Abstracts

WINTER MEETING

Unleashing the edge power of pathology

31 January – 2 February 2023

5th Joint Meeting with the Royal Society of Medicine

214th Scientific Meeting of the Pathological Society of Great Britain & Ireland

Hosted by

The University of Leeds

Venue

The Royal Society of Medicine

1 Wimpole Street, Marylebone

London W1G 0AE

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KEY TO SYMBOLS

℗ = Presenter

* = Supported by a grant from the Pathological Society of Great Britain & Ireland

ACKNOWLEDGEMENTS

This collection of abstracts is published jointly by the Royal Society of Medicine and the Pathological Society of Great Britain & Ireland.

Plenary Oral Abstracts

PL1

High-grade gliomas occurring in teenagers and young adult patients comprise novel molecular subgroups

Purpose of the study

High-grade gliomas (HGG) can occur in any central nervous system (CNS) location. They occur in adults and children and have a poor prognosis (20.8% 5-year survival for those diagnosed aged 0-19 years and 21.9% for those aged 20-44 years). This is better than older adults; those aged 45-54 and 55-64 years have a 5-year survival of 9.3% and 5.9% respectively. HGGs in teenagers and young adults (TYA) are understudied; this project aimed to characterise these tumours and identify therapeutic targets to improve survival.

Methods

HGG samples (n=178, FFPE/FF, aged 13-30 years) were collected from national/international collaborators, excluding well-characterised tumour entities (histone/IDH-mutant cases). DNA underwent methylation profiling (Illumina EPIC BeadArrays, n=178). iDat files were processed using the brain tumour classifier (MNP v12.5 R package), classifying cases against a reference cohort (n=2,800). Calibrated scores guided workflows to characterise the mutational landscape including RNA-based ArcherDx fusion panel (n=129) and whole exome sequencing (n=129). A histological review was undertaken in parallel.

Summary of results

Longitudinal samples (n=7) and well-characterised entities (n=34, including IDH-mutant tumours (n=19), PXA (n=8)) were excluded. 16% classified as either HGG subgroups characteristically seen in paediatrics (including DMG H3 K27-altered (n=3), pedHGG_RTK1/2, MYCN subtypes (n=25)) or those seen more frequently in adults (including GBM IDH-wildtype (mesenchymal) (n=8), DHG H3G34-mutant (n=10)). However, 51% did not classify highly as any recognised paediatric or adult-type subgroups, and some were assigned to novel recently identified and poorly characterised subgroups with distinct methylation profiles, molecular and histological features.

Conclusions

TYA HGG comprise novel subgroups with distinct methylation profiles and molecular characteristics, representing opportunities to refine future treatment and management.

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PL2

Multiple-instance-learning-based detection of coeliac disease in histological whole-slide images

We set out to develop a deep-learning-based classification method for the detection of coeliac disease (CD) in histological whole-slide images (WSIs) of duodenal biopsies. Typically, CD-diagnoses rely heavily on histological interpretations of duodenal biopsies, which are unavoidably subjective and often yield poor inter-observer agreement. The introduction of a deterministic, algorithmic approach offers the potential to mitigate this problem, and rapidly screen biopsies.
We use a state-of-the-art convolutional neural network to detect CD in a training dataset of 1841 (1163 normal; 680 CD-positive) WSIs of haematoxylin-and-eosin-stained duodenal biopsies. The data originate from two large UK hospitals. We trained the model under a weakly-supervised learning paradigm, which requires only slide-level labels, to determine which regions of WSIs contain evidence of disease and, in turn, to obtain slide-level diagnoses.
Using five-fold cross-validation in the training set, our developed model classified slides as normal with accuracy 96.7%, precision 98.0% and recall 96.1%, and as CD-positive with accuracy 96.7%, precision 94.9% and recall 96.5%. We tested our model on two independent test sets: one containing unseen data from the same labs|scanners as the training sources, and one from a truly independent, previously unseen, overseas source. On the same-source test set, the performance was essentially identical to cross-validation performance (each metric was within 1% of the cross-validation performance). On the different-source test set (17 normal; 17 CD-positive), the model correctly classified 94.1% (32|34) of all cases. Upon closer inspection, the two misclassifications were due to poor quality (out-of-focus) WSIs, and not a failure to generalise.
Our deep-learning-based approach for detecting CD classifies WSIs with high accuracy, precision and recall and exhibits strong evidence that it can generalise across different laboratories and scanner platforms.

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PL3

Artificial intelligence- (AI) assisted evaluation of tumour infiltrating CD3+ and CD8+ T cells in the tumour core (CT) and invasive margin (IM) for prognostication and prediction of benefit from adjuvant chemotherapy in early stage colorectal cancer (CRC): A retrospective analysis of the QUASAR trial

Background: High CD3 and CD8 T cell densities in the CT and IM of primary CRCs are associated with superior prognosis at all stages of disease. Their predictive effect on benefit from adjuvant chemotherapy in early stage CRC has not been tested.

Methods: FFPE samples from participants (pts) in QUASAR (adjuvant fluorouracil/folinic acid *vs* observation, stage 2/3 CRC) were analysed for CD3 and CD8 IHC. Pathologists annotated the core and peri-tumour areas on digital slide images. AI algorithms delineated the CT and IM and calculated the densities (cells/mm2) of each marker in each region. Pts were randomly partitioned into test and validation sets (1:1). In the test set, each measure's prognostic effect on recurrence-free interval (RFI) in each trial arm was assessed. Maximum-likelihoods methods were used to develop optimal cut-points. Analyses were repeated in the validation set. In predictive analyses, 2-year recurrence rate was the primary outcome; biomarker-treatment interactions were assessed.

Results: Tumour tissue from 868 pts (797 [92%] stage 2; 531 [61%] colon) was analysed. In the test set, optimal cut-points of 318, 798, 81 and 186 cells/mm2 were defined for CD3-CT, CD3-IM, CD8-CT and CD8-IM respectively. The recurrence rate in the high-risk group was twice that in the low-risk group for all measures (CD3-CT: rate ratio [RR] 2.00, [95%CI 1.33-2.94], p=0.0008; CD3-IM: 2.38, [1.59-3.57], p<0.00001; CD8-CT: 2.17, [1.59-3.57], p=0.0001; CD8-IM: 2.13 [1.43-3.23], p=0.0001), which was closely replicated in the validation set. CD3/8 counts were not predictive of benefit from adjuvant chemotherapy, with similar efficacy in the high and low risk groups.

Conclusions: AI-assisted CD3/8 counts were strongly associated with tumour recurrence rates. Proportional reductions in recurrence with chemotherapy were similar in high and low-risk disease. Hence, numbers of high-risk patients needed to treat to prevent one recurrence were about half the number for low-risk patients.

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PL4

Novel proliferation regulators identified using predictive bioinformatic approaches

Background: Improved understanding of breast cancer (BC) complex molecular pathways is required to predict prognosis and develop new therapeutic strategies. The purpose of this study is to apply an integrated approach that combines both clinical and bioinformatic data to reveal novel regulators of proliferation. Methods and Results: Whole slide images generated from haematoxylin and eosin-stained sections of The Cancer Genome Atlas (TCGA) BC database alongside their transcriptomic and clinical data, were used to identify differentially expressed genes (DEG) associated with cell division determined using mitotic scores. DEGs enriched in the cell cycle pathway were utilised to predict the protein-protein interaction (PPI) network. Ten hub genes (ORC6, SMC1B, CDKN2A, CDC25B, E2F1, E2F2, SKP2, ORC1, PTTG1, CDC25A) were identified using CytoHubba, a plugin of Cytoscape. The results were validated using the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) database. High expression of the hub genes was significantly correlated with worse survival. ORC 6 and SKP2 were predictors of survival independent of mitotic score or Ki67. ORC6 and SKP2 protein expression level in tumour tissue was significantly higher than in normal breast tissue. Conclusions: ORC6 and SKP2, play important role in BC proliferation and surpassed both Ki67 and mitotic scores in multivariate analysis, thus can serve as potential prognostic markers and therapeutic targets.

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PL5

Clonal trajectory to chronic myeloid leukaemia.

Purpose of the study: The clonal trajectories to most blood cancers remain unknown but are critical for enabling early intervention and prevention strategies. Here I investigated cancer latency and clonal expansion rates in chronic myeloid leukaemia (CML).

Methods: Whole genome sequencing of single-cell-derived blood colonies was undertaken. Over 400 colonies were grown from blood samples collected at diagnosis and in remission in 4 CML patients who responded to 1st line treatment. I reconstructed phylogenetic trees of blood using the pattern of sharing of somatic mutations across colonies to investigate clonal relationships in CML. Clonal expansion rates and timing of acquisition of BCR-ABL were inferred from the trees' branching patterns and mutation burden of the ancestral branch of the CML clade. *De novo* extraction of mutational signatures was performed using the HDP package and telomere lengths were analysed with Telomerecat.

Summary of results: CML phylogenies revealed that the clonal expansion of BCR-ABL-mutated clade occurred up to a maximum of 1-9 years before diagnosis, making CML one of the fastest growing blood cancers reported to date. *De novo* extraction of mutational signatures suggested that BCR-ABL-positive stem cells were rapidly cycling cells with higher mutation rates and a higher proportion of SBS1 mutational signature. BCR-ABL-positive colonies had shorter telomere lengths than BCR-ABL-negative colonies, despite the diagnosis sample being taken years earlier than the remission sample in keeping with their rapid growth.

Conclusions: These findings broaden our understanding of the spectrum of clonal dynamics of different blood cancers. Clinically, the rapid clonal expansion in CML might inform the management of BCR-ABL-positive healthy individuals, as well as inform therapeutic response and failure.

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PL6

Loss of key driver mutations in *IDH1 in metastatic chondrosarcoma*

Due to clonal expansion, driver mutations that are present during cancer initiation are typically present in every primary and metastatic cell in a tumour. In absence of counter evidence, it is assumed that clonal drivers contribute to disease progression, and thus represent potential therapeutic targets. Here we present evidence that the onco-metabolomic driver IDH1, an initiating event in chondrosarcoma and several other cancer types, can be deleted or disrupted in around 30% of cases with metastatic chondrosarcoma. In one case, we found large interstitial deletions that overlap the IDH1 locus causing loss of the mutant allele, while in another a whole arm LOH of chromosome 2 resulted in mutant IDH1 loss. We suggest that although IDH1 is required for cancer initiation, it may detrimentally affect fitness later in cancer evolution, and thus be subjected to negative selection. This has implications for the therapeutic inhibition of IDH1.

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Oral Free Abstracts

OF1

Predicting soft tissue tumours diagnoses with deep learning

Purpose of the study: diagnosing soft tissue tumours is challenging due to the large number (>100) of subtypes and their overlapping morphological features. Supplementary tests are often required, adding cost and extended turn-around times. Deep learning in digital pathology has shown promising results in the classification of more common cancers using whole slide images (WSI), but little has been achieved for soft tissue tumours. This study aims to develop a deep learning framework to aid pathologists reach a more accurate diagnosis in a reduced time frame and reduce the number of supplementary tests. However, ultimately the pathologist makes the diagnosis.

Methods: 754 digital WSI representing 10 soft tissue tumour subtypes classified based on characteristic molecular alterations were included in the study (desmoid and superficial fibromatosis, intramuscular myxoma, low grade fibromyxoid, dermatofibrosarcoma, neurofibroma, nodular fasciitis, solitary fibrous tumour, dedifferentiated liposarcoma, synovial sarcoma). Each slide is pre-processed via colour normalisation, and tumour tiles are extracted using a pre-trained convolutional neural network (CNN). A densenet-121 CNN is then trained on 654 out of the 754 WSI to classify each WSI as one of the 10 soft tissue tumour subtypes. The remaining 100 unseen WSI are used for independent testing.

Results: the proposed deep learning framework provides the correct diagnosis within the 10 subtypes in 85% of tested WSI Also, the correct diagnosis is found within the top 3 predicted diagnoses of the framework in 97% of WSI. Thus, the correct diagnosis could be confirmed/ reached efficiently with a limited number of immuno/molecular tests.

Conclusions: an accurate classifier of soft tissue tumours is provided in this work. The deep learning framework has the potential to assist pathologists in reducing time and cost to reach a diagnosis. Future work includes the evaluation of model performance against pathologists.

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OF2 *

Inflammatory Pathology in Human Motor Neuron Disease.

Purpose of the study.

Microglia are highly involved in the pathogenesis of Motor Neuron Disease (MND). Their activation correlates with several features of disease. However, how microglia contribute to human MND is not currently known. We aim to elucidate the role of immunity in sporadic MND by examining gene expression in post-mortem tissue.

Methods

Transcriptional analysis was performed using the nanoString Neuroinflammation panel of 770 genes. RNA was extracted from cervical spinal cord and motor cortex from MND patients (16 cases per location) and controls (8 cases per location). Immunohistochemistry (IHC) was used to further examine microglial pathology incorporating key pathways identified from the nanoString analysis.

Summary of results

In spinal cord, 89 genes were differentially expressed, including upregulation of genes linked to the TREM2, TYROBP, APOE and phagocytic pathways. Little immune gene expression was observed in the motor cortex. IHC analyses was performed to examine microglial pathology and validate the gene expression findings in the spinal cord, precentral gyrus and non-motor regions of the brain using tissue microarray technology. In sMND spinal cord, significant microglial reactivity, and an upregulation of APOE and TYROBP was observed. Motor white matter tracts showed significant immune activation, the severity of which equal to or exceeding the ventral horn. Expression of TREM2 did not differ between control and sMND cases, but increased TREM2 expression in the white matter, was associated with longer survival. The precentral gyrus of sMND cases showed little change in immune reactivity compared to control cases.

Conclusions

The spinal cord is a much more immune environment in sMND compared to the precentral gyrus, with the white matter tracts showing significant microglial activity in sMND. Expression of APOE and TYROBP in the spinal cord, highlight a role for disease-associated microglial signalling in sMND.

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OF3 *

Spatial evolution of Ductal Carcinoma In-Situ precludes accurate risk stratification on biopsy alone

Purpose: Ductal Carcinoma In-Situ (DCIS) is difficult to manage clinically as it is not possible to distinguish between lesions that are indolent and those that will progress to invasive breast cancer. Current strategies involve observation only in 'low risk' (low morphological grade) DCIS, based on biopsy diagnosis. This fails to take account of intra-lesion heterogeneity, which has important implications for management decisions made on biopsy samples. This study investigated the extent of whole lesion morphomolecular heterogeneity of known risk markers and genomic copy number alterations (CNA) in DCIS.

Methods: Whole lesions were formalin fixed and paraffin embedded (FFPE) in megablocks. Immunohistochemistry (IHC) for markers associated with risk (ER, PR, HER2, Beta6, Galectin-7, and ki-67) was used to map spatial heterogeneity across whole lesions. Areas of high heterogeneity were sampled to test for CNAs using shallow Whole Genome Sequencing (sWGS) at 0.5-1.5X. CNAs were analysed using ichorCNA and phylogeny was inferred using CNT-MD.

Summary of results: Whole lesions (n = 7) show marked inter- and intra-tumoural heterogeneity in IHC markers and CNAs (n = 5 (48 areas)). IHC did not predict CNA profile directly except in HER2 positive cases and amplification of ERBB2. Sub-clones appeared spatially distinct with inter-tumour variation in number of sub-clones (2-5).

Conclusion: We demonstrate heterogeneity across DCIS lesions both morphologically and genomically. There is evidence of spatial evolution of sub-clones with possible pathogenic CNAs present in small regions that may be missed when sampled by biopsy. As such accurate risk stratification via biopsy alone may not be possible due to inadequate sampling. Future work will investigate the clinical utility of the liquid biopsy, to establish if circulating tumour DNA can be used to sample CNAs and provide an additional tool for risk stratification.

This research was funded by a JSPS Research Training Fellowship.

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OF4

Label-free imaging of fresh human tissue and clinical application for histopathology

Remarkable technological advances have made it possible to observe cell dynamics and biological phenomena in experimental animals while they are still alive in recent years. Moreover, attempts have also been made recently to apply such novel technologies of tissue imaging to clinical histopathology. I have conducted the research to establish novel tissue imaging method with multiphoton excitation technique. In this method, we can get three-dimensional fluorescent histological images from fresh tissue in real time without fixation, thin slicing, or staining, only by near-infrared irradiation from the tissue surface. These fluorescent histological images enable not only histopathological evaluation by pathologists but also quantitative analysis involving deep learning modalities. I would like to report on the current state of fresh tissue visualization using multiphoton excitation as well as its potential for clinical application.

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OF5 *

Quantifying the accumulation of clonal mutations in colorectal epithelium from patients who develop cancer compared to those without

Around half of colorectal tumour mutations arise within normal epithelium. To become a fixed mutated clonal crypt, mutations occur within stem cells which then replace the crypt. Clones can be visualised by immunohistochemistry using X-linked marks. If loss of a protein gives a stem cell an advantage over wild-type stem cells it increases the likelihood of replacement and these are known as biased marks e.g. STAG2 and KDM6A. Those proteins with no advantage are neutral clonal marks e.g. MAOA. We aimed to investigate differences in clone dynamics in normal epithelium in patients with colorectal cancer compared to those without.

Normal colonic mucosa was collected from 42 cancer patients (CAN) and 18 patients who had resections for non-neoplastic and non-inflammatory indications (NNN). Slides underwent immunohistochemistry for MAOA, STAG2 and KDM6A and scanned. An artificial intelligence algorithm was used to quantify total crypt number and identify potential clones which were then manually curated. The number of clones divided by the total number of crypts sampled was plotted against age. A regression model was used to determine the rate of accumulation.

The average number of crypts sampled per patient was 222,957. There was positive correlation seen between clone frequency and patient age for all clonal marks. There was a significant difference seen in the rate accumulation of clones between the CAN and NNN groups for all 3 clonal marks.

This study has demonstrated that fixed clonal mutations increase in frequency with age and there is a significant increase in the rate of clone accumulation in colorectal cancer patients. This indicates that cancer patients either have more mutations due to increased carcinogen exposure or poorer repair mechanisms, increasing the likelihood of these mutations becoming fixed, or a combination of both. Another underlying mechanism may be increased promotional factors in cancer patients which increase the probability of mutation fixation.

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OF6 *

pTERT Mutation in the Risk-stratification of Solitary Fibrous Tumours

Background

Solitary fibrous tumours (SFT)(characterised by NAB2-STAT6 fusion) are soft-tissue tumours with unpredictable behaviour. Tumours classified as intermediate-risk by the WHO grading system are difficult to prognosticate, and patients may relapse after several years. The C228T TERT promoter (pTERT) mutation represents a potential predictor of aggressive behaviour, and our results demonstrate that it could be used to better stratify intermediate-risk patients.

Methods

We used a digital droplet PCR (ddPCR) assay on DNA derived from FFPE sections of 268 SFT from multiple institutions. We assessed pTERT methylation status in 90 representative SFT using ddPCR. Survival analysis was performed, and categorical variables were compared by logrank test.

Results

21%(56/268) of SFT had C228T pTERT mutations. Mutations were identified in 69%(9/13) of the high-risk group, 35%(25/72) of the intermediate risk-group, and 12%(16/134) of the low-risk group (WHO 3-tiered model). pTERT mutation was significantly associated with poorer outcome using a composite endpoint of recurrence, metastasis, and death from disease (log-rank $p<0.001$).

Within intermediate-risk tumours (N=58), pTERT mutation was associated with worse survival (log-rank $p=0.002$). In the first 5 years, 0%(0/44) in the wild-type group had experienced recurrence, metastasis, or death from disease, compared with 29%(4/14) in the mutated group.

pTERT mutation was associated with histological features of higher mitotic rate (6.0 ±6.6/10hpf vs 3.0 ±4.8/10hpf, $p<0.001$), and larger size (95 ±48mm vs 60 ±39mm, $p<0.001$).

1.1%(1/90) of SFT showed pTERT methylation suggesting methylation rarely represents an alternate mode of TERT activation in SFT.

Conclusions

pTERT mutation is associated with reduced survival in SFT. Detection of the pTERT mutation has the potential to improve current prognostication and guide management of patients with intermediate-risk tumours.

We thank the Jean Shanks and Pathological Society for funding this research.

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OF7 *

A mouse model of PTEN Hamartoma Tumour Syndrome (PHTS): insights into the pathology and genomics of PTEN as a tumour suppressor and utilisation for pre-clinical tumour prevention drug testing.

The PI3K/AKT signalling pathway controls cell growth, survival and metabolism. PTEN, the main negative regulator of the pathway, is a tumour suppressor, frequently inactivated in sporadic cancer. Germline *Pten* mutations underly the autosomal-dominant syndrome PHTS, characterised by benign tumours, neurocognitive defects, and increased cancer risk (namely of breast, thyroid and endometrium). PHTS is a unique context to study the temporal and oncogenic impact of PTEN loss. A PHTS relevant mouse model (*Pten*$^{+/-}$) was utilised for trans-species pathological comparison, PTEN status assessment and cancer prevention studies with quantitative pathology readouts. *Pten*$^{+/-}$ mice (Podsypanina et al. 1999) and *Pten*$^{+/+}$ were used on a mixed background. IHC performed via the Leica BOND Rxm. In-situ mRNA expression assessed via RNAscope^{TM}(ACD). DNA was extracted with the Qiagen minikit from laser micro-dissected tissue (Zeiss palm microbeam). Whole exome sequencing (WES) libraries were generated (Nonacus) and sequenced on the NovaSeq/NextSeq (Illumina). Histopathological analysis of *Pten*$^{+/-}$ mice reveals endometrial, thyroid and bowel lesions comparable to patients, and suggests PHTS-specific findings. Complete loss of the PTEN protein is seen (via IHC) at early timepoints in *Pten*$^{+/-}$ endometrium, in atypical endometrial hyperplasia (AEH) and in thyroid lesions (but not bowel polyps). This corresponds with increased PI3K/AKT signalling and absent PTEN mRNA expression. WES of the AEH detected no *Pten* gene mutations, suggesting a non-genomic, pre-transcriptional mechanism of *Pten* inactivation. Clinically-relevant PI3K pathway inhibitors for cancer-prevention were tested in this model. The mTOR inhibitor Rapamycin reduced AEH in the *Pten*$^{+/-}$ *mice*. The *Pten*$^{+/-}$ mouse model is substantiated as a faithful PHTS model, with relevance at a histopathological level and as model system to study *Pten* regulatory mechanisms. Therapeutic studies with this model identify candidate drugs for cancer prevention.

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OF8

Development of a comprehensive assay for the molecular profiling of cancer by target enrichment from FFPE specimens

Since June 2019, under the umbrella of the national health insurance system, Japan has started cancer genomic medicine (CGM) with comprehensive genomic profiling (CGP) tests. The Ministry of Health, Labour and Welfare (MHLW) of Japan constructed a network of CGM hospitals (a total of 233 institutes as of July 1, 2022) and established the Center for Cancer Genomics and Advanced Therapeutics (C-CAT), the national datacenter for CGM (Cancer Discov, 2022). We established a comprehensive assay, the Todai OncoPanel (TOP), which consists of DNA and RNA hybridization capture-based next-generation sequencing panels. A novel method for target enrichment, named the junction capture method, was developed for the RNA panel to accurately and cost-effectively detect 460 fusion genes as well as aberrantly spliced transcripts. The TOP RNA panel can also measure the expression profiles of an additional 1390 genes. The TOP DNA panel was developed to detect single nucleotide variants and insertions/deletions for 464 genes, to calculate tumour mutation burden and microsatellite instability status, and to infer chromosomal copy number. Clinically relevant somatic mutations were identified in 32.2% (59/183) of patients by prospective TOP testing, signifying the clinical utility of TOP for providing personalized medicine to cancer patients (Cancer Sci. 2019).

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Oral Undergraduate Abstracts

UG01

Relationship between the Warburg effect in tumour cells and the tumour microenvironment in colorectal cancer patients: results from a large multicentre study

Colorectal cancer (CRC) remains one of the most prevalent and deadly cancers worldwide. The tumour-node-metastasis (TNM) system is currently the only clinically used tool to predict prognosis for CRC patients. However, patients with the same TNM stage can have varying prognoses potentially requiring different treatment. The metabolic status of tumour cells (Warburg-subtype, low/moderate/high) has been proposed as potential prognostic factor in CRC. Potential biological mechanisms underlying the relationship between Warburg-subtype and prognosis have not been investigated in detail. One potential mechanism could be that the metabolic status of tumour cells affects the tumour microenvironment (TME).

Our objective was to investigate the relationship between Warburg-subtypes and the TME. Haematoxylin/Eosin stained tumour tissue microarray cores from 2171 CRC patients from the Netherlands Cohort Study were semiquantitatively assessed for tumour infiltrating lymphocytes (TILs) and relative tumour stroma content. The relationship between Warburg-subtype, TILs, and stroma content was investigated.

A significant association between tumour infiltrating lymphocytes and tumour stroma percentage was found ($p < 0.001$). There was no association between Warburg-subtype and tumour stroma content ($p = 0.243$) or between Warburg-subtype and TILs ($p = 0.480$). This is the first study to investigate the relationship between Warburg-subtypes and the TME in a large population-based series of CRC patients. Our data suggest that the prognostic value of Warburg-subtypes cannot be attributed to differences in TILs or tumour stroma content.

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UG02 *

Exposure to Enterotoxigenic Bacteroides fragilis (ETBF) bacteria found in association with colorectal cancer increases clonal mutations in mice

Enterotoxigenic Bacteroides fragilis (ETBF) is a gastrointestinal commensal bacterium that produces an enterotoxin and is associated with colorectal cancer. The Bacterioides fragilis toxin (BFT) has been shown to accelerate the impact of tumour mutations through DNA damage. In Apc+/- multiple intestinal neoplasia (Min) mice, exposure to ETBF greatly accelerates tumour formation, indicating a promotional mechanism, likely mediately by increased inflammation. We looked to investigate the role of ETBF in promotion by using X-linked clonal marks to visualise clonal mutations in mice with immunohistochemistry.

44 mice were split over 3 timepoints; 1 week, 4 weeks and 6 weeks. Each timepoint had 3 groups; sham unexposed mice, ETBF exposed mice, and mice exposed to ETBF with a mutated BFT gene and therefore a non-functional enterotoxin. Entire mouse colon was embedded enface to maximise crypt numbers. Immunohistochemistry was performed for 2 biased X-linked clonal marks; STAG2 and KDM6A. Slides were scanned and a deep learning algorithm applied to quantify crypt numbers and identify loss of expression to quantify clones.

The number of crypts examined ranged from 181,758 to 1,098,613. No fixed clones were detected in any sham mice group. There was a positive trend seen between the age of the mice and the clone frequency with both clonal marks. The highest clone frequency detected was in the ETBF group; 0.9 mutated clones per 100,000 crypts for STAG2. The highest frequency was 0.7 for the ETBF mutant group. At 4 weeks, there was an expanded patch of 2 mutant crypts found in an ETBF mouse.

