1

Udvikling af digital applikation til tælling af MUM1-positive celler i knoglemarvsundersøgelser

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Introduktion
Monoklonal gammopati af usikker signifikans (MGUS) estimeres at være tilstede hos ca. 5 % af befolkningen over 70 år. Den væsentligste måde at skelne forstadiet MGUS fra kræftsygdommen myelomatose er den monoklonale plasmacelleinfiltation i knoglemarven; over eller under 10 %. Dette estimeres af patologen med en væsentlig inter- og intraobservatør varians. Udover supplerende immunhistokemiske plasmacellefarvninger (bl.a. MUM1) findes der ikke nyere metoder til at assistere dette estimat. Vi forsøger her at udvikle en digital app til at finde andelen af MUM1-positive celler og derefter at validere den i en retrospektiv kohorte.
Materialer og metoder
På basis af et træningssæt udviklede vi en applikation i VisioPharm til tælling af MUM1-positive celler og negative baggrundskerner ved en blanding af manuel indstilling og AI-understøttet maskinlæring.
Vi indhentede 192 prøver diagnosticeret med MGUS eller myelomatose på Patologi, Aarhus Universitetshospital i perioden 2017-2018 og sammenlignede dels patologens samlede estimat og dels den digitale applikations tælling med kappa/lambda-ratioen og den prognostiske score ISS (aflæst fra biokemien) ved udregning af Spearman's rang korrelationskoefficient.
Resultater
Både i forhold til ISS-scoren og kappa/lambda-ratioen tenderede patologens estimat mod en bedre klinisk korrelation end den digitale tælling gjorde (laveste p-værdi 0,20).
Diskussion og konklusion
Vi har vist, at det er muligt at lave en app, der kan assistere i kernetælling i immunfarvninger. Muligt tællebias samt manglende inkorporering af kappa/lambda farvninger og morfologi kan være med til at forklare, hvorfor patologen tenderer mod en bedre korrelation med kliniske data.

2

THE PROGNOSTIC IMPACT OF HPV AND P16 STATUS IN VULVAR SQUAMOUS CELL CARCINOMAS – A POPULATION-BASED STUDY INCLUDING ~1,300 CANCERS

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Introduction:
Increasing evidence suggests that human papillomavirus (HPV)-associated vulvar squamous cell carcinomas (VSCCs) have a superior prognosis compared with HPV-independent VSCCs, but previous findings contain substantial variation. We established a large cohort of VSCC cases with the overall aim of identifying prognostic markers for VSCC. In the present study, we focus on p16/HPV status.
Material and Methods:
Our cohort includes all VSCC cases diagnosed at 13 Danish pathology departments during 1990–2017. Archived formalin-fixed, paraffin-embedded tumor tissue blocks were retrieved from the clinical biobanks and diagnoses were reviewed. HPV status was determined using the INNO LiPA Genotyping Extra test and the immunohistochemical CINtec p16 histology Kit. From nationwide registries, we obtained information on comorbidity and vital status. We estimate 5-year overall survival (OS) according to p16/HPV status by the Kaplan-Meier's method and hazard ratios (HRs) of death by Cox regression, adjusting for age, calendar year, stage of diagnosis, and comorbidity.
Results:
A total of 1278 (83%) cases had tissue blocks located and valid HPV and p16 results obtained. The prevalence of double positivity for p16/high-risk HPV was 31.0% (95% confidence interval (CI): 28.4–33.5) and preliminary results show that these women had better survival than women who were negative for both markers (5-year crude OS: 63.5% versus 47.4%, HR adjusted for age and calendar year=0.68, 95% CI, 0.52–0.90).
Discussion and conclusion:
Positivity for p16/high-risk HPV is a beneficial prognostic marker in VSCC. Further analyses will be presented at the annual meeting, including analyses adjusted for additional prognostic factors.

3

PRAME ekspressionen som redskab i den diagnostiske proces ved melanocytære læsioner

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Malignt melanom (MM) er en diagnose med stigende incidens, hvor tidlig og præcis diagnostik er afgørende for patienternes behandlingsforløb og prognose. Det er derfor af stor interesse at optimere og præcisere den differentialdiagnostiske proces ved melanocytære læsioner. Det er tidligere vist, at man ved immunhistokemisk undersøgelse af PRAME (preferentially expressed antigen in melanoma) ekspressionen, kan understøtte mistanken om diagnosen MM. I aktuelle retrospektive studie har man ved sensitivitets-, specificitets- og interobservatør analyser belyst, hvorvidt PRAME ekspressionen kan anvendes til at understøtte diagnosen MM. Således er inkluderet 100 cases med melanocytære læsioner, både af karakteren benign, dysplastisk, in-situ og malign. PRAME ekspressionen blev blindet vurderet af 4 læger, som for hver case afgav en score (0-4) på baggrund af procentdelen af positive tumorcellekerner. Resultatet blev defineret som ”positiv” ved > 75% farvede tumorcellekerner og "negativ" ved ≤ 75 % farvede tumorcellekerner. Man fandt at 27 ud af 29 MM var positive og at 56 ud af 71 ikke-invasive læsioner var negative. Herunder var alle benigne nævi, inklusive de dysplastiske, negative, mens kun under halvdelen af in-situ forandringerne var negative. Interobservatørkoefficienterne ved Cohens vægtede kappa var alle > 0,9. På baggrund af resultaterne vurderes PRAME ekspressionen anvendelig til at understøtte diagnosen MM, hvilket specielt kan være brugbart i forbindelse med differentialdiagnostikken mellem MM og de dysplastiske nævi, som morfologisk kan være vanskelige at skelne. PRAME-ekspressionen kan dog aldrig stå alene og må for alle læsioner sammenholdes med de kendte morfologiske kriterier - særligt ved in-situ forandringer, som fremstår med varierende PRAME-ekspression.

4

Unexpected finding of a liposarcoma mimicking a large angiomyolipoma

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Introduction:
Among fat-containing tumors of the retroperitoneum, differentiating the rare, well-differentiated perirenal liposarcoma from the more common and benign exophytic angiomyolipoma (AML) can be radiologically challenging. However histopathological evaluation usually provides the correct diagnosis, although this may depend on features only focally present.

Materials:
A 44-year-old healthy woman presented with a right abdominal mass. The CT scan showed a 14 cm well-circumscribed heterogenic fat-containing exophytic mass located in relation to the lower kidney pole. The initial impression was that of a hemorrhagic AML. The patient underwent two-step treatment with coiling followed by surgical resection.

Results:
Grossly, the tumor was composed of pale-yellow fatty tissue. In areas the tumor appeared more fibrous with a greyish colour. Microscopically, the tumor consisted of a major lipomatous and a minor sclerosing (fibromatous) component, clearly distinguishable from each other. In both components, bizarre tumor cells with pronounced nuclear pleomorphism (irregularity, hyperchromasia and multinucleation) could be appreciated. There was striking variation in size of adipocytic tumor cells. MDM2 nuclear immunopositivity was present. The case was signed out as a well-differentiated liposarcoma with a sclerosing component.

Conclusion:
AMLs of the kidney can usually be diagnosed by radiology alone. However, in cases with fat-depleted AMLs or very large heterogenous tumors, a biopsy may be necessary to obtain the correct diagnosis and not to overlook liposarcomas. Sampling of fatty areas with varying morphology is important. In doubtful cases, amplification of MDM2 should provide diagnostic certainty. It is important with this differential diagnostic awareness as management and prognosis differ considerably.

5

The value of two sections on lymph nodes from patients with colon cancer

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Introduction: Accurate staging of colon cancer is important and the number of regional lymph node metastases (LMNs) is a prognostic factor and also crucial in determining the need for adjuvant chemotherapy. The aim of the study was to examine the value of two consecutive sections of the lymph nodes compared with a single section. Material and Methods: 57 surgical specimens from patients diagnosed with colorectal carcinoma received at the Department of Pathology, Zealand University Hospital were included. Lymph nodes with only one section available was excluded. A resident and a pathologist evaluated all slides for the presence of LNMs and consequent change in staging due to one versus two sections. Results: In total 1638 lymph nodes were included. In 87 a LNM was identified. Two lymph nodes contained metastases and one isolated tumor cells in only one of the sections. All three LNMs originated from the same patient. Discussion and conclusion: In 3.4% of the examined lymph nodes a metastasis was present in only one of the sections. However, this had no influence on the final N stage. Assessment of lymph nodes from patients with colorectal cancer with two serial sections instead of a single section resulted in a small increase in the number of detected metastases with no clinical impact. The significance of occult lymph node metastases in colon cancer remains unclear, but studies including patients with other primary cancers i.e. esophageal, lung and breast have shown significant prognostic differences when occult metastases were revealed.

6

The topographical distribution of lymph node metastases in colon cancer

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Introduction: In accordance with current international guidelines all lymph nodes in colon cancer specimens must be examined in order to obtain an accurate staging. The aim of this study was to determine the localization of lymph node metastases in relation to the tumor and evaluate if a more limited approach would be feasible. Materials and Methods: Surgical specimens received at the Department of Pathology, Zealand University Hospital during a three month period were included. At the macroscopic examination each specimen was divided in up to four different segments; a tumor bearing, potentially a separate vessel bearing, an oral and anal segment. Number of lymph nodes and metastases were registered separately for each segment. Results: Resections from 57 patients were included. Of 1652 lymph nodes, 1020 were located in the tumor bearing segment, 193 in the vessel bearing, 205 in the oral and 234 in the anal segment. In 88 lymph nodes a metastasis was present, of which 83 were located in the tumor bearing segment, two in the vessel bearing segment, one in the oral segment and two in the anal segment. Discussion and conclusion: Patients with a metastasis in the vessel bearing, oral or anal segments all had metastases in the tumor bearing segment. As such, this segment could possibly be regarded as a sentinel segment, but this hypothesis would require further and larger studies.

7

The prognostic value of single or multiple human papillomavirus infections in cervical cancer patients

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Introduction: Infections with multiple human papilloma virus (HPV) genotypes are suggested to be associated with a higher risk of developing cervical dysplasia (CD) and cervical cancer (CC) compared to single infections. Thus, for cervical screening samples, detection of single/multiple HPV genotypes may be of prognostic value for HPV positive women.
Material and methods: In this prospective proof-of-concept cohort study, cervical smears (CS) and formalin- and paraffin embedded (FFPE) tissue from 15 women diagnosed with CC at Aarhus University Hospital (May 2021 to February 2022) were collected prior to treatment. All samples were analyzed with our targeted Next Generation Sequencing HPV Genotyping panel.
Results: When comparing HPV genotyping results between CS and corresponding FFPE samples from all patients, 100% agreement was found. Moreover, for all samples, only single genotype infections were detected. These were distributed as follows: 9 with HPV16 (60%), 4 with HPV18 (26.67%), 1 with HPV82 (6.67%) and 1 with HPV52 (6.67%).
Discussion and conclusion: Only single genotype infections were detected in CC patients. This may suggest that; 1) women with CC are infected with 1 genotype from the beginning of CD development, or 2) other and non-dominating genotypes are lost during progression of CD, leaving the dominating genotype in CC patients. However, further studies including HPV positive women with different grades of CD are needed to fully establish a possible association between presence of single/multiple HPV genotypes and grade of CD. Knowledge hereof may contribute to improvement of future risk stratification of HPV positive women in cervical screening.

8

Epiglottal metastasis of parotid salivary duct carcinoma one month post clinical remission of primary cancer.

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Salivary duct carcinoma (SDC) is a highly aggressive malignancy, known for frequent recurrence and metastasis. Distant metastases often affect lungs, bones and lymph nodes.
We present, with consent from next of kin, the case of a 58-year-old male, diagnosed with parotid SDC in the spring of 2022, treated with surgery and subsequent radiotherapy, leading to complete clinical remission in August 2022. The patient was admitted to the emergency room (ER) in September 2022 with face and lip edema. A CT scan showed suspected metastases in columna thoracalis, multiple costae, sternum, and liver. The patient desaturated and became hypotensive the same evening, and passed away the next morning, despite symptomatic treatment. An autopsy showed metastases in the epiglottis, lungs and liver. Cause of death was widespread metastases of SDC. Immunohistochemical staining of the epiglottal metastasis was positive for CK7, HER2, GATA3, E-CAD and Mammoglobin, confirming the patient's primary SDC as its origin. The metastatic cells had no expression of the androgen receptor, contrary to cells from the primary tumor.
Though there are cases of primary SDC of the larynx, this is, to our knowledge, the first documented case of SDC metastasis to the epiglottis. Recurrence one month after complete clinical remission is much earlier, than the median distant metastasis free survival of 26 months reported in the literature.

9

Histological characterization of normal tissue reactions in mice one year after proton irradiation

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Introduction: Radiotherapy-induced fibrosis is a common chronic side-effect, and understanding the radiobiological rationale is critical to improving patient care. This explorative study tested a procedure for comparing histological and stereological evaluation of radiation-induced late effects in mice legs receiving different radiation doses with a clinical leg flexibility assay.
Methods: The right hindlimb of 4 mice were irradiated with a single proton dose (27-45 Gy). The left hindlimb served as non-irradiated control. Legs were harvested one year after irradiation. In all legs, cross sections at three levels were cut: upper leg, ancle joint and foot, and all tissue sections were stained with Hematoxylin-Eosin and Masson's Trichrome. Total section area, area-fraction of connective- and adipose tissue, epidermal thickness, and total number of skin adnexa were evaluated. Fractions and total area were estimated using stereological point sampling and 2-dimensional nucleator.
Results: In comparison to the non-irradiated legs, connective tissue area-fraction was increased in the upper leg in the irradiated legs, whereas loss of adipose tissue and decrease in total section area was observed in both upper leg and ancle joint. Marked increase in epidermal thickness and loss of skin adnexa was found at all levels after irradiation. Mice with largest decrease in clinical leg flexibility showed most severe structural alterations.
Conclusion: Clinically verified leg atrophy and decreased flexibility after irradiation with proton therapy could be visualized histologically and quantified stereologically. Evaluation in a larger study with stratification according to radiation dose is needed to investigate if a dose-response relationship can be supported histologically.

