OP1

Linking Tumour Morphology and Biology To Deep-Learning Based End-to-End Prognostication in Colorectal Cancer Patients

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Oral Presentations Group 1A, Lecture theatre 1, June 18, 2024, 2:00 PM - 3:30 PM

Background: Several studies suggested recently, that deep-learning (DL) algorithms may be able to predict colorectal cancer (CRC) patients' prognosis directly from Haematoxylin/Eosin stained tissue sections. However, due to the mostly 'black-box' nature of DL algorithms, widespread implementation into routine diagnostics as well as into research pipelines is still lacking.

Methods: In this study, we investigate the relationship between a recently published DL-based end-to-end CRC patient prognosis prediction score, histomorphological and molecular features of two independent CRC cohorts (DUESSEL-CRC (n=164) and TCGA-CRC (n=207)). Histopathological features included Stroma AReactive Invasion Front Areas (SARIFA), tumor-adipose-feature (TAF), morphometrically measured proportion of tumor (PoT) data, grade of differentiation, lymph node status (pN), and depth of invasion (pT). Molecular features included mismatch repair (MMR) status, expression level of Ki67, BRCA1/2, ATM, consensus molecular subtypes (CMS) and pathway-derived subtypes (PDS).

Results: In DUESSEL-CRC and TCGA-CRC cohorts, presence of SARIFA and of TAF were associated with a higher prediction score, i.e. a higher risk of death according to the DL-based model (all $p<0.0001$). Higher intratumoral stroma content (PoT-low), immunohistochemical expression level of Ki67, BRCA1/2, ATM, and MMR status were variably related to the DL-based prediction score. Interestingly, the DL-based prediction scores for different transcriptional CMS and PDS groups did not differ significantly. Presence of metastatic lymph nodes or poor grade of tumour differentiation were also associated with a higher DL-based prediction score (all $p<0.05$).

Discussion: Our current study suggests that linking DL-based prediction scores to known histopathological or molecular features might increase the explainability of DL-based prognostication tools. Further studies need to demonstrate whether DL-based prediction score have added value to already known prognostic factors in CRC patients.

OP2

QuPath Quantification Identifies Distinct Cellular and Fibrotic Changes in Fibrostenosing Crohn's Disease

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Oral Presentations Group 1A, Lecture theatre 1, June 18, 2024, 2:00 PM - 3:30 PM

Background: Crohn's Disease (CD) affects up to 1% of the European population and is increasing in incidence. Well-established single nucleotide polymorphisms (SNPs) influence CD risk. CD effects are mostly due to chronic transmural inflammation, sometimes leading to progressive fibrosis of the wall with thickening and partial luminal obstruction.

Purpose: To accurately quantify cells and fibrosis involved in Crohn's fibrostenosing lesions, within each intestinal wall layer, which has not been previously reported.

Methods: Formalin-fixed, paraffin-embedded terminal ileal resections from 30 controls and 30 CD patients were stained using immunohistochemistry (IHC) to identify specific cell types: CD3, CD4 and CD8 T-cells, CD20 B-cells, CD68 macrophages, CD31 endothelial cells and smooth muscle actin-positive cells. Collagen deposits were visualised using Picro-Sirius Red (PSR) staining. The sections were captured digitally and analysed with QuPath, open-source imaging analysis software, to quantify the fibrosis and cell populations within each ileal layer (mucosa, muscularis mucosae, submucosa, muscularis propria, serosa). Collagen deposits were quantified using a machine-learning classifier, while the DAB-positive IHC-detected cells were counted using the built-in positive-cell detection function.

Results: The quantification of features showed partial loss of the mucosa layer due to widespread ulceration, with a notable expansion of the muscularis mucosae mostly due to increased fibrosis and smooth muscle hypertrophy. The serosa was also expanded by fibrosis. There was increased infiltration of all T and B lymphocytes (forming lymphoid aggregates in all cases), macrophages (forming granulomas in some cases) and endothelial cells, in all layers, except for the mucosa (due to its partial loss from ulceration).

Conclusion: Quantitative analysis showed marked cellular changes in all intestinal wall layers within Crohn's fibrostensing lesions, especially muscularis mucosae and serosa expansion. QuPath allows accurate quantification of fibrosis and cell infiltrations in all layers of the intestinal wall associated with CD and this may help to guide personalised therapy.

OP3

The Morphology of the Largest Tumour-negative Regional Lymph Node Reflects the Host Anti-tumour Immune Response in Oesophageal Cancer Patients

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Oral Presentations Group 1A, Lecture theatre 1, June 18, 2024, 2:00 PM - 3:30 PM

Background

Regional lymph node (LN) status is a key prognostic factor in patients with oesophageal cancer (OeC). Tumour derived antigens can lead to immune activation in LNs which might provide clinically useful information of the host's anti-tumour immune response. It is currently unknown whether the immune response is homogeneous across all tumour-negative LNs (LNneg) within a patient.

Purpose

We hypothesized that all LNneg within an OeC patient have a similar microarchitecture irrespective of their size and analysed the heterogeneity of LNneg morphology to established whether the largest LNneg can be used as a surrogate for the immune response of all LNnegs within a patient.

Methods

82 pN0 patients from the OE02 trial had at least two LNneg. The microarchitectural LN features (germinal centres (GC), lymphocytes outside GCs, histiocytes) were morphometrically quantified. Linear mixed-effects regression models, intraclass correlation coefficients (ICC) and Bland-Altman plots were used to determine systematic bias, reliability/variability and agreement of the LNneg microarchitecture measurements.

Results

Linear mixed-effects models revealed no systematic bias in LNneg morphology within a patient. The ICC indicated that variability was moderate for lymphocytes (ICC: 0.42; 95%CI: 0.11–0.63, p=0.007)) and GCs (ICC: 0.50; 95%CI: 0.23–0.68, p<0.001), and high for histiocytes (ICC: 0.07 (95%CI: -0.44–0.4, p=0.38). Bland-Altman analyses showed at maximum 8.5 % of values outside of the 95% limits of agreement.

Conclusions

This study is the first to systematically assess the agreement of the microarchitectural features in LNneg within an individual pN0 OeC patient. The absence of systematic bias supports the use of the largest LNneg as surrogate for a patient's overall immune response in OeC. Further studies are warranted to investigate whether the identified heterogeneity of the LNneg immune reaction within a patient is related to the distance between individual LNnegs and primary tumour or variation in lymphatic drainage routes.

OP4

Best-Practice Biomarker Testing of Oesophago-Gastric Cancer in the UK: Expert Consensus Recommendations Developed by Pathologists and Oncologists

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Oral Presentations Group 1A, Lecture theatre 1, June 18, 2024, 2:00 PM - 3:30 PM

Background
Oesophago-gastric cancers (OGC) are common malignancies with high disease-related mortality. Predictive biomarker testing can guide individualised treatment; testing for HER2, MSI/MMR, and PD-L1 is recommended, but implementation varies.

Purpose
To formulate consensus recommendations among pathologists and oncologists for OGC best-practice biomarker testing in the UK.

Methods
A pragmatic literature review was conducted to inform development of statements using a modified Delphi method. UK-practicing pathologists and oncologists were recruited for two rounds of online questionnaires, composed of topics relating to the overall pathway, pre-analytical, analytical, and post-analytical considerations. Statements were rated using a 7-point Likert scale, from 1, ‘strongly disagree’, to 7, ‘strongly agree’, and responses consolidated to quantify levels of agreement. Consensus was pre-determined at 80%. Responses were discussed during a virtual consensus meeting for validation and final recommendations.

Results
Participants from across the UK completed the first (n=26) and second Delphi rounds (n=18), with an even split between pathologists and oncologists. Consensus was reached (both 89%) that clear guidance and standardisation of the pathway across UK laboratories and Trusts is needed, and that coordination throughout the tissue journey is required for best practice. However, while oncologists reached consensus that reflex testing for all biomarkers should be implemented, pathologists did not, raising concerns relating to capacity. Consensus was reached that a minimum of 8 biopsies should be taken (92%), testing metastatic tumour samples was acceptable (100%), biomarker results should be reported in 5 working days (81-85%), and should be visible in the diagnostic pathology report (96%).

Conclusions
Best-practice recommendations for biomarker testing in OGC were formulated using opinions from UK pathologists and oncologists. These results support the need for standardisation and co-ordination throughout the tissue journey. Significant concerns were raised representing barriers to testing, highlighting challenges faced in clinical practice and a need to foster successful working relationships.

OP5

Overexpression of CUX2 is Related to Better Prognosis in Colorectal Cancer

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Oral Presentations Group 1A, Lecture theatre 1, June 18, 2024, 2:00 PM - 3:30 PM

Background: Colorectal cancer (CRC), the fourth most common cancer in the United Kingdom, differs in prognosis depending on the tumour stage and early (vascular invasion) or late metastatic events (nodal and distant metastases). Genomic analysis from the Cancer Genome Network (1) indicated that the transcription factor Cut Like Homeobox (CUX) 1, may be related to such metastatic events. This was corroborated locally and hence another member of the CUX family, CUX2, was investigated for similar correlations.

