Structured Reports for the Pathological Society Grant Awards: Career Development Fellowships (CDF), Path-Soc / J Shanks Fellowship, Cuthbert Dukes Award, PhD Studentships, International Collaborator Awards, Small Grant Scheme (several types) Awards, CRUK/Path-Soc Pre-Doctoral Research Bursary & Early Career Pathology Researcher (Hodgkin & Leishman) Grants.

Title:

Investigating the Tumour Microenvironment using Multiplex Digital Analysis in Non-Caucasian Breast Cancer

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BACKGROUND:

There are 55,200 new cases of breast cancer diagnosed in the UK per year, making up a total of 15% of all new cancer cases (2017)[1].

The world-wide literature reports differences in the incidence, pathobiology and prognosis of breast cancers according to ethnicity[2,3]. Studies have suggested a younger age of onset[4], more aggressive disease[5], a higher incidence of triple-negative breast cancer (TNBC)[6] and a less favourable outcome in African-American women[7], despite an overall increased breast cancer incidence within the European-American population[7]. Similar findings are echoed in the available, though limited, UK literature[8,9].

The tumour microenvironment is an exciting area of breast cancer research. Tumour infiltrating lymphocytes (TILs) and tumour associated macrophages (TAMs) have emerged as evolving areas of prognostic interest. TAM localisation, in particular, stromal infiltration has been shown to be an independent marker of reduced survival in breast carcinoma[10]. Higher TIL numbers are linked to improved pathological response following neoadjuvant therapy and increased treatment response in HER2+ and TNBCs[11-15]. There is much interest in the composition, density and localisation of TILs. For example, increased numbers of CD8+ cytotoxic TILs have been shown to be an independent marker of breast cancer-specific survival[16].

Data is emerging that TILs vary according to ethnicity. Stromal TILs significantly varied according to ethnicity in patients with advanced HER2+ breast cancer given pertuzumab or placebo plus trastuzumab and docetaxel[17]. The density of breast cancer TAMs and crown-like structures has been shown to vary significantly between ethnic groups[5].

However, information on the variability and impact of the tumour microenvironment of UK breast cancers according to patient ethnicity is limited.

AIMS:

This pilot study aims to explore the tumour microenvironment, namely the composition, density and localisation of TILs and tumour macrophages using novel digital imaging software and multiplex staining for CD4, CD8, CD20, FOXP3 and CD68 in UK breast cancer patients of differing ethnicity.

METHODS/RESULTS:

A total of 54 pre-treatment biopsies from women diagnosed with invasive breast cancer were selected retrospectively from our local NHS database (2004-2016) as an initial pilot study. The cases contained

a mixture of patients of different ethnicities including Indian, Pakistani and African. This pilot study focused on refining the methodology for future expansion of our cohort.

All cases were diagnosed as invasive carcinoma no specific type (NST), of grade 2 or 3 and a mixture of receptor types (ER+, HER+, and TNBC) where possible.

The cases underwent multiplex immunofluorescence staining with CD20, CD68, CD4, CD8 and FOXP3 antibodies using the Vectra Automated Quantitative Pathology Imaging System, which allows quantifiable multi-colour phenotyping of immune cell subsets. All cellular nuclei were stained with DAPI (figure-1, a).

Relevant fields showing the tumour/stromal interface were selected for each case using Phenochart® 1.0 (2018 PerkinElmer). Localisation, cell density and phenotypic analyses were achieved through utilisation of digital analysis software techniques (inForm® 2.4, 2019 Akoya Biosciences) in conjunction with control references.

Invasive tumour fields were identified by the investigator and reviewed by a specialist pathologist where required. Ductal carcinoma in situ and normal breast tissue were excluded from the analysis. The process of tissue segmentation (figure-1, b) was found to be greatly augmented with the generation of algorithms in addition to adding cytokeratin AE1/3 to the panel of immunohistochemistry. Fields containing background debris or significant tumour necrosis were removed from the analysis.

The images subsequently underwent the process of cell segmentation (figure-1, c) to delineate cell nuclei and membrane borders required for subsequent phenotyping of each tumour/stromal field into CD20+, CD4+, CD8+, CD68+, FOXP3+ and other 'DAPI+' cells (figure-1, d).