10

Mucoepidermoid carcinoma of the breast - a case report

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Mucoepidermoid carcinoma is a common malignant salivary gland tumor, but is exceedingly rare in the breast, with around 40-50 reported cases. We report on a 70 old woman who presented with an 8 mm tumor in the left breast. The patient was undergoing treatment for adenocarcinoma of the lung, and had a recent history of melanoma and ductal carcinoma in the right breast. Core needle biopsy showed a delineated tumor with groups of moderately pleomorphic cells, with an apparent epithelial and myoepithelial component. Adenoid cystic carcinoma was the primary differential diagnosis, but due to uncharacteristic findings with estrogen receptor positivity, invasive carcinoma with predominance of ductal carcinoma in situ was considered. A final diagnosis could not be given. The patient started Letrozole treatment, but no tumor response was observed. After the patient had completed treatment for her lung cancer, lumpectomy was performed. The histopathological examination showed a well-defined epithelial tumor consisting primarily of epidermoid and basaloid cells, with a small component of mucinous cells, arranged in solid and cystic patterns. The tumor cells were positive in GATA3, mammaglobin, estrogen receptor and with normal HER2 expression. SMMS-1 was completely negative. A CRT1-MAML2 gene fusion was demonstrated by NGS. The findings are consistent with a mucoepidermoid carcinoma. The tumor was radically excised and subsequent sentinel node procedure showed clusters of tumor cells. This case illustrates the difficulty of diagnosing MEC in the breast, especially on core needle biopsies, due to the unusual location whereby salivary gland like tumors are not considered.

11

Intraplacentært koriokarcinom

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Introduktion
De fleste koriokarcinomer er relateret til en molagraviditet. Intraplacentært koriokarcinom (IK), der opstår i løbet af en normal graviditet, er en yderst sjælden form af gestationalt koriokarcinom, som ses i ca. 1 af 50000 graviditeter.

Materiale og metode
En 27-årig rask førstegangsfødende med normal graviditet fødte ved gestationsalder 40 uger og 0 dage en rask dreng, som fik fuld APGAR-score. Pga. mistanke om infarkt blev placenta sendt til histologisk undersøgelse.

Resultater
Ved makroskopisk undersøgelse fandtes et 17 mm stort fast rødt område, som makroskopisk mistænktes at være et friskere infarkt. Mikroskopisk var det et koriokarcinom. Der påvistes invasion i villusstromaet og enkelte koriokarcinomembolier i den føtale cirkulation. Endvidere fandtes massiv perivilløs fibrinaflejring. 2 måneder post partum havde mor og barn S-hCG <2. Barnet havde en normal objektiv undersøgelse. Ved supplerende FDG-PET- og MR-skanning af moderen blev metastatisk sygdom udelukket.

Diskussion og konklusion
IK kan fremtræde asymptomatisk eller med metastatisk sygdom.
Den sande forekomst af IK er ukendt, idet kun en mindre del af alle placentae undersøges histologisk. Den alment accepterede holdning, at koriokarcinom er usandsynligt, hvis der i et evakueret materiale samtidig er repræsentation af korionvilli, gælder således ikke i alle tilfælde.
Det er muligt, at asymptomatisk/ikke identificeret IK kan give metastatisk maternel eller infantil sygdom ved en kommende graviditet.
Før anvendelsen af multiagent kemoterapi havde metastatisk koriokarcinom inkl. metastatisk IK en meget høj mortalitet. Siden har alle rapporterede tilfælde opnået komplet remission med kemoterapi, og de registrerede dødstilfælde er relateret til forsinket/manglende behandling.

12

Tubules with epididymis-like differentiation in the paratubal tissues of a postmenopausal woman.

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Introduction
Epididymis-like tubules in paratubal tissues is a rare finding described in a few case reports featuring female-to-male transgender patients (FTM) and a postmenopausal woman.
Material and Methods
We report the case of a 71-year-old postmenopausal female, who underwent bilateral salpingoophorectomy for a benign ovarian cyst.
Results
Histologic evaluation of paratubal tissues showed a group of tubules with round lumen and eosinophilic intraluminal contents. The lining of the tubules consisted of an inner layer of tall columnar cells with apical stereocilia, and an underlying basal layer of smaller cells. A thin rim of smooth muscle surrounded the tubules. The lining epithelium expressed PAX8, GATA3 and AR, and CD10 staining showed apical ekspression in the luminal cells, and cytoplasmic staining in the basal cells. The basal cells were in addition positive for p63. The surrounding smooth muscle layer was positive for desmin and WT1. The ducts were negative for ER and WT1. When compared to male epididymis and epididymis-like tubules of other sites, the morphology and the immune profile were nearly identical.
Discussion and conclusion
Not much is known about epididymis-like tubules in the female reproductive system; however, they could represent virilization of preexisting mesonephric remnants due to androgenic hormone exposure. In light of increasing FTM transgender patient population, this finding could become more commonplace, which could argue for their inclusion into future histology textbooks. Further research is necessary to prove the origin of these tubules.

13

A rare case of HPV-negative, P16-negative squamous cell carcinoma of the cervix

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Introduction
The large majority of squamous cell carcinomas of the cervix are HPV associated, and only recently WHO (World Health Organization) recognized HPV-independent squamous cell carcinoma as a separate entity. We present a rare case of HPV-negative, P16-negative squamous cell carcinoma of the cervix, with concurrent P16-negative preinvasive lesions.

Materials and methods
A 58-year-old woman was referred to the gynecological department at Aalborg University Hospital, with postmenopausal bleeding. She had no prior history of cervical dysplasia. At examination, a small, ulcerated lesion on the cervix was revealed and cervical biopsies and cytology were performed.

Results
HE-stained biopsies showed surface epithelium with high grade dysplasia (CIN3) and infiltration of invasive squamous cell carcinoma in the underlying stroma. Both the dysplastic and the invasive component showed negative reaction for P16 immunostaining. Testing for high-risk HPV was performed on both the liquid-based cytology (BD Onclarity HPV DNA test) and the paraffin embedded tissue (INNO-LiPA® HPV Genotyping Extra II). Both tests were negative. The patient was referred to Aarhus University Hospital where she underwent radical hysterectomy and pelvic lymphadenectomy. Tumor was confined to the cervix, with final FIGO-stage IB2.

Discussion
HPV-negative squamous cell carcinoma of the cervix is a rare entity, but with an increasingly HPV vaccinated population, the relative incidence is expected to increase.
With increasing dependence of HPV-testing in screening for cervical cancer, there is a risk of delaying diagnosis of women with HPV-negative lesions.

14

High-grade B-cell lymphoma with MYC and BCL6 rearrangements as an incidental finding in an appendectomy specimen

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Introduction: High-grade B-cell lymphomas (HGBL) comprise a heterogenous group of non-Hodgkin, mature B-cell lymphomas, which are generally considered clinically aggressive, and frequently exhibit overlapping features with diffuse large B-cell lymphomas and Burkitt lymphoma.
Case presentation: A 24-year-old, previously healthy male was admitted with classical clinical presentation of acute appendicitis. An uncomplicated laparoscopic appendectomy was performed. Histo- and molecular pathology examinations, including NGS, established a diagnosis of high-grade B-cell lymphoma with MYC and BCL6 rearrangements. After referral to the haematology department the patient underwent PET/CT and bone marrow biopsy, he was assigned stage IEA, IPI = 0, and chemotherapy was initiated.
Discussion: This case depicts a very uncommon occurrence of an accidentally discovered, fully localised HGBL in a patient of an unusually young age for this entity, where the only manifestations were those of acute appendicitis. Due to the localisation and the patient's age, differential diagnosis with Burkitt lymphoma was particularly important. HGBL represent a heterogenous group of primary lymphomas which are considered clinically aggressive. However, recent literature suggested that HGBL with MYC-BCL6 translocations differ both biologically and clinically from HGBL with MYC-BCL2 translocation and should therefore probably be classified as a separate entity.
Conclusion: However rare in incidence, this case demonstrates how a routine postoperative histopathologic examination of the appendectomy specimen allowed to diagnose a, generally considered, aggressive and rapidly growing lymphoid neoplasm at an early, fully localised stage of the development.

15

MUCOSAL EXPRESSION OF GENES PI3, ANXA1, AND VDR DESCRIMINATES CROHN'S DISEASE FROM ULCERATIVE COLITIS IN INFLAMMATORY BOWEL DISEASE PATIENTS

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The aim of this study was to develop a method aiding clinicians to distinguish between Crohn's disease (CD) and ulcerative colitis (UC), two subtypes of inflammatory bowel disease (IBD).
The study included a pilot study, testing in external cohorts, and a validation study. Formalin-fixed paraffin-embedded mucosal biopsies collected from IBD patients were used for both pilot and validation studies. In the pilot study, we used next-generation sequencing on 16 CDs, 17 UCs, and 10 healthy controls (HC). Subsequently, we tested the best 100 differentially expressed genes from the pilot study in two external cohorts from gene expression omnibus. The expression of the best 7 candidate genes was validated in an independent cohort of samples from 45 CDs and 53 UCs and 12 HCs using RT-qPCR. Finally, in situ hybridization (ISH) using RNAscope was performed on 11 cases.
We discovered that differential mRNA expression of three genes (ANXA1, PI3, and VDR) using RT-qPCR, in combination with the patient's age and sex, could distinguish CD from UC with an area under the curve (AUC) of 0.84 (P =.02). AUC of 0.77 (P =.01) revealed a significantly higher expression of VDR mRNA in CDs compared to UCs. ISH showed expression of PI3, ANXA1, and VDR mRNAs localized to mucosal epithelial cells.
In conclusion, we propose that the algorithm we developed using RT-PCR expression values of the 3 genes in combination with the patient's age and sex, could support clinicians in distinguishing CD from UC and be applied in a routine clinical setup.

16

Lymph node analysis utilizing artificial intelligence and multispectral imaging.

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Introduction: Multispectral imaging (MSI) is the process of illuminating an object at several wavelengths and capturing the resulting images which can be used for further analysis. This method has in recent years been investigated in clinical settings i.e. during endoscopy, in an effort to identify and characterize common disease processes such as Barrett's esophagus. Here we investigate the utility of MSI in combination with a convolutional neural network (CNN), to accurately categorize lymph nodes (LNs) with and without metastases.
Materials and Methods: Approximately 2000 tissue blocks containing formalin fixed paraffin embedded LN tissue were photographed at several wavelengths in the spectral range covering 450 – 980 nm, and the images were labeled as benign or malignant according to the reported diagnostic coding. Based on the images, a CNN was trained to identify as many true negative samples as possible with a minimum of false negatives. CNN classifiers were trained and validated on a training set, and confusion matrices and ROC curves validated on an independent test set of 903 samples from 217 patients.
Results: The CNN accurately identifies a subset of benign samples. We demonstrated the ability to identify 21% of the benign samples with a false negative error rate <2%.
Discussion and conclusion: This tool can aid in selecting tissue blocks for fast-track analysis, and avoid unnecessary examination of a considerable number of non-metastatic LNs. This could lighten the workload of laboratory personnel and pathologists and in turn reduce costs of i.e. expensive sentinel node protocols.

17

Infiltration of lymphocytes assessed by deep learning-based algorithms and the association with pathological response to neoadjuvant therapy in rectal cancer

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Introduction: The standard treatment strategy in locally advanced rectal cancer (RC) is neoadjuvant chemotherapy (nCRT) followed by surgery. Patients with RC receiving nCRT achieve varying pathological response. The ability to differentiate complete from other responders could potentially save patients from an ineffective treatment. Furthermore, organ sparing in RC is an emerging goal and tools to predict complete responders are warranted. This study aimed to investigate potential differences in histopathological features between complete responders vs. all other groups.

Material and Methods: We included 50 patients with RC treated with nCRT. Deep learning-based digital algorithms were developed to assess the epithelium tumor area percentage (ETP) based on hematoxylin and eosin-stained slides, and to quantify the density of CD3+ and CD8+ lymphocytes in immunohistochemically stained slides, from the diagnostic tumor biopsies. The ETP, and density of CD3+, CD8+ lymphocytes as well as the CD8/CD3-ratio were compared according to the Mandard tumor regression grade in the surgical specimens.

Results: When comparing the complete responders (n=7) to all other groups of response (n=43), there were no significant differences in the ETP ($P>0.05$). Densities of both CD3+ and CD8+ lymphocytes and the CD8/CD3-ratio in the biopsies were significantly higher in the group of complete responders ($P\leq0.05$).

Discussion and Conclusion: It is well-known that the infiltration of CD3+ and CD8+ lymphocytes in colorectal cancer is a prognostic marker. In the future, assessment of infiltration of CD8+ and CD3+ lymphocytes in diagnostic biopsies of patients with RC may be useful in predicting complete response to nCRT.

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Distribution and tumor cell proximity of immune cells in the tumor core, transition zone and periphery of glioblastomas

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Introduction: Glioblastoma is the most frequent and malignant brain tumor. The tumor cells quickly infiltrate the brain parenchyma and prevent total tumor resection, leading to recurrence. Immune therapies have limited effect but not much is known about the frequency and type of immune cells in the transition zone and periphery. In this study, we aimed to quantify the type and distribution of immune cells in glioblastomas.
Material and Methods: A cohort of 67 glioblastomas was established containing tissue from tumor core/transition zone/periphery. Patients were included if most tumor cells were positive in immunohistochemical staining for P53. Tissue sections were stained with P53 (tumor), FOXP3 (regulatory T cells), CD8 (cytotoxic T cells) and IBA1 (microglia/macrophages). Cells were counted by an app trained in Visiopharm software. The number of tumor cells with immune cells in proximity (30 µm) was quantified.
Results: The numbers of CD8+, FOXP3+ and Iba1+ cells were higher in core compared to periphery ($P < 0.001$), and in transition zone compared to periphery ($P < 0.001$). However, the immune to tumor cell ratio increased from core to transition zone and to periphery ($P < 0.001$). The highest amount of tumor cells with IBA1+ cells and FOXP3+ cells in proximity was found in the transition zone ($P < 0.001$).
Discussion and conclusion: Although levels of immune cells are highest in the core - the proximity and ratio of immune cells to tumor cells in the transition zone /periphery suggest immune-suppressive mechanisms to be active in these areas.

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A retrospective study of Incidence of synchronous epithelial appendiceal neoplasm, with colon adenocarcinoma

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[1]*Ouh*

Purpose
This study aimed to determine frequency of appendiceal neoplasm in right hemicolectomy samples in South-West Denmark with regard to possible therapeutic consequences.

