Abstracts

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KEY TO SYMBOLS

℗ = Presenter

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Plenary Oral Abstracts

PL1

Tracking T-cell clonal dynamics across time and space in metastatic colorectal cancer

Purpose: This study characterised T-cell dynamics in metastatic colorectal cancer (mCRC) using longitudinal samples from different metastatic sites and through chemotherapy.

Methods: We developed and validated a new FFPE-compatible TCR sequencing method called "FUME-TCRseq" and used it to track T-cell dynamics in 216 archival samples from 15 mCRC patients who had multiple resections (range 2-7) over a median period of 10 years (range 7-16 years). In parallel we characterised DNA and methylation alterations, gene expression, and immune cell composition using digital pathology.

Results: We detected a median of 348 unique TCRs per sample (range 69-9700) and revealed high levels of intra-tumour spatial heterogeneity in the TCR repertoire. No significant differences were detected in the number of T-cell clones or the TCR clonality between primary tumours (n=40), lung metastases (n=30) and liver metastases (n=50). We found that compared to chemo-naïve tumours (n=36), those that had been exposed to recent chemotherapy (n=41) had a significant increase in both the number of unique T-cell clones and Simpson's evenness, likely reflecting a broad T-cell response to DNA-damaging agents. This was supported by digital pathology analysis of H&E stained sections.

We tracked the most abundant T-cell clones through metastases, identifying ubiquitous clones which persisted across metastatic sites and through therapy. We found other expanded clones which were no longer present in metastases, although some of these were found to expand again in later metastases. Finally, we examined correlations with tumour genomics, finding evidence in some patients that the TCR repertoire tracks with tumour clones.

Conclusions: This work represents the first comprehensive analysis of T-cell dynamics through colorectal cancer metastasis, highlighting the changing T-cell landscape post-chemotherapy. Our results could have important clinical implications for personalised T cell-based immunotherapy in mCRC.

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PL2

DCIS-associated myoepithelial cells drive transcriptional alterations in macrophages through up-regulation of integrin αvβ6

Ductal carcinoma in-situ (DCIS) is a non-obligate precursor of invasive breast cancer, however, less than 50% of DCIS will ever progress to invasive cancer. There is therefore a need to understand how we can predict those DCIS cases that will progress. A key component in promoting tumour invasion is the immune microenvironment, though its role in DCIS is unclear. We previously have shown that up-regulation of integrin αvβ6 on DCIS-associated myoepithelial cells switches the tumour-suppressor properties of myoepithelial cells to tumour-promoter properties, leading to enhanced tumour cell invasion. We found that macrophages surrounding ducts positive for αvβ6; exhibited a more M2 tumour-promoter phenotype compared to DCIS ducts with myoepithelial cells negative for αvβ6. We therefore hypothesised that the altered phenotype of myoepithelial cells in DCIS may directly influence the peri-ductal immune infiltrate which could influence disease progression. Macrophages were isolated from fresh tissue from patients with DCIS. These were confirmed as either positive or negative for myoepithelial αvβ6. The macrophages were subjected to RNA sequencing. In parallel, primary myoepithelial cells were isolated from cosmetic mammoplasty samples and used to overexpress αvβ6 using a lentiviral inducible system. Conditioned Medium (CM) was collected and this was applied to macrophages which then underwent RNA seq. RNA-seq analysis demonstrated novel transcriptomic profiles revealing upregulation of genes, e.g. AREG and FOSL2, with potential prognostic significance in DCIS. Utilising conditioned medium generated from primary myoepithelial cells with inducible expression of αvβ6, we demonstrate induction of immune checkpoint inhibitor - CD274 (PD-L1) in monocyte-derived macrophages in vitro. We identified a novel macrophage gene signature, outside of the usual M1/M2 dichotomy, pointing towards a macrophage spectrum which is driven by an in vivo αvβ6-dependent tumour progressive ME. This work thus suggest a panel of microenvironmental markers could be used to predict progressive DCIS cases and may represent novel therapeutic targets to influence a tumour suppressive immune response.

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PL3

Characterisation of HER2-Enriched signature in breast cancer and prediction of the risk of recurrence using fine morphometric features.

Human epidermal growth factor receptor 2 (HER2) oncogenic signalling pathway is the key driver of HER2 positive (HER2+) breast cancer (BC). However, this molecular subtype is highly heterogenous with variable response to HER2 targeting therapy. We aimed to characterise the molecular signature of HER2+ tumours through assessment of histomorphometric features with highlighting their impact on response to therapy targeting HER2 signalling pathways. Methods: We identified subcellular morphometric features in 289 breast cancer cases, validated in 71 cases with available HER2-E gene expression profiles from TCGA dataset. Using Artificial neural network models, we predicted HER2-E signature and post-therapy recurrence risk. The morphometric model's performance was compared to PAM50 using ROC curves. We also studied the impact of intratumor heterogeneity and ER status on these morphometric features. Results: HER2-E tumours are significantly associated with larger nuclear and cellular area, less tumour cell density, open phase nuclear chromatin, larger cellular spatial distance, compared to other molecular ($p<0.001$) including luminal B/HER2+. HER2 positivity was significantly associated with nested tumour pattern, cells with abundant pale and foamy cytoplasm and distinct cellular membrane ($p<0.001$). HER2+/ER positive tumours are significantly associated with high intratumour heterogeneity. The HER2-E predictive model had high accuracy (AUC=0.85) compared to the mean AUC of 0.73 for PAM50 genes expression. Our model accurately predicted patient recurrence risk AUC=0.87 in the external test set). Patients with high-risk scores were significantly associated with increased 5-year distant metastasis (HR:9.21, 95%CI:4.8-17.7, $p<0.001$) independently from other clinicopathological parameters. Conclusion: Evaluating HER2+ tumours histomorphometric features can significantly characterise HER2-E signature and predict patients with high risk of recurrence post adjuvant anti-HER2 therapy.

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PL4

Timing copy number alterations in Barrett's oesophagus in absolute time

In Barrett's oesophagus, a precursor condition to oesophageal cancer, the burden and pattern of copy number alterations (CNAs) predicts the risk of progression to oesophageal adenocarcinoma (OAC). We hypothesised that the temporal ordering and absolute timing of occurrence of CNAs may hold additional prognostic information, and also reveal new information about the evolutionary dynamics of OAC development from Barrett's.

We have developed a new method to infer the absolute time of CNA occurrence from whole genome sequencing (WGS) data by using passenger single nucleotide variants (SNVs) as a molecular clock. This method incorporates information encoded in the "neutral tail" of low frequency mutations, which allows for estimation of the time since the most recent common ancestor (MRCA) of each sample. We called this method: "Timing Copy number Alterations with Bayesian Inference and Evolution" (CARBINE).

Using simulated data, we showed that CARBINE accurately infers both the timings of CNAs and a sample-specific MRCA time. Applying CARBINE to a cohort of multi-region Barrett's oesophagus samples, we found a characteristic time-lag between the accumulation of specific CNAs and the progression to cancer. Notably, loss of heterozygosity (LOH) of the short arm of chromosome 17 (17p) carrying a *TP53* mutation occurred roughly 30 years prior to OAC diagnosis, whereas 9p regions bearing a *CDKN2A* mutation typically underwent LOH over 40 years prior to diagnosis. Thus, CARBINE reveals the temporal CNA dynamics across the genome.

Hence, we have developed a novel method to time CNAs in absolute time, accounting for the neutral and subclonal evolution within the tissue. We have used the method to give new insights into the timings of CNAs in Barrett's oesophagus and its progression to cancer.

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PL5*

Spatial transcriptomic profiling reveals novel biomarkers in EBV+ lymphoproliferations with Hodgkin-like features: The next generation of diagnostics

Purpose of study: Epstein-Barr virus-positive (EBV+) lymphoproliferative disorders (LPD) with Hodgkin-like features show overlapping morphological features, rendering diagnostic distinction challenging. This study aimed to identify novel diagnostic biomarkers in EBV+ LPD

Methods: Nanostring GeoMX spatial profiling of the whole transcriptome in the HRS/HRS-like and macrophage compartments was performed in EBV+ mucocutaneous ulcer (MCU) (n = 6; 27 regions of interest (ROI)), EBV+ classical Hodgkin lymphoma (EBVcHL) (n = 8; 11 ROI) and EBV+ diffuse large B-cell lymphoma (EBVDLBCL) (n = 6; 12 ROI) on formalin fixed paraffin-embedded (FFPE) sections.

Results: Dimensionality reduction (tSNE) based on the transcriptomic profiles in the HRS/HRS-like and macrophage compartments showed that EBVMCU, EBVcHL and EBVDLBCL clustered distinctly. Differential gene expression using a linear mixed effects model and gene set enrichment analysis revealed expression pathways that discriminate between the Hodgkin-like EBV+ LPD. EBVMCU, in comparison to EBV+cHL, was characterised by marked antiviral responses, showing upregulation of pathways involved in T-reg chemotaxis in HRS cells (odds ratio (OR) 120; p <0.0001), and interferon-alpha; (OR 50; p <0.0005) and interferon-beta (OR 40; 0 < 0.0004) responses in macrophages. Similarly, EBVMCU HRS-like cells, in comparison to EBVDLBCL HRS-like cells, showed upregulation of type 1 interferon responses (OR 20; p< 0.00025). The EBVDLBCL macrophage compartment was distinctive from EBVMCU by negative regulation of pathways involved in T-cell activation (OR 80; p = 0.00001) and IL10 production (OR 40; p < 0.0001). Overall, EBVMCU showed evidence of active inflammation and anti-viral response, whereas EBV+cHL and EBVDLBCL showed evidence of immune permissive, and immune suppressive microenvironments, respectively.

Conclusion: Gene expression profiling targeting HRS/HRS-like and macrophage compartments diagnostically discriminates EBV+ LPD.

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PL6*

Multi-regional profiling of rare non-small cell lung carcinoma subtypes

Purpose of the study

Although the majority of non-small cell lung carcinomas (NSCLCs) are classified as adenocarcinomas or squamous cell carcinomas, around 10% of cases do not conform to these categories. Adenosquamous, large cell 'not-otherwise-specified' and pleomorphic carcinomas are poorly understood and are generally associated with an inferior prognosis. Multi-regional profiling of these rarer subtypes within the TRACERx 421 cohort may offer new insights into their pathology.

Methods

Multi-regional samples were taken from primary resection specimens with tumour types including 14 adenosquamous carcinomas, 14 pleomorphic carcinomas, 6 large cell carcinomas, and 1 carcinosarcoma. Genomic and transcriptomic profiling was then performed. Mutational driver events, copy number profiles, evolutionary metrics and gene expression profiles were compared between rarer carcinoma subtypes and adenocarcinoma and squamous cell carcinoma.

Summary of results

Adenosquamous carcinomas harboured genomic and transcriptomic features of both adenocarcinoma and squamous cell carcinoma, including segmental 3q gains, normally associated with squamous cell carcinoma, in regions with adenocarcinomatous histology. The majority of pleomorphic carcinomas harboured multiple whole-genome duplication events, suggesting that this may be a key driver of this aggressive phenotype. Pleomorphic carcinomas were largely separable into two transcriptomic clusters based upon the presence of squamous or adenocarcinomatous nondivergent histology.

Conclusions

The study revealed considerable heterogeneity in these rare tumour types, and reveals biological similarities between some of the rarer tumours with adenocarcinoma or squamous cell carcinoma. These findings suggest greater complexity in the relationship between these subtypes than is currently reflected by the histological classification, which larger future studies may help to clarify further.

This work was supported by a JSPS predoctoral bursary.

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Rapid Fire Oral Abstracts

RF1

Profiling the evolutionary history of giant cancer cells in undifferentiated pleomorphic sarcomas: Hopeful monsters or an evolutionary dead end?

Background: As tumours evolve they accumulate somatic mutations that serve as an archaeological record of their evolutionary past. Undifferentiated pleomorphic sarcomas (USARCs) are malignant soft tissue tumours that often contain scattered giant cancer cells, or potential 'hopeful monster cells', histologically. As USARCs frequently undergo whole genome doubling (WGD) and chromothripsis (Steele et al, 2019), both mutational events are likely to contribute to the formation of giant cancer cells, which frequently display atypical nuclear features such as polyploidy, micronuclei and extrachromosomal DNA. Aims: This study aims to leverage single cell sequencing approaches to determine the evolutionary history and genomic landscape of giant cancer cells in USARCs. Methods: 112 giant cancer cells identified in 10 USARCs were isolated via laser capture microdissection (LCM) and underwent WGS. In addition, single cell ploidy estimation was performed in one USARC using FACs. Following single cell WGS, copy number aberrations and structural variants were inferred in single cells. Paired bulk tumour-normal WGS was also performed. Results: LCM isolated giant cancer cells exhibited extreme sub-clonal heterogeneity with some demonstrating de novo chromothripsis whilst others lacked chromothripsis events identified in matched bulk WGS. FACS analysis and copy number inference of single cancer cells also revealed the presence of three ploidy populations (1n, ~2n & 2n+) and successive rounds of WGD following a genome wide haploidisation event in one USARC. Conclusions: Chromothripsis and WGD emerges as major contributors to intra-tumour heterogeneity in USARC giant cancer cells. Single cell LCM and WGS reveals that some giant cancer cells lack chromothripsis events identified in bulk WGS suggesting evolutionary antiquity. Multiple sub-clonal WGD events also permitted one USARC to continue to explore its fitness landscape contributing to ongoing tumour heterogeneity and lethality.

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RF2

Differentiation signals via Grainyhead-like transcription factor 3 induce expression of the DNA mutating enzyme APOBEC3A in healthy and cancerous epithelial cells: implications for somatic mutagenesis and drug resistance.

PURPOSE: Apolipoprotein-B mRNA editing catalytic polypeptide (APOBEC) 3A (A3A) restricts viral replication through its ability to convert cytosine to uracil in single-stranded DNA and RNA, thus altering coding sequences. Misdirected A3A activity against the cellular genome during DNA replication is a major source of somatic mutations in cancer but the triggers of A3A expression remain largely unknown.

METHODS: We employed single cell RNA sequencing (scRNA-seq) of 10 head and neck squamous cell carcinoma (HNSCC) cases and 7 matched normal tonsil samples, in vitro studies using immortalised keratinocytes and cancer cell lines, analysis of published scRNA-seq and chromatin immunoprecipitation (ChIP)-seq datasets and immunohistochemistry to study the expression and regulation of A3A in normal and transformed epithelial cells. Single-cell regulatory network inference and clustering (SCENIC) was used to predict transcription factors responsible for regulating A3A and predictions were validated using RNA interference in cultured cells.

RESULTS: A3A was highly expressed in healthy tonsil epithelium, wherein it was largely confined to differentiating cells displaying high Grainyhead-like transcription factor 3 (GRHL3) activity. Accordingly, induction of keratinocyte differentiation was accompanied by A3A upregulation in vitro and targeting GRHL3 with short-interfering (si)RNA blocked A3A induction. GRHL3 binds to an enhancer 33kb upstream of the A3A transcriptional start site in differentiating keratinocytes, suggesting direct regulation. A3A was also expressed in differentiated tumour cells displaying high GRHL3 activity in HNSCC and oesophageal SCC and in rare cases, high A3A expression and GRHL3 activity was observed in S-phase cells.

CONCLUSIONS: High A3A expression is normally confined to terminally differentiating keratinocytes but aberrant GRHL3-driven A3A expression during DNA replication may represent an important cause of APOBEC signature mutations in cancer.

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RF3*

Self-supervised AI guides discovery of morphologies associated with recurrence in lung adenocarcinoma

Purpose of the study: Lung cancer is the leading cause of cancer-related mortality worldwide and adenocarcinoma is the most common subtype. Predicting benefit from adjuvant chemotherapy remains elusive and current prognostic metrics are based on some pathological parameters such as tumour grade, size and stage.

Methods: Histomorphological phenotype learning (HPL) ingests unannotated whole slide images (WSIs) and discovers recurrent morphological features, encoding them as histomorphological phenotype clusters (HPCs). Each tumour can is then expressed as composition vector of HPCs. We trained a model using 4426 WSIs from 1007 patients with resected primary lung adenocarcinomas. Sample tiles from each HPC were histologically reviewed. Clusters were cross-validated using recurrence-free survival. We then projected our clusters onto a cohort of 23 TMAs comprised of patient triplicates. One core per patient was sequenced using TempOSeq.

Results: We discovered 52 HPCs which, without supervision, not only recapitulate existing gold standard grading but identify new prognostic groups with clear morphological and biological meaning. The most lethal HPCs were all defined by the presence of highly infiltrative single cells and ill-formed acini in dense collagenous and proliferative stroma. Conversely, prognostically favourable HPCs were characterised by low grade appearances and high lymphocyte burden. HPC-led prognostication exceeds that of grading and certain HPC interactions reveal associations with modes of recurrence.

Conclusions: HPCs defined by high stromal cellularity are associated with poor outcome and recurrence. Further work is required to quantify cellular content across HPCs and define phenotypic neighbourhoods to gain mechanistic insight.

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RF4

Evolutionary Dynamics of Phenotypic Plasticity in Metastatic Colorectal Cancer

Background: Metastasis is the main hallmark of cancer causing most cancer-related deaths. Efforts to precisely unravel the underlying evolutionary and molecular mechanisms of metastasis development so far had only limited success. Previously, we have found that phenotypic plasticity, the propensity to change a phenotype independent of the genotype, is established early in primary colorectal cancer (CRC).

Methods: To investigate its role in metastatic CRC and the impact on evolutionary trajectories we produced a comprehensive multi-omics dataset from patient-derived peritoneal metastatic sites. 133 lesions from 20 patients (median: 6 per patient) have been spatially dissected into 440 sub-pieces and co-isolated bulk DNA/RNA was subjected to whole genome/exome sequencing (WGS: n=432, WES: n=90) and matched RNA-Seq (n=148).

Results: Using genetic lineage tracing we find evidence for frequent genetically defined polyclonal seeding at distant metastatic sites but also within a single site. Within individual genetic clones, we observed largely absent karyotypic diversity. Single nucleus WGS in five metastatic lesions showed new CNAs at a detectable rate, but these did not seem to clonally expand, suggesting a high degree of stabilising selection. At the phenotypic level, matched RNA-Seq measurements revealed distinct gene expression clusters. However, these clusters did not necessarily correspond to metastatic sites of the same patient and were mostly not reflected by their genetic ancestry. Single nucleus ATAC profiling of sequential metastatic biopsies revealed profound changes in the phenotypic makeup of fibroblast and macrophage subpopulations. Following chemotherapy, these were partially reverted to patterns observed in normal peritoneum.

Conclusion: Our findings might indicate early evolutionary adoption of pervasive metastatic competence and phenotypic plasticity in metastatic clones as well as a propensity for extensive remodelling of the metastatic niche.

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RF5

An Investigation of Polygenic Risk Scores for Disease Susceptibility and Prognostic Prediction in an Inflammatory Bowel Disease Cohort

Polygenic Risk Scores (PRS), which describe an individual's genetic liability to a trait, are likely to play a key role in the development of personalised medicine approaches. Here we investigate the efficacy of using PRS to differentiate patients with Inflammatory Bowel Disease (IBD) from healthy controls and predict disease severity. Individuals were genotyped and imputed SNPs were used to calculate PRS. Predictive power of PRS, generated using a range of significance thresholds, were compared using area under the receiver operating characteristic curve (AUC). PRS was tested for associations with clinical characteristics and disease outcomes in followed-up patients. All PRS were associated with IBD status with the PRS calculated using all available variants having the strongest association with disease status (p=2.32x10-6) and the most predictive power (AUC=0.612). Patients with treatment escalations had statistically higher PRS (p=0.018). Patients with extensive disease and higher CRP at diagnosis showed a trend to higher PRS scores. Our results show including more variants in the PRS improves predictive accuracy, however an AUC of 0.61 limits diagnostic and clinical utility at present. PRS may be improved by including rarer susceptibility variants or clinical data. The results also show disease susceptibility and outcome are likely influenced by different underlying genetics and future directions should look to develop a useful PRS to predict worse disease prognosis using variants associated with outcome. Additionally, the results highlight the value of integrating PRS with diverse clinical data to understand the role of PRS in prognosis.

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Poster Abstracts

P1

A Homicide in Disguise: How the Autopsy Dug up Clues

An autopsy is performed in the occurrence of an out-of-the-ordinary manner of death where the cause of death is unclear. Through a detailed medicolegal investigation, it differentiates homicide from suicides or accidents. However, some people do not acknowledge its importance due to the conflict between science and religion. This is especially true for countries with a lack of education and awareness. The family of the deceased may be unmindful of medicolegal matters and hesitate to allow for an autopsy. In the instance that burial takes place before an autopsy was performed, the medicolegal officer requests for an exhumation. It is the act of digging up a body from its grave to be examined in more detail. Such was the case in our study. A dead body was retrieved from a water channel in the Sindh province, assumed to have accidentally drowned. The family held the funeral before an autopsy was performed. Later, suspicions arose surrounding the death, so the body was exhumed. The soft tissues were decomposed and unidentifiable. The examination suggested strangulation owing to the pivotal discovery of a fractured hyoid bone at the tip of the greater horn of the right cornu. Chemical tests came out negative for intoxication. Therefore, the cause of death was concluded to be asphyxia due to throttling, secondary to hyoid bone fracture. Currently, technology was developed to introduce advanced tests in forensic sciences to differentiate multiple causes of drowning. However, the dissatisfactory budget limits forensic experts in their work. There is little use in testing for diatoms to rule out drowning, as it has been proved to show discrepancies sometimes leading to a false-positive result. Hence, alternative methods need to be explored for a more efficient approach to find the cause of death.

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P2*

External validation of a digital pathology-based multimodal artificial intelligence-derived (MMAI) model in advanced prostate cancer patients starting long-term androgen deprivation therapy (ADT): an ancillary study of four phase III trials of the STAMPEDE platform protocol

Purpose of Study

Effective prognostication will improve targeting of treatment combinations in advanced prostate cancer. The ArteraAI Prostate MMAI prognostic test was recently developed in localised prostate cancer. We aimed to validate this test using final data from four phase III STAMPEDE trials (NCT00268476).

Methods

We included patients starting ADT in the docetaxel, docetaxel + zoledronic acid, abiraterone, or abiraterone + enzalutamide trials. The MMAI model used digitised prostate core biopsy slides, Gleason score, tumour stage, age & serum PSA. Fine-Gray/Cox regression adjusted for treatment allocation & cumulative incidence analyses were performed to evaluate associations with prostate cancer-specific mortality (PCSM) for continuous (per standard deviation increase) & categorical (quartile-Q) scores.

Results

Of 5132 eligible patients recruited 05/10/2005 - 31/03/2016, we included 3167 (1575 locally-advanced (M0), 1592 metastatic (M1); median follow-up: 6.9 years). MMAI score strongly associated with PCSM (HR 1.72 [95%CI 1.58-1.87], p<0.001). Ad hoc inspection of cumulative incidence curves identified MMAI Q4 (versus (v) Q1-3) as having the highest PCSM: 16% [12-19] v 5% [4-7] in M0 (HR 2.38 [1.82-3.12], p<0.001); 58% [53-63] v 39% [36-42] in M1 (HR: 1.66 [1.43-1.93], p<0.001). Quartile stratification by MMAI score significantly improved predicted 5-year PCSM in M0 patients over nodal status (node-negative: 5% [4-6] split Q1-3: 3% [2-4] v Q4: 11% [7-15]; node-positive: 13% [10-16] split Q1-3: 11% [8-14] v Q4: 20% [13-26]); in M1 patients, compared to metastatic volume (low-volume: 31% [27-34], Q1-3: 27% [23-31], Q4: 43% [36-51]; high-volume: 53% [50-57], Q1-3: 48% [44-52], Q4: 68% [62-75]).

Conclusions

MMAI score is prognostic in high-risk localised & metastatic prostate cancer, improving on disease burden for predicting death from prostate cancer in patients with & without intensified systemic treatment.

Project supported by a JSPS Clinical PhD Fellowship.

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P3

Comparing Coronial Post-mortem Data Pre- and Post-COVID: A Multilocational Analysis of Urban and Suburban Settings

Purpose of the study: We aim to review the characteristics of Coronial post-mortems since the end of COVID-19 pandemic-related restrictions and compare these to the pre-pandemic post-mortem landscape.
Methods: We reviewed 913 Coronial post-mortem reports produced from January to December 2019 and April 2022 to March 2023. One quarter of these was performed in a high-density urban setting, the other three quarters were performed in a semi-rural, suburban area, allowing further comparison between these different population cohorts.
Summary of results: There has been an increase in the mean length of time from death to post-mortem, which has more than doubled since 2019 (p=0.000). The frequency of severely decomposed bodies is greater than 2019 (p=0.000), driven by the suburban population group rising from 2% to 11% of cases, now a similar percentage to the urban group. There were fewer post-mortem cases which had a recorded pre-mortem history of mental health disease (p=0.002), and this was even in cases which were likely self-inflicted deaths (p=0.000); only 64% of these cases had a pre-mortem recorded mental health history in 2022/23 compared to 88% in 2019.
Conclusions: Our study has shown that some of the changes which affected the Coronial post-mortem landscape during the COVID-19 pandemic persist three years on. Some of these are likely to have a negative impact on national or local post-mortem service provision. The significantly increased delays between death and post-mortem are suggestive of a system in looming crisis. The increased frequency of severely decomposed bodies, especially in the suburban population, replicate findings from previous studies carried out during the pandemic, raising concerns about persistent social isolation.

