Oral presentation 1

0-1-1
Use of image analysis in external quality assessment of HER2 protein expression in breast cancer.
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Introduction: Visual assessment of immunohistochemical (IHC) HER2 is partly subjective. For the purpose of quality assurance, an objective and standardized method is desirable. The HER2-CONNECT algorithm (Visiopharm, Denmark), which gives a continuous measure of IHC HER2 membrane connectivity, has in a previous study of breast carcinomas showed a concordance rate with FISH based gene amplification status close to 100%. We wanted to evaluate the potential of HER2-CONNECT™ for external quality assessment of HER2 IHC.

Material and methods: Serial sections of tissue cores from 5 different breast carcinomas with well defined IHC HER2 expression and gene amplification status were stained by 232 laboratories participating in the NordiQC quality assessment programme run B12, 2011. Digital images of the scanned slides were analysed with HER2-CONNECT™ and compared with visual analysis and assessment scores given by the assessor group.

Results: When the staining quality was marked optimal, by the assessor group, the concordance rate with FISH was 100%. For stains marked as good sensitivity was 93.18% and specificity 94.64%.
When the staining quality was assessed as poor, the sensitivity was 52.38% and the specificity was 98.50%.
For the stains marked as good/borderline/poor the inter-laboratory variability increased six-fold.

Discussion and conclusion: Image analysis may provide an objective and standardized method for external quality assessment on the HER2 IHC quality on tissue sections.

0-1-2
Validation of Virtual Double Staining algorithm for estimation of Ki67 proliferation indices in Breast Carcinomas.
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Introduction: Ki67 is an important immunohistochemical marker of proliferation used in grading of e.g. breast cancer and endocrine neoplasms. However, manual counting and calculation of Ki67 proliferation index is labourious and prone to interobserver variance. Recently, a computerized algorithm that enables virtual alignment of two consecutive slides stained for pancytokeratin and Ki67 has been developed. Software analysis (Virtual Double Staining – VDS) of this image enables exclusion of stromal cells and calculation of Ki67 proliferation indices in tumour cells only. The purposes of this study were to compare manual counting and Virtual Double Staining for assessment of Ki67 proliferation indices in breast carcinomas.

Material and methods: Tissue Micro Arrays (TMA) were constructed with 158 cores of breast carcinomas. Two slides were cut from each TMA and immunohistochemically stained for pancytokeratin and Ki67. For each core, between 2-20% of the total core area were selected for exact manual counting of Ki67-positive and negative cells using the systematic uniformly random sampling principle. A minimum of 200 cells were counted. The same selected areas were counted using the VDS algorithm. Additionally, the VDS algorithm was used to calculate Ki67-proliferation index for each core. In order two analyze the importance of the distance between neighbouring slides, five consecutive slides were stained for PCK.
These slides were digitally fused and the percentage of overlap between stained and not stained areas calculated.

**Results:** There was good correlation (R² > 0.90) between manual counting in systematic randomized selected areas and virtual double staining of both the whole core and in selected areas. Comparison of the two methods using Bland-Altman plots did not reveal any skewness in certain data ranges. Overlap agreement between neighboring was on average above 88%. Additionally, diffusely infiltrating tumours was influenced by distance in a higher degree than more solid tumours. **Discussion and conclusion:** Virtual Double Staining may be an important future tool for improving accuracy and reproducibility of Ki67 proliferation indices in diagnostic and research settings.

**0-1-3**

**Validation of Virtual Double Staining algorithm for estimation of Ki67 proliferation indices in Breast Carcinomas.**

Rasmus Røge(1), Søren Nielsen(1), Rikke Riber-Hansen(2), Mogens Vyberg(1)

(1) Department of pathology, Aalborg University Hospital; (2) Department of Pathology, Aarhus University Hospital

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**0-1-4**

**Changes in numbers of mast cells and T-cells may play an important role in the development of necrotizing enterocolitis (NEC) in newborns**

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**Introduction:** Necrotizing enterocolitis (NEC) is one of the most common and devastating gastrointestinal diseases in premature neonates. The pathogenesis of NEC is not fully under-
stood and is probably multifactorial. Intestinal immaturity, particularly an excessive response to luminal microbial stimuli, is among the most frequently proposed hypotheses. Moreover, our knowledge of the numbers of immune cells in relation to gestational age (GA), including preterm infants, is still insufficient. Hence, our aims were to describe the counts of T and B cells, eosinophilic granulocytes and mast cells in the unaffected small intestinal mucosa and submucosa in relation to GA and to compare our findings with viable small bowel tissue from NEC patients.

**Material and methods:** 36 controls (GA 12 - 41 weeks) and 8 NEC specimens (GA 24 - 32 weeks) were obtained from autopsies and surgical resections and stained with giemsa, toluidine blue, CD3, CD79a, trypase and eosinophil basic major protein (BMK-13). Eight different cell counts were performed using the TissueGnost® software and the newCAST® software. Multiple linear regression analysis was performed with cell counts being transformed by natural logarithm and set as the dependent variable. GA (days) and NEC were set as the independent variables.

**Results:** Significant correlations between GA and cell counts were found in all cell types. NEC correlated significantly with all three submucosal mast cell counts and significantly negatively with the mucosal T-cell count. Adjusted R^2^ values (ratio of the counts that is explained by the independent variables, GA and NEC) were between 25.7-79.0 %. All F-tests (assessing the explanatory power) were significant. For all cell types there was a positive, significant, exponential correlation between GA and cell count, ranging between 0.009 and 0.029, signifying that a one day increase in GA would increase cells/mm^2^ of the corresponding cell type with 0.9-2.9%. P-values of these correlations were all p=

**Discussion and conclusion:** In conclusion, we found significant and strong evidence that eosinophilic granulocytes and mast cells of the human fetal small bowel increase exponentially during gestation. The exponential increase was less evident, yet equally significant in T- and B-cell estimations. Furthermore, our data indicate that mast cells may play an important role in the pathogenesis of NEC. The finding of decreased T-cells in NEC emphasizes the need for further studies.

0-1-5

**SSEA-4 and YKL-40 positive astroglial progenitors in subventricular zone of developing human neocortex**

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**Introduction:** The human cerebral cortex is one of the most complex structures of the human body. Detailed characterization of the proliferative zones during corticogenesis is essential to understand cortical development. Recent studies point toward purely gliogenic, purely neurogenic or multipotent distinct subpopulations of radial glial cells. The glycosphingolipid SSEA-4 is in a previous study found in human neural progenitor cells. The glycoprotein YKL-40 is recently associated with brain barrier sites of developing human foerebrain. Furthermore, intriguing small rounded YKL-40 positive cells are identified in the subventricular zone (SVZ). Here, we aimed to investigate the SSEA-4 positive neural progenitor cells in relation to the YKL-40 positive cell population in SVZ of developing human foerebrain, and examine its proposed astrogenic lineage.

**Material and methods:** Forebrain samples from one human embryo, 31 mm crown-rump length (CRL) and 12 fetuses (38–200 mm CRL) corresponding to 8th-21st weeks post conception were used. Immunohistochemistry and double-labeling immunofluorescence with antibodies against SSEA-4, YKL-40, and against developing forebrain specific cells (BLBP, GFAP, NeuN, calbindin, Tbr2, NG2, S100, Iba1, CD68) were applied and examined by confocal microscopy.

**Results:** Small rounded SSEA-4 and YKL-40 labeled cells were present in the BLBP positive proliferative zones adjacent to the lateral ganglionic eminence from 12th week post conception. With increasing age, a similarly stained cell population appeared more widespread in the SVZ. At mid-gestation, the enti-
Evaluation of the proliferation marker Ki-67 in gliomas: Interobserver variability and digital quantification

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Introduction: Tumour cell proliferation is associated with tumour aggressiveness in gliomas and other tumour types. The percentage of tumour cells labelled by the proliferation marker Ki-67, referred to as the Ki-67 labelling index (LI), has been recommended by WHO as an ancillary tool in glioma diagnostics. However no precise guidelines have been given on how to determine the Ki-67 LI. Nor is it known whether novel digital approaches will be an advantage. The aim of this study was to identify pathologist-related interobserver variability of the Ki-67 LI in whole slides and hot spots including digital quantification in the analysis.

Material and methods: Samples of 235 gliomas diagnosed between 2005 and 2009 were stained immunohistochemically with an antibody against Ki-67. Two observers (A and B) estimated a mean whole slide and hot spot Ki-67 LI for all tumours. For digital quantification, (C) whole slides were scanned with subsequent systematic random sampling of vital tumour areas. A software classifier was trained to identify positive and negative nuclei followed by calculation of the Ki-67 LI. A hot spot was defined as the sample image with the highest Ki-67 LI. The interobserver and the observer versus digital agreement were evaluated by kappa (κ) statistics.

Results: The mean (standard deviation) and median [range] Ki-67 LI for A, B and C, respectively for all whole tumour slides were: A: 12% (8%), 10% [0%-60%]; B: 13% (8%), 15% [0%-50%]; C: 16% (10%), 14% [0%-51%]. For hot spots the values were: A: 20% (13%), 20% [0%-90%]; B: 30% (16%), 30% [0%-80%]; C: 31% (17%), 31% [0%-81%]. The observed proportion of agreement and κ values for mean Ki-67 LI were: A/B: 46% (κ= 0.32); A/C: 37% (κ= 0.26); B/C: 37% (κ= 0.26). The observed proportion of agreement and κ values for hot spots were: A/B: 14% (κ= 0.04); A/C: 18% (κ= 0.09); B/C: 31% (κ= 0.21).

Discussion and conclusion: For whole slide Ki-67 LI there seemed to be better agreement between observers compared to hot spots when evaluating both the conventional and digital approach. We found a marked interobserver variability especially when interpreting hot spots, and for hot spots we found larger ranges of the values. Hot spot Ki-67 LI values should thus be used with caution. Digital quantification may be a promising method to standardize the Ki-67 LI, especially with standardized use of software classifiers between laboratories.
M2 polarized tumor-associated microglia/macrophages are associated with glioma prognosis
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Introduction: About 700 patients are diagnosed with glioma each year in Denmark. Glioblastoma multiforme (GBM, WHO grade IV) comprise 50% of these tumors making it both the most common and malignant primary brain tumor. Despite multimodal treatment with surgery followed by radiation and chemotherapy, patient survival remains poor with a 2-year survival below 30%. GBMs are highly infiltrative tumors and have a rich tumor microenvironment with tumor-associated microglia and macrophages (TAMs) constituting up to 30% of the total tumor tissue. Increasing evidence suggests that TAMs favor tumor progression by acquiring a protumorigenic M2 phenotype induced by the glioma cells and glioma cells with stem cell phenotype (GSC). The aim of this study was to investigate the prognostic impact of TAMs in gliomas.

Material and methods: Automated quantitative double immunofluorescence was performed on 242 tumor samples from a population-based patient cohort using ionized calcium-binding adaptor molecule-1 (IBA-1) to identify the general population of TAMs and CD204 to detect M2 polarized TAMs. Measurements obtained for each patient were area fractions (AF) and mean intensities (MI) of IBA-1 and CD204 in total tumor tissue as well as of CD204 within the IBA-1+ area (i.e. IBA-1(total), CD204(total), and CD204(IBA-1)).

Results: AF IBA-1(total), CD204(total) and CD204(IBA-1) increased with increasing malignancy grade (p<0.0001).

Discussion and conclusion: Our results suggest that TAMs are an important player in the tumor microenvironment and contribute to glioma aggressiveness. This may be explained by their polarization towards a M2 phenotype, which holds a prognostic value in high-grade gliomas.

Glioma cells in the tumor periphery have a stem cell phenotype
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Introduction: Gliomas are highly invasive tumors being incurable by surgery. Although surgery removes the bulk tumor, tumor cells in the periphery are left behind resulting in tumor relapses for most patients. Tumor stem cells (CSC) have been shown to be highly tumorigenic and resistant to both radiotherapy and chemotherapy. However, it is not known whether tumor cells in the tumor periphery have a stem cell phenotype. The aim of the present study was to characterize the phenotype of tumor cells in the periphery focusing on tumor stemness, proliferation and chemoresistance.

Material and methods: We identified 26 gliomas having the R132 mutation in Isocitrate DeHydrogenase 1 (mIDH1) and having tumor tissue from both core and a periphery present on the same tumor section. In total 18 WHO grade II tumors, 5 WHO grade III tumors and 3 WHO grade IV tumors were identified. A double immunofluorescence approach identifying mIDH1 positive tumor cells and a panel of markers was used combined with computer-based analysis. The trained software classifiers were designed to measure the IDH1 positive area-fraction of the markers in the chosen panel. The panel comprised five different stem cell markers (CD133, Musashi-1, Bmi-1, Sox-2 and Nestin), the proliferation marker Ki-67 as well as O6-methylguanin-
DNA-methyltransferase (MGMT), which is a DNA repair enzyme.

**Results:** The results showed that glioma cells in the periphery express stem cell markers, however at a significantly lower level than in the tumor core. The area fraction of the different stem cell markers were for core (C) and periphery (P): CD133 C: 0.3%, P: 0.03%; Musashi-1 C: 83.5%, P: 61.4%; Bmi-1 C: 90.1%, P: 75.0%; Sox-2 C: 78.5%, P: 66.0% and Nestin: C: 53.7 %, P: 29.3%. The area fraction of Ki-67 and MGMT were: Ki-67 C: 8.8%, P: 7.2% and MGMT C: 16.5%, P: 14.8%. This difference in the Ki-67 level was significant, whereas the difference in the MGMT level did not reach significance.

**Discussion and conclusion:** The results suggest that tumor cells in the periphery preserve a tumor stem cell phenotype. This may suggest that a high tumorigenic capacity is present in the tumor periphery although the proliferation index itself is reduced in peripheral tumor cells. Novel therapies aiming at preventing recurrence should therefore take tumor stemness into account.
Oral presentation 2

O-2-1
To submit, or not submit tissue from macroscopic normal cholecystectomy specimens.
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Introduction: Carcinoma of the gallbladder (GBC) is a rare malignancy that carries a very poor prognosis. It is a disease of the elderly, affecting mainly patients in their 60’s-70’s. Laparoscopic cholecystectomy is established as the gold-standard treatment for symptomatic gall stones. At present all specimens have routinely tissue submitted for histology regardless of the macroscopic finding and clinical setting. The aim of this study was to investigate the possibility of reducing the number of gallbladders examined histologically by addressing the incidence of GBC at a regional non-specialized hospital in correlation with the macroscopic findings and patient age.

Material and methods: Pathology reports of all cholecystectomy specimens examined at the Department of Pathology, Slagelse (and Holbæk) Hospital over a period of 12 years (2002 to 2013) were analyzed retrospectively. In all cases of GBC and biliary intraepithelial neoplasia (BilIN)/adenoma the reports were scrutinized with particular emphasis on macroscopic findings by the pathologist. It was registered if the gallbladder was normal, abnormal without a tumor/mass or with a tumor/mass.

Results: The total number of specimens was 6006. GBC was detected in nine (0.15%), BilIN/adenomas in 63 (1.04%) and metastatic malignancy to gallbladder in 2 (0.03%). The median age of patients with GBC was 73 years (range 61-84 years), except for one patient aged 34. In this case there were preoperative suspicion of malignancy and the patient was known with Primary Sclerosing Cholangitis (PSC) that is a well known risk factor for developing GBC. None of the 9 specimens with GBC was classified as normal on the macroscopic examination. 6 of the specimens had a tumor/mass and 3 had had other abnormalities like fibrosis, thickened wall or ulcerated mucosa. There was clinical suspicion of malignancy in 3.

In a two months period in 2013 of 148 specimens examined, 57 (38.5%) were macroscopically normal. The group of specimens with normal macroscopic findings and patient age under 50 years accounted for 28 (19%) of all cholecystectomy specimens in the 2 months period.

Discussion and conclusion: Our results show that GBC is very rare in young patients and that all cases of GBC were associated with abnormal macroscopic findings. Overall over one-third of the gallbladders had normal macroscopic features and could in principle be signed out without having tissue submitted for histology. To increase patient safety further and submitting tissue from all specimens of patients 50 years and older a significant cost saving would still be possible.

O-2-2
Preoperative prediction of macroradical primary surgery in ovarian cancer patients
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Introduction: Primary surgery is first choice of treatment, and complete tumor removal is an important prognostic factor for women with ovarian cancer, but neoadjuvant chemotherapy is an option when macroradical removal of the tumor is impossible. However, preoperative planning of optimal treatment strategy is a clinical challenge and no preoperative predicting tool for treatment decision is available.