Methods:
A retrospective review of the pathology report and histopathology was performed in patients undergoing right hemicolectomy for colorectal cancer. In total, 337 patients had right hemicolectomy due to the diagnosis of cancer between January 2013 and Maj 2021.
Results:
15 of 337 appendices had neoplasia: 10 SSL (Sessile serrated lesion) without dysplasia, 1 LMN (low grade mucinous neoplasia), 1 tubular adenoma with high-grade neoplasia, 1 villous adenoma with low-grade neoplasia and 2 neuroendocrine tumor. Appendiceal neoplasia were found in 15 (4.45%).
Conclusions:
Synchronous epithelial appendiceal neoplasia was found in 4.45% in this series. It could be the same in the left side colorectal cancer. Routine postoperative surveillance cannot assess the appendiceal mucosa. Thorough preoperative and perioperative evaluations should be performed in patients with colorectal cancer to exclude a synchronous or primary appendiceal tumor.

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Acute appendicitis as initial presentation of primary disseminated breast cancer

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Introduction
Breast cancer commonly metastasizes to the regional lymph nodes, bones, liver, lung, and brain. Metastasis in the gastrointestinal tract occurs and is usually more prevalent in invasive lobular breast cancer (ILC), however, involvement of the appendix is rare.
Material and Methods
A 59-year-old female presented with a 1-day history of strong pain in the right lower abdominal quadrant, fever, nausea, vomiting, and loose stools. The past 6 months, she had experienced lower abdominal pain. Diagnostic laparoscopy showed a necrotic, perforated appendix and appendectomy was performed.
Results
Pathology of the appendix revealed ILC metastasis. The tumour cells showed positive reaction for ER (100 %) and HER2 score 0 with negative reaction for E-cadherin. Subsequent mammography, ultrasound and PET-CT showed two BIRADS 5 lesions in the left breast and suspicion of axillary lymph node involvement. Diagnostic biopsies from the breast revealed invasive ductal carcinoma (IDC) with ER positivity (100 %), HER2 score 0 and 1+, and a strong positive reaction for E-cadherin.
Discussion and conclusion
The appendix is a rare site of metastasis from breast cancer. To our knowledge, metastatic breast cancer presenting as acute appendicitis without known primary tumour has only been reported in three other cases. This case emphasizes the importance of histological tumour subtype classification also at the metastatic site. Further, it shows the importance of identifying intra- and intertumour heterogeneity, which has major impact on treatment decision in the metastatic setting due to variation in pathogenic driver mutations in ILC as compared to IDC.

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LOWER INITIAL COLPOSCOPY REFERRAL IN HPV-SCREENING USING COMBINED GENOTYPE AND CYTOLOGY TRIAGE COMPARED TO LIQUID BASED CYTOLOGY(LBC)-SCREENING WITH HPV-TRIAGE

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Background
In 2021 Denmark initiated a phased implementation of HPV screening for women aged 30-59. In the Capital Region, HPV-screening is performed using extended genotyping and cytology to triage HPV-positive samples. A concern is whether HPV-screening result in higher direct referral to colposcopy after the index-screening round compared to the cytology-based screening.
Methods
Women aged 30-59 years participating in HPV or cytology-based screening in 2021 with a valid index-screening outcome were included. Data were retrieved from the Pathology DataBank. The colposcopy referral criterion of the index-sample was [HPV+ and any HSIL/ASC-H/AGC/AIS/CxCa] or [ASCUS/LSIL and any HPV16,18,31,33,52]. For cytology-based screening, the colposcopy referral criterion was [≥ASCH] or [ASCUS/HPV+].
Results
80.540 women participated in screening with a valid sample in 2021, 41.319 with HPV-screening versus 39.221 with cytology-based screening. In HPV-screening 2.1%(N=874) were referred to colposcopy on the index-sample, versus 2.6%(N=1001) for cytology-based screening. The distribution of referrals in HPV-screening was 1.3%(N=520) with HPV+/≥HSIL and 0.9%(N=354) ASCUS/LSIL and any HPV16,18,31,33,52. For cytology-based screening 1.7%(N=675) had ≥HSIL and 0.8%(326) ASCUS/HPV+. At data retrieval, 94% of all referred to colposcopy in both screening methods had completed the procedure. Histology showed 53%≤CIN1, 47%≥CIN2, and 33%≥CIN3 for those referred in HPV-screening(N=771). For cytology-based screening(N=886) results were 61%≤CIN1, 39%≥CIN2, and 26%≥CIN3. HPV-screening detected 22 Adenocarcinoma in situ and 9 CxCa. Cytology-based screening detected 21 Adenocarcinoma in situ and 11 CxCa.
Conclusion.
At the index-round, HPV-screening using an algorithm of extended genotyping/cytology reduce the initial colposcopy activity and increase the disease detection compared to cytology-based screening with HPV-triage.

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Udvikling i forekomsten af kolorektale serrate læsioner og polypper i den danske befolkning 2000-2021

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Introduktion
Kolorektale serrate læsioner og polypper (SP) omfatter, iht. nyeste WHO klassifikation fra 2019, hyperplastiske polypper (HP), sessile serrate læsioner uden og med dysplasi (SSL/SSL-D), samt traditionelle serrate adenomer (TSA). I dag antages 25-30% af kolorektale adenokarcinomer at udgå fra SP, hvorfor fokus på disse polypper er øget. De diagnostiske kriterier for HP og SSL har ændret sig flere gange i undersøgelsesperioden, hvorfor dette, udover øget fokus på SP og bedre endoskopisk udstyr, må antages at have en betydning for både forekomsten af SP og fordelingen mellem undertyperne.
Materialer og metoder
Vi brugte Patobank til at finde alle SP i perioden 2000-2021. SNOMED-koderne blev brugt til at bestemme polyptype og anatomisk lokalisation.
Resultater
I perioden 2000-2021 blev der fjernet 269.450 SP fra 164.077 patienter. Heraf var over halvdelen fjernet fra rektum og colon sigmoideum (56%). Medianalderen var 64.6 år [55.2-71.6] og 44.2% var kvinder. Fordelingen af undertyperne var SSL: 50.197, SSL-D: 5.924, HP: 203.237, TSA: 10.092. Vi så en generel stigning af SP fra 2.804 i 2000 til 25.846 i 2021, samt en stigning i andelen af SSL fra 1.1% (81) i 2006 til 27.7% (9891) i 2021. HP og TSA var hyppigst forekommende i rektum og colon sigmoideum, mens SSL og SSL-D oftest forekom i colon ascendens og colon sigmoideum, efterfulgt af de øvrige højre colonsegmenter.
Diskussion og konklusion
I løbet af perioden 2000-2021 blev der fundet et stærkt stigende antal SP, især SSL. Hvilket vil danne grundlag for fremtidige studier af SP og udvikling af kolorektal cancer.

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Case-cohort studies as a tissue- and time-reducing design for biomarker discovery studies allowing for time-to-event analysis

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<B>Introduction:</B>
Biomarker discovery studies in large cohorts can be costly and time-consuming if biological material is analyzed on all patients. The number of samples needed is traditionally reduced by applying case-control designs where cases are the individuals experiencing an event of interest within a given follow-up period. Recently, case-cohort designs have been developed as alternatives which similarly reduce the number of samples and, in addition, allow for time-to-event analysis. We describe a cancer cohort study where traditional case-control designs failed but where a case-cohort design successfully reduced the number of samples needed and allowed for robust estimations of recurrence and survival rates.
<B>Material and Methods:</B>
Initially, we considered a case-control biomarker study using FFPE-blocks from a cohort of 1481 breast cancer patients including 517 cases (defined as death and/or recurrence). With less than 2 controls available per case, traditional case-control designs failed. Instead, a case-cohort design was applied in which a subcohort was randomly selected from all cohort participants at start of follow-up. In this subcohort, some patients became cases during follow-up. All cases occurring outside the subcohort during follow-up were also included.
<B>Results:</B>
The cohort was reduced from 1481 to 999 patients with similar event rates compared to the full cohort: overall survival 64.4% vs. 64.3%, distant metastasis 26.7% vs. 26.6%, and loco-regional recurrence 4.6% vs. 4.6%.
<B>Discussion and Conclusion:</B>
The case-cohort design reduced the number of FFPE-blocks needed with 33% (482 patients) without compromising accurate time-to-event analysis. The case-cohort design is recommended for biomarker analysis in large cohorts.

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CERVICAL CANCER SCREENING ACTIVITY IN THE CAPITAL REGION OF DENMARK DURING THE COVID-19 PANDEMIC

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Introduction: We assessed the impact of the COVID-19 pandemic on cervical cancer screening activity in the Capital Region of Denmark. Denmark went through different levels of COVID-19 restrictions including periodic lockdowns March2020-January2022, however an unaltered continuation of all cancer screening programs was stipulated. Yet, access to clinician collected cervical screening was at times limited. Methods: Cervical screening activity was defined as screening by invitation, opportunistic screening, and screening participation by HPV self-sampling. Activity was monitored during the COVID-19 pandemic (2020-2022) and compared to a 3-year pre-pandemic reference (2017-2019). Results: The activity of cervical cancer screening by invitation was affected most in March-May 2020 with a monthly change in screening activity of -49%, -51% and -19%, respectively. This was offset throughout the remainder of 2020 resulting in a screening activity reduction of 8%. For 2021, <1% difference in activity of screening by invitation was observed. Opportunistic screening activity was reduced by 14% in 2020 and 25% in 2021. Participation in screening by HPV self-sampling increased significantly from 17% (2017-2019) to 21% (2020-2021) and increased by age ($p<0.001$). Overall screening activity decreased by 9% during 2020-2021. Conclusions: The COVID-19 pandemic impacted the activity of cervical cancer screening by invitation most in 2020 and mainly during the initial lockdown periods, resulting in a reduction of 8% in 2020. In 2021, the impact was minimal. Opportunistic screening activity decreased during the pandemic, however screening by HPV self-sampling was higher than pre-pandemic screening participation. Denmark has not instated any special post-pandemic cervical cancer screening initiatives.

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TIME AND AGE DEPENDENT HPV CLEARANCE AFTER HPV POSITIVE SCREENING INDEX SAMPLE: Health care policy implications for recommended follow-up

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Introduction
In the Capital Region of Denmark, women aged 30-59 undergoing HPV screening are recommended a re-test 12 months later if the index sample is HPV positive (any genotype) with normal cytology or ASCUS or LSIL with any of HPV35, 39, 45, 52, 56, 58, 59, 66, 68.

Materials and Methods
Women (30-59y) undergoing HPV screening from March-August 2021 with an index sample outcome of the above criteria (N=1317). Clearance was defined as a Ct score above the cut-off as well as a valid sample result (BD Onclarity HPV test). The association between clearance of HPV infection and age/time to follow-up was analyzed by logistic regression.

Results
Compliance to re-test recommendation was 61.5% and 75.5% at 12 and 18-months after index sample. The median number of days until follow-up was 364 days.
At re-test, 57% of the women remained HPV positive. Of these 12.7% had multiple infections. Of all HPV genotypes registered at index, 46% persisted with the same genotype at re-test, 9% were new types gained, and 45% was cleared. HPV clearance was significantly associated with time; the longer period until follow-up the higher clearance rate (p=0.035). No association between age and HPV infection clearance was found.

Discussion and Conclusion
The ability to clear HPV infections was age independent, while time to HPV follow-up test was significantly associated with HPV clearance, indicating 18-month follow-up time may be more optimal than 12 months to allow clearing of index sample HPV infection, reducing the number of unnecessary referrals after re-test.

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Immunohistochemical detection of p62 in liver biopsies: An important tool in diagnosing non-alcoholic steatohepatitis (NASH)

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The occurrence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide. The median prevalence in the adult population is currently estimated to about 20%, of which up to 25% suffers from the progressive non-alcoholic steatohepatitis (NASH). NASH is defined by steatosis in combination with lobular inflammation and hepatocellular ballooning, the latter characterized by enlarged, rounded cells with a clear, reticulated cytoplasm. However, the reproducibility of ballooning identification is low, which makes the diagnosis inconsistent. Other supportive, but inconstant features in NASH are centrilobular, pericellular fibrosis and Mallory-Denk bodies (MDBs). The latter typically appear in ballooned cells but are often difficult to discern in routine stains. Immunohistochemical detection of the ubiquitin-binding protein p62 is a highly sensitive method to detect MDBs as well as their precursor stages and, thus, a great help to discover ballooned cells. We analysed 100 consecutive liver biopsies from patients suspected of NASH. The diagnosis was histologically confirmed in 78, of which 72 (92%) revealed p62 positive, mostly ballooned cells. Among 22 cases without NASH but with steatosis (7) or fibrosis/cirrhosis of uncertain aetiology (5), few p62 positive cells were detected in 3 (25%), while in the remaining cases, normal or with other diseases (10), no p62 positive cells were found. Immunohistochemical staining for p62 in liver biopsies appears to be an important tool in diagnosing NASH.

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Automated quantification of the proliferation index in tumor tissue

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Background: The proliferation index is an important diagnostic and prognostic biomarker in many tumors. In this study we aim to develop a new method for automated quantification of the proliferation index using multiplex immunofluorescence (mIF) and digital image analysis (DIA) with artificial intelligence. We test the methods diagnostic performance in melanocytic lesions, breast carcinomas and neuroendocrine tumors (NET).
Methods: Tissue samples from 20 melanocytic lesions, 20 breast carcinomas and 13 NETs were retrieved from the archive at Department of Pathology. A tissue slide from each lesion was stained with mIF against Ki67 and a nuclear tumor marker, scanned digitally and then re-stained with Hematoxylin & Eosin (HE) and scanned. The two scans were aligned digitally, and DIA was performed using deep learning algorithms to identify all nuclei and quantify Ki67 positive tumor cells in different tumor regions. Ki67 indices of different tumor groups were compared.
Results: Mean Ki67 indexes were statically significantly different between breast Luminal A and Luminal B subtype (p: 0.0008) in breast cancers and between compound nevi and superficial spreading melanomas (p:0.0002) in melanocytic lesions. For NETs, no significant difference between mean Ki67 indexes of NET grade 1 and NET grade 2 was detected and the agreement between manual and automatically quantified Ki67 indices was poor.
Conclusion: Automated quantification of Ki67 indexes using artificial intelligence and mIF shows potential as a diagnostic aid in melanomas and breast cancers.