Purpose: This study aimed to explore whether expression of the transcription factor CUX2 correlated with clinicopathological variables and the metastatic phenotype of CRC.

Methods: Manual scoring of CRC tissue microarrays (n=380), immuno-stained for expression of CUX2 at the luminal, middle, and the advancing edge of each tumour was performed and analysed using SPSS software against clinicopathological variables to determine statistical significance. Survival analysis was assessed using Kaplan-Meier curves.

Results: Overexpression of CUX2 was significantly ($p < 0.05$) associated with better prognostication, such as lower grade, lower final stage, lower nodal stage, fewer recurrences, and metastases. When looking at MMR proficient and deficient cases it was also determined that there was significant correlation in the MMR proficient tumours with increased CUX2 expression at the advancing edge, correlating with better prognostic outcomes. Though survival advantage was observed for higher CUX2 expression at the advancing edge on univariate analysis, the significance was lost on multivariate analysis.

Conclusion: CUX2 appears to have a protective role in CRC, with loss related to poor prognostic variables. This seems to be a tissue specific effect within CRC as compared to other tumours (2) or may potentially be related to isoforms. Further research, including functional studies, would be necessary to fully delineate the specific role that CUX2 plays in CRC.

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OP6

High Frequency of Lynch Syndrome among Indonesian CRC is associated with KRAS and PIK3CA oncogenic mutations

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Oral Presentations Group 1A, Lecture theatre 1, June 18, 2024, 2:00 PM - 3:30 PM

Background: The incidence of young people <50 years old with colorectal cancer (CRC), termed as early onset colorectal cancer (EOCRC). is nearly 30% of the total CRC patients in Indonesia. Lynch syndrome (LS) is a hereditary type of CRC that is associated with a younger age of onset.
Purpose: Aim of this study was to investigate the frequency of LS in Indonesian CRC patients and its association with oncogenic mutation of KRAS and PIK3CA.
Methods: Archival (2016–2019) formalin-fixed, paraffin-embedded (FFPE) tumour tissues from CRC patients were collected from Sardjito General Hospital Yogyakarta, Indonesia. The previously described high-resolution melting (HRM)-based test called Nottingham Lynch Syndrome Test (N_LyST) was used in this project. The test consisted of five mononucleotide microsatellite markers (BAT25, BAT26, BCAT25, MYB, EWSR1), BRAF V600E mutation and MLH1 region C promoter methylation. Additionally, KRAS (exon 2 and 3), and PIK3CA (exon 9 and 20) were also tested.
Results: There was 50/231 (21.65%) EOCRC cases, with 15/50 (30%) regarded as MSI, as opposed to 29/181 (16.02%) within the older group. In total, 32/231 patients (13.85%) were classified as “Probable Lynch” (MSI, BRAF wildtype and MLH1 promoter unmethylated), which enriched in EOCRC as compared to older patients (24% vs. 11.05%, p=0.035). Nonetheless, 30/50 (76.00%) cases among EOCRC were non-LS (sporadic) and significantly associated with left-sided tumour. Overall survival of both “Probable Lynch” and non-LS (sporadic) groups (n=227) was comparable (p=0.59). KRAS mutation was significantly associated with LS status in 26/32 (81.25%) samples. PIK3CA mutation was present in a higher proportion in LS samples of 19/32 (59.38%), but not statistically significant. KRAS and PIK3CA mutations did not significantly affect overall survival (120 months) in LS and non-LS patients.
Conclusions: There is higher frequency of LS among CRC patients in Indonesia and associated with high frequency of KRAS and PIK3CA mutation.

OP7

Groove Pancreatitis: A Histopathological Perspective from a Single Tertiary Care Centre

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Oral Presentations Group 1B, Lecture theatre 1, June 18, 2024, 4:00 PM - 5:00 PM

Background

Groove pancreatitis (GP) is a rare subtype of chronic segmental pancreatitis, characterised by fibrosis within the pancreaticoduodenal groove. This small potential space permits passage of the pancreatic ducts, duodenal papillae and small vessels, and is a site where numerous benign and neoplastic entities arise. The similitude of clinicoradiological features of GP with malignant diseases make the management of pancreaticoduodenal groove lesions complex, often resulting in surgical intervention. Histological examination of pancreaticoduodenal resection specimens remains the definitive method of diagnosis1,2.

Purpose

We present an insight into our experiences of GP, a challenging and often misdiagnosed pathological entity, specifically the histopathological features and associated benign and malignant diseases.

Methods

A retrospective review of cases with identified GP from pancreaticoduodenectomy specimens was undertaken from a single tertiary centre between 2011 and 2024. Associations with GP were evaluated, including coexisting primary malignancies, and features of concurrent benign pathology.

Results

Groove pancreatitis was identified in 18 patients following pancreaticoduodenectomy for presumed cancer (10 male, 8 female, median age 59 years, range 42-77 years). Eleven (61%) were diagnosed histologically with a malignancy, where six (55%) were pancreatic neuroendocrine tumours, and five (45%), pancreatic adenocarcinomas. Median tumour grading was pT3. In three (43%) of seven patients with benign diagnoses, GP was the only pathology. All 18 patients had histological evidence of concurrent benign pathology. Seven (39%) had chronic cholecystitis and five (28%), pancreatic atrophy. Brunner's gland hyperplasia was observed three-times more frequently in patients without underlying malignancy.

Conclusions

Groove pancreatitis is a rare pathological entity with clinicoradiological semblances to pancreaticoduodenal malignancy. The histological diagnosis is essential for navigating ongoing care and features include medial duodenal wall thickening with cystic degeneration, fibro-inflammatory changes, and a varying incidence of Brunner's gland hyperplasia, which may be observed more frequently in benign pathology associated with GP1.

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OP8

Interpretable Machine Learning based detection of coeliac disease: the human way

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Oral Presentations Group 1B, Lecture theatre 1, June 18, 2024, 4:00 PM - 5:00 PM

Background: Coeliac disease (CD) diagnosis generally depends on histological examination of duodenal biopsies. However, the agreement between pathologist when diagnosing coeliac disease is estimated to be only 80%. This motivates the use of AI to improve the quality of diagnosis. However, most AI models are “black-box” methods – they do not justify or explain their outcome, hence hindering clinical adoption.
Purpose: We aim to improve the accuracy of coeliac disease diagnosis by developing an interpretable Machine Learning-based approach that imitates the approach taken by pathologists when diagnosing coeliac disease. Pathologists evaluate the level of crypt hyperplasia, villous blunting and intraepithelial lymphocytosis when diagnosing coeliac disease.
Methods: We present a semantic segmentation algorithm that can locate and classify villi, crypts, intraepithelial lymphocytes, as well as enterocytes in a Whole-Slide-Image of a duodenal biopsy. We use the segmentation masks to diagnose coeliac disease in an interpretable manner.
Results: Our model achieves a segmentation accuracy of 90% and manages to classify with an accuracy of 80%.
Conclusions: We highlight the potential for interpretable Machine Learning to be used to assist the pathologist and improve the accuracy of coeliac disease diagnosis.

OP9

Clinical-grade detection of coeliac disease with computational pathology

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Oral Presentations Group 1B, Lecture theatre 1, June 18, 2024, 4:00 PM - 5:00 PM

Background: Coeliac disease diagnosis generally depends on histological examination of duodenal biopsies. However, the agreement between pathologist when diagnosing coeliac disease is estimated to be only 80%.

Purpose: We aim to improve the accuracy of coeliac disease diagnosis by developing a Machine Learning-based diagnostic approach.

Methods: We present a machine learning model that can detect the presence or absence of coeliac disease from a set of representative duodenal biopsies. We train our model on a diverse dataset with biopsies from four different hospitals using the Multiple-Instance-Learning paradigm in a supervised manner.

Results: Our model successfully diagnoses coeliac disease on an independent test set from a previously unseen source with an accuracy of over 96%, achieving both sensitivity and specificity above 95%, thereby showing the potential to outperform pathologists.

Conclusions: We highlight the potential for Machine Learning to be used to greatly improve the accuracy of coeliac disease diagnosis.

OP10

Comparison of the Performance of the Whole Slide Image Quality Assessment Tool PathProfiler across Three Tissue Type Cohorts

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Oral Presentations Group 1B, Lecture theatre 1, June 18, 2024, 4:00 PM - 5:00 PM

Background
The advancement of artificial intelligence algorithms in histopathology has led to the establishment of quality assessment (QA) tools for whole slide image (WSI) quality. Image quality for accurate assessment is key for both research and clinical diagnosis as it may affect downstream treatment or AI model performance. PathProfiler is a previously developed QA tool for use in prostate biopsy cohorts, predicting the overall useability.

Purpose
We previously evaluated PathProfiler performance in a time-limited prospective study in bladder transurethral resection and kidney resection cases but these were limited in number in that assessment. In this study, PathProfiler was examined in larger cohorts, aiming to assess its generalisability in non-prostate histopathological images.

Methods
The latest version of PathProfiler was tested on three retrospective cohorts where 1052 slides had been scanned in the diagnostic histopathology laboratory comprising biopsies from prostate (331 slides), kidney (363 slides), and bladder samples (358 slides). The slide-level usability scores (0-1 with threshold = 0.5) with out-of-focus and staining quality scores (1-10 with threshold = 9) were generated and compared across the three cohorts.