The purpose of this study was to find a preoperative, non-invasive method to select between two treatment strategies, primary debulking surgery or neoadjuvant chemotherapy, in order to plan the individual, opti-
Material and methods: We evaluated the usefulness of the two serum biomarkers HE4 and CA125 in predicting complete tumor removal. Material and methods: Collection, handling, and storage of blood samples were performed according to strict guidelines by the Danish CancerBiobank. Clinical data was provided by the Danish Gynecologic Cancer Database (DGCD). Serum HE4 and CA125 analyses were performed using fully automatized kits from Abbott Diagnostics. Multivariate analyses included HE4, CA125, age, performance status and presence of ascites at preoperative ultrasonography.

Results: 181 patients were enrolled. Exclusion criteria was treatment with neoadjuvant chemotherapy (n=13) and performance status 4 (n=1). 167 patients with advanced epithelial ovarian cancer (138 FIGO stage III and 43 FIGO stage IV) were treated with primary debulking surgery. Complete removal of tumor was achieved in 50 cases (30%). The Receiver Operating Characteristics curves demonstrated an Area Under the Curve of 0.772 for HE4 and 0.684 for CA125. The multivariate model consisting of HE4, age, performance status and presence of ascites yes/no at ultrasound demonstrated an Area Under the Curve of 0.853. CA125 was excluded from the multivariate model by backward reduction because of insignificant contribution.

Discussion and conclusion: The new tumor marker HE4 was found superior compared to CA125 in predicting macroradical surgery in patients with advanced ovarian cancer. A multivariate model combining HE4, age, performance status and ultrasound assessment of presence of ascites may be a useful preoperative index for selecting patients to either primary debulking surgery or neoadjuvant chemotherapy.

Expected uterine malignancy after laparoscopic myomectomy or hysterectomy with morcellation
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Introduction: Uterine leiomyomas (LM) are benign neoplasms composed of cells demonstrating smooth muscle differentiation. They are common and often asymptomatic. No reliable method for preoperative distinction between LM and the rare leiomyosarcoma (LMS) is available. Myomectomy or hysterectomy is often performed laparoscopically. Power morcellation is a technique that divides tissue into small pieces and allows removal of larger tumors through the vagina or a laparoscopic port hole. Studies suggest that morcellation of occult LMS carries a risk of dissemination in the abdomen and peritoneal cavity, which may worsen prognosis. We examined -the number of patients in which an occult LMS or a smooth muscle tumour of uncertain malignant potential (STUMP) had been diagnosed after the use of a power morcellator during surgery at Herlev Hospital -Pathological findings and limitations caused by the morcellation technique

Material and methods: We conducted a search in our pathology database from the 1st of June 2008 until the 31st of December 2013, including all malignant smooth muscle tumours/STUMP. We read the pathology files and isolated cases where morcellation was performed. Microscopic findings and clinical records were reviewed.

Results: We isolated 17 cases with uterine LMS and 10 with STUMP. Morcellation technique was used in 6 cases: 5 LMS and 1 STUMP. The women were between 34 and 53 years old. Follow-up was 9-39 months. Tumour size or margins could not be determined in any of the cases because of tissue fragmentation. All cases displayed nuclear
atypia. All LMS displayed tumour cell necrosis and a high mitotic index, ranging from 8-20/10 high power fields (HPF) in hot spots. The STUMP case had 0/10 HPF and type of necrosis could not be determined. Immunohistochemical staining for desmin, smooth muscle actin 1A4, h-caldesmon and p16 was positive in all 6 tumours. Two patients were stage IV at the time of diagnosis. Two patients had no relapse at follow-up. The patient with STUMP had relapse 3 years after initial surgery, now diagnosed as a LMS, that was radically operated. One patient became terminally ill due to relapse.

Discussion and conclusion: In a 5½ year period six cases of occult morcellated LMS/STUMP were identified. The prognosis was not worsened for the 2 cases with stage IV, but it cannot be excluded that early relapse was caused by morcellation in 2 cases. Thorough and correct counseling of patients prior to morcellation of LM is essential. After January 2014 all patients with presumed benign LM are informed about the risk for sarcoma and morcellation. Pathologically, tumour staging was severely limited by morcellation as tumor size and margins could not be fully evaluated for LMS/STUMP.

O-2-4

Differentiating clear cell carcinomas in the ovary from serous and endometrioid carcinomas

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Introduction: Ovarian carcinomas have the highest mortality rate among the gynecological cancers and account for more than 90% of ovarian cancers. They are subdivided into: high-grade serous carcinoma (70%), endometrioid carcinoma (10-15%), clear cell carcinoma (5%), mucinous carcinoma (5%) and low-grade serous carcinoma. Clear cell carcinoma in advanced stage has a worse prognosis compared with other subtypes and can be rather difficult to differentiate from high-grade serous carcinoma and endometrioid carcinoma based on morphology alone.

Our objective was to apply 5 different immunohistochemical markers, HNF-1β, WT1, ER, GLYP3 and IGFBP-1 to the tree different ovarian carcinoma subtypes, to evaluate whether these biomarkers could differentiate the subtypes more accurately.

Material and methods: 30 cases of serous carcinoma grade II, 29 cases of serous carcinoma grade III, 22 cases of endometrioid carcinoma, grade II and III and 33 cases of clear cell carcinoma were included. TMA blocks were made for each subtype. The TMA blocks were stained with each of the immunohistochemical markers. The staining intensity and extension was evaluated using the H-score method.

Results: The median H-score for both GLYP3 and IGFBP1 were close to 0 in all of the carcinoma subtypes. WT1 showed a median H-score of approximately 250 in both grade II and III serous carcinomas, while close to 0 in the endometrioid and clear cell carcinomas. ER median H-score concerning serous carcinomas was 200 and 150 for the endometrioid carcinomas, but close to 0 in clear cell carcinomas. HNF-1β had a median H-score of 250 in clear cell carcinoma, approximately 90 in endometrioid carcinomas and close to 0 in serous carcinomas. For each biomarker one or a few of the H-score had a value far from the medial and must be considered an outlier.

Discussion and conclusion: The study showed that none of the chosen immunohistochemical markers solely could differentiate between the tree carcinoma subtypes. The median H-score for GLYP3 and IGFBP1 were close to 0 in all subtypes, and were thus not useful in the differentiation. ER, HNF-1β and especially WT1 proved more useful. However one has to keep in mind the possibility of outliers in all cases.
Stathmin-1 is a sensitive and specific biomarker for high-grade cervical intraepithelial neoplasia (CIN)

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Introduction: Most CIN1 and also CIN2 are due to transident infection with HPV and the risk for progression to cervical cancer is very low. However treatment with conization of high-grade lesions are important and effective to prevent progression. To avoid overtreatment of women with cervical precursor lesions it is therefore very important to distinguish between low and high-grade CIN.

The aim of this project was to investigate, whether immunohistochemical staining with Stathmin-1 on cervical biopsies could be used as a biomarker to differentiate between low-grade and high-grade lesions, and to compare the results with the immunohistochemical expression of p16 and HPV genotyping.

Material and methods: A total of 107 cervical biopsies were retrieved from the archives. Further 13 cervical biopsies with squamous carcinomas were identified and used as positive controls. Four my slides were cut for HE and immunohistochemical staining with Stathmin-1 on cervical biopsies could be used as a biomarker to differentiate between low-grade and high-grade lesions, and to compare the results with the immunohistochemical expression of p16 and HPV genotyping.

Results: Based on the HE stained slides, 26 samples were classified as benign, 32 as CIN1, 17 as CIN2 and 32 as CIN3. The agreement between the two pathologists was 62%.

Stathmin-1 was evaluated as positive in 0% of the benign biopsies, in 6% of CIN1, 76.4% of CIN2 and 100% of CIN3.

In contrast, 11.5% of the benign biopsies, 59% of CIN1 and 100% of both CIN2 and CIN3 were evaluated p16 positive. The agreement between the two pathologists for Stathmin-1 and p16 evaluation was very high with Kappa values of 92% and 96%, respectively.

All of the 13 carcinomas stained positive for Stathmin-1 and 12 out of 13 for p16, although the intensity and percentage of stained tumor cells varied in both cases.

The results of HPV analysis showed that 46% of the benign biopsies, 75% CIN1, 100% CIN2 and 96.8% CIN3 were high-risk HPV positive.

The sensitivity and specificity for CIN3 was 100% and 48%, respectively for p16 and 97% and 80%, respectively for Stathmin-1.

Discussion and conclusion: These results demonstrate that Stathmin-1 and p16 are sensitive biomarkers for CIN 3 lesions, and that Stathmin-1 is a more specific marker than p16. The evaluation of both the immunohistochemical stainings is highly reproducible. Immunohistochemical staining for Stathmin-1 might be useful as an adjunctive tool in a clinical setting to identify high-grade lesions. However, further and prospective studies are needed.

O-2-6
How does introduction of HPV-testing influence post-conization follow-up?

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Introduction: In 2012, new national recommendations introduced HPV-testing as a supplement to cytology in follow-up after conization. The objective of this study was to investigate the number of women returning to the screening programme within the first two controls, and to determine cytology and HPV-status in the group with signs of persistent disease.

Material and methods: Using data from The Pathology Data Bank, we included women who had been conizised in the Aalborg region in the period 01.01.2012 – 30.06.2013, with biopsy diagnosis of CIN 2, CIN 3 or AIS prior
to conization, and follow-up including both cervical cytology and HPV-testing. Women with invasive carcinoma were excluded from the study. Surgical margins, HPV-status and cytology at 1st and 2nd control, were registered. In case of negative margins, normal cytology and negative HPV-status, only one control was performed before returning to the screening programme. HPV-status was determined using Roche Cobas 4800. Follow-up time was 18-24 months.

**Results:** In the study period 426 women with CIN2+ lesions were conized. Of these, 278 women were included. Reasons for exclusion were mainly lack of HPV-testing in one or more of the controls (n=74) and insufficient follow-up, missing one or both controls (n=62).

Out of the 278 included women, 79.1 % (n=220) returned to screening programme within two controls. 33.8 % (n=94) returned after just one control. 20.9 % (n=58) had signs of residual disease after two controls. Of these, 62.1% (n=36) were positive for HR-HPV, but had normal cytology, 32.8% (n=19) were both positive for HR-HPV and had abnormal cytology, 5.2% (n=3) had abnormal cytology but were HR-HPV negative. In the follow-up period, 10 had histology verified recurrence or persistence of CIN2+. Of these, 9 had abnormal cytology and positive HR-HPV status, 1 was HR-HPV positive with normal cytology.

**Discussion and conclusion:** The new follow-up algorithm after conization allows a large majority of women to return to screening programme within few controls. We see this as a clear advantage, as it diminishes the psychological and financial burden of repeated controls. It is however necessary to conduct further studies, to determine long term outcome for the group returning to the screening programme.

In the group with signs of persistent disease, the majority was HR-HPV positive with normal cytology. It is noteworthy though, that all, but one, with biopsy verified recurrence or persistence of CIN2+ lesions in the control period, had both abnormal cytology and positive HR-HPV status. This raises the question if HR-HPV positive women with normal cytology risk unnecessary controls, and if the follow-up programme should be stratified even further.

**O-2-7**

**The value of adding cintecl plus dual staining for P16 (INK4A)/ KI-67 on COBAS HPV positive women for detection of high grade CIN**

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**Introduction:** HPV testing for high-risk HPV types for cervical cancer has a very high sensitivity and negative predictive value but the specificity is limited. There is a need for additional biomarkers to triage the HPV positive women in order to allocate the women to the right follow up and avoid unnecessary controls.

**Material and methods:** 2965 consecutive screening samples from women aged 30-65 years were included. The ThinPrep samples were evaluated by cytology and tested by the Cobas HPV Assay. The CINtec PLUS dual staining for p16(INK4a)/ki-67 was performed on the Cobas positive women. The dual staining was evaluated by two senior pathologists and in case of discrepancy also by a third pathologist. The follow-up after 6 month was recorded.

**Results:** The overall HPV positive rate was 12.0% by the Cobas Assay. Of the HPV positive women, 21.4% tested positive for HPV16, 7.3% for HPV18 and 79.4% for other high-risk types (including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). 4 samples were inadequate for CINTEC PLUS testing. After consensus 205 (58.4%) of the 351 HPV positive women were evaluated as having a positive CINtec PLUS test with a least one double stained cell. The Kappa value between the two pathologists was 0.47. 76 women had a histological diagnosis of CIN2+ and 58 women of CIN3+. Four carcinomas were detected and they were all evaluated positive by the CINtec PLUS test CINtec plus was evaluated as positive in 80%, 56 % and 52% of HPV16, HPV18 and
other high-risk positive samples, respectively. The sensitivity and specificity for CINtec PLUS to detect CIN2+ was 94.6% and 50.9%, and 94.8% and 48.8% to detect CIN3+. The negative predictive value (NPV) was 96% for CIN2 and 98% for CIN3.

Conclusion: CINtec PLUS seems to identify a subgroup of HPV positive women with a high risk for harbouring high-grade CIN with a sensitivity of 94.8 for CIN3+ % and a specificity of 48.8%.. In our preliminary data with short term follow up the NPV was high (98%) suggesting the CINtec PLUS dual staining might be used to further stratify Cobas negative HPV positive women. However, data on long term follow up are needed.

O-2-8
HPV-based self-sampling implementation with an opt-in strategy to improve cervical screening coverage.
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Introduction: In Denmark, 45% of cervical cancers are diagnosed in screening non-participants. The ongoing pilot implementation study in the Capital Region aims to improve the screening coverage rate by offering an HPV-based self-sampling test for free to non-attenders. Unlike in previous research studies, we rely on an opt-in strategy where invited women have to order the self-sample brush actively upon invitation. “Opt-in” was chosen to reduce the cost, and will probably be the most sustainable strategy for countries that will routinely roll-out self sampling. However, a concern is that an “opt-In” strategy might be potentially discouraging for participation. Therefore, we studied the actual response rates.

Material and methods: Non-attenders from the Capital Region (N= 54,585) were identified from the invitational module of the nationwide Pathology Data Bank. Women were randomly selected into batches of 1,000. They could respond by mail, webpage, mobile application, E-mail or phone. Reminders were sent after 8 weeks. Received kits are being analyzed with HC2, CLART, and Onclear HPV assays.

Results: So far, 20,000 invitations have been sent. The overall response rate for the first batch was 45% in 200 days after the invitation. Of the invited women, 39.6 % chose to receive the kit and 67.5 % of the latter returned a self-sample (mean age: 47.9 years). This increases the overall coverage rate by about 5-6%. So far, the overall prevalence of HR-HPV was 15.7% by Onclear.

Discussion and conclusion: Self-sampling with an opt-in strategy was well accepted among non-attenders, and might be a good supplement to the regular call-recall screening program.
Oral presentation 3

O-3-1

**Comparison of fixation of large intestine in Formaldehyde or Molecular Fixative**
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**Introduction:** Formaldehyde is the most commonly used fixative in pathology, but it is carcinogenic and allergenic. Some studies have found that an alcohol based fixative made especially for molecular analysis gives similar morphology, a more intense background staining in the HE stain and varied results in immunohistochemistry staining. DNA and RNA was consistently better preserved and yielded longer fragments.

**Material and methods:** The aim of this study was to test this on samples from large intestine cut into small biopsies measuring approx. 2x2x4 mm, and fix them for 4 and 6 hours in either 4% neutrally buffered formaldehyde or Tissue Tek® Xpress® Molecular Fixative. We also decided to process them in either Tissue Tek® VIP® 5 or Tissue Tek® Xpress®. We examined the stains HE and Alcian Pi KoSirius, immunohistochemical analysis CD117, Ki67, Actin SMM-1, CK20 and PMS2. Furthermore DNA quality and quantity were evaluated using fragment analysis and Real-Time PCR.

**Results and discussion:** We found that the tissue shrinks in Molecular Fixative in varying degrees; epithelium shrinks more than muscle tissue which shrinks more than connective tissue. This is in accordance with the theory which states that alcohol precipitates proteins and therefore the tissue shrinks. The background staining with Eosin in the HE staining is intensified and the competitive staining of muscle and connective tissue in Alcian Pi Ko Sirius is not optimal. This is also in accordance with theory, since formaldehyde uses the aminogroups for crosslinking, and these groups are also used by anion stains. Immunohistochemical staining varies and is largely dependent on the specific antibody; in the 5 antibodies we chose CK20 and PMS2 shows a decline in stainability in tissue fixed in Molecular Fixative, whereas Ki67, Actin SMM-1 and CD117 showed no difference between the different variables. We know from experience and tests that PMS2 is sensitive to poor formaldehyde fixation or alcohol fixation, but we did not expect CK20 to be. The molecular analysis performed showed a tendency toward a better quality and quantity using Molecular Fixative. Furthermore it has been observed that method of tissue preparation also influence the molecular results.