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2 cases of malakoplakia mimicking neoplasia of the urinary bladder

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Introduction
Malakoplakia is a rare granulomatous disorder, most commonly occurring in the genitourinary tract, which often is associated with recurrent UTI and hematuria. The etiology is unknown, but the condition is considered to result from a defective macrophage response to bacterial infection. Histopatologically, malakoplakia is characterized by the presence of von Hansemann histiocytes containing Michaelis-Gutmann bodies. Malakoplakia can present as nodules, plaques or ulcerations and may be mistaken for carcinoma.

Material & Methods
2 patients were investigated for macroscopic hematuria at the Department of Urology. On cystoscopy, neoplasia of the bladder was suspected and a TURB was performed. Specimens were sent for histological examination. Patient 1, a 71-year old female with no history of malignancy presented with broad-based exophytic tumors throughout the urinary bladder. Patient 2, a 61-year old female with no history of malignancy presented with ulcer-like lesions of the urinary bladder. The specimens were stained using H&E, CD68, AE1/3, iron, PAS, GMS, von Kossa and Ziehl-Nielsen stains.

Results
In both cases, histopathological examination revealed no malignancy, but the presence of neutrophils, plasma cells and sheets of round CD68+ histiocytes with abundant eosinophilic cytoplasm in the lamina propria. Suspected Michaelis-Gutmann bodies in the histiocytes were confirmed using PAS and von-Kossa stains, leading to the diagnosis of malakoplakia.

Discussion
Malakoplakia poses a diagnostic challenge and requires careful histological scrutiny to diagnose correctly, so malignancy or parasitic diseases are not overlooked. Furthermore, accurate diagnosis is important as it can be successfully treated with long term antibiotics.

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Glial Cells Distribution and Diversity in Neurodegenerative Protein Misfolding Diseases

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This explorative study aims to elucidate the distribution of glial cells in healthy and diseased human brain tissue and assert the diversity and reactivity of astrocytes described in other species. The main goal is to detect potential changes in the distribution pattern of these cells from a default physiological environment to different pathological settings and identify disease-specific morphological changes of astrocytes in Alzheimer's (AD) and Creutzfeldt-Jakob disease (CJD).
Using digital pathology and imaging software, immunohistochemically stained microglia (CD68) and astrocytes (GFA) were quantified, characterized morphologically, and compared between 5 molecular layers in both frontal (FC) and occipital (OCC) neocortices affected by either AD or CJD in comparison to healthy brain samples.
Preliminary data indicate that 1) CJD patients, unlike AD patients, had significantly increased CD68 staining in grey matter of both cortices compared to controls, 2) FC and OCC in AD and OCC in CJD patients had unique distribution patterns of GFA+ astrocytes compared to controls, 3) GFA+ astrocytes were found to be significantly different within a specific disease type, cortex region and layer compared to controls.
This indicates a complex nature of glial cells in the context of neuroinflammation and highlights the need for a more nuanced approach to these cells in the research of neurodegenerative proteinopathies.

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Methotrexate-associated EBV-positive mucocutaneous ulcer: A case report.

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EBV-associated lymphoproliferative disorders range from indolent disorders with spontaneous regression, to highly malignant cancers. EBV-positive mucocutaneous ulcer (EBV-MCU) is an EBV-associated lymphoproliferative disorder with histological features of malignancy, but showing an indolent clinical course, described in immunosuppressed patients (including patients with age-associated immunosenescence), where complete regression is often seen with decrease or termination of immunosuppressive treatment.
We present a case of EBV-MCU in a 91-year-old woman with methotrexate-treated rheumatoid arthritis. She presented with throat pain radiating to the right ear. Laryngoscopic examination revealed a fibrin coated ulcerating tumour at the ostium of the right tuba auditiva. MR and PET/CT showed a large tumour in the right side of rhinopharynx.
Biopsy material showed ulceration and necrosis, with underlying fibrotic tissue containing a dense polymorphic infiltration of inflammatory cells, but also accompanied by large atypical, Hodgkin- and Reed-Sternberg (HRS)-like cells with enlarged angular nuclei and prominent eosinophilic nucleoli. The area was surrounded by a rim of mature T-lymphocytes. The HRS-like cells showed positive expression for CD79a and CD30, but not CD20, with positivity for EBV (EBER and LMP1, but not ENBA2). Histopathology and the patient's medical history suggested EBV-MCU as the most likely diagnosis. Methotrexate treatment was discontinued and after three months, the patient's symptoms and tumour had completely regressed.
EBV-MCU may be hard to distinguish clinically and pathologically from malignant lymphoma. However, as illustrated by this case report, the effect of a correct diagnosis may be dramatic in preventing unnecessary treatment.

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Onkocytom i nyren – er det sikkert med active surveillance?

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Introduktion
Onkocytom er en benign renal neoplasi som billediagnostisk ikke med sikkerhed kan skelnes fra renalcellekarcinom (RCC) hvorfor især små nyretumorer biopteres.
Formålet med opgørelsen var at følge op på patienter med biopsiverificeret onkocytom i active surveillance med henblik på vurdering af forekomsten af RCC, herunder hybridtumorer, samt mortalitet af RCC for denne patientgruppe.
Materialer og metoder
Udtræk fra Patobank af patienter, der fik diagnosekoden M82900 (onkocytom) på Herlev Hospital i perioden 1/1 2010 til 31/12 2020. Oplysninger om histologi og follow-up blev indhentet via opslag i Patobank, Sundhedsplatformen og Sundhedsjournalen.
Resultater
213 patienter blev identificeret. Tre patienter udgik grundet utilgængelige journaloplysninger. 146 patienter (69,5 %) fik stillet diagnosen onkocytom ved ultralydsvejledt biopsi.
I opfølgningsperioden blev der fundet indikation for intervention hos 19 patienter, 2 ved radiologisk intervention og 17 ved kirurgi. Ud af 19 tumores (hvoraf 1 var en synkron tumor med biopsiverificeret RCC) blev der ved histologisk undersøgelse af resektaterne fundet yderligere 2 RCC. I begge tilfælde var den præoperative biopsi beskrevet med atypisk histologi. Resektaternes histologi var forenelig med den præoperative biopsi hos de 9 patienter, hvor biopsien viste onkocytom med klassisk histologi.
I alt 28 af de biopterede patienter er afgået ved døden. 1 patient er død af formodet dissemineret RCC. Patientens biopsi viste muligt onkocytom, men usikker histologi. Patienten frabad sig efterfølgende yderligere udredning.
Diskussion og konklusion
Når der ved biopsi findes renalt onkocytom med typisk histologi er risikoen for RCC eller hybridtumor minimal. Ved fund af atypisk histologi bør patienten tilbydes rebiopsi eller operation.

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Præstationsprøvning af danske lungepatologer, et kvalitetssikringsprojekt.

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Danske patologiafdelinger har stigende fokus på akkreditering. I forbindelse med akkreditering kræves deltagelse i præstationsprøvning eller lignende sammenligningsprogram. Der er kun få tilgængelige præstationsprøvningsprogrammer for lungepatologer, som overvejende synes suboptimale.
Projektets formål var at skabe en let adgang til et dansk præstationsprøvningsprogram, der bedst muligt imiterer det daglige diagnostiske mikroskopiarbejde.
Der deltog i alt 22 lungepatologer fra de 7 patologiafdelinger i landet, der varetager udredning af lungecancer. Prøvningen var en multiple-choice-test og forgik via Pathogates virtuelle platform. Den bestod af 10 cases. Det diagnostiske område og sværhedsgraden blev aftalt ved årsmødet 2022 for Danske lungepatologer. De deltagende afdelinger bidrog med en eller to cases. Prøvematerialet bestod af histologisk og cytologisk biopsimateriale samt resektater med de standard HE/MGG-farvninger og immunhistokemiske undersøgelser, der var udført ved den initiale diagnose. Prøvningen var sat op med en initial adgang til standardsnit. Efter afgivelse af en foreløbig diagnose blev der givet adgang til immunhistokemiske undersøgelser, hvorefter den endelige diagnose kunne stilles med angivelse af evt. obligatoriske supplerende undersøgelser.
Efter prøvningen blev fordelingen af svarene gjort op. Den hyppigst stillede diagnose i de enkelte cases blev betragtet som det korrekte svar. Der var 100% overensstemmelse i diagnosen i 3 ud af de 10 cases. Den laveste korrekte svarprocent var 68%, og den største spredning på diagnosen var 5 ud af i alt 20 mulige svar.
Endeligt blev deltagerne bedt om at svare på et spørgeskema vedrørende deltagelse i præstationsprøvningen. Overordnet syntes deltagerne, at prøvningens opsætning var god og ville kunne bruges til fremtidige præstationsprøvninger.

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The art of tissue slicing and stereological volume estimation – the generalized Cavalieri estimator and the Trapezoidal estimator

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The Cavalieri estimator is the gold standard stereological method for design-unbiased estimation of the volume of an organ and its subcompartments. Consequently, the method has been used in a very large number of quantitative microscopy and imaging studies. However, the method requires that the investigated structure is sliced by equidistant, systematic uniformly random cuts. I.e., that all slabs are of identical thickness. This is often not the case when an organ is sliced. Thus, visible variation in slab thickness occurs even when the organ is agar-embedded, and a cutting guide is used. Generally, this error is ignored, assuming that the resulting error in the volume estimate is insignificant.
We have developed a mathematical framework for assessing the consequences of irregular sectioning. Fortunately, we find that the generalized Cavalieri estimator based upon non-equidistant cuts is strictly unbiased. However, depending upon how the organ is sliced, the precision of the resulting volume estimate may be substantially reduced. Especially, if the used cutting method allows for frameshift relative to the intended positions of the cuts, i.e. with cumulative errors, the resulting variance of the volume estimates may be substantial. Unfortunately, this is the case when the pathologist cuts freehanded, without a guide, aiming at a certain slab thickness, when grossing. Finally, we have recently published the Trapezoidal estimator, which can eliminate the impact of irregular slicing, if an antithetic (non-frameshifting) cutting method is used, and if the positions of the cuts are recorded (e.g. by a photo) and taken into account.

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Implementering og validering af genomisk profilering på FFPE væv fra solide tumorer

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Introduktion: Mange kræfttumorer undersøges i dag som standard for udvalgte somatiske varianter, og kataloget af targeterbare varianter udvides hele tiden. Det kan dog være udfordrende at undersøge diagnostisk FFPE væv, da kvaliteten af nukleinsyrerne herfra ofte er lav. Formålet med dette studie var at implementere og validere et genpanel med 523 gener, som kan bruges til genomisk profilering af FFPE væv fra solide tumorer.

Materialer og metoder: 72 prøver bestående af kontrol DNA, RNA samt FFPE prøver fra solide tumorer blev sekventeret på et Illumina NextSeq 500 instrument ved brug af Trusight Oncology 500 genpanelet (TSO500; Illumina). Sekventeringsdata undergik bioinformatisk processering og blev efterfølgende brugt til at analysere kvaliteten af sekventeringerne, bestemme den nedre inputgrænse (ng) samt variant detektionsgrænsen.

Resultater: Ved brug af kontrol DNA og RNA blev minimumsinputgrænsen for TSO500 fastsat til at være 40 ng og variant detektionsgrænsen blev fastsat til en allelfrekvens på 5%; ved disse grænser var der en overensstemmelse på 99,3% for DNA og 100% for RNA mellem kontrol og varianterne detekteret med TSO500. Sekventeringen af nukleinsyrerne i FFPE prøverne var af høj nok kvalitet til pålideligt at kunne kalde varianter i 47/58 DNA prøver (81%) og 55/56 RNA prøver (98%). For FFPE prøver med kendt variant status genfandt TSO500 alle varianter i 28/32 DNA prøver (87,5%) og i 16/16 RNA prøver (100%).

Diskussion og konklusion: TSO500 kan anvendes til genomisk profilering af FFPE væv fra solide tumorer. I dette studie blev TSO500 TMB og MSI scores ikke valideret. Dette forventes gjort i nærmeste fremtid.

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Pludselig død efter uerkendt inferiort myokardieinfarkt

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En 74-årig mand, der tidligere i det væsentlige var rask, findes ukontaktbar på gulvet i hjemmet af pårørende.
Afdøde havde igennem 5 dage haft faldtendens og utilpashed samt smerter i ryggen mellem skulderbladene. Indlægges akut, hvor EKG gav mistanke om inferiort akut myokardieinfarkt (AMI) og 3. grads atrio-ventrikulært blok.
Ekkokardiografi viste svært nedsat pumpefunktion af venstre ventrikel samt muligt perikardieekssudat med koagler. Der foretages akut koronararteriografi (KAG) og anlægges stent i lukket højre koronararterie samt temporær pacemaker. Tilstanden forværres imidlertid og patienten afgår ved døden på KAG-stuen.
Der rekvireres obduktion på mistanke om myokardieruptur efter uerkendt inferiort AMI.
Ved obduktionen findes blodig væskeansamling og koagelmateriale på i alt 230 ml i perikardiet. På hjertets bagside, basalt nær septum, findes et hæmoragisk område på 21 mm i største diameter og en 1 mm cirkulær defekt i hjertet. På snitfladen ses her gulbrunlig misfarvning af myokardiet. Der findes tre-karssygdom (stent i højre koronararterie, kronisk okkluderet ramus circumflexus og op til 90% stenose i ramus descendens anterior).
Mikroskopisk undersøgelse viser nyligt (dage) transmuralt myokardieinfarkt med perforation og blødning i perikardiet. Det infarcerede område visualiseres tydeligt i Masson's trikomfarvning. Svarende til stenten findes højre koronararterie med aterosklerotisk plaque med ruptur og blødning.
Dødsårsagen vurderes at være hjertetamponade som følge af myokardieruptur på baggrund af transmuralt inferiort myokardieinfarkt efter plaqueruptur og trombedannelse i højre koronararterie.
Casen illustrerer at inferiore myokardieinfarkter kan have et anderledes symptombillede, samt nytten af Masson's trikrom farvning til mikroskopisk vurdering af myokardieinfarkt udbredelse.