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P4

Maintaining Standards in an Era of Vanishing Practice: Completion of Hospital Post-Mortem Authorisation Forms in Tayside, January 2022 - April 2023.

Purpose of the study: Relatively few adult hospital post-mortems are performed in Tayside annually. Local clinicians infrequently undertake post-mortem authorisation. There is no locally available tool for clinicians to refer to when completing authorisation. This audit evaluates completed adult hospital post-mortem authorisation forms, and recommends improvements to this process.

Methods: Authorisation forms for adult hospital post-mortems undertaken in Tayside between January 2022 - April 2023 were reviewed retrospectively. Documented information was compared to national guidance and legal standards set by The Human Tissue (Scotland) Act 2006. Resources on post-mortem authorisation used in other health boards were identified. Results were discussed with consultant pathologists who undertake hospital post-mortems.

Summary of results: 17 hospital post-mortems were undertaken between January 2022 - April 2023. 12 forms authorised a full post-mortem; 2/12 later stated specific organ systems to be examined only, despite authorising a full post-mortem. 5 forms authorised a limited post-mortem; 1/5 later ticked examination of "head, chest, abdomen" which is regarded as a full post-mortem. 9/17 forms did not authorise retention of organs; 2/9 later documented use of blocks and slides from retained organs. 1 form authorised removal/retention of the brain, but did not authorise examination of the head. 1 form authorised removal/retention of the brain; it was not stipulated that the brain was in fact to be returned to the deceased following examination. 12 authorisation forms demonstrated missing information.

Conclusions: It is challenging for clinicians to gain experience in authorising hospital post-mortems, in light of dwindling numbers being undertaken. In this audit, the majority of forms (71%) contained missing information; 7 forms (41%) demonstrated contradictory information. Local guidance to facilitate appropriate completion of authorisation forms is under construction.

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P5

Reassessment of SVUH Autopsy Blocking-Out Form Retention Compliance (Previously Audited in 2019)

A reaudit encompassing a review of 102 adult non-consented coronial autopsy reports at St. Vincent's University Hospital in the year 2021 was conducted on 10/07/2022, referring to Histopathology-Control of Process and Quality Records in Histopathology (Page 2 of 4)-MF-HISRECON and LF-IHS-AUTBL- to ensure each autopsy was accompanied by a corresponding blocking out form as requested by department SOP- The retention and storage of pathological records and specimens (5th edition) -Guidance from The Royal College of Pathologists and the Institute of Biomedical Science-(ref. paragraphs 39-41). The St. Vincent's Healthcare Group Department of Pathology and Laboratory Medicine Histopathology-Control of Process and Quality Records in Histopathology (Page 1 of 4) stipulates that autopsy blocking sheets should be preserved for two years. In 2021, 102 post-mortems were conducted, whereas in 2018, 132 post-mortems were performed. Among the autopsy reports, 63.7% included the additional post-mortem blocking-out forms, while 36.2% did not contain these supplementary forms and were not retained. The percentage difference between 2018 and 2021 for autopsy reports incorporating the supplementary post-mortem blocking-out forms was 15%. The percentage difference between 2018 and 2021 for autopsy reports that did not include the supplementary post-mortem blocking-out forms was 14%. To ensure compliance with post-mortem documentation, it is recommended to emphasize the retention of post-mortem forms during introductory NCHD training. Regular reminders and discussions about these procedures at weekly consultants' meetings and quarterly departmental QA meetings are also advised to ensure adherence to the guidelines. It is essential to audit the implementation of these recommendations in 12 months to ensure ongoing compliance and effectiveness.

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P6

A rare case report of frontal sinus meningoencephalocele in an adult presenting with cerebrospinal fluid leak

Meningoencephalocele is a condition characterised by the herniation of intracranial contents within a sac, including brain tissue and meninges, through a cranial defect. This rare condition is primarily seen in paediatrics, often due to neural tube congenital defects. Nonetheless, meningoencephaloceles have been reported in adults, as a result of trauma to the brain or skull, and in spontaneous cases.

This is a case report of an adult-onset meningoencephalocele of the frontal cortex and meninges into a frontal sinus. A 46-year-old female presented with left nasal rhinorrhoea with obstruction and frontal headache, who was previously investigated for idiopathic intracranial hypertension (IIH). The rhinorrhoea was confirmed to be cerebrospinal fluid (CSF) leak. MRI brain showed 2 areas of dehiscence and protrusion of brain - first in the anterior skull base within the left posterior ethmoidal air cells, and second in the left frontal sinus via inferior tracks through the frontoethmoidal recess. Both areas were surrounded by CSF. The appearances were considered representative of IIH decompression. The patient underwent an endonasal repair and the frontal sinus defect was biopsied.

Histopathological examination of the biopsies revealed fragments of neural tissue surrounded by a fibrous connective tissue showing foci of mixed inflammation. Focally, the cortical layers were seen. The features were in keeping with a meningoencephalocele from a frontal cortex protrusion and its meninges via the skull base defect into the frontal sinus, which led to a CSF leak and surrounding chronic inflammation.

Meningoencephaloceles extending through the base of the skull and facial bones have been well-documented, however, cases that extend through a frontal sinus defect are rarely reported in literature. Thus, this case report contributes to the limited body of current knowledge, with the aim to aid the diagnosis of this challenging and uncommon condition.

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P7

This abstract was withdrawn.

P8

Characterisation of HER2-Enriched signature in breast cancer and prediction of the risk of recurrence using fine morphometric features

Human epidermal growth factor receptor 2 positive (HER2+) breast cancer (BC) is highly heterogenous with variable response to HER2 targeting therapy. We aimed to characterise the molecular signature of HER2+ tumours through assessment of histomorphometric features with highlighting their impact on response to therapy targeting HER2 signalling pathways. Methods: Using digital image analysis, we identified a set of fine subcellular morphometric features associated with HER2 oncogenic signalling activity in a discovery cohort of 289 BC cases and validated them on 71 cases from the cancer genome atlas (TCGA) with available HER2-enriched (HER2-E) gene expression profile and PAM50 molecular subtyping. Artificial neural network models were then developed to predict both HER2-E molecular signature, and risk of tumour recurrence post adjuvant anti-HER2 therapy and the performance was compared against the PAM50 gene assay. The impact of intratumour heterogeneity and oestrogen receptor (ER) status on subcellular morphometric features was also assessed. Results: HER2-E tumours are significantly associated with larger nuclear and cellular area, less tumour cell density, open phase nuclear chromatin, larger cellular spatial distance, compared to other molecular ($p<0.001$) including luminal B/HER2+. HER2+/ER positive tumours are significantly associated with high intratumour heterogeneity. The HER2-E predictive model had high accuracy (AUC=0.85) compared to the mean AUC of 0.73 for PAM50 genes expression. Our model accurately predicted patient recurrence risk AUC=0.87 in the external test set). Patients with high-risk scores were significantly associated with increased 5-year distant metastasis (HR:9.21, 95%CI:4.8-17.7, $p<0.001$) independently from other clinicopathological parameters. Conclusion: Evaluating HER2+ tumours histomorphometric features can significantly characterise HER2-E signature and accurately predict patients with high risk of recurrence post adjuvant anti-HER2 therapy.

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P9

Histological types of invasive breast cancer in 830,000 women diagnosed in England during 1988-2016

Purpose of the study: There is some evidence that different breast cancer histological types have distinct characteristics, but few previous studies have been large enough to explore this systematically, or to consider rare histological types. We aimed to investigate the incidence and characteristics of different histological types of breast cancer in England.

Methods: We used national cancer registration data to describe trends in incidence of specific histological types of invasive breast cancer in women diagnosed when aged 18-89 in England from January 1988 to December 2016, and to investigate associations between breast cancer histological types and other patient and tumour characteristics.

Results: 838,776 women were diagnosed with a first primary invasive breast cancer in this 29-year period, including 614,698 (73%) cases of ductal carcinoma NST (no special type), 90,028 (11%) lobular carcinomas, and more than 16,000 (2%) each of tubular and mucinous carcinomas. Rarer histological types included medullary, metaplastic, papillary and cribriform carcinomas, with >1000 cases of each type. Data quality and completeness improved substantially during the study period. The different histological types of breast cancer showed different patterns in incidence by calendar period of diagnosis, age at diagnosis, and breast screening status, as well as different associations with tumour characteristics such as grade, stage at diagnosis, and molecular subtype.

Conclusions: This large national study provides an overview of incident invasive breast cancer in England over almost 30 years and gives an opportunity to investigate the characteristics of rare histological types, which smaller studies have been unable to explore. The results also reveal the value of histological types defined by microscopic morphology, in addition to newer molecular classifications.

Funding: Cancer Research UK (grants C8225/A21133, PRCRPG-Nov21\100001); National Institute for Health Research (CL-2017--13-001).

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P10

Gene regulated by oestrogen in breast cancer 1 (GREB1) as a surrogate oestrogen receptor biomarker

Purpose of the study: A significant proportion of ER-positive BCs fail to respond to endocrine therapy, which denotes that the response is not totally dependent on ER expression. We aimed to validate the expression of GREB1 as an ER-target gene using immunohistochemistry (IHC), and its possible prognostic significance.

Methods: A well-characterised BC cohort (n=1281) was stained. The cytoplasmic expression of GREB1 was tested in all BC molecular subtypes. Correlation with breast cancer-specific survival was carried out to test the outcome differences between ER-GREB1 co-expressing BCs and ER+/GREB1- tumours. Publicly available data on GREB1 mRNA expression were also retrieved from bc-GenExMiner, where correlation and prognostic analyses were performed. Summary of results: GREB1 was expressed in 64% of ER+ BC. In triple-negative BC, only 4 out of 76 cases were positive, while none of the HER2+/ER- cases showed GREB1 expression. Considering ER status, GREB1 showed 97% specificity to ER+ BC. Within ER+ /progesterone receptor (PR)+ tumours, positive GREB1 expression was noted in 71% of them. A significant association with favourable outcome was shown between ER+ BC expressing GREB1 when compared to GREB1- BC. GREB1 expression when analysed in ER+/PR+ tumours showed significantly favourable BCSS (p=0.02) in GREB1+ than GREB1- tumours. GREB1 was shown to be an independent prognostic marker in ER+ tumours when tested in a multivariate analysis adjusted for grade, tumour size and LN status (HR=0.6, 95% CI=0.4-0.8, p=0.001). Data on GREB1 showed a positive significant correlation (r=0.6, p<0.001) to ESR1. A significantly favourable overall survival and disease-free survival were shown in tumours expressing high GREB1 compared to low GREB1 expression.

Conclusions: GREB1 is a potential ER surrogate biomarker with high ER specificity. Identification of well-established ER-target genes could serve as a guide for patient risk stratification and targeted therapies.

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P11

The prognostic significance of stroma-tumour ratio combined with the immunity status in different molecular subtypes of breast cancer

Previous studies have demonstrated that the proportion of the stroma to tumour cells (stroma-tumour ratio (STR)) has a prognostic significance in breast cancer (BC). However, variable results were observed in the different molecular classes. In this study, we hypothesised that the mechanisms of stromal formation and composition are different in the various molecular classes, which could explain the different prognostic values. Material and methods: This study interrogated two large well-characterised BC cohorts. First, the public domain dataset (The cancer genome atlas data (TCGA), n=978) was used for stromal assessment including STR, transcriptomic profiling and encompassed tumour-infiltrating lymphocytes (TILS). Differential gene expression (DGE) analysis was performed to identify a set of genes associated with high STR in the three main molecular subtypes. The role of immune response measured as the contribution of TILs to the stroma has been also investigated. Correlation with patient outcome was carried out on TCGA and validated on an in-house BC cohort (n=834)

Results: High STR was associated with favourable patient outcomes while in luminal BC while high STR in TNBC showed an association with poor outcomes. DGE analysis identified various pathways in luminal and TNBC subtypes, with immune upregulation and hypoxia pathways evident in TNBC, unlike the luminal group whereas the pathways related to fibrosis and stromal remodelling. Low STR accompanied by high TILs were shown to carry the most favourable prognosis in TNBC. In line with the DGE results, TILs played a major prognostic role in the stroma in TNBC but not in the luminal or HER2-enriched subtypes. Our study provides insights into BC stroma and explains some of the conflicting results regarding the prognostic significance of the stroma in BC. While hypoxia and immune response are involved in the mechanism of stroma formation in TNBC, different mechanisms are involved in the luminal and HER2-enriched types.

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P12

Evaluation of tenascin-C expression in breast cancer tissue following neoadjuvant chemotherapy

Purpose

Tenascin-C is a matricellular glycoprotein involved in cancer invasion and metastasis. While its role in breast cancer has been investigated, it has not been studied exclusively in breast cancer patients receiving neoadjuvant chemotherapy (NACT). This is a group of patients who can benefit from detailed pathological assessment owing to an increased risk of local recurrence and poor prognosis.

Methods

Retrospective cohort analysis was conducted on twenty-four breast cancer patients who had undergone NACT. Immunohistochemical staining was performed using monoclonal anti-tenascin C antibody on formalin-fixed paraffin-embedded post-operative breast specimens. The distribution and intensity of tenascin-C expression was analysed and scored using pre-determined histological criteria. These scores were correlated with clinicopathologic characteristics and PREDICT breast cancer prognosis scores.

Results

Tenascin-C was well expressed by post-NACT breast cancer tissue, of which the tumour-associated stroma showed stronger expression than the tumour cells. More pronounced expression was seen in PR-positive patients. In the tumour-invasive border, greater expression of tenascin-C correlated with ER-negativity, triple-negative breast cancer and absence of lymph node involvement. In the central region of the tumour, greater expression correlated with better pathologic response to NACT. Areas of response to NACT and areas of residual cancer showed no significant difference in intensity of tenascin-C expression. Patients with better post-surgical PREDICT 10 scores showed greater tenascin-C expression in the tumour.

Conclusions

Tenascin-C is expressed in the stroma of post-NACT breast cancer tissue, and may be related to tumour phenotype, pathologic response to NACT and prognosis. Investigation with follow-up patient outcomes and a larger cohort can shed further light on the utility of tenascin-C as a predictor of response to NACT, or as a prognostic marker for breast cancer.

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P12

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P13

The Role of the Glutamine transporter SLC1A5 in breast cancer progression and immune interactions

Breast cancer affects a large number of women worldwide and is a major concern (Sun et al, 2017). SLC1A5 is a transporter that regulates the transport of amino acids, including glutamine (Kanai and Hediger, 2004). We hypothesised that the SLC1A5 gene could be used as a biomarker of breast cancer progression. The significance of SLC1A5 as a predictive and prognostic biomarker was assessed using online bioinformatic tools including KM plotter, ROC plotter, and TISDIB. SLC1A5 is a marker of poor prognosis at the protein level. The effects are dependent on the stage and the enrichment or depletion of immune compartments. Overexpression of SLC1A5 confers a negative prognosis in stage 1 when macrophages and natural killer T cells are enriched, and Type 1 and 2 T helper cells are reduced. Overexpression also confers a poor prognosis at stage 2 when basophils are depleted and at stage 3 when mesenchymal stem cells are depleted. However, there are specific situations in which overexpression of the gene confers a good prognosis, specifically enrichment of type 1 T-helper cells in stage 1 and enrichment of basophils at stage 2. SLC1A5 correlates with tumour infiltration of type 17 helper cells and the expression of the immunostimulatory genes CXCL12, ENTPD1, CD86, and PVR, the chemokine CXCL14 and the chemokine receptors CCR6, CCR4, and CX3CR1. Overexpression of SLC1A5 confers resistance to anthracyclines but has a sensitizing effect on the response to FEC in luminal A breast cancers. The gene also has a sensitising effect on the response to Tamoxifen, Aromatase inhibitors, and FEC in luminal B breast cancers. In triple-negative breast cancer (TNBC), the gene had a sensitising effect on response to CMF. In conclusion, SLC1A5 is a poor prognostic marker in breast carcinoma and its interactions with the immune system suggest that it may play a role in immune evasion.

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P14

The presence of internal controls for breast markers, not including breast biomarkers ER, PR, and Her2

Background: Immunohistochemistry (IHC) is important in breast pathology and is commonly requested in routine practice. The validity of IHC is usually based on the appropriate use of external control (EC). Here we speculate that internal control (IC) is a reliable indicator of the success of IHC and safely replaces EC.

Design/Methods: We assessed the presence/absence of internal controls for SMA, E-cadherin, p120, CK5/6, p63, SMM, CK14, MIB-1, D2-40, S100, SOX10, CD68, Inhibin, CD34, p16, Desmin, AE1/AE3, CAM5.2, + Androgen receptor on 100 consecutive breast specimens. The study does not include assessment of ER, PR, and Her2.

Results: 91/100 specimens contained appropriate internal control that could be assessed positive/negative and confirm satisfactory IHC staining. However, the role of EC simply confirmed the IHC results. In 9 cases no IC was present.

Conclusion: Most breast specimens (91/100) did contain IC. In the 9 cases IC was missing, and the pathology interpretation was not affected. We believe that EC could be safely omitted when performing IHC on breast specimens, saving pathologists and BMS time.

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P15

Assessing breast core biopsies for hormone receptors and HER-2 stains with and without external control

Purpose of study

Immunohistochemistry (IHC) for hormone receptors (ER, PR) and HER-2 in breast core biopsies is usually accompanied by external control (EC). This study aims to determine whether the assessment can be performed without the support of EC.

Methods

A total of 100 consecutive cases of invasive breast carcinoma core biopsies (B5b), already being reported according to gold standard guidelines for ER, PR, HER-2 and the EC, were reassessed without considering the EC results.

Summary of results

There was a 100% consultant agreement between the reassessment of cases without the contribution of the EC and the original assessment performed, in the presence of the EC, by the reporting pathologist.

Conclusions

In this study, normal ductal structures of the breast were used to assess the IHC reactions. Therefore, we believe that EC could be safely omitted for ER and PR IHC on the breast specimens thus, reducing biopsy turnaround times. HER-2 is an exception; this protein overexpression is not present in normal breast tissue and thus, requiring the EC.

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P16

Deciphering the Mystery: Investigating B3 Lesion Outcomes in a Single-Center Study

Background: In the age of breast cancer screening, the diagnosis of breast lesions with uncertain malignant potential, known as B3 lesions, is quite common. The approach to managing these lesions is a subject of ongoing debate. These B3 lesions can be removed surgically or by vacuum assisted excision. While the risk of these lesions progressing to breast cancer is acknowledged, there is a significant lack of data regarding their role as a risk factor for future breast cancer development.

Methods: We had a review on 100 B3 lesions that were diagnosed within our institution. All of them had follow-up information available. These B3 lesions encompassed various types, including flat epithelial atypia (FEA), atypical ductal hyperplasia (ADH), lobular intraepithelial neoplasia, papillary lesions, radial scars, cellular fibroepithelial/spindle cell lesions, among others.

Results: Immediate upgrade to breast cancer (either invasive or in situ) was observed in 23% of cases. Highest risk was associated with ADH diagnosis, where 55% of patients progressed to B5 in the subsequent resections. One out of 13 cases of cellular fibroepithelial lesions turned out to be a metaplastic carcinoma. Cases of flat epithelial atypia remained in B3 category. Radial scar/complex sclerosing lesions did not exhibit any progression to carcinoma. Intraductal papillomas displayed different outcomes: 65% showed no atypia, 16% progressed to B3 with atypia, 16% progressed to DCIS, and 3% advanced to invasive carcinoma. There were two cases of phyllodes tumors, with one being benign in subsequent resection and the other being malignant. A single case of lobular neoplasia was reclassified as DCIS during subsequent resections.

Conclusion: These findings lend support to the idea of surgical removal of B3 lesions and it also supports the idea of consideration of annual screening mammography for women with B3 diagnosis because of increased risk associated with it.

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P17

A case of primary angiosarcoma of the breast in a young woman

Introduction: Nonepithelial malignancies that arise from the connective tissue within the breast are extremely rare compared to the incidence of epithelial malignancies in the breast and account for less than 1 per cent of all breast malignancies. Of those, angiosarcomas of the breast are malignant endothelial cell derived tumours which may develop spontaneously as a primary breast malignancy (primary angiosarcoma), often in younger women aged 20--40 years, or may occur secondary to lymphoedema or radiotherapy in women treated previously for breast cancer. We report a case of a primary angiosarcoma of a young otherwise healthy female.

Case report: A 25-year-old woman was presented with a painful enlarging lump in her right breast for one year. There was no skin changes or nipple discharge. US scan showed a 11cm lesion with internal cystic areas and some hypoechoic elements. Core biopsy examination of the breast lesion showed a vascular lesion with some intervening adipose tissue and raised the possibility of a hamartoma and reported as B3 category. Excision and histological assessment were recommended at the multidisciplinary team meeting. Excision biopsy showed a haemorrhagic and fleshy soft tissue mass with a 70mm of maximum diameter. Microscopic examination showed an infiltrative vascular tumour with irregular vascular channels and solid areas comprising pleomorphic spindle cells with areas of necrosis and blood lakes. The tumour cells were positive for CD31, EGR and CD34 and negative for AE1/AE3. Mib1 proliferation index was high. The overall features are of a high grade angiosarcoma, and lesion involved the margins.

Conclusion: High grade angiosarcoma may contains low or intermediate grade elements, especially at the periphery of the tumour and these areas have deceptively benign appearance on histology. Therefore, solid-appearing vascular breast tumour in a young woman at the time of biopsy should be considered malignant until proven otherwise.

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P18

Investigation of differentially expressed genes in ER+PR- invasive breast cancer

Purpose of the study: A more robust understanding of the complex molecular pathways that lead to invasive breast cancer (IBC) development and progression is necessary to improve prognostication and develop more effective treatments for IBC. This study aimed to identify potential candidate genes and clarify the underlying molecular mechanisms that regulate estrogen receptor-positive (ER+) and progesterone receptor-negative (PR-) IBC.

Methods: We applied an integrated bioinformatic approach to analyze clinical outcomes data from the Cancer Genome Atlas (TCGA). Differentially expressed genes (DGEs) were identified using MultiExperiment Viewer. The functional enrichment analysis was performed using PANTHER, Metascape tool, and PANNZER. Protein- protein interaction network analysis for DEGs was established through STRING. The association between the expression of hub genes and the tumour features, and patient outcome was performed.

Results: A total of 33 upregulated and 88 downregulated genes were identified in ER+PR- IBC. The upregulated genes are mainly involved in regulating cell-cell adhesion, cell proliferation, cell cycle, cell division, mitosis, and autophagy. In contrast, the downregulated genes are responsible for the negative regulation of immune system processes and lymphocyte migration. The protein-protein interaction and gene-gene co-occurrence analysis revealed that the upregulated hub genes PIP4K2C, CDH1, and CLTC are densely connected to key nodes. High expression of PIP4K2C, CDH1, and CLTC were associated with aggressive tumour features including lymph node positive, group 3 Nottingham prognostic index, and histological type (all $P<0.05$). High PIP4K2C, and CDH1 expression were associated with short disease-free survival (all $P<0.05$).

Conclusion: This study provides insight into molecular-level mechanisms and related molecular biomarkers that could be exploited to improve the prognosis of patients with the ER+PR- IBC subtype.

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P19

A 5-year audit of the reporting of neoadjuvant breast cases against the ICCR reporting guidelines

There is an increasing role for neoadjuvant therapy in the treatment of breast cancer. In 2023, the International Collaboration on Cancer Reporting (ICCR) published a dataset for standardised reporting of breast cancer resection specimens following neoadjuvant therapy. These guidelines aim to standardise reporting and facilitate international data comparison. In our institution, a symptomatic breast centre, we have seen an increase in the number of specimens received following neoadjuvant therapy for breast cancer (18 in 2018, 60 in 2022). With the requirement for a new local reporting template, it was important to audit our reporting of these cases and compare to the new international guidelines prior to implementation of such a template.

A database search for excision breast specimens following neoadjuvant chemotherapy was performed for the years 2018-2023 and 166 cases were identified. These cases were then interrogated against the ICCR neoadjuvant dataset and the data was inputted into a spreadsheet where the data was analysed.

Of the 166 number of cases identified, 129 matched the search criteria and were analysed -- composed of a mixture of HER2 positive cases & triple negative breast cancers. There was excellent reporting of tumour type, presence/absence of DCIS and laterality. However, reporting of the residual cancer burden score & repeat hormone receptors (where appropriate) was lacking as well as variability in the description of tumour bed. In tandem, a local reporting template for neoadjuvant excision specimens, based on the ICCR dataset & in collaboration with the requirements of the clinical team, was introduced to encourage uniformity in reporting.

There is an ever increasing number of neoadjuvant breast excisions and with the introduction of the ICCR dataset, an audit of reported highlighted some deficiencies in reporting. A local reporting template based on the ICCR dataset was introduced and a plan for re-audit is planned, to close the audit cycle.

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P20

The role of capsule in Encapsulated papillary carcinoma of the breast: An image analysis study

Background: Encapsulated papillary carcinoma (EPC) is surrounded by a thick fibrous capsule-like structure, which is interpreted as a thickened basement membrane (BM). This study aimed to describe the geometric characteristics of the EPC capsule and to refine whether it is an expansion of the BM or a stromal reactive process.

