Increased lymph node germinal centre circularity is associated with improved invasive disease-free survival in triple-negative breast cancer patients of the GAIN-2 trial

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Background

Lymph nodes (LN) are highly organised secondary lymphoid organs playing a critical role in the adaptive immune response and reacting dynamically to antigens, including those from tumours.

LN are routinely assessed for metastases given their prognostic significance. However, other morphological features, such as presence, number and size of germinal centres (GC), immunological hubs for B cells, are not reported.

By applying *smuLymphNet*[1], a deep learning framework, we previously showed that invasive breast cancer patients whose axillary LN lacked GC formation had a higher risk of distant metastasis.

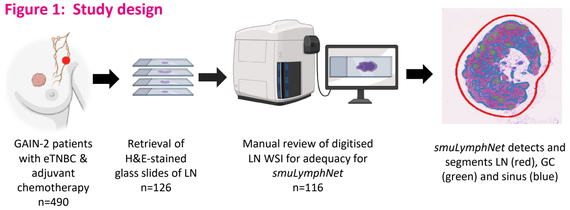
In this study we evaluated the generalisability of *smuLymphNet* and prognostic significance of GC morphology in LNs from patients with early triple negative breast cancer (eTNBC) enrolled in the GAIN-2 trial (NCT01690702)[2], a randomised phase III trial evaluating the optimal intense dose-dense therapy regimen in high-risk early breast cancer patients.

Material and Methods

Primary objectives of the study: The prognostic benefit of quantifying GC and sinuses within LN by *smuLymphNet* on patients' invasive disease-free survival (iDFS) and overall survival (OS).

- The GAIN-2 trial enrolled 2,857 patients with high risk early invasive breast cancer into the modified intent to treat (mITT) population. Of the mITT, 490 received adjuvant chemotherapy for eTNBC. Haematoxylin and eosin (H&E) stained slides of their LN were retrieved and digitised, totalling 149 whole slide images (WSI). WSI were manually reviewed for adequacy for *smuLymphNet* assessment (**Figure 1**).
- *smuLymphNet* is a multiscale deep learning framework that detects LN sections and segments GC and sinuses.
- A pathologist annotated germinal centres in 22 LN WSIs and the accuracy of the *smuLymphNet* segmentation was evaluated**.**

Figure 1: Study design



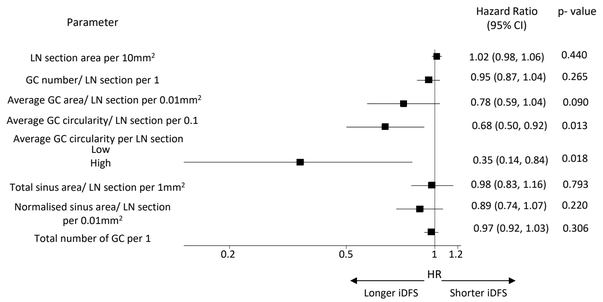
Quantification of GC in LN:

- Per LN section the GC number, area and circularity [OpenCV algorithm (4π * Area) / Perimeter^2)] were quantified.
- Mean values for the LN features were calculated per patient.
- The time to event outcomes iDFS and OS were analysed by Cox proportional hazard models and are presented using Kaplan-Meier method.
- Median follow-up time was estimated by the inverse Kaplan-Meier method.
- All reported p-values are two-sided, and $p \leq 0.05$ was considered statistically significant.

Results

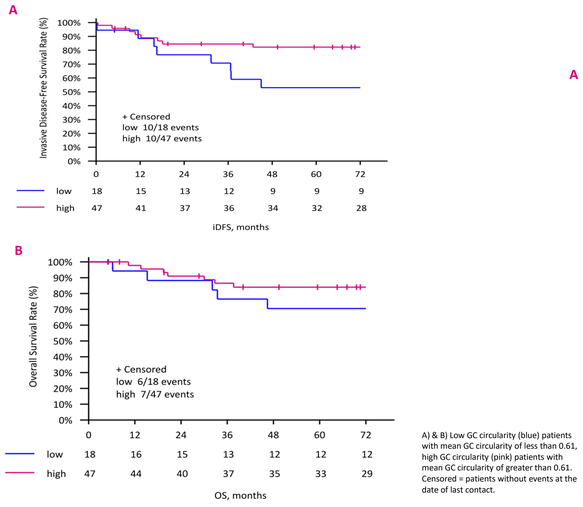
- Of the eTNBC patients assessed by *smuLymphNet,* 75/116 were LN positive. Median follow-up was 9.2 years (IQR, 5.5-11.2). Median age was 52.0 years (range 28.0- 69.0 years). There was no significant difference in clinicopathological characteristics between LN positive and negative patients (**Table 1**).
- *smuLymphNet* identified 513 GC in 281 LN sections (range, 0-28).
  - LN positive and LN negative patients had median GC numbers of 0.5 (range, 0-23.0) and 0.3 (range, 0-24.0), respectively.
  - GC area ranged from 0-0.1mm^2 in all patients.
  - Mean and median GC circularity was 0.7 for both LN positive and LN negative patients (range 0.2-0.9 vs 0.3-0.9).
- Strong positive correlation was found between *smuLymphNet* and pathologist identified GC (Spearman's rho was 0.876, $p < 0.005$) and a true positive detection of 55%.