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Pigmented epithelioid melanocytoma in a 9-year-old female.

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Pigmented epithelioid melanocytoma (PEM) is a rare melanocytic neoplasm with a predilection for young people, including children, but may occur at all ages. The etiology of PEM is unknown, but there is an association with the genetic disorder Carney complex. Clinically, PEM presents as a blue/black lesion, and is often mistaken for malignant melanoma. Histologically, PEM is composed of varying proportions of heavily pigmented epithelioid, spindled, and dendritic cells with characteristic vesicular nuclei, prominent nucleoli and melanophages. Recent molecular analysis allows for classification and subclassification of this melanocyte-derived skin tumor. The metastatic potential of PEM is predominantly limited to regional lymph nodes, and distant metastases are uncommon. The clinical significance, and the incidence of disease-related death, remain uncertain. We present a rare case of a PEM in the periorbital region of the right eye, in an otherwise healthy 9-year-old girl. Diagnosis was confirmed using immunohistochemistry and molecular analysis, and the lesion was excised with a narrow, but clear, margin of about 0.5 mm. Given its rare occurrence Danish clinical guidelines for treatment of PEM are unfortunately limited, and further treatment of this patient was discussed on a regional level. Subsequently consulting Professor Richard Scolyer at the Royal Prince Alfred Hospital in Sydney, Australia, a multidisciplinary team consisting of doctors of relevant specialized departments, reviewed the case and concluded there was no indication for further surgical intervention. Close monitoring on a regular basis, including ultrasound examination of regional lymph node stations, was instituted in the Pediatric Department of Odense University Hospital.

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Øger tilbud om selv-opsamlet prøve deltagelsen i livmoderhalskræftscreeningen?

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Introduktion
For at imødegå faldende deltagelse i livmoderhalskræftscreeningen, har Sundhedsstyrelsen besluttet at kvinder der modtager 2. geninvitation (2. rykker), skal tilbydes frit valg mellem en selv-opsamlet prøve (HPV-hjemmetest) eller en ordinær cervixcytologisk prøve hos egen læge. Tilbuddet skal gøre det attraktivt og lettere at deltage i screeningen. Region Hovedstaden tilbød, som de første i 2022, HPV-hjemmetest som valgmulighed, til alle kvinder der har modtaget 2. rykker.

Materialer & Metoder
I alt 55.507 kvinder modtog i perioden 2. rykker efter invitation til screening. Data er baseret på udtræk fra Cyres for aktivitet i Region Hovedstaden, dækkende perioden 2. marts til 19. december 2022.

Resultater
Blandt kvinder, der i perioden modtog 2. rykker, valgte 3.8% HPV-hjemmetest mens 14.6% valgte cervixcytologisk prøve. Den samlede tilslutningen var højest blandt 40-49-årige med 21.7% screenede efter 2. rykker, fordelt på 4.7% HPV-hjemmetest og 17% cervixcytologiske prøver. Kvinder 60-65 år havde den største tilslutning til HPV-hjemmetesttilbuddet med 6.2% mod 9% cervixcytologiske prøver. Kun 2.4% af de 23-29-årige valgte HPV-hjemmetest, hvor imod 13.5% valgte cervixcytologisk prøve.

Konklusion
Der opnås en højere deltagelse ved 2. rykker tilbud med valgmulighed. I alt 18,4% blev screenet efter 2. rykker mod 15% i tidligere år (DKLS-årsrapport 2021). Tilslutningen til HPV-hjemmetest er dog markant mindre, end de 20% der tilvælger HPV-hjemmetest, når Region Hovedstaden tilbyder HPV hjemmetest til u-screenede kvinder. Vi vurderer, at et komplekst 2. geninvitationsbrev betyder at mange kvinder reelt ikke får forholdt sig til tilbuddet, og at der fremadrettet er basis for en optimering af kommunikationen med borgerne.

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Disseminated mycobacterium genavense infection in the bone marrow mimicking a histiocytic neoplasm.

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Introduction
Mycobacterium genavense is an opportunistic pathogen, described as a difficult to diagnose, slowly growing nontuberculous mycobacterium that does not grow on routine media. The organism is a rare cause of disseminated mycobacterial infection in patients with immune suppression due to HIV, transplantation, hematologic malignancy or other reasons.
Material and Methods
A 64-year-old HIV negative male patient presented to the hospital with a 2-4 week history of worsening abdominal pain, anorexia and weight loss, fatigue, progressive dyspnea, and intermittent fevers. The past medical history was significant for rheumatoid arthritis treated with Rituximab and daily low-dose prednisolone. Initial blood workup revealed pancytopenia and elevated CRP. A CT scan showed hepatosplenomegaly, a thickened cecum, mesenteric lymphadenopathy, and bilateral apical lung infiltrates. The patient underwent a bone marrow biopsy due to pancytopenia, and later splenectomy due to splenic rupture.
Results
Histological evaluation of the bone marrow biopsy revealed a diffuse array of nodules of foamy macrophages, with the histiological resemblance of a histiocytic neoplasm. Ziehl-Neelsen and Wade-Fite stains showed myriads of acid-fast positive cytoplasmic coccobacilli. The spleen was completely effaced by mycobacteria laden macrophages. PCR examination of the bone marrow revealed mycobacterium genavense.
Discussion and conclusion
This represents a rare case of diffuse mycobacterium genavense infection in a HIV negative rheumatoid arthritis patient. It is not known if the infection was secondary to immune dysregulation associated with rheumatoid arthritis, immune suppressant medications, other hitherto not considered factors or a combination of these. This case supports the use of PCR for diagnosing mycobacterium genavense.

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Optimering og validering af immunhistokemisk påvisning af NPM1-mutationer hos patienter med akut myeloid leukæmi

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Introduktion: Nucleophosmin genet (NPM1) koder for et multifunktionelt transportprotein, nucleophosmin (NPM) som helt overvejende er lokaliseret i cellekerner. NPM1 er det mest almindelige muterede gen hos voksne med akut myeloid leukæmi (AML). Mutationen findes hos ca. 30% af nydiagnosticerede tilfælde af AML, og er både en behandlingsafgørende mutation og prognostisk markør. Den mest kendetegnede forandring ved NPM1 mutationer er en afvigende cytoplasmatisk ekspression af NPM.
Formålet med denne undersøgelse var at optimere og validere en immunhistokemisk påvisning af NPM1 mutationer hos AML-patienter så målrettet behandling hurtigere kan iværksættes og prognostisk subklassificering hurtigere kan foretages.
Materialer og metoder: Vi evaluerede 41 formalin fikserede materialer af knoglemarv fra patienter med nydiagnostiseret AML immunhistokemisk. 21 materialer var med NPM1-mutation (NPMc+) og 20 materialer var AML uden NPM1 mutation (NPMc-). Mutationsstatus var på forhånd påvist med Next Generation sekventering (NGS). Immunhistokemi for NPM blev kombineret med Ki-67 for at lette evalueringen. 4 erfarne hæmatopatologer lavede en blind scoring af alle prøver som værende enten NPMc+ eller NPMc-.
Resultater: I alt 164 scoringer blev foretaget (41x4), med korrekt scoring i 160 tilfælde (97,6%).
Diskussion og konklusion: Det er muligt at lave en pålidelig detektion af NPM1 mutationer immunhistokemisk. Immunhistokemi kan således være et godt supplement til NGS især set i lyset af at metoden er hurtigere end NGS. Kombinationen af immunhistokemi for NPM1 og Ki-67 øger den diagnostiske sikkerhed betragteligt.

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Fetal indications for late terminations of pregnancies: A descriptive study from 2008-2021 from Central Denmark Region

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Introduction: During the last years, several tools have been added to the prenatal diagnostics, including comparative genomic hybridization (CGH-array), trio exome sequencing and improved ultrasound techniques. The aim of this study was to describe the distribution of fetal indications leading to late terminations of pregnancies in the Central Denmark Region and to evaluate potential changes over time.
Material and Methods: A descriptive study based on records from the council of pregnancy termination in the Central Denmark Region containing all cases from 2008-2021. A total of 1971 cases of late terminations of pregnancies were identified.
Results: From 2008 to 2021 the total number of late terminations of pregnancies was stable at the level of 135±18 (mean±SD) cases per year. A small but significant increase in the number of genetic aberrations was seen over the time period, and this was mainly due to an increase in the detection of copy number variations or mutations, whereas the number of chromosomal aberrations was stable. Malformations leading to terminations of pregnancies were most commonly seen in the central nervous system (22±6 cases per year), followed by cardiovascular (18±5 cases per year), and musculoskeletal (10±3 cases per year).
Discussion and conclusion: Despite improved diagnostics tools, the total number of late terminations of pregnancies from 2008 to 2021 was stable. There was a small increase in the number of cases with genetic aberrations. Since the total number was stable, this may be due to an increased use of genetic testing in fetuses with detected malformations.

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Inter-rater reliability of PD-L1 scoring in biopsies from esophageal squamous cell carcinomas and adenocarcinomas in routine clinical pathology practice

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Introduction
PD-L1 testing is recommended in esophageal cancer and is a predictive biomarker in immunotherapy targeting the PD-L1-axis. Excellent inter- and intra-rater reproducibility has previously been published, but most often published data is performed on whole tumor-slides or TMA and do not reflect real-life use of PD-L1 testing on biopsy material. Our aim is to determine the inter-rater reliability of the Combined Positive Score (CPS) in biopsy material from esophageal cancer.

Material and methods
Eight gastro-pathologist consultants evaluated the CPS in ten esophageal biopsies - five squamous cell carcinomas and five adenocarcinomas. The inter-rater reliability was assessed by calculating the intraclass correlation coefficient (ICC) for continuous data and Krippendorff's Alpha for categorical data at clinically relevant CPS cutoffs of 1, 5 and 10.

Results
The ICC was 0.977 (95%CI 0.945-0.993) indicating "excellent reliability". At CPS ≥ 1, there was almost perfect agreement, with only two cases of disagreement, and the Krippendorff's Alpha couldn't be calculated. At CPS ≥ 5 and ≥ 10, the Krippendorff's Alpha was 0.002 (95%CI -0.478-0.480) and 0.127 (95%CI -0.134-0.426) both indicating lack of reliability.

Discussion and conclusion
The ICC was good across the full range of CPS levels, but this mainly indicates reliability in discriminating high from low PD-L1 expressers. The inter-rater reliability of the CPS was poor at clinically relevant CPS cutoffs of 5 and 10, although excellent agreement was observed at CPS cutoff of 1. The findings indicate that interpretation of CPS must be taken with caution when evaluating tumors with low PD-L1 expression.

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Frequency of positive superficial margins and subsequent re-resections in patients treated with mastectomy in a single institution from 2018-2022

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Skin-sparing (SSM) and nipple-sparing mastectomies (NSM) represent alternatives to simple mastectomy (SM) for premalignant/malignant disease and enable immediate breast reconstruction and improved aesthetic outcome. Regardless of mastectomy-type, the superficial margin is not considered a true resection margin, but an anatomical border, and indications for re-resection in case of positive margin is much debated.
Through a retrospective review of all mastectomies received at Department of Pathology, Aarhus University Hospital from 2018-2022, we aimed to evaluate superficial margin-status and re-resection rate according to mastectomy-type.
Using systemized SNOMED-search, 935 mastectomies were identified; encompassing 707 SM, 145 SSM and 77 NSM. Carcinoma was the most frequent indication for SM (82%), whereas SSM/NSM was more frequently used for ductal carcinoma in situ (DCIS) (25-41%). In 45/935 patients (4%), DCIS or carcinoma was present at the superficial margin ("on ink"), and in 56/935 (6%) the distance was < 2 mm (not “on ink”). There was a slightly higher positive margin-status for SSM (13-14%) compared to SM and NSM. Fifteen of 25 patients (60%) had a re-resection, if carcinoma was "on ink" (SM: 12/20, SSM: 3/4, NSM: 0/1), and 10 of 20 patients (50%) if DCIS was “on ink” (SM: 2/3, SSM: 6/13, NSM: 2/4). In subsequent re-resection specimens, residual disease was found in 33% and 50% for carcinoma and DCIS, respectively.
In conclusion, the rate of positive superficial margins was low. The high rate of residual disease in subsequent re-resections, however, calls for continuous attention of the superficial margin-status due to risk of local recurrence.

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Transition of Invasive Ductal Carcinoma to Metaplastic Squamous Carcinoma of the Breast During Recurrence: Case Report

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Introduction: Metaplastic carcinoma of the breast is a heterogenous group of rare neoplasms with prevalence of less than 1%. Pure squamous cell carcinoma (PSCC) represents around 0.1% of breast carcinomas. Literature related to its clinical presentation is sparse.
Material and Methods: Case: 81-year old woman was referred with a palpable mass in the lower lateral quadrant of the right breast. Biopsy material revealed invasive ductal carcinoma (IDC) with 100% estrogen receptor (ER) positivity and HER2 receptor borderline expression (score 2+) with HER2 gene amplification by FISH. The patient received endocrine therapy which was discontinued after 6 months because of side effects. Therefore, right breast mass resection was performed. Microscopic examination of the mass revealed IDC, ER positive and HER2 receptor overexpression. Further treatment was opted out due to comorbidities. 2 years after the resection the patient returns with a lump in the scar with retraction and ulceration of the skin. No lymph node metastasis was found in fine needle aspiration. Simple mastectomy was performed.
Results: Histopathological examination revealed that the recurrent tumor was PSCC with a diameter of 48 mm. ER positivity was reduced to 80%, whereas HER2 overexpression was maintained compared to the analysis of the breast mass. No other primary or metastatic disease was found.
Discussion and Conclusion: This case report shows that transition from IDC to PSCC in recurrent cancer after endocrine therapy is possible. Previously reported cases appeared with pathological transition after chemotherapy/radiotherapy. Further investigation is needed to clarify this transition to metaplastic cancer subtypes.

