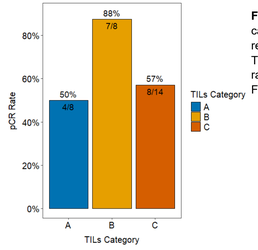


Figure 3 : pCR rate by TIL consensus category based on 30 patients who received neoadjuvant chemotherapy. There was no significant difference in pCR rate between TILs categories (p = 0.34, Fisher's exact test).

Figure 4: Recurrence-free survival by consensus TILs category (N = 43).

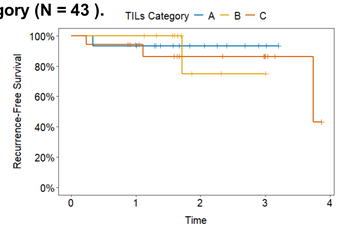


Figure 4: Recurrence-free survival by consensus TIL category from 43 evaluable patients. There were 5 recurrences over a median of 1.8 years of follow-up (1 death). There was no statistically significant difference in risk of recurrence by TILs category (p = 0.86, log-rank test).

Table 3: Patient Characteristics by TIL Category (N = 55*)

	TILs Consensus Category			
	A	B	C	
Variable	(N = 20)	(N = 14)	(N = 21)	P-value†
Age, years	64 (29 - 88)	52 (32 - 71)	47 (27 - 69)	0.036
Menopausal status				0.12
Premenopausal	5 (25%)	7 (50%)	11 (55%)	
Postmenopausal	15 (75%)	7 (50%)	9 (45%)	
Histologic type				0.45
IDC	18 (90%)	12 (86%)	20 (95%)	
ILC	1 (5%)	2 (14%)	0 (0%)	
Other	1 (5%)	0 (0%)	1 (5%)	
Grade				0.057
1	1 (5%)	1 (7%)	0 (0%)	
2	4 (20%)	2 (14%)	0 (0%)	
3	15 (75%)	11 (79%)	21 (100%)	
Ki67	45 (5 - 95)	60 (10 - 75)	58 (30 - 95)	0.43
Clinical stage				0.097
I	6 (32%)	2 (15%)	6 (30%)	
II	11 (58%)	5 (38%)	11 (55%)	
III	2 (11%)	6 (46%)	3 (15%)	
Systemic therapy				0.40
Neoadjuvant	10 (53%)	8 (67%)	15 (75%)	
Adjuvant	6 (32%)	3 (25%)	5 (25%)	
None	3 (16%)	1 (8%)	0 (0%)	
pCR (if NAC therapy)				0.34
Yes	4 (50%)	7 (88%)	8 (57%)	
No	4 (50%)	1 (12%)	6 (43%)	
Surgery type				0.95
Lumpectomy	10 (53%)	6 (43%)	10 (50%)	
Mastectomy	7 (37%)	6 (43%)	8 (40%)	
Chest wall WLE	0 (0%)	0 (0%)	1 (5%)	
None	2 (11%)	2 (14%)	1 (5%)	
Radiation therapy				0.92
Yes	14 (74%)	11 (79%)	16 (80%)	
No	5 (26%)	3 (21%)	4 (20%)	

*One patient excluded from this analysis due to lack of a consensus TIL classification; †Kruskal-Wallis test or Fisher's exact test.

Table 1: Selected patient and tumor characteristics based on TIL category. Includes 55 patients with evaluable disease for analysis.

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