**Conclusion:** The conclusion is that Molecular Fixative is not optimal for substitution of Formaldehyde as a routine fixative for traditional diagnosis on the examined tissue, but may be superior for molecular diagnostic.

O-3-2

**Impact of rapid tissue processing on subsequent molecular testing**
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**Introduction:** Due to the increasing demand for rapid pathology results new techniques for tissue processing have been developed. In contrast to traditional vacuum infiltration technology, which normally requires specimens to be loaded in batches and processed overnight, the Xpress from Sakura is able to process tissue in a few hours. However, it is not clear how this, microwave assisted, rapid tissue processing impacts the quality of nucleic acids in standard formalin fixed material and the subsequently use of molecular testing, which is increasing greatly in these years.

**Material and methods:** From 44 formalin-fixed specimens (9 breast, 16 lung, 17 colon and 2 kidney) paired tissue sections of similar dimensions, (1.0 x 0.4 x 0.4 cm) were taken. One of the paired samples was processed
using a conventional method (VIP, Sakura) and the other by an automated microwave-based rapid tissue-processing instrument (Xpress, Sakura).

DNA was purified from a 10 micron section of each sample using the QIAamp DNA FFPE Tissue-kit (Qiagen). DNA concentration and fragment length were determined using the TapeStation Instrument (Agilent) applying the Genomic DNA Screen Tape.

Functional studies were performed by SYBR green based qPCR (using 5 µl of sample DNA and 15 µl of SYBR Select Master Mix, Life Technologies). Amplicons of 100 and 200 base pairs were amplified and the Ct value was recorded. Paired tissue samples were purified and analyzed in the same batch.

Results and discussions: The average amount of purified DNA was 15.8 ng/µl for VIP samples and 7.5 ng/µl for Xpress processed samples. Four Xpress and one VIP sample could not be determined due to low amount of DNA. The average fragment length of VIP samples was 5,873 base pairs and for Xpress samples 5,542 base pairs.

In the functional test (qPCR) using the 100 base pair amplicon the Ct value was lower in the VIP processed material compared to Xpress in 34 out the 44 cases, indicating more amplifiable DNA in the majority of VIP samples. The same tendency was seen in the the 200 base pair amplicon where the Ct value was lowest in the VIP processed material in 36 out of 44 samples.

Conclusion: The results from our study indicate that less DNA can be purified form tissue samples processed using Xpress microwave based tissue processing compared to the conventional vacuum based method. Furthermore, it seems that DNA from VIP processed samples perform better in qPCR than DNA from Xpress samples do. Accordingly, before applying Xpress as a routine method for tissue processing it should be thoroughly considered whether subsequent molecular tests should be performed as these might be compromised by the processing procedure.

O-3-3

Comparison of two commercial kits for detection of codon 12 and 13 KRAS mutations in colorectal cancer

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Background: Patients carrying activating mutations of the KRAS gene do not respond to monoclonal antibody therapy targeting the epidermal growth factor receptor (EGFR). Therefore KRAS mutation testing is important in the clinical setting in order to select colorectal cancer (CRC) patients who will likely benefit from anti-EGFR treatment.

Aim: To evaluate the performance of two real-time PCR based commercial kits for detection of KRAS mutations using DNA extracted from archived formalin fixed paraffin-embedded (FFPE) CRC tissue.

Methods: DNA purified from 178 CRC patients was both subjected to the cobas® KRAS mutation kit and the Therascreen® KRAS mutation kit and analyzed according to the manufacturer’s instructions. Prior to DNA purification, all samples were subjected to tumour nuclei assessment according to the guidelines from Danish Molecular Pathology Group.

Results: While 81 specimens were found to be negative for KRAS mutations in both assays, three specimens were found Therascreen® KRAS negative but cobas® KRAS positive (concordance 96%). 71 specimens were detected as KRAS positive in both assays. One specimen was inconclusive using the cobas® KRAS kit whereas this sample was found mutated by use of the Therascreen® KRAS kit. Using the LightCycler® 480 (Hvidovre hospital) for Therascreen® KRAS analysis resulted in 18 inconclusive results. Of these, six specimens were KRAS positive while 12 specimens were KRAS negative using the cobas® KRAS system. In contrast, no inconclusive results were obtained using the ABI7500 platform (Herlev hospital).

Conclusion: Overall, both kits perform well
using FFPE samples with a tumour nuclei load ranging from 10% to 90%, thereby enabling the handling of the majority of clinical FFPE specimens. Both assay systems have been extensively used, though overall the laboratory performance of Therascreen is found to be limited compared to the cobas KRAS assay.

O-3-4
NGS analysis using the Ion AmpliSeq Colon and Lung cancer panel from Ion Torrent has a high sensitivity and good reproducibility.
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Introduction: Cancer is a multiage disease that arises as a result of mutational changes with activation of complex signaling networks. The use of mutation analysis as biomarkers for early cancer detection, staging and prediction has improved treatment of several cancer types. Next-Generation Sequencing (NGS) has made it possible to analyze multiple genes for clinically relevant somatic mutations. Using the PGM platform from Ion Torrent this analysis can be performed using a very small amount of DNA purified from tumor tissue and with a short analysis turnaround time. However, before implementing the NGS technology in a routine setting it is important to estimate the sensitivity of the analysis, and therefore the aim of the present study was to determine the limit of detection, which has a high reproducibility.

Material and methods: Analysis was performed using the Ion AmpliSeq™ Colon and Lung Cancer Research Panel, which contains 92 primer pairs covering 22 cancer related genes. As library template the AcroMetrix® Oncology Hotspot Control (Life Technologies) was used. This template library represents 53 genes at a frequency of 5-35 % and covers more than 500 mutations from the COSMIC database.
The Acrometrix control was diluted several times, using a colon cancer cell line, in order to determine the sensitivity of the analysis of all the different variants. All dilutions were made in 6 times replicates, and only considered positive, when all 6 replicates were positive. For each library 10 ng of DNA was used, and the analysis was performed on the PGM platform from Ion Torrent.

Results: For determination of the sensitivity 30 different libraries were analyzed, and the mean base coverage depth was 2636 (ranging from 1065-4570). 6 amplicons (genes PTEN, FGFR1, STK11 and ERBB2) were detected at a frequency of 5,1-5,5 %, but for the remaining variants tested a consistent frequency below 5 % was identified. For the target genes BRAF, EGFR, KRAS and NRAS the variants were detected at a frequency of 2,0-4,7 %.

Discussion and conclusion: The Acrometrix control is a useful tool to evaluate a large number of somatic variations in a large number of genes. Using 10 ng of DNA as template to the Ampliseq colon and lung cancer panel a sufficient mean base coverage depth was achieved. Furthermore, a high sensitivity and a good inter assay variation were observed. Studies analyzing the sensitivity and the specificity of the Ampliseq panel using formalin fixed, paraffin embedded tumor specimens are currently ongoing.

O-3-5
May miR-21 expression in HER2-positive breast cancer patients predict resistance to adjuvant trastuzumab?
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Introduction: Trastuzumab is established as standard care for patients with HER2-positive breast cancer both in the adjuvant and metastatic setting. However, approximately 50% of
the patients do not respond to the trastuzumab therapy, and therefore new predictive biomarkers are highly warranted. MicroRNAs (miRs) constitute an interesting group of biomarkers and their cellular expression can be determined in tumor samples by in situ hybridization (ISH) analysis. miR-21 is highly prevalent and up-regulated in breast cancer and has been linked to drug resistance in clinical and in vitro settings.

**Material and methods:** FFPE samples from high-grade breast cancers without clinical follow-up included 22 HER2-positive tumors and 15 HER2-negative tumors. Samples from patients with clinical follow-up included 16 cases with HER2-positive tumors that were treated with trastuzumab in the adjuvant setting of which 8 of the patients showed clinical recurrence and were considered resistant. Automated miR-21 ISH was performed using a chromogenic staining procedure, and the miR-21 ISH signal was scored in both tumor and stroma compartments.

**Results:** Histological examination indicated that patient samples could be divided into 3 major expression patterns: miR-21 predominantly in tumor stroma, predominantly in cancer cells or in both stromal and cancer cells. There was no obvious difference between the HER2-positive and HER2-negative tumors in terms of the miR-21 expression patterns and intensities. To explore the possibility that miR-21 expression levels and/or cellular localization could predict resistance to adjuvant trastuzumab in HER2-positive breast cancer patients, we analyzed the 16 HER2-positive tumors from patients that were treated with trastuzumab. Examination of the miR-21 expression patterns and intensities revealed no association between the miR-21 scores in the cancer cell population (p=0.69) or the stromal cells population (p=0.13) and recurrent disease after adjuvant trastuzumab.

**Discussion and conclusion:** Our findings suggest that elevated miR-21 expression does not predict resistance to adjuvant trastuzumab.

**O-3-6**

**MicroRNA expression differs between melanocytic nevi and melanoma – a microarray analysis.**


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**Introduction:** Melanoma is a potentially lethal cancer with increasing incidence. MicroRNAs (miRNAs) are short non-coding RNAs involved in diverse cellular functions - primarily by limiting the translation of different mRNAs. miRNAs are involved in the development and progression of a variety of cancers, including melanoma. To explore their role in melanoma, we used miRNA array analysis.

**Material and methods:** Tumour cores were sampled from 20 benign dermal melanocytic nevi and 20 melanomas (all formalin-fixed and paraffin-embedded) using a manual tissue arrayer. Tumour cores were stained by immunohistochemistry with melanocytic marker MART-1, to confirm that they contained adequate tumour material. Total RNA was purified from tumour core biopsies, and a dual-colour array analysis was conducted (Exiqon Services, Denmark), according to standard protocols. In brief, total RNA from all samples and a common reference pool were labelled with different fluorophores. The labelled samples and the reference RNA were mixed pair-wise and hybridized to the miRCURY LNA(TM) microRNA Array 7th Gen, containing capture probes targeting all miRNAs registered in the miRBase 18.0. Slides were then scanned and image analysis was carried out. The quantified signals were background corrected and normalized prior to statistical analysis.

**Results:** In principal component analysis, the samples mainly cluster according to their biological group (except for 2 samples). With an absolute log fold change larger than 1, an adjusted p-value below 0.05, and with standard signal intensities, the profiling analysis identified 77 differentially expressed miRNAs, e.g. miR-21-5p and miR-125b-5p.
Discussion and conclusion: Our study shows that miRNA expression differs between melanocytic nevi and melanoma. Many miRNAs show up- or (primarily) down-regulation in melanoma when compared to benign melanocytic nevi, suggesting an important epigenetic role in melanoma development. We will further validate these results by qPCR and in situ hybridisation in new tissue cohorts, in order to begin to establish their possible biological significance and tissue distribution.

O-3-7
Impact of sample tumor cellularity on microRNA expression levels in colorectal cancer tissue.
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Introduction: Numerous studies have identified several specific miRNAs linked to progression and prognosis in various cancers including colorectal cancer (CRC). However, different studies of specific miRNAs and CRC are not concordant, which may in part be attributable to intratumoral heterogeneity. Wang et al generated an algorithm including miR-92a, miR-375 and miR-424 expression levels to discriminate CRC from high-grade intraepithelial neoplasms in colorectal biopsies (1). The aim of the present study was to assess the variation of miR-92a, miR-375 and miR-424 expression levels in samples of CRC tissue, and to evaluate whether the variation and sample tumor cellularity would influence results.

Material and methods: A retrospective study on archived formalin fixed paraffin embedded tissue from 9 patients with early metastatic CRC (pT2 N+). From each tumor 5 selected areas representing luminal, central and invasive border zones were macro-dissected from 40 μm sections, giving 45 samples from which total RNA was extracted. Expression levels of miR-92a, miR-375 and miR-424 were analyzed by quantitative Real-Time PCR. Tumor cellularity was estimated from corresponding HE-stained sections.

Results: We found a considerable variation within tumors as well as between tumors for all three target miRNAs. For miR-92a and miR-375 we found a highly significant correlation between expression levels (ΔΔCt) and tumor cellularity (miR-92a: p=0.002, miR-375: p=0.002, miR-424: p=0.002).

Despite variation in miRNA expression levels, the majority of the investigated colorectal cancer samples were classified correctly as cancer, using Wangs algorithm. Samples at risk of being wrongly classified were all samples with a carcinoma cell proportion less than 50%.

Discussion and conclusion: miRNA profiling might aid in CRC diagnostics and prognostics. Our study shows that the expression levels of miRNAs may vary within the same CRC as well as between CRCs and that the proportion of carcinoma cells in tissue samples may further affect the measured expression levels of specific miRNAs. To ensure correct sampling and characterization of diagnostic tissue selected for molecular analyses, a close collaboration between molecular biologists and pathologists is mandatory. We found that knowledge of possible variation in miRNA profiles is crucial if miRNAs should be of future value in cancer diagnostics.

O-3-8
Do clinicians take action on the results of mismatch repair analyses in colorectal cancer?
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Introduction: The incidence of colorectal cancer (CRC) in Denmark is estimated to around 4200 per year. Lynch syndrome, defined by a hereditary defect in one of the four mismatch repair (MMR) genes, is the underlying cause in around 2 % of the cases. The Danish Colorectal Cancer Group recommends that immunohistochemical MMR protein analyses are performed on all CRCs to help identify hereditary cases. In case of lack of expression of the MMR proteins it is re-
commended that the patient should be referred to genetic counseling. However, in case of lack of expression of MLH1 the probability of the cancer being sporadic and caused by hypermethylation of the MLH1 promoter is high and a methylation- or BRAF- mutation analysis can be performed to further guide the clinicians. The aim of this study was to investigate whether the clinicians take action on the results of the MMR analyses.

**Material and methods:** All patients with a CRC with lack of MMR protein expression diagnosed at the departments of pathology at Hillerød and Herlev Hospitals during a 2-year period from 01.08.12 to 31.07.14 were included, in total 173 patients. In 76 of the 173 cases hypermethylation or BRAF-mutation had been shown, advocating for sporadic etiology, leaving 97 cases for further investigation. Case records on these patients were read to evaluate whether the clinicians had reflected on the results of the MMR analyses.

**Results:** Of the 97 cases 77 had loss of MLH1/PMS2, 2 cases had loss of PMS2, 8 cases had loss of MSH2/MSH6, 5 cases had loss of MSH6 and 5 cases had loss of MLH1/PMS2/MSH6. Of the 77 cases with MLH1 loss, lack of hypermethylation had been shown in 4 cases. Reflection was noted in the case record in 19/77 cases with MLH1/PMS2 loss, in 1/2 cases with PMS2 loss, in 4/8 cases with MSH2/MSH6 loss, in 2/5 cases with MHS6 loss and in 1/5 cases with MLH1/PMS2/MSH6 loss. In total 70 of 97 (72%) MMR results had not been reflected upon. In particular, 3/4 cases with proven lack of hypermethylation was not acted upon.

**Discussion and conclusion:** The decision whether a patient should be referred to genetic counseling rests with the clinician in agreement with the patient. MMR protein analyses including methylation analyses are performed to help clinicians in their assessment and guidance of patients, but the current study shows that in a substantial fraction of cases with potentially hereditary background, the results of the MMR analyses were not acted upon. The reasons for lack of action are various. A continuous and clear communication from pathologists to clinicians on performed molecular analyses and the possible impact of the outcome might increase clinicians' awareness and ensure that relevant ac-
O-4-1
Lesions of uncertain malignant potential (B3) on core needle biopsy of the breast
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Introduction: According to recommendations from DBCG core needle biopsies (CNB) of the breast are categorized in 5 groups ranging from unsatisfactory/not representative material (B1) to malignant (B5). The B3 category comprises different lesions with an uncertain malignant potential, which in the majority of cases are resected. In this study we want to compare the initial CNB with the pathology of the surgical specimen in order to evaluate whether we might be able to reduce both the use of this inconclusive pre-surgical diagnosis and consequently maybe reduce the excision rate of these often screening detected lesions.

Material and methods: A retrospective review of all patients diagnosed with B3-lesions on percutaneous image-guided CNB performed between January 1st 2010 and October 31st 2013 and subsequently undergoing surgical resection.

Results: 169 of 6544 patients who underwent CNB in this period had B3-lesions. Of these 56 (35%) were upgraded to either carcinoma in situ or invasive carcinoma after surgical excision: 21 of 73 intraductal papillomas, 25 of 29 atypical ductal hyperplasias, 9 of 17 LCIS, whereas only 1 of 29 atypical fibroepithelial lesions (phyllodes tumour or cellular fibroadenoma) was upgraded to a malignant diagnosis.