Results
PathProfiler demonstrated good accuracy in predicting usability scores for prostate (83%) and kidney (80%) WSIs. However, it displayed a higher false-negative rate (28%) and lower accuracy (71%) for bladder cohort, particularly struggling with distinguishing between out-of-focus areas and black ink artefacts. The useability threshold varies among cohorts and requires further tests to be calculated.

Conclusions
Our findings suggest that the prostate biopsy pre-trained PathProfiler has the potential to be applied to urological QA tests in kidney and bladder specimens, giving more insight into PathProfiler in these specimens. It has the potential to yield high performance in predicting usability beyond prostate samples despite not being trained previously on these tissue types. Further training may be needed to optimize its performance across diverse histopathological images.

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OP11

A Single-Institution UK Study Of Outcomes Of Pure Flat Epithelial Atypia Diagnosed On Breast Biopsy: Is Conservative Management Sufficient?

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Oral Presentations Group 2A, Lecture theatre 2, June 18, 2024, 2:00 PM - 3:30 PM

Background: Flat epithelial atypia (FEA) is defined as an intraductal proliferation of 3-5 layers of mildly atypical cells[1]. It is classified as a B3 lesion on biopsy[2]. Inter-observer variability in diagnosis exists and surgical/radiological management remains controversial due to lack of clarity on upgrade rates[3 4].
Purpose: To review all pure FEA lesions diagnosed on breast biopsy to identify upgrade rates and long-term outcomes.
Methods: 28,875 breast biopsy diagnoses were reviewed between 2010-2022. Cases of pure FEA in patients with no previous history of breast in situ or invasive carcinoma were re-reviewed by 2 specialised breast pathologists (PM, MVW; both members of UK NCCBP). Age of patient, imaging findings, excision findings, and long term radiological outcomes were recorded.
Results: 413 cases of FEA were diagnosed on biopsy in patients with no prior history of breast malignancy (1.4%). Of these, 195 (47.2%) were pure FEA without associated ADH, DCIS or in situ lobular neoplasia. Overall mean age at diagnosis: 51.9 years. Over 90% of these were associated with mammographic microcalcification; other imaging findings included textural changes or mass lesions. 74/195(37.9%) were managed by radiological follow up. Excision findings were: 56(28.7%) downgraded - benign; 55(28.2%) residual atypia; 10(5.13%) upgraded (3 IDC; 6 DCIS; 1 pleomorphic LCIS). 4/10 upgrades (40%) were associated with microcalcification only (size >9mm;/multifocality); 6/10 (60%) were associated with radiological textural changes/asymmetric density (ASD) +/- mass lesions/nipple discharge.
Conclusions: Pure FEA is a rare diagnosis (0.67%) on biopsy, of which 94.8% do not progress on surgical excision or radiological follow-up. Those that do progress are associated with radiological microcalcification size >9mm; multifocality; textural changes/ASD or clinical symptoms. We propose that all cases of pure FEA with microcalcification only and radiological/pathological concordance, lacking other clinical/radiological risk features should be managed conservatively.

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OP12

Glutamine Metabolism and DNA Repair in Breast Cancer

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Oral Presentations Group 2A, Lecture theatre 2, June 18, 2024, 2:00 PM - 3:30 PM

Background: Altered cancer metabolism and genomic instability are two key cancer hallmarks. Cancer cells reprogram their metabolic pathways to meet their necessary energy and cellular block demands. Changes in DNA repair protein expression can cause genomic instability through increased DNA damage caused by endogenous and exogenous factors, such as reactive oxygen species. An association between the glutamine metabolism protein solute carrier family 7 member 5 (SLC7A5) and the DNA repair protein flap structure-specific endonuclease (FEN1) was previously investigated in a large series of breast cancer (BC) using immunohistochemistry.

Purpose: This study aims to explore the potential association between glutamine metabolism and DNA repair in BC using in vitro models.

Methods: Western blot was used to assess SLC7A5 and FEN1 protein in BC cell lines previously transfected via siRNA to knockdown either SLC7A5 or FEN1. Co-immunoprecipitation (Co-IP) was used to investigate the protein-protein interactions involved between SLC7A5 and FEN1.

Results: SLC7A5 protein was decreased in BC cell lines with FEN1 knockdown and similarly FEN1 was decreased with SLC7A5 knockdown ($p<0.05$), as this was seen in both luminal A cell line MCF-7 and triple-negative cell line MDA-MB-436. using Co-IP and mass spectrometry co-partners were determined between SLC7A5 and FEN1 in both MCF-7 and MDA-MB-436 cell lines.

Conclusion: This study provides evidence that shows by investigating the relation between SLC7A5 and FEN1 there is an association between glutamine metabolism and DNA repair in BC which could have therapeutic implications.

Keywords: breast cancer, glutamine metabolism, DNA repair, SLC7A5, FEN1

OP13

The contribution of co-localisation of Solute Carriers in Breast Cancer

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Oral Presentations Group 2A, Lecture theatre 2, June 18, 2024, 2:00 PM - 3:30 PM

The contribution of co-localisation of Solute Carriers in Breast Cancer
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Background:
Breast cancer (BC) is a heterogeneous disease with a wide range of clinical outcomes. Glutamine is a key nutrient for tumour growth and progression in several BC subtypes. The expression of solute carriers associated with glutamine: SLC1A5, SLC3A2, and SLC7A5, in BC has been shown to predict poor prognosis.
Purpose:
To confirm the co-localisation and intra-tumoural heterogeneity of SLC1A5, SLC3A2, and SLC7A5 expression in BC, while also investigating their relationships with clinicopathological parameters.
Methods:
Multiplex immunohistochemistry (mIHC) was utilised to determine co-expression of SLC1A5, SLC3A2, and SLC7A5 in full-face BC sections (n=30). Homogeneity of SLC expression was assessed by analysing slides scanned at 40x magnification using the Nano-Zoomer Digital Scanner and subjected to quantitative digital image analysis employing Qu-Path version 0.5.0, an open-source digital pathology software. The annotations were generated to delineate regions of interest, facilitating the quantification of co-expression and spatial distribution. These findings were correlated with clinicopathological parameters.
Results:
In the invasive BC cells, there were variations in the homogenous staining of SLC1A5/SLC7A5 and SLC7A5/SLC3A2 ranging from 3.7-59.8%. The staining revealed areas of no expression, single expression, and dual expression. There were no regions showing co-expression of SLC1A5/SLC3A2. The co-expression of SLCs were associated with larger tumour size, poor NPI, age and TNBC.
Conclusion:
The co-expression of SLCs in BC correlates with some aggressive tumour features. This suggests their spatial distribution and co-expression potential role as indicators of tumour aggressiveness and may warrant further investigation as potential therapeutic targets.

OP14

Encapsulated Papillary Carcinoma of the Breast: An Institutional Case Review and Literature Review.

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Oral Presentations Group 2A, Lecture theatre 2, June 18, 2024, 2:00 PM - 3:30 PM

Background: Encapsulated papillary carcinoma (EPC) of the breast is a rare form of breast carcinoma, comprising only 0.5%-1% of all breast cancers (Tan et al., 2023). Its lack of a distinct myoepithelial cell layer surrounding the lesion has caused speculation about its invasive nature.

Purpose: To retrospectively investigate whether variables such as patient age and tumour characteristics have effects on EPC invasion.

Methods: 35 patient cases of EPC from Lincoln County Hospital were examined, retrospectively, reviewing data from January 2019 to December 2022. Basic demographic data was collected and examined using Microsoft Excel version 16.79.2. For statistical analysis, SPSS Version 29.0.1.0 was used, employing Descriptive statistics, Spearmen's Rank Correlation Coefficient, and an independent samples T-test. These aided in investigating associations between whole tumour size (WTS), patient age and invasive tumour components.

Results: Of the 35 cases the median tumour size was 18mm with an interquartile range of 19mm. One focally invasive case was identified, however, 42.86% (n=15) presented alongside an adjacent invasive carcinoma. Those with an associated invasive carcinoma had a significantly larger WTS in comparison to pure EPC (p=0.003). There was no statistically significant association between WTS and patient age (p=0.388) or size of invasive components (p=0.861).

Conclusions: EPC is an indolent neoplasm that often culminates in good clinical prognosis. Despite its architectural similarities to more invasive carcinomas, its biological behaviour aligns with in situ breast tumours. Clinical management should remain in line with current guidance. More comprehensive classification may enhance surveillance and understanding of rarer subtypes, necessitating respect for these lesions to be recognised as a distinct entity with specific subclassification. While no statistically significant associations have been identified regarding tumour behaviour, oestrogen receptor positivity is thought to play a role. Further research is required to confirm this.

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OP15

Assessing the Viability of Raman Spectroscopy as a Diagnostic Tool in Ovarian Cancer Surgery

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Oral Presentations Group 2A, Lecture theatre 2, June 18, 2024, 2:00 PM - 3:30 PM

Background:
Ovarian cancer is a leading cause of death in gynaecological cancers . Evidence suggests that complete macroscopic resection of cancer, or as close to as possible, confers a survival benefit. However, the intraoperative assessment of residual cancer volume is subjective as there is no existing objective tool for this assessment.