Material and methods: 100 cases were divided into 5 groups: EPC, ductal carcinoma in situ (DCIS), normal breast tissue and invasive tumours, with an additional (n=15) encapsulated papillary thyroid carcinoma (EPTC)as a control group. Representative slides from each case were stained with picrosirius red (PSR) stain and examined using polarised microscopy. Images were analysed using image analysis programs.

Results: Compared to the normal and DCIS BM, the EPC group showed a significant increase in collagen fibre width, straightness, and density, and a decrease in fibre length. The EPC capsule showed less alignment of fibres with more perpendicular arrangement, and it was enriched with disorganised collagen type I (stromal collagen) fibres. Compared to other groups, the EPC capsule showed significant variation intracapsular heterogeneity. Comparisons of measurements between the inner and outer parts of the capsule tissue were also undertaken in the EPC group. This showed that the outer part of the capsule had significantly higher collagen density with more collagen I compared to the inner part density. The outer capsule fibres showed more straightness and less aligned fibres. Compared to BM-like material in the invasive group, the EPC capsule showed a higher density of collagen fibres with longer, straighter, and more aligned fibres. Still, there was no difference in the distribution of both collagen types I and III. Conversely, compared to EPTC, there were no differences between both EPC and EPTC capsules except that the fibres in the EPC

Conclusion: This study provided evidence that the EPC capsule is a reactive process rather than thickened BM.

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P21

Unusual presentation of synchronous breast cancer and skin malignancy in a patient with Lynch syndrome: a case report and review of literature

Purpose of the study: Lynch syndrome is an inherited autosomal dominant condition leading to increased risk of various neoplasms, namely colorectal, endometrial, and ovarian cancer. In the United Kingdom, NICE recommends that patients with colorectal and endometrial cancer should be tested for Lynch syndrome via immunohistochemistry or microsatellite instability testing. There is conflicting evidence in the literature of the link between breast cancer and Lynch syndrome.

Methods: In this case report, we present to our knowledge the first report of a woman with co-existing breast and sebaceous carcinoma of the breast on a background of Lynch syndrome.

Summary of results: A 54-year-old woman presented with a lump in the right breast with a background of locally advanced colorectal cancer 15 year prior. She was previously found to have MLH1 gene mutation indicating Lynch syndrome. On examination of the breast lump, a P2 U5 nodule was noted. A core biopsy showed a grade 3, invasive, triple negative NST carcinoma. The patient underwent a bilateral mastectomy. The tumour was triple negative with patchy positivity for CK14 and CK5/6. A cystic skin lesion in the dermis and subcutaneous tissue of the contralateral breast was noted which comprised of lesional cells with a proliferation of clear cells and bland basaloid cells with evidence of sebaceous differentiation. Immunohistochemistry showed AR, podoplanin and p63 were positive. MSH1 and PMS2 deficiency was noted on MMR testing in the breast and skin lesions, consistent with Lynch syndrome.

Conclusion: In the background of Lynch syndrome, it is important to be aware of the increased risk of various other types of cancer thus requiring molecular testing of malignancy and cascade testing. Based on this case, it may be argued that breast cancer should be added to the list of Lynch syndrome associated malignancies.

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P22

Breast Cancer Now Tissue Bank Cell Culture Programme: A bespoke resource for the research community

The Breast Cancer Now Tissue Bank (BCNTB) Cell Culture Programme aims to make available a broad range of human primary breast cell populations and tissues to the research community to more accurately model normal and malignant breast for a wide range of functional studies.
Most frequently, breast developmental and cancer research focuses on animal models or on long-established breast cell lines. Whilst the latter offer ease of use and reproducibility, they fail to represent the complexity of the human breast and its cellular and micro-environmental interactions, or its heterogeneity. BCNTB routinely consents and curates tissues from women undergoing surgery for cosmetic, risk-reduction or therapeutic purposes. Tissue is harvested fresh and processed for different experimental systems. Normal, risk-reduction and malignant tissues are enzymatically digested to single cells and specific cell fractions purified using antibody-labelling techniques. Cells generated include normal epithelial, myoepithelial and fibroblast cells, as well as tumour cell-enriched populations. Types of fibroblast isolated include normal fibroblasts, tumour-associated fibroblasts and those isolated from matched morphologically normal adjacent (<2cm from tumour) and surround (>5cm from tumour) tissue. Live explants from malignant and non-malignant breast, ductal tree fragments and unsorted single cells are also banked. Isolated cells have been successfully employed in a variety of studies such as 3D organotypic culture and more complex 3D models using lentivirus-infected cells. They have also been used to study specific genes hyper-methylated in breast cancer using CRISPR. Other techniques using the primary cells include SiRNA, NGS and single cell patch clamping. All samples have linked comprehensive clinico-pathological information and can be matched to tissue samples from the Tissue Bank. Collection of specific sample groups is available following discussion.

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P23

Cystic Neutrophilic Granulomatous Mastitis -- a case series

Cystic neutrophilic granulomatous mastitis (CNGM) is an increasingly recognised form of granulomatous mastitis. It is believed that it is caused by Corynebacterium ssp. infection. Clinically and radiologically, it can be difficult to distinguish from invasive carcinoma. This case series describes several cases of CNGM identified in a symptomatic breast unit over a 2-year period.

A retrospective search for breast specimens with granulomatous inflammation was performed. Slides were reviewed for typical histological features of CNGM- lipogranulomas composed of central lipid vacuoles with neutrophilic rimming surrounded by an outer layer of epithelioid histiocytes. EMR was accessed for clinic letters, microbiology and radiology reports.

A total of 7 specimens were identified; 4 core biopsies and 3 excisions. All 4 patients identified were female, aged 32 to 59 years. Mammogram showed increased density, and ultrasound showed areas of altered echogenicity in each. 3 of these 4 cases were classified as BIRADS 5, one as BIRADS 4. In those who had multiple specimens examined, one showed contralateral CNGM. Cocci were seen in two specimens on H&E stain. All patients had subsequent swabs of pus sent for microbiological cultures and breast tissue sent for microbiological analysis, which did not isolate any pathogens. 3 patients had additional serological testing for possible systemic inflammatory disorders. Patients are being followed clinically by breast surgery & infectious diseases services. One patient received antibiotic treatment which led to resolution of symptoms.

CNGM may present as radiologically suspicious for carcinoma. Recognition of the features associated with CNGM should prompt a search for microorganisms, especially gram positive bacilli. The association with Corynebacterium is relevant, due to possible need for prolonged antibiotic and possibly steroid therapy, as is the difficulty in detecting this organism by gram stain or culture due to its fastidious nature.

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P24*

Are the syngeneic mouse Colorectal Cancer models MC38 and CT26 representative of human microsatellite instable and microsatellite stable Colon Cancer?

Purpose of the study: Approximately 15% of colorectal cancers (CRC) are Microsatellite Instable (MSI), with a subset responding to immunotherapy. The remaining 85% are Microsatellite stable (MSS) and showed poor response to immunotherapy in trials. Identifying which tumours respond to immunotherapy is challenging, highlighting an unmet clinical need. The extensively used syngeneic murine models MC38 and CT26 exhibit human MSI and MSS traits, respectively. We compared these models to CRC tumours to determine if they represent human disease as preclinical models for immunotherapeutic analysis. Methods: 20 formalin-fixed and paraffin-embedded human MSI and MSS tumours were compared, with 10 CT26 and 8 MC38 subcutaneous models grown in BALB/c and C57BI6 mice, respectively. Tumour morphology was analysed through haematoxylin and eosin-stained sections where tumour infiltrative lymphocytes (TILS) were quantified. Immunohistochemistry was utilised to detect the presence of PDL-1 macrophage (CD68 and F4/80). The slides were analysed digitally. Summary of results: PDL-1 expression in human and mice (MC38) MSI tumours were 2-fold and 1.5-fold (p=0.027, p=0.013) more than their MSS counterparts, respectively, suggesting similarities. No difference in TILS was observed between human MSI and MSS tumours (P =0.811). A 1.5-fold increase in TILS in the MC38 model (p=0.004) compared to CT26 (MSS) highlights disparities between the human tumour and models. Similarly, human MSI tumours expressed 2-fold more macrophages than MSS, whilst in mice, CT26 had 3-fold more. Conclusions: The murine models do not fully represent human tumours. The models shared similar traits to humans in their expression of markers key to immunotherapy response (PDL-1). However, discrepancies in characteristics of other immune cells (macrophage, TILS) showed the models could be suited for evaluating treatments targeting specific markers. Future work could benefit from using orthotopic models.

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P25

Using a small NGS panel to predict microsatellite instability in colorectal cancer samples

Purpose

Deficient mismatch repair (dMMR), and the associated microsatellite instability (MSI) is a subtype of colorectal cancer (CRC), with different optimal treatment and prognosis to the more common proficient mismatch repair (pMMR)/microsatellite stable (MSS) subtype. Diagnosis is routinely performed using immunohistochemistry (IHC) or PCR. MSI testing using analysis of next-generation sequencing (NGS) data appears to be comparable, usually with large gene panels or exome data. As small gene panels are being increasingly used in research and diagnostic settings, we wanted to explore the efficacy of testing MSI status using a minimal panel.

Methods

We sequenced 335 formalin-fixed, paraffin-embedded (FFPE) CRC resection samples using a Genefirst ATOM-seq panel, designed to cover CRC mutational hotspots across 20 genes, plus 29 microsatellite targets. We predicted MSI status using frequently cited software packages and compared this to IHC data.

We then reduced the size of the training data and gene panel by random subsampling, to explore the lower limits of the different algorithms.

Results

Under optimal conditions (29 regions, 112 training samples), the package mSINGS correctly called 39/43 MSI samples and 271/272 MSS samples with 20 samples failing due to low sequence depth. For MSIsensor-pro these values were 42/44 MSI samples, 271/274 MSS samples and 17 failures. Both packages achieved under the curve (AUC) of over 99%. Reducing to only three genomic regions and 22 training samples, mSINGS correctly called 35/42 MSI samples and 245/259 MSS samples with 34 failures (AUC 94%), compared to MSIsensor-pro with 37/43 MSI samples, 265/271 MSS samples and 21 failures (AUC 96%). Using non-random selection, six regions were enough to match the performance of the full set of 29.

Conclusions

A limited NGS panel can be used to accurately predict MSI status when undertaking routine gene testing of FFPE samples. This can easily be incorporated into research or diagnostic settings.

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P26*

Use of Stochastic Optical Reconstruction Microscopy (STORM) in the investigation of cardiac mitochondrial structure in diabetes

The mechanisms for impaired resting myocardial energetics observed in patients with diabetes and heart failure are incompletely understood. Mitochondrial dysfunction due to both over- and under-expression of the mitochondrial fission mediator dynamin-related protein 1 (DRP1) underpin this phenomenon but studies to visualise its subcellular localisation have been mainly performed in vitro. This study utilised stochastic optical reconstruction microscopy (STORM) to image mitochondria-DRP1 interactions in formalin-fixed and paraffin-embedded (FFPE) cardiac muscle from patients with and without type 2 diabetes (T2D).

Right atrial appendage biopsies were obtained from healthy or T2D patients undergoing aortic valve replacement surgery (REC ref. 18/YH/0442). Sections of FFPE tissue were labelled with primary antibodies against outer (TOMM20, VDAC1) and inner mitochondrial membrane proteins (COXIV) or DRP1 followed by STORM-compatible secondary antibodies. Longitudinally sectioned cardiomyocytes were identified by fluorescent staining of the cell border (wheat germ agglutinin) and nucleus (DAPI). Sections were imaged with 2D direct STORM (Nanoimager-S, ONI) or confocal microscopy.

Mitochondrial localisation of TOMM20 was determined by proximity to VDAC1 and COXIV signals. In both patient groups, STORM revealed punctate TOMM20 localisations and clusters surrounding areas of negative staining consistent with outer mitochondrial membrane distribution. In contrast, confocal microscopy showed continuous TOMM20 staining without observed puncta. Diffuse cellular DRP1 localisations shown by STORM exhibited occasional clustering potentially representing oligomerisation events.

STORM is superior to confocal microscopy in identifying sub-mitochondrial protein distribution in FFPE tissue. Further cluster and interaction factor analyses will elucidate TOMM20 and DRP1 co-localisations and their mitochondrial functional significance for the cardiac dysfunction seen in diabetes.

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P27*

Pannexin 1 regulates the development of pulmonary fibrosis following lung injury

Introduction: Pulmonary fibrosis is associated with epithelial damage, dysregulated inflammation and disordered tissue repair. Much of the pathogenesis remains uncertain and therapeutic options are limited. Pannexin 1 (Panx1) channels, which are widely found in organs in the body release small molecules during cell death. Despite recent evidence of their importance in the macrophage-mediated regulation of epithelial proliferation following acute lung injury, their role in fibrosis remains to be determined. We hypothesised that loss of Panx1 in a mouse model of pulmonary fibrosis would result in increased fibrosis.

Methods: Intratracheal bleomycin or crystalline silica was administered to wild type (WT) and Panx1-/- mice and culled after 14 or 28 days. Formalin-fixed lungs were removed en bloc with H&E and Masson's trichrome stained sections evaluated including automated fibrosis quantification (QuPath). From separate animals, alveolar lavage was collected and single-cell lung digest performed prior to immune cell phenotyping by flow cytometry, protein quantification by Luminex and mRNA profiling by qPCR. Statistical analysis was by unpaired t-test and two-way ANOVA.

Results: There was increased fibrosis in Panx1KO animals post-bleomycin and silica as assessed by the number of subpleural fibrotic foci (6 vs. 11 at D28 post bleomycin) and the percentage of collagen-positive area of the subpleural region (4% vs. 11%). There were also elevated levels of B-cell activating factor (BAFF), increased numbers of neutrophils and CD4+ T cells and a 4-fold increase in the expression of the collagen gene Col3a1. Conclusions: Our data demonstrate that Panx1 is an anti-fibrotic molecule in the lung. Characterisation of the initial inflammatory phase of Panx1KO response to bleomycin is underway alongside studies into which compartment Panx1 is acting.

Funding: Pathological Society & Wellcome Trust

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P28*

Combining histological and whole genome sequencing analysis of tumour infiltrating lymphocytes to predict response to immune checkpoint inhibitors

Purpose of study

Immune checkpoint inhibitors (CPIs) are effective therapies for some cancers. Currently, patient selection for such drugs is based on tumour mutational burden (TMB) but is suboptimal. Tumour infiltrating lymphocytes (TILs) can be assessed histologically and the T cell infiltrate has been predicted using DNA sequencing data to quantify T cell fraction and therapy response (Tcell ExTRECT: PMID:34497419). This study aims to test the premise that TILs are concordant with the Tcell ExTRECT algorithm and more useful than TMB in predicting CPI treatment response.

Methods

Whole genome sequencing was available from CPI treatment-naïve cancers from 318 patients in the Genomics England 100,000 genomes project, and who subsequently received CPIs. Digitised haematoxylin and eosin-stained whole slide images (WSI) from 74 of these patients were available. The cancers included 29 melanoma, 25 lung, 8 renal, 5 bladder, 4 breast, 1 colorectal, 1 glioblastoma and 1 ovarian.

Scoring of intratumoural and/or stromal TILs was performed of the WSI, based on published guidelines by The International Immuno-Oncology Biomarker Working Group. The Tcell ExTRECT algorithm was applied to these cases.

Summary of results

Histological TILs scoring was possible on 60/74 cases and included 218 WSI (between 1-5 WSI per patient). Stromal TILs were scored in 38/74 patients and there was a moderate/strong correlation with the Tcell ExTRECT signal (Rho=0.58, p=0.0004). This score also correlated with a high clonal TMB (p=0.0042). Kaplan-Meier and Cox regression survival analysis demonstrated no improved overall survival in patients with higher scores for any of the histological TILs measured.

Conclusion

This study highlights the potential complementary nature of histological assessment and genomic analysis of TILs and has potential clinical utility. However, the results should be cautiously interpreted until investigated in a larger cohort.

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P29

EVALUATION OF PAX2, PTEN AND BETA CATENIN IN EIN (Endometrial intraepithelial neoplasia/atypical hyperplasia)

EIN is a premalignant lesion which is associated with 30% risk progression to endometrial carcinoma. The imaging including MRI have low sensitivity and specificity for diagnosing this premalignant condition, the biopsy is considered as ascertainable for the diagnosis. Unfortunately, the diagnosis of EIN remains challenging and subjective. WHO 2020 have explained the morphological criteria along with the Loss of PAX2 and PTEN as desirable criteria for the diagnosis along with mutant beta catenin. Studies have shown that >90% of EIN as associated with aberrancy of either PAX2, PTEN and beta catenin. We report our departmental experience of evaluating these three markers as diagnostic asset for EIN/atypical hyperplasia. 14 random cases of endometrial biopsies and few cases of hysterectomy with a diagnosis of endometrial hyperplasia and atypical hyperplasia on H&E were collected in the Cellular Pathology department. After morphological diagnosis, PAX2, PTEN and beta catenin were applied on these cases. On evaluation, 50% of cases diagnosed as complex hyperplasia without atypia had aberrant expression of one of three IHC markers. 66.6% of cases diagnosed with atypical complex hyperplasia had aberrant expression of one of three IHC markers. In 22.2% cases there was aberrant expression of PTEN but it was in <10% of the glands. If we take those also as positive then total cases will become 88.8%. 11.1% of cases diagnosed as complex hyperplasia with atypia had no aberrant expression of any of the three IHC markers, as seen in other studies also. The results reveal that these markers in conjunction with the morphological features can be prudent in making a diagnosis of EIN/atypical complex hyperplasia.

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P30

Audit of MMR Testing In Endometrial Cancers

Lynch syndrome is an inherited disease that increases the risk of certain types of cancer, including endometrial and colorectal cancer. People with Lynch syndrome have a 42-60% risk of developing endometrial cancer. NICE recommends testing for Lynch syndrome after an endometrial cancer diagnosis as it is the first cancer in people with Lynch syndrome. So, Lynch syndrome can be identified earlier if tests are done after a diagnosis of endometrial cancer. Thereby, other cancers could be prevented. Familial Genetic testing for Lynch syndrome can be done with the aim of preventing Lynch syndrome-associated cancer developing or detecting it at an early stage. Guidelines for the use of immunohistochemistry (IHC) to identify tumours with mismatch repair (MMR) deficiency: * If IHC is abnormal with loss of MLH1, or loss of both MLH1 and PMS2 protein expression, do MLH1 promoter hypermethylation testing of tumour DNA. If MLH1 promoter hypermethylation is not detected, offer germline genetic testing to confirm Lynch syndrome. * If IHC is abnormal with loss of MSH2, MSH6 or isolated PMS2 protein expression, offer germline genetic testing to confirm Lynch syndrome. An audit of MMR testing in endometrial cancers diagnosed at DGH between January 2023 to July 2023 was performed. Thirty cases of endometrial carcinoma were diagnosed in which 5 cases had abnormal MMR results. Four cases had MLH hypermethylation . One case had insufficient material. Histological appearance in MLH1 hypermethylated cases were that of endometrioid type (2),mixed endometrioid and clear cell carcinoma (1) and p53 mutant adenocarcinoma(1).

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P31

An audit of HPV vaccination and infection status of CervicalCheck samples

Purpose of the study Vaccination against high-risk human papilloma virus (hrHPV) is highly effective in preventing persistent hrHPV infection, a precursor to high grade squamous intraepithelial lesions (HSIL) and invasive squamous cell carcinoma of the cervix. The aims of this study were to collate and analyse data of the cervical screening service regarding HPV vaccination status, hrHPV infection status and subsequent cervical cytology result if hrHPV was detected. Methods A retrospective audit and data analyses was conducted on samples of the cervical screening service in Ireland during the first quarter of 2023 (n=4635). Summary of results Vaccination rate: In this study 8.4% (391/4635) of all participants were identified as vaccinated against hrHPV by the test requestor. The vaccination rate was highest, 79%, among women aged 25-27 years. hrHPV infection status: The prevalence of hrHPV infection was 14.7% (684/4635) in the study group. Of these women, 13.7% (94/684) had been previously vaccinated. Cytology correlation: 18% with hrHPV (122/684) were diagnosed with HSIL in cervical cytology. 9% (11/122) of HSIL cases occurred in HPV-vaccinated individuals. Conclusion The quadrivalent HPV vaccine has been offered in Ireland since 2010, followed by the introduction of the nanovalent HPV vaccine in 2019. Women who received their HPV vaccinations within the framework of the vaccination programme have only started to enter the cervical screening programme. In this study, hrHPV infection and HSIL were associated with low rates of HPV vaccination. As more immunised individuals will enter the screening programme, continued auditing is required to monitor the impact of HPV vaccine on cervical lesions.

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P32

This abstract has been withdrawn

P33

Clinicopathological and functional significance of TP73 transcription factor in epithelial ovarian cancers

Background: TP73 belongs to the TP53 family of transcription factors. It has complex biological functions including roles during genomic instability, proliferative signalling, invasion, migration, angiogenesis, immune evasion, and neo-neurogenesis. The role of TP73 in ovarian cancer pathogenesis, response to therapy and prognosis is largely unknown.

Purpose: To understand the role of TP73 in ovarian cancer pathogenesis, response to therapy and prognosis.

Methods: a) Evaluation of TP73 depletion (by CRISPR/Cas-9 knock out) or TP73 overexpression (TP73 cDNA knock in) in a panel of ovarian cancer cell lines and evaluate cellular phenotype and response the cisplatin therapy. b) To evaluate clinicopathological significance of TP73 expression in 331 clinical ovarian cancer tissue microarray cohorts. Results: Pre-clinically, TP73 overexpression led to increased proliferation, migration, invasion and resistance to cisplatin therapy. On the other hand, TP73 depletion reduced proliferation and re-sensitized platinum resistance ovarian cancer cells. In clinical cohorts, TP73 protein overexpression was associated with higher stage, higher grade tumours and poor progression free survival.

Conclusion: Our data confirms that TP73 is a marker of aggressive phenotype. TP73 expression status could inform stratification of patients for personalized ovarian cancer therapy. Keywords: Ovarian cancer, TP73, platinum resistance, prognosis.

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P34

Discrepancy rates in reporting dermatopathology specimens

Purpose of the study: In this retrospective service evaluation at a tertiary centre, amended reports in Dermatopathology were analysed to identify discrepancies and harm.

Methods: All dermatopathology specimens reported in 2014 were identified in a retrospective search of recorded reports on the computer system. The amended reports were categorised based on the type of discrepancy/amendment made and whether harm occurred was established. Out of 11,519 cases, 135 (1.6%) generated an addendum, these cases were further analysed in detail. Summary of Results: 25.9% of the amendments were for margin re-assessment post Moh's excision, 16.3% results were changed for a more refined diagnosis following the Dermatopathology Clinico-pathologicial correlation meeting for inflammatory skin biopsies and 14.1% were typographical errors. Amendments were made to the RCPath dataset core items in 25.2% melanocytic and 15.6% BCC/SCC cases. There was an altered diagnosis in 2.9% of cases, of these two were benign and two were malignant cases. One small cluster of squamous cell carcinoma was missed in a punch biopsy and another case diagnosed as high-grade osteosarcoma in our department was diagnosed on review by a national expert as a Diffuse Large B cell Lymphoma.

Conclusion: No major harm was done as both discrepancies were identified prior to any definitive management. Overall we identified discrepancies in 1.6% cases and altered diagnoses in 0.03% cases. The evaluation of discrepancies does help to identify underlying problems and improve the performance of the team. The review of cases at MDT and clinico-pathological meetings is also a valuable tool for quality control in diagnostic pathology.

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P35

Audit of the clinical information provided on request forms for non-melanoma skin cancer excisions compared to the core data items listed in the Royal College of Pathologists dataset

Purpose of the study. Non-melanoma skin cancers (NMSCs) are the most common malignancies diagnosed in Caucasian populations. Basal cell carcinoma (BCC) is the most common skin cancer, followed by squamous cell carcinoma (SCC). The Royal College of Pathologists (RCPath) provides guidance on the minimum clinical information that should be recorded in pathology reports.

Methods. The clinical information provided for NMSC excisions was retrospectively compared to the core clinical data items listed in the RCPath minimum dataset (site of origin, type of specimen and maximum clinical dimension). Reports authorised from 1/9/22-30/9/22 (cycle 1) and 6/2/23-6/3/23 (cycle 2) were analysed. Changes implemented before re-audit included local presentation of the results at the dermatology morbidity and mortality meeting to educate and increase awareness and circulation of the presentation slides.

Summary of results. 200 NMSC excisions (168 BCC, 32 SCC) from 168 patients were included in cycle 1 and 167 excisions (136 BCC, 31 SCC) from 132 patients in cycle 2. 100% of forms from both cycles included the site of origin. Specimen type was included on 85% and 80.2% forms in cycles 1 and 2 respectively, $p=0.23$. Inclusion of the maximum clinical dimension improved from 22.5% to 42.5%, $p<0.01$. The proportion of forms including all core clinical data items increased from 18.5% to 34.1%, $p<0.01$.

Conclusions. The clinical information provided on NMSC excision request forms has significantly improved as a result of this audit. Further interventions including changes to the character limit on electronic histopathology request forms are currently being investigated. The importance of accurate and effective handover between dermatologists and pathologists via histology request forms should not be neglected. We recommend that our centre, and other dermatopathology departments, strive for continuous improvement, ongoing education and re-audit.