Discussion and conclusion: The total upgrade rate (35%) in the present study is somewhat higher compared with similar studies in the literature on this issue, reporting underestimation of malignancy in 20% on average. The highest upgrade rate was found in the subgroup representing ADH, 86.2% confirming the prudence of excising these lesions. The subgroup with intraductal papillomas turned out to have the lowest upgrade rate (22.3%), and consequently the group in which most benign lesions were unnecessarily surgically removed. The presence of atypia increases the upgrade rate. Even though we had used immunohistochemical staining in half (11) of the 21 core biopsies, that turned out to be malignant in this group, we still couldn’t predict the malignant outcome, neither could we be certain, that the histology remained benign in the large subgroup (73/94), which was not upgraded. Fibroadenomas and phyllodes tumour were difficult to differentiate on CNB, which is in keeping with the literature.

In conclusion, this study showed that B3 lesions in our institution represent a high number of malignant lesions after excision. Consequently surgical excision should be performed on the majority of B3 lesions for definite diagnosis. Finally it must be emphasized that correct pathological diagnosis is highly dependent on correct image guided needle localisation, the technical quality of the biopsy and both size and number of biopsies from the specific breast lesion.

O-4-2
Sentinel Node Biopsy in Breast Cancer Tumours ≤ 10 mm
Anne Marie Bak Jylling, Dept. of Pathology, Odense University Hospital, on behalf of the Multidisciplinary Breast Cancer Team OUH. Dept. of Pathology, Odense University Hospital

Introduction: Sentinel Node (SN) biopsy is well established in staging the axilla in breast cancer patients without clinical signs of metastasis. Frozen section during operation is normally done as to take advantage of the possibility of immediate axillary clearing in patients diagnosed with tumour spread. According to recommendations from Danish Breast Cancer cooperative Group (DBCG)
only patients with macrometastasis or involvement of three or more lymph nodes with smaller infiltration needs clearing.

Frozen section is time consuming and as metastatic spread from small tumours < 10 mm is rare, the purpose of this study was to evaluate a setup where frozen section for those small tumours was not performed.

**Materials and methods:** All patients from 1\textsuperscript{th} of January 2014 until 31\textsuperscript{th} of August 2014 with ultrasound estimated tumour size ≤ 10 mm with sentinel node biopsy performed without frozen section was evaluated.

**Results:** Of 40 patients with no frozen section performed, 33 had no spread to the sentinel node(s). The remaining seven patients showed two with single cell infiltration, one with micrometastasis and four with macrometastases. Two of those tumours ended up as being much larger than 10 mm (20 and 24 mm). 20 patients with “only” ductal carcinoma in situ (DCIS) had also SN performed without frozen section (and without tumourspread).

Of 213 patients with any kind of SN in the investigated period 16 had single cell infiltration, 14 micrometastasis and 27 macrometastasis. Of those 14 had single cell infiltration, 13 micrometastasis and 20 macrometastasis. Only one “Late positive SN” among these was discovered.

**Discussion and conclusion:** Avoiding frozen section for smaller breast cancer tumours is shortening anaesthesia for the patients and time sparing for surgery- as well as pathology-staff in far most cases. The risk of having macrometastasis with a tumour size > 10 mm almost doubles in our material. Taking in concern, that only very few patients needs reoperation in the smaller tumour size group and the above mentioned sparings in an economic as well as manpower challenged healthcare system, it seems to be justified not to perform frozen section SN on the smaller sized breast cancers. At the same time efforts to select the tumours properly in size and ultrasound staging of the axilla before operation have to be improved as much as possible. Not performing frozen section on patients with DCIS (other than specifically selected cases) also seems to be time sparing.

**Discussion and conclusion:**

Avoiding frozen section for smaller breast cancer tumours is shortening anaesthesia for the patients and time sparing for surgery- as well as pathology-staff in far most cases. The risk of having macrometastasis with a tumour size > 10 mm almost doubles in our material. Taking in concern, that only very few patients needs reoperation in the smaller tumour size group and the above mentioned sparings in an economic as well as manpower challenged healthcare system, it seems to be justified not to perform frozen section SN on the smaller sized breast cancers. At the same time efforts to select the tumours properly in size and ultrasound staging of the axilla before operation have to be improved as much as possible. Not performing frozen section on patients with DCIS (other than specifically selected cases) also seems to be time sparing.

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Avoiding frozen section for smaller breast cancer tumours is shortening anaesthesia for the patients and time sparing for surgery- as well as pathology-staff in far most cases. The risk of having macrometastasis with a tumour size > 10 mm almost doubles in our material. Taking in concern, that only very few patients needs reoperation in the smaller tumour size group and the above mentioned sparings in an economic as well as manpower challenged healthcare system, it seems to be justified not to perform frozen section SN on the smaller sized breast cancers. At the same time efforts to select the tumours properly in size and ultrasound staging of the axilla before operation have to be improved as much as possible. Not performing frozen section on patients with DCIS (other than specifically selected cases) also seems to be time sparing.

**Discussion and conclusion:**

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ods according to the national guidelines in Denmark and Sweden.

Material and methods: 15 breast pathologists evaluated the Ki67 staining (CONFIRM anti-Ki-67 Rabbit Monoclonal Primary Antibody 30-9) in a total of 120 circulated slides representing a consecutive series of malignant breast tumors. Each pathologist evaluated the slides by light microscopy. The staining interpretation was performed according to the Danish and Swedish guidelines, respectively. In brief, the Danish interpretation guidelines recommend a semi-quantitative evaluation of Ki67 hotspot areas with notation of the percentage of Ki67 positive invasive tumor cells (assessment score). The Swedish interpretation guidelines recommend calculation of 200 invasive tumour cells in hotspot areas with notation of the number of Ki67 positive tumour cells in percentage (counting score). Intraclass correlation (ICC) was used as a summary measure of inter-observer reproducibility and was determined for logit-transformed scores according to Polley et al. (2013). The ICC has a range of 0-1, with 1 denoting the highest agreement. The results were dichotomized with a Ki-67 cut-off at 20% according to St. Gallen 2013. Also, Light Kappa, which equals the average of all possible bivariate Kappas between observers, was determined.

Results: The ICC was 0.84 for the assessment scores and 0.86 for the counting scores (Ki-67 cut-off at 20%). For assessment scores Light Kappa was 0.65 and for counting scores Light Kappa was 0.64. The mean Ki67 positivity (%) for the assessment score was 30% and for the counting score 31%.

Discussion and conclusion: The results of this study demonstrate an only moderate agreement between observers. Further, no major difference was observed by applying either the assessment method or the calculation method. In conclusion, Ki67 interpretation by light microscopy is not reliable, resulting in altered sample classification which might have an impact on treatment selection.

Introduction: Diagnosis of prostate cancer at an early stage is crucial for patient prognosis. Identification and exploration of new immunohistochemical markers is necessary for optimizing the pre- and postoperative treatment of prostate cancer patients. A recent study demonstrated overexpression of PAX2 protein in human metastatic prostate tumors, and we therefore examined the correlation between PAX2 protein expression and prostate cancer stage.

Material and methods: Fifty patients diagnosed with different stages of prostate cancer at Department of Urology and Department of Pathology, Rigshospitalet, Copenhagen, Denmark, were included. These patients included 20 patients without lymph node metastasis at diagnosis, but with recurrence after radical prostatectomy, 20 patients without lymph node metastasis at diagnosis and without recurrence and 10 patients with lymph node metastasis at diagnosis. The diagnostic biopsies were reevaluated in order to ensure diagnosis and Gleason score. Clinical data, including treatment, biochemical recurrence and survival, were extracted from a clinical database containing all prostate cancer patients treated at Rigshospitalet since 1995. Immunohistochemical staining for PAX2 was performed on formalin-fixed and paraffin-embedded sections with a thickness of 3-4 µm, using a specific, monoclonal antibody and polymer visualization technique by Ventana/Roche. Normal human kidney tissue was used as control. All slides were assessed by two pathologists.

Results: Previous studies showed that normal prostate epithelial cells have no immunoreactivity for PAX2. In this study, PAX2 was evaluated as a prognostic marker of prostate cancer progression by immunohistochemical technique. The control group with normal kid-
ney tissue showed as expected positive PAX2 immunoreactivity. In contrast, none of the 50 patients demonstrate PAX2 immunohistochemical positive reactivity in areas with prostate cancer. This was irrespective of lymph node stage or experience of recurrence.

Discussion and conclusion: Our study demonstrated that dissemination of prostate cancer cells is not correlated to the expression of PAX2. Thus, PAX2 does not seem to be applicable as a prognostic marker of prostate cancer.

O-4-5

ERG protein expression over time - from diagnostic biopsies to radical prostatectomy specimens in clinically localized prostate cancer.

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Introduction: It has been shown that ERG protein expression harbours prognostic value in prostate cancer (PCa). However, the impact of sampling error and the extent of reclassification in ERG-status over time due to tumour heterogeneity is unknown. Consistency in ERG protein expression from diagnostic specimens through re-biopsies to radical prostatectomies (RPs) was evaluated in patients with clinically localised PCa initially managed on active surveillance to investigate the validity of ERG-status in biopsies. Moreover, the concordance between fluorescence in situ hybridization (FISH) assessment of TMPRSS2-ERG rearrangement and immunohistochemistry (IHC) analyses for ERG protein expression was analysed.

Material and methods: From the active surveillance program, 265 patients followed with PSA measurements, digital rectal examinations, and 10-12-core re-biopsies were included. Based on protocolled progression criteria, 86 patients underwent RP. Included tissue samples consisted of 625 biopsy-sets and 86 RP specimens. Five tissue micro array (TMA) blocks were constructed from malignant foci in the RP specimens. ERG protein expression was assessed by IHC in new sections from tumour-containing biopsies and RP samples (anti-ERG, clone: EPR3864; Roche/Ventana). Patients were labelled ‘ERG-positive’ if all foci demonstrated ERG expression and ‘ERG-negative’ if all foci were negative. Patients with both positive and negative foci were labelled ‘ERG-heterogeneous’. A triple-colour TMPRSS2-ERG FISH assay (FISH ZytoLight® TriCheck™ Probe, SPEC ERG/TMPRSS2; Zytovision) was applied to 74 biopsies according to manufactures instructions. FISH results were dichotomised into presence or absence of ERG rearrangement and were correlated with the IHC findings.

Results: The concordance between FISH (+/- ERG rearrangement) and IHC (+/- ERG protein expression) was 97.3%. IHC demonstrated a sensitivity and specificity for ERG rearrangement of 100% and 95.5%, respectively. Applying IHC in diagnostic specimens showed that 38.1% of patients were ERG-positive, 53.6% were ERG-negative, and 8.3% were ERG-heterogeneous. If ERG-status was dichotomized (ERG-positive or heterogeneous versus ERG-negative), 88.2% of patients did not experience ERG reclassification during up to four rounds of re-biopsies. The concordance in ERG-status between biopsies and RP specimens was 89.5-94.2% depending on the number of re-biopsies included. Sampling bias was assumed to explain most (81.3%) of the mismatches in ERG-status.

Discussion and conclusion: Consistency in ERG-status ranged from 90-95% for patients undergoing serial biopsies and RP. This indicates that biopsies can be used reliably to investigate ERG’s prognostic and predictive value.
Myc protein expression is significantly correlated to plasmablastic morphology, tumor burden and high proliferation index in 193 untreated patients with Multiple Myeloma.

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Introduction: Prognostic markers in Multiple Myeloma (MM) are t(4;14), t(14;16), del(17p) and del(13q) detected at metaphases, International Staging System (ISS) score (based on serum levels of albumin and beta-2-microglobulin), proliferation index (PI) and plasmablastic morphology. Myc oncogene has a pivotal role in cell growth and proliferation, but the prognostic impact of myc activation in MM is controversial. Myc activation is reported in 10-67% with MM depending on the method used e.g. karyotyping, fluorescence in situ hybridisation (FISH) or gene expression analysis. A commercial myc antibody is now available, which makes myc protein expression easy to detect. The aim of this study was to describe the interdependency of myc protein expression and prognostic features in MM.

Material and methods: We retrospectively analysed bone marrow samples from 193 untreated patients diagnosed with MM between January 1st 2006 and June 30th 2010 in the Region of Southern Denmark. Clinical, karyotyping and FISH data was collected. Myc protein expression was assessed using immunohistochemical double staining for the plasma cell marker CD138 (red membrane staining) and myc (brown nuclear staining). Plasmablastic/plasmacytic morphology, percentage of plasma cell infiltration in bone marrow and PI was assessed, the latter using immunohistochemical double staining for CD138 and Ki-67.

Results: Myc protein was expressed in 177 cases (92%) with a range of 1-65% MYC-positive plasma cells. In 74 cases (38%) ≥ 10% myc-positive plasma cells was present and correlated significantly with plasmablastic morphology (pmyc-positive plasma cells compared to 44% of symptomatic MM (p=0.001).

Discussion and conclusion: In this study, we assessed myc expression in 193 untreated patients with MM and found significant correlation of myc with adverse prognostic features e.g. high ISS score, plasmablastic morphology, high PI and high percentage of plasma cell infiltration in bone marrow. Thus, myc overexpression appears to be a feature of aggressive disease. Analysis of survival data is ongoing and will be presented.

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O-4-7
Ex vivo dermoscopy with “derm dotting” in routine dermatopathology practice: our experience in 1 year
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Introduction: Correlation to clinical information and dermoscopy is important in accurate histopathology diagnosis of atypical melanocytic lesions. The idea of good collaboration between physician and pathologist is sadly often remote from reality. Ex vivo dermoscopy (EVD) is an alternative for this missing link. The aim of this presentation is to share our 1-year experience of applying EVD.

Material and methods: We capture images of formalin fixed skin specimens of atypical pigmented lesions and lesions suspect for melanoma. A Dermlite II dermoscope mounted on a Nikon Coolpix 995 camera is used. We prefer non-contact way of imaging. Different colours of nail varnish (“derm dotting”) is applied to mark focal hyperpigmented or depigmented areas as well as areas exhibiting a diffuse pattern of deeply located pigment. This aids selection of cut level and links gross examination to microscopy.

Results: We have saved EVD images of over 300 specimens until now. Derm dotting was applied in approx. 20% of cases and in
approx. 20% of these, the marks helped highlight crucial focal changes such as deepest invasion level or focal development of melanoma or dysplastic nevus. A difficulty to adjust focus in some areas of the lesion is a limitation we sometimes experience.

Discussion and conclusion: Dermoscopy is known to increase sensitivity and specificity when diagnosing melanocytic lesions clinically. The dermoscopy images as supplement to histopathology examination provides useful information and helps localizing focal changes. There have been different attempts to highlight such focal changes: ink, sutures or punch incisions. EVD on formalin fixed specimens is shown to be as effective, when accepting that vascular structures cannot be demonstrated. In our lab we routinely dot focal changes with multiple colour nail varnish as described by Haspeslagh et al. (2013). The method is cheap, simple and not time consuming. It increases overall interobserver agreement, helps assessing surgical margins, minimizes sampling errors and possibly reduces recut rate. Furthermore it gives us a better insight in clinical suspicion and request as well as the nature of melanocytic lesions. In our opinion the benefit of using EVD and derm dotting outweighs the resources used. And not the least, we think it is fun!

O-4-8

Patternning of renal medullary vasa recta bundles take place in a narrow developmental window during early third trimester of the human pregnancy

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Introduction: Renal medullary microvessels consist of vasa recta bundles and peritubular capillaries. The structural organization of the vasa recta bundles is necessary for normal urinary concentration of solutes and blood pressure regulation. Only little is known about fetal development of this microvascular bed. In a rat model, we have shown that vasa recta bundle formation takes place in a narrow developmental window during postnatal kidney development corresponding to the third trimester of the human pregnancy. The aim of the study is to investigate the developmental time course of vasa recta bundle formation in human fetal kidney tissue.

Material and methods: The treatment biobank at Department of Pathology, Odense University Hospital was searched for autopsies of fetuses with a gestational age (GA) within late second (GA 21-26 weeks) and third trimester (GA 27-42 weeks). A total of 95 second trimester and 121 third trimester autopsies performed within the time period 2000-2013 were identified. Fetuses with known chromosomal abnormalities or kidney developmental defects were excluded. Maceration was evaluated in hematoxylin and eosin stained tissue sections and only autopsies with viable kidney tissue samples were included. Immunohistochemical staining of the endothelial cell marker CD31 was performed and kidney tissue evaluated for vasa recta bundle formation.

Results: A total of 13 kidneys from late second and 29 kidneys from third trimester were evaluated. During late second trimester (GA 21-26), single vasa rectae were observed in the renal outer medulla, but no vascular bundle formation was seen at any time point. At the beginning of third trimester (GA 27-28), immature vascular bundles containing a low number of vasa rectae appeared in the outer medulla. At later developmental stages, expansion of bundle size was observed with an increase in microvascular number within each bundle. In late third trimester, no difference in bundle size from adult kidney tissue was observed.