Purpose:
In this work we evaluate the viability of Raman spectroscopy for assessment of ovarian cancer and thus, consideration as the answer to this important clinical need.

Methods:
Raman spectroscopy measurements were taken from ex vivo ovarian and peritoneal tissue from seventy-three participants (n=20 benign, n=11 borderline and n=42 cancer). The spectra were analysed using a multivariate analysis model and compared to histology of consecutive frozen sections from the same tissue block. Classification and leave one out cross validation performance were recorded.

Result:
In ovarian tissue this model achieved 94% sensitivity and 98% specificity for prediction of cancer from benign (AUC 0.97) and 98% sensitivity and 89% specificity for prediction of cancer from borderline (AUC 0.99). In peritoneal tissue, the model achieved 78% sensitivity and 84% specificity for prediction of cancer from benign in participants who had primary surgery (AUC 0.86) and 68% sensitivity and 81% specificity in participant who had post chemotherapy surgery (AUC 0.79).

Conclusions
It is unclear whether the difference in the primary and post chemotherapy group performance infers an effect of chemotherapy, and this needs further study. Raman spectroscopy was able to classify cancer from non-cancer with high cross-validation accuracies and to our knowledge, this is the first time borderline ovarian tumours have been assessed using Raman spectroscopy.
Raman spectroscopy is a viable technique for the assessment of ovarian cancer.

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OP16

A Streamlined Approach to Placental Histopathology in a District General Hospital (Rationalising the placenta workload in a District General Hospital)

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Oral Presentations Group 2A, Lecture theatre 2, June 18, 2024, 2:00 PM - 3:30 PM

Background and purpose:
Placental pathology comprises a substantial portion of histopathology workload in the UK. Due to increasing accessions and limited consultant reporting time, our department implemented the Royal College of Pathologists' placental tissue pathway for selective examination. This study evaluates the impact of this approach in a District General Hospital in Essex, UK.

Methods:
We audited placenta specimens received in 2020 and retrospectively assessed their triage status using RCPath guidelines. Implementation occurred in January 2023 to streamline reporting and processing time. We analyzed the workload, including consultant reporting time, registrar bench time, and laboratory processing time, as well as cost implications.

Results:
Results showed a 200% increase in placenta specimens from 2019 to 2020. Applying RCPath guidelines in 2020 would have reduced the number of specimens requiring full examination by over half. From January 2023 to February 2024, 332 placentas were received, with 104 not examined, 79 macroscopically examined only, and 149 fully examined including histology. Turnaround time for fully examined placentas decreased from 42 to 12 days, with 90% reported within 12 days. Placentas not examined or macroscopically examined had a report turnaround time of 8 days on average. No clinical requests were made for further examination of triaged placentas during the audited period.

Conclusion:
In conclusion, implementing RCPath guidelines effectively rationalized reporting and processing time for placental pathology, resulting in cost savings and improved efficiency in our department.

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OP17

Immunological targeting of frameshift neoantigens in MSI-H endometrial cancers

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Oral Presentations Group 2B, Lecture theatre 2, June 18, 2024, 4:00 PM - 5:00 PM

Background:
Microsatellite instability-high (MSI-H) cancers are cancers with variability in short repeat sequences in numerous loci in the genome. The MSI-H phenotype is commonly a result of deficient DNA mismatch repair (dMMR), which may be caused by hereditary cancer syndromes such as Lynch Syndrome (LS). LS is characterized by MMR mutations in the germline and predisposes carriers to multiple cancers. EC is the most common extraintestinal malignancy predisposed by LS. LS screening for ECs patients is clinically useful but is a time-consuming and labour-intensive process. Checkpoint inhibitors (CPIs) such as PD-1 inhibitor pembrolizumab showed higher response in dMMR patients. Generation of frameshift neoantigens from second-hit mutations and subsequent T cell responses is hypothesized to be a contributor to the effectiveness of CPIs.
Purpose:
This study aims to select frequent frameshift neoantigens from cancer driver gene mutations, and to analyse their T cell binding properties and changes in TCR repertoire. These insights allow for further development of early diagnostic strategies and novel immunotherapies.
Methods:
A review of studies reporting somatic frameshift mutations in MSI-H ECs was conducted to shortlist the most frequent frameshift neoantigens. These will be screened against patient-derived T cells using the barcode-enabled antigen mapping T cell assay (BEAM-T) to detect the presence of compatible T cells in MSI-H tumours. The results will then be validated using bulk TCR sequencing.
Results:
The most frequent frameshift neoantigens are from RPL22 (52%), RNF43 (38%), DNA-PKcs (22%), JAK1 (21%) and PTEN (18%) among other cancer driver genes. These neoantigens will be further investigated using BEAM-T assays and TCR sequencing.
Conclusion:
In this ongoing project, frequent frameshift neoantigens were identified and will proceed into T cell assays, which will reveal neoantigen-specific T cells that can be further investigated for their effector functions, and in the development of early diagnostic strategies and novel immunotherapeutics.

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OP18

The Continuing Role of Transmission Electron Microscopy in the Diagnosis of monogenic Ehlers-Danlos Syndromes.

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Oral Presentations Group 2B, Lecture theatre 2, June 18, 2024, 4:00 PM - 5:00 PM

Background: Ehler-Danlos syndromes (EDS) is an umbrella term describing 14 types of which 13 are monogenic with overlapping features including generalised joint hypermobility, skin and vascular fragility and generalised connective tissue friability. As DNA analysis has become the gold standard for a diagnosis of EDS, use of transmission electron microscopy (TEM) in clinical practice is decreasing. However, due to increased use of next generation sequencing, the frequency of variants of uncertain significance (VUS) is increasing as well. In certain cases of suspected monogenic EDS, TEM analysis of collagen structure may have the potential to contribute to variant classification.

Purpose: To demonstrate the added value of TEM in patients with suspicion on a monogenic EDS type.

Methods: The patient presented clinically with hyperextensible skin, skin fragility, mild atrophic scarring and generalised joint hypermobility, leading to a suspicion on monogenic EDS, in particular on classical or classical-like EDS. Whole Genome Sequencing revealed a COL5A1 variant c.3551C>T; Pro1184Leu which was classified as a cold VUS, unlikely to cause classical EDS. TEM analysis of a skin biopsy was performed to assess for abnormalities of collagen fibrils.

Results: Transmission electron microscopy studies on the patients' skin biopsy showed ultrastructural alterations in collagen fibril diameter and appearance consistent with collagen flowers. The clinical features of the patient together with the presence of abundant collagen flowers supported the suspicion on a rare EDS type in particular classical EDS or classical-like EDS despite the identified cold COL5A1 VUS. As a result, RNA sequencing in skin fibroblast is undertaken to assess for decreased expression of genes involved in monogenic EDS

Conclusions: Clinical assessment in combination with TEM analysis of collagen structure may have the potential to aid to identification and/or classification of variants in genes underlying specific rare, monogenic EDS types.

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1-Ehlers-Danlos syndromes:importance of defining the type
Fleur S van Dijk,Neeti Ghali, Arvind Chandratheva
2-Electron microscopy in the diagnosis of Ehlers–Danlos
syndromes: correlation with clinical and genetic investigations
C. Angwin, N. Ghali, D. Baker, A.F. Brady, F.M. Pope, A. Vandersteen,B. Wagner,D.J.P. Ferguson and F.S van Dijk

OP19

Assessment of the efficacy of an extended gene-panel using participants recruited to the Yorkshire Cancer Research Bowel Cancer Improvement Programme.

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Oral Presentations Group 2B, Lecture theatre 2, June 18, 2024, 4:00 PM - 5:00 PM

Purpose:
Medium-sized gene panels have a role in both research and diagnostic settings, particularly when they can be extended to include clinically relevant information such as microsatellite instability (MSI) and DPYD status. In conjunction with our commercial collaborator GeneFirst, we have optimised their medium-sized gene panel and tested its efficacy on a cohort of colorectal cancer (CRC) patients recruited to the Yorkshire Cancer Research funded Bowel Cancer Improvement Programme (YCRBCIP).

Methods:
We retrieved 515 formalin-fixed, paraffin embedded (FFPE) CRC resection specimens from participants recruited to the YCRBCIP, and following DNA extraction, sequenced these samples using the GeneFirst ATOM-Seq panel. The panel covers known mutational hotspots in 20 genes, with known clinical relevance in CRC, in addition to five DPYD SNPs and 29 microsatellite targets.

Results:
Analysis of known CRC driver mutation hotspots demonstrated that 19/515 (3.7%) of samples contained an NRAS mutation, 195/515 (37.9%) contained a KRAS mutation, 288/515 (55.9%) contained a TP53 mutation, 71/515 (13.6%) contained a PIK3CA mutation and 59/515 (11.5%) contained a BRAF mutation. In addition, 33/515 (6.4%) showed the presence of a DPYD SNP, indicating sensitivity to 5FU-based chemotherapy and 76/515 (14.8%) were classed as microsatellite unstable. Taking the cut-off for assay failure at a median coverage of <50X, 34/515 (6.6%) of samples failed the assay. Excluding DPYD, the variant allele frequencies (VAF) observed, ranged from 1.04-94.98. Time taken from extracted DNA to library prep completion was six hours.