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P36*

A Quantitative Measurement of Cells in Melanoma Tissue as a Novel Prognostic Marker

Purpose of the study: Breslow thickness (BT) is the gold standard measurement of melanoma tumour size and is a one dimensional prognostic marker. A two dimensional estimate, calculated tumour area (CTA), has been shown to be a better prognostic marker but is semi-quantitative. We have devised a semi-automated method to measure the novel feature Melanoma Nuclear Count (MNC), a quantitative estimate of invasive cell number.

Methods: 102 cases with BT >1.0mm were stained with SOX10 and scanned to produce whole slide images (WSI) using a Hamamatsu NanoZoomer S210. The images were imported into QuPath for pre-processing and the region of interest was selected. In situ disease was removed. MNC was detected using StarDist, a deep learning algorithm used to identify nuclei and cells. Measurements were exported and analysis conducted in R.

Summary of results: A StarDist script was adapted to derive MNC and the count was internally validated. MNC showed a strong positive correlation with BT (0.82) and CTA (0.89) suggesting it was a plausible surrogate for tumour burden. In a Cox Proportional Hazards Regression univariate analysis MNC, BT and CTA were all significant ($p < 0.0001$) for overall survival (OS), melanoma specific survival (MSS) and metastasis free survival (MFS). In the multivariate analysis MNC remained significant for OS (HR 2.29, C.I 1.33-3.95, $p = 0.003$) and MSS (HR 3.7, C.I 1.47-9.31, $p = 0.006$) whilst BT became non-significant.

Conclusions: The results suggest that MNC is a better prognostic feature for survival than BT. It is likely that MNC is a closer estimate of tumour size than BT as MNC is a closer approximation of true invasive cell number. This is a small scale study limited by melanoma size, exclusion of cases with extensive melanin pigmentation and a small number of events. Further investigation is required to increase the strength of analysis, validate the method and MNC as a prognostic feature.

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P37

Scar Re- excision in cutaneous malignant melanomas- one year (2022) audit study

Melanoma scar re-excision takes place even if the tumour is excised histologically when margins are smaller than 0.5cms for stages 0-2. As per 2014 NICE guideline, clinical margin of 0.5- 2cm should be around the histological biopsy scar. The RCPath 2019 Skin dataset guidance on handling these specimens is to include a record of the scar at macroscopy and consider sampling the specimen in its shortest transverse axis, incorporating the area where the scar appears closest to the margins. The guidance believes this generally can be achieved in one to four cassettes. We report our departmental experience with 2022 melanoma scar re-excisions. 86 specimens were identified, 10mm was the smallest and 110mm was the largest specimen received. The number of blocks sampled ranged between 2 -14 blocks. In 24 cases, the entire specimen was processed. In 4 cases, there was no macroscopic abnormality but at microscopy, lentigo maligna and benign naevi in 2 cases each were found, despite clear histological margins in the primary excision. In the absence of explicit guidelines for processing melanoma scar specimens, a pragmatic approach would mean the immediate skin adjacent to scar margins at both polar ends and along the radial surface that represents the new clinical margin only needs to be sampled. A discretionary approach would mean more sampling for previous positive margins at primary excision or presence of a pigmented abnormality seen grossly. 6 cases with a previous incomplete primary excision showed negative microscopy and 7 cases with a pigmented abnormality showed reparative changes of haemorrhage and granulation tissue at microscopy making these approaches futile Hence one size fits all option does not take into account peculiarities of the individual lesion. Extending margins of excision for inapparent metastases that are rare and whose localisation cannot be predicted should make way for personalised rather than standardised excisions.

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P38

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P39

This abstract has been withdrawn

P40

Potential and Ethical pitfalls of AI integration in Dermatology

Purpose of the study: The integration of artificial intelligence (AI) in the field of dermatology promises a revolutionary transformation in our approach to this medical domain. However, the use of AI in dermatology is still in its early stages, and with limited scope of applications. While some of the existing research performed has highlighted the capabilities of AI in diagnosing lesions comparable to those of a dermatologist, the implementation of AI brings about its own ethical challenges that affect both the clinician and the patient. This article aims to explore the ethical issues and future prospects surrounding AI implementation in dermatology by drawing insights from the current literature.

Methods: A qualitative analysis was conducted on articles focusing on AI usage in medical care, with a specific emphasis on dermatology.

Summary of results:The analysis unveils four main ethical issues that emerge from the implementation of AI in dermatology: selective bias and unequal treatment based on skin tone differences, intrusion of privacy brought about by the contribution of images on an open-access database, the potential erosion of patient autonomy as AI takes center stage in decision-making, the risk of unwarranted harm to patients as a result of unnecessary biopsies to potential misalignment of treatment priorities between AI systems and patients.

Conclusion: While AI's integration in dermatology promises to streamline the diagnostic process , it also sparks ethical issues that affect the main stakeholders. These findings indicate the need for further development and enhancement of AI before they are capable of being implemented. Introduction of a transparent, explainable AI model would alleviate concerns regarding patient autonomy. Simultaneously, the establishment of a global open-access database will serve to mitigate selective bias. Furthermore, additional research comparing algorithms would be useful in establishing a standardized validation tool.

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P41

An unusual palatal tumour: A case report of clear cell acanthoma of the palate

Clear cell acanthoma (CCA) or 'Degos acanthoma' is a rare benign lesion, the pathogenesis of which is uncertain, with some reporting that it arises as part of a reactive/inflammatory process or may represent a true benign epithelial neoplasm (Usmani 2020). It occurs in the 5-6th decade and has a propensity for the lower extremities, with very few reports of cases occurring in the mucosal regions of the head and neck (Argyris et al. 2020). We present a case of palatal CCA and the use of D2-40 and EMA immunohistochemistry with PAS special stain as adjuncts to diagnosis.

A 55-year-old male presented with a 5mm papillomatous lesion on the right posterior palate, with well-defined borders, which was non tender to palpation and not associated with lymphadenopathy.

Microscopically the lesion showed abrupt transition from adjacent normal to lesional epithelium, with acanthosis and broad, elongated rete processes containing large cells with pale to clear cytoplasm. Neutrophilic microabscesses were also seen. There was no evidence of cytological atypia or mitoses.

Immunohistochemistry was positive for EMA, with basal and suprabasal cells selectively highlighted by D2-40. Glycogen granules were also present on PAS stain.

A diagnosis of CCA of the palate was made after local review.

CCA of the palate is an extremely rare lesion, with unknown pathogenesis. Although the diagnosis is largely morphological, ancillary tests may prove useful adjuncts, particularly strong EMA staining and PAS special stain highlighting the presence of glycogen within keratinocytes. Treatment depends on the number and size of lesions and surgical excision is curative for most lesions. Others may spontaneously regress and some malignant variants have been infrequently reported, characterised by cytological atypia and a high Ki67 index (Melson 2022).

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P42

This abstract has been withdrawn

P43

Warthin-like Mucoepidermoid Carcinoma of the Salivary Gland Diagnostic Challenges: A Case Study

Introduction: Mucoepidermoid carcinoma (MEC) is the most frequent malignant salivary gland neoplasm in both adults and children, accounting for approximately 30% of all salivary gland malignancies. These lesions are characterised by mucous, intermediate and epidermoid cells and characteristically harbour a specific MAML2 gene rearrangement. Various subtypes have been described, including Warthin-like MEC, which shares features with Warthin tumour and pose diagnostic challenges especially on cytology.

Case Report: A 60-year-old woman presented with a lump in her left parotid gland. Fine-needle aspiration of the lesion was performed twice, with both showing a polymorphous lymphoid population and oncocytic cells. The lesion was described as suspicious for, but not diagnostic of, Warthin tumour. A core biopsy of the lesion was performed, which was inconclusive and the differential diagnosis was between low-grade MEC with oncocytic change, and Warthin tumour showing squamous and mucinous metaplasia. No MAML2 rearrangement was identified on this specimen, and complete excision was recommended.

The excised lesion revealed a 38mm thinly encapsulated lesion composed of ducts lined by low grade mucinous and intermediate cells, admixed with squamous nests. These structures were surrounded by extensive lymphoid tissue with germinal centre formation. The lesion was again sent for genetic testing and a MAML2 rearrangement was detected, and the diagnosis was given as low-grade MEC, Warthin-like pattern.

Conclusion: Warthin-like MEC is a rare variant of MEC and can be a diagnostic challenge on cytology and core biopsy. There is particular difficulty in Warthin tumour with squamous and mucinous metaplasia. Detection of a MAML2 rearrangement supports the diagnosis of Warthin-like MEC, but is not always reliable. Awareness about this relatively new entity and a high index of suspicion can help in succinct diagnosis.

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P44

A rare salivary tumour in the nasal cavity

Carcinoma ex-pleomorphic adenoma (CXPA) is an uncommon malignancy of salivary tissue usually associated with the parotid gland. Primary sinonasal CXPA is vanishingly rare, with only 27 cases reported in the world literature. We present a case of CXPA of the nasal cavity in a 50-year-old woman which posed diagnostic challenges. The patient was referred to our head and neck tertiary referral centre with suspected poorly-differentiated sinonasal carcinoma. Imaging showed a soft tissue mass expanding the left nasal cavity, involving the nasal bone, septum and maxilla, with FDG-avid neck nodes at levels 1 & 2. The original biopsy of the nasal mass was very small; on review of the H&E stained sections, the morphological differential included chondrosarcoma. However, on immunostaining the tumour was diffusely cytokeratin positive with only focal S100 positivity, favouring a carcinoma. Subsequent review by a specialist sarcoma team excluded sarcoma, and sarcomatoid carcinoma was included in the differential. With a working diagnosis of primary sinonasal carcinoma, the tumour was resected. Histological examination showed a biphasic tumour with similar morphology to the original biopsy. In addition, a central sclerotic nidus was found, showing remnants of atypical ducts, compatible with the site of an antecedent pleomorphic adenoma. Thus, the diagnosis of CXPA was made. Pleomorphic adenoma (PA) often shows a characteristic PLAG1 (>50%) or HMGA2 (10-15%) gene rearrangement. These can be detected by immunohistochemistry or FISH, and serve as a useful marker of pre-existing PA in suspected cases of CXPA (result pending for this case). Management depends on the extent of the tumour, and can include surgery and chemoradiotherapy. This case highlights the difficulties encountered in diagnosing a rare lesion in a rare location. The diagnosis required careful assessment of the morphology combined with immunohistochemical and molecular testing as adjuncts in the setting of a specialist centre with an experienced multidisciplinary team.

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P45

Meningothelial meningioma masquerading as a nasopharyngeal mass

Introduction

Meningiomas are the commonest intracranial neoplasms and skull base lesions account for about 25% of them. They tend to pass through natural foramina such as cranial nerve foramina and can reach orbital and nasal cavities. When presenting as a nasopharyngeal mass, the differential diagnoses include a wide variety of neoplasms in the head and neck region.

Case report

An 84-year-old female presented with nasal blockage. Nasal endoscopy revealed a postnasal papillomatous lesion. CT scan report indicated a mass involving the left nasopharynx and infiltrating the pterygoid plate and left sphenoid bone. Endoscopic biopsy was undertaken. The received specimen comprised six pale tan pieces of tissue ranging from 2 to 4mm in diameter. Microscopy revealed mucosal fragments lined by benign respiratory epithelium with the corium infiltrated by whorls of epithelioid cells having eosinophilic cytoplasm, oval nuclei and Intranuclear inclusions. Scattered Psammoma bodies were identified. Mitotic figures were inconspicuous, and no necrosis or anaplastic features were seen. Immunohistochemically, the lesional cells were positive for EMA and PR. MIB-1 proliferation index was low and tumour was negative for Cam 5.2, p40, S100, SOX10, CD34. The above features were consistent with WHO Grade 1 meningothelial meningioma. Subsequent MRI scan confirmed appearances in keeping with intraosseous meningioma involving left greater wing of sphenoid and Meckel's cave with perineural spread along V1 and V2 trigeminal branches into the nasopharynx. Conservative management was agreed in the MDT meeting.

Comment

Skull base meningioma with extracranial extension can pose diagnostic challenges clinically, radiologically and histologically. The awareness of this common neoplasm in an unexpected site will help avoid difficulties in diagnosis and management.

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P46*

Profiling the complex rearrangement architecture of sarcoma

Genomic rearrangements are key mutational processes in bone and soft tissue tumours, used for both disease classification and as biomarkers. However, the mutational processes and rearrangement architecture underlying many of these events remain poorly characterised. Recent data have indicated that sarcomas show particularly high frequencies of complex rearrangement events, including patterns which do not fit those of known mutational mechanisms. As the largest whole genome sequencing (WGS) cohort of sarcomas to date, the Genomics England (GE) 100,000 Genomes project represents a unique dataset in which to profile these events.

Structural variants (SVs) were identified in WGS data from 978 GE samples using an optimised approach based on 5 SV callers. SVs identified by at least two callers following quality control and germline and panel of normal filtering were taken forward for further analysis. Chromothriptic events, extrachromosomal DNA (ecDNA) and genes enriched for SVs were identified using established algorithms.

Consistent with previous reports, the prevalence of SVs varies by tumour type, with particularly high rates observed in liposarcoma: these tumours also showed the highest rates of chromothripsis. We have examined some tumour types at higher granularity than previously available, demonstrating directly that the rates of complex rearrangement events vary across subtypes. Similarly, the presence of ecDNA varies by tumour type, with the novel observation of particularly high rates in angiosarcoma. Using these data, we have catalogued the rearrangement patterns generating canonical gene fusions in fusion-driven soft tissue tumour types.

Despite more recent advances in histological classification, survival for patients with sarcoma has remained largely unchanged for 40 years. Characterisation of the mutational processes underlying these rearrangements will shed light on the pathogenesis of these tumours.

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P47*

Biomarkers and anthracycline induced cardiotoxicity, a systematic review and meta-analysis

Purpose of the study Breast cancer is an extremely prevalent condition, consistently been recognised amongst the most frequent cancers worldwide. With the diagnosis of breast cancer comes a mortality rate of 33.3% in females. Anthracyclines are a mainstay in the treatment process of breast cancer patients. Whilst the use of anthracyclines has made significant strides in reducing mortality, they have been associated with a number of side-effects -- most notably among these are reports of cardiotoxicity. Several studies have used the measure of left ventricular ejection fraction (LVEF) to investigate potential cardiotoxicity. Methods A literature search was performed on 5 databases -- Google scholar, OVID, MedRvix, Medline and Clinicaltrials.gov. The search terms used were (Anthracyclines and/or doxorubicin and/or epirubicin) AND (Cardiotoxicity) AND (Breast cancer or malignancy) AND (Biomarker). Through screening and subsequent elimination of less relevant papers, 5 observational studies were found to be eligible for inclusion in this meta-analysis. Summary of results Our meta-analysis of 5 studies therefore investigated 8 biomarkers associated with cardiotoxicity. Of these, GDF15 and GAL3 demonstrate positive hazard ratios of 3.73 [95% CI 2.69, 5.17] and HR 4.48 [95% CI 3.27, 6.13] respectively. Conclusions Our studies highlight novel biomarkers not previously assessed. Furthermore, future research is required to investigate whether standardised imaging or the use of biomarkers would prove to be a safer, more cost-effective measure of cardiotoxicity in breast cancer patients undergoing anthracycline chemotherapy.

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P48

HER2 positive mucinous carcinoma of the breast; a case series and review of literature

Purpose of Study: Invasive mucinous carcinoma is an uncommon type of breast cancer with an incidence of 2-4%. The tumour is generally of the luminal phenotype and carries a good prognosis. A rare variant of this subtype expresses the human epidermal growth factor receptor 2 (HER2). We aim to describe the radiological, pathological features and outcome of a series of this type and review the limited literature pertaining to it.

Methods: 6 cases of HER2 positive breast carcinoma from a single tertiary UK institution over a 9 year period were identified. Presentation, histopathological grade, nodal and hormone receptor status, management and survival data were collected then compared to the recent literature.

Results: All of the patients included in this study were female. Patients' age, at diagnosis, ranged from 55 to 75 with a median of 74. 5/6 patients (83.3%) presented with a breast or chest wall lump. 2/6 (33.3 %) of cancers were ER positive and one (16.7%) was PR positive (the only triple positive case). All cases were of grade 2 mucinous carcinoma with a micropapillary component noted in 2/6 (33.3%) of cases and lymph node metastasis in 50% of cases at the current presentation. 5/6 (83.3%) of patients were managed with adjuvant chemotherapy/anti- HER2 therapy following surgery with no neoadjuvant chemotherapy given. Follow up was up to 84 months (median 42) with one patient dying at 42 months.

Conclusion: HER2 positive mucinous mammary carcinoma is extremely rare and generally treated with surgical resection and adjuvant anti-HER2/chemotherapy. This series supports that this is an aggressive phenotype distinct from the more common HER2 negative mucinous carcinoma; The variant is more likely to be of a higher grade, metastasise to lymph nodes and exhibit micropapillary features -- all found to be significant indicators for a worse prognosis.

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P49

Is the "maximum manual Ki67 score" using TMAs an independent prognostic factor for overall survival in lung adenocarcinoma?

Our aim is to deeply understand the role of Ki-67 as prognostic marker in lung adenocarcinoma, by using manual Ki67 score on TMA samples and investigate whether Ki67 may be an independent prognostic factor when is evaluate with other classical prognostic markers such as tumour size, pleural involvement and nodal status. Our objectives are focusing on investigating whether Ki67 is a prognostic factor in lung adenocarcinoma using manual score on TMA samples. In addition, to investigate whether Ki67 is an independent prognostic biomarker in lung adenocarcinomas. This study contains 794 surgical specimens from primary pulmonary adenocarcinoma surgeries performed with curative intent in Leicester between 1998 and 2014. TMAs were constructed with three 1mm cores of lung adenocarcinoma tumour per patient case. Manual Ki67 score was obtained from TMAs. Because of each patient case has three cores, the maximum Ki67 score was the data included for our analysis. Our results show that maximum manual Ki67 score, as continuous variable predicts poor outcome (HR: 1.009; p<0.001). Grouping by tertials, The Kaplan-Meier curve shows that patients with low manual Ki67 had the best prognosis with a median of OS at 4.5 years. By contrast, the median of OS for the medium and high manual Ki67 groups was 2.8 and 2.3 years, respectively. The Cox regression model shows that patients with high manual Ki67 predicts the worst overall survival (HR: 1.73; p<0.001). Likewise, patients with medium manual Ki67 predicts significantly poor prognosis (HR: 1.52; p<0.001). Furthermore, a multivariate Cox regression model including manual Ki67 score, pleura involvement, nodal status, largest tumour size, vascular invasion, sex, and age shows that all these variables are independent prognostic markers in lung adenocarcinoma. This study demonstrates that manual Ki67 score is a poor prognostic factor in lung adenocarcinoma, by using the maximum Ki67 score obtained among all patient's three cores tissue samples. Patients with high manual Ki67 score (>60%) had the worst prognosis in term of overall survival. Maximum manual Ki67 score, as continuous variable, is an independent prognostic factor for overall survival in lung adenocarcinoma, when the model includes other clinical-pathological variables such as pleura involvement, nodal status, largest tumour size, vascular invasion, sex, and age.

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P50

The Comparative Pathology Workbench: An Integrated Online Tool for Interactive Visual Analytics for Biomedical Data

The Comparative Pathology Workbench (CPW) is a software tool to help pathologists gather and share related histopathological image and other data in a single place, in order to allow them to arrange such data into a meaningful structure for visual comparison and analysis.

The CPW provides a visual analytics platform, that offers shared access to an interactive "spreadsheet" style presentation of images and associated analysis data, using a grid layout of rows and columns of images in cells, termed a Workbench. All image data is hosted by other resource applications such as OMERO, with the exception of locally cached thumbnail images.

The CPW allows for easy visualisation of any image data to further enhance understanding discussion, and shared observations between users. Within the CPW, each Workbench and the Cells within them, have associated discussion threads, hosted by a WordPress server, to allow collaborative analysis and consensual interpretation of the data.

Integration with the metadata held in the European Bioinformatics Institute's Single Cell Expression Atlas has been developed. A further integration has been developed with analyses generated by QuPath, software for digital pathology image analysis, where generated analyses can be stored in the link between two images.

We have proven the applicability and effectiveness of the CPW through 3 exemplar applications: a Crohn's Disease Fibrostenosing Lesion Comparative Analysis for the Edinburgh Gut Cell Atlas Project; a Skin Tumour Expert Diagnosis Review for the DERMATLAS project; and finally, a Coeliac Disease Duodenal Biopsy Diagnosis Concordance Audit.

Finally, an integration will be built to reference the Human Cell Atlas Data / Helmsley Gut Cell Atlas Data Repository from within the CPW, to allow image data to be shared from these sources too.

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P51

Analysis of cMYC as an adjunct to p53 in predicting concurrent or subsequent development of colorectal carcinoma in chronic-inflammatory bowel disease associated low grade dysplasia

Purpose of study: p53 is an established biomarker for CIBD-dysplasia, with a mutated phenotype associated with higher risk of progression to colorectal carcinoma. cMYC has not been evaluated as a biomarker for CIBD-associated dysplasia. Our aim was to investigate if cMYC amplification in CIBD-associated low grade dysplasia correlates with p53 and is associated with concurrent or subsequent colorectal carcinoma. Methods: Specimens coded for IBD and low grade dysplasia from 2015-2020 were analysed. The first low grade dysplasia biopsy was retrieved with the block and stained for both cMYC and p53. Stains were read by a single observer blinded to outcome. Moderate to strong nuclear positivity in >50% of dysplastic epithelium was considered positive for cMYC. p53 was considered positive if overexpressed or not expressed (null) in dysplastic epithelium. Correlation with subsequent surgical resections/biopsies was performed. Results: 17 patients fulfilled the inclusion criteria. 14 had relevant surgical resections and 17 had subsequent colonic biopsies. 9 were diagnosed with colorectal carcinoma or high grade dysplasia in subsequent resections and biopsies. Of these, cMYC was positive in 7 and p53 was mutated in 6. Of 3 wild-type p53 stains, 2 were positive for cMYC. 4 of 9 cases were discordant. 10 of 17 had at least one initial biopsy positive for CMYC. 7 of these 10 were diagnosed with colorectal adenocarcinoma within 2 years. Of 7 patients with negative cMYC biopsies, 2 were diagnosed with colorectal adenocarcinoma or high grade dysplasia within 1 year. 1 of these had a mutated p53 biopsy. 5 did not progress to malignancy. Conclusions: Our preliminary study suggests that strong nuclear positivity for cMYC in CIBD-associated low grade dysplasia may predict concurrent or subsequent development of colorectal carcinoma and may act as an adjunct to p53 staining in this context. Large scale, multi-institutional studies are required to fully investigate these preliminary results.

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P52

Gastric biopsy protocols for dyspepsia and suspected H.pylori infection - one year experience in District General Hospital

Following the inclusion of H.Pylori infection as an infectious disease in the 11th revision of the International Classification of Diseases, the indication for treatment is no longer reserved for patients with clinical manifestations of infection. However, this has resulted in widespread prescription of proton pump inhibitors (PPIs) for dyspepsia symptoms, often a significant amount of time before investigations into H.pylori begin. PPIs can alter the morphology of H.pylori from spiral to coccoid shape, hampering their identification in gastric biopsies. Hence, PPI therapy along with histological features such as atrophy, intestinal metaplasia and reactive gastropathy are recognised as hostile environments that can produce a false negative result for H.Pylori in biopsies. To avoid this, the sixth edition of the Maastricht/Florence 2021 Consensus Report, Public Health England and NICE Clinical Knowledge Summaries have prescribed recommendations for testing of H.Pylori and protocols for endoscopic sampling of gastric biopsies. We analysed our one-year departmental experience in reporting gastric biopsies sampled for initial investigation of H.Pylori and dyspepsia in 2022. 928 cases were identified in the study period. Both antrum and body compartments were sampled and sent in separate pots in 16.9% cases from Endoscopy. The formal advice to stop PPI therapy for minimum 2 weeks before endoscopy was not evident in 59.4% cases, with continued/ongoing PPI treatment identified whilst being biopsied. Only 4.2% cases were reported as H.Pylori positive by the Pathologist. 7% of cases were found to have both endoscopic and histological features of PPI therapy and 13.2% of cases were found to have a histological hostile environment other than PPI changes. There is a need to balance the timing for PPI prescription and cessation, planning endoscopy and submitting appropriate gastric samples to optimise H Pylori identification in gastric biopsies to curtail false negative results.

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P53*

Utility of T-cell Receptor Repertoire Sequencing in the Diagnosis and Subclassification of Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) can be challenging to diagnose and subclassify as ulcerative colitis (UC) and Crohn's disease (CD), especially at the early stage. Given the increasing incidence of IBD, and the importance of its diagnosis and subclassification for clinical management, it is important to develop an objective, robust method to diagnose and subclassify IBD. The T-cell receptor repertoire (TCRR) can be different in IBD compared to non-IBD individuals, and in UC compared to CD individuals. A machine learning algorithm developed to distinguish between 2 diagnostic groups based on differences in the TCRR was trained and validated for its use in diagnosing and subclassifying quiescent IBD, which serves as a good model for early IBD. In addition, detailed analysis of the T-cell receptor repertoire of quiescent IBD samples was performed to identify signatures in the TCRR which can also aid in diagnosis and subclassification of early IBD. Though the machine learning algorithm developed did not achieve a high accuracy, and largely no significant trends in the TCRR were identified, it is possible that this study was limited by its sample size. Future work would include repetition of the study with a larger sample size.