This study has demonstrated that after ETBF exposure, whole fixed clones can be detected in mouse colon after a minimum of 1 week. This is seen with 2 independent clonal marks and there is positive correlation with age. This is likely demonstrating that ETBF exposure promotes fixation of clonal stem cell mutations. Furthermore the patch of 2 crypts indicates there may be an effect on fission.

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UG03 *

Investigating the effects of irradiation on the peritumoral microvasculature in Glioblastoma Multiforme

Glioblastoma (GBM) is characterised by microvascular proliferation (MVP) and vastly abnormal microvasculature. Investigations into these microvascular abnormalities are often focussed on the tumour bulk itself, but the peritumoral area (PTA) has been shown to be of significant importance in GBM. Indeed, it is within this area that GBM usually recurs, indicating the need for research into the peritumoral microenvironment. We therefore set out to characterise the microvasculature of the PTA using a CT2A glioma mouse model.
6 mice were injected intracranially with CT2A glioma cells. 3 of the subsequent tumours were allowed to grow without treatment while 3 were irradiated and allowed to regrow post-irradiation. Mice were injected with BSA-Alexfluor555 to trace extravasation from blood vessels. Control and irradiated mice were sacrificed on days 18 and 26 post-implantation respectively and brains were excised. Mouse brains were formalin fixed and OCT-embedded ready for sectioning. Sections were stained using CD34/Endomucin antibodies to mark the microvasculature, and a-SMA antibodies to mark pericytes. The PTA was defined as 200um around tumour bulk and Images were taken using the Nikon A1R confocal microscope at 10x till the entire PTA was viewable. ImageJ was used to count blood vessels, SMA-positive blood vessels, and extravasation was measured by comparing tracer positive pixels between the PTA and an area of 200um within the tumour bulk.
A significant increase in microvascular density (P=0.03), but not SMA-positive vessels (P=0.62), was found between irradiated versus control tumours. Extravasation was found to be significantly greater in the PTA versus the tumour bulk in irradiated tumours (P=0.02) but not in control tumours (P=0.38). This could indicate that peritumoral microvasculature is reactive to radiotherapy or its microenvironmental consequences, such as hypoxia, and is relatively functional when compared to the tumoral microvasculature.
Funded by PathSoc

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UG04 *

Morphological Comparison of Colon Cancer Resection Specimens Derived from Two Leading Surgical Techniques: Japanese D3 and European Complete Mesocolic Excision with Central Vascular Ligation

Patient outcomes following treatment for colon cancer have not improved to the same degree as for rectal cancer. Leading colorectal surgical centres have improved oncological outcomes -- Japanese D3 resection and European complete mesocolic excision (CME) with vascular ligation -- but an international standard is elusive. We compared resection specimens of centres that employ such techniques through the international T-REX study and aimed to find any significant morphological differences.

High-definition photographs from 270 colonic resections, across 14 surgical centres taking part in the T-REX study, were morphologically analysed. The research team remained blinded to centre origin and outcomes. Four parameters were measured: area of mesentery, length between the tumour and high-tie, length between the nearest bowel wall and high-tie and length of large bowel.

The length of large bowel resected was significantly different across centres. Some showed a longer length (suggesting CME), while others showed a shorter length (suggesting D3). Left sided tumours -- median: 297.70 mm [IQR: 233.80-350.00 mm] vs 160.42 mm [143.96-205.31 mm], p=0.024). Right sided tumours: 199.74 mm [182.93-263.37 mm] vs 136.29 mm [131.22-166.18 mm], p=0.011. The area of mesentery for left sided tumours was significantly greater in centres likely undertaking CME (14,198 mm2 [10,435.00-16,278.00 mm^2] vs 6,537.00 mm^2 [5,687.33-8,394.67 mm^2], p=0.027). Median distance between tumour and high tie was 96.08 mm for right sided tumours and 100.62 mm for left sided.

While a significant difference was found in the resected length of large bowel for left and right sided tumours and thus area of mesentery, the measures of central radicality were equitable between centres. This demonstrates, high quality surgery has taken place across the study when compared to the published literature. Further work will unblind the centres and their operative approaches and link to short/long-term patient outcomes.

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UG05 *

Deep Learning Segmentation of Picrosirius Red Staining for the Assessment of Collagen Deposition in Pre-Clinical Models of Cancer

Purpose of the study: A hallmark of cancer is increased tumour stiffness, with collagen being the principal component contributing to this biomechanical phenotype. Picrosirius Red staining quantification (a common technique to visualise collagen deposition) is mainly conducted by thresholding red pixels to distinguish collagen from its background. These methods are hindered by extensive pre-processing and increased sensitivity to staining and collagen appearance variations.

Methods: We used a deep learning network, based on the U-Net architecture, and combined the commonly used RGB colour space with the a* channel of the CIELAB colour space, which is sensitive to the red colour. A cohort of orthotopically-propagated mouse models from 13 cancer types was used. Our training and validation sets consisted of 2,240 and 1,216 sub-images (256x256 pixel) respectively from 9 samples. The test set had 1,088 sub-images from 4 samples from cancer types not seen during training/validation. The training set consisted of 7% positive pixels and 93% negative (no collagen) pixels, so we selected the Tversky Loss to account for the skewness. The collagen segmentation was assessed by the specificity, sensitivity and accuracy metrics.

Summary of results: Our network combining the RGB with the a* channel scored 99.14% specificity, 70.14% sensitivity and 97.5% overall accuracy, outperforming the models trained only on RGB (97.39% specificity, 90.63% sensitivity and 97.1% overall accuracy) or a* channel (97.33% specificity, 91.74% sensitivity and 96.9% overall accuracy). Visual inspection showed that specificity below 98.5% was not acceptable, as it contained many false positives which do not allow for an accurate quantitative assessment of collagen deposition.

Conclusion: Deep learning applied on both RGB and CIELAB image colour spaces can robustly quantify collagen deposition from Picrosirius Red staining.

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UG06

Vascular Remodelling and Structural Changes in Placentas of Pregnant Mice Infected with Various Pathogens

Purpose of the study: Infection in pregnancy is associated with various adverse outcomes including preeclampsia and intrauterine growth restriction. We aimed to investigate whether defects in maternal spiral artery remodelling and placental labyrinth zone formation might account for the changes in fetal and placental weights observed in infection in mouse pregnancy.
Methods: Pregnant C57BL/6 mice were infected with vaccinia virus (VACV), toxoplasma gondii (T. gondii), mouse cytomegalovirus (MCMV), influenza virus A (IAV), or mock-infected. Success of vascular remodelling was assessed in mid-gestation implantation sites using two measures: firstly, the thickness of spiral artery walls was determined through total vessel:lumen area ratios in H&E-stained sections. Secondly, smooth muscle actin levels were determined using HRP-DAB-based immunohistochemistry. Labyrinth formation was analysed in mature placentas stained using double-label cytokeratin and vimentin immunohistochemistry. Relative labyrinth size was determined using labyrinth:junctional zone area ratios.
Summary of results: We found that maternal spiral arteries from VACV-infected mice had increased wall thickness compared to mock-infected animals. Additionally, spiral arteries from T. gondii-infected mice had increased levels of smooth muscle actin compared to controls. Furthermore, we found that relative labyrinth size was unchanged in VACV, T. gondii, MCMV and IAV-infected dams compared to mock-infected mice.
Conclusions: Our results suggest that maternal vascular remodelling is impaired in VACV and T. gondii -infections in mouse pregnancy, which may, in part, account for the changes in fetal and placental weights observed. The impact on placental structure is currently less well-defined. These findings provide a strong foundation for future research into the factors contributing to outcomes of pregnancies complicated by infection, and the cellular mechanisms underlying them.

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Poster Abstracts

P1

The clinical value of progesterone receptor expression in luminal breast cancer using a large cohort of patients with long-term follow-up

Background: The routine assessment of progesterone receptor (PR) expression in breast cancer (BC) remains controversial. Here, we aimed to evaluate the role of PR expression in luminal BC, with emphasis on the definition of positivity and its prognostic significance as compared to Ki67 expression. Methods: A large cohort (n=1924) of oestrogen receptor (ER) positive/HER2 negative BC was included. PR was immunohistochemically (IHC) stained on full face sections and core needle biopsies (CNB) where the optimal scoring cut-off was evaluated. In addition, the association of PR with other clinicopathological factors, cellular proliferation, disease outcome and response to adjuvant therapy were analysed. Results: Although several cut-offs showed prognostic significance, the optimal cut-off, to categorise PR expression into two clinically distinct prognostic groups on CNB was 10%. PR negativity showed a significant association with features of aggressive tumour behaviour and poor outcome. Multivariate analyses indicated that the association between PR negativity and poor outcome was independent of tumour grade, size, node stage, and Ki67. PR negativity showed independent association with shorter survival in patients who received endocrine therapy while Ki67 was not. Conclusion: PR IHC expression provides independent prognostic value superior to Ki67. Routine assessment of PR expression in BC using 10% cut-off in the clinical setting is recommended.

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P2

Refining the definition of HER2 low class in invasive breast cancer

Purpose of the study: Emerging evidence indicates that breast cancer (BC) patients whose tumours express HER2 protein without HER2 gene amplification (HER2-low), can benefit from antibody-drug conjugates (ADC). However, the current definition of HER2-low BC remains incomplete with low rates of concordance. This study aims to refine HER2-low definition with emphasis on distinguishing HER2 score 0 from score 1+ to identify patients who are eligible for ADC. Methods: BC cohort (n=363) with HER2 IHC scores 0, 1+ and 2+ (without HER2 gene amplification) and available HER2 mRNA was included. HER2 staining intensity, pattern, and subcellular localisation were reassessed. Artificial neural network analysis was applied to cluster the cohort and to distinguish HER2 score 0 from 1+. Reproducibility and reliability of the refined criteria were tested. Results: HER2 IHC score 1+ was refined as membranous staining in invasive cells as either: 1) faint intensity in ≥20% of cells regardless the circumferential completeness, 2) weak complete staining in ≤10%, 3) weak incomplete staining in >10%, 4) moderate incomplete staining in ≤10%. Based on this, 63% of the HER2 negative cases were reclassified as positive (HER2-low). The refined score showed perfect observer agreement compared to the moderate agreement in the original clinical scores. Similar results were generated when the refined score was applied on the independent BC cohorts. A proposal to refine the definition of other HER2 classes is presented. Conclusion: This study refined the definition of HER2-low BC based on correlation with HER2 mRNA and distinguished between HER2 IHC score 1+ and score 0 tumours.

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P3

Differential Response of HER2 positive breast cancer based on HER2 protein expression level

Background: Human epidermal growth factor receptor 2 (HER2) positive breast cancer (BC) is defined either by protein overexpression or equivocal protein with evidence of HER2 gene amplification by in situ hybridisation (ISH) technique. Emerging data indicate that there is a differential response to targeted anti-HER2 therapy between these two classes, however, the clinicopathological characteristics and underlying mechanisms for this difference are not well understood which we aimed to investigate in this study. Methods: A large cohort of BC (n= 7254) was used. The clinical and pathological characteristics of HER2 immunohistochemistry (IHC) classes were assessed and correlated with pathological complete response (pCR) rate and oestrogen receptor (ER) status. Differential gene expression (DGE) analysis was carried out to decipher the molecular and biological signature of HER2 positive BC classes. Results: HER2 IHC 2+ /ISH amplified BC shows a significantly lower pCR rate than IHC score 3+ tumours regardless of HER2 copy number (61% versus 24%, p<0.001). Compared to IHC score 3+ tumours, HER2 IHC 2+ /ISH amplified BC are less frequently classified as HER2 enriched molecular subtype (49% versus 16, p<0.001), show upregulation of trastuzumab resistance genes, downregulation of genes responsible for HER2 oncogenic pathway activation and response to trastuzumab and more enriched with ER signalling pathway and coactivated genes. ER, positivity was significantly associated with lower pCR rates in HER2 IHC2 2+/ISH amplified than HER2 IHC 3+. Conclusion: Overexpression of HER2 protein is the driver of the oncogenic pathway in HER2 positive BC and it is the main predictor of response to anti-HER2 therapy. ER pathway is more dominant in BC with equivocal HER2 expression.

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P4

The clinical value of progesterone receptor expression in luminal breast cancer

Background: The routine assessment of progesterone receptor (PR) expression in breast cancer (BC) remains controversial. Here, we aimed to evaluate the role of PR expression in luminal BC, with emphasis on the definition of positivity and its prognostic significance as compared to Ki67 expression. Methods: A large cohort (n=1924) of oestrogen receptor (ER) positive/HER2 negative BC was included. PR was immunohistochemically (IHC) stained on full face sections and core needle biopsies (CNB) where the optimal scoring cut-off was evaluated. In addition, the association of PR with other clinicopathological factors, cellular proliferation, disease outcome and response to adjuvant therapy were analysed. Results: Although several cut-offs showed prognostic significance, the optimal cut-off, to categorise PR expression into two clinically distinct prognostic groups on CNB was 10%. PR negativity showed a significant association with features of aggressive tumour behaviour and poor outcome. Multivariate analyses indicated that the association between PR negativity and poor outcome was independent of tumour grade, size, node stage, and Ki67. PR negativity showed independent association with shorter survival in patients who received endocrine therapy while Ki67 was not. Conclusion: PR IHC expression provides independent prognostic value superior to Ki67. Routine assessment of PR expression in BC using 10% cut-off in the clinical setting is recommended.

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P5

Ki67 assessment in ER positive / HER2 negative breast cancer: A comparative study between different scoring methods

Background: Ki67 reflects the proliferation activity in breast cancer (BC). However, an optimal method for its assessment in clinical settings has yet to be robustly defined. In this study, we compared several methods to score Ki67 to identify a reliable and reproducible method for routine practice. Methods: Sections from luminal BC cohort (n=1662) were immunohistochemically stained with Ki67 and were assessed for the percentage, pattern, intensity of expression and heterogeneity index, which means the difference in Ki67 expression between the quantification Ki67 in hotspot and average Ki67. Ki67 positivity was evaluated using three methods: (i) quantification of Ki67 positive cells among 1000 invasive tumour cells within hotspot, (ii) average estimation of Ki67 within a defined hotspot, and (iii) average estimation of Ki67 positivity within the whole section. Time required for scoring, inter-observer agreement and association with outcome were determined. Results: The mean percentage of Ki67 expression per 1000 cells method was 16%, while the mean value of Ki67 scores using the average estimation within hotspot and whole slide were 14% and 12%, respectively. Quantification of Ki67 positive cells within 1000 cells had the highest degree of consistency between observers, and the highest hazard ratio predicting patient outcome when compared to using different common Ki67 cut-offs, which was independent on the other two methods. Granular pattern of Ki67 expression was associated with poorer outcome as compared to the other patterns. High heterogeneity index showed strong association with shorter survival in patients who received endocrine therapy. Conclusion: Assessment of Ki67 expression using quantification positive cells among 1000 tumour cells is an optimal method to achieve high reliability and reproducibility. Comment on the predominant Ki67 expression pattern add prognostic value.

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P6

Evaluation Oncotype DX® 21-Gene Recurrence Score and Clinicopathological Parameters in Early Stage Breast Cancer

Background: Oncotype DX recurrence score (RS) is a clinically validated assay, which predicts the likelihood of disease recurrence in oestrogen receptor positive, HER2 negative (ER+/HER2-) breast cancer (BC). In this study we aimed to compare the performance of Oncotype DX against the conventional clinicopathological parameters using a large BC cohort diagnosed in a single institution. Methods: A cohort (n=430) of ER+/HER2- BC patients who were diagnosed at the Nottingham University Hospitals NHS Trust and had Oncotype DX testing as a part of their routine care was included. Correlation with the clinicopathological and other biomarkers including the proliferation index was analysed. Results: The median Oncotype DX RS was 17.5 (range 0-69). There was a significant association between high RS and grade 3 tumours. None of grade 1 BC or grade 2 tumours with mitosis score 1 showed high RS. Low RS was significantly associated with special tumour types where none of the patients with classical lobular or tubular carcinomas had a high RS. There was an inverse association between RS and levels of ER and progesterone receptor (PR) expression, and a positive linear correlation with Ki67 labelling index. Notably, 6 patients who developed recurrence had an intermediate RS, however, 4 out of these 6 cases (67%) were identified as high-risk disease when the conventional clinical and molecular parameters were considered. Conclusion: Oncotype DX RS is strongly correlated with the conventional clinicopathological parameters in BC. Some tumour features such as tumour grade, type, PR status and Ki67 index can be used as surrogate markers in certain scenarios.

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P7

The Prognostic and Predictive Value of Polo-like kinase-1 (PLK1) Expression in Breast Cancer; is it Oncogene or Tumour Suppressor Gene?

Background: Polo-like kinase-1 (PLK1) plays a crucial role in cell cycle progression, and it is overexpressed in many tumours, including breast cancer (BC). However, the characteristics of PLK1 expression in different molecular subtypes of BC remain unclear. In this study, we aimed to evaluate the prognostic and predictive role of PLK1 in BC with emphasis on its role in molecular subtypes. Methods: A large BC cohort (n=1208) were immunohistochemically stained for PLK1. The association with clinicopathological data, the molecular subtypes and survival outcome was analysed. Transcriptomic expression of PLK1 was evaluated in publicly available datasets including The Cancer Genome Atlas and the Kaplan--Meier Plotter tool. Results: High expression of PLK1 was significantly associated with parameters characteristic of aggressive tumour behaviour, including triple negative (TN) molecular subtype (p=0.02). Lower PLK1 expression was significantly associated with worse outcome in the whole cohort (p=0.032), luminal (p=0.030) and HER2 enriched BC (p=0.045). In TNBC, high PLk1 expression was associated with poor outcome (p=0.026). While lower PLK1 was an independent risk factor for poor prognosis in luminal BC, higher PLK1 expression was independent poor prognostic factor in TNBC. On the mRNA levels, PLK1 mRNA expression was higher in TNBC compared with luminal BC, consistent with protein expression. Low PLK1 mRNA expression showed strong association with poor outcome in luminal BC in some of publicly available datasets whereas in others, high expression of PLK1 showed a significant association with poor survival in luminal subtype. Conclusion: The prognostic and predictive roles of PLK1 in BC is molecular subtype dependent. As PLK1 inhibitors enter clinical trials for several cancer types, our study supports evaluation of pharmacological inhibition of PLK1 as an therapeutic approach in TNBC. However, in luminal BC, PLK1 role remains controversial.

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P8

The clinical and biological significance of atypical mitosis in breast cancer

Atypical mitosis is considered a feature of malignancy, however, its significance in breast cancer (BC) remains elusive. Here, we aimed to assess the clinical value of atypical mitoses in BC and to explore their underlying molecular features. Atypical and typical mitotic figures were quantified and correlated with clinicopathological variables in a large cohort of primary BC tissue sections (n = 846) using digitalised haematoxylins and eosin whole-slide images (WSIs). In addition, atypical mitoses were assessed in The Cancer Genome Atlas (TCGA) BC dataset (n = 1032) and were linked to the genetic alterations and pathways. In this study, the median of typical mitoses was 17 per 3 mm2 (range 0--120 mitoses), while the median of atypical mitoses was 4 (range 0--103 mitoses). High atypical mitoses were significantly associated with parameters characteristic of aggressive tumours behaviour. The total number of mitoses, and a high atypical-to-typical mitoses ratio (>0.27) were associated with poor BC specific survival (BCSS), (p = 0.04 and 0.01, respectively). The atypical-to-typical mitoses ratio dichotomised triple negative-BC (TNBC) patients into two distinct groups in terms of the association with the outcome, while the overall number of mitoses was not. Moreover, TNBC patients with high atypical-to-typical mitoses ratio treated with adjuvant chemotherapy were associated with shorter survival (p = 0.003). Transcriptomic analysis of the TCGA-BRCA cohort dichotomized based on atypical mitoses identified 2494 differentially expressed genes. These included genes linked to pathways involved in chromosomal localization and segregation, centrosome assembly, spindle and microtubule formation, regulation of cell cycle and DNA repair. To conclude, the atypical-to-typical mitoses ratio has prognostic value independent of the overall mitotic count in BC patients and could predict the response to chemotherapy in TNBC.

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P9

Combined proliferation and apoptosis index provides better risk stratification in breast cancer

Introduction: Breast cancer (BC) risk stratification is critical for predicting behaviour and guiding management decision-making. Despite the well-established prognostic value of cellular proliferation in BC, the interplay between proliferation and apoptosis remains to be defined. In this study, we hypothesised that the combined proliferation and apoptosis indices can provide a more accurate prognostic indicator. Methods and Results: Apoptotic and mitotic figures were counted in whole slide images (WSI) generated from haematoxylin and eosin-stained sections of 1545 BC cases. Counts were carried out visually within defined areas. Our results showed a significant correlation between mitotic and apoptotic scores. Similar to mitotic scores, high apoptotic counts were associated with features of aggressive behaviour including high grade, high pleomorphism score, and hormonal receptor negativity. Although apoptotic index (AI) was an independent prognostic indicator, the prognostic value was synergistically increased when combined with the mitotic index. BC patients with a high combined mitotic and apoptotic index had the shortest survival. Conclusion: Apoptotic figures count provides additional prognostic value in BC when combined with mitotic index. within the era of artificial intelligence, such a combination can be implemented to assess the behaviour of BC and provides an accurate prognostic indicator.

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P10

The role of Assembly Factor for Spindle Microtubules (ASPM) in breast cancer and its prognostic significance

Background: Assembly Factor for Spindle Microtubules (ASPM) plays a critical role in mitotic spindle regulation and normal mitosis. This study aims to evaluate the clinical and prognostic significance of ASPM in breast cancer (BC). Methods: ASPM expression was evaluated on tissue microarray of a large BC cohort (n = 1314) using immunohistochemistry. ASPM transcriptomic expression was assessed using the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) and Cancer Genome Atlas (TCGA) BC cohorts (n=2834). The correlation between ASPM expression, clinicopathological parameters and outcome was assessed. Results: ASPM expression was detected in the nucleus and cytoplasm of the tumour cells. It was significantly associated with aggressive BC features including high mitotic score, high tumour grade, and poor Nottingham Prognostic Index. In luminal BC, ASPM cytoplasmic expression was associated with shorter BC specific survival (BCSS) (HR 2.8, CI 95% 1.5-5.2; p=0.001) and high ASPM nuclear expression was associated with shorter BCSS and distant metastasis free survival (DMFS) (HR 4.8, CI 95%2.3-9.9; p<0.001 and HR 2.1, CI 95%;1.4-3.32p=0.001). In the endocrine therapy-treated group, high ASPM cytoplasmic expression was predictive of a higher risk of death from BC (HR= 2.7, CI 95% 1.5-5.1; p=0.001). However, such association was not observed in patients who did not receive endocrine therapy. Conclusion: ASPM expression is a poor prognostic biomarker in BC and its expression can help in predicting recurrence after endocrine therapy. Further investigations are warranted to reveal its underlying mechanisms and potential therapeutic benefit in various BC subtypes.

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P11

Kinetochore-Associated Protein DSN1 is associated with aggressive characteristics and predicts poor prognosis in patients with invasive breast cancer

Background: DSN1 is a dosage inhibitor of NnF1 that forms part of the kinetochore protein complex necessary for proper chromosome assembly during mitosis and plays a key role in tumour cell proliferation and malignant transformation. However, the role of DSN1 in breast cancer is still unknown. This study aimed to evaluate the clinical and prognostic significance of DSN1 in invasive breast cancer (BC). Methods: DSN1 expression was evaluated using immunohistochemical analysis of tissue microarrays of a large BC cohort (n=1161), and at the mRNA level utilising the Cancer Genome Atlas (TCGA) BC cohort (n=854). The correlation between DSN1 expression and clinicopathological parameters and patient outcome was investigated. Results: DSN1 expression was detected in tumour cell cytoplasm and was significantly associated with aggressive BC characteristics including high tumour grade and high Nottingham Prognostic Index scores. Elevated DSN1 expression predicted poor BC-specific survival (BCSS) and distant metastasis-free survival (DMFS) in the whole cohort and luminal subtypes. In endocrine therapy-, chemotherapy- and radiotherapy-treated patients, high cytoplasmic DSN1 expression was significantly associated with shorter survival. Conclusion: High DSN1 expression is a prognostic biomarker for poor patient outcome and its expression can predict patient response to hormonal therapy, radiotherapy, and chemotherapy.

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P12

AI in Pathology: A Medical Student's AI Assisted Crash Course in Diagnostic Histopathology of Large Bowel

Purpose of the study

The digitalisation of histopathology in recent years, has provided a catalyst for advancements in the applications of artificial intelligence (AI) within pathology, raising questions among pathologists today for the future role of pathologists within histopathology.The aim of this case study was to pilot AI technologies in providing efficient and effective education on the histopathology of large bowel specimens.

Methods

A case study design, involving a singular 5th year medical student, from the University of Exeter, engaging within a two-week placement, with no prior teaching of large bowel histopathology within her medical course. Whole slide imaging technologies at Musgrove Park Hospital allowed all large bowel specimens received by the unit over the two-week period to be inputted into the AI technology, which in turn generated diagnoses. The student analysed individual slides using whole slide imaging technology and determined a diagnosis, then comparing this to the AI's diagnoses. A confusion matrix and total percentage accuracy was generated for each test. The primary outcome of the study aimed to measure the progression of total percentage accuracy over the two-week course.

Summary of Results

Eighty-seven slides were generated over the two-week course, divided over the course of seven tests. Prior knowledge was assessed through the first test, which reached a total percentage accuracy of 0%. Total percentage accuracy increased as days of training increased. Furthermore, a negative correlation was found on the confusion matrices between the occurrence of disagreement and with days of training. A case study format allowed collection of qualitative data on the perceptions of student learning experiences. Student's predicted features at the end of the study were deemed accurate by a consultant histopathologist in the unit.

Conclusions

Larger studies will need to provide further evidence of the true effectiveness of AI in medical education.