Conclusions:
We have demonstrated that this novel medium-sized gene panel developed by GeneFirst, can detect the expected mutation frequencies across multiple driver genes, in a large cohort of CRC patients recruited across the Yorkshire and Humber region. Being able to also derive clinically relevant information pertaining to DPYD status and MSI status, makes this panel one which could be incorporated to either clinical diagnostic or research environments.

OP20

In Too Deep? Non-Forensic Diving Death Investigation

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Oral Presentations Group 2B, Lecture theatre 2, June 18, 2024, 4:00 PM - 5:00 PM

Background
Between the years of 2012 and 2022 there were 136 recreational diving deaths in the UK, an average of 12.4 per year, with around 3 to 5 of those occuring in the South West. The investigation of non-suspicious diving deaths falls to the Coroner, and due to the complexity of these cases they are often pushed, potentially unnecessarily, to forensic pathologists. The process requires a general understanding of the physiology of diving and how this interacts with natural disease, the interpretation of ancillary testing including toxicology and equipment testing reports, and the ability to synthesise the information into a logical sequence of events.

Purpose
This explores the processes in a non-forensic diving death investigation service in the South West of England which attempts to provide a greater level of understanding around recreational diving deaths than afforded by a routine Coronial autopsy.

Methods
With assistance from the outgoing regional diving pathology expert, we developed a service in conjunction with the HM Senior Coroner for the area filling a need in this coastal community.

Results
This includes a protocolised approach to ensure consistency and reproducibility of post mortem examination. This will also address some of the challenges and controversies within this area of practice.

Conclusions
The investigation of recreational diving deaths does not require a forensic approach, but rather a considered and informed approach incorporating knowledge of the processes involved in diving and how they interact with natural disease. With this in mind, we may be able to reduce the number of deaths certified simply as “drowning”, and improve the safety of the sport for all involved.

References

BSAC Annual Diving Incident Reports 2012-2022 (https://www.bsac.com/safety/diving-incidents/annual-diving-incident-report/)

OP21

Unexplained Haematuria and the Role of the Pathologist in Medical Renal Disease

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Oral presentations Group 3A, Lecture theatre 3, June 18, 2024, 2:00 PM - 3:30 PM

Background

This 32 year old female presented with blood and protein in her urine. Her left kidney was small on ultrasound with scarring at the upper pole. She also had a recent history of pulmonary embolisms and a radiological diagnosis of focal nodular hyperplasia of the liver. Her glomerulonephritis screen was negative, and the clinical team were considering IgA nephropathy. A renal biopsy of her right kidney was undertaken.

Results/Findings

The biopsy showed glomerulomegaly, ischaemic changes, and one glomerulus contained a hyalinotic scar. Mild patchy interstitial fibrosis and tubular atrophy was present, and there was also mild fibrointimal thickening of interlobular sized arteries. Immunofluorescence was negative. The features raised the possibility of focal segmental glomerulosclerosis (FSGS). Review of the laboratory information system showed a placenta from a previous miscarriage.

The limited renal biopsy findings were not sufficient to explain the level of blood and protein in the urine, but the history of pulmonary embolisms and miscarriage was suggestive of antiphospholipid syndrome (APS). Warfarin anticoagulation prevented routine assessment for the lupus anticoagulant, but a Taipan Snake Venom Time (TSVT) test showed a result at the upper limit of the reference range. Anti-cardiolipin and anti-beta-2 glycoprotein-1 antibodies were negative. The ultrasound appearance of a small left kidney could be indicative of renal artery stenosis, which is associated with APS.

Conclusion

The classic renal involvement in APS is thrombotic microangiopathy, which was not present in this biopsy, but the microscopic features did not explain the clinical presentation. This case highlights the role of pathologists in bringing together the clinico-pathological correlation. We raised the possibility of APS to the renal physicians given the clinical history as a potential unifying diagnosis, allowing for appropriate investigation. Sero-negative APS or another coagulopathy remains a possibility.

OP22

Expression of NKX3.1 in Sertoli Cell Tumors

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Oral presentations Group 3A, Lecture theatre 3, June 18, 2024, 2:00 PM - 3:30 PM

Background:
Sertoli leydig cell tumors are rare tumors(<0.5% of ovarian neoplasms). Most of the tumors present in a young age. Patients present with virilization or isosexual precocity. Tumors can be solid (fleshy pale yellow) or cystic with a mean size of 12-14 cm. These tumors usually affect young adults and are seen in one or both testicles with microcalcifications. Sertoli leydig cell tumors express FOXL2 in 50% of cases. Mutations in DICER1 are present in 60% of cases.

Purpose: To evaluate the NKX3.1 expression by immunohistochemistry in normal testicular parenchyma, in Sertoli cell tumors and Sertoli Leydig cell tumors of testes and ovary.

Methods: Retrospective observational study.
Place and Duration of Study: Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore 2011-2021.
Methodology: We used immunohistochemistry to evaluate the positivity and loss of nuclear expression of NKX3.1 in sertoli cell tumor (11 cases), sertoli Leydig cell tumor (31 cases) and in normal testicular parenchyma (7 cases).

Results: 10 out of 10 cases of benign testicular parenchyma expressed positivity with nuclear staining of NKX3.1 in Sertoli cells. 2 out of 11 Sertoli cell tumors expressed positivity with nuclear positivity of NKX3.1 in Sertoli cell component (18.18%) and 9 of the cases showed loss of staining of NKX3.1 (81.8%). All Sertoli Leydig cell tumors showed loss of staining of NKX3.1.

Conclusion: Nuclear expression of NKX3.1 is seen in Sertoli cells of normal testicular parenchyma. This staining is lost in Sertoli cell tumors and Sertoli Leydig cell tumors.

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OP23

EXPRESSION OF BAP1 IN CLEAR CELL RENAL CELL CARCINOMA

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Oral presentations Group 3A, Lecture theatre 3, June 18, 2024, 2:00 PM - 3:30 PM

Background:
Clear cell renal cell carcinoma generally occurs in adults and comprises 70% of all epithelial renal tumours. BAP1 (BRCA1 associated protein 1 on chromosome 3) is a commonly mutated gene in clear cell renal cell carcinoma. Aim of the study was to evaluate the prognostic significance of BAP1 by immunohistochemistry in clear cell renal cell carcinoma.

Methods:
It was a descriptive case series in which data was retrospectively collected. In this study, there were 142 cases of which 110 cases were of clear cell renal cell carcinoma, 16 cases were papillary renal cell carcinoma and 16 cases were of chromophobe renal cell carcinoma. Immunohistochemistry was used to evaluate the loss of nuclear expression of BAP1.

Results:
Loss of BAP1 was observed in 60% of cases of clear cell renal cell carcinoma. 27% of grade 1 tumours, 62% of grade 2 tumours, 65% of grade 3 tumours and 66% of grade 4 tumours showed loss of BAP1. Loss of BAP1 was observed in 54% cases of stage 1 tumours, 72% of stage 2 tumours and 66% of stage 3 tumours. Our study showed loss of BAP1 in 67% of cases with tumour necrosis, in 75% of cases with sarcomatoid features and in 60% of patients with distant metastasis.

Conclusions:
We conclude that the loss of BAP1 nuclear expression is associated with poor prognostic features. i.e., higher grade, higher stage, tumour necrosis, sarcomatoid features and distant metastasis leading to death of patients. These finding lend support to the idea of doing BAP1 immunohistochemistry in routine labs for determining prognosis.

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OP24

Theranostic Potential of Ultrasound Activated Microbubbles for Glioma Diagnosis and Treatment: A Review

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Oral presentations Group 3A, Lecture theatre 3, June 18, 2024, 2:00 PM - 3:30 PM

Background: The poor prognostics of central nervous system tumours are a clinical problem that is in part due to the tight substance control from the blood-brain barrier. Microbubbles combined with focused ultrasound is a developing method to overcome this issue with a strong evidence base for effectiveness and safety, a topic that will be discussed.
Purpose: This review will cover a collection of pre-clinical and clinical trials from the past twelve years, looking at the uses of different microbubbles. Trials using different bubbles, including Definity, Optison, and Sonovue, will be discussed with uses in sonobiopsy/liquid biopsy, chemotherapy drug delivery and immunotherapy. It will also look at current discussions around the safety and tissue effects of microbubble use on the blood-brain barrier, covering any changes noticed in histology and radiology or adverse effects experienced by patients in clinical trials.
Method: A search was conducted on Web of Science to locate clinical trials using microbubbles in central nervous system tumour diagnosis and treatment.
Results: The search located sixteen clinical trials on the uses of microbubbles in central nervous system tumours, covering drug delivery, immunotherapy and liquid biopsy. This includes clinical and pre-clinical trials with a focus on the implementation of microbubbles and assessing the safety of opening the blood-brain barrier.
Conclusion: Central nervous system tumours are a big area of development with microbubbles and focused ultrasound regimes being developed for diagnosis and treatment methods. It’s a promising area with published early clinical trials and many ongoing or proposed clinical trials happening to explore clinical implementation.