Discussion and conclusion: Patterning of human renal medullary vasa recta bundles take place in a narrow developmental window in early third trimester (GA 27-28) followed by expansion of bundle size to the adult configuration by the end of third trimester.
P-1-01
How to improve the endoscopists’ recording of colorectal (CR) polyp-data.
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Introduction: The Danish CR cancer screening program follows national guidelines. Those guidelines were supplemented with a check list on the requisition form to be filled in by the endoscopist in order to provide the pathologist with relevant data. This template was developed by the DCCG (Danish Colorectal Cancer Group) Pathology Group in order to ensure standardization in requisition and reporting of any CR polyp removed, including nonscreening polyps. The template contains 6 parameters (screening status, procedure, location, gross profile, size, entire polyp removed/submitted), necessary for correct risk stratification and patient surveillance, and for collection of true statistical data. In our initial assessment upon the onset of screening, only about one fourth of requisitions provided all relevant data. Our aim is to assess how to help clinicians in providing the correct data, thereby facilitating the pathology reporting, ultimately optimizing the patient management.

Material and methods: Material consists of all requisitions from CR polyp specimens (n=272; 151 from Hospital staff (HS), 121 from practitioners (PR)), processed in our department, Sept. 2014. All HS and PR requisition forms were reviewed and it was examined to what extent required data were present with/without use of template. Our method follows the Shewhart/Deming quality cycle “Plan-do-study-act”, in which we use an intervention centered on “the good HS” as a role model followed by a direct dialogue with all available HS. PR intervention has been based on meetings and dialogue. To record any improvement, a review medio Dec. followed the intervention. This produced 20 HS and 14 PR requisitions.

Results: Before the intervention, the template was used consistently/sporadically, respectively, by only 2 HS, both correctly (12% of HS requisitions). 16% of PR requisitions provided all parameters correctly. In our dialogue with HS during the intervention, 76% mentioned lack of knowledge as the reason for not following the guidelines. At follow up after the intervention, HS used the template correctly in 90% of requisitions, and PR provided all relevant data in 50% of requisitions. In 10% of cases HS still failed to use the template, which implied lack of some relevant data. The dialogue also produced a proposal to improve the template (adding a box to record whether the endoscopy was triggered by a prior screening colonoscopy).

Discussion and conclusion: This investigation shows that the quality cycle can be of help to produce a meaningful intervention in close contact with clinicians, thereby responding to the expectations in the guidelines. We conclude that adhering to guidelines is generally accomplished by dialogue, and further demonstrate the utility of the template.

P-1-02
The prevalence of serrated lesions of the appendix in women with mucinous or serous neoplasm of the ovary
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Introduction: Ovarian epithelial cancers have been found to be associated with specific mutations including KRAS and BRAF as well as mismatch repair deficiency. Some of these mutations have also been linked to the serrated neoplastic pathway in colorectal cancers. A high prevalence of serrated lesions (SLs) in the appendix in case of an ovarian epithelial cancer could thus be expected theoretically. The aim of this study was to determine the prevalence of SLs in the appendix of women with a primary ovarian mucinous or serous neoplasm.

Material and methods: A retrospective
cohort of all patients from Herlev, Glostrup and Gentofte Hospitals with resection of a primary ovarian mucinous neoplasm (M84803, M84703, M84701, M84700, M90150, M90151) or a primary ovarian serous neoplasm (M84611, M84613, M84411, M84410, M90141, M90140, M84413) and an appendectomy at time of surgery between 2000 and 2013 was identified from the histological database Patobank. Microscopy of the appendix was revised by two pathologists with respect to the presence of serrated lesions, which were classified pathologically according to the WHO’s criteria as hyperplastic polyp (HP), sessile serrated adenoma/polyp (SSA/P) with or without cytological dysplasia, or traditional serrated adenoma (TSA) regardless of initial diagnosis.

A total of 162 patients were identified, of which 73 patients had a mucinous neoplasm and 88 patients a serous neoplasm of the ovary.

**Results:** Among patients with a mucinous ovarian neoplasm 10/73 (14%) had a SL in the appendix, subclassified as 2 hyperplastic polyps, 2 TSA and 6 SSA/P (one with dysplasia), whereas 5/89 (6%) of patients with a serous ovarian neoplasm had a SL, subclassified as 1 hyperplastic polyp, 1 TSA and 3 SSA/P (none with dysplasia). The prevalence of the 2 groups was not significantly different (P=0.10).

**Discussion and conclusion:** A recent study of patients who had undergone appendectomy due to inflammation showed a prevalence of SLs of 7%. This percentage corresponds to our findings among patients with an ovarian neoplasm indicating that there might not be any association between ovarian epithelial neoplasms and the presence of SLs in the appendix. Few studies have addressed the SLs of the appendix, however two recent studies have concluded that SLs differ from their colorectal counterparts and one of these found a low prevalence of BRAF and a high prevalence of KRAS mutations indicating a different serrated neoplastic pathway in the appendix. Undertaking molecular analysis of the SLs of the appendix and the corresponding ovarian serous and mucinous neoplasm in our study may elucidate whether the molecular pathways correspond.

**P-1-03**

**A questionnaire on the diagnosis of microscopic colitis in Denmark**

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**Introduction:** The diagnosis of microscopic colitis (MC) is a triad of chronic diarrhea, macroscopically normal endoscopy and characteristic histological findings. The histological criteria of MC (collagenous colitis (CC) and lymphocytic colitis (LC)), defined in the 1980s, are based on HE-stained sections, which according to recent European guidelines is sufficient to diagnose MC. Recently, another subset of MC, named incomplete MC (MCi), has been introduced, covering patients with prolonged diarrhea and normal endoscopy, but with biopsies not fulfilling the histological criteria of MC.

The purpose of the questionnaire is to register the gastrointestinal (GI) pathologists’ practice of staining and coding, when handling colorectal biopsies, with focus on MC/MCi. An additional purpose is to investigate awareness of the MCi subset.

**Materials and methods:** A questionnaire was mailed to 15 pathology departments in Denmark, addressed to those consultants responsible for the diagnostics of GI specimens. All 15 departments participated and a total of 48 GI pathologists contributed.

**Results:** Initial routine stainings applied to non-neoplastic colorectal biopsies: 71% of respondents use HE only, the remaining use HE combined with a connective tissue staining and / or mucous staining. Colorectal biopsies with histological suspicion of CC: 67% use a non-IHC connective tissue staining, 23% apply the tenascin (IHC staining) in addition to HE staining. Colorectal biopsies with histological suspicion of LC: 85% use IHC staining for T-lymphocytes (CD3) in addition to HE staining, 15% do not apply additional stainings.
Awareness of MCi: 50% report consistent use of the term “microscopic colitis obs. pro” indicating knowledge the MCi subset. SNOMED codes of MC / MCi: Codes for CC / LC are used consistently. Codes for MCi are presently not available, necessitating the use of less specific codes.

**Conclusion:** A 100% response rate was obtained, illustrating the respondents’ interest in this field. Histological suspicion of MC conspicuously increased the use of special stains/IHC. Considering the recommendations of the European guidelines the wide use of particularly CD3 is noteworthy. Awareness of the concept of MCi is widespread; however, in order to learn more about LC and LCi, we recommend an assessment of the utility of CD3 in differentiating LCi from LC and from non-specific colitis, respectively. We also emphasize the need for a code for LCi. It is our anecdotal experience that CCi less often is a differential diagnostic issue. To document or reject this statement a code for CCi is likewise warranted. Such undertaking could be the pathologists’ contribution to reduce the risk of missing patients with a treatable cause of diarrhea.

P-1-04

**Collagen colitis – do the clinicians provide relevant data?**

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**Introduction:** The diagnosis of collagen colitis (CC), which is based on the triad chronic non-bloody diarrhoea, no abnormality at endoscopy, and histological alterations (broadened (>10 µ) collagen table beneath the surface epithelium) is often straightforward. However, given the diversity of the microscopic findings, including more subtle features approaching those of incomplete CC (CCI) (collagen plate >7-10 µ), clinical information (symptoms, endoscopic findings, suggested diagnosis) become pivotal elements on the requisition form, as is detail on sampling site(s). Thus, a single or a few non-informative biopsies do not exclude CC and should in the appropriate clinical context prompt more extensive sampling. Additionally, a diagnosis of CCI should only be made in case of diarrhoea and normal endoscopy, especially if therapy decisions are influenced by the histological evaluation. Here we assess the extent of this issue, and further compare the performance of hospital staff with practitioners.

**Material and methods:** The pathology file of Hvidovre Hospital was searched for cases coded as CC and/or hyalinised fibrosis for the 30 month period January 2011 through June 2013, totaling 190 reports, all issued by GI-pathologists. Biopsies were provided by hospital staff (Medical Gatriunit) in 100 cases (group 1), by practitioners (GI-surgeons) in 90 cases (group 2). The pathology forms were consulted for clinical information.

**Results:** A clinical diagnosis was proposed in group 1 and 2 in 73% and 59% of the cases. Information on symptoms/endoscopic findings was recorded in 67%/61% in group 1 and in 62%/44% in group 2. Biopsy sites (any kind, i.e. distance from the anus, right vs. left colon, specification of segment(s)) were registered in 94% by group 1, in 87% in group 2. Specification of the subanatomical segments sampled was given in 16% and 13% by group 1 and 2.

**Discussion and conclusion:** The requisition forms uncommonly fulfilled all requirements. The hospital staff performed somewhat better than did the practitioners regarding information on endoscopic findings and suggested clinical diagnosis. In this context it may be relevant that contributors of the two groups differ in specialty. For both groups information on sampled segments was rarely provided. It may be reasoned that determination of the segment biopsied could be more problematic to ensure than the other site indications. Though the clinical data would not influence the diagnosis of histologically full-blown CC, these data may facilitate the interpretation of biopsies with subtle alterations, which in the appropriate context may sum up to CCI. Furthermore, symptoms often precede the fully developed histological picture. Hence, the need to encourage gastroenterologists to provide such data.
The Intestinal Stem Cell Marker SOX9 Predicts Relapse of Stage II Colon Cancer Patients
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Introduction: Approximately 25% of patients with stage I and stage II colon cancer relapse. To this date no optimal biomarkers can identify colon cancer patients with elevated risk of relapse. The aim of this study was to investigate the intestinal stem cell marker, SOX9, as a biomarker for identification of stage II colon cancer patients with high risk of relapse.

Material and methods: Paraffin-embedded tissues of primary tumors from 154 patients diagnosed with stage II colon cancer from January 2005 to August 2008 were consecutively included. The samples were further stratified by location of tumor and tumor mismatch repair (MMR) deficiency. MMR deficiency was analyzed by immunohistochemistry (IHC) and a methylation assay. The level of SOX9 was investigated by IHC both at the invasive front of the primary tumor and at the luminal surface.

Results: 24% of the included patients had relapse. MMR deficiency was shown in 23% of the tumors and primarily due to methylation of the MLH1 promoter. Tumor MMR deficiency was negatively associated with relapse (p=0.02). SOX9 levels at the luminal surface of the tumors were not associated with relapse. Low levels of SOX9 at the invasive front of the tumor was an independent predictor of relapse (p=0.02) when adjusted for age, gender, postoperativean chemotherapy, pathological risk factors, and MMR deficiency.

Discussion and conclusion: MMR deficiency is negatively associated with relapse of stage II colon cancer patients. Low level of SOX9 at the invasive front of the primary tumor is an independent predictor of relapse of stage II colon cancer patients and may justify more intensive follow-up of these patients.

Mesenteric Fibromatosis in a patient with a history of GIST: Report of a case
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Introduction: Though a rare mesenchymal neoplasm occurring in only 2-3 people/million/year mesenteric fibromatosis (MF) is the most common primary tumor of the mesentery. MF may form large tumors and tend towards aggressive, locally invasive growth. High rates of recurrence after surgical removal have been documented, whereas MF does not metastasize. Grossly MF may closely resemble gastrointestinal stromal tumors (GISTs) but at the microscopic level separating these entities is usually straightforward. Here we report a case-history of a patient previously diagnosed with a GIST of the esophagus now presenting with a mesenteric tumor.

Material and methods: A 72 year old male previously diagnosed with a GIST of the esophagus and urothelial carcinoma of the bladder (T1N1). Follow-up computerized tomography after chemotherapy treatment revealed a small bowel mesenteric mass and a tumor in the right kidney. Biopsies showed the renal tumor to be a papillary renal cell carcinoma but were inconclusive with regards to the mesenteric mass. The patient underwent surgery and had both tumors removed. After formalin fixation and cut-up, paraffin-embedding and tissue sectioning the mesenteric tumor was examined microscopically.

Results: Gross appearance: The cut surface of the tumor was homogenous, whitish with a whorl like pattern. It seemed circumscribed and well defined, had a firm consistency and measured 42 mm in diameter.

Histology: The tumor was fibromatous with varying cellularity and an infiltrative margin. Tumor cells were bland spindle or stellate cells with no or little atypia. They formed sweeping fascicles separated by dense collagenous stroma. Scattered keloid-type fibres and foci with myxoid stroma were seen. Thin-walled, dilated veins were quite prominent.
and a few thick walled arteries were noted. Inflammation, haemorrhage, cystic degeneration and necrosis was absent. Immunohistochemically tumor cells showed no expression of DOG1, CD117, CD34, actin, desmin, caldesmon, cytokeratins or S-100 (reaction for β-Catenin awaits). Proliferation by Ki-67 reactivity was

**Discussion and conclusion:** We present findings of mesenteric fibromatosis in a patient with a history of both a GIST of the esophagus, disseminated urothelial carcinoma of the bladder and papillary renal cell carcinoma. Radiologically and peroperatively the mesenteric tumor was considered another GIST and in fact MF is quite often misdiagnosed as GISTS. This case-report sheds light on the MF entity and reminds us that as populations live longer we will encounter more patients with histories of multiple types of tumors.

P-1-07

**Perivascular epithelioid cell tumor (PEComa) of the liver – a case report**

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**Introduction:** The perivascular epithelioid tumor (PEComa) family is a group of mesenchymal tumors which have perivascular epithelioid cells (PEC) in common. The WHO classified PEComas as a separate tumor family in 2002, comprising angiomyolipoma (AML) of the kidneys, clear cell "sugar" tumor and lymphangioleiomyomatosis of the lungs and PEComas not otherwise specified (PEComa-NOS), meaning similar tumors at various extrarenal and pulmonal sites. Classical AML shows PECs admixed with fat tissue, smooth muscle cells and abnormal thick-walled blood vessels and, in the liver, often also extramedullary haematopoiesis.

**Material and methods:** A 53-year-old woman with the BRCA-2 mutation had two years earlier undergone left-sided mastectomy due to estrogen receptor positive ductal breast cancer combined with prophylactic right-sided mastectomy. She was now scheduled for prophylactic salpingo-oophorectomy. At the preoperative contrast-enhanced computed tomography (CT) of the abdomen, a 3x2 cm hypovascular process in the liver was found. There was strong enhancement in the arterial phase and decreasing enhancement during the venous phase. The tumor was resected surgically.

**Results:** Macroscopically, a well-demarcated, non-encapsulated tumor of light grey colour measuring 2.3 cm in maximum diameter was observed. At microscopy, the tumor consisted of a monomorphic proliferation of relatively large epithelioid cells with an often clear, but focally eosinophilic granular cytoplasm. These cells were intermingled with spindle-shaped cells. Tumor cell nuclei were pleomorphic with often distinct nucleoli. The tumor cells were accompanied by numerous blood vessels. Fat tissue, thick-walled blood vessels and extramedullary haematopoiesis were lacking. Immunohistochemically, there was strong expression of HMB45, Melan A, smooth-muscle actin, vimentin and HNF-1B in most of the tumor cells. The following immunohistochemical markers were negative: CD10, CD34, CD45, CD56, CD117, CDX2, CK7, CK20, cytokeratin CAM5.2 & CKAE1/AE3, desmin, GFAP, glypican 3, hepatocyte antigen, inhibin, MDM2, myogenin, Pax8, p63, synaptophysin, S100, TTF1, villin, WT1 and estrogen receptor. Based on these findings, the diagnosis PEComa-NOS was made.

**Discussion and conclusion:** In this report of PEComa-NOS of the liver, there was a monomorphic proliferation of epithelioid cells, distinguishing this tumor from a classic AML. Such monomorphic tumors are rare in the liver. PEComas showing an infiltrative growth pattern, pleomorphism, atypical mitoses or necrosis should be regarded as borderline or malignant sarcomas. The significance of the association with the BRCA-2 mutation remains unclear, but we assume that it probably is unlikely that the BRCA-2 mutation predisposes to a non-epithelial malignancy.
Histological features of the pancreas in a patient with congenital hyperinsulinism due to Beckwith-Wiedemann syndrome
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Introduction: Beckwith-Wiedemann syndrome (BWS) is a genetic disorder with typical features such as macroglossia, abdominal wall defects, macrosomia, visceromegaly and embryonal tumors. Hypoglycemia is reported in about half of all newborns with BWS, usually resolving spontaneously within the first month of life. However, 5% suffer from severe and prolonged hypoglycemia necessitating intensive medical treatment, and a small number of these patients need a near-total pancreatectomy. Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycemia in newborns, most frequently caused by changes in the genes ABCC8 and KCNJ11, coding for the ATP-dependent potassium channel of the pancreatic B cell.