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P13

The prognostic significance of tumour to stroma ratio in risk stratification of the luminal breast cancer patient: An artificial intelligence study

The tumour microenvironment (TME) has been shown to play an important role in the prognostication of epithelial tumours. However, its assessment is often dependent on subjective visual assessment of stroma to tumour ratio (STR). Using artificial intelligence (AI) based algorithms on whole slide images (WSIs) could produce a detailed and more objective assessment of STR, with the potential to discover new features beyond human eye's ability. In this study, we used the power of AI to assess STR utilising a large cohort of well-characterised luminal (ER+/HER2-) early breast cancer patients. Methods: AI-based algorithm was applied to the WSI of 1,312 luminal BC cases with long follow-up data. Automated quantification of tumour cells and stromal cells was carried out. STR was calculated in the WSI in terms of surface area and cell count ratios. The intratumoral STR heterogeneity and spatial distribution were assessed. Tumour volume, a parameter that expresses the actual tumour size and tumour cell density, was also calculated. Results: High STR was associated with good prognostic BC parameters (low tumour grade, good Nottingham prognostic index (NPI), small tumour size, p<0.001) and longer survival in the whole cohort and in a subgroup of endocrine-only treated patients. Tumour volume was associated with tumour behaviour and patients' survival better than tumour size. Multivariate cox regression model revealed that larger tumour volume was independent predictor of worse patients' outcome (BC specific survival; HR:1.9, P=0.01, 95%CI:1.17-3.11 and distant metastasis free survival; HR:1.86, P=0.008, 95%CI:1.18-2.92). Conclusion: Our objective STR is a good prognostic parameter that can be used for risk stratification and proper treatment of ER-positive early-breast cancer patients. Tumour volume is reliable in the prediction of patients' survival.

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P14

Using deep learning-based approaches to characterise ageing in different tissue components of normal breast tissue of women with different risk of developing breast cancer

Ageing, a breast cancer risk factor, can be reflected in histologically normal breast tissue (NBT). However, the relationship between age-related histological features and cancer risk is not fully understood. We hypothesise that risk-predictive age-related features could be learned from digitalised whole slide images (WSI) of NBT using deep learning (DL) methods.
A total of 2,000 WSIs from 1,404 women were collected from 5 cohorts from 6 NBT resources, including healthy consented women, reduction mammoplasties, risk-reducing mastectomies and contralateral, ipsilateral and peri-tumoral NBT. The patient's age ranges from 16~79. DL-based pipelines were implemented to predict three tissue types and age. Class activation maps (CAM) and graph-based analyses were used to visualise local histological features and extract global features from WSIs, respectively.
Across 6 NBT resources, 70 selected WSIs were manually annotated for epithelial cells, fibrous stroma and adipocytes, resembling the "ground truth". Extensive comparisons of different tile sizes, tile overlapping ratios, stain normalisation techniques and DL feature extractors were conducted to implement a Tissue-Classifier, which achieved 95% accuracy in 3-fold cross-validation. DL pipelines for age prediction were trained in each tissue type. Tissue-specific and age-related local histological features were visualised on tiles and confirmed by pathologists. Global variations were observed in the predicted tissue age heatmaps of WSIs across different risk groups.
Our DL-based models robustly captured local histological patterns to predict tissue components and age. The global organisation of these patterns showed variations in WSIs of women with different risk of developing breast cancers, which may provide new insights into premalignant alterations in NBT.

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P15 *

Comparing the effect of histopathology section thickness on tumour classifier generation and cell detection with Digital Pathology Methods

Digital pathology solutions have the potential to augment and in some cases replace, traditional pathology techniques. However, it is important to have quality assurance for examination of whole slide images. Understanding what factors affect its ability to identify tumour is crucial in understanding how we may employ the technology in routine clinical settings. One factor that may affect performance is section thickness as highly cellular, thick sections with high levels of background noise may impair accurate cell detections and whether the stain used influences this. The aim of the current study was to analyse the effect of section thickness and staining (H&E versus haematoxylin only staining) on whole slide cell detection and tumour percentage. We analysed two tumour types, a colorectal carcinoma and melanoma using QuPath, an open-source digital pathology software. The two blocks, one colorectal cancer and one malignant melanoma were irrevocably anonymised. Parallel sections, 3µm, 4µm and 5µm each were cut and stained. One section from each thickness group was stained with H&E, the other was stained with haematoxylin only. Scanned, whole slide images were analysed on QuPath. Classifiers were created for each image to detect the number of tumour, stromal, and necrotic cells. Our study showed that classifiers created for a specific section thickness cannot be applied to sections of different thickness with confidence due to variable results. Staining sections with haematoxylin only compared to show the importance of contrast also produced different cell detection results within the same thickness group. On this basis classifiers created on an image with one staining technique cannot be used to accurately detect tumour in an image of another staining type. We conclude that section thickness and staining affects the percentage of tumour cells detected when whole slide images are analysed using digital pathology methods and is an important factor for quality assurance.

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P16

Identification of Lewy body pathology propagation patterns in Lewy body disorders, using automated digital image analysis

Purpose of the study: Regional Lewy body propagation patterns are still a subject of debate years following the discovery of Lewy bodies as a hallmark pathology of Parkinson's disease. The body first hypothesis, in which Lewy body propagation originates in the enteric nervous system and ascends to the brainstem and then spreads across the cortical areas, is the conventional theory for Lewy pathology propagation. More recently, another propagation pattern known as the brain first hypothesis stipulates that aggregation may begin in the limbic system and expand to the forebrain and brainstem simultaneously. If regional differences in Lewy pathology burden are influenced by genetic background, such as APOE4 is not known.

Methods: Using machine learning-based automated pathology burden quantification, we determined Lewy body burden across six brain regions (medulla, pons, amygdala, anterior cingulate cortex, anterior frontal cortex and cortex of the inferior parietal lobule), in patients with Parkinson's disease, Parkinson's disease dementia and dementia with Lewy bodies.

Summary of results: We show distinct regional distribution patterns of Lewy pathology depending on the disease state. Whilst Lewy pathology burden in the analysed cohort was not affected by age at death or disease duration, APOE4 carriers had consistently higher Lewy body burden in the cortical and limbic, but not brainstem regions.

Conclusions: The provisional findings show the importance of computational quantitative analysis in identifying differences in regional pathology distribution, which, when combined with other multi-omics data in future studies, may reveal previously unknown molecular signatures that drive Lewy body disorder progression.

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P17

Automatic Mitosis Counting for Mesenchymal Tumour

Purpose of the study: Mitotic activity is an important feature for grading mesenchymal tumours. This is achieved by counting mitotic figures in the 10 most mitotically active high-power fields. This is a time-consuming, and laborious task; it is prone to inter-observer variation, and inaccurate recognition of mitoses can lead to misgrading of tumours. In this study, we propose an artificial intelligence (AI)-aided approach to detect mitotic figures in digitised pathology slides.

Methods: (1) Haematoxylin and eosin-stained sections (n=94) were destained and restained using an anti-phosphorylated histone H3 (pHH3) antibody to detect mitoses and create a large-scale dataset of mitotic figures contours and positions. (2) An AI algorithm was trained on the dataset (training/validation = 84, hold-out = 10) and applied on whole slide images (WSIs) for detecting suspected mitotic figures: these were reviewed and labelled as true and false positives by two pathologists; the annotations were then used to augment the dataset. (3) The two-stage model was applied on new WSIs with AI-generated tumour contours for generating mitotic counts position and quantify the mitotic index. The results were compared to those provided by pathologists.

Results: The proposed mitotic detection model yielded a sensitivity of 0.859, a specificity of 0.655 and a F1 score of 0.743 on the hold-out dataset. The sensitivity indicates the algorithm successfully detected most of the mitotic figures, but the lower specificity is explained by neutrophils, lymphocytes, and apoptotic figures being called as mitotic figures.

Conclusions: A large-scale database for mitotic figures in mesenchymal tumour was established. This was then used to develop a mitosis detection model which gave excellent sensitivity but lower specificity. The model has the potential to assist in the grading of mesenchymal tumours, although further annotation by pathologists is required to improve the specificity.

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P18

Inference of germline and somatic variation using weakly-supervised deep transfer learning in an experimental model of chemical carcinogenesis

Purpose of the study
Most human cancer genomes exhibit multiple mutational signatures, reflecting the complex milieu of cellular damage and repair occurring during carcinogenesis. We used a robustly controlled, highly powered in vivo experiment and investigated genotype-phenotype correlates by applying machine learning approaches to digital pathology images.

Methods
Inbred mice were exposed to a single dose of diethylnitrosamine shortly after birth. Resultant liver tumours were isolated and submitted for whole genome sequencing, total RNA sequencing, and histopathology. This cohort was used to discover the phenomenon of lesion segregation, a unifying property of exogenous mutagenesis which has important implications for cancer genome evolution (Aitken et al, Nature, 2020). We have previously modelled quantitative nuclear morphology in these tumours to predict whole genome duplication, germline variation, and driver oncogene mutations. Here, we orthogonally validate this using weakly-supervised deep transfer learning.

Summary of results
This approach can robustly predict germline variation between mouse strains, somatic driver mutations within strain, and heterozygosity of Ctcf, with performance significantly in excess of class-weighted null expectations. We compare a range of technical choices including neural network architectures, tuning base model weights, data partitioning, and image normalisation methods. Finally, we use attention ranking to investigate the local features which drive model predictions.

Conclusions
Germline and somatic genetic variation shape the histological phenotype of liver tumour tissue even when the major covariates of carcinogenesis are stringently controlled. This study emphasises that clinical-grade molecular inference models must be parameterised on ancestrally-diverse populations to ensure generalisability. It additionally raises the possibility that actionable germline variation may be predicted from routine tumour pathology.

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P19 *

Does batch testing impact the quality of multiplex immunofluorescence staining in large epidemiological cohorts of formalin-fixed paraffin-embedded tissues?

Purpose of the study

To validate novel multiplex immunofluorescence (mIF) staining to HRP-DAB immunohistochemistry (IHC) and to compare the mIF images produced across multiple staining batches in order to identify sources of bias that could influence accurate biomarker quantification.

Methods

Two novel mIF biomarker panels were assessed (NIB18-0282); multiplex panel 1 (MP1) which detected CD3, CD4, CD8 CD20, Ki67 and synaptophysin and multiplex panel 2 (MP2) for CD3, CD68, CTLA-4, PD-L1, STING and synaptophysin. Staining for each mIF panel was conducted in batches of 15 slides. Each batch of whole-face sections was run with a multi-tissue TMA as a mIF staining control. Slides were scanned using either an Aperio AT2 or PhenoImager HT. QuPath software was used to analyse both IHC and mIF images.

Summary of results

Correlation matrices show moderate-strong positive correlations (Rs>0.60) for mIF vs HRP-DAB IHC biomarker quantification in MP1 and MP2, validating panel design for all biomarkers except CTLA-4. Review of the slides found non-specific CTLA-4 staining present in HRP-DAB IHC but not mIF thus contributing to poor correlation (Rs=-0.11). Differences in cell mean intensities produced across control TMAs by staining batch in MP1 and MP2 was found to be acceptable (<2 Gy). However, evidence of dye-specific batch bias was observed in both MP1 and MP2 for Opal 520 antibody-opal pairings.

Conclusions

Staining using mIF sufficiently replicated biomarker analysis using HRP-DAB IHC validating MP1 and MP2 for the purposes of digital quantification. Use of mIF to target CTLA-4 produced cleaner reaction product for digital image analysis of the biomarker highlighting a strength of non HRP-DAB based detection systems. Staining batch effects were minimal across both panels; however, this study provides novel evidence that dye-specific batch effects can occur and may affect downstream image processing and biomarker quantitation.

A JSPS summer studentship supported this work.

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P20

DIVE IN to Immune Niches: Combining high-dimensional human tissue phenotyping and AI-based immune microenvironment analysis to identify and quantify immune cell niches

Advances in multiplexed imaging have enabled simultaneous detection of multiple cellular markers at single-cell resolution using fluorescence-based antibody staining and mass cytometry methods. The Cell DIVE platform provides a powerful tool to investigate immune microenvironments and interactions of immune cells with stroma and vasculature in spatially preserved niches using whole-slide widefield immunofluorescence imaging. The multiplexing process involves cyclical rounds of antibody staining, image acquisition and fluorophore bleaching, allowing simultaneous visualisation of up to 60 markers on a single tissue section. Using the Cell DIVE platform, we have validated 90 markers across 12 different normal and pathological human tissues, in addition to over 20 markers for mouse tissues, allowing rapid multiplexed imaging of broad immune panels across sites and species. The Cell DIVE platform further provides automatic removal of background autofluorescence, enabling sensitive and reliable multi-marker visualisation for downstream single-cell analysis. This has enabled the identification of immune cell populations and their stromal niches within human lymph nodes and provided spatial context to microenvironment changes in response to vaccine adjuvants. We have developed a custom multiplexed image analysis pipeline that employs the open-source AI-based DeepCell algorithm for nuclear and cell segmentation. Quality control metrics, including comparison with background staining, are used to appraise each marker. Following the generation of a cell by marker intensity matrix, downstream analysis becomes possible for cellular immunophenotyping, providing an end-to-end multiplexed imaging toolkit for translational research. This approach has been used to stratify patients with inflammatory bowel diseases and rheumatoid arthritis based on the tissue composition and location of immune cells to inform therapeutic responses.

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P21 *

Exploring Digital Image Analysis Methods to Investigate Pathological Slide Images

Purpose of the study: to investigate different digital methods to analyse whole slide images, including using QuPath and Stardist extension to quantify Beta-2-microglobulin (B2M) immunohistochemistry (IHC) from Head and Neck Cancer slides, and to quantify the spatial localisation of Tumour-Infiltrating lymphocytes (TILs) in undifferentiated sarcoma (USARC) slides and its impact on prognosis. Methods: B2M study: QuPath digital image analysis software used to perform cell detection, B2M/DAB staining detection, and classification by cell type (e.g. tumour, stroma, lymphocytes e.t.c.) on each of the 9 Head and Neck Tumour slides. Measurements recorded from these detections used to calculate the percentage of tumour cells in each slide that stained positively for DAB staining, thus positive for B2M. TILs study: QuPath digital image analysis software and Stardist extension (deep-learning Python implementation of star-convex object detection) used to perform cell and lymphocyte detection and classification at the tumour centre and periphery on 37 USARC slides (from 30 patient). Once data collected, R studio used to compare the quantification and spatial localisation of the TILs with the patient survival data. Results: B2M study: The average positive percentage of B2M expression in the tumour cells across the 9 Head and Neck cancer slides was 5.46%, which is very low and could be linked to DNA methylation data to suggest a method of how the tumour cells are evading the immune system. TILs study: Across the 37 USARC slides there was a significant increase in the number of TILs from the centre to the periphery of the tumour. Additionally, the survival probability of patients with a TILs density greater than 4.8% was significantly higher. Conclusions: Both of these individual studies have suggested methods using digital image analysis software QuPath and Stardist to efficiently investigate pathological slides. Larger studies repeating these methods will be needed for validation.

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P22

WNT-associated protein expression correlates with detection of metachronous polyps in a post-polypectomy surveillance population

To reduce incidences of colorectal cancer, pre-malignant colonic polyps are excised by polypectomy. The current British Society of Gastroenterology (BSG) guidelines for post-polypectomy surveillance stratifies patient risk of metachronous polyps by histology, grade of dysplasia, size and number of polyps removed at index polypectomy. However, only 50% of patients will develop metachronous polyps resulting in a significant number of unnecessary, invasive, and expensive surveillance colonoscopies. There are currently no biomarkers for predicting metachronous polyps. We investigated the utility of using E-Cadherin (surrogate marker for WNT signalling), SOX9 (cell stemness), and Ki67 (cell proliferation) to predict metachronous polyps and complement existing surveillance guidelines.

A Tissue Microarray (TMA) was constructed from formalin-fixed paraffin-embedded left-sided colonic polyps previously resected from 279 patients between 2009 - 2016, as part of the Scottish Bowel Screening Program. Immunohistochemistry (IHC) was used to detect the expression of the E-Cadherin, SOX9, and Ki67. Protein expression was assessed by both manual scoring and the digital pathology.

Statistical analysis showed that high expression of E-cadherin, SOX9, or Ki67 in luminal cores are independent prognostic factors for metachronous disease and are significantly associated with earlier detection of metachronous polyps or CRC (HR 2.3, 95% CI 1.6-3.3, p<0.001; HR 2.3, 95% CI 1.3-3.0, p=0.001 and; HR 1.7, 95% CI 1.2-2.4; p =0.005, respectively). A new risk score was created using a combination of BSG risk score and expression of E-cadherin, SOX-9 and Ki67 (HR 3.2, 95% CI 2.3-4.4, p<0.001).

The new combined risk score is better at stratifying patient risk of future metachronous polyps than the current BSG guidelines alone.

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P23

Investigating the prevalence and quantification of Escherichia coli polyketide synthase (pks) toxin and Bacteroides fragilis toxin (BFT) carriage within the faecal microbiome of a NHS Bowel Cancer Screening Programme cohort and a developing-country colorectal cancer cohort

Purpose: We previously performed 16SrRNA amplicon profiling of the faecal microbiome of guaiac faecal occult blood test (gFOBT) cards from the NHS Bowel Cancer Screening Programme (NHSBCSP), and showed the potential for microbiome analysis to improve colorectal cancer (CRC) screening accuracy. Using the same methodology, we profiled the microbiome of four developing countries (Chile, Argentina, India, Vietnam) and showed that the collective CRC-associated microbiome of this cohort resembled that of developed countries. In this current study, we set out to determine the carriage of pks and BFT (two CRC-associated toxins) within the NHSBCSP and developing-country cohorts. Methods: DNA was extracted from gFOBT samples (NHSBCSP cohort n=931; developing-country cohort n=84). TaqMan qPCR to pks and BFT toxins was performed in duplicate. Positivity was defined as Cq mean of duplicates <35. Results: NHSBCSP cohort BFT: 1 sample failed. Overall BFT prevalence was 9%. Carriage (prevalence)(median copy number) was: cancer (18/192=9%)(604), adenoma (22/191=12%)(305), colonoscopy-normal (13/190=7%)(255), non-neoplastic (17/167=10%)(382), blood-negative (15/190=8%)(124). pks: 155 samples failed(17%). Overall pks prevalence was 31%. Carriage (prevalence)(median copy number) was: cancer (54/158=34%)(1139), adenoma (54/161=34%)(2334), colonoscopy-normal (41/161=25%)(2156), non-neoplastic (44/138=32%)(1517), blood-negative (44/158=28%)(327). Developing-country cohort BFT: Overall BFT prevalence was 8%. pks: 5 samples failed (6%). Overall pks prevalence was 6%. Conclusions: Prevalence rates of BFT and pks toxins in the NHSBCSP cohort match existing CRC literature. There is no appreciable difference in toxin prevalence according to colonoscopy group. Median copy number of BFT is higher in the cancer group. pks prevalence is lower in the developing-country cohort (6%) compared with the NHSBCSP cohort (31%) and existing literature. This finding will be validated using an additional Indian cohort.

℗Young, C.A.[1]; Wood, H.[1]; Ramakrishnan, A.[2]; Nang, P.V.[3]; Vaccaro, C.[4]; Contreras Melendez, L.[5]; Bose, M.[2]; Doi, M.[3]; Piñero, T.[4]; Tapia Valladares, C.[5]; Arguero, J.[4]; Fuentes Balaguer, A.[1]; Bottomley, D.[1]; Wilkinson, L.[1]; Gallop, N.[1]; Benton, S.[6]; Burtonwood, C.[6]; Brealey, M.[6]; Campodonico, V.[7]; Sears, C.[7]; Quirke, P.[1]

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P23

Investigating the prevalence and quantification of Escherichia coli polyketide synthase (pks) toxin and Bacteroides fragilis toxin (BFT) carriage within the faecal microbiome of a NHS Bowel Cancer Screening Programme cohort and a developing-country colorectal cancer cohort

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℗Young, C.A.[1]; Wood, H.[1]; Ramakrishnan, A.[2]; Nang, P.V.[3]; Vaccaro, C.[4]; Contreras Melendez, L.[5]; Bose, M.[2]; Doi, M.[3]; Piñero, T.[4]; Tapia Valladares, C.[5]; Arguero, J.[4]; Fuentes Balaguer, A.[1]; Bottomley, D.[1]; Wilkinson, L.[1]; Gallop, N.[1]; Benton, S.[6]; Burtonwood, C.[6]; Brealey, M.[6]; Campodonico, V.[7]; Sears, C.[7]; Quirke, P.[1]

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P24

Association between artificial intelligence- (AI) assisted tumour AREG and EREG immunohistochemistry (IHC) and outcomes from anti-EGFR therapy during the routine management of metastatic colorectal cancer (mCRC): an observational cohort study

Background: AREG and EREG are ligands of EGFR that predicted benefit from anti-EGFR therapy in an analysis of the PICCOLO trial (irinotecan ± panitumumab). Here, we sought to validate those findings through an analysis of patients who received anti-EGFR therapy during routine care for mCRC.

Methods: Patients (pts) who received panitumumab or cetuximab ± chemotherapy at any time for treatment of mCRC at 8 UK Cancer Centres were eligible. Central *RAS* testing by next generation sequencing (NGS) was performed for pts where extended *RAS* testing had not been previously undertaken. *RAS*-mutant (mut) and *RAS*-unknown pts were excluded. AREG and EREG positive tumour cells were identified by IHC. Pathologists annotated tumour areas on digital images of glass slides. AI algorithms calculated the percentage of tumour cells staining positive for AREG and EREG. More than 20% AREG and/or EREG tumour cell positivity was regarded as high biomarker expression, the optimal cut-point identified in PICCOLO. Study endpoints were progression-free survival (PFS) and overall survival (OS).

Results: 541 pts were recruited. 494 (91.3%) had adequate archival tissue for analysis. Central *RAS* testing was successfully performed in 255 of 393 (64.9%) pts without existing extended *RAS* results, leading to 45 exclusions, leaving 449 pts in the primary analysis population. After adjustment for additional prognostic factors, high AREG/EREG expression (n=360; 80.2%) was associated with significantly prolonged PFS (HR 0.73; 95% CI, 0.56-0.95; p=0.02) and OS (HR 0.66 [0.50-0.86]; p=0.002). Median PFS in the high *vs* low biomarker groups was 8.5 *vs* 4.4 months; median OS 16.4 *vs* 8.9 months. The significant difference in OS (high *vs* low) was maintained in the subgroup with right-sided primary tumour location (n=107; 23.8%) (HR 0.56 [0.37-0.86]; p=0.007).

Conclusions: High tumour AREG/EREG expression was associated with significantly prolonged PFS and OS among a cohort of pts treated with anti-EGFR therapy for mCRC.

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P25

Glasgow Microenvironment Score as a Prognostic and Predictive Biomarker

To better predict prognosis and response to treatment regimens there has been a focus to develop colorectal cancer (CRC) subtypes, culminating in Consensus Molecular Subtypes (CMS). However, CMS is expensive and time-consuming. The Glasgow Microenvironment Score (GMS) was devised to provide a histological subtyping method for CRC. Using standard diagnostic H&E sections, patients are assigned to three independently prognostic groups based on inflammatory infiltrate and stromal invasion (GMS 0, 1,2). The inflammatory infiltrate is measured using Klintrup Mäkinen grade which assesses immune cell presence at the invasive margin and stromal invasion which assesses tumour stroma percentage. GMS has an independent prognostic power in a stage I-III colorectal cancer diagnosed in Glasgow Royal Infirmary (1997-2007). The majority of patients had colon cancer (76.9%), were stage III (55%), N stage 0 (62.5%) and MMR proficient (82.1%) and 32% had adjuvant chemotherapy. GMS was significantly associated with cancer-specific survival (HR= 1.9, 95%CI; 1.5-2.2, p<0.001). Patients classified as GMS0 (immune) had the best outcomes with mean survival of 173 months (95% CI 165-181) compared to 145 months (95% CI 137-153) for GMS1 (intermediate). Patients with GMS2 (stromal) exhibited the worst prognosis with a mean survival time of 112 months (95% CI 97-127). GMS has potential as a predictive marker for standard and novel therapies; in the TransScot clinical trial cohort, GMS0 group has a significantly better clinical response to FOLFOX chemotherapy over CAPOX. Furthermore, GMS2 is enriched for JAK/STAT3 signalling, with GMS2 patient-derived organoids responding better to JAK inhibitors than GMS0/1 organoids. Therefore, GMS as a subtyping method is easily translatable to routine diagnostics and represents a promising tools for providing prognostic and predictive value for a fraction of the cost and turnaround time associated with sequencing modalities.

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P26

SARIFA (Stroma AReactive Invasion Front Areas) proofs to be prognostic biomarker also in pancreatic adenocarcinoma.

Background: SARIFA (Strooma AReatctive Invasion Front Areas) is a newly described histological prognostic biomarker, defined as a direct contact between a cluster of tumorous glands/cells and inconspicuous surrounding adipose tissue at the invasion front, which showed its significance in gastric and colon cancer, previously. The assessment of this parameter is easily applicable, requires no additional stainings and shows very low interobserver variability. Despite the progress of oncology in many entities, pancreatic adenocarcinomas (PDA) still belong to the group of hard-to-treat cancers. Because of its functional link to tumour metabolics SARIFA could be an interesting biomarker in PDA. Methods: We compiled a collective of patients, who have undergone pancreatic tumour resection between years 2005 and 2015 due to PDA. SARIFA-positivity has been defined as occurrence of a direct contact between tumorous glands or cells and surrounding adipose tissue at the invasion front by at least 2/3 of given HE-stained tumour slides. Results: 980 slides have been evaluated which corresponds to 176 patients, with mean age of 66 ± 10 and a female:male ratio of 1:1.2 in this collective. SARIFA classification proved to be easily and fast to evaluate and showed remarkably low interobserver variability. SARIFA-positivity was identified in 53 cases (30%). Compared to SARIFA-negative cases it was associated with a significantly reduced overall survival time (median 16.0 versus 22.0 months, p = .018, n = 176). Conclusion: By overall poor survival rates of patients with a diagnosis of pancreatic cancer, SARIFA could be shown to be a promising prognostic factor. The evaluation of SARIFA-status can be easily applied in pathologic diagnostics of pancreatic cancer. Moreover, based on what could be shown on gastric cancer, it offers a new therapeutic perspective targeting lipid metabolics. Further investigations and statistical analyses are currently ongoing.