OP25

Understanding Epigenetic Mechanisms in Oral Dysplasia and Early Invasive Carcinoma: Using Tissue-Engineered Models for Epigenomic Profiling

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Oral presentations Group 3A, Lecture theatre 3, June 18, 2024, 2:00 PM - 3:30 PM

Background: Oral potentially malignant lesions (OPML) manifest as white or red lesions, exhibiting histological alterations. While many OPMLs revert to normal epithelium, 9-33% may progress to oral cancer. Over the past decade, UK oral cancer cases surged by 58%, with a continued upward trend projected. In 2020, there were 200,000 OPML referrals, resulting in 8,722 oral cancer diagnoses.

Purpose: The key molecular events leading to OPML and progression to oral cancer are poorly understood. Much of the published research uses cells cultured in monolayer which does not physiologically represent the in vivo situation. We aimed to validate tissue-engineered mucosal constructs as a tool for defining key molecular events involved in cancer progression.
Methods: Normal, dysplastic, and OSCC keratinocytes were used to generate full-thickness tissue-engineered models, validated against native tissue. Next-generation RNA sequencing and DNA methylation profiling identified differentially expressed and methylated genes. Bioinformatics analysis evaluated gene set pathways and identified genes of interest. Validation was conducted using Sanger sequencing and qPCR, with the hypomethylating agent decitabine assessed for potential gene function restoration.

Results: The tissue-engineered constructs closely resembled native tissue, with dysplastic models exhibiting disorganized epithelial architecture and cellular atypia. RNA and DNA methylation sequencing revealed 1070 downregulated and 1907 upregulated genes, and 1209 hypermethylated and 1791 hypomethylated promoter regions in dysplastic models compared to normal. Integration analysis identified 59 genes downregulated and hypermethylated, associated with key pathways including choline metabolism, p53 signaling, antigen processing, and cytokine signaling. Decitabine effectively restored the function of specific tumor suppressor genes in the dysplastic model.

Conclusion: The study demonstrates the reliability of tissue-engineered models in reproducing cancer progression in vitro. Dysplastic models showed significant molecular alterations associated with oral cancer progression. These findings may offer insights for developing therapeutic targets to improve outcomes for early-stage OSCC patients.

OP26

Quantitative and Automated analysis of Head and Neck Cancers Using Artificial Intelligence

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Oral presentations Group 3A, Lecture theatre 3, June 18, 2024, 2:00 PM - 3:30 PM

Background: Artificial Intelligence (AI) has been shown to help in the identification and quantification of digital tissue biomarkers in several cancers. However, there has been little exploration of its role in head and neck squamous cell carcinoma (HNSCC).
Purpose: To determine whether morphometric analysis using a custom-trained classifier is useful in the objective assessment of clinicopathological, molecular, mutational, and survival correlation in HNSCC.
Methods: The retrospective study used whole slide images (WSI) of HNSCC from the publicly available datasets (The Cancer Genome Atlas and The Cancer Imaging Archive) and an AI-based open-source software (QuPath) for image analysis. In the morphometric study, epithelial, stromal, and immune cells in WSI of normal oral mucosa (NOM), dysplasia (OED), and HNSCC were annotated, followed by morphological features extraction and comparison using ANOVA and Student's t-test. For classifier development, two classifiers, artificial neural network-multilayer perceptron (ANN-MLP) and Random trees (Rtrees) were trained with epithelium, stroma, immune cells, and mitotic figures in WSI of NOM, OED, and HNSCC WSIs. The performance of the classifiers was tested on unseen HNSCC WSIs for automatic segmentation followed by downstream analysis.
Results: Student t-test and ANOVA revealed statistically significant differences between most morphological features of epithelial, stromal, and immune cells between NOM, OED, and HNSCC ($p<0.05$). The ANN-MLP classifier performed better than Rtrees for the automatic segmentation of epithelial, stromal, and immune cells, with F1 scores of 0.78, 0.79, and 0.82 respectively. The downstream analysis showed a significant correlation between morphometric features and prognosis in HNSCC ($p<0.05$). The F1 score of the ANN-MLP classifier for atypical mitosis was 0.79 and both the automatic and manual counts were significantly correlated with prognosis in HNSCC ($p<0.05$).
Conclusion: Morphometric assessment using a custom-trained classifier can potentially serve as a tissue biomarker for objective differentiation between dysplasia and cancer, prognosis determination, and risk stratification.

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OP27

Quantifying Infiltration of Macrophages with CD163 Marker in Head & Neck Tumour Microenvironment Post-Radiotherapy

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Oral presentations Group 3B, Lecture theatre 3, June 18, 2024, 4:00 PM - 5:00 PM

Background:
Head and neck squamous cell cancers (HNSCC) rank as the 6th most common cancer globally, resulting in approximately 300,000 deaths worldwide [1]. Radiotherapy (RT) stands as a prominent treatment modality. Extensive research has been conducted to assess the efficacy of this treatment, aiming to minimise the side effects experienced by patients. Among the factors influencing treatment response, M2 macrophages have emerged as significant contributors, given their pro-tumour and anti-inflammatory characteristics. Consequently, greater infiltration of M2 macrophages within the tumour post-RT could exacerbate resistance to treatment.
Purpose:
This project aims to quantify M2 macrophages, using the macrophage marker CD163, within the entirety of head and neck tumours.
Method:
Tumours were generated using the murine head and neck cancer cell line MTCQ1, with separate groups left untreated or subjected to radiation (8 Gy dosage). Tumour collection occurred on days 1, 3, 5, 7, and 10, followed by therapeutic intervention. The creation of the slides involved the fixation of the collected tumours in 4% paraformaldehyde, processing and embedding in paraffin. Utilising ImageJ, the percentage of positively stained CD163 cells was determined across the entire tumour.
Results:
On ANOVA analysis, a significant interaction was observed between days and treatment (P=0.0325). In the irradiated group (n=15), there was a progressive 2.87% increase in the percentage of positively stained cells up to day 7. Notably, the interaction between untreated and irradiated tumour groups on day 7 exhibited strong statistical significance (P=0.0027), the irradiated group being 2.54% greater than the untreated group.
Conclusion:
The findings suggest potential implications for guiding RT in HNSCCs. Day 7 presented the most notable disparity between untreated and irradiated groups, highlighting a potential window for targeting post-RT resistance and specifying the optimal radiation dosage. However, these preclinical observations warrant replication in clinical trials to validate their relevance for patient care.

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OP28

Too Much Skin In the Game: A Dermatopathology Audit of Cancer Services at Broomfield Hospital, MSE NHS Trust

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Oral presentations Group 3B, Lecture theatre 3, June 18, 2024, 4:00 PM - 5:00 PM

Background

In the East of England there has been an 89% increase in pathology cases clinically labelled as possible cancer between 2018 to 2022 (1). Dermatology is a specialty driving this increase in urgent workload (1). The two week wait (2ww) concept is a pathway for patients with suspected cancer which is focused on providing rapid diagnoses and is outlined under the NHS constitution (2).

Purpose

To audit the cancer dermatopathology service and identify cases labelled as possible cancer/2ww that could be downgraded to non-urgent.

Methods

Laboratory records identified all dermatology cases that arrived in the laboratory in October 2023. Urgency, clinical impression, and histological diagnosis were recorded for each sample. Samples sent as a 2ww were isolated. Cases were graded as appropriate and inappropriate for the 2ww pathway. Appropriate cases included clinically possible melanomas, squamous cell carcinomas and basal cell carcinomas (BCC) in high-risk areas (eyes, lips, nose). Re-excisions were defined as removal of a lesion that had previously been proven to be malignant histologically.

Results

981 patients were processed in the laboratory in October 2023. 634 patients (65%) were reported on the 2ww pathway. Of these 634 2ww patients, 189 (30%) were deemed inappropriate for the 2ww pathway based on clinical impression alone. Re-excisions (67: 35%) and BCCs in low risk areas (25: 13%) made up 92 (48%) patients of this inappropriate cohort of samples on the 2ww pathway. Removing these 92 patients from the initial 634 patients would reduce the 2ww case load by 15%.

Conclusions

Many dermatology samples are inappropriately labelled as a 2ww suspected cancer sample based only on clinical impression. This short abstract does not include final histological diagnoses. Groups of inappropriate samples should be identified and removed from this pathway with an aim to reduce urgent workload.

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OP29

Novel quantitative digital pathology pipeline unveils distinct regional patterns of misfolded protein pathologies in Parkinson's disease

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Oral presentations Group 3B, Lecture theatre 3, June 18, 2024, 4:00 PM - 5:00 PM

Background: The characteristic α-synuclein inclusions in Parkinson's disease (PD) are variably accompanied with concomitant amyloid-β and tau neurofibrillary tangle pathology, which may influence the rate to dementia progression in PD. However, the extent of misfolded protein pathologies across the brain in PD is not fully described, due to challenges of current semi-quantitative assessment approach. There is therefore a need to establish novel pipelines for consistent and scalable quantification of misfolded protein pathologies.