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P54

Validation of the 'APIS Breast Cancer Subtyping Kit' mRNA Assay to Test for ER, PR, HER-2, and Ki67 in Invasive Breast Cancer

Purpose of the study

The APIS mRNA assay is marketed as a fast and highly accurate method for the molecular detection of standard biomarkers routinely assessed during treatment decisions for patients with invasive breast cancer (IBC). A four-gene signature for measuring proliferation is also generated. Here we compare current histological methods, immunohistochemistry (IHC) and in situ hybridisation (ISH), with RNA-based analysis using the gene expression of four markers: ESR1 (ER), PGR (PR), ERBB2 (HER-2) and MKI (Ki-67).

Methods

100 consecutive FFPE breast core biopsies with IBC, categorised as B5b, were selected. IHC/ISH for ER, PR, HER-2 and Ki-67 (the latter not routinely reported in our laboratory) were assessed. RNA was extracted and the APIS assay was run. Results generated were in a binary manner (positive/ negative or high/low). The two methods were compared. Ki-67 results were compared with the number of Ki-67 positive cells on IHC.

Summary of results

ESR1 was discordant in 3 of 100 cases: 1 scored ER and PR positive (7/8, HER-2 negative) but triple negative on APIS, even after repeating the assay. 1 was a recurrent breast cancer, ER positive and APIS ESR1 negative. The third was ER positive and APIS ESR1 negative. PGR was discordant in 10 of 100 cases. Of these, 8 had PR low scores (2-6/8) and APIS PGR negative. 2 were PR negative (0/8) but APIS PGR positive. ERBB2 results were 100% concordant with IHC/ISH. Ki-67 was discordant in 3 of 100 cases when compared with MK167 but discordant in 24/100 when compared with four novel proliferation biomarkers (MKI67, PCNA, CCNA2, KIF23).

Conclusions

The APIS assay may offer a quicker and viable alternative for assessing ER, PR, HER-2 and Ki-67 in IBC. It could alleviate pressure on histopathology laboratories. Moreover, it removes the subjective interpretation of these markers and the need for histopathologist input at this stage.

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P55*

Multiple disease mechanisms underlying autophagy deficits in ATG7-related neurological disease

Autophagy is a highly conserved catabolic process, key to maintaining cellular homeostasis. It relies on a number of core autophagy (ATG) genes, very few of which have been associated with disease. We recently described the consequences of rare, damaging bi-allelic ATG7 variants in a cohort of twelve patients from five unrelated families affecting a core autophagy protein involved in the two ubiquitin-like conjugation systems required for autophagic vesicle expansion (PMID: 34161705). All patients presented with developmental delay and ataxia with neuroimaging showing atrophy of the corpus callosum and cerebellar hypoplasia. Analysis of patient-derived fibroblasts showed undetectable or greatly decreased ATG7 protein, resulting in a complete loss or severe depletion of autophagic flux. Here we present data on an expanding ATG7 patient cohort; trio whole exome sequencing has identified a first patient with a de novo heterozygous ATG7 variant, leading to a missense change in a conserved amino acid within the protein's ATPase domain. Biochemical analyses indicated decreased ATG7 levels, resulting in a mild autophagy flux deficit. Moreover, a foetal case with segregating loss of function ATG7 variants associated with polymalformative syndrome demonstrated no detectable ATG7 protein in a frozen liver sample, with analysis of brain tissue identifying migrating neurons in the corpus callosum and purkinje cells in the cerebellum as some of the susceptible cell types. To further understand the cell types involved, we are developing an iPSC-derived neural model to dissect the role of autophagy in the context of neuronal-astrocyte interactions.

While the original patient cohort shared a unifying clinical phenotype and disease mechanism, the expanding cohort indicates that the underlying disease pathology may be more variable. Further expansion of the patient cohort will provide better insight into these mechanisms underlying ATG7 related neurological disease. (Path Soc Funded)

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P56*

Investigating Whole Genome Doubling In Undifferentiated Sarcomas Using Flow Cytometry

Purpose of the study:

Whole genome duplication (WGD) estimates derived from whole genome sequencing (WGS) data are up to 30% incorrect. WGD is associated with poor prognosis in many cancer subtypes, and accurate ploidy estimation is crucial for understanding WGD in tumour progression and facilitating biomarker identification. This study demonstrated the effectiveness of flow cytometry (FC) in accurate determination of ploidy from formalin-fixed paraffin-embedded (FFPE) tissue of undifferentiated sarcoma (USARC).

Methods:

50 FFPE USARC tissue curls (50µm) were dissociated into single nuclei. The sample cell density was standardised and stained with DAPI before being analysed with FC. The tumour ploidy was calculated using diploid controls and compared to image cytometry (IC) data and WGS derived ploidies (ASCAT).

Results:

FC vs IC showed very strong correlation for tumour ploidy ($R^2=0.9371$, $p<0.0001$)- 92% (35/38) samples exhibited concordant ploidy. FC vs ASCAT displayed a statistically significant relationship with weak correlation ($R^2=0.1227$, $p=0.0493$), 69% (22/32) of samples showing concordant ploidies. The remaining 31% were discrepant, reflecting ASCAT ploidy overestimation and underestimation by a factor of 2. Similarly, IC vs ASCAT showed 64% concordance (25/39) and 36% discrepancies.

Conclusion:

These results show that FC is an effective, accurate method to determine ploidy from FFPE tissue. Moreover, the findings suggested that FC and IC could be used interchangeably for ploidy calculation. We validated previous work by Van Loo et al. (2010), demonstrating that approximately 30% of WGS ploidy solutions are incorrect. Employing these "gold standard" derived ploidies could be used to correct copy number caller inaccuracies and improve understanding of WGD in USARC and other cancers. Our lab will build upon this study with an aim to train a deep neural network for identifying WGD in digital pathology.

This research was kindly supported by PathSoc PhD grant 185734.

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P57

Analysis of Rates and Sub-types of Dysplasia in a Large Cohort of Sessile Serrated Lesions of the Colorectum

Purpose of the study: We aim to describe rates of dysplasia, subtype them by type, adenomatous and serrated and grade (high and low) and evaluate the use of MLH1 immunohistochemical ancillary testing in SSLs diagnosed in a tertiary endoscopy referral centre and a National Bowel Screening Centre.

Methods: We identified SSLs by searching the laboratory information system for all cases in a two year period between 01/01/2021 and 31/12/2022. Traditional Serrated Adenomas were excluded. We noted the total number of SSLs, any dysplasia and whether MLH1 testing took place. In cases where dysplasia subtype was not included in the report (68) the slides were reviewed by two observers (JM and DG). Cases were divided into 2 cohorts based on if they were referred for endoscopy via the National Bowel Screening Program or from a 'symptomatic' pathway.

Summary of Results: 1805 SSLs were identified. Overall dysplasia rate was 5.1%, (92/1805). Low grade adenomatous dysplasia was seen in 2.9%% (53/1805), high grade adenomatous dysplasia in 0.22% (4/1805), low grade serrated dysplasia in 1.94% (35/1805) and no case of high-grade serrated dysplasia (0/1805) was identified. There were similar levels of dysplasia in the 2 groups, 5.08% (83/1633) in the symptomatic cohort and 5.23% (9/72) in the screening cohort. Two SSLs had both adenomatous and serrated dysplasia. MLH1 testing was performed on 15% (14/92) of dysplastic polyps and loss of expression was seen in two, both with low grade serrated dysplasia.

Conclusions: We describe rates of dysplasia and its subtypes in a large series of SSLs in order to inform quality standards for pathologists.

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P58

An audit into all Reports of Adenocarcinoma Diagnoses from Colorectal Cancer Resection Specimens during the calendar year of 2022 at University Hospitals Coventry and Warwickshire NHS Trust

Purpose of the Study: This audit was done using data from all Colorectal Cancer Reports which were signed off during 2022. Adenocarcinoma diagnoses were of interest for this audit. Audit criteria was based on the guidelines as set out by the Royal College of Pathologists (RCPath) on reporting of colorectal cancer specimens. First, to observe if all reports followed guidelines on macroscopic and microscopic dataset reporting regulations. The criteria for audit are as follows: minimum of 15 lymph nodes examined per resection specimen, at least 20% of total cases to have peritoneal involvement, at least 30% of total cases to have venous invasion and all cases to be reported and signed out within 10 days of specimen reception at the laboratory. Methods: Data for this audit was kindly provided by the IT department in Pathology. They provided me with all colorectal resections done at UHCW within the year of 2022. This totalled over 1900 cases. I then filtered through all these cases whereby only Adenocarcinoma cases which were colorectal resections were included in my audit. I narrowed the total number of cases for audit down to 155. I then analysed each case report one by one against the RCPath guidelines and their audit criteria. Summary of Results: 83% of case reports demonstrated at least 15 lymph nodes examined. Peritoneal involvement and Venous invasion passed the Audit with 25% of total cases showing evidence of peritoneal involvement and 66% showing venous invasion respectively. 97% compliance with macroscopic and microscopic dataset guidelines, respectively. Only 24% of reports were signed out within 10 working days of reception by pathology lab. Conclusions: Overall positive outcome from this audit but room for improvement. RCPath proforma should be complied with to ensure all reports aesthetically mimic the dataset criteria. Try to achieve at least 15 lymph node examinations in 100% of cases. Encourage quicker signing out of reports to achieve 10 working day target.

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P59

Emerging Histopathological Risk Factors for Lymph Node Metastases in pT1 Colorectal Cancer: Immune Response and Tumour-Stroma Interface

Colorectal cancer (CRC) is a common malignancy worldwide and tumour stage is closely related to clinical outcome. A small but significant proportion of submucosal-invasive (i.e., pT1) CRC are associated with regional lymph node metastases (LNM) and a worse prognosis. Histopathological risk factors for LNM that are recognised within national guidelines include lymphovascular invasion, perineural invasion, tumour size, tumour grade, histological subtype, tumour budding and proximity to the surgical margin. Calculation tools developed to aid the prediction of LNM risk using these parameters alone have variable concordance with real-life patient data in validation sets.

The immune cell infiltrate associated with neoplasms and the tumour-stroma interface are hypothesised to play an important role in the aetiology of metastases. In pT1 CRC, a deficient mismatch repair (dMMR) phenotype is associated with the presence of tumour infiltrating lymphocytes (TILs) and a lower incidence of LNM compared to mismatch proficient tumours. Similarly, greater numbers of CD8+ TILs, specifically in the tumour centre, are associated with lower rates of LNM. Studies have demonstrated that higher PD-L1 expression in TILs is associated with lower LNM risk in pT1 CRC. The relationship, if any, between PD-L1 expression and factors such as the pattern of immune cell infiltration and MMR status in pT1 CRC is currently unknown. Immature, myxoid stroma at the tumour periphery is associated with absent TILs, a high degree of tumour budding and, in pT1 CRC, LNM. In pT1 CRC, a greater proportion of tumour stroma has been associated with an increased risk of LNM. A peri-tumoural stroma (PTS) score has been proposed as another parameter predictive of LNM but has not yet been validated in a pT1 cohort. Although early studies are promising, methodological standardisation and large-scale validation are required before these results are integrated into diagnostic practice.

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P60

Emerging Histopathological Risk Factors for Lymph Node Metastases in pT1 Colorectal Cancer: Immune Response and Tumour-Stroma Interface

Colorectal cancer (CRC) is a common malignancy worldwide and tumour stage is closely related to clinical outcome. A small but significant proportion of submucosal-invasive (i.e., pT1) CRC are associated with regional lymph node metastases (LNM) and a worse prognosis. Histopathological risk factors for LNM that are recognised within national guidelines include lymphovascular invasion, perineural invasion, tumour size, tumour grade, histological subtype, tumour budding and proximity to the surgical margin. Calculation tools developed to aid the prediction of LNM risk using these parameters alone have variable concordance with real-life patient data in validation sets.

The immune cell infiltrate associated with neoplasms and the tumour-stroma interface are hypothesised to play an important role in the aetiology of metastases. In pT1 CRC, a deficient mismatch repair (dMMR) phenotype is associated with the presence of tumour infiltrating lymphocytes (TILs) and a lower incidence of LNM compared to mismatch proficient tumours. Similarly, greater numbers of CD8+ TILs, specifically in the tumour centre, are associated with lower rates of LNM. Studies have demonstrated that higher PD-L1 expression in TILs is associated with lower LNM risk in pT1 CRC. The relationship, if any, between PD-L1 expression and factors such as the pattern of immune cell infiltration and MMR status in pT1 CRC is currently unknown. Immature, myxoid stroma at the tumour periphery is associated with absent TILs, a high degree of tumour budding and, in pT1 CRC, LNM. In pT1 CRC, a greater proportion of tumour stroma has been associated with an increased risk of LNM. A peri-tumoural stroma (PTS) score has been proposed as another parameter predictive of LNM but has not yet been validated in a pT1 cohort. Although early studies are promising, methodological standardisation and large-scale validation are required before these results are integrated into diagnostic practice.

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P61

Comparison of Models for the Calculation of Lymph Node Metastasis Risk in pT1 Colorectal Cancer

Purpose of the study: Colorectal cancer (CRC) is a common malignancy worldwide and diagnosis at an early stage is associated with improved prognosis. In pT1 CRC, lymph node metastases (LNM) are associated with a worse outcome and prompt consideration for surgical resection. Many risk factors have been proposed for the presence of LNM and these have been combined into several different calculator models, mainly presented as nomograms. The aim of this study was to compare the utility of published calculator models and the LNM probabilities derived from each.

Methods: Nine published risk calculator models were identified -- eight nomograms and one online tool. Data from two virtual patients (VPs) were input into each model -- one low-risk and one high-risk, created using risk factors derived, where possible, from published guidelines and meta-analyses. The ease of use of each model in this setting was assessed and the calculated LNM probabilities were compared.

Summary of results: The nature of the data items was solely histological in four models, demographic plus histological and clinical plus histological data in two models each and all three data types in one. Eight of the nine calculator models were applicable to both VPs. Within these, the most heavily weighted data item was tumour grade in four models, with tumour depth, vascular invasion, tumour budding mitoses and imaging-reported node involvement most weighted in one model each. All eight applicable models indicated a low LNM probability (<0.002-0.06) for the low-risk VP and a high LNM probability (0.58->0.99) for the high-risk VP.

Conclusions: Multiple published assessment calculators exist for LNM probability assessment in pT1 CRC and use different combinations of histological and non-histological data. All models include at least some individual histological features from published guidelines. Further comparisons using VPs not at combined risk factor extremes may allow greater discrimination between calculators.

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P62

Radical Cholecystectomies, a decade's experience in review...

Background: Gallbladder carcinoma (GBC) is a rare disease with dismal prognosis. The majority of cases are identified at an advanced state that preclude curative approaches which would usually necessitate removal of the gallbladder with liver resection and portal lymphadenectomy. The aim of this study was to determine the outcomes of patients undergoing surgery for suspected GBC.

Methods: A retrospective analysis of patients who underwent radical cholecystecomies at Queen's Medical Centre, Nottingham, UK between 1st January 2014 and October 2023 was performed. Patients with concomitant primaries, incidental GBC or completion liver resection following index cholecystectomy were excluded.

Results: 41 patients were eligible for analysis, those were comprised of 13 males and 28 females with a median age of 67 (IQR 57-72). The incidence of malignancy on final histology was 31.7% (GBC n=11, metastatic renal carcinoma n=1, metastatic melanoma n=1). The pathologies encountered in benign cases (n=28) were chronic cholecystitis (53.5%, n=15), chronic cholecystitis and adenomyomatosis (21.4%, n=6), xanthogranulomatous cholecystitis (10.7%, n=3), adenomyomatosis (7.1%, n=2), follicular cholecystitis (3.5%, n=1), benign GB polyps (3.5%, n=1). Cystic margin frozen sections was performed in 41% while median tumour size was 30mm in cases of GBC. Positive portal lymph nodes were identified in two patients while R0 resection was achieved in all cases. Median length of stay was 5 days (IQR 5-7) with Clavien Dindo complication grade ≥ III of 12%.

Conclusions: Radical cholecystectomy remains the standard of care for suspected GBC however sufficient preoperative counselling is prudent given likelihood of benign disease on final histology.

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P63

Primary Hepatoid Carcinoma of the Gallbladder With Traditional Adenocarcinoma, Squamous and Neuroendocrine Differentiation: A Case Report

Introduction: Hepatoid carcinoma is a rare type of adenocarcinoma of the gallbladder. We reported a rare case of primary hepatoid carcinoma of gallbladder with areas of traditional adenocarcinoma and focal squamous and neuroendocrine differentiation. Case Summary: An 81-year-old male presented with gastric outlet obstruction, which was found to be secondary to gallstone ileus resulting from a complex cholecystoduodenal fistula. He underwent cholecystectomy and repair of fistula and retrieval of stone. On gross examination, a tumour was noted within the gallbladder lumen.

Results: Histological examination revealed a non-polypoid adenocarcinoma showing predominantly areas of hepatoid morphology with areas of traditional adenocarcinoma and foci of neuroendocrine and squamous differentiation. The background gallbladder mucosa showed high grade dysplasia. The tumour stained diffusely positive for CK7 within the glandular, traditional adenocarcinoma component, and showed variable staining within the solid hepatoid area which was also positive for Glypican 3 but negative within the area of glandular differentiation. CD34 highlighted focal neovascularisation in the hepatoid solid areas. CK8, CK19 were positive in both traditional and hepatoid tumour areas. CEA and CA19.9 were positive in the glandular component only. Synaptophysin, p40 and p63 showed focal positive staining in one of the solid areas with neuroendocrine-type morphological features, but not in the glandular or hepatoid components. Occasional cells within the dysplastic epithelium showed positive staining with P40. The tumour was MMR proficient.

Conclusion : We present a rare case of hepatoid carcinoma in the gallbladder with areas of traditional adenocarcinoma, squamous and neuroendocrine differentiation confirmed by immunohistochemistry. To the best of our knowledge, these are rare tumours and highlight the possibility of multidirectional differentiation arising from stem cells within the gallbladder mucosa.

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P64

Pancreatic Collision Tumours: A Meeting Of Two Distinct Entities

Background

Collision tumours are a rare entity composed of at least two different types of cancer within the same anatomical site, in which there is no mixed area within the collision zone. These type of lesions are challenging to diagnose with the majority not identified on pre-operative biopsy and usually have poor prognosis.

Methods

We report the case of a 73 year old male who presented with a head of pancreas mass in which the biopsy showed a poorly differentiated adenocarcinoma, however, on resection we identified two distinct tumours with unique immunohistochemical profiles. The literature regarding these lesions is limited and our case adds weight to the published data.

Results

The first tumour showed a syncytial growth pattern with pushing expansile borders and positive staining for CD10, EMA, CEA, CK7 and CA19.9 but negative for CK20 and CDX2; this was in contrast to the second tumour which showed a glandular and infiltrative appearance. This tumour was positive for CD10, EMA, CDX2 and CEA but negative for CK20 and CA19.9. Furthermore, we assessed for mismatch repair which showed loss of PMS2 in the first tumour and loss of PMS2 in the second. We concluded based on morphological and immunohistochemical pattern the first tumour was a pancreatic medullary carcinoma and the second a poorly differentiated adenocarcinoma arising from the duodenum. K-RAS mutation was detected in the first tumour.

Conclusion

Pancreatic Medullary carcinoma is a rare subtype of the pancreatic ductal carcinoma; to date there have only been 26 reported cases in the literature. These lesions have a unique genetic profile with KRAS mutations; a small group demonstrates microsatellite instability. Our case highlights the importance of effective sampling and utilising immunohistochemistry to diagnose pancreatic lesions. Furthermore, we highlight the essential role of the pathologist in identifying pancreatic medullary carcinoma and the need of further investigations and genetic counselling.

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P65

A comparative One Health assay analysis of Luminescence immunoprecipitation system (LIPS) and Radioimmunoassay (RIA): which is most specific at detecting islet autoantibodies to identify those at risk of developing Type 1 Diabetes (T1D) in both medical and veterinary settings?

Purpose of the study: Four islet autoantibody markers can be detected years prior to T1D onset: glutamate decarboxylase 65 (GADA), islet-antigen 2 (IA-2A), insulin (IAA) and zinc transporter 8 (ZnT8A). RIA is the current gold standard test for autoantibody detection. However, LIPS offers improved affordability, convenience, sustainability, and safety. We aimed to determine the specificity of LIPS and its potential to replace RIA. The benefits of LIPS could be applicable to autoimmune diabetes in animals such as Bank voles, which can be housed for laboratory research.

Methods: Blood sera (n=1459) from relatives of people with T1D from the Barts Oxford study to test for all four autoantibodies by RIA and LIPS. Small volume samples from 48 bank voles were tested for islet autoantibodies by LIPS alone. Use of labelled antigens designed for detection in humans was assessed by comparison between species' protein sequences. In RIA and LIPS, the residual radiation/luminescence detected was proportional to the presence of autoantibodies.

Results: After selecting one sample per individual where samples had ≥3 LIPS and RIA results, 83 samples remained [median 31.3yrs (range 2.7-57.4yrs); 46 (55.4%) males; 32 (38.6%) individuals were later diagnosed with T1D]. Autoantibody status was highly concordant between methods (agreeing in 83-96% of samples; some discrepant samples between methods for IA-2A/ZnT8A/IAA were observed; Fisher's exact tests p<0.0001), and autoantibody levels were highly correlated (Spearman's r:≥0.72, p<0.0001). Overall, lower LIPS positivity thresholds allowed for autoantibody detection at lower concentrations. When comparing antigen sequences recognised by islet autoantibodies between human human and vole, no epitope differences were observed for IA-2 and insulin, with 21% of voles positive for IA-2A and 6% for IAA, suggesting IA-2A and IAA LIPS are compatible tests. Conclusions: Our data set shows LIPS has comparable specificity to RIA.

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P66

Pathologist compliance to reporting guidelines for colorectal resections and analysis of MMR pattern (2021-2023)

Purpose of study: The primary aim was to assess pathologist compliance to RCPath guidelines on reporting of colorectal cancer (CRC) resections from September 2021 to April 2023. Secondary aims included conformity with mismatch repair (MMR) guidelines and molecular profiling in colorectal cancers reported.

Methods: CRC resections reported on CoPath (LIMS) between September 2023 and April 2023 were searched using the following SNOMED codes: Gastrointestinal resection(P1100I), Rectum(T68000), Colon(T67000), Adenocarcinoma(M81403). The following was extracted: pathologist, resection type, stage, site, total lymph node (LN) number, positive LN, margins (R0/R1), MMR status, NGS results. Compliance regarding reporting of serosal involvement (<20%), number of LNs (<12) and extramural invasion (<30%) was analysed as well as frequency of MMR deficiency and NGS mutations found.

Summary of results: 244 cancers (87 rectal, 157 colonic) were reported between September 2021 and April 2023. Serosal involvement was present in 22.98% of cases, extramural invasion in 39.24% and the median number of LN analysed was 22. 29 cases were MMR deficient, the most common pattern lost was MLH1/PMS2 expression. 11 of these cases had MLH1 promoter hypermethylation and 2 had BRAF V600E mutations. 86% of MMR deficient cancers were right sided and in patients over 60. 49 MMR proficient metastatic cases underwent NGS. 50% had mutations identified: 16 KRAS mutations, 7 BRAF mutations, 2 NRAS mutations. 12% of our cases had MMR deficiency (vs. 15% in the literature), 35% had RAS mutations (vs. 40% in the literature) and 8% had BRAF mutations (vs. 10% in the literature).

Conclusions: Our institution hits targets on key pathology indicators and has good adherence to NICE guidelines on the testing for MLH1 hypermethylation and BRAF mutations when MLH1/PMS2 deficiency is present. The cohort of patients analysed reported frequency of MMR deficiency and RAS/BRAF mutations in keeping with c…

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P67

A rare case of bowel perforation in a 25 year old patient with vascular Ehlers Danlos Syndrome

Introduction: Ehlers Danlos syndrome, vascular type (vEDS) is an autosomal dominant connective tissue disorder caused by mutation in the type III collagen gene. Adult patients with vEDS usually present with vascular rupture/ dissection, gastrointestinal tract perforation or uterine rupture as blood vessels, intestines and uterus are rich in type III collagen.

Case history: This 25-year-old diagnosed patient with vascular Ehlers Danlos syndrome presented with features of bowel perforation. CT scan showed splenic flexure perforation. Subtotal colectomy with end ileostomy was performed. Macroscopically, colon showed perforation and multiple diverticulae like out pouched areas.

Microscopy showed localized acute peritonitis around the site of perforation. Diverticulae like foci showed gross attenuation of the muscularis propria with areas where the bowel wall was completely devoid of muscularis propria with replacement by fibrous connective tissue. Cause of bowel perforation was attributed to vEDS.

He also has history of right internal carotid artery dissection and bilateral vertebral artery aneurysm.

Discussion: Around 35 cases of bowel perforation in vEDS has been reported in the literature. In one review study, mean age of perforation was 28 years and common site of perforation was sigmoid colon. Focal complete absence of muscle layer as in our case was described in only a few cases.

Conclusion: vEDS should be suspected in young patients with gastrointestinal perforation without any obvious cause. Careful microscopic examination of abnormal looking areas away from perforation will help to identify defective muscular layer and therefore raise suspicion of vEDS in undiagnosed patients. vEDS is different to other benign forms of EDS and should be managed carefully.