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Mesenkymal dysplasi i placenta kan klinisk imitere mola

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Kasuistik: 30-årig kvinde, G3, P0, fik ved førstetrimesterskanning påvist et foster med omfalocele indeholdende lever og tarm. I forbindelse med placentabiopsi (CVS) bemærkedes stor, 'mølædt' og molalignende placenta. Array-CGH var normal, og der påvistes ikke triploidi. Ved tværfaglig konference rejstes mistanke om mesenkymal dysplasi (MD) i placenta, som sammen med omfalocelet gav mistanke om Beckwith-Wiedemanns syndrom (BWS). Parret valgte provokeret abort i uge 16+2.
Ved obduktion fandtes et hunligt foster med omfalocele og cyto- og nukleomegali i binyrecortex. Placentas vægt var 2 gange forventet gennemsnitsvægt, og der sås makroskopisk blærer. Mikroskopisk fandtes store, forgrenede og ødematøse stamvilli og normale perifere villi. Immunhistokemisk fandtes p57 bevaret i den villøse trofoblast og tabt i villusstromakernerne. Disse fund bekræfter mesenkymal dysplasi. De samlede forandringer tyder på BWS.
BWS skyldes genetiske og epigenetiske forandringer i den imprintede region på kromosom 11p.15.5. Det overvejende maternelt udtrykte gen CDKN1C findes i denne region. På oprenset DNA blev påvist en variant i CDKN1C i heterozygot form. Varianten er ikke tidligere rapporteret. Den prædikteres at medføre et præmaturt stopcodon og klassificeres som sandsynlig patogen. Varianten bekræfter med stor sandsynlighed diagnosen molekylærgenetisk, og der afventes forældreanalyser.
MD er i ca. 20% af tilfældene associeret med BWS. Ved MD er der øget risiko for negativt graviditetsudfald i form af vaksthæmning, intrauterin død og præterm fødsel, og kun 9% af graviditeterne ender med et rask barn.
Ved klinisk mistanke om partiel mola bør MD i placenta overvejes som differentialdiagnose, idet behandling/intervention og kontroller præ- og postpartum er forskellige.

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Flowcytometrisk analyse af monocytpopulationen i perifert blod til diagnosticering af kronisk myelomonocytær leukæmi

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Introduktion
Monocytose kan ses på baggrund af en række tilstande og sygdomme herunder ved kronisk myelomonocytær leukæmi (CMML). En ny flowcytometrisk screeningsmetode har vist, at en relativ andel af klassiske CD14+/CD16- monocytter (MO1) på ≥94% af alle monocytter i perifert blod kan skelne CMML fra andre årsager til monocytose. Knoglemarvsundersøgelse vil dermed kunne undlades på den større gruppe af patienter med reaktiv monocytose, som typisk har en MO1 på <94%. Vi har lavet en retrospektiv opgørelse af den nyligt implementerede undersøgelse på vores afdeling for at se, om denne lever op til de forventede standarder.
Materialer og metode
Vi inkluderede 26 patienter, der har fået lavet subtypering af monocytter med flowcytometrisk analyse af perifert blod, og som enten er kendt med CMML eller er udredt for persisterende monocytose i perioden 23.09.2020-17.06.2022. Som kontrolgruppe inkluderede vi 10 raske bloddonorer. Vi sammenholdte patienternes MO1-værdi (≥94% eller <94%) med diagnosen ved knoglemarvsundersøgelsen (CMML eller anden diagnose).
Resultater
Vores opgørelse viste, at 15 ud af 16 patienter med CMML havde en MO1 på ≥94% og at 8 ud af 10 patienter uden CMML havde en MO1 på <94%. I kontrolgruppen havde 10 ud af 10 bloddonorer en M01 på <94%. Screeningsmetodens sensitivitet er dermed 93,8% mens specificiteten er 80,0%. Den positive prædiktive værdi er 88,2% og den negative prædiktive værdi er 88,9%.
Diskussion og konklusion
Den flowcytometriske analyse af MO1 på vores afdeling har en tilfredsstillende standard som et klinisk screeningsværktøj til at skelne patienter med CMML fra patienter med anden årsag til monocytose.

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Simpel analyse til bestemmelse af MLH1 promotor methylering ved kolorektalcancer

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Introduktion
MLH1 promotor methyleringsanalyse benyttes til vurdering af arvelig komponent ved kolorektalcancer diagnostik. Tumorer med manglende MMR-protein ekspression og negative for MLH1 promotor methylering er altovervejende arvelige. Hidtil er MLH1 methyleringsanalyse primært foretaget ved probebaseret fragmentanalyse (SALSA MS-MLPA, MRC Holland) som er resourcekrævende. Her valideres en real-time smeltepunkts MLH1 methylerings PCR-analyse som alternativ (Epimelt MLH1, MethylDetect, Århus).
Materialer og Metoder
109 kolorektalcancer vævsprøver blev analyseret med MLPA (Rigshospitalet) og Epimelt (Hvidovre). Yderligere 14 prøver med normal MMR IHC ekspression blev testet med EpiMelt. MLPA kræver 50-250ng DNA med min. 20% tumorkerner. Epimelt kræver min. 10% tumorkerner og 10-100ng bisulfitkonverteret DNA. Epimelt detekterer methylering i ét site i MLH1, mens MLPA undersøger 4 sites i MLH1 genet samt identificerer methylering i 7 gener (MGMT, MLH1, MLH3, PMS2, MSH2, MSH3, MSH6).
Resultater
Epimelt identificerede 91 prøver som MLH1 methyleret, heraf fandt MLPA 80 (88% konkordans). Overordnet konkordans mellem analyserne var 89%, med en kappaværdi på 0.68. Intern reproducerbarhed for Epimelt var 94% (N=18). Af 12 prøver med normal MMR-status fandt EpiMelt 100% ikke methyleret. To prøver var analytisk inkonklusive.
Diskussion og konklusion
Epimelt MLH1 analysen var reproducerbar, med høj konkordans til MLPA-analysen. Epimelt giver mulighed for et hurtigere laboratorie workflow på generiske realtime PCR-instrumenter, kræver ikke special apparatur, og er dermed mindre ressourcekrævende end MLPA.
Epimelt MLH1 analysen er et alternativ til MLPA-analyse, og giver mulighed for hurtigt og præcis bestemmelse af MLH1 methyleringsstatus sammenholdt med MLPA ved kolorectalcancer. Inklusion pågår stadig og vi forventer at præsentere yderligere resultater og subgruppe analyser.

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ANALYTICAL AND DIAGNOSTIC PERFORMANCE OF HPV SELF-SAMPLES COMPARED TO CLINICIAN-COLLECTED SCREENING SAMPLES IN THE CAPITAL REGION OF DENMARK

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HPV self-sampling is gaining acceptance as an equal to clinician-collected cervical screening samples, but whereas HPV testing on clinician-collected samples has internationally recognized criterions for validated use no such consensus is established for HPV self-collected samples. Here we provide quality assurance on HPV self-sample analysis as conducted in the Capital Region of Denmark.
PCR Ct scores for HBB (internal control) and HPV genotype readouts from 20,567 self-collected samples (Evalyn brush, 3 ml CBD media) and 39,701 clinician-collected samples (Combi-brush, 10 ml SurePath) were analyzed. All samples had been routinely analyzed using the BD Onclarity HPV assay in the screening program. Concordance between HPV positive self-collected samples and subsequent clinician collected follow-up (FU) samples were analyzed for 1380 self-sampling HPV positive women.
HPV prevalence was 15.1% and 8.5% for self-collected and clinician-collected samples, respectively. On self-samples, the average HBB Ct score was 21.2 ± 1.8 compared to an HBB Ct score of 24.4 ± 1.5 for clinician-collected samples. Comparing HPV positive self-samples to the recommended clinician-collected FU sample, 60% (N=829) remained HPV positive, whereas 40% (N=551) were HPV negative. The index CtHPV score for consistently HPV positive at FU was 25.9 ± 4.7 versus 30.5 ± 3.5 for those FU negative.
Jointly, these observations indicate that preanalytical factors such as resuspension medium, volume, and brush type impacts the analytical test outcome on self-collected samples, and that protocols for analysis of self-samples should undergo the same rigorous validation from the scientific community and manufacturers as has been performed for the clinician-collected samples.

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Ileocecal intussusception årsaget af et tubulovilløst adenom i tyndtarmen.

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Introduktion
Ileocecal invagination (intussusception) er en klinisk tilstand, hvor tyndtarmen invaginerer i caecum. Tilstanden forekommer oftest hos børn, og ses meget sjældent hos de voksne patienter. Intussusceptioner hos voksne behandles med et kirurgisk indgreb i form af tarm resektion, da hovedparten af alle intussusceptioner skyldes læsioner i tarmvæggen (karcinomer og benigne tumorer). Læsionerne kan forekomme i hele tarmens udstrækning, men er hyppigst lokaliseret i tyktarmen med nogle enkelte tilfælde observeret i tyndtarmen. Tumorer er karakteriseret af høj grad af malignitet og diagnosticeres oftest i højere stadier.
Metode
Det er en klinisk case af ileocecal intussusception hos en 67-årig kvinde. Patienten ankom akut med 12 timer af intermitterende mavesmerter og havde ingen tidligere medicinsk historik af relevans. Der blev foretaget billeddiagnostik vurdering efterfulgt af kirurgisk indgreb og slutteligt foretaget histopatologisk undersøgelse.
Resultater
Ved billeddiagnostisk undersøgelse konstateredes ileocecal intussusception. Ved efterfølgende kirurgisk behandling blev der fjernet en neoplastisk proces i ileum. I den slutlige histopatologiske undersøgelse diagnosticeres denne neoplastiske proces som et tubulovilløst adenom i terminale ileum.
Konklusion
I denne case studium præsenterer vi et sjældent tilfælde af tubulovilløst adenom som årsagen til ileocecal invaginering af tyndtarmen op i caecum.

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Kollagen gastrit hos patient med persisterende dyspepsi

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Kollagen gastrit er en histopatologisk tilstand defineret som subepitelial aflejring af kollagen, med en minimumtykkelse på 10 mikrometer, ofte ledsaget af inflammation i ventrikelslimhinden og epitelskade. Der er i litteraturen beskrevet to typer af kollagen gastrit: en pædiatrisk og en voksentype. Den pædiatriske type ses typisk som en isoleret tilstand, imens voksentypen oftere er associeret med kollagen kolit og autoimmune sygdomme. Den kliniske præsentation varierer afhængig af type, men er oftest relateret til GI-kanalen.
Aktuelle case omhandler en 36-årig kvinde med globulusfornemmelse og vedvarende dyspepsi gennem 6 måneder, trods behandling med protonpumpeinhibitorer og antacida. Der blev foretaget en gastroskopi som viste slimhindeforandringer ved den gastroøsofagale overgang (GEJ), suspekte for Barrett's øsofagus. Biopsier fra cardia viste kollagen gastrit med et 40 mikometer tykt subepitelialt kollagent bånd. Gastroskopien blev gentaget 1,5 måned senere, hvor symptomerne var uændrede, med fund af makroskopisk upåfaldende slimhinde. Randombiopsier fra ventrikel og GEJ viste igen kollagen gastrit, om end mindre udtalt end ved første biopsitilfælde.
Kollagen gastrit er en sjælden tilstand med færre end 100 publicerede cases. Ætiologien og patogenesen er ukendt, og der er ikke konsensus omkring standardbehandling. Udover de to kendte typer; pædiatrisk og voksentype, er der beskrevet få tilfælde af kollagen gastrit som tilfældigt fund hos asymptomatiske patienter. Aktuelle case falder ikke indenfor nogle af disse kategorier. Man kan overveje hvorvidt kollagen gastrit er en manifestation af persisterende dyspepsi, som man derfor skal være opmærksom på.

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Automated annotation of virtual dual stains to generate high-performing convolutional neural network for detecting cancer metastases in H&E-stained lymph nodes

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The technology of visual recognition is improving rapidly, and with a commencing digitalization of pathology, it shall soon help pathologists in making faster and more precise diagnoses. Various studies have created algorithms that identify tumor cells; however, these algorithms are often trained by manually annotating whole slide images (WSI), a labor-intensive and cumbersome task. This proof-of-concept study aims to train a convolutional neural network (CNN) that detects lymph node metastases (LNM), solely using immunohistochemical WSIs as the ground-truth-mask. From 40 colorectal cancer (CRC) patients diagnosed with LNM, a hematoxylin-and-eosin (H&E) slide with and one without metastasis were scanned. The same slides were subsequently stained with pan-cytokeratin and rescanned. The two WSIs were aligned digitally as a virtual double stain. Pan-cytokeratin-positive cells and the normal tissue was both labeled using color thresholding. Based on these automated annotations, a CNN was trained. The CNN was tested on 388 H&E WSIs of lymph nodes from 20 different patients with CRC. Our CNN makes pixel-level predictions on each WSI, labeling tissue as malignant or benign. Based on the malignant area of each WSI, our CNN achieves an area under the curve of 0,9917 and a sensitivity of 100% and a specificity of 94%, when classifying slides as benign or malignant. The study shows that this technique has promising potential. The performance of a CNN is largely dependent on the size of the training data, and with this method it seems manageable to create large, annotated data sets with high quality within a reasonable timeframe.

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Case Report of IgM Multiple Myeloma, a Rare Hematologic Entity

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Introduction: Multiple myeloma (MM) is a neoplastic proliferative disorder of plasma cells resulting in production of monoclonal immunoglobulin, called M protein. Thus the plasma cells produce only one type of light chain, often with an associated heavy chain (IgG or IgA) or no heavy chain. IgM as heavy chain is extremely rare and comprising less than 0,5% of all MM cases.
Material and methods: A 74-year-old man presented with fatigue, weight loss and backpain. Laboratory results showed a high M protein at 40 g/l (IgM/lambda). Furthermore, he had anemia but had no enlargement of lymph nodes or spleen. He had a bone marrow examination done.
Results: The bone marrow showed 60-70% immature plasma cells and only few lymphocytes. There were large aggregates of plasma cells but no lymphocytic infiltration. Flow cytometry showed 1% polyclonal B-lymphocytes and 10% monoclonal plasma cells. The plasma cells were marked in CD138, MUM1, CD38, partial CD20, IgM and lambda. They were negative in CD45, CD19, PAX5, cyclinD1, CD56 and CD117. There was no mutation in MYD88.
Discussion and conclusion: An IgM M protein is most often associated with lymphoplasmacytic lymphoma (LPL) / Mb. Waldenström (WM) and IgM MGUS, which are indolent diseases, while MM is a very aggressive disease, hence they need very different treatment. The majority of LPL / WM have MYD88 mutation. This case illustrates a rare entity, where the use of new molecular diagnostics can help in the differential diagnosis.