Purpose: To use machine learning-based tools for quantitative image analysis on digital whole slide images across multiple brain regions to generate automatically quantified data for further downstream integration with clinical phenotypes, genetic profiles and regional transcriptomic signatures for identifying disease subtypes.

Methods: Digitally scanned regional brain sections stained for alpha-synuclein, amyloid-beta and hyperphosphorylated tau from PD and control cases were segmented into regions of interest to quantify the pathology in specific anatomical regions. Using open-source pathology image analysis software (QuPath), algorithms have been developed for large-scale high-throughput quantification of misfolded protein pathology.

Results: We have quantified misfolded protein load across multiple brain regions in over 300 cases. Comparative analyses of these pathologies within the same region and between regions has identified distinct patterns of pathology between different presentations of PD, based on clinical and genetic data.

Conclusion: We report a novel digital pathology pipeline that is reproducible and robust for quantification of misfolded protein pathology in PD. Such approaches can now be developed to include other pathological markers and established for other disease profiles.

OP30

EWSR1-SMAD3 positive fibroblastic tumour: a series of three cases.

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Oral presentations Group 3B, Lecture theatre 3, June 18, 2024, 4:00 PM - 5:00 PM

TITLE:

EWSR1-SMAD3 positive fibroblastic tumour: a series of three cases.

BACKGROUND:

EWSR1-SMAD3 fibroblastic tumour is a rare cutaneous soft tissue neoplasm, most commonly arising in acral sites. The tumour has a well-circumscribed, nodular architecture and consists of uniform, bland, fibroblastic spindle cells set within a collagenous to myxoid stroma.

PURPOSE:

We present three cases of EWSR1-SMAD3 fibroblastic tumour with the aim of elucidating the morphological features and immunoprofile and determine when to consider it in the differential diagnosis and consider further molecular testing.

METHODS:
A search of the electronic record (tertiary soft tissue pathology referral service).

DISCUSSION & CONCLUSION:

All three cases occurred in the women aged between 20-69, all three occurred on the feet and ranged between 6mm to 10mm in maximum dimension.

Microscopically common features included fascicles of bland spindle cells set in fibrous or fibromyxoid stroma. The cells contained eosinophilic cytoplasm and oval blunt-ended nuclei. Stromal hyalinisation and dystrophic calcification were noted in one case. No atypia, significant mitotic activity or necrosis was present.

All three tumours demonstrated strong diffuse expression of ERG and CD34 negativity.

One tumour showed recurrence after 9 months.

Fluorescence in-situ hybridisation analysis showed an abnormal signal pattern indicating a rearrangement involving the EWSR1 gene and expression of fusion gene EWSR1:SMAD3.

Our series confirms the findings from the literature and further elucidates the histological spectrum. EWSR1-SMAD3 fibroblastic tumour is a little known entity and should be considered in this morphological context combined with ERG positivity and CD34 negativity.

PL1

Modelling ETV6::RUNX1+ B-cell ALL: Secondary loss of the wild-type ETV6 allele releases the B-cell developmental block imposed by first-hit ETV6::RUNX1

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Plenary Session 1, Lecture theatre 1, June 19, 2024, 2:00 PM - 3:30 PM

Introduction:
ETV6::RUNX1 is the most frequent genetic aberration in childhood B-cell acute lymphoblastic leukemia (B-ALL), the most common pediatric cancer, and acts as a 'first-hit'; arising in utero but resulting in a clinically silent pre-leukemia. Additional post-natal mutations are necessary for leukemic transformation, but how they collaborate with the initiating event has not been fully characterized. iPSCs recapitulate fetal lymphopoiesis, affording an opportunity to model leukemogenesis in developmentally relevant cell types. In this system, engineered ETV6::RUNX1 corrupts B-myeloid (CD34+CD19-IL7R+) progenitors and blocks B-cell differentiation[1].

Methods:
Loss of the wild-type ETV6 allele is the most frequent second-hit in ETV6::RUNX1+ B-ALL. Using CRISPR/Cas9-mediated mutagenesis, ETV6::RUNX1/ETV6-/- lines were established; subsequent cre-mediated reversion of ETV6::RUNX1 derived ETV6-/- clones. In vitro B-lymphopoiesis was assessed by flow-cytometry and immunophenotypically-defined B-myeloid, proB and preB compartments were sorted for RNA-sequencing, then subjected to differential gene expression and pathway analyses.

Results:
Secondary loss of the wild-type ETV6 allele resulted in expansion of the B-myeloid progenitor and downstream proB and preB populations. Transcriptomic analysis revealed upregulation of genes enriched in signatures of cell cycle, B-cells and inflammatory pathways following additional mutation of ETV6 in the B-myeloid progenitor compartment. Reversion of ETV6::RUNX1 revealed that B-myeloid progenitor expansion and inflammatory signature upregulation are dependent on a first-/second-hit interaction. Additionally, a significant upregulation of RAG1/RAG2 gene expression is observed in ETV6::RUNX1/ETV6-/- preB cells.

Conclusion:
Our results suggest that secondary loss of ETV6 promotes leukemogenesis by: i) upregulating cell cycle and B-cell associated signatures, amplifying the B-myeloid progenitor compartment and releasing the ETV6::RUNX1 block on B-cell differentiation; ii) activating or sensitizing to inflammatory signaling which may additionally iii) collaborate with RAG upregulation in preB cells to promote subsequent genomic rearrangements. Work is ongoing to characterize the role of other second-hit mutations (PAX5+/- and CDKN2A-/-), individually and in combination, in childhood ETV6::RUNX1+ B-ALL.

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PL2

Super-resolution fluorescence microscopy and AI for renal diagnosis: a replacement for electron microscopy?

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Plenary Session 1, Lecture theatre 1, June 19, 2024, 2:00 PM - 3:30 PM

Super-resolution fluorescence microscopy (SRFM) can provide data on the spatial distributions of specific proteins at a level of detail well beyond the capability of standard immunofluorescence or confocal microscopy (~20 nm). Compared with electron microscopy (EM), it is also easy to fit into standard FFPE tissue processing protocols [1, 2], faster overall as an assay and highly specific for molecules of interest. It may also conveniently image more regions of interest in a tissue section than EM, potentially allowing greater sensitivity to pathological changes.

Renal disease is the most common use case for EM as a diagnostic assay. In particular, focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) currently require EM to inspect the morphology of podocytic structures on the glomerular capillary basement membrane.

We have acquired SRFM data on nephrin, which localises to the filtration slits between podocyte foot processes, and collagen, part of the basement membrane. We have obtained more than 300 fields of view (FOVs) in total for each protein, from FFPE tissue sections from several MCD, FSGS (both diagnosed according the current gold standard) and normal control cases (sections from donors with no known renal disease).

We have trained an AI algorithm to classify the FOVs as MCD, FSGS or Normal, using the gold standard diagnoses as ground truth. We partition the FOVs into multiple tiles and apply an attention-based multiple-instance learning pipeline, to make use of and identify salient areas in a FOV [3, 4]. We have obtained a mean AUROC > 0.90 in 5-fold cross-validation for predicting all three conditions.

SRFM has the potential to provide a wealth of new information relevant to medical decision-making. We also anticipate that it will save time and cost, and in some cases improve sensitivity, where EM is currently used.

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PL3

Identifying Morphological Biomarkers for Progression of Ductal Carcinoma in Situ of the Breast Through Computational Analysis

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Plenary Session 1, Lecture theatre 1, June 19, 2024, 2:00 PM - 3:30 PM

Background
Ductal Carcinoma in situ (DCIS) is a pre-invasive breast cancer that may or may not progress to invasive cancer. Despite this uncertainty, all women diagnosed with DCIS undergo surgical excision and additional therapy. However, there are no reliable markers to differentiate between DCIS lesions that will progress or recur and those that will not.

Purpose
This study aims to distinguish morphological features across various DCIS nuclear types, to lay the groundwork for prognostic models.

Methods
We utilised HoVer-Net, fine-tuned with 50,000 manually annotated nuclei, for nuclear segmentation/classification in whole slide images. Additionally, the DeepLab model with a ResNet backbone facilitated 9-class region segmentation, distinguishing benign ducts, DCIS, fibrous stroma, among others. Using selected regions from 320 cases of the UK/ANZ DCIS trial, we assessed nuclear morphology and applied logistic regression and random forest models to identify prognostic features for DCIS progression.

Results
The DeepLab model, enhanced with a ResNet backbone, achieved a Dice score of 0.87 in the validation set, while the fine-tuned HoVer-Net exhibited an F1 score of 0.81 for nuclear classification. Our analysis discerned 17 morphological distinctions with statistical significance ($p<0.05$) between progressors and non-progressors, particularly in cellular orientation, eccentricity and solidity of lymphocytes and nuclear radii, perimeter and orientation within neoplastic nuclei. The logistic regression and random forest findings were consistent. The convergence of these analytical models highlighted consistent predictive features, thereby refining our predictive capacity for DCIS progression and recurrence.

Conclusions
Through the development of a 9-class region segmentation and the refinement of HoVer-Net for nuclear segmentation and classification, we have identified significant morphological variations that serve as potential prognostic indicators for the risk of DCIS progression. The integration of these features into an interpretable computational model, subject to external validation, represents the next phase of research, aimed at enhancing the prediction of DCIS progression.