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P27

The Integrated Technologies for Improved Polyp Surveillance Study (INCISE) cohort: post-polypectomy metachronous polyp detection assessed against British Society of Gastroenterology 2020 surveillance guidelines

Introduction The British Society of Gastroenterology guidelines (BSG2020) recommend surveillance colonoscopy to high-risk patients following colorectal polypectomy. While 50% of patients develop metachonrous polyps, the remaining patients undergo further unnecessary, invasive colonoscopies, a significant burden on the NHS. Here we describe the Integrated Technologies for Improved Polyp Surveillance (INCISE) cohort, evaluate metachronous lesion risk and compare outcomes by BSG2020. Methods A retrospective study was performed from 2643 NHS Greater Glasgow & Clyde patients undergoing polypectomy between 2009 and 2016 followed by surveillance colonoscopy. Demographics, index polyp number, histology, location, size and BSG2020 risk were compared by metachronous lesion outcome using X2/ANOVA. Multivariate polynomial regression identified independent predictors of advanced future lesions. Results Analysis showed older age predicted advanced metachronous lesions, while male sex, polyp number, right-sided index polyps and BSG2020 high-risk predicted non-and advanced metachronous lesions($p \leq 0.001$). Older age, villous, advanced index polyps and polyp number predicted early (<2 years) lesions. Male sex, right-sided index polyps and BSG2020 high-risk predicted early and late (>2 years) lesions ($p<0.001$). Comparing BSG2020 high to low-risk patients, the rate of non-and advanced metachronous polyps was higher ($p<0.001$), but CRC was similar and no differences in lesions found >2 years (non- 23% vs 21%, advanced 8% vs 7% and CRC 0.9% vs 0.4%; $p=0.140$). Conclusion BSG2020 high-risk is associated with metachronous lesions but cannot differentiate advanced/non-advanced and is not predictive >2 years. Advanced polyps/CRC rate in BSG2020 high-risk patients was only slightly higher than low risk. Current guidelines requires improvement.

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P28

A Molecular Audit of Colorectal Adenocarcinoma in Southeast London

Introduction Defining a strategy for Lynch Syndrome testing in colorectal adenocarcinoma in the United Kingdom is central to the genomic medicine service rollout. The Advanced Diagnostics Laboratory at King's College Hospital (KCH) has been testing for mismatch repair (MMR) deficiency and hotspot mutations since 2016. We therefore sought to characterise our patient population in preparation for centralisation with the South East Genomics Laboratory Hub. Methods We audited all colorectal adenocarcinoma samples that underwent molecular testing (MMR immunohistochemistry, microsatellite instability (MSI) testing and next generation sequencing for BRAF, KRAS and NRAS) from January 2019 to September 2020. Results In total, 300 samples were analysed, of which 36 (12%) were MSI-high. Within this group, 22 samples (61%) had a BRAF (p.Val600Glu) mutation; all of these showed MLH1 and PMS2 loss on immunohistochemistry. One case showed concurrent focal loss of MSH6 expression. Two cases (9%) had a KRAS mutation. MSH2 expression was lost in one of these tumours on immunohistochemistry; the other showed loss of MSH6 expression. No mutations were identified in the remaining 12 cases (39%). Of these, seven showed loss of MLH1 and PMS2 on immunohistochemistry. PMS2 was solely lost in one sample; MSH2 was lost (with or without MSH6) in two cases; MSH6 was solely lost in one case; and a null pattern was seen in one case. Interestingly, only one case was found to be MMR proficient on immunohistochemistry i.e. was discordant with MSI testing. Thus, only seven cases (2%) required referral to a Clinical Geneticist for the exclusion of Lynch syndrome, and immunohistochemistry performed as well as MSI testing in identifying possible Lynch cases. Conclusion MMR immunohistochemistry coupled with BRAF mutational analysis remains an effective screening tool for Lynch syndrome in colorectal adenocarcinoma. We aim to compare this to MLH1 promotor hypermethylation testing in the future.

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P29

Integrating renal cell carcinoma tissue samples into a large prospective cohort: A feasibility study

PURPOSE OF THE STUDY Prospective cohort studies are a powerful epidemiological tool for investigating cancer risk factors, but may have limited information on histopathological subtypes of cancer from linkage to registry data. We investigated the feasibility of retrieving more detailed histopathology information and samples for participants in a large cohort study who were diagnosed with renal cell carcinoma (RCC). METHODS The Million Women Study (MWS) is a prospective cohort of 1.3 million UK women, recruited via the NHS breast screening programme in 1996-2001. Information on incident cancers and treatments comes from linkage to routine national datasets, including the National Cancer Registration and Analysis Service, and Hospital Episode Statistics. Over 5000 cases of RCC have accrued after 20 years of follow-up. We identified MWS participants who were diagnosed or treated for renal cell carcinoma in Oxford, and retrieved their original histopathology reports, archival slides and tissue blocks. Where possible, we scanned H&E stained slides to obtain digital whole-slide images of diagnostic biopsies and/or representative blocks of resection specimens, and took cores of tissue to make tissue micro-arrays. SUMMARY OF RESULTS We identified 74 MWS participants with a diagnosis of renal cell carcinoma who were diagnosed or treated in the Oxford area, according to Hospital Episode Statistics information. 65/74 had a record in the Oxford histopathology database with an available report. Of these, 57/65 had consented for use of their tissue in research, and 50/65 had a suitable tissue sample available (n=2 biopsy only, n=48 resection specimen). CONCLUSIONS It is feasible to obtain original histopathology reports, and retrieve archival slides and tissue blocks, for cases of renal cell carcinoma from a large population-based cohort study. Work is ongoing for further characterisation of the tumour samples using immunostaining, and for similar work at other sites.

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P30 *

The application of single cell analysis to understand clear cell renal cell carcinoma

Purpose of the study Clear cell renal cell carcinoma (ccRCC) is the commonest type of kidney cancer. Patients with metastatic disease have relatively poor outcomes, despite use of novel immunotherapy drugs. It is not known why these immunotherapy agents fail with an absence of biomarkers to identify non-responders. Increasing awareness of the tumour microenvironment (TME) is shaping our understanding of treatment resistance with myeloid cells of particular interest.

Methods

Fresh surgical tumour tissue and matched normal tissue was retrieved from six patients undergoing nephrectomy for curative intent of primary ccRCC. Fresh/frozen tissue and formalin-fixed paraffin-embedded blocks were generated alongside isolated single cells for flow cytometry and single-cell RNA sequencing. Downstream analysis was performed using Seurat. Potential targets were validated using immunohistochemistry (IHC).

Summary of results

>8000 single cells were sequenced across tumour and matched normal tissue. Tumour cell clusters, alongside immune cell populations unique to the TME were identified when compared to matched normal tissue. The macrophages identified in the tumour samples had a C1QA+/TREM2+/APOE+ transcriptional profile associated with tumour-associated macrophages, different to macrophages in the background samples. Flow cytometry confirmed higher proportions of CD45+ cells in the tumour than in background tissue. IHC showed infiltration of CD68+/CD163+ macrophages and CD3+ lymphocytes within the tumour, with relative absence in background samples.

Conclusions

This study has successfully demonstrated isolation of tumour and myeloid cells from a ccRCC primary tissue pipeline. A robust platform has been established for further work in a larger cohort with a focus on biomarker identification. This work was supported by a joint Jean Shanks/ Pathological Society pre-doctoral bursary.

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P31

Squamous Cell Carcinoma of the Penis in a Ugandan Referral Hospital

Background: Uganda has an age adjusted incidence of penile cancer of at least 3 times the global average. High risk HPV has been reported to play a major role in its oncogenesis. No previous studies have been done in Uganda to establish the histologic subtypes of penile squamous cell carcinoma (pSCC), p53 or p16INK4a status and their relationships to the histologic subtypes of pSCC.

Objectives: To evaluate the histologic subtypes, the p53 and p16-INK4a expression in archived pSCC formalin fixed paraffin embedded blocks in a public laboratory from 2009 to 2020. P16 expression is used a surrogate for high risk human papilloma virus (HR HPV).

Methods: 87 tissue specimens previously histologically diagnosed with penile squamous cell carcinoma from 2009 to 2020 were identified. The specimens were examined under H&E stain to establish their histologic subtypes and their p53 and p16INK4a was status established by immunohistochemistry.

Results: Of the 87 specimens, the conventional subtype made up the majority of the specimens with 68(78.1%) specimens followed by the basaloid subtype 7(8.0%), papillary subtype 4(4.6%), warty subtype 3(3.4%) and adenosquamous subtype 2(2.3%). The prevalence of p16INK4a and p53 positivity was 57.5% and 41.4% respectively. There was no significant difference in p16INK4a or p53 staining within the individual histologic subtypes of pSCC.

Conclusion: The most common histologic subtypes of pSCC present were the keratinizing and basaloid subtypes. The prevalence of p16INK4a and p53 positive pSCC specimens is high and there was no association between p16INK4a, p53 status and the histologic subtypes of pSCC. The distribution of p16 expressing pSCC (57.5%) is not significantly greater than that reported in a global metanalysis (49%) suggesting that the high incidence may be due to other factors as well as the prevalence of HR-HPV..

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P32

Histopathologic reporting of penile cancer, PeIN and use of p16 immunohistochemistry

Background: Penile intraepithelial neoplasia(PeIN) and invasive squamous carcinoma are classified into HPV-associated and HPV-independent tumours. In a survey conducted by the ISUP (International Society of Urological Pathology) in 2020, 80% of the respondents reported they use p16 immunohistochemistry (IHC) to differentiate HPV-associated from HPV-independent PeIN and invasive squamous carcinoma. ISUP recommends the use of p16 IHC to differentiate pleomorphic variant of PeIN from HPV-associated PeIN. It also recommended the use of p16 IHC in cases where the tumoral histologic features of the SCCs are not classical for HPV-associated tumours, especially if poorly differentiated.

Purpose: We performed an audit to evaluate our use of p16 immunohistochemistry in penile pathology and our reporting practice.

Methods: All samples processed at our department labelled as penile/foreskin in the period from 01/01/2019 -- 31/12/2020 (2 years) were assessed (260 cases).

Results: There were 191 foreskin specimens and 69 glans specimens. These included 59 biopsies and 201 excisions and resections. p16 IHC was used in 50 cases. 23 cases showed presence of abnormal expression (positive), 26 cases showed absence of abnormal staining (negative) and 1 equivocal result. 34/260 cases were diagnosed as PeIN (23 undifferentiated and 11 differentiated). 20/260 cases were diagnosed as invasive squamous carcinoma (12 NST, 6 keratinising, 1 basaloid and 1 pseudohyperplastic). In the squamous carcinoma cases 5 were well differentiated, 13 moderately differentiated, and 2 poorly differentiated. 6 cases were staged as pT1, 4 cases as pT1a, 3 cases as pT2 and 2 cases as pT3. Perineural infiltration was seen in 5 cases and 1 case showed lymphovascular invasion. p16 IHC was used in 18/20 SCC diagnoses.

Conclusion: p16 IHC is a useful adjunct in the diagnoses of PeIN and penile squamous carcinoma. In our department we now recommend the use of p16 IHC in all cases of penile invasive SCC and PeIN.

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P33

A comparative study of the role of ATM mutations on the radiobiological and immune response in Metastatic Castration Resistant Prostate Cancer treated with X-rays or Radium-223

Purpose of Study:
Metastatic castration-resistant prostate cancer (mCRPC) is an aggressive and incurable disease with a 30% 5-year survival. Approximately 25% of mCRPC patients possess mutations in DNA damage repair (DDR) genes, with ATM mutations making up 5% of total incidences. These patients present with aggressive phenotypes and worse prognoses than patients without mutations. 223Ra is the only radiopharmaceutical showing efficacy in reducing symptomatic skeletal metastases whilst prolonging overall survival. These benefits are more significant in men who harbour DDR mutations. Evidence suggests DDR deficiencies also impact the immune response. Observations found irradiation to have significant effects on the cGAS/STING pathway. Despite radiotherapy's importance in prostate cancer (PCa) treatment, some patients' disease will become resistant and metastasise. There is urgent need for safer and more effective treatment.

Methods:
Human PCa cells, DU145, PC3, 22Rv1 and LnCaP ATM isogenic CRISPR/Cas9 were used. The cells were exposed to 225kVp X-rays, with doses ranging from 0.5Gy-8Gy at a dose rate of 0.57Gy/min; or exposed to 223Ra for 24 hours, with concentrations from 0.1-0.5mBq/ml. Clonogenic survival assay, flow cytometry and DNA damage were used for radiobiological assessment. Western blots, polymerase chain reaction, and immunofluorescence staining for PD-L1 were used in immunological assessments. Two-tailed Student's t-test was used for statistical analysis.

Summary of Results:
223Ra showed greater radiosensitivity, damage and G2/M cell cycle arrest than X-rays in ATM-deficient cells. 223Ra upregulated STING-driven inflammatory genes and PD-L1 in ATM-deficient cell lines.

Conclusion:
223Ra has significant impacts on the radiobiological response in ATM-deficient PCa cells. Observations of an interplay between ATM deficiency and 223Ra are novel. The study provides a rationale for 223Ra to be used as a therapeutic approach for mCRPC patients who harbour ATM mutations

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P34

Discrepancy rates in reporting uropathology specimen in referral cases

Purpose of the study: To identify and classify discrepancies in regional MDT Urology referral specimens. Methods: All external referral cases of uropathology specimens for MDT discussion in the calendar year 2018 were retrieved from the computer system and the final report was compared with the original report. Cases with discrepancies were further subcategorised into groups and any discordant cases were studied in detail, using the internal audit trail and as per the system proposed by the RCPath. The relevant RCPath datasets were taken as standards for reporting specimens. Slides were not reviewed further. Opinion cases and testicular supra-regional MDT cases were excluded from the study. Summary of results: A total of 223 bladders, 477 prostate, 23 kidney, and 15 cytology/metastatic tumour cases were studied. The major discrepancy cases included two bladder cases which were downgraded from sarcomatoid carcinoma and CIS respectively to florid reactive changes. One case of prostate reported as 3+4=7 was florid basal cell hyperplasia on review. There were no major discrepancies in reporting of the kidney and cytology/metastatic tumour category. The overall histological review resulted in a major discrepancy in 0.4% of cases, a minor discrepancy in the primary report in 34.6% of cases, and total agreement in 65% of cases. Conclusions: In our opinion, there is no cause for concern about the standard of practice in the uropathology team within the region. All of the discrepancies noted are not unexpected in clinical practice when cases are referred to a specialist centre for review. There was no harm to patients, as any resultant modifications to treatment or follow-up were discussed at the MDT meetings. Review of cases at MDT is a valuable tool for quality control in diagnostic pathology and can yield valuable information regarding departmental diagnostic performance. The information generated can be used to improve team performance and patient management.

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P35

Histopathologic reporting of penile cancer, PeIN and use of p16 immunohistochemistry

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Conclusion: p16 IHC is a useful adjunct in the diagnoses of PeIN and penile squamous carcinoma. In our department we now recommend the use of p16 IHC in all cases of penile invasive SCC and PeIN.

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P36

Crescentic Glomerulonephritis Due To Amyloid; A Case Report

Renal amyloidosis usually presents with nephrotic-range proteinuria. Other presentations are rare and amyloid is not usually suspected as a cause of crescentic GN presenting as RPGN.

We present a case of a 64 year old man with a 40 year history of inherited cutaneous amyloid, who presented with progressive severe acute kidney injury, nephrotic syndrome and microscopic haematuria. Initial serologic testing for causes of rapidly progressive glomerulonephritis were negative. Renal biopsy demonstrated the presence of 90% active cellular crescents with Congo red positive staining in glomeruli on light microscopy and amyloid fibrils in glomerular basement membrane on electron microscopy.

Further investigations demonstrated 10-fold excess of serum lamda light chains and 11% plasma cell infiltration on bone marrow aspirate. Serum amyloid P-component (SAP) scan confirmed amyloid deposit limited to the kidneys.

He was initially treated with high dose prednisolone and then Velcade, cyclophosphamide and dexamethasone. Renal function continued to deteriorate and he was commenced on haemodialysis four months after initial presentation.

Only 5 previous cases of renal amyloid presenting as RPGN and crescentic GN have been reported; although retrospective review of amyloid biopsies and PMs suggest that crescents can be found in 1 to 13% of amyloid cases. Although the specific type of amyloid in the kidney in our case has not been confirmed the association of cutaneous amyloid with crescentic GN appears to be unique.

In previous cases the crescentic GN is postulated to be due to amyloid infiltration of the GBM resulting in rupture of the membrane and serum exudation into the urinary space. In this case amyloid fibres are seen traversing the GBM, supporting this aetiology.

Our case illustrates that renal amyloidosis can present as rapidly progressive progressive glomerulonephritis with crescent formation. This needs to be considered in crescentic GN without serological markers of vasculitis.

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P37

Expression of p73 isoforms in normal and tumour tissue

p73 belongs to the p53 family of transcription factors but, unlike p53, p73 is rarely mutated in cancer. Transcription from two distinct promoters in the *TP73* gene in combination with alternative splicing gives rise to several p73 isoforms that have distinct functional properties. We have produced and characterized four novel isoform-specific p73 antibodies; mouse monoclonals to TAp73, ΔNp73, and p73α, and rabbit polyclonal to p73α, recognizing the N-terminal regions of TAp73 or ΔNp73, or the C-terminal region of p73α isoforms. These reagents have been assessed for cross-reactivity with p53 and p63 isoforms and immunohistochemistry in normal tissues and tumours. TAp73 is restricted to the nuclei of multiciliated cells in normal tissues, suggesting that it may act as a marker of differentiation in tumours such as endometrial and ovarian carcinomas. p73α is seen in the most basal undifferentiated cells in squamous and in urothelial epithelia. That basal squamous cells are strongly positive for p73α but not for either TAp73 or ΔNp73 indicates the presence of other N-terminally truncated isoforms in these tissues. These well-characterized mono-specific antibodies will be useful for functional studies of p73 and its interactions with the p53 family of transcription factors, and may have prognostic value in specific human pathologies. This work was supported by grants CZ.02.1.01/0.0/0.0/16_019/0000868 and MMCI 00209805.

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P38

Histopathology reporting of temporal artery biopsy specimens for giant cell arteritis: results of a modified Delphi study

Purpose of the study: The temporal artery biopsy (TAB) is regarded as the gold standard test in the diagnosis of giant cell arteritis (GCA). Emerging evidence highlights that there is a lack of agreement amongst experienced pathologists on the diagnostic features observed in TAB sections for the diagnosis of GCA. The aim of this research study was to reach consensus on the key parameters for the reporting of TAB specimens to be included in a standardised reporting proforma. Methods: A modified Delphi process, comprising 3 survey rounds and 3 virtual consensus group meetings, was undertaken to reach consensus by 13 UK-based consultants. Statements were formulated following a comprehensive literature review and sent to participants as an online survey. Participants were required to rate their level of agreement with each statement on a nine-point Likert scale. Consensus was defined a priori as an agreement of ≥70% and individual feedback was provided after each round, together with data on the distribution of group responses. Summary of results: Overall, 67 statements reached consensus and 17 statements did not. The participant response rate was 100% (13/13) for all 3 rounds. The expert participants agreed upon the key factors pertaining to clinical information, specimen handling and microscopic pathological features for the reporting of TAB specimens. Consensus was achieved on all the microscopic features that should be included in the reporting of TABs for the diagnosis of GCA. Areas for future research were identified, such as if, and how, the histological patterns of GCA correlate with various clinical parameters, and how rapidly steroid therapy influences microscopic findings. Conclusions: We aimed to identify which clinical and histological parameters are of diagnostic value for the reporting of TABs for GCA diagnosis. By means of a Delphi study, consensus was reached by an expert panel on the key items to be included in a standardised reporting protocol.

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P39 *

Striatal neuron changes in post-stroke and other age-related dementias

Purpose of study Dementia has a significant effect on memory and daily activities, with a nine-fold increased risk in stroke survivors. Striatum plays a key role in the above functions but has yet to be explored in dementia patients. Hence this study was performed to evaluate changes in the neuronal density and neuronal volume in caudate, putamen and striatum and their relationship with age, brain weight, cognitive score (MMSE and CamCOG), and vascular pathology score.

Methods Thirty-eight patients were included in this study and were controlled for age, gender and brain weight. Significant differences were only seen in cognitive scores and vascular pathology scores. 3D stereo-investigator was used to assess the density of neurons and neuronal volume in the caudate, putamen and striatum.

Summary of results No significant difference was observed between the density of neurons in the 3 brain regions($p=0.775$) and within each group($p>0.05$). There was a significantly lower neuronal density in the caudate nucleus than in putamen in PSD ($p=0.024$). Significantly higher neuronal volume in dementia cases was observed in all 3 regions of the brain($p<0.05$). Correlation between neuronal volume and density was only significant in the striatum($r=0.345, p=0.02$). Positive correlation was observed in post-stroke non-demented putamen between neuronal volume and cognitive scores: MMSE($r=0.889, p=0.01$) and CamCOG($r=0.714$, $P=0.031$). A significantly negative correlation was observed between putamen neuronal density in PSND and vascular pathology score ($p=0.009$). No correlation was observed between density and neuronal volume with age and brain weight across the 3 brain regions.

onclusion Patients with dementia were observed to have enlarged neurons and a possible reduction in the density of neurons, which might have relations to the changes in cognitive function.

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P40 *

DNA damage response is altered in arginosuccinate synthetase negative glioblastoma upon arginine depletion therapy, potentiating current standard of care

Glioblastoma (GBM) is considered a cancer of unmet clinical need due to it being the most prevalent and aggressive primary brain malignancy. Current median overall survival is 15 months despite standard of care (SOC) comprising of maximal safe resection, temozolomide and radiotherapy. A certain subset of GBM exhibits a unique metabolic vulnerability where 30% lack transcriptional expression of arginosuccinate synthetase 1 (ASS1). These 'ASS1 negative' tumours, despite being more aggressive, become auxotrophic to arginine, rendering them susceptible to ADI-PEG20 which hydrolyses arginine to citrulline peripherally in the blood. This removes the requirement for blood brain barrier passage. We show that this increased aggressiveness may partly be due to an enhanced DNA repair response following radiotherapeutic damage. Increased expression of *CHEK1* was seen in these ASS1 negative tumours. With phenotypic and qPCR studies, we observed that treatment with ADI-PEG20 significantly decreased proliferation and altered expression of key DNA repair genes. Downregulation of *PCNA, PARP1, ATR* and *CHEK1* was apparent following treatment. Immunohistochemical analysis of intracranial human GBM xenografts for PCNA protein of saline and ADI-PEG20 treated mouse models revealed 10% decreased median positive cell staining in ADI-PEG20 treated sections. Since DNA repair gene upregulation correlates with treatment resistance, DNA damage response inhibition can be used to overcome this issue and potentiate current therapy. Our results help elucidate the mechanistic rationale for the use of ADI-PEG20 in a combinatory approach with SOC, particularly helping explain its role in radiosensitisation. Moreover, this provides the opportunity to investigate the use of DNA damage response inhibitors alongside the pleotropic effects of arginine depletion as a therapeutic strategy for this devastating malignancy.

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P41

Determining immunoglobulin G subclass profiles of tertiary lymphoid structures in a post-mortem multiple sclerosis case

Purpose of study: Multiple Sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS). The relative grade of meningeal inflammation and the presence of tertiary-lymphoid structures (TLS) in MS is associated with a more severe and progressive disease course. The immune cell composition of TLS in MS is unclear and it is not known whether the immune profiles of TLS are distinct in different CNS compartments that harbour inflammatory cells. This study aims to characterise the immunoglobulin profiles of TLS and other CNS inflammatory areas in post-mortem brain tissue. Methods: Using formalin-fixed paraffin-embedded post-mortem tissue from the Dame Ingrid Allen Tissue Collection at Queen's University Belfast, in-depth molecular profiling of TLS and other CNS inflammatory compartments was undertaken. Nanostring digital spatial profiling whole transcriptome analysis was performed on 2 MS cases harbouring TLS. Selected RNA candidates were subsequently validated using the RNAscope multiplex assay. Immunohistochemical (IHC) staining of IgG4 and IgG3 was undertaken to determine if there is a distinction between these IgG subclasses in different inflammatory CNS compartments. Summary of results: Highest expressed genes in TLS were IGHG4 and IGHG3. IHC analysis revealed that relative to other meningeal inflammatory sites there was a high expression of IgG4 within TLS. No IgG4 detection was observed in grey or white matter perivascular spaces. IgG4+ cells did not co-localise with CD138+ plasma cells which represented 10% of cells within TLS. Conclusions: These findings demonstrate that TLS may have unique immune signatures in comparison to other areas of compartmentalised inflammation in MS. Determining the immune signatures of TLS and if there are distinctions between other inflammatory CNS spaces will be critical to increase our understanding of pathological mechanisms in progressive disease.

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P42

Visualising microglial cell apoptosis using DARC (Detection of Apoptosing Retinal Cells)

Purpose of study: DARC (Detection of Apoptosing Retinal Cells) is a non-invasive diagnostic technology developed in our laboratory that enables the detection of apoptotic cells using a fluorescently labelled modified form of Annexin. It has previously been demonstrated that DARC can identify unhealthy cells in neurodegenerative disease models including those of glaucoma, AMD, Parkinson's disease, and Alzheimer's disease *in vivo*. As many recent papers increasingly indicate that microglial cells have an important role in neurodegenerative diseases, the aim of this study was to assess whether DARC technology could also be used to visualise the apoptosis of microglia *in vitro*.

Methods: The rat retinal precursor cell line R28 was used for its high degree of reproducibility and cellular makeup, which has been observed in the Cordeiro lab to include retinal ganglion cells and microglia. Cells were grown to 80% confluency in a 96-well plate with DMEM with Pen-Strep, at which point 0.5 mM of hydrogen peroxide was added as an insult and incubated for 24 hours. DARC labelled with DyLight 488 (ANX488) was added to each well after 22 hours and incubated for the remaining 2 hours. To visualise microglia and retinal ganglion cells, the R28 cells were stained using Iba-1 (ionised calcium-binding adaptor molecule, a microglial marker) and RBPMS (an RNA-binding protein specific to retinal ganglion cells) which were both independently labelled with fluorescent secondary antibodies.

Summary of results: Fluorescent microscopy identified Iba-1 suggesting microglial expression in R28 cells. DARC performed as expected, with DARC signal exclusively, and in high proportions only in cells that received 0.5 mM of hydrogen peroxide exposure. Areas where only Iba-1 and DARC spots were colocalised with no contribution from RBPMS were found.

Conclusions: DARC technology can be used as a biomarker not only for detecting the apoptosis of neuronal cells but also microglial cells.

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P43

Investigating the role of the phosphatase DUSP4 in uveal melanoma

Purpose:
Dual-specificity phosphatase 4 (DUSP4) is a member of a subfamily of phosphatases which inactivate target kinases by dephosphorylating phosphoserine/threonine/phosphotyrosine residues. In uveal melanoma (UM), DUSP4 may be involved in regulating the mitogen-activated protein kinase (MAPK) signalling cascade; activated in >90% UM by mutations in upstream G-protein alpha subunits GNAQ/11. This study examined whether DUSP4 protein expression in primary UM (pUM) was a biomarker of metastatic risk, and whether DUSP4 knockdown sensitises UM cells to, selumetinib or doxorubicin, as in other cancers.

Methods:
DUSP4 mRNA data from The Cancer Genome Atlas and DUSP4 protein expression examined using immunohistochemistry in 28 pUM were examined for association with clinical, genetic and histological features. In-vitro cytotoxic drug assays tested the efficacy of selumetinib and doxorubicin in a panel of UM cell lines with/without siRNA DUSP4 gene silencing.