Material and methods: A premature girl born at 33 weeks of gestation had severe, early-onset hypoglycemia, non-responsive to intravenous glucose, diazoxide, octreotide and glucagon, necessitating 95% pancreatectomy at 20 days of age. The patient also had an abdominal wall defect, hemihypertrophy and visceromegaly. Today, at the age of four years, she only needs a small dosage of diazoxide.

Results: A preoperative F-18-DOPA PET/CT scan showed diffuse uptake of the radiotracer throughout the entire pancreas. Genetic testing revealed paternal uniparental disomy of the entire chromosome 11, consistent with BWS, while ABCC8, KCNJ11 and other known CHI genes were normal. The left-sided resection specimen measured 10x20x70 mm. Histologically, confluent small islets and trabeculi of endocrine cells with uniform nuclei and sparse cytoplasm were observed throughout the pancreas. Most of the endocrine cells expressed insulin, while cells positive for glucagon and somatostatin were observed at the periphery of the confluent trabeculi and islets. The endocrine cells occupied more than 50% of the parenchyma. The entire resected tissue showed these changes, as opposed to classical focal CHI which usually presents as a focal adenomatous hyperplasia measuring 2-10 mm. Acinar cells and small ducts were mainly confined to the periphery of the pancreatic lobules. In addition, a focus of pancreatoblastoma measuring 3x1 mm was noted.

Discussion and conclusion: In this report of a premature neonate with severe CHI due to BWS requiring near-total pancreatectomy, we found diffuse adenomatous hyperplasia of endocrine cells. These microscopic features differ from the focal, diffuse and atypical forms of CHI. We were able to identify only 11 similar cases in the published literature. The combination of these changes associated with a focus of pancreatoblastoma, however, has only been reported once before. The patient is on continuous follow up, mainly due to the risk of development of other embryonal tumors.

P-1-09
Mixed acinar-neuroendocrine carcinoma of the pancreas: a case report and a review
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Introduction: The majority of malignant pancreatic neoplasms can be categorized into three distinct types: ductal adenocarcinoma, acinar cell carcinoma (ACC) and neuroendocrine neoplasm. Mixed exocrine and neuroendocrine pancreatic tumors are rare, and in most instances the exocrine component consists of an adenocarcinoma. We report a case of a mixed acinar-neuroendocrine carcinoma (MAEC) of the pancreas which is a particularly rare entity. Moreover, we reviewed the previously published cases, focusing on...
age and gender of the patients and localization and size of the tumors.

**Material and methods:** A 62-year-old woman presented with abdominal discomfort. Pancreatic exocrine and endocrine symptoms were absent. Abdominal contrast-enhanced CT showed a tumor in the pancreatic tail. The patient underwent a left-sided pancreatic resection with splenectomy. She is currently undergoing postoperative adjuvant chemotherapy with gemcitabine targeting the acinar cell carcinoma component. At present, 5 months after her operation, there is no evidence of recurrence.

**Results:** Macroscopically, a tumor in the pancreatic tail was observed, measuring 5.2 cm in diameter. The cut surface revealed cystic, solid, and hemorrhagic areas. At microscopy, the tumor was well-circumscribed and entirely encapsulated. Some of the tumor cells in the cystic areas were reminiscent of acinar cells. The majority of the tumor cells were arranged in a solid growth pattern. Immunohistochemistry revealed > 30% positivity for chromogranin, synaptophysin, CD56, CD10, chymotrypsin, CK8 and, nuclearly, beta-catenin. The Ki-67 index was 22%. There were 5 mitoses per 10 HPF. The following immunohistochemical markers were negative: alpha-1 antitrypsin, CK7, CK20, gastrin, glucagon, insulin, progesterone receptor, serotonin, somatostatin, somatostatin receptor 2, vasoactive intestinal polypeptide (VIP) and vimentin. The postoperative diagnosis of a MAEC was made. Review of the English literature revealed 40 previously published cases of resected MAECs. These patients had a mean age of 61.8 years (range 16-89 years) and 56% were men. In 56% the tumor was located in the head and in 32% in the tail. The mean tumor size was 7.1 cm (range 1.2-19.5 cm).

**Discussion and conclusion:** Compared to classical ACC, patients with MAECs have a slightly higher age, are less frequently males and the tumors are more often located in the pancreatic head. The histogenesis of MAEC is still controversial. Due to the small number of cases it is not possible to define an optimal treatment for these patients, but chemotherapy directed against the acinar cell carcinoma component is used in most patients.

**P-1-10**

**Sporadic desmoid tumor of the pancreas**

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**Introduction:** Desmoid tumors, also known as deep fibromatoses or desmoid-type fibromatoses, are clonal fibroblastic proliferations arising in the deep soft tissues and characterized by infiltrative growth and a tendency towards local recurrence. These tumors do not metastasize. About half of the cases are located intra-abdominally, most often in the pelvis and mesentery.

**Material and methods:** A 63-year-old female complained of nonspecific and intermittent abdominal pain for 6 months. A CT urography was performed which showed a 5.1 x 4.8 cm solid tumor in the pancreatic tail. There were no radiological signs of metastasis. The patient was successfully treated by a left-sided, spleen-preserving pancreaticectomy. The resected tissue was examined by conventional microscopy and immunohistochemistry. Gastroduodenoscopy and colonoscopy revealed no polyps.

**Results:** The resected mass appeared spherical and well-circumscribed, measuring 4.8 x 5.8 x 5 cm. It had a homogenous and pale, grey-white color and did not show any signs of bleeding or necrosis. The cut surface had a whirling texture of the tissue. At microscopy, the tumor cells had a spindled morphology with indistinct cell membranes and were organized in whirled and storiform formations. The nuclei were elongated and cigar-like with tapered ends. The tumor cells were accompanied by thin fascicles of collagenous tissue. No giant cells or increased inflammatory cells were observed. Less than 1 mitosis per 10 high power fields was observed. The tumor was non-encapsulated but clearly delimited from the pancreatic parenchyma. Only focally, a few tumor cells infiltrated around islets of Langerhans. Numerous small- to medium-sized vessels were observed throughout the tumor. By immunohistochemistry, the tumor cells showed strong nuclear expression of beta-catenin and were
positive for vimentin and CD-10. There was weak expression of Factor XIIIa, calponin, osteonectin and, very focally, nestin. The tumor cells were negative for the following markers: ALK-1, Bcl-2, CD-34, CD-68PG, CD-117, cytokeratin CK AE1/AE3, desmin, DOG1, EMA, GFAP, HMB45, MDM2, Melan A, smooth-muscle myosin heavy chain (SM-MHC) and S100.

**Discussion and conclusion:** This report of a sporadic desmoid tumor of the pancreas shows that this entity has to be considered in the differential diagnosis of mass-forming lesions in the pancreas. It is, however, a rare tumor in the pancreas, highlighted by the fact that only 16 cases have been previously reported. Sometimes, desmoid tumors are associated with familial colorectal polyposis (Gardner’s syndrome). However, colonoscopy revealed no colorectal polyps in our patient. As opposed to the syndromal types, recurrences are unlikely in resected sporadic pancreatic desmoid tumors with clear margins.

**P-1-11**

**Different KRAS statuses in synchronous colorectal cancer**

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**Introduction:** Treatments with monoclonal antibodies targeting the epidermal growth factor receptors (EGFR) have improved the prognosis of metastatic colorectal cancer (CRC). Analysis of KRAS status allows the selection of patients with KRAS wildtype (WT), who will benefit from the targeted therapies.

**Material and methods:** A 55 year old man presented to his general physician a history of rectal bleeding and change of bowel habits through a year. No familial history of cancer was reported. Sigmoidoscopy revealed two polyposous tumours (oral tumour, T1 and anal tumour, T2) in sigmoidum, only 5 mm apart. Biopsy revealed adenocarcinoma. On computed tomography (CT) scan of the body there was no sign of disseminated cancer. The patient underwent laparoscopic rectosigmoid resection.

**Results:** Pathological examination revealed 2 well-differentated adenocarcinomas, both T3N2V0M0. Immunohemistry for mismatch repair genes for both tumours did not reveal any evidence for hereditary non-polyposis CRC. The patient underwent 4 cycles of adjuvant chemotherapy, unexpectedly with increasing CEA. The first evaluation revealed several liver metastases on CT scan. Analysis of KRAS status of T1 was KRAS WT. The patient received treatment with FOLFIRI + Bevacizumab before he underwent hemihepatectomy and RFA treatment of the liver metastases. Despite further treatment with FOLFIRI evaluation scan showed relapse with new liver metastases. The oncologist required KRAS status of T2, which surprisingly revealed a different status: KRAS mutational (MT) status. Analysis of KRAS status of the new liver metastases was KRAS MT status as well. The patient resumed Bevacizumab treatment combined with FOLFIRI. Following CT scan showed stable disease. He then underwent new RFA treatment of the liver metastases and is still chemotherapy treatment.

**Discussion and conclusion:** Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. It is the third most common cancer worldwide and the fourth most common cause of death. Activating KRAS gene mutations are detected in approximately 40% of CRC. Synchronous colorectal cancers are rare events, they represent up to 5% of CRC. Patients with synchronous colorectal cancers have a worse prognosis. KRAS status can be different in synchronous tumours and KRAS mutation can be acquired or lost during tumour metastatic dissemination. In up to 10% of cases there are no consistent mutation patterns between the primary tumours and the metastatic lesions. The need for biopsy and analysis of KRAS status in all primary tumours – even those in close proximity - and in metastases is essential to optimise treatment.
P-2-01
HPV prevalence findings in the danish HPV self-sampling implementation compared to routine screening.
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Introduction: In Denmark, 45% of cervical cancers are diagnosed in screening non-participants. The ongoing pilot implementation study in the Capital Region aims to improve the screening coverage rate by offering an HPV-based self-sampling test for free to non-attenders. Here we report on the HPV prevalence in the returned self-sampling brushes, with an age-stratified comparison to routine physician-taken samples from screening participants.

Material and methods: Non-attenders from the Capital Region (N= 54,585) were identified from the invitational module of the nationwide Pathology Data Bank. Women were randomly invited in batches of 1,000, and the recruitment is still on-going. Reminders were sent after 8 weeks. HPV prevalence was determined by testing with the BD Onclarity assay. For comparison, we used data from a BD Onclarity study on unselected routine samples, including predominantly primary screening samples.

Results: In 2488 analyzed self-samples, the overall HPV prevalence was 15.7 %. Age-stratified data showed that 26.9% of women aged 30-39 years had a positive test result; at 40-49 years, this was 13.9%; at 50-59 15.0%; and at ≥60 years 13.7%. For comparison, in a regular screening population (N=811), HPV prevalence was 20.4%, 13.2%, 7.5%, and 4.7% respectively.

Discussion and conclusion: HPV prevalence in women aged 30-49 years was similar between non-participants who accepted self-sampling and regular screening participants. In women aged ≥50 years, the prevalence rates were higher in the self-sampling group. These preliminary data suggest that the older self-sampling group has a higher risk of CIN, and might particularly benefit from self-sampling.

P-2-02
Cross-reactivity to untargeted, low-risk HPV genotypes by cobas, APTIMA and HC2
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Introduction: Cervical cancer screening is increasingly changing from cytology-based to HPV-based testing. However, HPV screening displays lower specificity than cytology due to false-positive test results partly caused by cross-reactivity to untargeted, low-risk genotypes. Cross-reactivity is costly as it may cause unnecessary follow up procedures and anxiety in women. We studied cross-reactivity in three widely used clinical HPV assays with a full genotyping assay as a reference.

Material and methods: Within the Danish Horizon study cross-reactivity to low-risk genotypes in consecutive routinely evaluated samples from 5,022 women was examined using Hybrid Capture 2 (HC2, Qiagen), cobas (Roche), and APTIMA (Hologic-GenProbe) with CLART HPV2 (Genomica) as reference assay. Complete histology follow-up was retrieved after 2.5 years.

Results: Of 1505 HPV positive samples, 157 samples showed cross-reactivity to low-risk genotypes: 109 on HC2, 62 on cobas and 35 on APTIMA. The three assays had only 10
samples with cross-reactivity in common. Absolute cross-reactivity was 2.2% on HC2, 1.2% on cobas, and 0.7% on APTIMA. Relative cross-reactivity was 10.6% on HC2, 4.6% on cobas, and 4.2% on APTIMA. Cross-reactivity was most common in young women, in women with abnormal cytology, and in follow-up samples. The number of assay Positive samples with no genotypes detected on CLART varied from 49 on HC2 to 162 on cobas and 56 on APTIMA with only 12 samples in common. The most frequent cross-reacting genotypes were HPV70, HPV53, HPV82, and HPV66 (HC2 only). Cross-reactivity was associated with low viral input. In total, 6 cross-reacting samples were associated with ≥CIN2, but none from primary screening at age 30-65 years.

Discussion and conclusion: All three assays showed cross-reactivity to low-risk genotypes. Cross-reacting samples had lower viral load than samples with high-risk genotypes. Absolute cross-reactivity was lower in primary screening samples than in referred samples. Though cross-reactivity added to the problem of false positive test results in HPV screening, cross-reactivity was not common and did not tend to be associated with ≥CIN2 in primary screening.

Material and methods: Women with cytological abnormalities were managed according to routine recommendations. Those with cytology-normal/HPV-positive samples (on any of the four assays) were invited for repeated cytology and HPV testing in 1.5 year.

Results: HPV testing detected more ≥CIN3, but the differences were not significant; the sensitivity of HC2 was 90% (95% CI: 76-97), of cobas and CLART 95% (95% CI: 83-99), of APTIMA 87% (95% CI: 73-96), and of cytology 79% (95% CI: 64-91). The specificity for ≥CIN3 varied between 85% (95% CI: 84-86) for cobas and 97% (95% CI: 96-97) for cytology. The specificity of cobas and CLART was significantly lower, and that of APTIMA higher, than of HC2. Detection of CIN1 was increased with all HPV assays, but only for cobas the increase was statistically significant. With APTIMA combined with cytological triage, about 20% more women were referred for colposcopy than with cytology screening. With the three DNA assays, the increase was ≥50%. The number of women with repeated testing was twice as high with APTIMA and almost five times as high with cobas compared to cytology.

Discussion and conclusion: HPV-based screening of Danish women aged 30-65 detected slightly more high-grade CIN but decreased the screening specificity, and increased the demand for additional testing. In conclusion, differences in the detection of high-grade CIN, false-positive test results, and the number of follow-up procedures were observed between HPV assays (HC2, cobas, CLART, and APTIMA) on the one hand, and cytology on the other hand in Danish women undergoing primary screening at age 30-65 years. The differences were small between the HPV assays alone, although HC2 and particularly APTIMA were associated with smaller increases in false-positive tests, number of needed colposcopies, and frequency of repeated testing than cobas and CLART.

P-2-03
Human Papillomavirus assays and cytology in primary cervical screening of women aged 30 years and above
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Introduction: In women aged ≥30 years, Human Papillomavirus testing will replace cytology for primary cervical screening. We compared Hybrid Capture 2 (HC2), cobas, CLART, and APTIMA HPV assays with cytology on 2869 SurePath samples from women undergoing routine screening at 30-65 years in Copenhagen, Denmark.
High risk-HPV prevalence in women aged 60-65 years and follow up of high risk-HPV positive women
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Introduction: According to the new 2012 national recommendations for screening for cervical cancer, HPV-DNA testing should be used as the primary screening test for women aged 60-64 years. As triage test, genotyping and/or cytology should be used. If no HR-HPV is found, women should be withdrawn from the programme. It is known that the incidence of HPV infection is low among women aged 60-64 years, but the number of HPV-positive women that have CIN2+ lesions or persistent HR-HPV infection over time is still not clear.

The aim of this study was to determine the HPV prevalence among women aged 60-65 years, and in a smaller group of HR-HPV positive women, investigate how many of those that have CIN2+ lesions or persistent HPV infection in short term follow-up.

Material and methods: By using data available in The Pathology Data Bank we included women aged 60-65 years who had been primary screened by HPV testing (Roche Cobas 4800) in the Institute of Pathology, Aalborg University Hospital, in the period 01.07.2012 – 01.12.2014. Women with a history of abnormal cervical cytology or histology, 6 years prior to inclusion, were excluded from the study.