PL4

A Self-Supervised AI Learning Approach Extracts Morphological Meaning From H&E Images of Colorectal Cancer

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Plenary Session 1, Lecture theatre 1, June 19, 2024, 2:00 PM - 3:30 PM

Background: Diagnosis and treatment of colorectal cancer (CRC) is dependent upon microscopic assessment of information contained within tissue by pathologists. This is time and resource demanding and prone to inter- and intra-observer variability. Recent development in self-supervised learning approaches provide new opportunities to better understand the morphological characteristics of cancer on histological slides[1]. We have applied a self-supervised learning artificial intelligence (AI) approach called Histomorphological Phenotype Learning (HPL) to whole slide images (WSIs) of CRC.

Purpose: To apply the self-supervised learning AI approach HPL to CRC in order to build a library of morphological clusters that represent regions of WSIs and assess their predictive potential.

Methods: We have trained a self-supervised learning Barlow Twins method on 2772 H&E WSIs of colorectal adenocarcinoma from the TranSCOT cohort. The WSIs were split into tiles that are 224x224 pixels at 10x magnification. The model was trained on a subset of 500,000 tiles and morphological features from these were extracted. We utilised Leiden community detection to identify histomorphological phenotype clusters (HPCs) in a features space defined by the learned representations, free from any clinical or pathological labels. A Cox proportional hazards model was applied over patient vector representations, defining each patient as a composition of HPCs. Tiles within each HPC have been examined by pathologists and the morphological features documented.

Results: Resulting HPCs display distinctive histomorphological patterns that highlight tissue type and architecture. HPCs achieve a concordance index range of 0.59 to 0.66. HPCs associated with poorer outcome are stroma rich and display poorly differentiated tumour. HPCs associated with better outcome display high immune cell infiltration.

Conclusions: Self-supervised learning AI approaches have the ability to predict outcome and provide a library of histomorphological features within tumour and tumour microenvironment.

This research has been supported by the Jean Shanks & Pathological Society Pre-Doctoral Research Bursary.

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PL5

Experimental modelling of unicortical internal surface laryngeal fracturing, identified by histological processing, in a fatal case of applied neck pressure.

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Plenary Session 2, Lecture theatre 1, June 19, 2024, 4:00 PM - 5:00 PM

Pathological findings can often be subtle or limited in cases of fatal neck compression at autopsy. We previously presented the value of using a more detailed approach to examination of the larynx involving decalcification, transverse sectioning and complete histological processing using mega block cassettes in a case which aided to reveal five internal laryngeal fractures providing sufficient evidence that the most likely mechanism of death was by strangulation. Considering this, this we have since explored the nature of and mechanism of these poorly recognised “buckling” type internal fractures which can easily be missed using a standard forensic autopsy approach.

We undertook simple experiments using 3D printed pliable models of the thyroid and cricoid cartilages coated internally with hardened and cooled Isomalt (a sugar baking product) subjecting them to various types of compression (anterior, bi-lateral and combined anterior-bilateral compression) and documenting any resulting surface material stress cracking produced. Compression in all three forms was confirmed to cause internal surface material cracking to both the thyroid and cricoid cartilage models, including in the locations found in our previously presented case. Certain patterns of surface material cracking were found to be more commonly associated with particular forms of compression. Over 90% of all the surface material cracking were obliquely or vertically orientated on the models, supporting a transverse (rather than longitudinal) sectioning approach of a decalcified larynx in relevant forensic autopsy cases.

PL6

Building a Morphomolecular Dictionary of Lung Adenocarcinoma Using Self-Supervised Learning

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Plenary Session 2, Lecture theatre 1, June 19, 2024, 4:00 PM - 5:00 PM

Background: Self-supervised learning is a versatile deep learning methodology which can encode histological features as quantitative vectors. This approach requires no up-front hypothesis or manual annotation. Our pipeline, histomorphological phenotype learning (HPL), breaks whole slide images (WSIs) into small tiles and learns features from each tile, encoding them numerically. These vector representations from each tile are clustered, to construct groups of morphological similar tiles. Each patient's tumour can then be expressed as a proportion of clusters. Crucially, representative images from each cluster can be reviewed by eye, providing a layer of interpretability.

Purpose: We demonstrate the ability to construct a dictionary of recurrent phenotypes spanning the spectrum seen in lung adenocarcinoma. Furthermore, we integrate spatially resolved morphological features with multiplex immunofluorescence.

Methods: We trained HPL using 4427 WSIs from 1007 patients. These were consecutive surgical resections of lung adenocarcinoma from a single centre. We used Cox proportional hazards to generate risk scores for overall and recurrence-free survival. We ran images of 23 TMAs through the trained model to assign cores to clusters and applied an immune-focused multiplex immunofluorescence panel on a serial section.

Results: There were 64 morphological clusters. Some of these are defined by classical growth patterns, while others by recurrent stromal appearances. Appearances with high fibroblast burden were associated with poor outcome and those with increased lymphocyte burden with favourable outcome. For each cluster we calculated cell phenotype fractions and linked these to prognosis.

Conclusions: Self-supervised learning is a versatile deep learning methodology which can enable discovery of clinically useful prognostic features and tumour biology. Our approach demonstrates a way to identify and quantify histological features and integrate them with spatial proteomic data.

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PL7

An Interpretable Classification Model Using Gluten-Specific TCR Sequences Shows Diagnostic Potential in Coeliac Disease

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Plenary Session 2, Lecture theatre 1, June 19, 2024, 4:00 PM - 5:00 PM

Background: Specific HLA-DQ molecules, presenting deamidated gliadin peptides (with origin in dietary gluten) underpin the pathogenesis of coeliac disease and promote T-cell mediated duodenal injury. Current diagnostic methods require gluten consumption and use observation by pathologists of small intestinal biopsies, a process sometimes affected by subjective interpretation, leading to poor interobserver concordance (73% using only duodenal biopsies and 80% using duodenal biopsies with IgA tissue transglutaminase and haemoglobin data). The difficulty in ensuring patients with coeliac disease are diagnosed is exacerbated by the diverse and non-specific presentations of coeliac disease including anaemia, intestinal symptoms, osteoporosis, lymphoma, intestinal cancer and sometimes the lack of obvious signs or symptoms.
Purpose: With the increasing prevalence of coeliac disease and rate of diagnosis around 36%, there is an unmet need for a new gold-standard of testing.
Methods: We investigated the use of T-cell receptor (TCR) repertoire characteristics as a diagnostic tool. To build an interpretable machine learning model, we sequenced mucosal CD4+ T-cell TCR repertoires from 20 patients (12 with coeliac disease of which 5 were on gluten-free diets; 8 healthy controls) as a training dataset. We tested the diagnostic potential of our machine learning model using independently published TCR sequence data.
Results: Diagnosing coeliac disease showed a training accuracy of 100% (using 44 TCR alpha sequences alone and when combined with 28 TCR beta sequences), including patients on a gluten-free diet (using TCR alpha sequences alone). Testing accuracy was 80% (using TCR alpha sequences alone).
Conclusions: We identified the set of 20 CD4+ TCR sequences (10 TCR alpha and 10 TCR beta) with the greatest potential to discriminate between duodenal biopsies from patients with coeliac disease and healthy controls, thus developing a prototype for a more objective, gluten-independent, diagnostic test for coeliac disease.
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PL8

Using spatial transcriptomics to investigate the molecular underpinnings of selective neuronal vulnerability in α-synucleinopathies

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Background: Parkinson's Disease (PD) and multiple system atrophy (MSA) are two α -synucleinopathies caused by different strains of abnormally folded α-synuclein (α-syn) (1,2) and present with differential neuronal vulnerability. In PD, the substantia nigra, amygdala and locus coeruleus are consistently severely affected with characteristic neuronal Lewy bodies, which may also be detectable in other brain regions depending on the stage of the disease. While in MSA, neurones in the caudate nucleus and putamen or the inferior olivary nucleus (ION) and pontine base are selectively severely affected with other brain regions affected to a lesser degree (3). However, the exact mechanisms underlying differences regional vulnerability across different forms of α -synucleinopathies remain elusive.
Purpose: Our study aims to identify the molecular basis of neurons of the ION, which are severely affected in MSA, but spared in PD using cellular and spatial resolutions of a novel spatial transcriptomics platform to analyse the differentially affected cells of the ION.
Methods: We analysed fresh-frozen human post-mortem brain tissue from the Queen Square Brain Bank using the Nanostring GeoMx spatial transcriptomic platform (5) to profile the transcriptome of neurons, astrocytes and microglia in the ION from cases of MSA and PD. Briefly, regions of interest (ROIs), were manually marked and appropriately segmented for each cell type, and automated molecular profiling performed in the cells for each ROI by photocleaving index oligomers from respective probes.
Results: Our data reveal a successful enrichment of regional cell-specific transcriptomes and that there is significant differential expression of genes between ION cells across control, PD and MSA cases indicating that specific disease mechanisms are active in each disease.
Conclusions: These novel findings greatly improve our understanding of the pathomechanisms underlying disease pathogenesis and how these are affected in two different α -synucleinopathies.

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