Results:
DUSP4 protein was observed in >90% of cases, with strong nuclear positivity in 79%. Despite higher DUSP4 mRNA levels in disomy 3/wild type BAP1 UM, there was no significant association of nuclear DUSP4 (nDUSP4) protein with metastatic risk predictors or outcome. Normal choroidal melanocytes present in 14/14 tumour eyes with areas of non-involved choroid distant from the tumour, were negative for nDUSP4. DUSP4 expression was observed in all 5 cell lines examined although levels varied. DUSP4 silencing in the GNAQ/11 mutant cell lines Mel202, MP46, MP41 cells did not affect ERK1/2 or phospho-ERK levels but increased the levels in the GNAQ/11 wild type cell line, Mel285. None of the cell lines showed enhanced sensitivity to selumetinib/doxorubicin.

Conclusions:
DUSP4 protein expression is not a biomarker of UM metastatic risk. DUSP4 plays a complex role in oncogenesis as reported in other cancers and further work is required to fully understand its functional role in the MAPK pathway in this tumour.

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P44

Identification of Educational Training Needs in Whole Viral Genomic Sequencing in Asia, Africa, and Latin America.

Whole Viral Genome sequencing is an essential tool to identify and track the emergence and spread of viruses such as SARS-CoV-2 variants as the COVID-19 pandemic progresses. Sequence data informs public health interventions and the further development of diagnostics, therapeutics and vaccines. However, the existing inequity of access to pathogen sequencing worldwide warrants identification of training and educational needs within genomic sequencing. We conducted qualitative analysis of key focus groups with stakeholders across Asia, Africa and Latin America. This work was performed in collaboration with COG-Train, an international educational initiative providing open-access learning in SARS-CoV-2 genomics, developed jointly by COG-UK consortium and Wellcome Connecting Science (WCS). We present the results summarising key training needs in each region and the education gaps identified by stakeholders. These critically include translationality of data into policy; generation of online accessible analytical resources; and cross-disciplinary skills training. Our work contributes towards efforts to close the inequity gap of global genomic sequencing, enhance international SARS-CoV-2 sequencing and surveillance, as well as prepare for the rapid adaptive response to future potential pathogens that arise.

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P45

Development of a novel educational theoretical framework for approaching histopathology case microscopy work, with discussion of theoretical considerations for optimising the student learning environment in this.

Purpose of the study Microscopic examination of specimens is central to tissue diagnosis. Few studies exist examining how expert pathologists approach, and how novices learn, microscopy. We take a theory informed approach to describe how pathologists might perform a microscopic examination. The aim was to create a novel theoretical model for learning from microscopy, which can then be used to map trainee learning goals. Methods A standard approach to microscopy was developed from authors' experiences of working in histopathology. Selected educational theories were applied to this. These were then adapted into a working model of approach to microscopy and used to identify ways to optimise trainee learning. Summary of results Histopathologists examine microscopy slides at low then high power. At low power, they perform pattern recognition, and activation of illness scripts from schema, by scanning the slide to look for salient features. They perform a systematic examination of the slide at low and high power utilising type 2 thinking, through selective attention and active inhibition. This means they filter out normal histology and focus on the features which most influence the diagnosis. The histology is correlated with clinical information. These processes require a feedback loop where, if microscopy is not in keeping with clinical or previous pathological information, further review and reconsideration is undertaken. Trainees develop declarative and procedural knowledge about microscopy and common features of diseases. Trainers should consider how to convert this knowledge to longer-term memory while balancing cognitive load. Conclusions Approach to microscopy is often a subconscious process. By understanding this process, the learning environment can be optimised for trainee learning. This framework has potential to lead to theory informed research and development of multi-pronged educational initiatives for trainees/trainers.

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P46 *

QuPath Edu - Use of open-source digital microscopy in histology teaching for medical students

QuPath is open-source software that is widely used for digital pathology research (https://qupath.github.io/). Despite being designed primarily for viewing and analysing images, QuPath's open architecture and versatility mean that it can be adapted for other purposes, such as education. If such an analysis tool is used to teach, students can use the same setup for research projects in the future. Here, we describe how we have used QuPath as teaching tool at the University of Oulu Medical Faculty since 2018, training 800 medical and dentistry degree students to date. During this time, we have created a new toolkit called OpenMicroanatomy and a QuPath compatible open-source extension called QuPath Edu, that enhances the software and adds many new features designed for education.

QuPath Edu is a free teaching tool that can be used both by teachers and students for learning basic histology and histopathology as well as advanced image analysis. QuPath Edu supports a guided learning experience and facilitates practical groupwork. Teachers can create annotated guided tours around slides and incorporate both explanations about the structures and multiple-choice questions. QuPath Edu supports rich text, such as adding links, images, and videos in the description of the slide to provide additional information. QuPath Edu can be deployed on individual computers (from a hard drive), within a local network, or for remote use from a cloud service. When used on the cloud, all materials are also compatible with a lightweight browser-based image viewer, without the analysis tools provided by QuPath.

We have recently been awarded an Education Grant from the Pathological Society to support our ongoing work on QuPath Edu, and to ensure that it can be used by teachers and students at other institutions. We are working on making QuPath Edu easier to install and setup, improving existing features such as the guided tours, and adding new features for both researchers and students.

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P47

Introducing the Consortium for Analytic Standardisation in Immunohistochemistry

Immunohistochemistry has become the mainstay of clinical practice in the diagnostic histopathology setting in recent decades. Today there are a plethora of relevant IHC markers that are either specific or highly selective for some important diagnostic cellular components. There are important companion diagnostic IHC assays that can identify cancer patients that may respond to effective targeted therapies. Unlike with most other clinical assays, there are still no universally available validated reference standard controls for IHC testing centres to use. Without them there are risks of unseen errors creeping into every IHC testing laboratory across the world. False results can go unnoticed. During 2021, a group of experts in the field of pathology and immunohistochemistry led by Dr Steve Bogen, an eminent Biochemist in Boston, USA, came together to form the Consortium for Analytic Standardisation in Immunohistochemistry. The mission of the CASI team is to create validated reference standard controls for all the important biomarkers using specific proteins and microbeads. In addition, software is being developed to assist with reading the protein expression within these controls in a digital slide setting. This work is supported by a grant from the National Cancer Institute, in the USA. Some of these controls have already been approved by the FDA. Who is involved, how the work is being done and why these controls are required will be explained.

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P48

Working in partnership to implement genomic medicine into the cancer pathway

The evolution of genomic medicine has beneficially impacted the management of cancer. Genomics is now integral to the diagnosis and classification of some tumours as illustrated in the latest World Health Organisation tumour classifications. Also there has been a revolution in personalised medicine, with a move away from a "one size fits all" cancer treatment to targeting key genomic targets that drive the tumour. We have also seen an increase in testing for hereditary cancer disease where the underlying genomic abnormality not only has implications to family members but in some instances may also provide a treatment target. The 100,000 Genomes Project was a mammoth undertaking that has shown the value of interrogating the whole genome providing deeper insights into genomic drivers of cancer and in identifying treatment targets that would not have been available using existing standard of care (SOC). The use of whole genome sequencing (WGS) is no longer a research tool but has become an established SOC in certain clinical scenarios. The increasing role of genomic testing in the diagnosis and management of cancers has had a significant impact on Cellular Pathology. Histopathologists are central to delivering testing in this new genomic age. There is a requirement to optimise use of small samples to meet both the diagnostic challenges and clinical need. Furthermore, they need the knowledge to understand, interpret and integrate genomic findings into the Cellular Pathology report. In the cancer setting they are the interface between genomics and the clinical teams. NHS England is working with the Genomic Medicine Service Alliances and Pathology leads from the Genomic Laboratory Hubs (GLH) to identify areas of change, identify best practice and define the shape of Cellular Pathology for genomic testing.

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P49 *

Investigation of newly developed CD1d and TACI antibodies as novel markers for small B cell non-Hodgkin lymphoma subtypes.

Aims: The study aims to validate the diagnostic usefulness of CD1d and TACI proteins in small B-cell non-Hodgkin lymphoma diagnostics. Measuring expression may provide insights into the potential of these markers for development of targeted immunotherapies.

Background: CD1d is an antigen-presenting protein structurally related to MHC, binding lipid antigens for presentation to iNKT cells. CD1d expressed on mature B-cells indicates their ability to stimulate iNKT cells: in disease, this promotes iNKT-induced leukaemic B-cell death. TACI is a TNF receptor family surface protein, commonly expressed on transitional, memory, and active B-cells. Distinct proteins interact through TACI to regulate T-cell independent B-cell immune responses.

Methods: Single immunohistochemistry staining was used on routine biopsies. The use of newly developed primary antibodies against CD1d and TACI enabled immunoreactivity localisation of proteins within entities, with over 30 cases were stained for each protein.

Summary of results: Positive CD1d expression was seen in some small B-cell lymphoma, such as MZL. However, negative expression was consistent across CLL cases. MCL cases are of particular interest as mantle cells in tonsil controls were CD1d positive but negative in MCLs. TACI expression was found in almost all cases of MZL (nodal and extranodal) whereas this expression was more heterogeneous across MCL and CLL cases, varying from negative to, more rarely, strong positive.

Conclusions: The preliminary results suggest CD1d and TACI as potential novel biomarkers in subsets of small B cell lymphoma. Downregulation of CD1d is a consistent finding of CLL. Strong expression of CD1d and TACI in some lymphoma subtypes highlights directions amenable for iNKT cell-based immunotherapies and other cell-based immunotherapies, respectively.

Funded by a Pathological Society Grant under the JSPS Studentships.

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P50 *

CD85a, a myeloid cell checkpoint: an immunohistochemical study in samples of leukaemia and lymphoma.

Aim: To measure the expression of CD85a in a selection of lymphoproliferative disorders, with the hope it may provide useful diagnostic and immunotherapeutic results.

Background: CD85a, a leukocyte immunoglobulin-like receptor, is thought to be a myeloid cell checkpoint involved in inhibiting and controlling the immune response, via recruitment of SHP-1 and -2 (tyrosine phosphatases), disrupting the NF-κB pathway. However, much remains unknown about its function and role. It is highly expressed on macrophages and other myeloid cells, but there is uncertainty over its expression in lymphoproliferative disorders.

Methods: A CD85a monoclonal antibody was used for single immunohistochemistry staining in more than 30 cases of acute myeloid leukaemia (AML) and small B cell lymphoma. Anti-rat was the secondary stain. The stains were scanned, and images assessed for CD85a expression.

Summary of results: CD85a expression largely varied among cases of small B cell lymphoma. Expression was confined to macrophages, highlighting them either as scattered cells or small bundles of macrophagic collections. Chronic lymphocytic leukaemia, MALT lymphoma and Mantle cell lymphoma all showed heterogeneous macrophage content (CD85a positive) and the highest number of CD85a positive macrophages was found in Marginal zone lymphoma (MZL) cases. Similarly, in the cohort of AML including cases of low, moderate and poor risk (assessed by cytogenetic and molecular profile), expression of CD85a was heterogeneous, and localised to marrow macrophages, with the exception of 9/33 cases, in which a great proportion of the leukaemic cells were CD85a positive.

Conclusion: Increased expression of CD85a in MZL in resident-macrophages suggests a potential role of tumour-associated macrophages might be aid to immune evasion. The findings prompt further studies (i.e. multiplex immmunolabelling) to immune-profile the type of CD85a macrophages enriching the tumour microenvironment of MZL.

Funded by a PathSoc grant.

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P51

Thyroid gland adenoma with uncommon spindle cell morphology: A case report.

Background: Primary spindle cell neoplasms are rare in the thyroid gland and comprise spindle cell variants of papillary, follicular, medullary and anaplastic carcinoma, mesenchymal tumours and rarely spindle cell follicular adenoma (SCFA). Therefore, careful exclusion of common lesions is important. Method: Here, we describe a clinico-pathological analysis of an encapsulated thyroid nodule showing predominant spindle cell morphology. Results: A 38-year-old male presented with a right thyroid nodule that on ultrasound scan was reported as a U3/4 lesion. FNA was non-diagnostic. Diagnostic thyroid lobectomy was performed and there was a well-circumscribed nodule, 60 mm with variegated cut surface. Microscopy showed an encapsulated tumour composed predominantly of sheets of spindle cells with a focal area of colloid poor follicular architecture. Nuclear features of papillary carcinoma were not identified. The stroma was collagenous with hyalinised blood vessels. There was no evidence of capsular or vascular invasion. The lesional cells were positive for TTF1, Thyroglobulin and Cam 5.2, consistent with follicular and epithelial differentiation. The MIB 1 proliferation index was <5%. Conclusion: This case demonstrates the diversity in follicular thyroid neoplasms especially with unusual occurrence of spindle cell morphology. Differentiation from other conditions with similar morphology, such as medullary and anaplastic carcinoma and mesenchymal lesions is of paramount importance clinically. Use of ancillary investigations such as immunohistochemistry and molecular diagnostics would help in accurate diagnosis.

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P52

The role of different forensic injuries in disaster victim identification.

Introduction: Disaster victim identification (DVI) is an important process due to the legal and ethical issues involved. This process is also used in humanitarian and missing cases. There are 3 main types of trauma: ante-mortem, peri-mortem, and post-mortem. We attempt to understand their role in DVI as well as some challenges involved.

Methods: To understand more, we performed an advanced literature search using terms like forensic, anthropology, mortem, and disaster. We identified 136 eligible sources, but only 13 were included in the final report. Our inclusion criteria were: a full paper, peer-reviewed, in English, and be from between 2012-2021.

Results: We have found that the International Criminal Police Organisation (INTERPOL) has a specific DVI 4-step process which allows for the most accurate identification with high evidence threshold of DNA, fingerprints, and odontology. Step one involves scene examination, step two performs a post-mortem examination of the body to find any pathologies and devices, while taking biological and DNA samples, and performing various radiological examinations. The next stage compares found data with previous medical and ontology records, to find a match in the last stage. In some cases, the threshold is lowered due to a lack of evidence or specific circumstances. There have been cases where the match was only based on identical radiological scans or medical implants. Other methods can be used instead of an autopsy if children, religion or disfigurement are involved, such as craniofacial superimposition. Many economic, cultural, and ethical challenges affect DVI. Many areas of improvement have been identified like automated scans and lowering the DVI threshold.

Conclusion: DVI is a lengthy process which can be adapted depending on the circumstances and evidence. On some occasions, identification can only be made using radiology. This area still needs improvements and further research, but the ethics involved can be the main challenge.

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P53

Sudden Cardiac Death in Cardiac Transplant Recipients. Differences in post-transplant survival

Although advances have greatly improved long-term survival rates in cardiac transplant patients, sudden cardiac death (SCD) occurs in a considerable number of cases. While these cases are well defined in clinical studies, autopsy studies which can provide crucial insights into the cause of death are lacking. Cases referred to the CRY national cardiovascular pathology centre at St George's University London were prospectively added to our database. We extracted these and identified those of SCD in cardiac transplant recipients to identify the causes and outline the key pathological changes. All cases underwent systematic examination as per guidelines on autopsy practice including transverse dissection of the coronary arteries at 2 mm intervals. Measurements and macroscopic descriptions of the heart were entered into the CRY database at the time of dissection. 27 cardiac transplant cases were identified, of these 22 (82%) were male. The average age at transplant was 32.04 ± 15.4 years. The most frequent cause of death was cardiac allograft vasculopathy (CAV) (n=19, 70.4%). All female cardiac transplant recipients died of CAV (n=5) at an average age of 25.4 ± 8.9 years, and an average survival post-transplant of 3.5 ± 3.9 years. While males who died of CAV (n= 14) at an average age of 45.4 ± 17.9, had a significantly higher average survival post -- transplant of 12.4 ± 8.4 years (p=0.007**). Key pathological findings in CAV cases were concentric, diffuse thickening of the coronary arteries and microvasculature by intimal hyperplasia with lymphocytic intimitis. This resulted in fibrosis with lymphocytic infiltrates located around the vessels and throughout the myocardium. Our data agreed with the literature that males are more frequently develop CAV. However, we have shown that females with CAV had a significantly lower average survival time post- transplant which warrants further investigation. CAV is an important cause of SCD in cardiac transplant recipients.

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P54

A service evaluation of Post Mortem Computed Tomography (PMCT) Radiology reports in adults for non-forensic deaths

PMCT is an evolving area in autopsy practice and increasingly may act as an alternative to open autopsy. Currently there is no nation-wide standardised proforma for reporting PMCTs, thus the contents of reports can vary between radiologists. The purpose of this service evaluation is to review current PMCT reporting practice and highlight significant omissions, with a view to informing a structured reporting tool.

Methodology: 100 consecutive PMCT reports produced in November 2021 were analysed and compared. A standardised reporting proforma was designed, the contents of which were extrapolated from the literature and based on expert opinion.

Results: The tissues of the brain, heart and lungs were reported on consistently. The majority (>90%) commented on the skull, mediastinum, coronary artery calcification, skeletal system, abdominal and pelvic tissues. Areas that were less frequently commented upon included the large vessels, upper airway, cervical spine and skeletal frame and limb soft tissue. A cause of death was formally provided in 21% of reports, and in two cases this was judged to be inaccurate.

Conclusions: The findings demonstrate marked variability in the data provided in PMCT reports. There were common themes whereby certain tissues were consistently omitted. A significant proportion of reports formally stated a cause of death, which is arguably beyond the scope of the radiological examination. The cause of death should ideally be reserved for the pathologist conducting the external examination, taking into account the clinical history, PMCT and invasive autopsy findings. There may be confusion and potential delays to a coronial investigation should the cause of death provided by the pathologist differ from that in the PMCT report. A reporting tool with both structured headings and free text space would standardise PMCT reports and bring them in line with best practise.

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P55

A retrospective audit of adherence to national autopsy reporting guidelines in 137 autopsies conducted in the Leicester Royal Infirmary during 2020

There is variability in the structure of autopsy reports between hospital Trusts and histopathologists in the UK. The purpose of this study was to compare the extent to which autopsies conducted by a single Histopathology consultant in 2020 across 3 HMC judiciaries adhered to the Coroners Rules 1984 and the Royal College of Pathologists good autopsy practice guidelines.

140 autopsies were conducted by the Consultant Histopathologist between 7th January 2020 - 24th December 2020 and searched using iLaboratory, of which 137 were eligible for further analysis. Hospital post-mortems were excluded. Information from each case report regarding the clinical history, internal examination, external examination, and CT imaging were extracted manually, collated, and analysed using Microsoft Excel.

Age, date of birth, and sex were included in all cases (100%). Ethnicity (96%), weight (kg; 97%), height (cm; 95%), past medical history (96%), BMI (kg/cm2; 93%), and medication history (85%) were included most of the time, and anecdotally contained new information in addition to the coroner's case history from accompanying information from the HMC office. Social history (3%), occupational exposure (0%), and allergies (0%) were included less frequently.

Information easily obtained from the coroner's summary, by direct observation or by estimated measurements with support from the mortuary staff, was reported most frequently, and should be documented to provide a more coherent and convincing clinicopathological correlation. This is especially true when the additional information requested reveals compounding risk factors, particularly in the case of cardiovascular-related deaths, such as BMI, ethnicity, treatment effects, or genetic conditions, which may be useful to families. Together with any imaging correlates and internal examination features, detailed and standardised autopsy reports can be used as teaching tools for trainees and students.

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P56

This Abstract has been withdrawn

P57

Unicortical internal laryngeal fracturing in fatal applied neck pressure; the value of a detailed histological approach.

The application of significant pressure to the neck sufficient to cause death, can leave limited findings at autopsy. A 34 year old man was found in cardio-respiratory arrest after an argument had been heard. He was initially resuscitated but died after a period of intensive care management with no clinical explanation for the collapse becoming available.

There were no external marks of injury at autopsy. A moderate number of conjunctival and peri orbital petechial haemorrhages were evident along with a single undisplaced fracture to the left superior thyroid horn. The remainder of the larynx and the hyoid bone were entirely normal to inspection and palpation, with no bruising in the strap muscles. The remainder of the autopsy, histology of the organs and the toxicology revealed no relevant positive findings.

This paper presents the value of decalcification, transverse sectioning and complete histological processing using mega blocks of the larnyx, that was undertaken. Histology of the multiple sections revealed an undisplaced fracture to the contra lateral superior horn, along with a series of subtle unicortical buckling - type fractures to the internal surfaces of both sides of the cricoid cartilage, the anterior aspect of the prominence of the thyroid carlilage and to the posterior third of the thyroid lamina, with bleeding, fibrin deposition and an early neutrophilic infiltrate.

Significant specialist histopathology technical laboratory time was required to optimise the material for the histological assessment which documented a further five laryngeal fractures which provided clear pathological evidence of forceful neck compression likely over a broad area of the neck. An individual subsequently admitted placing the subject in a neck/head lock precipitating the cardiac arrest.

Pathologists should consider this detailed approach to examination of the larynx in selected potentially suspicious deaths where fatal subtle neck pressure may have occurred,

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P58

Autopsy by imaging: the last 10 years (a review)

Virtual autopsies (VAs) are non-invasive, bypassing many of the challenges posed by traditional autopsies (TAs). This is a literature review about the sensitivity of the main VA techniques: post mortem (PM) computed tomography (PMCT) and PM magnetic resonance (PMMR). This could help to identify the most appropriate uses for VA, and where future research should focus. A review was performed, searching for literature from the last 10 years regarding how sensitive VA is at detecting common lesions that could cause or contribute to death. 33 studies were included. There was strong agreement that PMCT had strengths in detecting: free air; fractures; large fluid accumulations; and calcifications. PMCT's weaknesses included missing: pulmonary emboli; myocardial infarctions; and visceral/soft tissue lesions. The strengths of PMMR were less widely agreed, but included detecting: large fluid collections; myocardial infarctions; and visceral/soft tissue lesions. There were no wide agreements on PMMR's weaknesses due to a lack of literature. Therefore, VA is a useful adjunct to TA; however, its drawbacks in reliably detecting common causes of death restrict its ability to fully replace TA. Novel imaging techniques are being developed in order to bridge the current gaps of VA, and make autopsies even less invasive.

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P59

A retrospective audit of the utility of non-invasive Computed Tomography in determining the causes of death in 137 autopsies conducted in the Leicester Royal Infirmary during 2020

Using Computed tomography (CT) imaging for autopsies is a growing area of histopathology. The purpose of this study was to evaluate the utility of CT for non-invasive post-mortems using the audit recommendations in the Royal College of Pathologists 2011 Guidelines for post-mortem cross-sectional imaging in adults for non-forensic deaths.

140 autopsies were conducted by the Consultant Histopathologist between 7th January 2020 - 24th December 2020 and searched using iLaboratory, of which 137 were eligible for further analysis. Hospital post-mortems were excluded. Information from each case regarding the clinical history, internal examination, external examination, and CT imaging reports were extracted manually, collated, and analysed using Microsoft Excel. In addition, the percentage of cases triaged to post-mortem CT and the proportion of these cases that required an invasive procedure following imaging were assessed.

67% (92 cases) of all post-mortems conducted in 2020 were triaged for CT imaging. CT was sufficient to determine the likely cause of death in 96% (88 cases) of these reported autopsies, most of which were due to cardiovascular disease. Only 4 cases required a limited post-mortem after CT imaging to obtain samples for histology, toxicology, and virology. These deaths were attributed to coronary artery thrombus, hypertensive heart disease, alcohol misuse and pregabalin intoxication, and required additional tests to support the likely cause of death.

Some pathophysiology cannot be detected by imaging alone and may warrant invasive post-mortems or magnetic resonance imaging to determine the cause of death. However, this study highlights the utility of CT for this purpose, especially in cardiovascular-related deaths, with features visible on plain cross-sectional imaging such as vascular narrowing, calcifications, and atheroma. Increasing usage of non-invasive CT imaging during the pandemic helped protect mortuary staff from the risk of exposure to COVID-19.

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P60

Medico-legal importance of defined tyre impression marking, from lifted superficial skin components on the internal aspect of clothing material, in fatal pedestrian road traffic collisions

A detailed autopsy approach is needed to optimise the investigation of fatal pedestrian road traffic collisions, to determine, where possible, the likely dynamics of the incident. This includes documenting any primary vehicular (bumper-type) contact injury to the legs, and evidence that the individual was run over, potentially whilst already positioned on the ground. Marks that can help identify the vehicle involved are especially valuable.

Whilst patterned marks can be found on the skin surface, these are uncommon and may be indistinct and/or obscured by abrasion and developing bruising, particularly if there is a significant post incident survival period, or if the clothing is thick. The value of identifying any vehicular fragments such as paint and glass on the clothing surface is well known, but the value of assessing the internal material surface does not appear to be reported in the literature.

This report concerns a middle-aged female who died after sustaining multiple injuries in a collision with an agricultural vehicle. The external surface of her denim jeans showed no helpful marks, but the internal surface revealed a very well defined and preserved large vehicle tyre impression mark, that was photographed (with angled scale) for forensic comparison work. The mark was formed by lifted superficial skin components that had been crushed against the clothing material. Other areas of the internal surface of the jeans showed similarly formed marks left by forceful impaction with the road surface. Similar but smaller areas of internal surface markings were also found in a subsequent autopsy case where the trousers were of tracksuit material rather than denim.

It is therefore recommended that the clothing should, whenever feasible, be recovered and inspected carefully, including internally, as an important part of the post mortem investigation of pedestrian vehicular fatalities.

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P61

The Utility of Toxicology in Coronial Post Mortem Examinations

Purpose of the study Toxicological analysis of post mortem samples is often imperative to determine the cause of death. The aim of this audit was to assess the utility of toxicology in a coronial post mortem service in a Model 3 hospital in Ireland, where toxicological analysis was performed on in 82% (170) of cases.

Methods A retrospective audit was conducted of coronial post mortem reports (207) issued in 2020, with detailed assessment of the toxicology reports.

Summary of results In our cohort 21% (36/170) of the cases yielded negative toxicology results, while in an additional 14% (24/170) only 'over-the-counter' drugs were identified in non-toxic concentration. In 19% (36/170) of deaths use of illicit drugs or excess alcohol consumption was the direct cause of death, while foreign substances played a strong contributory role in the cause of death in an additional 7%). The female:male ratio of the deceased was 1:1.85, while it was slightly lower: 1:1.67 in fatalities associated with substance misuse. The mean age of death was 60.3 years, compared to 44.1 years in the cohort succumbed to substance abuse.