We made a follow-up study of the HR-HPV positive women primary screened by HPV testing in the period 01.07.2012 – 31.12.2013, by searching in The Pathology Data Bank for the first subsequent HPV-test, after primary screening, that also included diagnosis of cytology and/or histology. Follow-up time was 12-29 months.

Results: Within the study period 4587 women aged 60-65 years were primary screened by HPV testing. The prevalence of HR-HPV was 5.7% (n=260). The prevalence of HPV 16 was 1.2% (n=56), HPV 18 0.5% (n=23) and other HR-HPV types 4.0% (n=181).

In the follow-up study we found 161 HR-HPV positive women out of a total of 3124 screened women. Of those, 23% (n=37) had no or insufficient follow-up and were excluded. Of the 124 included women, 10.5% (n=13) had CIN2+ lesions in the first subsequent follow up, and went on to further treatment. In the remaining group, 64.9% (n=72) were HR-HPV positive, and of those, 7 had abnormal low-grade cytology/histology. 35.1% (n=39) were HR-HPV negative, and of those 2 had abnormal low-grade cytology/histology.

Discussion and conclusion: By using the sensitive HPV-DNA testing as the primary screening test, it is expected that more women have to be investigated further. After exclusion of women with high-grade lesions, we found that 64.9% of the women with a primary positive HR-HPV screening test, were still HR-HPV positive in the first subsequent follow-up. This raises the question how to forwardly manage women aged 60-65 years without high-grade lesions, but with an ongoing positive HR-HPV test.

Ki67/p16 immunocytochemical double staining as a supplementary staining used in screening for cervical cancer.
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Introduction: In screening for cervical cancer differential diagnostic issues can be Atypical Squamous Cells cannot exclude High-grade squamous intraepithelial lesion + (ASC-H +) and adenocarcinoma in situ + (AIS+) towards atrophy, reactive changes, atypical tissue repair, endometriosis and tubar metaplasia. Studies have showed that a Ki67/p16 double immunocytochemical staining increases the specificity and maintains high sensitivity for the diagnosis of HSIL+/AIS+ compared to normal cytology and high risk (HR) human papilloma virus (HPV) testing alone. WHO recommend the use of p16 at histological materials in some HPV-associated lesions. We wanted to develop a double immunocytochemical staining for Ki67/p16 used
at cervical cytology samples as an alternative method to commercially available kit. **Material and methods:** Material consists of 24 BD SurePath fixed cervical cytology samples included 14 samples negative for HR-HPV (normal, reactive changes, atrophy, tubar metaplasia) and 9 samples positive for HR-HPV (4 samples with ASCUS/LSIL, 5 samples with HSIL). The samples were stained for Ki67/p16 with in house developed double immunocytochemical (ICC) method. The reaction for Ki67/p16 were detected with MACH 2 Double Stain 1 kit (Biocare) and we're visualized with chromogens Deep Space Black / Red Warp (Biocare) on Dako Autostainer Link 48. A cell pool consisting of HR-HPV-positive BD SurePath fixed material was used as control. **Results:** Ki67/p16 immunocytochemical double staining was seen as distinctive black Ki67 nuclear reaction and strong red-colored cytoplasm p16 staining in the same cell. All 14 HR-HPV-negative samples with benign changes were negative for the immunocytochemical double staining for Ki67/p16, and all 9 HR-HPV-positive samples with abnormal cellular changes were positive. **Discussion and conclusion:** Immunocytochemical double staining for Ki67/p16 set up with the chromogenic color combination Deep Space Black (Ki67) and Warp Red (p16) provides convincing and clear results. The method shows both high sensitivity and specificity and can be used as a supplementary triage method especially in the differential diagnosis of ASC-H+/AIS+ in the screening program for cervical cancer.

**P-2-06**

**Co-existence of choriocarcinoma and a viable pregnancy – a case report.**

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**Introduction:**

Choriocarcinoma is a rare, highly malignant neoplasm composed of biphasic cellular components of multinucleated syncytiotrophoblastic- and mononucleated trophoblastic cells. It is accompanied by necrosis and characterized by production of human chorionic gonadotropine (hCG). Choriocarcinomas can be gestational or non-gestational. Gestational choriocarcinoma (GC) develop from gestational tissue and thus contain paternal genomic material. Non-gestational choriocarcinoma (NGC) develop from gonadal- or extragonadal germ cells or from dedifferentiation of somatic carcinomas, all with a genomic content similar to that of the patient. Although GC and NGC have similar histomorphologic appearances, the prognosis and treatment differ immensely. GC reacts well to chemotherapy and is usually curative, whereas NGC is less chemosensitive with a poorer prognosis. **Material and methods:** A 33 year old woman pregnant with a viable fetus (gestational week 22), presented with a 8cm tumor in the uterus, abnormally elevated serum hCG, and tumors in the lungs and liver. Do to a strong clinical suspicion of choriocarcinoma, an abdominal hysterectomy was performed. DNA purified from biopsies of the placenta and the uterine tumor was analyzed with polymorphic DNA markers and compared to those of the patient. The placenta and uterus were subjected to histomorphologic examination. **Results:** The DNA profile from the placenta-biopsy was consistent with a normal female fetal genotype with a biparental contribution to the genome. Surprisingly, the DNA profile from the tumor-biopsy was similar to that of the patient, thus either representing NGC or maternal contamination. The histomorphologic appearance of the placenta was normal. At gross examination of the uterus, nodules of grayish tumor masses infiltrating the endometrium intermixed with necrosis and hemorrhage were found. Microscopically, the classic biphasic pattern of neoplastic syncytiotrophoblasts and trophoblasts were seen along with extensive necrosis and hemorrhage. As the origin of choriocarcinoma is important, DNA purified from formalin-fixed paraffin-embedded micro-dissected tissue, microsco-
pically diagnosed as choriocarcinoma, was analyzed. Here, the DNA profile was consistent with a normal diploid male fetal genotype. The pregnancy was thus interpreted as a twin pregnancy with one normal twin (female) and a GC (male).

**Discussion and conclusion:** This rare case illustrates the difficulties in gaining representative material in choriocarcinomas with extensive necrosis and hemorrhage. Here, micro-dissection can be of crucial assistance. Choriocarcinoma co-existing with a viable pregnancy is an extremely rare condition, which only has been reported 36 times previously.

P-2-07

**Adenocarcinoma with intestinal differentiation occurring in endometrial biopsy: A report of two cases.**

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**Introduction:** Intestinal differentiation (ID) within the endometrium whether it be a benign change, in hyperplasia or adenocarcinoma, is extremely rare and only a few cases have been reported in the literature. Hence if ID is identified in an endometrial biopsy, one has to consider whether the changes arise from a primary endometrial lesion or is a metastasis from an occult gastrointestinal cancer. We present 2 cases of ID diagnosed in endometrial biopsies, in which the final diagnoses were an endometrial metastases from a low grade mucinous neoplasia of the appendix (LAMN) and an endometrioid adenocarcinoma with areas of ID, respectively.

**Material and methods:** *Case 1:* A 58-year-old woman, referred for removal of an endometrial polyp. She subsequently underwent a hysterectomy with bilateral salpingoooforectomy and appendectomy.

*Case 2:* A 79-year-old woman presented with postmenopausal bleeding. She underwent an endometrial aspiration biopsy and subsequently a hysterectomy and left-sided salpingooophorectomy.

**Results:** *Case 1:* The initial biopsy showed lakes of mucin with fragments of mucinous adenocarcinoma of intestinal type. The background endometrium was atrophic. The resection specimen showed an uterus with signs of previous biopsy but otherwise unremarkable. The appendix was dilated with a perforation of the wall and mucus on the serosa. Histologically the fallopian tubes had mucin within the lumen. Adjacent to the endometrial biopsy site was foci of atypical epithelium with ID. The appendix had the classical morphology of LAMN, showing a proliferative mucin epithelium with low grade atypia.

**Discussion and conclusion:** Endometrial ID identified in endometrial biopsies is almost invariably associated with premalignancy or malignancy. Immunohistochemistry will reveal an intestinal profile whether it is a primary endometrial or a secondary gastrointestinal adenocarcinoma. When associated with typical endometrioid adenocarcinoma as in case 2, a primary endometrial adenocarcinoma is most probable.

P-2-08

**Recurrence of Ovarian Granulosa Cell Tumour 34 years Following Initial Diagnosis**

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**Introduction:** Granulosa cell tumours of the ovary have potential for aggressive behaviour
and can develop late recurrences, even 20-30 years following initial diagnosis. We report a case with recurrence of an ovarian granulosa cell tumour 34 years following initial diagnosis.

**Material and methods:** A 75 year old woman with left side lumpectomy 2006 due to breast cancer, and complete hysterectomy 1980 due to ovarian cancer, presented with intermittent colic pains and the feeling of a left side abdominal mass. Examination revealed a palpable indolent smooth mass under the left curvature and the patient was referred to a computer tomography scan which showed a 16 x 12 x 11 cm solitary tumour in the left hypochondrium in relation to splenic vessels but with no suspicion of splenic origin. Three needle biopsies were obtained and submitted for pathologic review.

**Results:** Microscopic examination with hematoxylin-eosin slides of the needle biopsies demonstrated a tumour of small cells with sparse cytoplasm and round or ovoid nuclei with inconspicuous nucleoli. Some cells had nuclear grooves. The growth pattern was diffuse with small foci of microfollicular growth. No significant mitotic activity was observed. Immunohistochemical staining showed positive reaction for CD99, WT-1, inhibin, vimentin, podoplanin, CD56 and progesterone-receptor. The tumor showed negative reaction for desmin, actin, epithelial membrane antigen (EMA), CD45, CD3, CD4, CD5, CD8, CD10, CD20, CD79, carcinoembryonal antigen (CEA), S-100, CD34, CD117, synaptophysin, chomogranin, estrogen-receptor, CK7 and CK20.

After the needle biopsies the patient was referred to a total excision of the tumour.

**Discussion and conclusion:** The morphological and immunohistochemical findings were characteristic for granulosa cell tumour and examination of the patient’s old medical records revealed, that she was treated for an ovarian granulosa cell tumour in 1980. This case thus shows the importance of long term follow up of granulosa cell tumours.

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**P-2-09**

**High grade ovarian immature teratoma in a 10-year-old girl**

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**Introduction:** Immature teratoma is a relatively rare tumour which is most common in the first three decades of life. The majority of immature teratomas are localised in the ovary with the sacrococcygeal region as the second most common site. Immature teratomas are usually unilateral and predominantly solid with a median diameter in the range of 16-20 cm. Microscopic features include immature embryonal-type tissues dominated especially in higher grade tumour by immature neuroectodermal tubules, pseudorosettes and mitotically active glia. The tumour is graded according to a three-tiered grading system (grade 1-3) based on the relative amount of immature neuroepithelial tissue, but a two-tiered system (high/low grade) is gaining acceptance. Surgical resection without chemotherapy is curative for the majority of children.

**Material and methods:** A 10-year-old girl presented with intermittent abdominal pain and abdominal discomfort. The child had a history of severe asthma with daily symptoms and recurrent pneumonias. MRI scan and ultrasound examination of the pelvis and abdomen showed an approximately 14x7 cm suspected malignant tumour with cystic and solid areas in relation to the left ovary. Serum HCG, alpha-fetoprotein and CA-125 were within normal limits. A left oophorectomy was performed. At the operation no apparent abdominal macroscopic metastases were identified.

**Results:** The gross examination of the tumour showed an ovary measuring 13.5x8.5x7 cm with intact capsule. The ovary was partially cystic with two solid areas with maximal diameters of 7.5 cm and 4.5 cm respectively. The cut surface was heterogeneous with grey and yellow areas and focal hemorrhagic discolorations. Microscopically the sections were dominated by neurogenous tissue with a mixture of mature glia tissue, groups of ganglion cells, and choroid plexus. However, a large amount (occupying >3 LPF (x40)) of immature neuroepithelial tissue was also identified.
The tissue consisted of neuroectodermal tubules with partially ciliated columnar cells with hyperchromatic, elongated nuclei and neuroectodermal rosettes with relatively small cells with scant cytoplasm and hyperchromatic nuclei. A maximum of 34 mitoses per 10 HPF were counted in the immature areas. Other parts of the tumour showed elements of bone, cartilage, adipose tissue, respiratory and squamous epithelium together with areas of necrosis. There were no foci of yolk sac tumour. The findings were consistent with grade 3 immature teratoma FIGO stadium IA.

Discussion and conclusion: Ovarian immature teratomas are rare in childhood, and the literature is sparse on the subject. The prognosis is favourable and surgery is curative in the most of the cases while chemotherapy is reserved for malignant recurrence.

P-2-10
Combined Collagen IV and Laminin-5 (γ2) double immuno-staining: Discriminating high grade dysplasia from microinvasive lesions of the uro-gynaecological tract.
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Introduction: Laminins are basement membrane glycoproteins. In most basement membranes laminins form a double polymer with type IV collagen. The functional properties of laminins include cell adhesion, proliferation, differentiation, growth and migration. In normal tissue, the Laminin-5 (γ2) chain has a restricted expression pattern: it is only expressed in basement membranes under basal epithelial cells of the exocervix, endocervix, endometrium, intestine, stomach, urinary bladder, lung, skin, tongue and esophagus. In early malignant transformation, the basement membrane is disrupted and Laminin-5 (γ2) chain is up-regulated and often expressed strongly in the invasive front by the tumor cells. The aim of this study was to evaluate if double immuno-staining for Collagen IV + Laminin-5 (γ2) could be used as a tool to differentiate between the two challenging entities of high grade dysplasia and microinvasive lesions in a small cohort of patients diagnosed with squamous cell carcinoma and urothelial carcinoma.

Material and methods: Formalin fixed and paraffin-embedded tissue samples from 12 patients were evaluated including 9 cases from cervix and 3 cases from urinary bladder (males). All tissue specimens had normal, dysplastic and invasive areas based on HE morphology. Sequential double immuno-staining technique was applied to detect Collagen IV and Laminin-5 (γ2).

Results: Strong Laminin-5 (γ2) expression as well as disrupted Collagen IV staining of the basement membrane was seen in the invasive front of all squamous cell carcinomas and urothelial carcinomas. In non-invasive (normal or dysplastic) areas, all cases showed intact basement membranes and Laminin-5 (γ2) staining was negative in the vast majority of dysplastic areas. One case of urothelial carcinoma and two cases of squamous cell carcinomas of the cervix displayed a weak staining intensity for Laminin-5 (γ2) in adjacent dysplastic epithelium.

Discussion and conclusion: Although the number of cases included in this study is low, our work demonstrated that it is beneficial to use a double immuno-staining protocol for Collagen IV and Laminin-5 (γ2) discriminating high grade dysplasia from microinvasive lesions in cervical or urological tissue specimens. The double immuno-staining combination for Collagen IV and Laminin-5 (γ2) may be applied in other challenging diagnostic situations differentiating pre-invasive from invasive lesions, but its fully diagnostic potential needs to be elucidated.
P-3-01  
Pigmented epithelioid melanocytoma: A report of two cases  
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Introduction: Pigmented epithelioid melanocytoma (PEM), a tumor indistinguishable from epithelioid blue nevus of Carney Complex, is an unusual melanocytic neoplasm with a borderline malignant potential. We describe two cases of PEM with regional lymph node metastasis in one of these cases.  

Material and methods: The skin and regional lymph node specimens were processed according to routine protocol for melanocytic lesions and regional lymph nodes. Sentinel lymph nodes (SN) were examined in five levels with immunostains for S100 and MLA according to the national Danish guidelines. In one of the cases primary tumour tissue was available for immunohistochemical analysis for Ki67/MLA markers as well as Vysis Melanoma FISH 4 Probe Kit analysis.  

Results: A 15-year-old female and a 21-year-old male underwent excision biopsies of dark pigmented nodular lesions on their backs, 3 and 7 mm in diameter, respectively. Microscopical examination revealed pigmented epithelioid and spindle cell melanocytic tumours with abundant melanophages. Signs of maturation and focal blue nevus-like pattern was noted. No mitoses, ulceration or features of conventional melanoma were identified. The tumours were respectively 1.3 mm and 2.5 mm in thickness with involvement of reticular dermis. The results of the FISH analysis were within normal ranges. Metastasis was identified in the SN of the second case.  