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PRAME and HMB-45 panel for distinguishing primary melanocytic tumors

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Introduction: The utility of a single immunohistochemistry for PRAME (PReferentially expressed Antigen in MElanoma) can vary, with published literature showing sensitivity between 67% and 83%. Previously, one technical study explored the use of double staining for HMB45 and PRAME on a small subset of melanocytic tumors suggesting a potential diagnostic benefit. The aim of this study was to assess the value of a dual panel of PRAME and HMB45 in primary melanocytic tumors.
Methods and materials: A total of 259 tumors from a two-year period span were retrieved from the department's archives. All tumors were reviewed by two experienced dermatopathologists and classified as one of the following: benign nevi, dysplastic nevi, and malignant melanoma. Only the cases with agreement on the classification were included. Specimens included 141 nevi, 32 dysplastic nevi, and 86 malignant melanomas. New sections were stained with PRAME and HMB45, respectively. For PRAME, a nuclear, and for HMB45, a cytoplasmic staining, was considered positive and scored as described in the literature on a scale from 0 to 4+.
Results: We report superior sensitivity and specificity for diffuse 4+ PRAME positivity, 73% and 96% respectively, when compared to HMB45, 57% and 86% respectively. No nevi showed double 4+ positivity, however, sensitivity for the double positivity was only 50%.
Discussion and conclusion: Our results confirm the superiority of PRAME over HMB45 in differential diagnosis of melanocytic tumors. However, a combined staining can significantly increase specificity rendering a benign diagnosis more unlikely in a setting of double 4+ diffuse positivity.

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En moderne stadium II colon cancer kohorte

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Den kirurgiske og onkologiske behandling af colon cancer (CC) har udviklet sig og i 2014 blev screeningsprogrammet indført. Patienter med stadium II CC behandles primært med operation samt adjuverende kemoterapi til udvalgte patienter med høj recidivrisiko. Der foregår derfor megen populationsbaseret forskning i biomarkører, der kan forudsige, hvilke stadium II patienter der har høj risiko for tilbagefald. Med indførslen af screeningsprogrammet diagnosticeres flere patienter tidligere, og deres overlevelse fra diagnosetidspunktet er længere. Herudover har standardiseret og grundig lymfeknudesampling samt moderne radiologiske modaliteter gjort en stadium II CC kohorte meget "ren". Derfor er der behov for en tidssvarende stadium II CC kohorte repræsentativ for den nuværende patientpopulation til vurdering af prognostiske biomarkører.
Patienter operativt behandlet for stadium II CC i Region Syddanmark fra 2014-2016 identificeres i Danish Colorectal Cancer Groups (DCCG) database og i patologisystemet. Inklusionskriterierne omfatter patienter med ikke-arvelig, histologisk verificeret adenocarcinom stadium II, der ikke har modtaget neoadjuverende behandling, haft maligne lidelser indenfor 10 år fra diagnosetidspunktet eller synkrone tumorer på diagnosetidspunktet.
En forskningsbiobank med vævssnit fra den invasive front og tilhørende forskningsdatabase med kliniske data herunder overlevelsesdata og mikroskopidata etableres.
Dataudtræk fra DCCG og patologisystemet identificerer 739 patienter. Inklusionskriterierne er opfyldt for 499 patienter og de udgåede patienter fordeler sig med 16 ikke-radikalt opererede, 30 non-stadium II, 6 med utilstrækkeligt vævsmateriale og 188 patienter med eksklusionsberettigede kliniske data. Men kun 43 påviste recidiver afskiller kohorten sig væsentligt fra tidligere og vi konkluderer, at det derfor er særdeles relevant at teste prognostiske biomarkører i moderne, screenede kohorter.

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NGS-analyse til bestemmelse af ERBB2 og ESR1 mRNA-ekspression og molekylær subtypebestemmelse af brystkræft

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Introduktion: NGS transkriptom-analyse til bestemmelse af mRNA niveauer af ERBB2 (HER2), ESR1 (ER) og PGR (PGR) blev implementeret som supplement til den immunhistokemiske (IHC) bestemmelse af proteinekspression. Assayet anvendes også til bestemmelse af molekylære subtyper (HER2-enriched, Luminal A, Luminal B eller Basal-like) som ellers bestemmes med Prosigna-assay (Nanostring teknologi). Her præsenteres sammenhængen mellem mRNA niveauer, IHC og molekylære subtyper for rutineanalyser udført i perioden juni 2021 til december 2022.
Materiale og metode: I alt indgår 1.207 konsekutive analyser i kvalitetsopgørelsen. NGS blev udført med Ion Torrent NGS transcriptionsarray; et amplikonbaseret sekvensassay for > 20.000 transkripter. Til molekylær subtypebestemmelse anvendes en publiceret algoritme (AWCA), der ud fra ekspression af 50 gener (PAM50) bestemmer tumorens molekylære subtype ved korrelationsberegning. Sammenligningen mellem molekylære subtyper inkluderer data fra yderligere 149 prøver fra analysevalideringen.
Resultater: Overordnet sås overensstemmelse mellem IHC-klassifikation og mRNA niveau for HER2 i 1149/1207 (95,2%) af prøverne og for ER i 1182/1207 (97,9%). De fleste uoverensstemmelser kunne forklares med lav IHC positiv fraktion og/eller tumorheterogeneitet. HER2 2+/FISH-negative og HER2 2+/FISH-positive prøver kunne ikke adskilles på basis af ERBB2 mRNA-ekspression. Der fandtes overensstemmelse mellem den molekylære subtypebestemmelse med AWCA og Prosigna i 87,7% af prøverne (n=236). Størst forskel fandtes for AWCA Luminal A, men graden af overensstemmelse for disse kan øges til 97,6% ved anvendelse af et QC-kriterie på forskellen mellem korrelationsberegningen for Luminal A og Luminal B.
Diskussion og konklusion: Med transkriptom-analyse og AWCA-algoritmen dannes grundlag for rapportering af mRNA-niveauer og molekylære subtyper. Optimeret AWCA-algoritme kan potentielt erstatte Prosigna-analyse på visse rutineprøver.

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BRCA2 deletioner i metastatisk prostatakræft

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Introduktion
I december 2021 blev Olaparib (PARP-inhibitor) godkendt som behandling til patienter med metastaserende kastrationsresistent prostatacancer (mCRPC) med BRCA-mutationer. På Sygehus Lillebælt bliver patienterne henvist til BRCA-analyse når andre behandlingsmuligheder er udtømte og der ses progression. Størstedelen af patienterne har udelukkende knoglemetastaser og der bliver derfor udført analyse på biopsier fra primær diagnose.

Materialer og metoder
I perioden 15/5-2022 til 31/12-2022 er 27 patienter med mCRPC er blevet undersøgt for varianter i bl.a. BRCA1 og BRCA2 med NGS-panelet TSO500 (Illumina) med henblik på mulighed for behandling med PARP-inhibitorer. Panelet dækker alle exons i BRCA1 og BRCA2, samt giver en indikation for amplifikationer/deletioner. Der er primært anvendt diagnostiske biopsier (taget i 2006-2022), men enkelte patienter har haft tilgængeligt materiale fra metastaser. BRCA2 deletioner er bekræftet med en valideret Multiplex ligation-dependent probe amplication analyse.

Resultater
Af de 27 patienter har det været muligt at lave komplet analyse for 24, for de resterende 3 har materialet været af for dårlig kvalitet. Der er ikke fundet patogene single nucleotide variants eller mindre indels i BRCA1 og BRCA2, men der er påvist BRCA2 deletion i 3 patienter (0-1 kopier), svarende til 11% af patienterne. I skrivende stund er første patient (testet i juli) i behandling med PARP-inhibitor og har god effekt af behandlingen, de to andre har fået svar på analysen i december 2022.

Diskussion og konklusion
Der er påvist BRCA2 deletion i 11% af de testede patienter, hvilket viser behovet for at sikre at BRCA deletioner er inkluderet i testen for denne patientgruppe.

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Digital whole slide imaging for histological determination of Helicobacter pylori infection is non-inferior to light microscopy

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Digital whole slide imaging (WSI) as a diagnostic tool has potential advantages for improving pathology service a.o. the application of digital diagnostic tools to support the pathology evaluation. Before WSI can be considered an alternative to microscopic evaluation, basic non-inferiority studies to light microscopy (LM) must be conducted. Here we compare the diagnostic accuracy and inter-observer variability by two observers of WSI compared to LM.

In total, 120 archival ventricle biopsies with suspected helicobacter pylori were identified and included. Of these 36 had an original H. pylori diagnosis registered. Two expert GI pathologists each examined the 120 H&E and immunohistochemically stained slides using 1) light microscope, and after at least 14 days, they re-examined the samples digitally as 2) WSI. The diagnosis of H. pylori was recorded, and concordance calculated. Inter-observer concordance of LH and WSI was calculated.

For both observers, WSI had a sensitivity of 94% to LH. For observer 1 WSI had a specificity of 98% whereas observer 2 had a specificity of 95% to LM. Overall agreement and kappa were above 95% and >0.88 respectively for both observers. Inter-observer concordance was 96% for WSI and 98% for LM.

We conclude that WSI is equivalent to LM for detecting Helicobacter pylori in biopsies. Moreover, differences between LM and WSI was mainly driven by inter-observer variability, not choice of technology. In perspective, adaptation to WSI require time before it becomes as natural as LM, and thus the inter observer concordance could be speculated to increase as routine is acquired.

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Assessment and development of local HRD testing in ovarian cancer

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Introduction
The discovery that PARP inhibitors (PARPi) are efficient in patients harboring.homologous recombination deficiency (HRD), has opened up a new therapeutic avenue for high-grade ovarian cancers (HGSC). Approximately 50% of HGSC cases are HRD-positive Testing for HRD prior to treatment has until now been done abroad at Myriad/MyChoice. Apart from the high cost and time, this is not transparent and compromise local autonomy and patient data sharing. Here, we assessed and compared HRD results from different methods together with results obtained from Myriad in order to establish a local HRD test.

Material and Methods
A total of 58 samples from HGSC patients, with estimated tumor content of ≥30% were included. The material was subjected to genome-wide single nucleotide polymorphism (SNP) array, covering over 900 cancer-associated genes. For HRD calculation, we employed publicly available algorithms, and compared with Myriad results.

Results
Our data showed high correlation between results from SNP-array and Myriad technologies (R2= 0.91, p<2.2-16), where the former presented an overall higher HRD score than the latter. Six samples (10.3%) were inconclusive because the algorithm could not estimate the aberrant cell fraction in those cases.

Discussion and Conclusion
Our current findings showed similar results between the 2 methods, indicating the feasibility of implementation of a local HRD testing. .Thus, we are currently assessing more samples in order to benchmark the local HRD against Myriad, and properly adjust the threshold.

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A rare case of malignant adenomyoepithelioma of the breast

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Introduction
Adenomyoepithelioma (AME) of the breast is a rare biphasic tumour with an epithelial and a myoepithelial component. Both AME and its malignant counterpart primarily affect elderly women and malignant transformation can arise from either component or both. To date around 200 cases of AME have been reported mostly casuistically and of these only few malignant. Here, we report a case of malignant AME (MAME) with a metaplastic carcinoma arising from an AME.

Materials and methods
A 74-year-old woman presented with a tumour in her right breast. A core needle biopsy was obtained and the patient underwent neoadjuvant chemotherapy and subsequent lumpectomy.

Results
The core needle biopsy revealed metaplastic carcinoma, most likely spindle cell carcinoma. Microscopy of the lumpectomy showed a radically resected 35 mm residual biphasic tumour with a peripheral invasive component and a centrally located non-invasive component. The non-invasive component comprised of tubular formations of monomorphic cells with preserved myoepithelial cell layer, whereas the invasive component presented as small solid nests with squamoid diffentiation, cords and tubular formations. Immunohistochemically, both components were estrogen receptor negative and without HER2 overexpression. The infiltrative part showed p63 positivity and a Ki67 index of 20 %. MAME was suspected and external consultation confirmed the diagnosis of metaplastic carcinoma developed within an AME.

Discussion and conclusion
AME and MAME are rare entities most often treated with wide surgical excision. In this case, the diagnosis was established only after total excision. Awareness of the existence of AME and MAME is important for optimizing treatment.

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Kaposi´s sarcoma in a liver graft: a case report

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We present the clinical history and histology of Kaposi´s sarcoma developed in a liver graft.
A 50-year-old man with non-alcoholic steatohepatitis received an orthotopic liver transplant and was 5 months later admitted with abdominal pain, diarrhea, headache, and fatigue. Initial diagnostic tests, including ultrasound and CT, showed multiple hypoechoic lesions in the liver but no extrahepatic lesions. The differential diagnosis included both graft versus host rejection, metastasis, and infection. Histology showed a vascular tumor. Immunohistochemical staining revealed the presence of Human Herpes Virus 8 (HHV8) compatible with Kaposi´s sarcoma (KS). HHV8 can be transmitted with the graft or be latent in the lymphocytes of the patient and reactivated after immunosuppression. DNA analysis of the tumor tissue did not match with the DNA of the donor, however analysis of blood from the recipient of the lungs from the same donor showed HHV8. We therefore concluded that the HHV-8 infection in this case was transmitted from the donor.
The fundamental treatment of post-transplantation KS is reducing the immunosuppressive therapy. Tacrolimus was replaced with everolimus and mycophenolate mofetil and prednisone were discontinued. A PET scan 2 months later showed complete metabolic remission of the tumors, but a biopsy showed active KS and acute cellular rejection BANFF 2-3. Eventually a combination of tacrolimus and everolimus was found balancing the immunosuppression that prevented further rejection of the graft while still effectively treating KS without the need for chemotherapy.