Figure 2. Forest plot of univariate cox regression analyses with GC features for iDFS



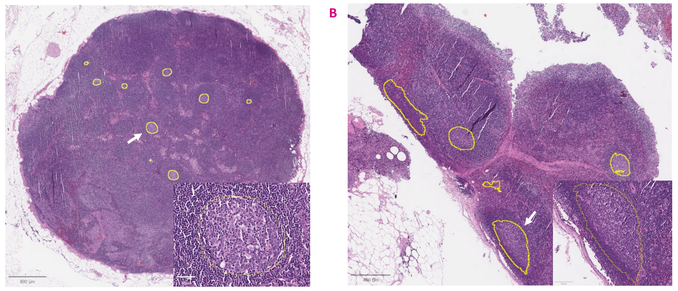
- Higher GC circularity (continuous at 0.1 increase and dichotomised at 0.61) was significantly associated with improved iDFS (HR=0.68 and 0.35, 95% CI 0.50-0.92 and 0.14-0.84, p=0.013 and 0.018, respectively) (**Figure 2 & 3A**).
- This remained significant after adjustment for age at diagnosis, pN status and sTILs (HR=0.64 and 0.28, 95% CI 0.46-0.87 and 0.11-0.70, p=0.005 and 0.007, respectively).
- OS results were consistent with iDFS; higher GC circularity (per 0.1 increase) significantly associated with improved survival when adjusted for age at diagnosis, pN status, and sTILs (HR=0.65, 95% CI 0.43-0.97, p=0.037) (**Figure 3B**).
- GC circularity did not correlate with sTILs, Ki67 index, age at diagnosis, histological grade and stage.

Figure 3. Kaplan-Meier curves for A) iDFS and B) OS, low vs high mean GC circularity per LN section



A) & B) Low GC circularity (blue) patients with mean GC circularity of less than 0.61, high GC circularity (pink) patients with mean GC circularity of greater than 0.61. Censored = patients without events at the date of last contact.

Figure 4. Histology examples of A) high and B) low GC circularity with *smuLymphNet* GC detections overlaid



A) Representative H&E-stained LN section with high mean GC circularity, B) Representative H&E-stained LN section with low mean GC circularity. *smuLymphNet* GC detections in yellow. White arrow indicates germinal centre visualised at high power (inset)

Table 1. Baseline clinicopathological characteristics

Parameter	Category	LN Negative N= 41 N (valid %)	LN Positive N= 75 N (valid %)
Age, years	Median age (range)	54 (28-66)	52 (29-69)
pT	pT1	19 (46.3)	34 (45.3)
	pT2	20 (48.8)	34 (45.3)
	pT3	2 (4.9)	7 (9.3)
	pT4	0 (0.0)	0 (0.0)
pN	pN0	41 (100.0)	0 (0.0)
	pN1	0 (0.0)	43 (57.3)
	pN2	0 (0.0)	23 (30.7)
	pN3	0 (0.0)	9 (12.0)
Histological tumour type	Ductal invasive	24 (58.5)	63 (84.0)
	Lobular invasive	0 (0.0)	0 (0.0)
	Other*	17 (41.5)	12 (16.0)
Tumor grading	G1	0 (0.0)	1 (1.3)
	G2	13 (31.7)	11 (14.7)
	G3	28 (68.3)	63 (84.0)
Ki67, central	≤20%	2 (4.9)	4 (5.3)
	>20%	39 (95.1)	71 (94.7)
sTILs, central	Low (1-10%)	19 (46.3)	30 (40.5)
	Intermediate (11-59%)	17 (41.5)	27 (36.5)
	High (60-100%)	5 (12.2)	17 (23.0)
	Missing	0 (0.0)	1 (1.3)

*The category other' comprises predominantly non-specific histological diagnoses (about 50% NST/NOS variants), as well as rare invasive subtypes (e.g. metaplastic, medullary, papillary carcinomas and mixed histologies).

Conclusions

smuLymphNet enabled reproducible, automated assessment of GC morphology, capturing variation in circularity that cannot be reliably quantified by visual inspection. Across 281 lymph node sections, the tool demonstrated strong concordance with pathologist assessments and accurately identified GC structures. Higher GC circularity was consistently associated with improved iDFS, even after adjustment for clinical covariates. These associations extended to OS, reinforcing GC circularity as a prognostic morphological feature. The results align with previous findings in treatment-naïve TNBC and position GC circularity alongside established features such as GC number and sinus area[1]. Further evaluation in neoadjuvantly treated TNBC cohorts is warranted to support clinical translation and enhance prognostication.

References

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2.Möbus V, Luck HJ, Ladda E, et al. Phase III randomised trial comparing intense dose-dense chemotherapy to tailored dose-dense chemotherapy in high-risk early breast cancer (GAIN-2). Eur J Cancer. 2021;156:138-48.

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Biorender.com was used to generate Figure 1 Study Design