Region-Specific Collagen Morphometric Parameters in HER2+ Breast Cancer versus TNBC: Insights from SHG/TPE Imaging and AI-Based Analysis

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Abstract

BACKGROUND: Triple-Negative Breast Cancer (TNBC) and HER2-positive (HER2+) breast cancer represent biologically distinct subtypes with differing molecular drivers, clinical behaviours, and responses to therapy. Despite these differences, both subtypes may be associated with significant tumor-stroma interactions and fibrotic remodelling, which may contribute to disease progression and therapeutic response differences. However, comparative spatial characterization of collagen architecture across different tissue compartments in these subtypes remains limited. Second harmonic generation/two-photon excitation (SHG/TPE) microscopy, coupled with AI-based image analysis, enables quantitative assessment of collagen morphometry beyond conventional histopathology. This study aims to compare region-specific collagen features between TNBC and HER2+ breast cancer biopsies and identify distinguishing fibrosis patterns across tumor and non-tumor regions.

METHODS: Unstained breast cancer biopsies from TNBC (n=21) and HER2+ (n=158) patients from Dept of Surgery, National Taiwan University Hospital were imaged using SHG/TPE microscopy. Collagen morphometric features were identified using a proprietary AI-based analysis platform (Genesis®200, HistoIndex Pte Ltd, Singapore). Four anatomically distinct tissue regions were assessed: tumor, lobule and duct, stroma-fat, and stroma-fibrosis. Each region comprised of 65 collagen morphometric parameters, and was compared between TNBC and HER2+ groups within each region using normalized Median Relative Differences. Statistical comparisons were performed using the Wilcoxon rank-sum test to evaluate feature-level differences between the two subtypes.

RESULTS: Region-specific collagen morphometric profiles demonstrated distinct differences between TNBC and HER2+ breast cancer biopsies. Within the tumor region, collagen parameters were largely comparable between the two groups, with no major differences observed. In the stroma-fibrosis compartment, TNBC samples showed elevations in parameters related to collagen fibres but these trends were not statistically significant (Figure 1A). Statistically significant reductions ($p < 0.05$) of specific collagen parameters were observed in lobule and duct regions of HER2+ biopsies versus TNBC biopsies; 25% decrease in number of thick strings per unit tissue area, a 17% reduction in string perimeter, and decrease of 20% and 18% in width of all strings and width of aggregated strings per unit tissue area, respectively (Figure 1B).

CONCLUSIONS: Quantitative SHG/TPE imaging combined with AI-based collagen morphometric profiling reveals distinct fibrosis patterns between TNBC and HER2+ breast cancer subtypes. The lobule and duct region in HER2+ biopsies demonstrates a unique collagen architecture when compared with rest of TNBC biopsies. These spatially localized differences in extracellular matrix structure may reflect subtype-specific stromal remodelling and could offer utility in subtype classification, prognosis, or therapeutic response prediction. Further validation of these imaging-derived biomarkers in larger cohorts is warranted.

Introduction

- Triple-Negative Breast Cancer (TNBC) and HER2-positive (HER2+) breast cancers represent biologically and clinically distinct subtypes [1]
- Despite their differing molecular drivers, both subtypes exhibit tumor–stroma interactions and fibrotic remodelling that could potentially influence progression and treatment response
- Fibrosis and collagen architecture are key components of the tumor microenvironment (TME) that modulate cell behaviour, invasion, and drug resistance [2,3,4]
- Conventional histopathology provides limited quantitative assessment of these collagen features
- Second Harmonic Generation (SHG)/Two-Photon Excitation Fluorescence (TPEF) microscopy and AI-analysis based qFibrosis® [5] allows stain-free, high-resolution visualization of fibrillar collagen structure
- AIM: This study investigates region-specific collagen morphometric differences between TNBC and HER2+ breast cancers using SHG/TPEF microscopy and AI-based image analysis

Methods and Materials

- **Samples:** Unstained breast cancer biopsies — TNBC (n = 21), HER2+ (n = 158) - from Department of Surgery, National Taiwan University Hospital
- **Imaging and Analysis Platform:** Genesis®200 (HistoIndex Pte Ltd, Singapore) for automated SHG/TPEF microscopy and AI-based collagen quantification
- **Tissue segmentation:** Four anatomical regions were analyzed, defined based on expert pathologist-guided annotations established from prior training cohorts:
  a) Tumor region
  b) Non-tumor regions: lobule & duct, stroma-fat, stroma-fibrosis
- **Parameters:** >60 collagen morphometric features per region (e.g., string width, perimeter, aggregation, orientation)
- **Statistical Analysis:** Normalized Median Relative Differences and Wilcoxon rank-sum test ($p < 0.05$ threshold for significance)

Results

- **Tumor Region:** No significant differences in collagen morphometric features between TNBC and HER2+ biopsies
- **Stroma-Fibrosis Region:** TNBC showed higher collagen fibre-related parameters, but trends did not reach statistical significance
- **Lobule and Duct Region:**
  - HER2+ biopsies have significantly lower collagen morphometrics compared with TNBC:
    - 25% fewer thick strings per tissue area
    - 17% lower string perimeter
    - 20% and 18% decreases in average and aggregated string width, respectively ($p < 0.05$)