Conclusions In 38% (19/50) of deaths associated with foreign substances, there was neither history of misuse nor raised suspicion by the police after investigation of the scene of death. Although there are no national guidelines available, recent US recommendations (Davis, 2014) offer guidance when to order toxicological tests based on history, scene of death, and autopsy findings. These help to minimise the unnecessary tests ordered, decrease costs and improve turnaround time on autopsy reports. However, our cohort demonstrates that consideration should be given to avail with toxicological tests in a wider variety of cases, especially in younger individuals, to avoid missing fatalities attributed to substance misuse, even when the history or circumstances of death does not raise the suspicion.

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P62

A retrospective audit into the effectiveness of FNA as a diagnostic tool in salivary gland tumours within the South West London Pathology group.

Fine needle aspiration (FNAc) plays a major role in the diagnostic pathway of salivary gland pathology, in both benign and malignant lesions and is the most effective and non-invasive investigative tool. The primary and differential diagnoses in cytopathological review of salivary gland FNAc is also central to risk stratifying and managing patients with salivary gland neoplasms. In this audit, the objective was to identify the trends and challenges in FNA diagnoses of salivary cytopathology.
A total of 218 cases with FNAc were retrieved that had subsequent histology resection specimens and the data were collated using the formal cyto and histopathology reports. The list of differential diagnoses generated from FNAc were compared to the histological findings, in addition to reviewing whether or not the definitive histological diagnosis was mentioned in the primary, or differential diagnoses generated from the FNA reports.
In summary, the findings demonstrated the specificity for FNA in this cohort was 96.9% whilst sensitivity was 78.6%. A total of 17 cases were identified with discrepancies and both the cytology and histology were re-reviewed. The main diagnostic challenges observed were regards to Warthins tumours misdiagnosed as mucoepidermoid carcinomas, underdiagnosis of in-situ carcinoma ex-pleomorphic adenoma and metastatic malignancies being diagnosed as primary malignant salivary neoplasms. Additionally, the subtyping of the salivary gland malignancies on FNAc alone were observed to be challenging, with 34 of the 56 malignant cases being offered differential diagnoses as opposed to 149 of the 162 of benign cases being offered a definitive diagnosis on FNAc.
This audit identified that the reporting strategies used in our centre are largely in keeping with national standards; the challenges of salivary FNAc are multi-fold and are dependent on the radiological expertise, adequate sampling as well as thorough understanding of the salivary pathology.

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P63 *

Quantification of PD-L1 in normal, potentially malignant and malignant oropharyngeal tissues using image analysis

Purpose of the Study Oropharyngeal cancers develop mainly as squamous cell carcinoma (OPSCC) and have variable prognosis with HPV-associated OPSCC having a significantly better prognosis. Anti-PD-L1 immunotherapy has shown promise for oropharyngeal cancer patients. However, the response to this treatment depends on PD-L1 level in the tumour microenvironment. The aim of this study was to quantify PD-L1 expression in normal, premalignant, and malignant oropharyngeal samples using image analysis. Methods Samples of normal, dysplasia, HPV+ and HPV- OPSCC were obtained from NHS Grampian Biorepository and underwent immunohistochemistry staining to detect PDL-1. Whole-slide scans were obtained and analysed using QuPath and ImageJ to detect positive cells. Results Our data showed that the mean percentage of PD-L1 positive cells in the tissue sections of HPV+ tumours and normal tissues were comparable but significantly lower than HPV- tumours. Additionally, the percentage of PD-L1-positive cells was increased in dysplasia samples, but the difference was not significant. Together, these results indicate that the expression of the immune checkpoint PD-L1 is significantly higher in HPV- cancers compared to HPV+ cancer samples. Due to the biological role of PD-L1 as an inhibitor of cytotoxic T cell responses, this would lead to a significantly more immunosuppressive tumour microenvironment in HPV- OPSCC, which correlates with their worse prognosis. This carries significant potential for clinical applications since such PD-L1-expressing tumours could be more susceptible to treatment with PD-L1-targeting immune checkpoint inhibitors. In addition, the increased PD-L1 expression in pre-malignant lesions indicates its potential role as a marker of malignant changes. Conclusion These results indicate the important role of PD-L1 in oropharyngeal carcinogenesis and support additional clinical research into PD-L1 inhibitors in these diseases. Supported by Path Soc Undergraduate Elective Bursary

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P64

Are Tertiary Lymphoid Structures Reliable Biomarkers in Squamous Cell Carcinomas of the Head and Neck?

Purpose of the study

Tertiary Lymphoid Structures (TLS) are ectopic lymphoid structures in inflamed or tumour tissues. The study aimed to determine whether TLS can be reliably identified using H&E stained slides and if TLS formation is influenced by anatomical differences in the head and neck.

Methods

The Northern Ireland Biobank (NIB21-008) provided access to diagnostic H&E stained slides and their digital images for patient cohorts representing 263 squamous cell carcinoma cases of oropharyngeal and 16 cases of lateral boarder of tongue (total number of H&E slides across sites n=326). All patient slides were initially reviewed using a light microscope for TLS. Digital annotation of the representative slide from each case was carried out using QuPath. Statistics were carried out using Microsoft Excel and Python to compare manual vs digital TLS detection, inter-observer digital vs digital TLS detection, and TLS detection across sites as well as across all slides for one patient vs assessment using a single representative slide.

Summary of results

Fisher's Exact test demonstrated robust detection of TLS between intra and inter-observer review ($p<0.0207$). The Intersection over Union test indicated high agreement in area of TLS annotations produced between independent digital annotators for TLS structures (0.957) highlighting spatial reproducibility. Differences in quantity (25.1% vs 6.25% of oropharyngeal and lateral tongue samples with at least one TLS present respectively) and no correlation of TLS results ($R_s=-0.12$) within patient samples indicates likelihood of site-specific bias for TLS quantification as a biomarker.

Conclusions

As a biomarker, TLS are robust and reproducible by manual and digital methods. However, whilst TLS were present and identifiable in oropharynx, there was limited evidence of their presence in the tongue. This study provides an early indication that assessment for TLS may benefit some head and neck cancers but only at specific sites.

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This Abstract has been withdrawn

P66 *

Exploring the Prognostic Significance of TERT Promotor Mutations in Clear Cell Chondrosarcoma

Purpose of Study: Chondrosarcoma (CS) is a rare malignant tumour showing chondroid differentiation. Cases are grouped into conventional and non-conventional types, with non-conventional CS consisting of clear cell (CCCS), mesenchymal and dedifferentiated CS. A recent paper (Zhang et al. 2021) correlated promotor mutations in telomerase reverse transcriptase (TERTp) with a higher grade and poorer prognosis in conventional CS. We therefore extended this research to non-conventional CS by investigating the prognostic impact of TERTp mutations on CCCS. *Methods:* We accessed 37 CCCS samples including 18 from formalin-fixed paraffin-embedded (FFPE) macrodissected tissue sections, 13 from FFPE blocks and 6 frozen samples. DNA extraction was carried out using QIAgen. DNA purity and concentration was measured using Nanodrop and Qubit. FFPE samples had low concentrations and purity while frozen samples had higher concentrations. Ethanol precipitation was conducted in cases of low DNA yield (26 of 37 samples), to improve purity and concentration. We carried out digital droplet PCR (ddPCR) to detect the canonical TERTp mutation in CCCS cases with wild-type, positive and no-template controls. *Results:* The positive control set a threshold of confidence which defined the number of called droplets >98 as high confidence results, 30-97 called droplets as low confidence and <30 as failed. CCCS cases were considered 'mutant-positive' when called droplets contained >5% mutant template. Using these criteria, 4/18 high and low confidence CCCS cases (22%) had mutant droplets, including 3/10 high confidence results (30%). *Conclusion:* These preliminary results do not implicate TERTp mutations as a significant marker of CCCS. However, DNA quality was poor for some of the samples and 9 of the failed or low confidence results can be repeated (with re-extraction).

We thank the Jean Shanks and Pathological Society for funding our studentships*; we learnt a great deal and had fun. We hope to bring this work to fruition.

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P67

Conventional Giant Cell Tumour of Bone and Osteoclast-Rich Tumours in Pagetic Bone Disease - genetically distinct entities

Purpose of the study
Paget disease of bone (PDB) is a metabolic disorder with a strong genetic component, characterised by pronounced disorganised bone remodelling. Complications of PDB include an increased risk of developing primary bone neoplasms, mainly osteosarcoma but not conventional giant cell tumour of bone (cGCTB) as defined by H3F3A/B mutations. Recently GCT-like lesions have been reported in patients affected by PDB with germline mutations in the zinc finger protein 687 (ZNF687) and PFN1 genes. Here we report on a 60-year-old man with known polyostotic PDB who presented a large iliac osteoclast-rich mass and later with a similar lesion at C4, both with features of malignancy on imaging.

Methods
We performed whole exome sequencing (WES) on tumour and matched normal tissue.

Summary of results
Histology showed a GCT-like lesion with osteoclasts much larger than usual, emperipolesis and an inconspicuous stromal component. WES revealed a germline heterozygous mutation in ZNF687 (Exon 6 -- c.2810C>G) in blood and in all tumour samples analysed from the ilium (n=4) and the C4 lesion (n=1). Our analysis did not reveal any cancer driver alterations, and crucially no H3F3A/B mutations, the hallmark of cGCTB. However, two intronic, non-pathogenic, somatic mutations were identified solely in the pelvic lesion, findings confirmed by ddPCR, implying an absence of a clonal relationship between the two anatomically distinct lesions.

Conclusions
We conclude that osteoclast-rich lesions in PDB are genetically distinct from cGCTB. Given the lack of driver alterations detected in osteoclast-rich lesions in PDB these are likely to represent a non-neoplastic exuberant form of PDB. As a direct result of this case, PDB patients in England have access to genetic testing for ZN687 germline mutations with immediate clinical impact. Early detection of those harbouring these mutations could potentially benefit from preventive treatment, by using osteoclast-blocking agents.

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P68 *

Recurrent *FOSL1* rearrangements in desmoplastic fibroblastoma.

Purpose of the study: The rare fibrous tumour desmoplastic fibroblastoma is characterised by overexpression of FOSL1. However, previous studies using cytogenetic and molecular techniques have not identified an underlying somatic change involving the *FOSL1* gene to explain this finding. Based on a novel finding in an index case, we sought to investigate the presence of *FOSL1* rearrangements in desmoplastic fibroblastoma.

Methods: Whole genome and targeted RNA sequencing was performed on our index patient as part of clinical care. We then investigated 15 desmoplastic fibroblastomas and 15 fibromas of tendon sheath using immunohistochemistry, in-situ hybridisation and targeted RNA sequencing.

Results: Rearrangements in *FOSL1* and *FOS* were identified in 10/15 and 2/15 desmoplastic fibroblastomas respectively, which mirror the pattern of *FOS* rearrangements observed in benign bone and vascular tumours. Fibroma of tendon sheath, which shares histological features with desmoplastic fibroblastoma, harboured *USP6* rearrangements in 9/15 cases, and did not demonstrate rearrangements in any of the four FOS genes. Overall concordance between FOSL1 immunohistochemistry and RNA sequencing results was 88%.

Conclusion: These findings illustrate that *FOSL1* and *FOS* rearrangements are a recurrent event in desmoplastic fibroblastoma, establishing this finding as a useful diagnostic adjunct and expanding the spectrum of tumours driven by FOS gene family alterations.
This work was supported by a Jean Shanks Foundation and Pathological Society Clinical PhD Fellowship to author SDN.

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P69*

TP53 Mutation in the Risk Stratification of Solitary Fibrous Tumour - a JSPS Path Soc Summer Studentship Project

Purpose of the study

Solitary Fibrous Tumour (SFT) is a rare connective tissue tumour characterised by fusion of the NAB2 and STAT6 genes. The clinical outcome of SFT can be difficult to predict even when employing the two risk stratification models published. TP53 mutations are reported to occur in SFT, but the correlation with clinical outcome is unclear. The most common TP53 mutations are concentrated in exons 5 to 8 encoding the DNA Binding Domain. Our study aims to establish if there is a correlation between TP53 mutations, clinical outcome and morphological features.

Methods

We acquired 343 SFT samples from the RNOH biobank, including frozen and formalin-fixed paraffin-embedded material. These were classified into high, intermediate, and low-risk groups on two models based on mitotic count, patient age, tumour size, and necrosis. We extracted DNA from the tumour samples, designed primer sets for the TP53 exons 5-8 (PCR products from 149-196 bp), and performed PCR and gel electrophoresis. PCR products were purified, underwent Sanger sequencing, and then analysed.

Results

TP53 mutations were sought in exon 7 from 12 SFT samples, including seven intermediate-risk, three high-risk and two mixed intermediate and high-risk tumours (models yielded different grades). Only one high and one intermediate-risk tumour harboured a pathogenic mutation. Some sequences were difficult to analyse, potentially due to contaminating non-tumour cells.

Conclusions

Once exons of interest (exons 5-8) are sequenced for all 343 samples, we will determine the clinical impact of TP53 mutations in SFTs. To improve the sequencing quality of samples, punch biopsy could be used to target tumour cells and reduce wild-type DNA.

We thank the Jean Shanks and Pathological Society for funding our studentships and providing us with this fantastic opportunity. Following university holidays, we are planning to finish the project, ultimately learning more about the disease.*

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P70

Giant cell tumour of bone -- integrated methylation and Copy number profiles in diagnostic setting

Purpose of the study: We present a small series of cases (n=3) from a referral centre for bone tumours where methylation and copy number profiles (CNP) are integrated in the diagnostic flow. Methods: 3 cases of Giant cell tumour of bone (GCTB) with confirmed H3F3A G34W mutation, arising in 2 females and 1 male, where malignancy entered the differential diagnosis were subjected to DNA methylation analysis based on the Infinium Human Epic Array (850k) platform (Illumina) from FFPE samples. Anatomic sites included femur (n=2) and 4th lumbar vertebra (n=1). Age of the patients was 31, 33, and 44 (median 33 years). Summary of results: In two out of three cases, the histopathological features and the clinic-radiological findings were contradictory, precluding a final robust diagnosis on histology alone. For both cases the methylation profile was able to assign the tumour to the GCT class (matching score 0.84 and 0.98031 respectively). A flat CNP allowed a conclusive diagnosis of a conventional GCTB in the first case, whereas the second one presented chromosomal aberrations underpinning a malignant transformation. The 3rd case represents a MGCTB with the methylation profile of a conventional osteosarcoma (matching score 0.93) and few long-range aberrations on CNP, which were less pronounced than what is expected in conventional osteosarcoma. The discrepancy between the methylation class and low amplitude of events, in addition to the H3F3A G34W mutation were consistent with the diagnosis of MGCTB. Conclusions: A small number of cases (n=3) selected from a bone tumour reference centre that integrates DNA methylation profiling in routine diagnostic flow are discussed. The information given by the CNP has the potential to predict aggressive behaviour in challenging diagnostic cases with direct impact on patient's management and care.

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Spatially resolving the dynamics of immunoediting in advanced colorectal cancer

Purpose: To establish the relationship between spatially resolved neoantigen burden and spatial heterogeneity of the tumour microenvironment (TME) in advanced colorectal cancer (CRC). *Methods:* Targeted sequencing (130x) of the immunopeptidome (genomic regions that generate neoantigens binding to the ten most common HLA alleles) was performed on 82 superficial, invasive and lymph node deposits of colorectal cancer (region of interest (ROI)) from 11 stage III microsatellite-stable CRCs. Cyclic immunofluorescence (CyCIF) was performed on 34 matching ROIs (13 superficial, 11 invasive and 10 node) and 10 normal ROIs. H&E-stained tissue of the invasive margin (500um either side of the tumour-normal interface) and remaining tumour was analysed using a deep-learning based classifier to estimate immune densities for 6 CRCs. *Results:* Lymphocyte densities were significantly greater in the invasive margin compared to the remaining tumour (Wilcoxon signed rank test, lymphocytes p=0.0007). Fraction of PDL1+ cells was elevated in invasive ROIs compared to superficial ROIs (Wilcoxon rank sum test, p=0.0001), possibly due to increased immune pressure from greater lymphocyte densities. Increased PDL1+ fraction may help tumour cells escape from the immune system and consequently accrue neoantigens. However, ROIs with increased PDL1+ fraction did not show increased subclonal neoantigen burden (Spearman correlation, missense: mutations: rho=0.01, p=0.93, peptides: rho=-0.04,p=0.8, indels- mutations: rho=0.02, p=0.91, peptides: rho=0.1,p=0.52). Proportional neoantigen burden (defined as the proportion of non-synonymous mutations that produce strong-binding neoantigen peptides) was not significantly different across sample types (missense: Kruskal-Wallis, p=0.18; indels: Kruskal-Wallis p=0.51). *Conclusions:* Despite ongoing TME remodelling during the shift to an invasive phenotype, no differences in neoantigen burden were noted, suggesting a lack of ongoing immunoediting in advanced CRC.

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P72

Loss of CLIC4 in colorectal cancer is associated with advanced tumour stage.

Purpose of the study: Chloride intracellular channel 4 (CLIC4) has previously been shown to play an important role in the regulation of multiple cellular processes including proliferation, differentiation, and angiogenesis. This study aimed to investigate CLIC4 expression in a cohort of colorectal cancer (CRC) patients and assess for clinicopathological correlations.

Methods: Tissue microarrays (TMA) containing 852 CRC cases were stained with CLIC4 antibody. Slides scanned and digitised, images imported into QuPath, tissue annotated and automated classifiers constructed for the assessment of CLIC4 staining. H-scores were analysed using SPSS for clinicopathological correlations and survival analysis.

Results: CLIC4 showed staining in the stromal compartment only. Expression was shown to be higher in the tumour associated stroma rather than the adjacent normal tissue stroma ($p<0.001$). Loss of CLIC4 expression was associated with later stage ($p=0.002$), positive vascular invasion (VI) ($p=0.005$), high grade ($p<0.001$), right sided tumours ($p=0.003$), and MMR deficiency ($p=0.003$). Following subgroup analysis of MMR proficient (MMRp) and deficient (MMRd) cases, later stage ($p=0.028$), high grade ($p=0.029$) and inconspicuous peritumoral lymphocytes ($p=0.014$) were associated with MMRp status whilst only positive VI ($p=0.005$) was associated with MMRd status. Kaplan Meier analysis showed no significant difference in overall survival in all categories. Aberrantly, the survival status (alive) at 5 years and no recurrence was associated with lower CLIC4 expression.

Conclusion: CLIC4 protein expression shows complex associations in CRC tissue. Being overexpressed in tumoural stroma compared to normal, it seems CLIC4 may be associated with stromal remodelling for colorectal cancer development. However, loss at later stages of cancer progression indicates involvement of other molecular drivers at advanced stages of CRC

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Stroma Areactive Invasion Front Areas (SARIFA) in gastric carcinoma as a new biomarker implicating an interaction between adipocytes and tumor cells -- a validation study

Introduction: Recently, we presented Stroma Areactive Invasion Front Areas (SARIFA) as a new biomarker in gastrointestinal cancer. We defined it as the direct contact between a cluster of at least five tumour cells and local adipose tissue at the invasion front. In our initial study, including 480 cancers of the stomach and the gastroesophageal junction, SARIFA was found to be a negative independent prognostic factor with low interobserver variability. Moreover, we provided first evidence for an interaction between adipocytes and tumour cells. Using digital spatial profiling (DSP), the most upregulated genes in SARIFA positive cases were those associated with lipid metabolism indicating potential underlying mechanisms related to tumour-promoting adipocytes. Our next objective was to evaluate the prognostic value of SARIFA in an independent external collective. Methods: An independent external validation collective consisting of 489 adenocarcinomas of the stomach and the gastroesophageal junction was independently classified according to SARIFA by two pathologists. Association of SARIFA status with clinicopathological characteristics and overall survival was evaluated. Results: SARIFA-positivity was found in 38%. Patients who presented SARIFA positive tumours had a significantly lower overall survival (OS) (median: 24 vs. 56 months; p = 0.002, n = 489). SARIFA positivity proofed as a negative independent prognostic factor for OS (HR 1.335, 95% CI 1.026-1.736, p = 0.03) in Cox regression analyses, which were adjusted for known prognostic parameters (age, pTNM). Regarding the pT stage, only advanced-stage (pT3/4) SARIFA-positive patients showed a worse OS (p = 0.011). SARIFA positivity was associated with positive lymph nodes and advanced pT stage. Conclusions: SARIFA combines low interobserver variability, minimal effort, and remarkable prognostic relevance in an external validation collective. The proposed metabolic mechanism could offer a new target for therapy.

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P74

Inter-observer agreement in digital pathology reporting of duodenal biopsies: normal, coeliac disease and indeterminate enteropathy

When patients with coeliac disease (CD) consume gluten, an autoimmune response in the duodenum effects characteristic inflammatory changes which, in a histological setting, are used to diagnose the disease. Multiple small studies have shown a range of levels of concordance between pathologists for making this diagnosis, with Cohen's kappa coefficients varying widely between zero and one, raising the question of reproducibility in CD diagnoses. We therefore examined the inter-observer agreement in CD diagnoses across a group of 17 gastro-intestinal pathologists who each reviewed 100 duodenal biopsies. Each observer independently examined 100 whole-slide images (WSIs) of haematoxylin-and-eosin-stained duodenal biopsies using a user-friendly online platform. The observers were given no supporting metadata for any of the images. The dataset contained 40 cases previously diagnosed as normal, 40 cases diagnoses as CD and 20 cases reported histologically as indeterminate. The observers were asked classify each image as CD, indeterminate or normal. The WSIs renamed, so that observers did not know how they had scored them the first time and a subset of 13 participants reviewed each WSI again, this time with the inclusion of serological data and the patient's haemoglobin level (where these had been available to us at the time of obtaining the WSI). In the absence of haemoglobin and serological data, the probability of two observers agreeing was 0.74 (s.d. 0.08), with a Cohen's Kappa efficient of 0.60 (s.d. 0.11). With the inclusion of serological metadata, the probability of two observers agreeing was 0.80 (s.d. 0.06), and Cohen's Kappa increased to 0.67 (s.d. 0.09). Commensurate with results of previous smaller published studies, this study shows relatively poor inter-observer agreement for the histology-based diagnoses of CD. The levels of concordance are more similar to those seen for agreement about histological grading than overall diagnosis, in the diagnosis of cancer.

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P75

CUX1 expression in tumour infiltrating chronic inflammatory cells promotes early progression events in colorectal cancer

Purpose of Study: CUX1, a haplo-insufficient tumour suppressor gene has been shown to be involved in vascular invasion and the distant metastases in colorectal cancer (CRC). Previous analysis from our patient cohort showed CUX1 protein expression in the epithelial compartment to be associated with poor prognosis in CRC. As lymphocytes and plasma cells also express CUX1, this study aimed to assess the clinicopathological correlations of CUX1 expression in CRC infiltrating chronic inflammatory cells. Method: CRC tissue microarrays (TMAs) (n=408) were stained for CUX1 and assessed for the density of tumour-infiltrating cells overall as well as for CUX1 staining and results analysed for correlations on SPSS. Results: Results showed a correlation between a high chronic inflammatory cell density and an early overall stage (stage 1), T1 stage, and absence of vascular invasion. Low immune cell density was associated with vascular invasion ($p=0.004$), whereas high immunological density was detected in younger individuals ($p=0.034$). CUX1 expression in chronic inflammatory cells correlated with CUX1 expression in the tumour ($p<0.001$). Higher CUX1 expression (above the lowest quartile) in immune cells corresponds with the presence of perineural invasion ($p< 0.05$). Conclusion: Results confirm that immune cell infiltration in CRC promotes tumour containment at an early stage. Higher levels of CUX1 expression in the immune infiltrate is associated with early progression events such as perineural spread and therefore may abrogate the protective effects of the tumour-associated immune response.

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P76

Microarchitecture of lymph nodes and its prognostic value in patients with upper gastrointestinal cancer: a systematic review and meta-analysis

Background: The most important prognostic factor in patients with oesophageal (OeC) or gastric cancer (GC) is the status of regional tumour draining lymph nodes (TDLNs). It has been suggested that the anti-tumour immune response can trigger TDLN microarchitecture changes like follicular hyperplasia (FH), sinus histiocytosis (SH) or paracortex hyperplasia (PH).

Methods: Systematic search in Embase, MEDLINE, PubMed, CINAHL and Cochrane up to June 2021 including cohort studies, randomized controlled trials and case control studies. Outcome measure: prognostic value (overall survival) of the TDLN microarchitecture changes assessed on histopathological slides. The review was registered in the PROSPERO Database.

Results: 5256 publications were initially identified. After full-text review of 159 articles, 32 articles matched all inclusion criteria analysing a total of 5242 TDLN from 1898 OeC and 3982 GC patients.

10 different TDLN reactive changes were described which we grouped into three categories: TDLN compartment hyperplasia (SH, FH, PH), cell-specific infiltration (dendritic cells, Tregs, neutrophils, macrophages, epitheloid reaction), TDLN morphology changes (diameter size, stromal reaction). The first category was studied in a sufficient number of TDLNs to allow meta-analysis. Meta-analysis results suggest presence of SH or FH as positive survival indicators (pooled odds ratios 1.91 and 1.59, 95% confidence interval 1.12-3.24 and 1.17-2.15, respectively).

Conclusions: Our systematic review with meta-analysis suggests that SH and FH could be clinically useful biomarkers of a favourable host anti-tumour response in OeC and GC patients. However, currently available evidence is limited and results for other TDLN reaction patterns remain inconclusive warranting further studies.

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P77

Endoscopic ultrasound-guided fine-needle aspiration: service evaluation at a tertiary center

Purpose of the study: This service evaluation aims to review EUSFNA used as a diagnostic tool in the assessment of hepatopancreatobiliary, lymph nodes, and intra-abdominal masses. Methods: All consecutive EUSFNA samples reported from January 2018 and December 2021 were identified in a retrospective search conducted on the internal computer system. External referral cases were excluded from the study. The data search included age, sex, site of lesion, and diagnostic adequacy. Summary of results: The study comprised 1100 patients. There were 483 hepatopancreatobiliary, 469 lymph nodes, and 148 intra-abdominal masses. Hepatopancreatobiliary lesions was classed as insufficient (8.5%), benign (14.7%), neoplastic (12.2%), atypia (3.1%), suspicious for malignancy (1.2%) and malignant (60.3%). Lymph node cases showed insufficient (8.3%), benign (45.6%), Neoplastic (0.2%), atypia (0.6%), suspicious for malignancy (0.2%) and malignant (45.1%). The intra-abdominal masses were subdivided into insufficient (10.1%), benign (14.9%), neoplastic (42.6%), atypia (0%), suspicious for malignancy (0.6%) and malignant (31.8%). Excision was not available in a large number of cases that were followed up by oncology or radiology. Amongst the hepatopancreatobiliary neoplastic and malignant excision, the positive predictive value was 100%. Conclusions: The overall adequacy of specimens obtained during EUSFNA is 91.4%. There was no false positive diagnosis. Results are in line with similar studies published in the literature.