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P68

Constrained karyotypes and immune predation may explain the low incidence of small intestinal cancer

Tumours of the small bowel are rare and represent the smallest fraction of all gastrointestinal (GI) cancers despite being the largest organ in the GI tract. As a result, they have not been molecularly well characterised and consequently are often clinically managed similarly to colorectal cancers. Here we performed shallow whole-genome sequencing (sWGS) on 125 cases of the most common adenocarcinomas in the small bowel, duodenal adenocarcinomas, and compared their somatic copy number aberrations to 880 upper and lower GI primary adenocarcinomas from the TCGA. Furthermore, we reanalysed published whole exome sequencing (WEX) from a further 91 small intestinal carcinomas to assess the immune landscape. In our sWGS cohort, we found that duodenal adenocarcinomas have a significantly lower incidence of genome doubling (20%) than elsewhere in the GI tract (colon=43.6%, stomach=44%, rectum=55%, oesophagus=64.3%). Duodenal cancers also had less fractured genomes, as measured by the number of copy number alterations distinct to baseline ploidy, than colon (p=6.3x10-4, ratio=0.176), rectal (p=3x10-5, ratio=0.279), stomach (p=1.5x10-8, ratio=0.309) and oesophageal (p=1.2x10-11, ratio=0.546) adenocarcinomas. Within the previously published WEX cohort of small intestinal adenocarcinomas, we found that escaped small intestinal tumours were associated with a significantly decreased overall survival compared to non-escaped tumours (HR 2.197 (95% CI, 1.736-- 46.63); p=0.009). Further, dN/dS analysis indicated strong immune-mediated negative selection on clonal non-synonymous mutations in the immunopeptidome in most tumours. The decreased frequency of genome doubling amongst small bowel cancers compared to other GI cancers could indicate more restricted routes for karyotype evolution in the small bowel. Our data also suggest a more pertinent role for immune-predation than elsewhere in the GI tract. Together this may explain their lower incidence.

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P69

Assessment of histopathological reporting standards in colorectal carcinoma

Background: Datasets provided by the Royal College of Pathologists outline fundamental data elements essential for inclusion in histology reports. The 2007 Dataset for Histopathological Reporting of Colorectal Cancer (2nd Edition) introduced specific reporting criteria for three vital prognostic factors: Median number of lymph nodes, frequency of peritoneal involvement and frequency of venous invasion. Objective: Our objective is to assess the department's ability to meet these stringent reporting standards.

Methods: We conducted a retrospective study of cases covering the period from January 1, 2022, to March 16, 2023, encompassing a total of 133 colon, 92 rectal resections and 265 biopsy specimens.

Results: In our study, findings revealed variations in lymph node counts with median number of 18. Extramural venous invasion was present in 29% of cases while intramural venous invasion was seen in 4% of cases. About 28% of cases exhibited peritoneal involvement. Out of 21 treated cases, 7 achieved TRG1, and 14 achieved TRG2/3. MSI status was checked in 385 cases and it was unstable in 51 cases (15% of cases). 92% of cases showed loss of expression of MLH-1 and PMS-2. In cases with loss of MLH1-PMS2, BRAF analysis was performed. The presence of BRAF V600E mutation indicates a sporadic tumor. BRAF mutation was seen in 27 cases (29% of cases). If BRAF mutation is not identified, MLH1 hypermethylation studies are done for those cases. In 5 cases, MLH1 promotor hypermethylation analysis was performed. Of these hypermethylation was identified in 2 cases indicating a sporadic tumor. The other 3 cases were referred to the genetic service for Lynch syndrome evaluation. KRAS gene mutation was seen in 46% of cases. NRAS gene mutation was seen in 7% of cases. The presence of a KRAS/NRAS activating mutation indicates non-sensitivity to anti-EGFR therapy.

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P70

A case report of juvenile-like (inflammatory/hyperplastic) mucosal polyposis of the gastrointestinal tract in neurofibromatosis type 1

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease, is a common autosomal dominant inherited disorder characterised by pigmentary abnormalities and neurocutaneous tumours. The disease can feature various gastrointestinal (GI) manifestations, most commonly gastrointestinal stromal tumour (GIST). Juvenile-like (inflammatory/hyperplastic) mucosal polyps of the GI tract are extremely rare but have previously been suggested to be a manifestation of NF1.

The patient was a 65-year-old male with NF1 who presented with symptoms of subacute small bowel obstruction. The patient exhibited NF1 manifestations, including café au lait pigmentation and multiple sebaceous cysts. A CT scan showed multifocal ileal tumours with associated lymphadenopathy, which were suspected to be neurofibromas. A capsule endoscopy showed sessile polyps in the ileum with surface erosions and a broad base. The patient underwent an ileocaecal resection for symptomatic reasons.

Histopathological examination of the resection specimen revealed approximately 20-30 polyps in the terminal ileum. Microscopy showed ulceration, severe crypt distortion, and pseudopyloric metaplasia. The lesions had cores containing numerous inflammatory cells, but only scanty neural elements and ganglion cells were present. These features were in keeping with a diagnosis of juvenile-like (inflammatory/hyperplastic) mucosal polyps.

This case report adds to the growing body of evidence suggesting that juvenile-like (inflammatory/hyperplastic) mucosal polyps of the GI tract are a manifestation of NF1, emphasising the importance of recognising and characterising rare manifestations within the spectrum of NF1-related pathologies. In contrast to most previous case reports of this entity, we describe presentation with multiple polyps in the small bowel.

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P71

Sex determinants of clinical outcome in oesophageal carcinoma 2022-2023: a single centre study

Incidence of oesophageal carcinoma in the UK has increased by 4% over 30 years. Rates in females have decreased by 16%, and in males have increased by 11%. 5-year survival rates for oesophageal carcinoma remain unchanged. Health equality differences are stark with 43% higher incidence in the most deprived quintile compared with the least. Males incidence is 50% higher in the most deprived quintile compared with the least. We set out to understand the demographic and outcome data of oesophageal carcinoma cases in our diverse centre.

An audit of new histopathological oesophageal carcinoma diagnoses over 1 year was performed. Data spanned one primary referral centre including cases from Northeast Scotland and the Northern Isles. Data extracted from health records included risk factors such as: BMI >25, sex, smoking status, GORD, Alcohol intake >14 units/week, and family history. TNM stage, mortality and precursor lesion data was also collected.

We identified 122 cases of oesophageal carcinoma; 89 (73%) male and 33 (27%) females. Males and females show distinct age-of onset distributions with female incidence peaking between 80-84 and males 65-69. Squamous cell carcinoma accounted for over 50% of oesophageal carcinoma diagnoses in females compared with 22% in males. Alcohol excess (>14 units/week) was seen in 6% of females but over 25% of males. A higher percentage of males (71%) had a BMI > 25kg/m2 compared with females (58%). The risk profile for GORD and smoking was similar between sexes.

Understanding sex differences may improve outcomes by addressing the needs of individuals within at-risk populations. Importantly, whilst females present later, they have a worse 5-year survival. Understanding the molecular underpinnings of these sex differences is needed. Our data indicates that a precision medicine approach is important in oesophageal carcinoma, with distinct sex differences in risk profiles identifying targeted areas for research, public prevention, and funding.

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P72

Risk factors and clinical outcome in oesophageal carcinoma -- A single centre study

Background: Oesophageal carcinomas are aggressive tumours with a high mortality rate. Despite this poor outlook, 59% of oesophageal carcinomas are thought to be preventable. Therefore, a better understanding of risk factors is a priority to inform precision prevention strategies at the local authority level. We have therefore performed an audit of risk factors and associated outcomes for oesophageal carcinomas diagnosed within our primary referral centre to gain a better understanding of how prevention strategies could be targeted in our local population.

Methods: This study presents data from an audit evaluating 122 oesophageal carcinomas diagnosed between July 2022 and August 2023 in a single primary referral centre covering cases from all of Northeast Scotland and the Northern Isles. The aim is to gain a better understanding of the risk factors determining survival to identify areas of concern for precision prevention and local authority management.

Results: We identified 122 cases of oesophageal carcinoma diagnosed between July 2022 to August 2023. 27 % (33) were females and 72% (88) males. The median age of onset was 73 (age range from 41 to 93). There were 85 adenocarcinomas, 32 squamous cell carcinoma and 5 other tumours including poorly- or undifferentiated carcinomas and neuroendocrine carcinomas. The most common risk factor for adenocarcinoma in our data was BMI> 25 and for squamous cell carcinoma it was smoking. Importantly, we identified that the mortality was higher in the population with BMI<25.

Conclusion: By performing this audit, we have a better understanding of the risk factors driving oesophageal incidence in our local population. These data will facilitate the identification of at-risk populations, enabling early detection and targeted management and treatment strategies.

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P73

Evaluation of Human Epidermal Growth Factor Receptor 2 (HER2) expression in gastric and gastroesophageal junction adenocarcinoma- a single center experience

Purpose of the study

Gastric and gastro-oesophageal junction(GOJ) adenocarcinoma is the fifth most common cancer and fourth leading cause of cancer death globally. HER2 overexpression occurs in 12-17% of oesophageal, 13-22% of gastric and up to 30% of GOJ adenocarcinoma. We evaluated the incidence of HER2(4B5) expression and predictive pathological features in gastric and GOJ adenocarcinoma reported at Royal Surrey Hospital.

Method

This is a 9-year retrospective study. HER2(4B5) immunohistochemistry(IHC) and in situ-hybridization(ISH) status and pathological data were collected from January 2014 to December 2022.

Results

Overall 375 biopsies and 206 resections specimens fulfilled inclusion criteria. HER2 overexpression was identified in 10.7%(22/206) of resection specimens(RS) and 14.4%(54/375) of biopsies. The overall incidence in biopsies and resection specimens was around 13%. The median age of patients at the time of surgery was 67.7 years, with 68.2% of being male. In RS most HER2(4B5) positive tumours were G3 intestinal(50%) followed by G2 intestinal(40.9%) and were situated at GOJ/cardia of stomach(77.2%). Diffuse type tumours have not shown positivity for HER2(4B5) in RS in our study. Most HER2 positive tumours were in pT1b(40.9%) stage. Nodal positivity (pN1) was identified in 45.45% of HER2(4B5) positive cases. HER2(4B5) IHC/ISH had been performed on both biopsies and subsequent RS in 100 patients in which 94% cases showed concordance and six (6%) cases were discordant.

Conclusion

HER2 positivity is associated with intestinal morphology rather diffuse pattern. HER2 testing should be performed on resection specimens when the initial biopsy has a negative HER2 IHC/SISH if patients are being considered for HER2 targeted therapies due to tumour heterogeneity.

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P74

High-Grade Upper Urinary Tract Urothelial Carcinoma in an Incomplete Duplex Kidney: A Case Report

Introduction

Urothelial carcinoma of the upper urinary tract is a relatively uncommon neoplasm compared to the urothelial carcinoma arising in the bladder. The development of urothelial carcinoma within a duplex kidney is even rarer. Here we report a case of multifocal high-grade urothelial carcinoma in a duplex kidney involving the renal pelvis, duplex ureters as well as the conjoint distal ureter.

Case Report

An 84-year-old gentleman presented with an episode of painless visible haematuria without any associated lower urinary tract symptoms (LUTS) or B-symptoms. He was an ex-smoker with a past medical history of non-tumour orchidectomy at age 19. CT urogram revealed a left duplex kidney and ureters with extensive left ureteric lesion resulting in left hydronephrosis of both moieties of the duplex left kidney. A nephroureterectomy was performed. Macroscopic examination showed an incomplete duplex collecting system, with the two ureters draining the upper and lower moieties of the kidney joining up distally to form a single ureter. It also supported the radiological findings of dilated renal pelvises, with tumour being identified throughout one of the duplex ureters and the proximal part of conjoint distal ureter. Microscopic examinations revealed multi-focal urothelial carcinoma. The carcinoma was invading the sub-epithelial connective tissue in the ureter draining the upper moiety with non-invasive high-grade urothelial carcinoma in the calyces of the upper moiety, the distal part of the ureter draining the lower moiety, and the proximal part of the conjoint distal ureter.

Conclusion

This is one of the very few documented cases of urothelial carcinoma arising within the duplex kidney and urinary tracts. Chronic irritation and urine stagnation are known risk factors of urothelial carcinoma and may have contributed to the development of carcinoma in these cases.

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P75

Service evaluation of Frozen Section Analysis during cystectomy procedure

Purpose of the study In this retrospective service evaluation at a tertiary centre, frozen sections undertaken during cystectomy are analysed. Methods All cystectomy specimens reported from January 2017 to March 2023 were identified in a retrospective search of recorded reports on the computer system. Amongst the 112 cystectomies performed, 20 cases (18%) generated a frozen section request. Summary of Results The average age of the patients was 53, with nine female and eleven male patients. Eleven cases (55%) were for surgical margins in partial cystectomy, and five (25%) had pre-surgical mapping biopsies. The final histology for the partial cystectomy cases included seven urachal adenocarcinomas, two leiomyomas, one paraganglioma and one urachal mucinous cystic tumour of low malignant potential with intraepithelial carcinoma. For the cystectomies, five (25%) urethral, three (15%) ureteric and one (5%) peritoneal margin were sent. The final histology included three urothelial carcinomas, two paragangliomas, two with no residual tumours and one each of carcinoma-in-situ and squamous cell carcinoma. On frozen section analysis, nineteen cases showed no malignant cells. One case had detached malignant cells. However, the possibility of carry-over precluded the surgeon from changing the operative plan. On final histology, this case had malignant cells at the margin and subsequently, the patient had a total cystectomy which showed residual adenocarcinoma. Conclusion Over the six years of data analysis, frozen sections for surgical margin assessment were infrequent. There was no change to the planned surgical procedure following the frozen section analysis, indicating surgeons' preference for initial wider margins. Concordance between the frozen section and final histology was 100%. In selected cases, the frozen section may contribute to patient management, reducing positive margins and the risk of disease progression, but it is not advocated as a routine procedure.

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P76

The impact of focal vs. established extra-prostatic extension on prognosis in patients who undergo a radical prostatectomy: a narrative systematic review

Purpose of study: Extra-prostatic extension (EPE), the extension of prostate cancer beyond the confines of the prostate, is subdivided into focal (F-EPE) and established (E-EPE). Measurement of EPE is difficult to determine and several methods have been devised, such as Wheeler's and Epstein's. It is a well-recognised adverse prognostic factor. The purpose of this systematic review was to investigate the impact of F-EPE vs E-EPE on prognostic outcomes in patients who have undergone a radical prostatectomy.

Methods: A review of Embase, Medline (R) and Pubmed databases was conducted. Studies were eligible for full text screening if they included prostate cancer patients who underwent a radical prostatectomy (RP) and investigated extent of EPE and prognostic factors (ie. biochemical recurrence, metastasis, cancer progression, overall survival) as a primary or secondary outcome. Data extraction was carried out with the CHARMS-PF checklist, the quality of studies was determined using the Newcastle-Ottawa Scale for cohort studies and a narrative summary was carried out.

Summary of results: A total of 23 studies, including 37,367 men, were included. Only six of the studies were of high quality. 19 studies reported how they measured EPE; nine used Epstein's criteria, three used Wheeler's criteria, three used both, two studies used radial distance and four provided their own criteria. 11 studies showed that extent of EPE was associated significantly with prognosis, one study showed a marginal significance and nine studies did not show a significant correlation.

Conclusions: This is the first systematic review to investigate extent of EPE on prognostic outcomes in prostate cancer patients. Extent of EPE impacts prognosis; however due to the lack of a standardised consensus on EPE measurement and variation in prognostic outcome definitions provided by the studies, larger prospective studies need to be carried out to determine the true impact extent of EPE has on prognosis.

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P77

A rare case of low grade epithelioid malignant mesothelioma arising from tunica vaginalis in a 37 year old male

Introduction: Mesothelioma of tunica vaginalis(TV) is rare accounting for less than 1% of all mesothelioma cases with only around 300 cases reported upto now.

Case history: This is a 37-year-old male presented with persistent hydrocoele. Ultrasound scan showed left hydrocoele with tumour nodules studding the wall. Excised TV was thickened and studded by multiple nodules many with a papillary appearance.

Microscopy revealed complex tubulopapillary structures arising from the tunica vaginalis visceralis and projecting into the lumen of the TV space. These were covered by a simple cuboidal mesothelial cells invaginating to form an arborizing network forming the expanded papillary structures. Focally the cells infiltrated underlying stroma of the tunica focally extending into the dartos muscle. The cells were positive for CK7, calretinin and WT1. Proliferative index was low(2-3%).

Differential diagnoses included well differentiated papillary mesothelial tumour(WPMT) and florid mesothelial hyperplasia. WPMT doesn't show stromal invasion and nuclear atypia which were present in this case. Mass with typical studding of hydrocoele sac was incompatible with mesothelial hyperplasia which shows fibrotic thickening only.

Although nuclear staining for BAP1 and cytoplasmic MTAP expression were retained, features such as complex architecture, intermediate grade nuclear atypia and focal stromal invasion favoured a low grade epithelioid malignant mesothelioma (confirmed by expert pathologist opinion).

Discussion and conclusion: Mesothelioma of TV commonly presents with hydrocoele. In patients with recurrent hydrocoele, mesothelial tumours need to be excluded. Mesothelioma shows CDKN2A homozygous deletion and BAP1 loss.

Local recurrence commonly occur within 2 years. It frequently spread to peritoneum (implantation if processus vaginalis is patent), regional lymph nodes, scrotal and perineal skin.

Prognosis is better with early diagnosis, in patients <60 years and with epithelioid morphology..

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P78

Ketamine Cystitis: A Case Report and Meta-analysis of the Histopathological Features

Purpose of the study: Recreational ketamine usage is an increasing world-wide problem. Ketamine cystitis (KC) is a debilitating side effect, for which histopathological pathognomonic documentation is lacking. Past literature regarding KC, predominantly comprises of independent case reports and only 2 small case series from over 5 years ago. This is the first KC meta-analysis, compiling all case reports and series with our experience of a classic index case to surmise the key histopathological features of KC. Methods: A meta-analysis of the key KC papers was performed from 2007 to 2023, yielding 125 KC cases in total. The pertinent histopathological findings, alongside the case numbers observed, were tabulated and compared to see which features were most specific and sensitive for the diagnosis of KC. We compared these features to our case report of a 29 year old gentleman who underwent cystectomy for lower urinary tract symptoms (LUTS) secondary to ketamine usage. Summary of results: 79% of the cases published reported mucosal denudation as a feature of KC. The second most predominant feature (found in 44% of cases) was scattered inflammation, of varying degrees and type. Less than 1/3 of cases found the features of; a layer of reactive cells and fibrotic lamina propria. Previously characterised pathogenomic features of KC such as predominant stromal eosinophils and the presence of mast cells were only found in less than a quarter of reports. Other histopathological findings such as squamous metaplasia, intravascular eosinophils, oedematous lamina propria, focal calcification, and blood vessel changes were found in less than 15% of reports. Interestingly, nerve hyperplasia and accompanying ureteric changes were seen within our case and the largest case series to date, (20% of cases) but not commented upon or evaluated in the other cases published. Conclusions: In conclusion, there is considerable overlap with the features of KC with that of cystitis secondary to; drugs,..

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P79

Fibrothecoma of the Testis: A Case Report, Literature Review and Diagnostic Utility of SF1

Purpose of the study: Sex cord stromal tumours (SCSTs) are an uncommon neoplasm of the testis, with tumours of the fibro-thecoma group an even rarer entity. We describe a case of Fibrothecoma of the Testis (FT), and literature review with particular analysis of the immunohistochemistry (IHC) utilised to diagnose this unusual entity. Methods: A 33 year old man presented with a confined unilateral testicular mass, without evidence of raised tumour markers or metastasis. He underwent a radical orchidectomy and dissection at the referring institution. An initial IHC panel and report was issued, but referred for external consultation. Results: The testis sections showed an intraparenchymal lesion, comprised of bland spindle cells, with tapered ends and open chromatin with occasional nucleoli. There was no mitotic activity or necrosis. Within the lesion there was entrapped collagen and abundant small, compressed vessels. IHC showed focal positivity for SF1 and very scanty Calretinin positivity. There was diffuse positivity for Calponin and Actin. There was negativity for S100, CD34, Inhibin, Desmin and DOG1. Reticulum stain showed no nested component. Within the literature, important differential diagnoses to exclude were; Unclassified Sex Cord Stromal Tumour (USCST) due to the potential for malignancy, and Myoid Gonadal Stromal Tumour. The prominent collagen component and negative Reticulum, and S100 negativity and actin positivity helped to exclude these differentials. Also in the literature, the majority of FT cases were inhibin positive. In those that were negative, SF1 was not utilised. In our case the morphology, alongside SF1 and smooth muscle marker positivity, were in keeping with a diagnosis of FT. Conclusion: This case report demonstrates 3 valuable points; (1) FTs are a rare entity that requires a high index of suspicion; (2) IHC can be variable, and SF1 is a useful marker; (3) the distinction between FT and USCST is important for prognosis and follow up.

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P80

Should we report the distance to the anterior margin for PSA recurrence prediction?

Background & Objectives: Since the anterior aspect of the prostate tissue is irregular, measuring the distance to the anterior margin is controversial. Our aim is to investigate the relationship between PSA recurrence and the distance to the anterior margin in anterior dominant Grade Group 1 (GG1) prostate cancers. Methods: A total of 20 GG1 radical prostatectomy specimens (RPs) with anterior dominant tumours were included in this study from 2010 to 2022. PSA recurrence was defined as PSA level greater than or equal to 0.2 ng/mL. Slides were reviewed in order to select the closest margin. Scanned slides were marked by pathologists to acquire digital measurements. Results: 20/10 cases had tumours located within 1 mm of the margin. Among these 10 cases, 3 (33.3%) had PSA recurrence. In the other 10 cases where the tumour-to-margin distance was greater than 1 mm, none (0%) had PSA recurrence. The mean and median follow-up for cases with PSA recurrences were 6.8 and 6.5 years, respectively, with a range of 5-11 years. In total, PSA recurrence was observed in 3 (15%) of the cases, and notably, all 3 of these cases had margins closer than 1 mm. When looking at the specific distances, the mean distance for the 3 cases with recurrent PSA was 0.6 mm (median of 0.437; range of 0.4 to 0.98 mm). In contrast, the mean distance for cases without PSA recurrence was 1.8 mm (median of 1.12; range, 0.135 to 5 mm). Conclusion: Although there are few numbers of anterior-dominant tumour cases, the surgical margin distance at the anterior aspect of the prostate may have an impact on the prognosis of such cases. The long-term follow-up of the patients with GG1 P-Ca may reveal that the tumour distance to the anterior margin can be an important prognostic feature, which should take place in the radical prostatectomy reports in the future.

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P81

Intraoperative Frozen Section Analysis of Ureteric Margins in Patients with Urothelial Carcinoma undergoing Cystoprostatectomy/Cystectomy -- A 5-year retrospective analysis

Intraoperative frozen section (FS) analysis of ureteric margins is a frequent laboratory request during cystoprostatectomy/cystectomy (CPT/CT) procedures in patients with urothelial carcinoma (UC). However, the efficacy of achieving a negative ureteric margin is under debate since many studies have shown that FS negative margins do not confer a decreased subsequent upper tract recurrence or an improved long-term survival. Furthermore, it has been suggested that FS may not be necessary in all patients undergoing these procedures, and that a risk-based assessment, with FS analysis intraoperatively being limited to high-risk patients, is the best approach. High-risk patients include ones with biopsy proven, concurrent UC with carcinoma in-situ (CIS).

With the aim of evaluating the FS ureteric margin occurrence rate and result concordance in our institution, a 5-year retrospective search was carried out using the laboratory information system. Clinicopathologic features studied included patients' age, pathologic tumour stage, presence of CIS, intraoperative FS diagnosis and concordance with the permanent section. 66 cases were identified. The mean age of patients undergoing CPT/CT procedures was 68.8 years old. FS showed CIS in 1.5% (1 patient), in this case, further margins were sent intraoperatively, and this further margin was shown to be benign. Atypia was seen in 6% (4 patients), of these cases 50% sent further margins and these were shown to be benign. Concordance between the FS and permanent section was excellent at 98.5%. In terms of risk stratification of patients, pre-operative biopsies showing CIS were present in 48% of patients who underwent FS ureteric margin assessment.

Further study and analysis of the utility of FS ureteric margins in CPT/CT is needed, as well as collaboration with Urology colleagues, to ensure patient safety and highest possible patient survival rates, while also efficiently utilising histopathology & laboratory services.