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Profiling and prognostic role of circular RNAs in pancreatic cancer

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Introduction: The median survival of pancreatic cancer (PC) is only 8 months. New diagnostic, prognostic and predictive biomarkers are needed. Circular RNAs (circRNAs) have gained interest in different types of cancer, but only few studies have evaluated their role in PC. The putative oncogene ciRS-7 is of particular interest but has not been studied in PC. We aimed to identify the most differentially expressed circRNAs in PC compared to controls and to explore their prognostic role and spatial expression.
Material and Methods: We included 108 surgical PC specimens. Using RNA sequencing (RNA-seq) on frozen specimens from a subset of the tumors and controls, we detected 20.440 circRNAs, 137 of which were identified as most differentially expressed. A custom code set of capture and reporter probes was designed to target 152 circRNAs, 15 of which were additionally identified in the literature. CircRNA expression was analyzed in all tumors using NanoString nCounter platform. Chromogenic in situ hybridisation (CISH), tissue microarrays and digital imaging analysis (Visiopharm) were used to examine spatial ciRS-7 expression.
Results: Based on their circRNA profiles, we identified different PC subclusters. The 15 most differentially expressed circRNAs showed fold changes from 8.0 to –169.6, and 6 of 15 held significant prognostic value in univariate analyses (HRs 0.59-1.81 (SEs 0.05-0.3)). CiRS-7 was absent in PC cells but highly expressed in tumor microenvironment.
Discussion and Conclusion: In this explorative study, we identified six new circRNAs with prognostic value in PC. ciRS-7 may play an oncogenic role in PC, particularly in stroma-rich tumors.

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Paraganglioma. Two cases.

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Introduction: Paragangliomas are rare neuroendocrine neoplasms of the extra-adrenal ganglia. Sympathetic paragangliomas are usually catecholamine- secreting and occur along the sympathetic chain from the base of the skull to the prostate. There is no accurate data about the specific prevalence of paragangliomas. The combined prevalence of pheochromocytomas and paragangliomas is 64.4 per million inhabitants in Denmark. Paragangliomas are more common in females, occur at a mean age of 43.3 years and can be part of genetic syndromes. Malignancy defines by metastatic disease, which can appear with long latency.
Material and methods: Here we present two cases of paragangliomas. Our laboratory received needle biopsies from a tumor of the soft tissue of the abdomen and resection from a tumor of the bladder. Both samples were proceeded according to current standards.
Results: Histopathologic findings showed nests of tumor cells separated by thin stroma. The cells had rounded nuclei and abundant lightly granulated and eosinophilic cytoplasm. Immunohistochemical staining showed strong positive reactions for vimentin, GATA3, chromogranin, synaptophysin, CD56 and diffuse positive reaction for S100.
Discussion and conclusion: Paragangliomas are very rare, but potentially life threatening. It is important to recognize paragangliomas histologically and immunohistochemically as they cannot be differentiated from other abdominal tumors clinically. Histologically it can be difficult to differentiate from especially bladder cancer due to positive reaction for GATA3 emphasizing the importance of awareness of this rather rare entity. The only cure for paraganglioma is surgical resection. Currently, there are no standardized histologic criteria for differentiating malignant and benign paragangliomas.

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Clinical and molecular characterization of extracranial metastases in glioblastomas

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Glioblastoma (GBM) is the most malignant primary brain tumor in adults, but in contrast to many other malignancies, they rarely metastasize. Since metastasizing GBMs cause both diagnostic and therapeutic challenges, our aim was to characterize these tumors clinically and molecularly. We therefore collected and examined the largest cohort to date of 16 glioma patients (14 GBMs and 2 lower-grade gliomas) with extracranial metastases (ECMs), including 10 distant metastases (DMs) to lymph node(s), bone, liver, neck, and scalp, and 6 extracranial extensions (EEs). Clinically (gender, age, treatment, and survival), the GBM patients were comparable with other GBM patients, while MRI assessment revealed proximity of the brain tumors to dura, large vessels, and ventricles in a high proportion of the patients. Paired samples from primary tumors, recurrences and metastases were analyzed by genome-wide methylation profiling and copy number analysis, overall confirming the histological diagnoses and revealing a patient-specific molecular pattern, although differentially methylated regions in EEs and DMs vs. primary tumors, respectively, were identified. A higher methylation-based stem-cell division rate in DMs vs. primary tumors was revealed, while cell-type deconvolution showed no major changes in immune cell composition. Immunohistochemistry followed by AI-based quantification of immune- and stem cell markers did not reveal different levels in ECMs vs. primary tumors. In conclusion, patients with metastasizing GBMs are clinically comparable to other GBM patients. The molecular analyses overall confirmed the histological diagnoses and showed a patient-specific pattern over time. Surprisingly, immune cell proportions appeared to be similar in primary tumors and ECMs.

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Syfilis: Et casestudie af en fortsat aktuel differentialdiagnose

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Introduktion

Syfilis er en venerologisk sygdom, som man i Danmark 1990´erne stort set betragtede som udryddet. Op igennem 00´erne, og i særdeleshed igennem de seneste år er incidensen dog igen steget betydeligt fra 365 tilfælde i 2019 til 634 tilfælde i 2021. Langt størstedelen af tilfældene ses blandt mænd der har sex med mænd.

Materialer og metoder

En 42-årig heteroseksuel mand henvises til Øre- næse- halsafdelingen via egen læge grundet forstørrede tungetonsiller på mistanke om malignt lymfom. Ultralyd viser cobblestone relief, og tonsillerne lyser kraftigt op ved PET-scanning.

Resultater

Biopsier fra tungetonsil viser uspecifikke fund, herunder slimhinde med hyperplasi, og markant intraepitelial betændelsescelleinfiltration. Stedvist med indtryk af kondylomatøse forandringer. Basalt ses cellulær atypi og atypisk udseende epitelceller samt spredte mitoser. På denne baggrund stilles diagnosen dysplasi. Grundet usikkerhed om diagnosen, konfereres der yderligere med klinikerne, hvorefter der foretages supplerende undersøgelse og IHC (immunhistokemi). Disse viser normal P16 epitelekspression, adskillige CD38-positive plasmaceller og massiv forekomst af spiralformede bakterier basalt i epitelet med kraftig positiv reaktion for Treponema Pallidum.

Diskussion og konklusion

Syfilis har en stigende incidens, og er samtidigt kendt som ”the great imitator”. Sygdommen kan være svær at erkende da anamnesen kan være behæftet fejl når informationerne er følsomme for patienten, samtidigt er det klassiske histologiske billede med plasmacelleinfiltrater fraværende i op mod 1/3 af biopsierne. Det er derfor en vigtig diagnose at have i tankerne for både kliniker og patolog, også når placeringen eller præsentationsformen er atypisk.

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Diagnostic and clinical outcome at 12-months re-test after HPV+ index sample in HPV-screening using extended genotyping and cytology as triage

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Background
In the Capital Region of Denmark, new HPV screening is conducted using extended genotyping and cytology as triage of HPV+ samples. Index screening samples with concurrent ASCUS or LSIL in combination with HPV35,39,45,51,56,58,59,66,68 and samples with concurrent normal cytology are referred to a re-test after 12 months. Here we evaluate the diagnostic and clinical outcome of the 1st 12 months re-test after HPV positive index sample.
Methods
All women aged 30 to 59 years underwent HPV screening from March to August 2021 with recommended follow-up of re-test at 12 months was included (N=1317). Re-test data at 12 months were retrieved from the Pathology DataBank.
Results
Overall, 42% of the women cleared the index HPV infection. Of the re-test of HPV positive women (N=545), 11% had concurrent ≥ASCH, 18% ASCUS or LSIL (any HPV genotype), 39% had HPV16,18,31,33,52 and NILM cytology. All these were referred to colposcopy. The remaining 32% were referred to a 2nd re-test as per guideline.
In total, 263 women had colposcopy (71%). For HPV+/ASCH, AGC, HSIL or AIS, 41% showed ≥CIN2 and 27%≥CIN3; for HPV+/ASCUS or LSIL, 17% ≥CIN2 and 7% ≥CIN3, and for HPV16,18,31,33,52 and NILM cytology, 15% showed ≥CIN2 and 6%≥CIN3.
Conclusion
Most disease were detected in the group of HPV-positive/≥ASCH. The triage groups HPV+/ASCUS or LSIL and HPV16,18,31,33,52/NILM cytology yielded very few histology confirmed ≥CIN3. The cautious conclusion is that the latter two triage groups potentially could benefit from a 2nd re-test recommendation rather than referral to colposcopy.

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IMPACT OF JOINT HPV33/HPV58 PROBE ON REFERRAL AND OUTCOME AFTER HPV POSITIVE INDEX SAMPLE USING BD ONCLARITY HPV ASSAY

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Background
Women aged 30 to 59 are offered HPV screening. All HPV positive index samples undergo combined triage of extended genotyping and cytology, where high-grade cytology and HPV 16/18/31/33 /51 with concurrent ASCUS or LSIL are referred for colposcopy and biopsies. Samples with HPV 35/39/45/52/58/56/59/66/68 and concurrent ASCUS/LSIL or normal cytology is referred for a re-test in 12 months. Using the BD Onclarity HPV assay, HPV 33/58 genotypes are by design bulked together. Here we evaluate the impact of the joint HPV33/58 probe on referral and clinical outcome.

Methods
All women aged 30-59 undergoing screening with HPV test from January-June 2022 with BD Onclarity HPV assay HPV33/58 index sample result (N=161) were re-tested for HPV33-58 separation using the Seegene Anyplex II HPV28. Register based follow-up through the Danish Pathology Databank for histology outcome was retrieved October 2022.

Results
Of the joint HPV33/58 probe positive samples, 37% were HPV33 upon genotyping, 2 % were HPV33 and 58 co-infections and 61% were HPV58 positive. Of resulting histologies 50% were ≥CIN2 for HPV33 index sample positives, compared to 5% for HPV58 index sample positive women.

Conclusion
The risk of developing ≥CIN2 is significantly higher for HPV33 than HPV58. Currently HPV33/58 are analyzed pairwise, thus women in the ASCUS or LSIL triage category with HPV58 are unnecessary referred for colposcopy and biopsies instead of a secondary HPV analysis in 12 months, indicating a general overdiagnosis and overtreatment. It would therefore be beneficial for the patient to analyze HPV33 and HPV58 separately.

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Comparing results of versions 11 and 12 of Heidelberg's DNA methylation-based classification tool.

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Introduction: DNA methylation-based classification of CNS tumours has proven to be a valuable diagnostic tool for improved brain tumour diagnostics. The classification tool (classifier) was recently updated to include additional methylation classes encompassing new tumour types as well as refinement of existing tumour types. In this quality assurance study, we compare the results of versions 11 and 12 in a series of glial tumour cases to explore which version (if any) is the most compatible with the final integrated pathological diagnosis.
Material and Methods: We examined 28 glial tumour cases on which methylation profiling (MP) was performed. The results of both versions were then correlated with additional findings (initial histopathology, immunohistochemistry, Next-Generation Sequencing and copy number variations). This led to a stratification of the cases based on whether the final diagnoses were supported by the results from either of the two classifiers or both.
Results: 19 (67.8%) cases were supported by the results from both classifiers. The result of classifier version 11 was favoured in 6 (21.4%) cases, while the result of version 12 was favoured in 2 (7.1%). In 1 (3%) case the result of either classifier failed to support the final pathological diagnosis.
Discussion and conclusion: There is an overall good correlation between the results of versions 11 and 12 when used on gliomas, with version 11 being slightly favoured in a few cases.

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Immunohistochemical expression of interferon-stimulated genes in virus-infected histopathological specimens

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When cells contain intracellular viral components, this normally leads to increased expression of type-I interferons. These acts autocrine and paracrine and induces expression of interferon-stimulated genes (ISG). We hypothesized that upregulation of ISGs can be used as a screening marker for many viral infections in histopathological specimens. The purpose of this study was to compare the immunohistochemical expression of the following ISGs MxA, RIG-I, PKR, and PD-L1 in virus-infected and normal formalin-fixed paraffin-embedded tissues.
Archival tissues with proved viral infection with the following viruses were included: SARS-CoV2 (n=3), HPV (n=5), CMV (n=3), EBV (n=5), HSV (n=5) and molluscum contagiosum virus (MCV, n=5). 5 normal samples of each tissue type were included as references. The tissues were incorporated in tissue microarrays (TMA). After vigorous optimization of the immunohistochemical staining protocols, sections of the TMAs were stained with antibodies against the ISGs. The stained slides were qualitatively assessed whether infected cases showed distinct upregulation of the individual ISGs as compared to non-infected normal controls.
MxA provided the highest sensitivity for the detection of viral infection (0.80), followed by PD-L1 (0.68), PKR (0.56), and RIG-I (0.48). The HPV and MCV cases generally showed little or no inflammatory response, and if these cases were omitted, the sensitivities increased to 1.00, 0.87, 0.80, and 0.63, respectively.
In conclusion, the immunohistochemical expression of MxA, PD-L1, PKR, and RIG-I was upregulated during most of the investigated viral infections, but with large variations. ISGs either alone or in combination could be a potential screening marker for viral infection.

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Spatial proteomics profiling of NSCLC biopsies – characterization of treatment resistance pathways and discovery of biomarker panels.

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Lung cancer remains one of the most common cancer types and leading cause of cancer death worldwide with a 5-year survival rate of approximately 24%. In Denmark alone, almost 5000 patients are yearly diagnosed with lung cancer. 75% of patients are typically diagnosed at a disseminated stage, when tumor is no longer surgically removable and requires systemic treatment. Thus, methods to stratify patients in responder/non-responder-groups on biopsy material early on are crucial. Targeted therapy with tyrosine kinase inhibitors (TKIs) is effective, but treatment resistance inevitably occurs. Especially, it remains unexplained how patients with identical mutational profiles respond differently to this treatment, and why virtually all at some point become resistant. In this project, we couple a unique comprehensive clinicopathological and molecular database of 4500 lung cancer patients in the Capital Region with our unprecedented ultrasensitive laser capture microdissection (LCM)- and mass spectrometry (MS)-based proteomics fingerprinting on archival diagnostic biopsies. We will use this workflow to 1) characterize the spatial global proteome, phospho-proteome, and single-cell proteome in subgroups of patients with identical unique mutational profiles (UMPs) but different response to TKI treatment, and 2) also characterize TKI-resistance mechanisms in a subset of patients, using re-biopsies taken at progression on TKIs. We expect that in-depth, spatially resolved proteomic profiling of these patient sub-cohorts will identify specific protein expression and signaling pathway signatures that can explain differences in treatment response and resistance mechanisms and that may represent novel predictive biomarkers and/or therapeutic targets.