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P78 *

Evaluating the use of immunohistochemistry and multiplexing of collagen Type I, Type III and elastin to histologically distinguish between Acute and Chronic Liver Injury

There have been several discussions probing how well a strict dichotomist approach works in classifying patients with acute or chronic liver injury works for the middle spectrum of liver injury. We set out to explore how single and double epitope immunohistochemistry (IHC) could complement the conventional histochemical stains when examining morphological changes of the extra cellular matrix at sites of hepatocyte loss. A retrospective study consisting of 48 livers was used and stained according to the following protocol: haemotoxylin and eosin, IHC anti elastin antibody stain, Victoria Blue (VB) stain with and without an oxidation step, and an IHC double epitope for collagen I and collagen III. A Nuance multispectral digital camera was used to supplement the analysis. This allowed us to convert the resulting images to a digital signal of different colours to better visualise the two epitopes: active profibrogenic deposition of connective tissue and pre-exiting connective tissue fibres condensed from hepatocyte collapse. Our findings suggest that changes to the extra cellular matrix make up part of a continuum. This relates to the balance between a range of factors including the severity of parenchymal injury and the possibility and progress of regeneration. Staining for collagen I, III and elastin was rewarding: it permitted us to better demonstrate the dynamics of connective tissue formation in liver fibrosis. We use these observations to advocate for the use of a scale to classify changes to the ECM ranging from a normal liver where collagen I,III and elastin are restricted to vessels and normal to end stage liver with diseasemature elastic fibres. In conclusion, we were able to use our observations to confirm the little researched but, amongst hepatopathologists, well known, fact that elastification is a sign of chronic liver injury. In addition, we found the deposition of collagen I often appears prior to early elastification. Supported by Jean Shanks Foundation.

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P79

Quantification of plasma cells using digital pathology software in bone marrow trephine biopsies of multiple myeloma.

Background: Assessment of plasma cell infiltrate in bone marrow biopsies is essential for effective diagnosis and prediction of treatment response in multiple myeloma. However, current methods carried out manually by clinical pathologists can be time consuming, prone to error and are semiquantitaive. Digital pathology offers a faster and potentially more accurate method of plasma cell count. We aimed to compare two different digital pathology softwares, Visiopharm® and QuPath, and assess their accuracy against manual scoring by a clinical pathologist. Method: Ten sample slides stained for CD138 and were used to optimise positive cell classification applications on the digital pathology softwares Visiopharm® and QuPath. Plasma cells were assessed using both apps and compared to pathologist scoring, using SPSS to calculate Pearson correlation for manual scoring vs Visiopharm® (M-V) and manual scoring vs QuPath (M-Q) Results: Overall concordance for M-V and M-Q were r=0.96 p<0.001 and r=0.69 p<0.05 respectively. For cases with diffuse plasma cell infiltrates, there was significant correlation associated with M-V (r=0.99 p<0.001) however M-Q showed no significant correlation (r=0.43 p>0.05). Similarly, for cases with patchy infiltrates and aggregates, significant correlation was associated with M-V (r=0.95 P<0.05) however M-Q showed no significant correlation (r=0.41 p>0.05). Interpretation: These results demonstrate that Visiopharm® shows greater correlation with pathologist compared to QuPath. There is also greater significant correlation when scoring plasma cell infiltrates compared to patchy infiltrates and aggregates. This suggest that digital pathology does offer a potentially viable alternative to pathologist scoring in the enumeration of plasma cells. Visiopharm® is a more sophisticated software than QuPath and contains a greater variety of functions and adjustable parameters. QuPath also incorrectly identified both positive and negative cells in background and artefact areas. This could explain the difference in correlation between the two softwares.

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P80 *

Stain Normalization Gives Greater Generalizability than Stain Jittering in Neural Network Training for the Classification of Duodenal Biopsy WSIs

Around 1% of the population of the UK and North America have a diagnosis of coeliac disease (CD), due to a damaging immune response to the small intestine. Assessing whether a patient has CD relies primarily on the examination of a duodenal biopsy, an unavoidably subjective process with poor inter-observer concordance. In the past, neural networks have been used to classify duodenal biopsies with CD with varying degrees of success. However, neural networks tend to overfit when trained on data that lacks diversity. This can make the neural network unreliable when attempting to classify biopsies prepared in different laboratories and/ or scanned with different equipment from that represented in the training data. The application of either stain normalization or stain jittering to the data before training the neural network can mitigate this. In this study, three neural network models were trained to determine whether a WSI of a biopsy showed features of CD. One was trained on WSI data with no stain pre-processing, one with stain normalization and one with stain jittering. The generalizability of these three models was compared by assessing their performance on four datasets: WSI of biopsies prepared in the same laboratory and scanned on the same Ventana scanner as the training data, WSI of the same biopsies rescanned on a Hamamatsu scanner, WSI of the same biopsies rescanned on an Aperio scanner, and WSI of different biopsies from a different laboratory scanned on an Aperio scanner. We found that the accuracies and F1 scores on the different datasets ranged from 95.3%-47.5% and 0.947-0.619 respectively when trained without stain processing, 98.4%-84.6% and 0.982-0.847 respectively when trained with stain normalization, and 100.0%-69.3% and 1.000-0.747 respectively when trained with stain jittering. Stain normalization greatly improves the generalizability of neural network models between different laboratories and scanners. Research was supported by a PathSoc grant.

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P81*

Convolutional neural network-based normalisation scheme with U-Net architecture for more efficient stain pre-processing in diagnostic classification of whole slide images

The growing uptake in the digitisation of pathology, paired with rapid development in artificial intelligence, has paved the way for extensive research into the automated detection of disease processes, with potential for the creation of decision support tools to aid pathologists in making diagnoses, as a stepping stone to full automation. Such tools might prevent delays and backlogs and mitigate some risks of human error. Generalisability of analytical methods between different data sources is hampered by slides prepared in different centres or scanned with different slide scanners giving markedly different scanned whole slide images (WSIs). A key technique, known as stain normalisation, addresses this limitation by providing a standardisation step for images before analysis, but has a high computational cost. We therefore aimed to replace the stain extraction part of such a pipeline with a separate neural network. A convolutional neural network with U-Net architecture was trained for 30 Epochs on the Camelyon-7 sentinel lymph node dataset (500 WSIs) to approximate the conventional Macenko's stain normalisation method, in order to produce a model capable of standardising the concentrations of stains and normalising the colour of WSIs much more rapidly than Macenko's method. We used the output of this normalisation step to train a U-Net to identify nuclei in the Lizard dataset (238 WSIs), training for 30 Epochs and achieving a maximum validation dice coefficient/ F1 score of 70.1% compared with 66.43% for the Macenko method and 54.73% without stain normalisation. The U-Net neural network-based approach provides normalisation of stain concentration comparable with Macenko's method, but uses parallel computing to make the process faster and more computationally efficient, indicating its potential utility in both research and clinical diagnosis. Part of this work was supported by a PathSoc PhD studentship.

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P82

Nuclear and Region Segmentation of the Pre-invasive Ductal Carcinoma In Situ of the Breast for the Development of a Risk Progression Model

Purpose of the study Whilst most invasive breast cancers are thought to develop from Ductal Carcinoma In Situ (DCIS), up to 50% of DCIS will never progress to invasive cancer. Despite this, all women with DCIS receive surgical excision, radiotherapy and/or endocrine therapy. Currently, there are no reliable markers to distinguish between those lesions that progress and those that do not. Despite the recent developments in machine learning (ML), the potential of ML algorithms for prediction of malignant transformation for pre-cancerous conditions remains largely unexplored. In this study, we aim to employ ML algorithms to investigate the hypothesis that the integrated analysis of morphological features from tumour and its microenvironment would better stratify patients with DCIS allowing more tailored patient management. Methods Three well-curated independent DCIS patient cohorts are used in the study. These cohorts include rich clinicopathological and outcome data derived from Barts and Nottingham Biobanks and from the UK/ANZ DCIS trial. For nuclear segmentation and classification, we used a deep learning tool HoVer-Net while the FCN-8 VGG fully-convolutional network was used in our experiments for region segmentation. Summary of results We have optimised Hover-Net, pretrained on the PanNuke dataset, to segment and classify nuclei into neoplastic epithelium, inflammatory, connective, dead and non-neoplastic epithelium classes. The FCN-8 VGG was used to segment neoplastic epithelium, non-neoplastic epithelium, fibrous stroma, adipose stroma, necrosis, calcification and other regions. Conclusions We have achieved promising region and nuclear segmentation of the breast tissue. This will be used to extract a range of morphological features, which will be utilised in building a binary classification model stratifying patients into high- and low-risk groups.

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P83 *

Determination of the pattern of diagnoses in duodenal biopsies as an essential step in developing an artificial intelligence approach to diagnostic classification

When developing a new classification approach in diagnostics, it is very important to understand what expected results might look like following "real life" deployment. We are developing an artificial intelligence-mediated computational diagnostic classifier for duodenal biopsies, to help mitigate the shortage of pathologists and to improve the accuracy of diagnosis of biopsies taken for suspected gluten sensitivity (coeliac disease). We therefore chose to audit 18 months' worth of duodenal biopsies received in our centre to determine the exact proportions of different diagnoses. 4588 endoscopic duodenal biopsies were audited. 73.57% were normal. 6.91% of biopsies fell within the spectrum of coeliac disease, gluten-sensitive enteropathy and refractory coeliac disease, while 6.51% were diagnosed as showing non-specific inflammation, with 1.84% showing adenomas, 0.4% showing duodenal carcinoma and 0.06% showing neuroendocrine tumours. Rarer diagnoses included ulceration, Helicobacter, Giardia, telangiectasia, transplant rejection and lymphoma. Results indicate that a fully automated system that could identify normal duodenal biopsies and biopsies within the spectrum of coeliac disease/ gluten-sensitive enteropathy, could decrease pathologists' endoscopic duodenal biopsy workload by up to 80%. Some of the authors of this work were supported by PathSoc undergraduate elective bursaries.

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P84

Machine-learning based image analysis algorithms improve inter-pathologist concordance for scoring PD-L1 expression in non-small cell lung cancer.

Introduction Programmed death ligand 1 (PD-L1) expression on tumour cells is the only predictive biomarker of response to immuno-modulatory (IM) therapy for patients with non-small cell lung cancer (NSCLC). Accuracy of this biomarker is hampered by its challenging interpretation. Here we explore if the use of machine-learning derived image analysis tools can improve inter-pathologist concordance of assessing PD-L1 expression in NSCLC and so improve the accuracy of this vital biomarker. Method Five pathologists who routinely score PD-L1 expression on NSCLC specimens at a major regional referral hospital for thoracic surgery participated. The SP263 clone was used to stain for PD-L1 and a tumour proportion score (TPS) was given for each case as both an absolute number (0-100%) and as clinical categories of negative (<1%), weak positive (1-49%) or strong positive (≥50%). 13 NSCLC small biopsies were selected to show a range of PD-L1 expressions and the H&E and SP263 slide for each case was scanned using the DP200. Each pathologist independently scored each case using the Roche-Ventana PD-L1 image analysis algorithm. A wash-out period of 6 weeks was followed by each pathologist re-scoring the same cases on a digital pathology platform but without the use of the image analysis tool. Scores were captured as continuous data (TPS) and categorical data (clinical categories) and inter-pathologist concordance assessed using intraclass coefficient correlation (ICC) and Fleiss' kappa respectively. Results All five pathologists scored all cases with and without the algorithm. Fleiss' kappa when using the image analysis algorithm was 0.841 (very good agreement p<0.0001) and without the algorithm was 0.622 (good agreement p<0.0001). The ICC when using the image analysis algorithm was 0.991 (p<0.001) and without the algorithm was 0.975 (p<0.001). Five cases (38%) were potentially classified into clinically meaningful different categories by two or more pathologists when not using the im

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P85

The Influence of Neoadjuvant Treatment on Predicting Microsatellite Instability Status in Gastric Cancer Resection Specimens with Deep Learning

Background: In gastric cancer (GC), microsatellite instability/mismatch repair deficiency (MSI/dMMR) has been predicted from Haematoxylin & Eosin (H&E) stained tissue of resection specimens using deep learning (DL). However, whether this marker can equally well be predicted after neoadjuvant chemotherapy is currently unknown.

Purpose: We aimed to compare MSI/dMMR predictability by DL-based classifiers between pretreated and non-pretreated resection specimens from two independent GC cohorts.

Methods: A Multiple-Instance-Learning (MIL) based classifier was trained to predict MSI/dMMR status from digitised H&E whole slide images of GC resections from Augsburg, Germany (AUGSB, n=180, 27% pretreated, 9% MSI/dMMR) and Munich, Germany (TUM, n=592, 52% pretreated, 10% MSI/dMMR). We performed a subgroup-specific AUROC-analysis for pretreated vs. non-pretreated cases.

Results: A baseline assessment of MSI/dMMR predictability yielded AUROCs of .49 for AUGSB and of .82 for TUM using the full cohorts. In AUGSB, the MSI/dMMR prediction AUROC was 0.65 for pretreated cases and 0.42 for non-pretreated cases. In TUM, the MSI/dMMR prediction AUROC was 0.84 for pretreated cases and 0.80 for non-pretreated cases. Interestingly, pretreatment itself was not detectable in AUGSB with an AUROC of 0.56, but detectable in TUM with an AUROC of 0.83.

Conclusions: Neoadjuvant treatment does not seem to negatively affect predictability of MSI/dMMR from H&E WSI with MIL. Instead, we detected a slight performance increase in pretrated cases. The signature that neoadjuvant treatment leaves on WSI should be further investigated with possible extension to other biomarkers.

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P86

Self-learning artificial intelligence provides a comprehensive dictionary of regional lung cancer phenotypes linking histology to molecular biology and patient outcomes

Purpose To date almost all applications of deep learning artificial intelligence have been 'supervised', ie they have relied upon the provision of expert annotations for algorithm training. This is time-consuming, and confines the algorithm to a human-defined gold standard. Instead, we have taken an unsupervised approach, training an algorithm to discover recurrent histomorphological phenotypes within non-small cell lung cancer. Methods Who slide H&E tumour image sets from TCGA and NYU were used for training and validation. Images were divided into 0.5mm tiles. Tiles were subjected to minor modifications (eg rotation, zoom, colour balance) and, using a deep learning AI architecture, a Barlow Twins algorithm was applied to learn discriminatory imaged features, which themselves define the dimensions of high-dimensional latent space. Individual tiles were mathematically projected into in this space, and a Leiden algorithm was used to define spatial clusters. Tile image clusters were histopathologically examined, correlated with RNASeq and cell lineage data, and used as variables in survival models. Summary of results 47 recurrent histomorphological phenotype clusters were identified in lung adenocarcinoma. The predominance of several clusters in whole slide images is related to patient outcome. Good outcome clusters are characterised by low-grade appearances and lymphocytes, while poor appearances are poorly differentiated and immune-cold. Furthermore, the algorithm predicts survival in an external training set with a C-index of 0.74, as accurate as the latest grading recommendations. Furthermore, clusters show distinct molecular phenotypes in linked RNASeq data, notably in expression signatures related to tumour cell proliferation and to immune cell infiltration. Conclusions This unsupervised method reveals a 'dictionary' of tumour appearances, with the potential to revolutionise the automated assessment of whole tumour images and to aid biological discovery.

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Baseline Audit of Prostate Biopsy Reporting Patterns in 3 Histopathology Laboratories - Preparation for Deployment of Artificial Intelligence in the Articulate Pro Study

Purpose of Study There are often significant inter and intra pathologist and lab variations in prostate biopsy reporting practice. It becomes particularly important in multicentre studies to analyse what the baseline reporting patterns of sites are, before attempting to understand the impact of AI reporting assistance in reaching diagnoses and making assessments such as grading. This study audited the baseline prostate biopsy reporting practices (diagnosis and grading) at the 3 laboratories involved in the Articulate Pro (Artificial Intelligence for Cellular Pathology Transformation in Prostate Practice) study evaluating prospective clinical deployment of a market authorised prostate biopsy diagnostic assistance technology. Methods Data from 1st January 2021-- 31st December 2021 were collected from 3 NHS Hospital Trusts in the Articulate Pro study. This included all prostate biopsies reported during this period. The results were categorised as "benign", "malignant", "atypia" and "other". One site provided Gleason Gradings, which were categorised as Grade Group 1 or Groups 2-5. Results 2485 prostate biopsies were reported: 1189, 652 and 644 from sites 1, 2 and 3 respectively. Of these, 664 (27%) were benign (24%, 38% and 21% from sites 1, 2 and 3). 1695 (68%) were malignant (71%, 55% and 76% from sites 1, 2 and 3), 88 (4%) were atypical (5%, 1% and 3% from sites 1, 2 and 3) and 38 (1%) were other (0%, 6% and 0% from sites 1, 2 and 3). Site 3 additionally provide Gleason Grading information. Of these, 5 (1%) were ungraded, 49 (10%) were Grade Group 1, and 432 (89%) were Groups 2-5. Conclusions The outcome of the baseline audit showed that there were some differences between sites in reporting patterns with rates of reported malignancy from 55-76%. Possible reasons include population base or clinical urological practice differences. Understanding this baseline landscape before deployment of AI assistance in reporting is key to understanding subsequent impact of AI.

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Self-learning artificial intelligence provides a comprehensive dictionary of regional lung cancer phenotypes without requiring annotations

Purpose To date almost all applications of deep learning artificial intelligence have been 'supervised', ie they have relied upon the provision of expert annotations for algorithm training. This is time-consuming, and confines the algorithm to a human-defined gold standard. Instead, we have taken an unsupervised approach, training an algorithm to discover recurrent histomorphological phenotypes within non-small cell lung cancer. Methods Whole slide H&E tumour image sets from TCGA and NYU were used for training and validation. Images were divided into 0.5mm tiles. Tiles were subjected to minor modifications (eg rotation, zoom, colour balance) and, using a deep learning AI architecture, a Barlow Twins algorithm was applied to learn discriminatory image features, which themselves define the dimensions of high-dimensional latent space. Individual tiles were mathematically projected into in this space, and a Leiden algorithm was used to define spatial clusters. Tile image clusters were histopathologically examined, correlated with RNASeq and cell lineage data, and used as variables in survival models. Summary of results 47 recurrent histomorphological phenotype clusters were identified in lung adenocarcinoma. The predominance of several clusters in whole slide images is related to patient outcome. Good outcome clusters are characterised by low-grade appearances and lymphocytes, while poor outcome clusters are poorly differentiated and immune-cold. The algorithm predicts survival as accurately as the latest diagnostic grading recommendations. Clusters show distinct molecular phenotypes in linked RNASeq data, notably in expression signatures related to tumour cell proliferation and to immune cell infiltration. Conclusions This unsupervised method reveals a 'dictionary' of tumour appearances and biology, with the potential to revolutionise the automated assessment of tissue images for both research and clinical purposes.

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Phosphohistone-H3 (PHH3) antibody counterstained with hematoxylin and eosin as part of breast cancer grading: increasing mitotic cell counting precision and efficiency

Background: Mitotic count in breast cancer is an important prognostic marker. Unfortunately, substantial inter and intra-observer variation exists when pathologists manually count mitotic figures. To alleviate this problem, we developed a new technique incorporating both H&E and PHH3 a marker highly specific to mitotic figures, and compared it to visual scoring of mitotic figures using H&E only. Methods: Two full-face sections from 97 cases were cut, one stained with H&E only, the other was stained with PHH3, and counterstained with H&E (PHH3-H&E). Counting mitoses using PHH3-H&E was compared to traditional mitoses scoring using H&E in terms of reproducibility, scoring time, and ability to detect mitosis hotspots. We assessed the agreement between manual and image analysis-assisted scoring of mitotic figures using H&E and PHH3-H&E-stained cells. The diagnostic performance of PHH3 in detecting mitotic figures in terms of sensitivity and specificity was measured. Finally, PHH3 was replaced with mitosis score in a multivariate analysis to assess it is significance. Results: Pathologists detected a significantly higher mitotic figure using the PHH3-H&E (median ± SD, 20 ± 33) compared with H&E alone (median ± SD, 16 ± 25), $p<0.001$. The concordance between pathologists in identifying mitotic figures was highest when using the dual PHH3-H&E technique, in addition, it highlighted mitotic figures at low power, allowing better agreement on choosing hotspot area (k= 0.842) in comparison with standard H&E (k= 0.625). A better agreement between image analysis-assisted software and the human eye was observed for PHH3 stained mitotic figures. When mitosis score was replaced with PHH3 in a Cox regression model with other grade components, PHH3 was an independent predictor of survival (HR 5.66, 95% CI 1.92-16.69; p=0.002), and even showed a more significant association with BCSS, than mitosis (HR 3.63, 95% CI 1.49-8.86; p=0.005) and Ki67 (p=0.27). Conclusion: Using PHH3-H&E-stained slides can reliably be used in routine scoring of mitotic figures and integrating both techniques will compensate for each other's limitations and improve diagnostic accuracy, quality, and precision.

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PKMYT1 overexpression is associated with aggressive tumour features and a poor prognosis in breast cancer

Background: Protein kinase, membrane-associated tyrosine/threonine 1 (PKMYT1) has been implicated in the development and progression of several malignancies, including colorectal, gastric, lung, and hepatocellular carcinoma, and has been suggested as a good target for enhancing radio sensitivity in lung cancer, however, its role in breast cancer (BC) is still unclear. This study aims to evaluate the clinical and prognostic significance of PKMYT1 in BC. Methods: PKMYT1 expression was evaluated on a tissue microarray of a large BC cohort (n = 1124) using immunohistochemistry. PKMYT1 transcriptomic expression was assessed using the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (n=1980) and Cancer Genome Atlas (TCGA) BC cohorts (n=850). The correlation between PKMYT1 expression, clinicopathological parameters and outcome was assessed. Results: PKMYT1 expression was detected in the nucleus of the tumour cells. It was significantly associated with aggressive BC features including high tumour grade, and poor Nottingham Prognostic Index. high PKMYT1 nuclear expression was associated with shorter BC specific survival (BCSS) (HR 4.09, CI 95%2.093-7.99; p<0.001) and distant metastasis-free survival (DMFS) (HR 1.79, CI 95%;1.2-2.68; p=0.004). In the radiotherapy-treated group, high PKMYT1 nuclear expression was predictive of a higher risk of death from BC (HR= 2.06, CI 95% 1.3-3.26; p=0.001). However, such association was not observed in patients who did not receive radiotherapy. Conclusion: PKMYT1 expression is a poor prognostic biomarker in BC and its expression can help in predicting radiotherapy response. Further investigations are warranted to reveal its underlying mechanisms and potential therapeutic benefit in various BC subtypes.

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The role of the Neurotrophic tyrosine kinase receptor-1 gene (NTRK-1) in the progression of breast cancer

Purpose of the study: The Neurotrophic tyrosine kinase receptor-1 gene (NTRK-1) belongs to a group of three genes, NTRK1, NTRK2, & NTRK3, which encode for the Neurotrophic tyrosine kinase receptor proteins, TrkA, TrkB, & TrkC, respectively. High expression of NTRK-1 has been associated with neuroplasticity, resulting in improved memory and cognition, particularly in response to psychological trauma/stress. NTRK gene fusions have been associated with various cancers, including breast cancer. The aim of this study was to determine how wild type NTRK-1 influences progression of breast cancer, as a first step to investigating its role as a potential molecular link between breast cancer progression & psychological stress.
Methods: The bioinformatics tools KM Plotter, TISIDB, cBioPortal & Human Protein Atlas were used to produce data relating to NTRK-1 expression & breast cancer.
Results: NTRK-1 improves overall survival of breast cancer. However its effects become more complex, depending on stage. It is equally expressed in each subtype of breast cancer, as well as each stage. However, it only improves survival at stage 1 & 3 & has no significant effect at stage 2 or 4. This effect is independent of mutation burden. The interaction of NTRK-1 with the immune system is complex & the prognostic effect shifts depending on which specific components of the immune system are enriched or depleted in the tumour microenvironment. One example of an effect found was that, at stages 1 and 3, enrichment of Mesenchymal & Natural killer cells results in a poor prognostic effect.
Conclusions: NTRK-1 has a good prognostic effect in breast cancer at stages 1 & 3, regardless of mutation burden, but can have a poor prognostic effect in specific immune microenvironments. Further study into the immune system interaction is required to substantiate these observations & establish whether NTRK forms a molecular link between the risk of cancer progression and psychological stress.

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Oestrogen receptor low positive breast cancer: A validation study

Purpose of the study: Oestrogen Receptor (ER) status in breast cancer (BC) is determined using immunohistochemistry (IHC) with nuclear expression of ≥1% is defined as ER positive. BC expressing ER in 1-9% (ER low positive), is a unique subgroup mostly classified as basal-like or HER2-enriched by intrinsic subtype classification. In this study, we hypothesised that BC with ER-low positive represents a heterogeneous group with a mixture of ER positive and ER negative tumours, which may explain their clinical behaviour and response to endocrine therapy. Methods: A large primary BC cohort (n=8171) was investigated. Cases were categorised into 3 groups: ER-low (1-9%), ER positive (≥10%) and ER negative (<1%). Clinicopathological and outcome characteristics were compared between the 3 groups. ER-low cases with available core biopsy and excision specimen tissues were studied for IHC staining using different ER staining assays, *ESR1* mRNA was assessed using RNAscope and RT-PCR. PAM50 molecular subtyping and *ESR1* mRNA expression levels were also assessed in The Cancer Genome Atlas (TCGA) dataset. Summary of results: The originally reported ER low positive tumours constitute less than 2% of BC and showed significant clinicopathological similarity with ER negative tumours compared to ER positive tumours. Most of ER low positive cases were of non-luminal types showing low levels of *ESR1* mRNA expression difference from ER negative cases. However, further validation revealed that 45% of these tumours were negative with repeated staining, confirmed by RNAscope and RT-PCR in 82% of them. The ER-low positive tumours were enriched with false positive ER staining. Conclusions: ER low positive BC diagnosed includes a proportion of ER negative cases. We recommended repeating testing of cases showing 1-9% ER expression to help guide management decision.

