sequencing often gave only low TCR repertoire diversity. Therefore, an RNA based approach was taken, given the presence of multiple rearranged TCR transcripts, but only a single rearranged TCR genomic DNA copy in any given cell.
FUME-TCRseq is a nested, multiplex, reverse-transcriptase polymerase chain reaction (RT PCR) method for TCR repertoire sequencing designed to amplify TCR-beta sequences from FFPE RNA. We modified this PCR system for TCR sequencing, using gene-specific primers from the EUROCLONALITY TCR PCR system. Primers were re-designed for cDNA compatibility, moving them to be entirely within the coding region of the constant or junctional segment. Following sequencing, the amplified products were analysed with in-house machine learning methodologies.
The new PCR system successfully amplified sequences using gene-specific primers.
This study developed a novel approach to bulk TCR repertoire sequencing from FFPE RNA. Now that we have a suitable T-cell sequencing methodology, bioinformatic analysis can be applied to compare repertoires between patients with or without various pathological conditions, including CeD, with the long-term aim of providing better diagnostic tests for these conditions.

OP9 The impact of deploying an ai solution for supporting breast cancer diagnosis in multiple NHS hospitals: DEBORAH study accuracy

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Background: Breast cancer is the most common cancer in the UK, with over 59000 cases a year projected to rise further. The declining pathology workforce poses challenges in meeting diagnostic demand whilst maintaining turnaround times. When accurate, artificial intelligence (AI) tools have the potential to support pathologists and alleviate pressures on pathology departments.
Purpose: This study evaluates the diagnostic performance of a commercial AI solution, IBEX Breast, on a large patient cohort from 5 NHS hospitals across the UK.
Methods: This non-interventional, observational prospective study includes 2,600 female patients who underwent breast core biopsy. Initial diagnoses were made by pathologist review unsupported by AI as per standard of care. After composing a preliminary report, the pathologists had access to the AI findings. Diagnostic outcomes were compared and discrepancies were assessed in classifications of Benign, Cancer, Invasive Cancer, and DCIS diagnoses. Cases with potential discrepancies were further discussed within the pathologist teams, before a final diagnosis was determined through adjudication.
Results: In discriminating between cancerous and benign cases, the AI demonstrated a sensitivity of 100%, specificity of 99.7%, negative predictive value (NPV) of 99.7%, and positive predictive value (PPV) of 100%. In identifying invasive cancer, it exhibited sensitivity of 99.6%, specificity of 99.9%, PPV of 99.7%, and NPV of 99.8%. For DCIS diagnosis, the AI achieved sensitivity of 98.7%, specificity of 99.9%, PPV of 98.7%, and NPV of 99.9%.
Conclusions: The AI solution demonstrated excellent diagnostic performance across all the diagnostic categories investigated, showing very high sensitivity, specificity, and predictive values in distinguishing between cancerous and non-cancerous cases, as well as in discriminating between invasive cancer and DCIS. These findings suggest the AI solution has potential to improve accuracy and support patient care.

OP10 TP53 mutations in ductal carcinoma in situ of the breast are associated with ipsilateral invasive recurrence.

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Purpose of the study: Ductal carcinoma in situ (DCIS) of the breast is regarded as a non-obligate precursor of invasive breast cancer. The natural behaviour of DCIS is unknown, since most patients undergo surgery after a DCIS diagnosis. This study aimed to identify biomarkers for the prediction of ipsilateral invasive recurrence after surgery, by comparing the features of DCIS with an in situ recurrence versus DCIS with an invasive recurrence.
Methods: Representative tissue blocks from primary DCIS and their ipsilateral recurrence were retrieved. Brightfield immunohistochemistry (IHC) was performed for oestrogen receptor (ER), progesterone receptor (PR) and HER2. Immunofluorescence multiplex IHC was performed for EGFR, p53, decorin, versican, CD3 and CD20. All IHC stains were performed using an automated slides stainer. All DCIS were subjected to next-generation sequencing (NGS) to determine TP53 status. Statistics comprised univariate Cox survival analyses.
Summary of results: In total, 44 DCIS patients with an ipsilateral recurrence after surgery were identified: 23 in situ recurrences and 21 invasive recurrences. Stromal expression of decorin and versican and stromal tumour-infiltrating lymphocytes were not associated with the time to invasive recurrence. Expression of ER, PR, HER2 and EGFR in the neoplastic cells were not predictive for invasive recurrence. TP53-mutated DCIS showed a significantly shorter mean time to ipsilateral invasive recurrence (26 months) than DCIS without TP53 mutations (89 months; $p = 0.016$; hazard ratio 3.537; 95% confidence interval 1.267–9.869). These NGS-based findings were confirmed by the p53 IHC staining pattern.
Conclusions: TP53 mutation status is a promising predictive marker to identify DCIS patients at high risk of developing ipsilateral invasive recurrence, and p53 IHC can be used as a surrogate marker. This observation requires validation in larger, independent patient cohorts.

OP11 How to predict upstaging to invasive breast carcinoma after a biopsy diagnosis of pure ductal carcinoma in situ of the breast?

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Purpose of the study Around 20% of patients with biopsy-diagnosed pure ductal carcinoma in situ (DCIS) present an invasive tumour in the subsequent resection. We aim to find reliable prognostic markers to predict upstaging to invasive breast cancer (IBC). Methods Archived biopsy slides of 272 patients with pure biopsy-diagnosed DCIS were assessed. Nuclear atypia, DCIS architecture, necrosis, calcifications, periductal myxoid stroma, lobular cancerization, and HER2 were scored as categorial variables. Tumour-infiltrating lymphocytes (TILs), oestrogen receptor (ER), and progesterone receptor (PR) were scored as continuous variables. Statistical analysis included Chi-square tests for categorical data, Mann-Whitney tests for continuous data, univariate and multivariate logistic regression. Summary of results Fifty-four of 272 women (19.9%) were upstaged to IBC after surgery. In univariate analysis, solid architecture and lack of calcifications are significantly associated with upstaging ($p = 0.008$ and $p = 0.02$). Multivariate analyses show that solid architecture and lack of calcifications are independent predictors ($p = 0.004$ and $p = 0.001$). Some parameters show a trend towards upstaging without being statistically significant: high TILs ($p = 0.063$) and periductal myxoid stroma ($p = 0.084$). Cribriform architecture, ER, PR, HER2, nuclear grade, solid or micro-papillary architecture and lobular cancerisation are not statistically significantly associated with upstaging ($p > 0.05$). Conclusions We identified the absence of calcifications and solid DCIS architecture as significant predictors for upstaging to IBC. The present complementary immunohistochemistry for ER and PR realized in clinical practice does not help to distinguish DCIS with synchronous IBC from pure DCIS. We currently analyse the expression of 12 different proteins by multiplex immunofluorescence immunohisto-chemistry, aiming to explore their expression in the extracellular matrix, the neoplastic cells and TILs.