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P82

A Rare Case Report: Extraneural Renal Haemangioblastoma and Concomitant PEComa

Purpose of the study: Haemangioblastomas (HB) are a mesenchymal tumour most frequently found within the central nervous system. Rarely, extraneuraxial HB can arise in locations such as the lung and bladder. We report the first case to our knowledge of a HB with concomitant PEC-oma within the kidney. Methods: A 64 year old underwent a partial nephrectomy for a solid lesion in the kidney highly suggestive for renal cell carcinoma (RCC). After an initial IHC panel at the referring hospital, the case was sent to the regional referral centre, wherein additional IHC, Renal RNA fusion gene and RNA Fusion Transcript NGS panel were performed. Summary of results: Microscopically the solid, nested lesion was comprised of oval cells with abundant eosinophilic to clear cytoplasm, encapsulated by a fibrous capsule. There was a prominent network of arborising thin-walled capillaries and a few haemangiopericytoma-like blood vessels. Tumour cells stained positively for CAIX, PAX 8, Vimentin, Inhibin and S-100. EMA, AE1/3 and CD10 were focally positive. CD31 and CD34 highlighted an intra-lesional vascular network. RCC marker, Melan-A, HMB45, CD68, SMA, Desmin, CD117, CK20, GATA 3, CK7, Synaptophysin, Chromogranin, SF1 and AE1/3 were negative. In 3 sections, small sub-capsular proliferations of bland spindle cells embedded in collagenous stroma were seen, that showed strong positivity for Desmin and SMA, with focal staining for HMB45/Melan-A. CAIX, AE1/3, S100 EMA, PAX-8 and CD68 were negative. NGS showed no TFE3 or NTRK fusion. The RNA fusion gene panel showed 2 nonsense variants; TSC1 c.1165G>T p.(Gly389Ter) and TSC1 c.2227C>T p.(Gln743Ter). Overall, the appearances were diagnostic of a renal HB and PEC-oma. Conclusions: Renal HB is a benign, rare, challenging entity requiring a high index of suspicion and diligent IHC utilisation, to avoid misdiagnosis of a RCC. Co-existent haemangioblastomas and PEC-omas are rarer still, and require a keen eye to diagnose both within a single specimen.

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P83*

Improving AI-based cell subtype identification for accurate Neutrophil to Lymphocyte Ratio (NLR) calculation as a diagnostic and prognostic instrument in cancer

Purpose of the study: Peripheral blood myeloid expansion, indicated by elevated Neutrophil-to-lymphocyte Ratio (NLR), is linked with poor prognosis, tumour progression and treatment resistance in malignancies including colorectal cancer. Therefore, NLR can be a valuable prognostic tool and aid in cancer treatments such as reversing therapy resistance by targeting myeloid chemotaxis. This project aimed to re-train an existing cell detection model to identify granulocyte subtypes, neutrophils and eosinophils enabling the calculation of Neutrophil-to-Lymphocyte Ratio (NLR)

Methods: HeteroGenius MIM Cell Analysis Add-on was used which implements a UNET based cell detector and type classifier. 176 images were selected from the original model training data which grouped all granulocyte subtypes into a single class. 19 additional images were annotated for this new model to include neutrophils and eosinophils to give a total of 10 classes. The weights of the model were bootstrapped from the original model which significantly accelerated the model's learning process. Annotations were reviewed for reliability by a histopathologist.

Summary of results: The model is still training and is currently at 13K epochs. Before current training the model was unable to identify the new targets. After 13K epochs the new model is successfully automatically identifying neutrophils and eosinophils. The first pilot study on 87 colorectal cancer images within the tumour revealed mean Neutrophil density to be around 180 per mm2 with average NLR ratio of 0.172. Mean Eosinophil density was around 2 per mm2. The new model deep learning loss is currently 1.15 suggesting the bootstrapping procedure applied allows us to obtain a usable model more rapidly than training a model from scratch.

Conclusions: With further training this approach will enable in depth exploration of the clinical value of NLR and eosinophil counts within tissues.

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P84

Evaluating the use of dual mRNA Kappa/Lambda In Situ Hybridisation in the assessment of B-cell clonality in Marginal Zone Lymphoma

Purpose of Study: The assessment of cell clonality plays a vital role in the diagnosis of B-cell lymphomas: to distinguish cancer from hyperplasia or inflammation. This is often performed by using flow cytometry (FC), immunohistochemistry (IHC) or polymerase chain reaction (PCR) to identify any immunoglobulin light chain restriction. However, these methods have practical limitations, such as the need for unfixed tissue, poor background contrast and the need for specialist equipment respectively. In this study, we compare the use of dual mRNA Kappa/Lambda In Situ Hybridisation (ISH) in the assessment of B-cell clonality in marginal zone lymphoma (MZL) with FC and IHC and assess its utility as a diagnostic tool.

Methods: Using the VENTANA BenchMark Ultra, ISH was performed on 44 cases of MZL. A consultant histopathologist analysed the slides to identify the type of restriction. We compared these results with FC/IHC to determine the concordance. A PubMed literature review was conducted to identify other studies using similar methods on MZL. The key selection criterion was the use of ISH on low-grade non-Hodgkin's lymphoma (NHL), including MZL, after 2013. Five suitable studies were identified and analysed with our data.

Summary of Results: Light chain restriction was identified in 37 of the 44 cases. IHC/FC data was available for 21 cases, and the concordance with ISH was 95%. Combined with other data, the mean concordance against reference methods in MZL is 97.6%, and 97.5% in NHL. Some of the practical advantages of ISH include the ability to use fixed tissue, good background contrast and a fully automated staining process. Some concerns of ISH were noted as well, such as the risk of technical failure and over-interpretation of the results.

Conclusion: Dual mRNA ISH can accurately identify light chain restriction and has several advantages in its application, making it a powerful and useful tool in the diagnosis of low-grade NHL, such as MZL, in healthcare environments.

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P85

Primary Breast Lymphoma - a rare clinical entity

Primary breast lymphoma (PBL) is a rare type of non-Hodgkin lymphoma (HNL). As a clinical entity, it represents 0.05%-0.53% of all breast malignancies and 2.2% of extranodal malignant lymphomas. It is defined as the existence of a breast lymphoma without evidence of systemic disease. The majority being diffuse large B-cell lymphomas. PBL most commonly presents as a rapidly-growing palpable mass, with non-specific imaging characteristics. Diagnosis is confirmed by biopsy and subsequent histological analysis, including immunohistochemistry and molecular testing. Accurate diagnosis of these rare entities is required. In this report, we present a case of primary breast lymphoma.

An 84-year-old lady was admitted to Our Lady of Lourdes Hospital, Drogheda, Ireland, with back pain, weight loss and malaise. CT TAP was carried out which reported a 5.5cm left-sided breast mass. The patient underwent Triple Assessment in Beaumont Hospital. US was performed and an R4 score was given, followed by core biopsy. The biopsy showed histological features highly suggestive of a Non-Hodgkins B cell lymphoma. Immunohistochemistry was carried out which showed CD20, MUM1 and Bcl6 positivity. CD10, Bcl2 & CD23 were negative. The case was referred for molecular testing in St James's Hospital, Dublin, Ireland. FISH analysis was negative for double hit lymphoma. Final diagnosis was breast primary, diffuse large B-cell lymphoma, non-germinal centre type.

Due to the rarity of PB-BLBCL, data regarding the appropriate management is lacking. As breast clinical triple assessment usually detects breast carcinomas, we would like to discuss the features which may suggest a lymphoma clinically, radiologically and histologically. Treatment regimens are based on combination of radiotherapy, chemotherapy and immunotherapy. We would like to discuss the current treatment regimen as well as emergent targeted therapies, as well as the potential role of surgery in cases of primary breast lymphoma.

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P86

Hepatic Mass Leading to Spontaneous Haemorrhage: A complication of a rare haematological disorder

Background Spontaneous hepatic haemorrhage is a rare surgical emergency commonly encountered in the setting of hepatocellular neoplasm; benign or malignant apart from coagulation disorders.

Case history A 77-year-old lady presented with acute abdominal pain, vomiting, dark-coloured urine, and watery diarrhoea. Contrast-enhanced CT abdomen and pelvis revealed a large, 14.6mmx10.3mm, intrahepatic subcapsular haematoma involving liver segment 6, haemoperitoneum, and splenomegaly. An emergency laparotomy and excision of liver segment 5/6 was performed. Gross examination revealed a large haemorrhagic lesion. On microscopy, the lesion showed sheets of immature myeloid precursors predominantly of monocytic series admixed with neutrophils, eosinophils, and an occasional micro-megakaryocyte. These atypical myeloid cells were positive for MPO and CD68 and were negative for CD34 & CD117. MIB-1 was ~70-80%. On further inquiry, the clinician revealed a history of chronic myelomonocytic leukaemia (CMML) diagnosed many years ago. In view of this history of CMML, the diagnosis of a deposit of CMML within the liver parenchyma was offered.

Discussion & Conclusion CMML is a rare clonal haematological malignancy with overlapping myeloproliferative and myelodysplastic features with an inherent risk for leukemic transformation. Common extra-medullary manifestations of CMML include splenomegaly, hepatomegaly, lymphadenopathy, and leukemic cutis. However, manifestation as a haemorrhagic hepatic mass is an exceptional complication. Thorough history taking is the key to accurate diagnosis in such cases.

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P87

Synchronous occurrence of primary breast cancer and renal diffuse large B-cell lymphoma with central nervous system involvement -- an unusual presentation

We present this case of a 72-year-old lady who presented initially in May 2023 with a breast lump. A core biopsy demonstrated invasive ductal carcinoma. She had subsequent definitive surgery (mastectomy) which was successful. However, a month later she presented to the A&E department with seizures, presumed related to her recent diagnosis of breast carcinoma. Brain imaging showed a mass with local vasogenic oedema. At this point, the patient was given a full staging and a further 2cm mass was identified in her right kidney as well as mastoid process enhancement. Initially it was suggested that this was metastatic disease from her known breast carcinoma. However, at the MDT it was decided that a renal biopsy was required given that the grade 1 breast cancer was node negative. The renal biopsy was consistent with diffuse large B-cell lymphoma (DLBCL), non-germinal centre subtype. A subsequent biopsy from the mastoid process was consistent with DLBCL similar to the previous kidney biopsy. Molecular testing has been requested. This case underscores the fact that the presence of synchronous malignancies may pose both diagnostic and treatment challenges. Accurate staging of both malignancies and multidisciplinary team discussion is of utmost importance to guide an optimal therapeutic approach. Histopathological evaluation in this case was essential for both tumours, given the treatment options for widely metastatic carcinoma differs greatly from a high-grade lymphoma and a localised low grade carcinoma. In addition, although DLBCL is the most common lymphoma, only 30-40% will present as extra-nodal disease. Renal DLBCLs account for <5% of extranodal lymphomas. It is usually associated with a poor outcome due to central nervous system involvement. Therefore, we will discuss the importance of molecular testing in risk stratification and multidisciplinary involvement in management plans.

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P88

An unusual case of BCL2-negative follicular lymphoma harbouring a t(14;18)(q32;q21) rearrangement: A case report and review of the literature

Up to 90% of follicular lymphomas harbour a t(14;18) translocation, ordinarily corresponding to BCL2 expression in neoplastic follicles on immunohistochemistry. This is often used as a diagnostic aid when distinguishing from reactive follicles. However, a small subset of t(14;18) positive follicular lymphomas show BCL2 pseudonegativity.

To highlight this phenomenon, we present one such case of a grade 2 follicular lymphoma, where the neoplastic follicles showed a lack of BCL2 expression on immunohistochemistry, despite the presence of a t(14;18) translocation.

A 50 year old male presented with enlarged groin nodes and a retroperitoneal mass. A core biopsy revealed loss of normal lymph node architecture with nodularity and sclerosis. The follicular dendritic cell meshwork was partially retained, but lost in the sclerotic areas. The neoplastic follicles showed a mixed small lymphoid and centrocytic appearance with occasional centroblast cells. Some of these cells were also present within interfollicular spaces. They were positive for pan-B markers and expressed a follicle centre cell immunophenotype, but were negative for BCL2. Despite the lack of BCL2 expression, FISH studies with IGH/BCL2 Dual Fusion probe for detection of the t(14;18)(q32;q21) translocation associated with follicular lymphoma showed an abnormal pattern confirming an IGH::BCL2 rearrangement. There was no evidence of BCL6 rearrangement on break apart probes at 3q27.

The mechanism by which t(14;18) positive follicular lymphomas exhibit BCL2 pseudonegativity is not fully understood but there is evidence in the literature to suggest that missense mutations in the BCL2 gene are more likely to be associated with this, potentially as a result of structural modifications to the protein which interfere with the binding to conventional diagnostic antibodies. This case highlights a potential diagnostic pitfall and emphasises the utility of molecular investigations as a diagnostic adjunct in lymphoma cases.

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P89

Primary intracranial small lymphocytic lymphoma arising on a background of pachyonychia congenita

Introduction: Low-grade lymphoma arising in the central nervous system is very rare. When it occurs, most cases are marginal zone lymphomas or there is a history of another systemic low-grade lymphoma. We would like to report a case of small lymphocytic lymphoma (SLL) presenting as an intracranial mass.

Case: The patient, a 41-year-old female, presented with an 18-month history of unsteadiness, left-sided hearing impairment, tinnitus and vertigo. An MRI performed at the time demonstrated a left cerebellopontine angle mass with features suggestive of a meningioma. Systemic disease was not identified on imaging and blood indices were normal. As the patient was symptomatic, a retro-sigmoid craniotomy and excision of the mass was undertaken. Histologically, the lesion was composed of a dense proliferation of small round blue cells with oval nuclei and indistinct nucleoli, expressing CD20, BCL6 and CD23. There was no evidence of granulomata or histiocytic disease. Due to the unusual presentation, molecular studies were undertaken, however, specific translocations (t(11:14) or t(14:18)) were not identified. B cell clonality studies demonstrated a clonal B cell population. The histological, immunohistochemical and molecular features were most in keeping with a low-grade B cell lymphoma, favouring chronic lymphocytic leukemia/small lymphocytic lymphoma, arising intracranially. The patient had a history of pachyonychia congenita (PC), a rare autosomal dominant keratin disorder affecting the skin. A single case of a low-grade B-cell lymphoma in a young PC patient has been described in the literature.

Conclusion: CLL/SLL arising in the central nervous system is extremely rare. It is unclear whether there is an association between intracranial SLL and the patient's history of PC due to the rarity of both diseases. We discuss the histological and clinical features, initial and ongoing management of intracranial CLL/SLL and the morbidity and mortality associated with same..

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P90

Extracavitary Pleural Effusion Lymphoma: A Rare Case Report

Purpose of the study: Primary Effusion Lymphoma (PEL) is a rare but aggressive large B cell lymphoma associated with Human Herpesvirus 8 (HHV8). PEL presents as a serous effusion within a body cavity in the absence of a known tumour mass. However rarely it can also present as an extracavitary mass, in locations such as the bladder, ureter, and bowel. To our knowledge this is the first known UK case report of an extracavitary PEL within a groin lymph node. Methods: A 54-year-old gentleman presented with a right groin mass and underwent a core biopsy following which he was referred in to the regional SIHMDS diagnostic lymphoma service where comprehensive assessment (IHC, FISH and clonality studies) was undertaken. Summary of results: The core biopsy showed singly scattered and aggregates of large atypical pleomorphic malignant cells, some of which had an anaplastic morphology while others had Reed-Sternberg cell-like morphology. There was a patchy background of small benign B- and T- lymphocytes.CD45 was difficult to assess, however it appeared to focally stain a few tumour cells. The tumour cells were negative for: CD20, CD79a, PAX-5, CD22, CD30, CD15, CD2, CD3, CD5, CD7, CD4, CD8, TdT, ALK-1, p63, CD57, CD43, MPO, CD68, CD163, OCT3/4, S-100, SOX-10, AE1/3, MNF-116, Desmin, CD56, kappa and lambda. The large tumour cells were immuno-positive for EMA, CD138, MUM-1, EBER-ISH, HHV-8, with a Ki-67 of 40-50%. Cytogenetic analysis showed no evidence of MYC, BCL2, or BCL6 rearrangement. Clonality was established for clonal B cell receptor gamma chain gene rearrangements. Conclusions: This case report demonstrates 3 important points, viz., (1) extracavitary PEL is rare and requires a high index of suspicion in highly atypical large B cell neoplasms; (2) extensive IHC panels and ancillary testing are crucial in its diagnosis; and, (3) clonality studies should be considered in establishing the cell lineage.

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P91

Gingival ulcer in a background of immunosuppression; EBV related distinct entity

Introduction

Oral lesions are common in immunocompromised patients and can be classified as infectious (bacterial - syphilis, mycobacteria, virus- herpes simplex virus, cytomegalovirus, fungal and parasitic) and non-infectious aetiologies. The non-infectious aetiologies include aphthous stomatitis and malignancy, particularly squamous cell carcinoma, Kaposi sarcoma and non-Hodgkin lymphoma. EBV associated lymphoproliferations comprises a broad spectrum of disease ranging from indolent localized lesion to highly aggressive systemic lymphoma.

Case report

A 61-years old gentlemen with a history of renal transplant on immunosuppressive therapy for three years was presented with 4 weeks history of pain and swelling of gingiva. Examination revealed an erythematous, friable, indurated ulcer adjacent to a mobile tooth. The incisional biopsy revealed an ulcer with necroinflammatory infiltrate in which ulcer base containing a dense polymorphic lymphoid infiltrate composed of large atypical cells, small lymphocytes, plasma cells, neutrophils and histiocytic. The large atypical cells were positive for CD20, CD30, EBER, PAX5, OCT2, MUM1 and were negative for CD15, CD10, BCL6 and EMA in keeping with Epstein-Barr virus positive mucocutaneous ulcer (EBV-MCU). MIB-1 proliferative index was 40-50%. There is no evidence of systemic lymphadenopathy or hepato-spleenomegaly.

Conclusion and Discussion

EBV-MCU most commonly involves oral cavity including gingiva. It has a very good prognosis with regression or complete remission. The main differential diagnosis to be considered are other EBV associated malignancies including Burkitt's lymphoma, Hodgkin lymphoma, post transplantation/human immunodeficiency virus associated lymphoproliferative disease, T cell lymphomas and nasopharyngeal carcinoma. High clinical suspicion is required, as failure to diagnose EBV-MCU may results in significant morbidity and mortality.

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P92

Hepatic involvement in Multiple Myeloma- a case report

Introduction

Multiple myeloma(MM) is a malignant plasma cell disorder characterized by plasma cell infiltration of the bone marrow and overproduction of immunoglobulin or light chains. Liver lesions involving MM may present with hepatomegaly, jaundice, ascites, fulminant liver failure, or are totally asymptomatic incidentally found by autopsy or image examination.

Case Report

A 69-year-old gentleman, with a history of MM in sternum since 2018 was presented with right shoulder pain after 3 years of remission. Follow up PET-CT revealed progressive disease involving scapula, spine, ribs and humeri and a lesion in left valleculae with bilateral cervical lymph node metastasis which was diagnosed to be poorly differentiated squamous cell carcinoma(SCC) on biopsy. PET-CT after the treatment revealed complete response in laryngeal tumour but progressive disease in bone and incidental finding of two liver lesions measuring 4.8cm in segment V and 2.5mm in segment VII /VIII. Biopsy of the liver lesion revealed sheets of abnormal plasma cells showing positivity for CD138, CD 38 and CD 56 and negativity for P40 consistent with MM deposit rather SCC metastasis.

Discussion and Conclusion.

Hepatic involvement of MM can manifest as light chain deposition disease, amyloidosis or as plasma cell infiltration. Plasma cell infiltration of the liver occurs in 40% cases. It has two distinct pathological variant comprising diffuse infiltrative pattern (sinusoidal/ portal or mixed) and macroscopic nodular form. Diffused hepatic infiltration is the predominant pattern of liver involvement and nodular liver lesions involved by MM is a rare condition. This has a poor prognostic and an aggressive clinical course.

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P93

Artificial intelligence in histopathological image analysis of brain tumours: a systematic review

Purpose of the Study: This systematic review examines the utility of artificial intelligence (AI) in the analysis of digitised brain tumour slides with the aim of informing its potential clinical application in neuro-oncology. Methods: Comprehensive searches were conducted across EMBASE, Medline, and the Cochrane Library up to June 2023 using relevant keywords. Risk of bias was evaluated using the PROBAST criteria. Summary of Results: A total of 68 pertinent studies were identified and qualitatively analysed. These studies were predominantly retrospective and preclinical, with gliomas being the most frequently analysed tumour type. Convolutional neural networks and support vector machines were the commonly used AI algorithms, with the primary objective of the AI models being tumour classification and grading. The majority of the research was conducted under legacy WHO classifications, which mainly relied on histo-architectural features and have been surpassed by advances in molecular biology. A high risk of bias was observed, primarily stemming from issues such as opacity in the reporting of patient cohort and image dataset characteristics, lack of external validation, and inadequate recognition of batch effects in multi-institutional datasets. Conclusions: Based on the study's findings, practical recommendations for future research are offered, including the development of a framework for clinical implementation. We outline the importance of enhancing communication between the AI community and neuropathologists and aligning AI approaches with the specific needs of neuropathologists. The study also emphasises the potential of AI-based analysis in the evaluation of selected immunohistochemical stain results and classical morphological features, such as mitotic count.

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P94

Investigating of the role of Piwil2 gene in Medulloblastoma (childhood brain tumour) development

Introduction: Medulloblastoma is the most common childhood's malignant brain tumours which is about 15 -- 20%. Mortality due to this disease is high (~40%) and successful treatment is associated with significant neurological and cognitive consequences, making new therapies desirable. Indeed, drivers identification for this devastating tumour will allow development of more precise targeted therapies. Piwil2 gene as a stem cell maintaining factor that trigger spermatogenesis process has shown to be expressed in different tumour tissues and cell lines. Including, MB primary tumours, in mice. In the other hand, normal tissues showed no expression of piwil2. The tumorigenic effects of piwil2, owing to the activation of STAT3/BclX pathways. This study emphasized that overexpression of piwil2 leads to NIH3T3 highly proliferation and differentiation. However, genomic silencing piwil2 leads to decrease cellular proliferation and differentiation even leads to apoptosis.

Methods: 1-Expression analysis of the Piwil2 gene in human MB cancer tissues. 2- Expression analysis of the Piwil2 gene in medulloblastoma tissue using immunohistochemical (IHC) analysis. 3- Investigate the effects of Piwil2 gene expression upon patients survivals.

Results: Immunohistochemical (IHC) analysis was performed using PIWIL 2 polyclonal antibody was stained both cytoplasmic and nuclear pattern in patient with medulloblastoma tissue. Strong staining pattern was detected effects of Piwil2 gene expression upon patients survivals was done and the high PIWIL2 expression linked significantly with high rate of death, whereas the low PIWIL2 expression correlate with high rate of survival

Conclusions : In this study , we have shown that Piwil2 is positive in patient with medulloblastoma. the Piwil2 gene has highly expressed with MB patient tissue , which link to high rate of death. However, the low expression of Piwil2 gene in MB patient showed high rate of survival.

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P95

Potential and Pitfalls of AI integration in Dermatology

Introduction The integration of artificial intelligence (AI) in the field of dermatology promises a revolutionary transformation in our approach to this medical domain. However, the use of AI in dermatology is still in its early stages, and with limited scope of applications. While some of the existing research performed has highlighted the capabilities of AI in diagnosing lesions comparable to those of a dermatologist, the implementation of AI brings about its own ethical challenges that affect both the clinician and the patient. This article aims to explore the ethical issues and future prospects surrounding AI implementation in dermatology by drawing insights from the current literature. Methodology A qualitative analysis was conducted on articles focusing on AI usage in medical care, with a specific emphasis on dermatology. Results The analysis unveils four main ethical issues that emerge from the implementation of AI in dermatology: selective bias and unequal treatment based on skin tone differences, intrusion of privacy brought about by the contribution of images on an open-access database, the potential erosion of patient autonomy as AI takes center stage in decision-making, the risk of unwarranted harm to patients as a result of unnecessary biopsies to potential misalignment of treatment priorities between AI systems and patients. Conclusion While AI's integration in dermatology promises to streamline the diagnostic process , it also sparks ethical issues that affect the main stakeholders. These findings indicate the need for further development and enhancement of AI before they are capable of being implemented. Introduction of a transparent, explainable AI model would alleviate concerns regarding patient autonomy. Simultaneously, the establishment of a global open-access database will serve to mitigate selective bias. Furthermore, additional research comparing algorithms would be useful in establishing a standardized validatation tool.

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P96

Incidental Diagnosis of Tuberous Sclerosis Complex in a Patient with Endometrioid Carcinoma Revealing PEComas: A Case Report

Purpose: This case report highlights the incidental diagnosis of Tuberous Sclerosis Complex (TSC) in a postmenopausal woman initially diagnosed with FIGO grade 2 endometrioid carcinoma. It underscores the diagnostic challenges of TSC and emphasizes the significance of comprehensive evaluations and genetic assessments, even in atypical clinical presentations. Methods: A 66-year-old woman with postmenopausal bleeding underwent a total abdominal hysterectomy, bilateral salpingo-oophrectomy, and bilateral pelvic nodal resection. Imaging studies revealed kidney angiolipomas and pulmonary lymphangioleiomyomatosis (LAM), leading to a histopathological examination and immunohistochemistry analysis. Results: The patient was diagnosed with TSC due to the presence of bilateral angiolipomas, pulmonary LAM, and characteristic skin lesions. Histopathological analysis revealed grade II endometrioid carcinoma infiltrating the myometrium, along with myometrial nodules displaying diverse characteristics. Immunohistochemistry indicated positive staining for desmin, H-caldesmon, HMB45, and melan A, and negative results for ALK1, S100, and SOX10, confirming the presence of perivascular epithelioid cell neoplasms (PEComas). Pelvic nodes exhibited spindle cells consistent with LAM, and mature adipocyte aggregates were observed. Conclusions: This case emphasizes the importance of recognizing the diverse manifestations of TSC, including the presence of PEComas within the female reproductive system. The diagnostic challenges associated with TSC necessitate a high index of suspicion and comprehensive evaluations. It also underscores the importance of regular organ function monitoring in affected individuals. Differential diagnoses, such as Von Hippel-Lindau (VHL) and Neurofibromatosis Type 1 (NF1), should be considered in cases with similar tumorous presentations.