Discussion

This Pathological Society grant ward has facilitated this pilot study which is still ongoing. The study has provided the pilot feasibility data on the application of novel digital analytical techniques in studying the tumour microenvironment. Through this, we have been able to explore the tumour microenvironment including the cell density, location and phenotype at the tumour/stromal interface.

We hope to expand our cohort and relate our data to clinical parameters so that we may add to the scientific literature on this topic. Understanding the tumour microenvironment may provide valuable information on those who may benefit from targeted immune therapies.

How Closely Have the Original Aims been Met:

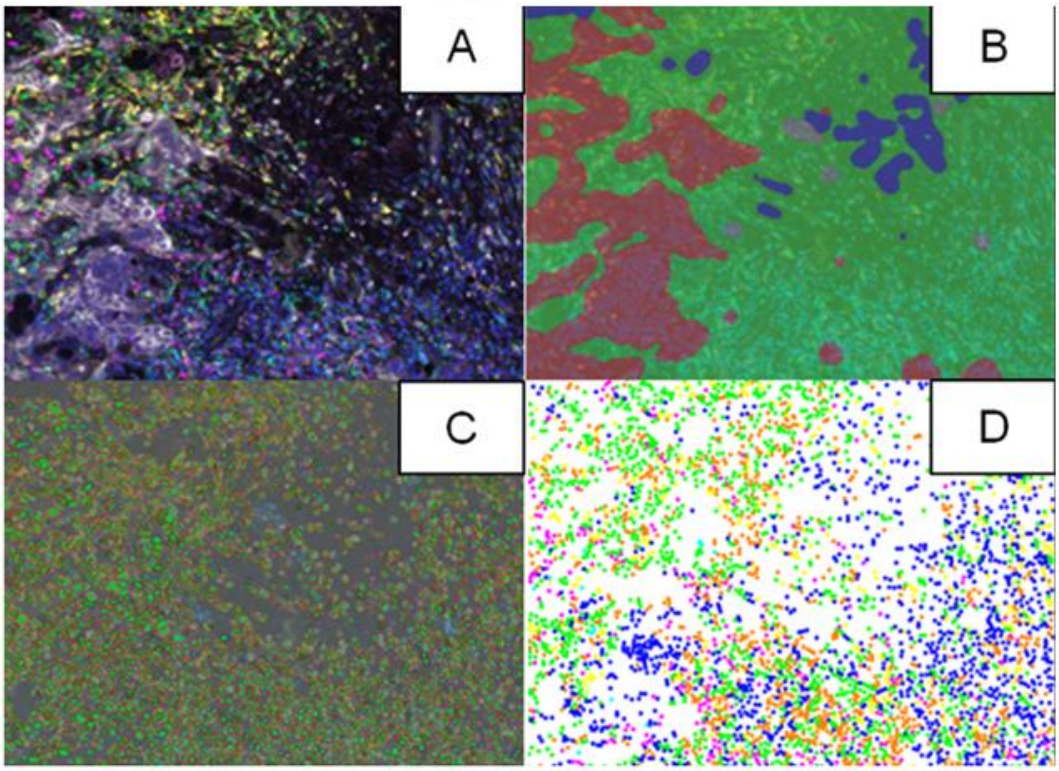
This project has successfully utilised digital software analysis techniques to study the tumour microenvironment in breast cancer in patients of differing ethnicities. This pilot study has allowed refinement of our methodology. We hope to expand our cohort and relate our findings with clinical parameters, the work of which is currently ongoing.

Outputs (including meeting abstracts, oral presentations, original papers, review articles) from the study in which the Pathological Society has been acknowledged:

An abstract entitled 'Multiplex immunophenotyping of breast cancer tumour microenvironment' has been submitted by our group to the 32nd Congress of the European Society of Pathology and the XXXIII International Congress of the International Academy of Pathology meeting.

Figure-1:

Digital images courtesy of inForm® software



Digital images A-D: Tumour-stroma interface

A: Fluorescence multiplex view
Pink: Cytokeratin AE 1/3. Magenta: CD68. Yellow: CD20. Green: CD4. Orange: CD8. Cyan: FOXP3. Blue: DAPI
B: Tissue segmentation
Red: Tumour. Green: Stroma. Blue: Background
C: Cell segmentation
Green: Nuclei. Red: Cell membrane.
D: Cell phenotype (colours as per A)

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