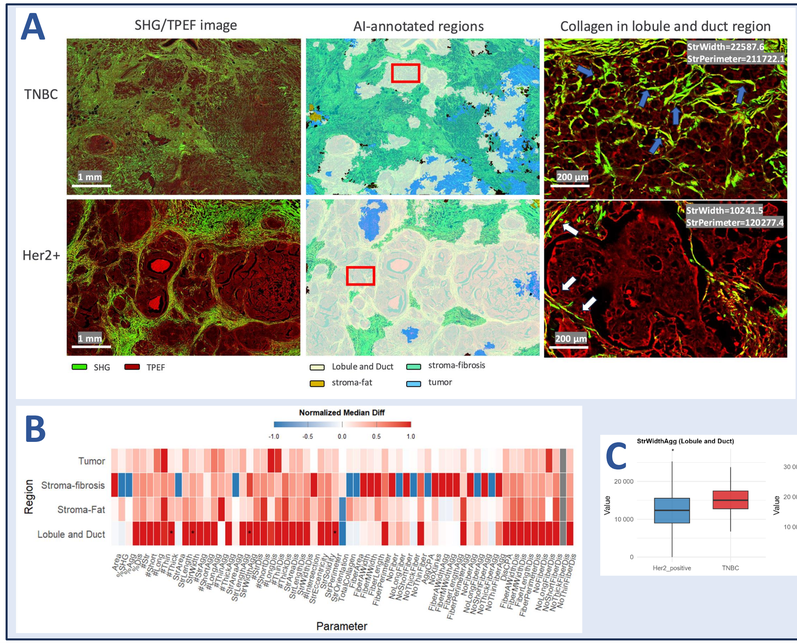


Figure 1. [A] Representative SHG/TPEF image showing tumor (red) and collagen (green) annotations. The middle panel displays AI-generated zonal segmentation into tumor, lobule & duct, stroma–fat, and stroma–fibrosis regions (color legend shown below). The right panel illustrates differences in collagen string width and string perimeter distribution between TNBC and HER2+ biopsies. TNBC biopsies on SHG/TPEF imaging and AI-based quantitative assessments shows higher values of collagen string width and perimeter (blue arrows) compared to HER2+ biopsies (white arrows). These fibers correspond to the ROIs highlighted by red boxes in the middle panel.

[B] TNBC vs HER2+ group Normalized Median Relative Difference by region (Parameter-wise normalization scaled median differences to within the scale of -1 to +1; Wilcoxon test, *p<0.05, **p<0.01). **[C]** Box plots illustrating statistically significant differences in collagen string-related parameters in the lobule and duct region between TNBC and HER2+ breast cancer biopsies; #Thick (number of thick strings per unit tissue area), StrPerimeter (string perimeter), StrWidth (width of all strings per unit tissue area), StrWidthAgg (width of aggregated strings per unit tissue area).

Discussion

- **Subtype-Specific Stromal Remodelling:**
  - Distinct collagen organization reflects underlying stromal activation and mechanical signalling patterns specific to TNBC vs. HER2+ tumors [6]
  - Our results show that TNBC is associated with denser and more organized collagen, which could promote invasiveness and poor prognosis as previously described [6,7]
- **Diagnostic and Prognostic Potential:**
  - Alterations in collagen fibre width, alignment, and density correlate with breast cancer aggressiveness and survival outcomes [8]
  - Quantitative collagen morphometry could complement immunohistochemical and molecular profiling by providing a structural signature for tumor subtype classification [9]
- **Therapeutic and Drug Trial Implications:**
  - Spatial ECM heterogeneity influences drug penetration, immune infiltration, and mechanical resistance [10,11]
  - Fibrosis-targeting therapies or anti-stromal agents could in future require subtype-specific evaluation, based on differences in collagen remodelling patterns [12]
  - Imaging-based collagen metrics could serve as quantitative endpoints in therapeutic trials assessing ECM-modulating or combination therapies
- **Broader Applications:**
  - SHG/TPEF-based collagen profiling can potentially be extended to longitudinal biopsy analysis in neoadjuvant chemotherapy trials to assess stromal response
  - May aid in developing AI-assisted diagnostic models integrating morphological fibrosis features with clinical and genomic data for predictive analytics

Conclusions

- SHG/TPEF imaging combined with AI-based collagen morphometry analysis (qFibrosis®) reveals region-specific stromal and fibrotic differences between TNBC and HER2+ breast cancers
- Significant reductions in collagen thickness and density parameters in the lobule and duct regions of HER2+ cases could suggest subtype-specific ECM remodeling patterns
- These quantitative ECM descriptors could support subtype differentiation, prognostic modeling, and therapy response assessment
- Future work should validate these findings in larger, multi-cohort studies and explore integration with genomic and clinical outcomes

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