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Evaluation of the Prognostic Significance of Anterior Gradient 2 (AGR2) Expression in Invasive Breast Cancer

Purpose of the study: Anterior Gradient 2 (AGR2) is a member of the disulfide isomerase family of endoplasmic reticulum proteins, and it is an estrogen-related protein. It is involved in cellular activities which increase cell proliferation and survival, and promotes carcinogenesis. It has been under the research scope to elaborate its role in invasive breast cancer (BC) with a subsequent therapeutic potential. Our purpose was to examine the clinico-pathological and prognostic significance of AGR2 in BC.
Methods: AGR2 expression was evaluated at the proteomic level utilising a large BC cohort (n=1695) by immunohistochemistry (IHC). The online dataset Breast Cancer Gene Expression Miner (GenExMiner) was used for validation at the genomic level.
Summary of results: High AGR2 expression was associated with adverse outcome in terms of shorter breast cancer-specific survival, distant metastasis-free interval, and disease-free interval, and these significant associations were independent of other key prognostic variables as tumour size, grade, and stage, beside lympho-vascular status (HR=1.441, 95%CI=1.168-1.778, p=0.001), (HR=1.372, 95%CI=1.112-1.692, p=0.003), and (HR=1.373, 95%CI=1.128-1.673, p=0.002), respectively. In addition, high AGR2 expression showed significant association with upregulated estrogen (ER) and progesterone (PR) receptors, and with lympho-vascular invasion ($p< 0.05$ for all). The worse survival potential was also obtained by GenExMiner dataset (HR=1.16, 95%CI=1.05-1.27, p=0.0031).
Conclusions: AGR2 confers a poor prognostic value in invasive BC. Further functional assessment is recommended to evaluate its potential therapeutic utility in BC.

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DCIS-associated myoepithelial cells drive tumor progressive inflammation through up-regulation of integrin ?v?6.

Background: Ductal carcinoma in-situ (DCIS) is a pre-invasive stage of breast cancer, however <50% will progress to invasion. A key component in promoting tumour invasion is inflammation, though its role in DCIS progression is unclear. We previously, showed αvβ6 expression on DCIS myoepithelial cells promotes tumour cell invasion. We hypothesized that altered myoepithelial phenotype in DCIS may modulate periductal inflammation to contribute to disease progression. Methods: The inflammatory infiltrate in DCIS cases (20 αvβ6+ and 20 αvβ6-) was characterized by immunohistochemistry (IHC) and immunofluorescence-IHC, focusing on Tumour Associated Macrophage (MΦ) (TAM) markers. A myoepithelial cell line over-expressing αvβ6 (β6-1089) and a control counterpart (N-1089) were analysed for cytokine and signalling molecule expression with proteome profilers and western blotting. The monocyte cell line (THP-1) was exposed to conditioned media from β6-1089 or N-1089 followed protein expression analysis. 3D models were used to examine if the in vivo changes to macrophages can be modelled in vitro. RNA-seq was carried out on MΦ from patients. Results: IHC analysis revealed a significant association between myoepithelial αvβ6 expression and increased presence of TAM (p=0.0026). αvβ6 positive DCIS exhibited a microenvironment comprising 15% TAMs compared to 3% in αvβ6 negative DCIS cases. This shift in MΦ phenotype was recapitulated in 3D organotypic assays incorporating MΦ and myoepithelial cells. Proteome profiler analysis of THP-1 cells grown with conditioned media from αvβ6 positive myoepithelial cells demonstrated increased expression of CCL5 while all other proteins analyzed where generally suppressed. Conclusion: DCIS myoepithelial cells upregulate αvβ6 and exhibit changes in the periductal inflammatory infiltrate indicating a switch to a tumor promoting phenotype. Further investigation is required to determine the prognostic value and underlying mechanisms of this chan

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Evaluation of tumour infiltrating lymphocytes in luminal breast cancer using deep learning approaches

Purpose of the study: Tumour infiltrating lymphocytes (TILs) are recognised as a prognostic parameter in triple negative and HER2 positive breast cancer (BC), while their role in the luminal (ER+/HER2-) BC remains unclear. In this study, we utilised the power of artificial intelligence (AI) to assess the clinical significance of TILs in the luminal BC. Methods: A large cohort (n=1,312) of early-stage luminal BC was included in this study. A supervised deep learning model was used to assess stromal (sTILs) and intratumoural (tTILs) count, and their spatial distribution to the surrounding cells in terms of co-occurrence describing their proximity to stromal or tumour cells and co-localisation defining the overlap between TILs and tumour cells. Correlation of the AI based scores with other clinicopathological parameters and patient outcome was also analysed. Summary of results: sTILs showed strong positive linear correlation with tTILs (r=0.6). Both high sTILs and tTILs counts and co-occurrences were associated with unfavourable clinicopathological parameters including high grade, lymph node metastasis, large tumour size and young age, and with shorter patients' survival. High tTILs-tumour cells overlapping was associated with shorter distant metastasis free survival. Automated assessment of stroma area occupied by TILs corresponding to routine visual score was not significantly associated with outcome. Conclusions: Automated assessment of TILs counts, and spatial distribution are associated with markers of poor prognosis and shorter survival in luminal BC. The utilisation of AI algorithms would add to the limited information pathologist can retrieve from visual eyeballing assessment.

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Using bioinformatics analysis to screen for triple-negative breast cancer biomarkers

Background: Due to its heterogeneity, high treatment resistance, poor prognosis, and lack of therapeutic targets, triple-negative breast cancer (TNBC) is a particularly deadly subtype of breast cancer. Epigenetic therapies targeting histone modifying enzymes hold a promise for cancer therapies. This study used genomic datasets and bioinformatics analysis to study the value of histone methylases (HMTs) and histone demethylases (HDMs) as novel prognostic biomarkers for TNBC. Methods and results: A bioinformatic analysis was carried out to reveal the candidate epigenetic modifiers that may be used as prognostic biomarkers in TNBC subtypes. The Breast Cancer Gene-Expression Miner and UALCAN databases were used to compare the expression of 41 different HMTs and 21 HDMs between normal tissues and major TNBC subtypes. Subsets of HMTs and HDMs highly expressed genes were identified. Subsequent analysis of overall survival associated with the expression of these biomarkers was identified by Kaplan-Meier Plotting. High expression of SMYD2 was associated with a shorter patients' survival, whereas PRMT1, CARM1, and KDM1B were correlated with longer survival ($p<0.01$). Importantly they also demonstrated differential expression in different TNBC subtypes and therefore their importance in disease stratification. Conclusions: Based on the bioinformatic datasets, the histone modification SMYD2 showed a prognostic value in TNBC. This highlights the value of bioinformatic datasets in cancer studies for a better understanding of TNBC and for the discovery of novel biomarkers and therapeutic targets.

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KDM5B is a prognostic marker of triple-negative breast cancer (TNBC)

Background: Histone methylation plays a crucial role in the control of gene expression; alterations of this process have been seen in several malignancies including triple-negative breast cancer (TNBC). KDM5B is an H3K4 demethylase family that regulates gene transcription and is involved in cancer development and proliferation. This study aimed to investigate KDM5B expression in TNBC tissue and determines its clinicopathological significance. Methods: The transcriptional levels of KDM5B were evaluated using multiple well-characterised TNBC cohorts including METABRIC dataset, BC-GenExMiner and Kaplan-Meier Plotter cohorts. The protein expression of KDM5B was investigated by immunohistochemistry in 600 TNBC cohort. The associations between KDM5B expression and clinical pathological parameters of TNBC patients were analyzed. The expression of KDM5B was further assessed with other epigenetic genes. Results: The public databases showed the level of KDM5B mRNA was significantly upregulated in TNBC molecular subtypes. High transcriptomic KDM5B expression was an independent predictor of shorter BC-specific survival and distant metastasis-free survival in TNBC. KDM5B mRNA overexpression was significantly correlated with high expression of histone methylation genes KDM7A and KDM1. The IHC results showed that the High expression of KDM5B protein level worse overall survival (P=0.040). Conclusion: This study indicates that KDM5B is an independent prognostic biomarker in TNBC. These results warrant further functional validation for KDM5B as a potential therapeutic target in TNBC.

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P98

The characteristics and prognostic significance of immunohistochemical expression of Histone H1 in breast cancer

Background: Breast cancer (BC) is the second leading cause of cancer-related deaths in women. The need for new clinical biomarkers in BC is crucial to further predict prognosis and therapeutic response. Histone H1 is involved in chromatin organization and gene regulation, and it is overexpressed in many cancer types including BC. In this study, we aimed to evaluate the prognostic and predictive role of histone H1 in BC and in the different molecular subtypes. Methods: A large BC cohort (n=1311) were immunohistochemically stained for histone H1. H. score of histone H1 expression was calculated and the association with clinicopathological data and survival outcome was evaluated. Results: The mean ± SD of histone H1 expression was 88 ± 24%. Stromal expression was found 85% of tumours. High histone H1 expression showed a significant association with worse survival in BC patients (p=0.019). With classification into molecular subclasses, there was strong association between high histone H1 expression and triple negative (TN) subtype (p=0.007). In addition, multivariate cox regression analysis showed that high histone H1 expression was associated with poor outcome independent of tumour size, histologic tumour grade and stage. Conclusion: These results indicate that histone H1 has a poor prognostic value in BC and could act as a potential therapeutic target for TNBC patients who show resistance to therapy.

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Title: Idiopathic Granulomatous Mastitis (IGM)- A Diagnostic Challenge

Introduction Idiopathic is a rare, benign, inflammatory breast disorder of unknown aetiology. It typically presents in parous women with a recent history of lactation. Differential diagnoses include breast malignancy and granulomatous diseases. The pathological criteria for diagnosis of IGM includes granulomatous inflammation with the presence of multinucleated giant cells and fat necrosis, abscesses, sinus tract and eosinophils.

Methods All clinically detected cases of idiopathic granulomatous mastitis over a 5 year period (2017-2022) were identified using HIPE data. Patient demographics, clinical and radiological data as well as histology and microbiology results were recorded. A retrospective review was undertaken of the histological features including; 1. Periductal or perilobular inflammation 2. Sheet like or well formed granulomas 3. Presence of neutrophils/eosinophils 4. Presence of necrosis 5. Special stains.

RESULTS The average age of patient was 44.9. A new breast lump was the most common clinical presentation, 72.5% of patients (n=29). 61% of samples were biopsies of the left breast (n=25), with the remainder (n=15) from the right. 19.5% (n=8) were post-partum within 24 months from presentation. 14.6% (n=6) were current smokers. 2 patients had a history of diabetes mellitus. All were R2 lesions.
All 40 samples sent for histological analysis had granulomatous inflammation present. 32 were described as in sheets, 7 well-formed, and 1 combination of both. Neutrophils were seen in 34 samples, foreign body giant cells seen in 29, histiocytes seen in all 40 samples, and necrosis was visualised in 2 samples only. A positive gram stain was reported in 2 samples, which correlated with microbiology results.

Conclusion IGM is difficult to distinguish clinically from other inflammatory breast diseases or cancer. Clinicopathological correlation with radiology and microbiology are important, but the gold standard of diagnosis remains with histopathology.

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Different clinical and pathological profiles of sudden cardiac death victims caused by coronary artery dissection or myocardial infarction with non-obstructed coronary arteries (MINOCA): insights from a UK nationwide autopsy-based registry

Spontaneous coronary artery dissection (SCAD) and myocardial infarction with non-obstructed coronary arteries (MINOCA) are increasingly recognized cause of acute coronary syndrome and potentially of sudden cardiac death (SCD). SCAD has been correlated to coronary fibromuscolar dysplasia (FMD), but the prevalence of SCAD and FMD among SCD victims is unclear. We aim to assess characteristics of decedents with SCAD found at autopsy, and to compare their clinical and pathological profile with MINOCA victims. We reviewed a database of 5325 consecutive cases of SCDs referred to our cardiac pathology center between 1994 and July 2017. We identified 21 (0.4%) cases with SCAD and 37 (0.7%) with MINOCA. No signs of coronary FMD were found among SCAD and MINOCA victims. Compared to MINOCA, SCAD decedents were mostly females (81% versus 38%, p=0.02) and SCD occurred during peripartum more frequently in SCAD rather than MINOCA female victims (29% versus 7%, p=0.02). Infarcted myocardium was identified in all cases of MINOCA but only in 8 (38%) of SCAD decedents (p<0.001). Pre-mortem cardiac symptoms were present in 100% of SCAD and 49% of MINOCA victims (p<0.001); substances use or abuse was reported in none of SCAD versus 46% of MINOCA decedents (p=0.001). SCAD and MINOCA are rare causes of SCD. At autopsy coronary FMD is not present among SCAD victims. Compared to MINOCA, SCAD victims are more frequently females, are linked to pregnancy, and always experienced pre-mortem cardiac symptoms. Among MINOCA victims' substances use or abuse is common.

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P101*

Cytokine therapy for cardiac protection in Friedreich's ataxia

Purpose of study: Friedreich's ataxia (FA) is a devastating disease currently lacking any proven treatment. It is the most common hereditary ataxia caused by a homozygous GAA/TTC tri-nucleotide repeat expansion within intron 1 of the FXN gene. This triplet-expansion results in transcriptional repression of frataxin; a small mitochondrial protein involved in iron-sulphur cluster biosynthesis and antioxidant protection. Symptoms generally begin in childhood and affected individuals experience insidious accumulation of disability with progressive ataxia, dysarthria, neuropathy, pyramidal weakness and cardiac dysfunction. Congestive cardiac failure is the leading cause of death in patients with FA. We have recently described the neuroprotective properties of cytokines granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF) in models of FA. We studied the cardioprotective effects of G-CSF and SCF in a humanised murine model of the disease.

Methods: Transgenic YG8R mice, which carry a human genomic FXN transgene containing a GAA expansion within intron 1, received monthly subcutaneous injections of G-CSF and/or SCF. After 6 months of treatment, mice were sacrificed for mRNA, protein and histological analysis of heart tissue.

Summary of results: A combination of G-CSF and SCF had pronounced effects on frataxin protein levels. Elevations in antioxidant regulators (Nrf2 and PGC1-alpha) and defences (SOD1 and catalase) were detected in response to treatment. Abrogation of disease-related histiocytic inflammation and oxidative damage were also apparent in mice treated with G-CSF and SCF.

Conclusion: These experiments provide evidence that the cytokines G-CSF and SCF may offer a cardioprotective treatment for patients with FA. Funded by a Pathological Society Postdoctoral Collaborative Small Grant

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Synchronous and Metachronous Primary Cancers: Case series of experience in a Pathology Laboratory

Background: Occurrence of another primary cancer in individuals with prior cancer diagnosis is increasingly common due to improved survivor indices with modern diagnostic techniques and treatment options globally. Materials & Method: A clinico-pathological analysis of 9 patients with histopathological diagnosis of multiple primary cancers over a 15years period. Cases with tumours affecting contiguous structures such as the urinary bladder, prostate and cervix uteri with similar tumour morphology are excluded. Results: Nine patients were histologically diagnosed with synchronous and metachronous multiple primary cancers from 2008 to 2022. The patients' ages ranged from 35 to 70 years and they presented with varying symptoms of breast mass, abdominal swelling, facial plaques, thigh mass, weight loss, vaginal bleeding and haematuria. Three patients aged 57, 50 and 47years had renal cell carcinoma in association with prostatic adenocarcinoma, penile squamous cell carcinoma and lipomatosis. Three patients had colonic adenocarcinoma, eosophageal squamous cell carcinoma and anal adenosquamous carcinoma in association with vulva basaloid carcinoma, thigh skin porocarcinoma and infiltrative ductal carcinoma of the breast respectively. The remaining three patients aged 35, 72 and 62years respectively had combinations of facial basal cell carcinoma with infiltrative ductal carcinoma, squamous cell carcinoma and hidradenocarcinoma and ovarian cystadenocarcinoma with uterine leiomyosarcoma. Treatments received included surgery, radiotherapy and chemotherapy. Two patients are alive after a 5year follow up, 3 died within 6months of second tumour diagnosis while 3 were lost to follow up and one is within a month of diagnosis. Conclusion: The primary cancers involved the Kidney, Ovary, Uterus, Vulva, Eosophagus, Skin while the second tumours involved the colon, skin, prostate, penis and breast. The metastatic deposits affected the chest wall, left femur and supraclavicular lymph node.

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Two cases of non-mass forming ALK positive lung adenocarcinomas diagnosed on cytology

Background

Anaplastic Large cell lymphoma Kinase (ALK) positive lung adenocarcinomas are known to have aggressive clinical behaviour with advanced tumour stage at initial presentation. Histopathological diagnosis and detection of ALK mutation are crucial for the treatment of these patients using targeted therapy. Here we present two cases of ALK positive lung adenocarcinomas where the patients presented without lung masses visible on imaging and the diagnosis and predictive biomarker testing were made solely based on cytology.

Case report

Both patients were young males (32 and 40 years respectively), with progressive shortness of breath. Patient 1 was a smoker, and was found to have a large unilateral pleural effusion on CT. Patient 2 a non smoker, had pleural effusion, nodular pleural thickening and mediastinal lymphadenopathy on CT chest. There were no suspicious masses in lung parenchyma in both. The differential diagnosis were wide based on these findings. Both underwent pleural tapping, and patient 2 underwent EBUS FNA from mediastinal node. Cytology samples from both patients showed atypical cells with features of adenocarcinoma. The diagnosis was confirmed by immunohistochemistry of the cell block preparation. Molecular testing for ALK, ROS1, PDL-1, EGFR and BRAF were performed on the cytology samples. ALK testing done using Ventana D5F3 rabbit monoclonal antibody showed positive staining. The other tests were negative. They both have been receiving Alectinib therapy for more than a year and are responding well clinically.

Conclusions

Absence of tumour mass on imaging studies is rare in invasive adenocarcinomas of lung, especially if ALK mutation is present. In cases like this, where the lung tumours are not detectable or reachable, cytology can be used as an effective tool for tissue diagnosis and predictive biomarker testing.

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A Case of Metastatic Melanoma With Cartilaginous Differentiation: variation of PRAME expression and other changes following targeted therapy

Introduction

Melanoma with cartilaginous differentiation is a very rare histological subtype with only a few cases described in the literature. Cartilaginous differentiation can occur in metastatic melanomas with or without cartilaginous areas in the primary melanoma. Distinguishing them from other chondroid tumours can be challenging, especially when cartilaginous differentiation is found in the metastasis. Immunohistochemistry becomes crucial in such cases to arrive at the right diagnosis.

Case report

A 70-year-old gentleman presented with a 2 month history of an enlarging axillary mass. Core biopsy of the mass showed an undifferentiated melanoma composed of sheets of rounded, pleomorphic cells with a high mitotic rate and areas of necrosis. The tumour was positive for melanoma markers S100, Melan A and HMB45 with strong and diffuse nuclear expression of PRAME. Molecular testing revealed BRAF mutation, and he was treated with targeted therapy using BRAF and MEK inhibitors. A suspicious pigmented skin lesion on the back showed clinical regression following the therapy with no evidence of residual primary melanoma on subsequent excision biopsy. However, the axillary mass showed enlargement and 6 months after initial diagnosis an axillary dissection was performed. Histology from the enlarged axillary lymph node showed complete necrosis of the undifferentiated melanoma but persistent nodules of cartilaginous tissue with atypical chrondrosarcomatous morphology. These cartilaginous areas expressed S100 strongly with weak and focal SOX10 and PRAME expression but were negative for HMB45 and Melan A.

Conclusions

Our case describes a rare histological subtype of melanoma where only the chrondosarcomatous elements persisted and enlarged following immunotherapy. In addition, to our knowledge, this is also the first documentation of variation of PRAME expression in melanoma with cartilaginous differentiation.

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Cutaneous actinomycosis of the foreskin; A case report

Actinomycosis is a relatively rare chronic granulomatous infectious disease caused by different species of *Actinomyces* bacteria. Cutaneous actinomycosis of the penis and foreskin is very rare, with only a handful of prior case reports published within the literature.

Here we present the case of a 22 year old uncircumcised male patient with no significant past medical history who presents with a palpable lump within the foreskin. He underwent a circumcision and the excised tissue was sent for histology. Within the sub-epithelial tissue was a large cavity with associated suppurative granulomatous inflammation which was filled with necrotic material and scattered bacterial colonies. Special stains including PAS and Grocott's methenamine silver stains highlighted gram-positive filamentous organisms within sulphur granules consistent with actinomyces species. After surgical excision, the patient was treated with a course of antibiotics and made a full, uncomplicated recovery.

Most previous cases of actinomycosis of the penis reported in the literature were associated with pilonidal sinus formation. In our case, whilst no definite sinus tract was present, ingrown hair shafts were seen within the surrounding tissues. It has been postulated that the accumulation of hair shafts beneath the skin surface induces a chronic inflammatory state which may predispose to secondary infection. Although the incidence of penile actinomycosis is extremely rare, it should be considered within the differential diagnosis of any palpable lump in this area.

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A case of Rosai Dorfman disease mimicking IgG4 related disease: A diagnostic conundrum.

Introduction: Rosai-Dorfman disease (RDD) is a rare histiocytic disease characterized by S-100 positive histiocytes exhibiting emperipolesis and involving lymph nodes and many other extra nodal sites. Recently, cases of RDD with increased number of IgG4 positive plasma cells have been described in literature, posing diagnostic challenge. Case presentation: A 41-year-old female presented with right sided nasal obstruction and level II cervical lymphadenopathy. Core biopsy examination of the lymph node revealed expansion of the interfollicular zone with increased number of IgG4 positive plasma cells (>100/ hpf), scattered eosinophils and S100 positive histiocytes. A possibility of RDD and IgG4 related lymphadenopathy was raised. Biopsy of the nasal polyp showed a dense inflammatory cell infiltrate with numerous plasma cells, lymphocytes and S100 positive histiocytes showing emperipolesis. The background contained IgG4 positive plasma cells, but these were not significant. The overall appearances were consistent with RDD with patchy increase in IgG4 positive plasma cells, involving cervical lymph node and nasal region. Conclusion: This case emphasizes the importance of excluding conditions like RDD, which can mimic IgG4 disease clinically and histologically before arriving at the diagnosis of IgG4 related disease. Emperipolesis, the hallmark feature of RDD, might not be seen abundantly in all biopsies. Likewise, IgG4 disease biopsies might not show characteristic features like obliterative phlebitis and storiform fibrosis. Therefore, multidisciplinary approach with correlation with clinical, serological, radiological, and histological features is advisable to diagnose these rare entities

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Impact of Covid-19 on the quality of reporting of colorectal mucosal biopsies in the diagnosis and assessment of inflammatory bowel disease

Background: Histopathology is essential in the diagnosis and classification of inflammatory bowel disease. Accurate reporting of histopathology ensures an optimum standard of patient care. The Covid-19 pandemic caused significant disruption to global healthcare practices that impacted resource availability and workflow. Aim: To assess the quality of reporting of colorectal mucosal biopsies taken for the diagnosis and assessment of inflammatory bowel disease. Method: Compliance with RCPath guidelines was measured retrospectively at Manchester Royal Infirmary over four study periods: pre-pandemic (February - April 2019), first wave (March - May 2020), between waves (June - August 2020) and second wave (December - February 2021). Results: Total number of specimens reported drastically decreased from 133 pre-pandemic to 32 during the first wave, and increased again by the second wave (n=85). Insufficient biopsy samples were provided to pathologists during the early pandemic: 37.5% were deemed adequate compared to 96.1% pre-pandemic. The quality of histopathology reports was greatly impacted by the pandemic. During the intermediate period: 13.3% of reports did not mention the number of tissue fragments and 16.7% did not describe the microscopic distribution of inflammation (both parameters achieved 100% pre-pandemic). The presence or absence of dysplasia was described in 84.4% of cases during the first wave and 90% of cases during the intermediate period, compared to 94.7% pre-pandemic. During the second wave, most parameters were comparable to and sometimes exceeding measurements taken before the pandemic, although the total number of specimens reported remained lower. A 7-day turnaround time during the second wave was met in 69.4% of cases, compared to 32.3% of cases pre-pandemic. Conclusion: The quality of tissue sampling and histopathological reporting was greatly impacted during the Covid-19 pandemic but started to improve by the second wave of infection.

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A Comparison of Breslow Thickness Measurement Using Light and Digital Microscopy

Purpose

Breslow thickness, as measured using a Vernier scale or eyepiece graticule on a light microscope, remains the strongest prognostic biomarker of cutaneous melanoma to date. Digital pathology affords opportunities to measure Breslow thickness digitally using a measurement tool within viewing software, but the accuracy of digital Breslow measurements has not been evaluated. This work aims to compare traditional Breslow thickness measurements on glass slides with digital measurements and assess their respective strengths as prognostic biomarkers. This work was funded by the Pathological Society's Undergraduate Elective Bursary.

Methods

63 specimens were selected at random from a sub-sample of a larger existing dataset, the Leeds Melanoma Cohort. Measurements were taken using a Vernier scale on a light microscope and on the corresponding digital slide. Measurements were validated by a board-certified pathologist using a graticule. Median difference between the two methods is presented, alongside a Bland-Altman plot for assessment of agreement. Multivariate cox proportional hazards regression models were conducted to assess overall survival and melanoma specific survival for the two measurements and their fit compared using Akaike's and Bayesian information criteria.

Results

The digital measurements were larger by a median of 0.03mm (95% CI: 0.02, 0.08). Most cases were within standard limits for agreement and had similar performance in modelling overall and melanoma specific survival, based on information criteria.

Conclusions

This small study has demonstrated similar measurements between each modality, with digital slide measurements being greater than glass measurements by 0.03mm. As such, digital slide measurements are unlikely to impact the current TNM8 AJCC staging system, which requires measurements to be provided to the nearest 0.1mm. This work does not provide evidence that digital measurement affects the strength of Breslow thickness as a prognostic biomarker.

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Metastatic skin sarcoma: A rare cause of intestinal obstruction

Background: Pleomorphic dermal sarcoma (PDS) is a rare primary skin malignancy on sun-damaged skin of elderly, possibly related to atypical fibroxanthoma. It carries 20-30% risk of metastasis to skin, lymph node and lung. Method: Here, we present a clinico-pathological analysis of a metastatic malignant spindle cell tumour causing small intestinal obstruction. Results: A 78-year-old male presented with features of intestinal obstruction and iron deficiency anaemia. CT showed acute small bowel obstruction with possible intussusception. Small bowel resection was performed and macroscopic examination revealed two large polypoid masses in the bowel. Histopathological examination of polypoid mass revealed a malignant spindle cell tumour that was positive for CD68, CD10 and Factor XIIIA. Later upper GI endoscopy revealed a prepyloric malignant polyp and on biopsy, the polyp showed a spindle cell tumour with similar morphology. Further clinical information identified that the patient had an exophytic lesion removed from the right forehead two years back, diagnosed as PDS on histological examination. The morphological appearances of the current small bowel and gastric tumour were the same as the previous skin sarcoma and the features were consistent with metastatic deposit of PDS causing small bowel obstruction. Conclusion: Though the occurrence is rare, PDS carries a significant metastatic potential. It is extremely rare to have deposits in gastrointestinal (GIT) system, causing anaemia and intestinal obstruction. This case emphasises the need to include metastatic sarcoma in the differentials of a pleomorphic spindle cell tumour in GIT without any known lineage.

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