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P97

ESTABLISHMENT OF FLUORESCENCE IN SITU HYBRIDISATION (FISH) FOR EWSR1 AND MDM2 AT ST JAMES HOSPITAL, DUBLIN, IRELAND

EWSR1 (Ewing sarcoma RNA binding protein 1) located on 22q12 is a translocation partner in diverse sarcomas, encoding a multifunctional protein involved in various cellular processes, including gene expression, cell signaling, and RNA processing/transport. EWSR1 is a promiscuous gene and can fuse with many different partner genes. MDM2 (Murine Double Minute Clone 2), located at 12q15, encodes a negative regulator of the p53 tumor suppressor. Increased levels of MDM2 inactivate the apoptotic and cell cycle arrest functions of p53, as do deletion or mutation of p53. Fluorescence in situ hybridization (FISH) requires little tissue and can be performed on 4µm sections of formalin-fixed paraffin- embedded tissue. With the increasing use of core biopsy for diagnosis, which yields limited material, the ability to perform FISH in paraffin-fixed tissue is essential for diagnosis. FISH for both EWSR1 and MDM2, using Zytovision probes, was fully validated and implemented at St. James &'s Hospital, Dublin, in September 2022. The validation included analysis of cases with confirmed positive FISH results (10 EWSR1 cases (6 SVUH, 4 SJH) and 12 MDM2 cases (6 SVUH, 6 SJH)). We also conducted inter-laboratory comparisons with Leeds Teaching Hospitals NHS Trust for EQA (2 MDM2 and 2 EWSR1 cases, with concurrence of results) and joined the GenQA Sarcoma FISH scheme. Cases are signed out by a specialist medical scientist and a consultant histopathologist. To date, 27 cases for MDM2 (7 positive), of which 16 were referred cases, and 7 cases for EWSR1 (3 positive), of which three were referred cases. Referred cases are charged €200 per FISH probe test. We share our positive experience about the introduction of FISH for EWSR1 and MDM2 at SJH in collaboration with SVUH. This represents a significant step forward in diagnostic capabilities for pathologists in Ireland. Expanding FISH diagnostics requires a national funding strategy.

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P98

Integrating Whole Genome Sequencing for Triple Negative Breast Cancer Diagnosis: Potentials and Challenges

In 2022, the NHS Genomic Medicine Service in England launched a pilot study trialling whole genome sequencing (WGS) for patients with triple negative breast cancer (TNBC), irrespective of disease stage. WGS allows identification of pathogenic genetic alterations, including 'mutational signatures', and facilitates patient stratification for targeted therapies though clinical trials. However, implementing fresh tissue pathways for WGS has proven challenging, and the clinical utility of WGS has been questioned by the multidisciplinary team.

Herein, we present four cases of TNBC of varying morphology that underwent WGS as part of this pilot programme at King's College Hospital (South East Genomics Laboratory Hub). In all cases, WGS identified new potential treatment options. In one patient with locally advanced, pleomorphic invasive lobular carcinoma, we identified an activating ERBB2 mutation and low-level ERBB2 amplification. The tumour was thus reclassified as 'human epidermal growth factor receptor 2 (HER2) low', making the patient eligible for HER2-targeted therapies. In another patient with a grade 3, node-negative metaplastic carcinoma, we identified a potentially actionable PIK3R1 variant, CCND1 amplification and CDKN2A loss. However, clinical trials targeting this mutation and these aberrations are currently only available for oestrogen receptor positive patients. In three patients, one with a known germline BRCA1 variant, we identified a homologous recombination deficiency signature, indicating that they might respond to Poly (ADP-ribose) polymerase (PARP) inhibitor therapy. However, Olaparib is currently only approved for inherited, and not sporadic, TNBC.

Thus, while WGS did identify novel treatment options for each patient, getting access to relevant targeted therapies in each case was not straightforward. Further work, with multidisciplinary input, is needed across our diverse patient population to refine how best to implement and use WGS in routine practice.

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P99

Optimising CRISPR epigenome editing to upregulate CXCR4 for the enhancement of HSPCs engraftment

Gene editing therapies have advanced significantly in the last decade, particularly in hematopoietic stem and progenitor cell (HSPC) autologous transplants. However, engrafting genetically modified HSPCs into the bone marrow of animal models remains challenging due to potential immune responses hindering engraftment. The homing receptor CXCR4 is a principal up regulator of HSPC engraftment. This project aims to enhance and optimise CXCR4 expression using CRISPRa epigenome editing technologies. For the CRISPRa system, we retrieved the CXCR4 promoter sequence from the Eukaryotic Promoter Database and designed three sgRNAs targeting it using Benchling. The sequence encoding the dCas9-VPR and sgRNAs were cloned into expression plasmids and was transiently transfected into HEK293T cells. After three days, the total RNA was extracted from the cells, converted into cDNA, and analysed by RT-qPCR. The fold increase in CXCR4 expression was calculated by comparing transfected cells to untreated samples using the 2-ΔΔCt method. The relative quantification of the gene expression by RT-qPCR was repeated three times on each sgRNA combination. All sgRNA combinations led to an increase in CXCR4 expression in HEK293T cells. We found that, the combination of two sgRNAs closer to the transcription start site (TSS) results in a fold increase in the CXCR4 expression of 8.07. The highest fold increase observed using a single sgRNA was 5.98. The triple sgRNA combination only produced a 2.64 fold increase. The preference for two sgRNAs suggests that single sgRNA combinations has low sensitivity for dCas9-VPR due to the low number of recruitment sites. Conversely, employing three sgRNAs may lead to excessive recruitment, impacting specificity for CXCR4. Thus for our target, the use of two sgRNAs in two distinct locations can produce a site both sensitive and specific for dCas9 recruitment. Future steps involve replicating optimised sgRNA combinations with HSPCs using a new delivery approach.

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P100

Possible Clonal Evolution of Chronic Lymphocytic Leukaemia (CLL) to ALK-Positive Anaplastic Large T-Cell Lymphoma with TRB and IGH rearrangements: A Lethal Instance of Lineage Infidelity?

Introduction Richter transformation is the sudden transition of CLL to a more aggressive form of large cell lymphoma, usually of B-cell lineage; transformation to T-cell lymphoma is rare and must be differentiated from a second primary lymphoma. Recombination of both TRB and IGH in the same clone is a rare phenomenon, referred to as "lineage infidelity", and occurs in <5% of mature B-cell lymphomas. We present a case of Richter transformation from CLL to ALK+ Anaplastic large T-cell lymphoma (ALCL) confirmed by co-existing IGH, IGK and TCR rearrangements. Case presentation A 75 year old man presented with inguinal lymphadenopathy and was diagnosed with CLL. Clonality studies demonstrated IGH and IGK rearrangements. The disease progressed five years later and the patient received 6 cycles of Obinutuzumab/chlorambucil, achieving disease stabilisation. A year later, the patient presented with bowel obstruction. Diagnostic laparoscopy revealed a large retroperitoneal mass adherent to jejunal and ileal bowel loops. A peritoneal biopsy showed a dyscohesive population of large cells with pleomorphic nuclei and prominent nucleoli resembling "hallmark cells" which infiltrated the fat. CLL was not identified. Tumour cells expressed T-cell lineage markers (CD45, CD30, CD5), and ALK-1; the following markers were not expressed: CD20, CD79a, CD3, CD7, CD34, TdT, cKIT, CD138, CD23. Molecular testing detected the same IGH and IGK clonal rearrangements identified in CLL cells and a newly acquired TCR rearrangement, which confirmed the diagnosis of ALK+ ALCL arising from CLL (Richter transformation). The patient died due to post-surgical complications. Discussion Molecular studies enable the identification of unusual cases of lineage infidelity and allow confirmation of clonal evolution from CLL to aggressive ALK+ ALCL. This can have important implications for patient management.

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P101

Thyroid lymphoma: molecular insights by mutation profiling

Purpose of the study

Primary thyroid lymphomas commonly originate from a background of Hashimoto's thyroiditis and comprise largely extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (EMZL) and diffuse large B-cell lymphoma (DLBCL), followed by follicular lymphoma (FL). Thyroid EMZL has a distinct mutation profile from those of other sites, but it is unknown whether their mutation profile differs from those of thyroid FL and DLBCL, valuable in their differential diagnosis.

Methods

A total of 102 thyroid lymphomas were histologically reviewed and the final diagnosis included 43 EMZL (10 BCL6-tr+ve), 22 FL (5 BCL2-tr+ve, 10 BCL6-tr+ve, 1 both BCL2/BCL6-tr+ve) and 37 DLBCL. DNA from FFPE tissue was investigated for mutation in 174 lymphoma genes by NGS.

Summary of results

All three lymphoma groups had a considerable overlap in their mutation profiles, including frequent mutations in somatic hypermutation targeted (TNFRSF14, IGLL5) and non-targeted genes (TET2, CD274, FAS, GNA13). Subset analysis according to chromosome translocation revealed that BCL2-tr+ve FLs were enriched for CREBBP, KMT2D, EZH2, but lacked TET2, CD274 and IGLL5 mutations, while BCL2-tr-ve FL including those with BCL6 translocation displayed few differences from EMZL and DLBCL. There were also few differences in the mutation profile between EMZL with and without BCL6 translocation.

Conclusions

The common mutation profile among thyroid EMZL, BCL2-tr-ve FL and DLBCL may result from their common aetiology (Hashimoto's thyroiditis) and hence similar pathogenic processes. The distinct mutation pattern in BCL2 tr+ve FL may evolve due to selection of cooperative oncogenic events with aberrantly expressed BCL2.

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P102

Audit Evaluating the Thy3A Thyroid Cytology Category Within our Institution and the Correlation Between Cytology and Histology

Purpose of the study: Thyroid cytology can be a difficult area with some subjectivity when assessing follicular lesions. The Royal College of Pathologists have stated criteria for when the category of Thy3a should be used and advised that the average risk of malignancy in Thy3a specimens is 25%. In this audit our aim was to determine how often the Thy3a category is used, in what instances and the subsequent resection histology.

Methods: A search was conducted on our laboratory information management system from 01/01/22 to 01/05/22 to identify thyroid cytology cases.

Results: Of 90 cases, 27% were reported at Thy1/1c, 28% as Thy2/2c, 22% as Thy3a, 14% as Thy3F, 3% as Thy4 and 6% as Thy5. For those cases reported as Thy3a (20 cases) the reasons were: paucicellularity (5 cases), cytological atypia (3), architectural atypia (10) and both cytological and architectural atypia (2). Of the Thy3a cases, 8 had a repeat cytology in which 1 was downgraded to Thy2c, 4 remained as Thy3a, 1 was called Thy3F and the last was called Thy4. Only 11 had subsequent resections. 4 of these were diagnosed as follicular adenoma, 1 was a papillary carcinoma (the corresponding cytology had shown cytological atypia) and the remaining were non-neoplastic follicular lesions. In comparison, 9 of the 13 Thy3F cases had resections. Of these 5 were follicular adenomas, 1 was a papillary carcinoma, 2 were non-neoplastic follicular lesions and the last was a child with a non-invasive poorly differentiated follicular neoplasm of uncertain malignancy potential.

Conclusions: In this limited study we show a malignancy rate of 9% for Thy3a specimens. We show there is overlap between resection specimen diagnoses when comparing Th3a and Th3F categories which has been demonstrated in a previous multi-centre study. This highlights the difficult nature of assessment of follicular lesions on cytological preparations and emphasises the importance of MDT discussion with monitoring and repeat sampling as necessary.

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P103

The single cell atlas of clear cell sarcoma

Purpose of study: Clear cell sarcoma of tendons and aponeuroses (CCS) is a rare, fusion-driven, soft tissue sarcoma that predominantly arises in the limbs and extremities of 20-40-year olds. The histology reveals spindled to epithelioid cells with generally uniform nuclei arranged in nests, few mitoses, prominent nucleoli, and occasional presence of multi-nucleated wreath like cells. Here we present our efforts to create a cellular map of CCS to identify tumour and normal cell niches which might prognosticate response to therapies.

Methods: 10 tumour regions from 8 patients (5 primary/5 relapses) have been processed for single nuclear RNAseq on the 10X Genomics 3' gene-expression platform. Data was processed using cellranger v6.0 then analysis conducted using standard Seurat pipelines. Cell cluster identities were assigned using a consensus of 3 databases on Enrichr from the top 50 highly-expressed genes per cluster.

Summary of results: The cell atlas of CCS comprises mostly of tumour cells which distinctly cluster based on underlying cell signalling perturbations, plus small clusters of stromal and immune cells. The tumour roughly consists of 6 populations each of which is contributed from each of the different patient samples, implying shared evolutionary processes across the patients. Only 7% of the cells within CCS can be confidently assigned as being normal, with each patient contributing a different cohort of cells depending on the tumour's anatomical location. The most commonly shared cells between all tumours appear to be M2 macrophages, histiocytes, vascular endothelial cells, and neutrophils. The lymph node metastases possessed a greater diversity of immune cells, including plasmocytic dendritic cells and lymphoid cells.

Conclusion CCS has a spectrum of distinct transcriptional populations despite a homogenous histology, and a seemingly immune exclusive phenotype with small macrophagic/neutrophil populations and very few dendritic, NK, lymphoid or T-cells.

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P104

The Genomic Landscape of Clear Cell Sarcoma

Purpose of the study Clear cell sarcoma of soft tissue (CCS) is a rare soft tissue tumour of tendons and aponeuroses mostly affecting the lower extremities. CCS tumours are quite aggressive with high local recurrence and metastasis rates. In this study, we aim to investigate the longitudinal whole genome data (WGS) of CCS in combination with bulk transcriptomics and methylation data to gain a comprehensive understanding of CCS.

Methods Current study utilizes WGS data of 45 CCS samples, bulk RNA-seq data of 37 samples and methylation data of 46 samples. WGS data was processed using bcbio-nextgen toolkit while RNA-seq and methylation data was processed using DESeq2 and sesame package in R.

Summary of results Analysis revealed the prevalence of diploidy and low tumour mutation burden across all CCS cases. There was absence of any specific recurrent non-synonymous mutation, however, the spectrum of somatic non-synonymous mutations identified converged on common signaling pathways like NTK-RAS and PI3K-Akt. Frequent copy number alterations were observed in chromosomes 8, 7, 9 and 17 and unexpectedly identified significant subclonality. Recurrent focal copy number variations were identified at loci-1p22.1, 8q24.12, 9p21.3 and 17q25.1 which harbour cancer genes like MYC, CDKN2A and CDKN2B. Unexpectedly, complex structural events of chromothripsis and chromoplexy were identified in 10 primary CCS samples and nealry 30% of them were also associated with the EWSR1 fusion.

Conclusions Despite all CCS showing recurrent driver fusion genes and a paucity of recurrent point mutations, the spectrum of genetic heterogeneity underpinned by subclonal copy number changes chromothripsis and chromoplexy is unexpected. Evaluation of the longitudinal data of CCS will further shed light on their evolution patterns. Our analysis encompassing WGS data along with transcriptomic and methylation could provide valuable insights to advance treatment strategies and improve survival in CCS patients.

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P105*

Retrospective Machine-Learning-Assisted Analysis of the Immune Infiltrate in Osteosarcoma

Purpose of the study: Major treatment breakthroughs, including checkpoint inhibitors (CPIs), have seen a marked increase in survival in several cancers. However, the impact of CPIs on osteosarcoma (OS) has been limited, benefiting <10% of patients. The response to CPIs may be related to the density of tumour-infiltrating lymphocytes (TILs). Our study aims to investigate the profile of the immune infiltrate in osteosarcoma and correlate it with clinical outcomes.

Methods: 112 whole slide images (WSI) of biopsies were obtained from 99 osteosarcoma patients. Using QuPath, >300 annotations of tumour cells and TILs were manually made on randomly selected segments from 30 slides, serving as a training dataset for QuPath's machine-learning detection algorithm. A blinded TIL count was performed by two experienced pathologists to confirm the accuracy of the algorithm, before applying it across all WSIs. Data on the tumour area and the number of TILs were collected from QuPath. TIL density was calculated and correlated with patient survival, response to chemotherapy and age at diagnosis.

Summary of results: Our analysis showed TIL density ranging from 0.892 to 551 TILs/mm2 across all WSI (mean = 68.9 TILs/mm2). Slides from 82.8% of patients (n = 82) showed a low TIL density (<85 cells/mm2) and 17.2% (n = 17) showed a high TIL density (>85 cells/mm2) ($p = 0.023$). We found no correlation between TIL density and patient survival ($p = 0.228$), patients' response to chemotherapy ($p = 0.360$) or age at diagnosis ($p = 0.078$, $R = 0.210$).

Conclusions: Our results show a low prevalence of TILs in osteosarcoma, relative to other solid tumours, a finding consistent with the outcome of patients treated with CPIs. However, we found a subset of osteosarcoma patients with a significantly higher TIL density. As the density of TILs may be a predictor of survival and response to CPIs, this could prove an efficient means of patient selection for immunotherapy.

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P106*

Identifying Quiescent Osteosarcoma Cells Through Gene Expression Analysis

Study purpose: Osteosarcoma, the most common primary bone tumour affecting young individuals, is typically treated with neoadjuvant chemotherapy and surgery. However, about 45% of patients exhibit poor histological responses, mostly associated with worse outcomes. Our research explores the hypothesis that quiescent (cell cycle arrested) cells, irrespective of their genetic make-up, may contribute to treatment resistance. Our aim was to confirm in vitro quiescent osteosarcoma cells through gene expression analysis.

Methods: Osteosarcoma cell lines (CAL72 and HOS) were treated with CellTracker-Red, a fluorescent dye to distinguish the dye-retaining non dividing (quiescent) and dye-negative (proliferative) cells. Using Flow Cytometer-activated cell sorting, we separated the quiescent and proliferative populations (CellTracker positive and negative respectively) and analysed the expression of eight selected genes previously associated with quiescence, on four independent experiments. RNA was extracted, followed by cDNA synthesis and quantitative real-time Polymerase Chain Reaction (qPCR). Gene expression fold changes were calculated, normalised to the reference gene GAPDH, and presented as bar charts using R programming.

Results Summary: Among the analysed genes, PSAP, TMEM, GSN, YPEL3 (typically upregulated in quiescence) showed elevated expression, while HSP90AA1, CAD, ILF3, CCT5 (typically downregulated) showed reduced expression in the quiescent cell populations compared to the proliferative cell populations.

Conclusion: Consistent results were observed across replicates and both cell lines, despite some discrepancies, overall supporting the findings that quiescent cells can be detected in osteosarcoma cell lines and coherently display quiescence-associated genes. In future work we aim to analyse patient-derived tumour cells to validate our findings on heterogeneous primary osteosarcoma cell populations.

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P107*

Investigating the methylation landscape of clear cell sarcoma of soft tissue

Purpose:Clear Cell Sarcoma (CCS) of soft tissue, is a rare sarcoma subtype primarily seen in young adults. It has a proclivity for localising to tendons and aponeuroses.The tumour has a high local recurrence and metastatic rate despite surgery, radiotherapy and chemotherapeutic interventions. The key driver event in CCS is a genetic translocation resulting in the EWSR1::ATF1 fusion oncoprotein which upregulates a melanocytic transcriptional program. In other fusion driven sarcomas DNA methylation plays an important role in gene regulation and can be used for disease classification. In order to determine if CCS also showed clinically relevant epigenetic programs, the DNA methylation patterns of CCS were investigated.

Methods: The sample cohort consisted of 37 primary tumours, 4 local recurrences and 15 metastasis. DNA extractions were conducted on both FFPE and frozen tumour tissue samples and matched to normal (blood and adjacent tissue) and melanoma controls. The extracted DNA was subjected to bisulphite conversion, processed on Illumina Infinium Methylation EPICv2 arrays and the resultant methylation data was analysed using the SeSAMe package in R Studio.

Results: Dimensionality reduction showed distinct clustering of CCS samples compared to normal tissue and melanoma and a global hypomethylation phenotype. Differential methylation analysis showed specific patterns of methylation of the promoters of SOX10 and MITF which are known neural crest factors. Hypermethylation of the TERT promoter was seen in 13 samples and was associated with increased gene expression.

Conclusion: The CCS methylation landscape is distinctive from melanoma despite sharing a similar transcriptional program that includes activation of SOX10 and MITF and TERT promoter methylation. The clinical and biological implications of these findings are now being examined to determine if methylation could be useful for prognosis.

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P108

Abnormal signal patterns of Break-apart FISH probes Used in the Diagnosis of Bone and Soft Tissue Tumours

Break-apart FISH (fluorescence in site hybridisation) probes are used as fast and reliable ancillary method to support the diagnoses of many bone and soft tissue tumours. The signal patterns of the break-apart probes are usually easily interpreted. However, some cases may show abnormal signal patterns leading to equivocal results. The present study aims to explore the incidence of abnormal signal pattern by tumour type to raise awareness of this occurrence. Methods: A total of 979 patients were collected from our files. All cases analysed by FISH using break-apart probes were reviewed to identify the frequency and type of the abnormal signal patterns. These were classified as deletion, amplification, and extra copy of the gene locus. Results: Abnormal signal patterns were detected in 9% (8/92 cases) of alveolar rhabdomyosarcomas (FOXO1), 2% (1/51 cases) of clear cell sarcoma (EWSR1), 6% (25/459 cases) of Ewing sarcoma (EWSR1), 33% (6/18 cases) of low grade fibromyxoid sarcoma (FUS), 7% (12/168 cases) of nodular fasciitis (USP6) and 8% (15/191 cases) of synovial sarcoma (SS18). The interpretation of these abnormal signal pattern may be challenging. Confirmatory test using a different technique - FISH Fusion probes, RT-PCR, Next Generation Sequence (NGS) or Whole Genome Sequencing (WGS) - was useful to validate these uncertain results. Summary: This is a large cohort with systemic review of break-apart probes in bone and soft tissue tumours. Our retrospective analysis highlighted the frequency of abnormal signal patterns by disease type and demonstrated the need of confirmatory results by a different molecular technique.

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P109

Atypical presentation of rib osteomyelitis

A 22 year-old male fell on a rocky precipice at the age of 11 and injured the right side of his chest. He continued to experience intermittent bouts of pain in the following years. He consulted various doctors during this time but was only prescribed painkillers. He presented with a 1 month history of increasing pain and swelling over the right chest and had no contact history of TB.

On examination, the patient had a small fluctuant swelling over the right inframammary region with localised tenderness over the right 4th rib. ESR was modestly elevated, and MRI showed marrow oedema with fluid collection over the affected rib. A decision was made to do surgical exploration, debridement and biopsy.

During surgery, a collection of pus was found overlying the right 4th rib with associated lytic destruction and granulation tissue replacement. The involved portion of the rib was fully resected.

Aerobic culture yielded MSSA. TB GeneXpert, AFB smear and fungal microscopy were negative. Tissue biopsy of the affected rib showed skeletal muscle bundles and fibrocollagenous tissue with sheets of chronic lymphoplasmacytic infiltrates and granulation tissue. No granumolmas or atypical cells were seen. The findings strongly suggested a chronic inflammatory pathology, consistent with osteomyelitis.

The patient was treated with IV ceftriaxone for 6 weeks and oral cotrimoxazole for 3 months based on antibiotic sensitivity data. The wound healed well and the patient made a full recovery.

Chronic rib osteomyelitis is rare. It usually presents with fever, chest pain and other constitutional symptoms. Occasionally, it presents in an atypical fashion, making diagnosis a formidable challenge.

Our case illustrates the high degree of clinical suspicion needed to diagnose chronic rib osteomyelitis. Recognition is critical for appropriate investigation and successful therapeutic intervention.

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P110

Defining Osteosarcoma Cell Types By Single Nuclei RNA Sequencing

Objectives: Osteosarcoma is the most common primary bone sarcoma of young adults. Genomic studies have reported heterogeneity within osteosarcoma, however the transcriptional consequences of this are unclear. In this work, our aim was to characterise osteosarcoma cell types using single nuclei RNA sequencing in relation to differentiation states along mesenchymal developmental lineages.

Methods: 400,000 high quality nuclei were obtained from multiregional single nuclei RNA sequencing (snRNAseq) of 20 osteosarcoma resection specimens on the 10x Genomics platform. Cell types were annotated based on differential expression analysis and copy number profiles. Recurrent transcriptional phenotypes were identified using hierarchical clustering and gene set enrichment analysis. A logistic regression-based method was used to directly compare osteosarcoma cell transcriptomes to a reference foetal bone single cell dataset.

Results: Categorisation of osteosarcoma tumour cell clusters at the individual patient level led to the identification of common gene marker profiles shared across tumour cells from multiple tumours. Grouping of tumour clusters expressing similar marker genes reveal the common active pathways across tumours including MYC targets, cell motility and matrix remodelling, hypoxia, and regulation of bone differentiation. Interrogation of bulk RNA profiles from a larger cohort of 81 tumours confirm that these are highly active pathways in high grade osteosarcoma compared to low grade subtypes. Expression of canonical osteoblast stage markers and direct tumour to normal comparison with foetal osteoblasts demonstrate that osteosarcoma cell phenotype is arrested before the mature osteoblast stage.

Conclusion: This work resolves the diversity of osteosarcoma cell types whilst demonstrating common trends of active tumourigenic pathways across tumours. Furthermore, snRNAseq enables a qualitative assessment of osteosarcoma cell maturation using the global cell transcriptome.

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