Discussion and conclusion: PEM is a rare melanocytic neoplasm with the potential to recur and metastasize to regional lymph nodes. Although few cases with liver metastases have been reported, overall prognosis is good. Molecular studies confirm PEM as a distinct genetic entity characterized by loss of the expression of protein kinase, regulatory subunit 1 alpha. Histological features of PEM are often indistinguishable from epithelioid blue nevus. The latter can be associated with the Carney Complex, which should be considered in lesions with this morphology. The lesions in combination with Carney Complex are usually benign. We have recommended genetic consultation to our patients. Treatment of PEM should include local excision. Width of the surgical margins is not clearly defined yet. Sentinel node (SN) biopsy could be an appropriate staging procedure. However, the necessity of SN biopsy is debatable according to some authors.

P-3-02  
Multiple Wiesner nevi: A case report  
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Introduction: Wiesner nevus or BAP'oma is a recently described entity characterized by distinct histological features and BRAF and BAP1 germline mutations with a predisposition to internal neoplasia. We describe a patient with multiple Wiesner nevi.  

Material and methods: The skin specimens were processed according to the routine hi-
Histology sections were stained with Hematoxylin & Eosin. Immunohistochemical examinations for Ki67/MLA, HMB45, p16 and pBRAF were performed and BRAF mutational status was assessed by PCR as well. Sequencing of BAP1 was carried out for two lesions and on perilesional skin of one of the lesions.

Results: A 29-year-old female presented with multiple skin lesions on her trunk and extremities. The lesions were clinically consistent with Spitz nevi. Five lesions on the left shoulder, the left side of the back, the trunk and the hip were excised over a period of two years. Microscopy of all the lesions revealed unusual dermal and junctional melanocytic proliferation with epithelioid features, a lack of maturation and of mitotic activity. A BRAF V600E mutation was identified in the lesions. A BAP1 T351Lfs*11 mutation was identified in both lesions and perilesional skin.

Discussion and conclusion: Unusual histology makes the diagnosing of Wiesner nevus challenging for the dermatopathologist. Wiesner nevi are classified by some authors as a premalignant condition. The development of malignant melanoma in pre-existing Wiesner nevi is, however, unlikely. It is important to recognize the Wiesner nevus as a potential marker for germline BAP1 mutation. Mutations in BAP1 are associated with cancer susceptibility syndrome with a predisposition to malignant mesothelioma, uveal melanoma and some other tumours. It is, therefore, important to provide the patient with an individual follow up plan. Although sporadic Wiesner nevi may occur, multiple lesions raise a suspicion of syndrome association. In such cases, BAP1 mutation can be detected in other tissue. We recommended that the patient be seen at a department of clinical genetics.

P-3-04
Extra mammary invasive Paget’s disease misinterpreted as urothelial metastasis: A case study
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Introduction: The labia major, scrotum and perineum are the most frequent sites of extra mammary Paget’s disease (EMPD). EMPD results from the presence of carcinoma cells in the epidermis, nearly always involving eccrine glands and hair follicles. Dermal invasion is rarely seen. The histogenesis of EMPD remains controversial, and may not be uniform. About 25% of all cases have an underlying cutaneous adnexal carcinoma. 10-15% have an internal
carcinoma involving the rectum, prostate, bladder cervix or urethra, which appears to have etiological significance. In the case of no underlying carcinoma, several pathogenic explanations have been proposed: an underlying in-situ adnexal carcinoma, an origin from the dermal or poral portion of the sweat glands, origin from apocrine or eccrine cells or pluripotent cells in the epidermis.

**Material and methods:** A 4mm biopsy of a tumor in the posterior part of the scrotum of an 85-year-old male and later a larger resection were routinely processed.

**Results:** The biopsy showed a proliferation of epithelial tumor cells in the dermis, some infiltrating the overlying epidermis. The tumor cells were positive for BER-EP4, EMA, CEA, GATA3, Cytokeratin AE1/AE3 and an estimated 30% positive for Ki-67. The cells were negative for PSA, CD68, S100, HMB45, Melan A CAD17 and P63. Based on morphology and the immunohistochemical profile, with special weight on the GATA3 positivity, the tumor was interpreted as likely to derive from an urothelial carcinoma. Examination of the urogenital tract and colon showed no tumors. The larger resection held an elevated 10mm tumor. Microscopically there was diffuse pagetoid spread of tumor cells, positive for CK7, EP4, CEA, GCDFP-15 and GATA3, negative for melanocyte markers, PSA, PCC, CK20, CDX2 and Villin. The tumor grew into adnexal structures and tumor cells surrounded by a basal lamina presented in a small area in dermis. There was a focus of ulceration and invasion of the dermis.

**Discussion and conclusion:** GATA3 has recently been introduced in our department as a useful marker for breast and urothelial tumors, being expressed in 100% of breast and skin adnex tumors, >90% of urothelial carcinomas and the trap here was a small biopsy from an unusual invasive Paget’s disease, and the pathologist SB not being familiar with the new marker.

**Introduction:** Myeloid sarcoma is a rare condition characterized by the occurrence of an extra-medullary tumor mass consisting of myeloid blasts with or without maturing. Most common locations are the skin, bone and lymph nodes. Myeloid sarcoma of the placenta is an extremely rare entity with only two prior examples of decidual involvement described in the English-language literature.

**Material and methods:** In 2014 a 27-year-old primigravida presented at 32 weeks gestation with influenza-like symptoms, swelling of the gums and leukocytosis of 52x10^9/l. Smear of peripheral blood and bone marrow aspirate showed 25% and 25-30% blasts, respectively. The blastpopulation consisted of both myeloblasts (CD34+, CD117+, CD33+, MPO+, CD7+,CD123+, HLA-DRw, CD11b-, CD64-) and monoblasts (CD34-, CD117-, CD33+, CD123+, HLA-DR+, CD11b+, CD64+, CD14-, CD35-). Based on morphology and immunophenotyping (flowcytometry and immunohistochemistry) the diagnosis of AML-M4 was made. Karyotype: 47,XX,+8. NPM1 A mutation was found by quantitative PCR and FLT3 ITD was found by PCR followed by capillary electrophoresis. The patient was treated with Hydroxyurea due to blast crisis and steroids for enhancement of fetal lung maturity before subacute section with delivery of a premature girl. The baby was examined by the obstetric team and was found healthy and showed no evidence of hematologic compromise. Postoperatively the patient developed bleeding and disseminated intravascular coagulopathy. Post-partum she was treated with chemotherapy consisting of Daunorubicin and Ara-c.

**Results:** On histopathologic examination the placenta was grossly unremarkable except for the impression of condensations in the luminal vessels. On microscopic examination the maternal surface revealed a minor tumor mass composed of myeloid blasts and maturing myeloid cells. Immunohistochemically the myeloid blasts were positive for CD117,
CD33 and CD123 and negative for CD34. In the intervillious space there were scattered, individual myeloblasts with identical immune profile. The diagnosis of myeloid sarcoma was given. 30-days follow-up showed complete remission in peripheral blood and bone marrow by morphology, immunophenotyping and karyotyping. Quantitative PCR, however, revealed 0.07% minimal residual disease.

**Discussion and conclusion:** The frequency of myeloid sarcoma in placenta may be underestimated because placentae may appear normal at gross examination and therefore may not be adequately examined histologically. The present case report highlights the importance of detailed histopathologic examination of the placentas from leukemic patients.

P-3-06

**Congenital dyserythropoietic anemia: a case report with differential diagnostic considerations**

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**Introduction:** The congenital dyserythropoietic anemias (CDA) comprise a group of rare hereditary disorders that are characterised by ineffective erythropoiesis as the predominant mechanism of anemia and by distinct morphological abnormalities of late erythroblasts in the bone marrow. In the majority of CDAs, inheritance is autosomal recessive, and due to the small number of offspring in most European families, single cases in one family are the rule rather than the exception. The rarity of the disorder, the need to obtain a bone marrow specimen for diagnosis and the several differential diagnoses all explain why correct diagnosis is difficult and often delayed.

**Material and methods:** An otherwise healthy baby boy was born following a normal full-term pregnancy. He was no. 2/2, the sibling being healthy. The family was of Danish ethnicity.

The boy was born with severe metabolic acidosis, circulatory collapse with biventricular failure and anaemia, hemoglobin 6.1 mmol/l (9.1-14.9 mmol/l). He was successfully treated with inotropic agents and blood transfusion but was hospitalized again 5 weeks old with recurrence of unexplained anemia, hemoglobin 3.5 mmol/l (5.5-10.5 mmol/l). He also had an inadequate reticulocyte count for the degree of anaemia. Because of slightly elevated lactate, Pearson syndrome was suspected but mutational screening of mitochondrial DNA was normal. Instead, characteristic morphologic abnormalities in peripheral blood and bone marrow indicated CDA. The patient is now 10 months old and remains stable with a normocytic anemia, haemoglobin 4.5 mmol/l (5.8-9.4 mmol/l) without transfusion.

**Results:** Peripheral blood smear showed pronounced aniso- and poikilocytosis and a few binucleated mature erythroblasts. The neutrophil count was decreased but with no dysplastic changes or blasts and the number and morphology of the platelets was normal. The bone marrow was hypercellular due to an erythroid hyperplasia with pronounced increase of erythroblasts with increased erythropoietic/granulopoietic ratio. The proerythroblasts and the basophilic erythroblasts were normoblastic, however, more than 10% of the late precursors were binucleated. There was no vacuolization of the haematopoetic precursors and no ringed sideroblasts were found.

**Discussion and conclusion:** The combined evidence of congenital anaemia, ineffective erythropoieses, typical morphology of bone marrow erythroblasts and the exclusion of relevant differential diagnoses should lead to the diagnosis of CDA. The differential diagnoses are many and information about clinically associated dysmorphology, biochemistry, bone marrow morphology and findings at the molecular level must all be compared. In our patient, SEC23B gene mutational studies are in progress.
**P-3-07**

**Primary extranodal marginal zone B-cell lymphoma of MALT in the thymus as an incidental finding in a caucasian woman: A case report**

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**Introduction:** Primary thymic extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (TML) is an extremely rare disease strongly associated with autoimmune disease, especially Sjögren’s syndrome. There is a marked female preponderance and there seems to be a predilection for Asians. The treatment is surgical excision and/or chemotherapy with an excellent outcome.

**Material and methods:** A 60-year old Caucasian woman presented with a 6 week history of muscle pain and arthralgia in arms and legs accompanied by swelling and morning stiffness. The initial laboratory examination revealed anemia, leucocytosis and thrombocytosis and significant increase in CRP. ANA, anti-CCP and RF were negative. PET-CT scan was performed and incidentally a mass in the anterior mediastinum with relation to thymus was detected. Thymectomy was performed and submitted for pathological examination. Postoperative adjuvant therapy was not given. Postoperative tests showed abnormalities suggestive of systemic lupus erythematosus (SLE) with elevated ESR, Anti-Śm antibodies and RNP antibodies. Currently rheumatological examination is ongoing to confirm the diagnosis of SLE.

**Results:** Specimen examination revealed a semi-lobulated mass that measured 7x3x0.8 cm and 7x2.5x1 cm and weighed 17 grams. The mass was contained within the thymus without signs of invasion in the surrounding structures. The cut surface showed a homogenous pale appearance with no cystic spaces. The tissie was solid without a well-defined tumor. Histologically, the normal thymic lobular architecture was effaced by a dense lymphoid infiltrate and few residual Hassall corpuscles. Cortical areas could be identified. The lymphoid infiltrate consisted of small to medium sized lymphocytes with a pale cytoplasm. There was a prominent plasmacytic differentiation and scattered reactive lymphoid follicles. The thymic epithelial network was highlighted by cytokeratin staining and was found distorted due to the infiltrate. The neoplastic lymphoid cells were positive for CD20, PAX5 and BCL2, and the plasma cells showed clear lambda light chain restriction. Flow cytometry showed plasma cells and a population of B-cells with lambda light chain restriction. A diagnosis of TML was made.

**Discussion and conclusion:** We report a case of TML discovered incidentally in a middle-aged Caucasian woman with autoimmune disease. Current rheumatologic workup is suggestive of SLE. To our knowledge, this is the first case report of TML in a Caucasian woman accompanied by SLE. Diagnosis of TML in a biopsy from an anterior mediastinal mass can be difficult, and pathologists should be aware that appropriate immunohistochemistry is necessary in the histopathological work-up in order to identify the thymic origin.

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**P-3-08**

**Case report: Nonamyloidotic Monoclonal Light Chain Restrictive Cardiomyopathy (RCM): Verification using Mass Spectrometry**

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**Introduction:** Tissue deposition of monoclonal immunoglobulins (Ig) can be seen in be-
nign or malignant proliferations of plasma cells or B cells. These deposits may be composed of only a single class of Ig light chains, heavy chains or both. Fibrillar deposits are seen in cases of amyloidosis, while granular deposits may be found in nonamyloidotic conditions. Immunohistochemistry (IHC) and electronmicroscopy have hitherto been used to typify the monoclonal component. We present a rare case of Nonamyloidotic Restrictive Cardiomyopathy due to light chain deposition of Kappa type, which was diagnosed with the aid of Mass spectrometric (MS) analysis of endomyocardial biopsy.

**Material and methods:** Endomyocardial biopsy from the right ventricle of a 68 years old man with hypertrophy, restrictive mode and EF of 45% were assessed microscopically, supplied with IHC for Kappa(κ) and Lambda(λ), Congo red staining and subsequently tandem MS for protein sequencing.

**Results:** Myocytes were regularly arranged, of normal shape and slightly hypertrophic, interstitial widening due to deposition of pinkish eosinophilic material, which was negative for Congo-red reaction. IHC – kappa reaction was stronger than lambda reaction but there was significant background staining.

The raw data from tandem MS was analyzed by label free quantification through MaxQuant using Human reference proteome database (26/11 2014), and a generated Ig reference database. The Ig κ/λ ratio was approximated to 3300 using the intensity based absolute quantification (IBAQ) value. This data supports that there is a very high proportion of κ light chain in the heart biopsy. Hence the conclusion that there is deposition of κ light chain. Concomitant marrow biopsy showed about 10 % abnormal κ restricted plasma cells.

**Discussion and conclusion:** RCM due to light chain deposition disease (LCDD) is a rare entity. Studies have shown predominance of κ over λ deposits in LCDD and λ light chain overexpression in amyloid associated light chain deposition.

MS directly analyses the actual protein peptides and has the capability for high throughput, identification of the entire proteome, and has been used in typing of amyloid in various tissues including formalin-fixed paraffin-embedded specimens. It has also been used to typify glomerular deposits and characterise immunoglobulins in the nervous system. MS based platforms have been applied to peptide profiling in carcinomas of ovary, prostate and glioblastomas.

In our case, although the IHC pointed towards the kappa chain restriction, actual verification was only possible with subsequent MS analysis.

**P-3-09**

**Aggressive myocardial and pulmonary calcifications after lung transplantation of a Cystic Fibrosis Patient: A case report.**

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**Introduction:** Severe myocardial and pulmonary calcifications occurred after transplantation in a 33-years-old female with cystic fibrosis, who received a double lung transplantation because of end-stage lung failure. She suffered from severe complications such as bleeding, cardiac tamponade and cardiac arrest after the operation. She died 12 days postoperatively.

**Material and methods:** Autopsy revealed a pale brown myocardium with prominent diffuse yellow-white striations, which were not detected on x-rays before transplantation. Representative sections of myocardium and lung were fixed in formalin, embedded in paraffin and stained for calcium by the Von Kossa method.

**Results:** Von Kossa staining of the representative sections of the myocardium and lung showed diffuse extravascular calcium deposits. No calcification foci were displayed on chest x-ray taken on the last week, which demonstrates calcification process happened quickly.

**Discussion and conclusion:** It is speculated if these severe calcifications significantly contributed to cardiac arrest and were the major cause of death. Calcifications due to hyperphosphatemia, treatment with vitamin D supplementation, altered calcium metabolism in cystic fibrosis, sequela of extensive necrosis are well described as causes of metastas-
tic or dystrophic calcifications but there are no reports of calcifications so early or extensive after transplantation as in this case. Further investigation is needed to determine the risk of extrasosseous calcification after transplantation in cystic fibrosis patients.

P-3-10
Carcinosarcoma of the lung, a rare diagnosis
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Introduction: Pulmonary carcinosarcoma is a rare neoplasm accounting for 0.3-1.3% of lung malignancies. The tumour has a biphasic histopathological pattern consisting of both carcinoma and a sarcomatous element. In the WHO classification of tumours of the lung, it is included in the group of sarcomatoid carcinomas which is defined as poorly differentiated non-small cell lung carcinomas which contain a component of sarcomatoid differentiation. The average age of diagnosis is 60 years, it mostly affects men and it is mainly associated with smoking.

Material and methods: A 73-year old male, smoker, with hemoptysis and recurrent pneumonias. CT-scan revealed an elongated tumour in the right upper lobe with ingrowth to the right main bronchus and small nodular changes all over the right lobe. Furthermore an enlarged lymph node in the mediastinum was seen. A bronchoscopy was performed and the tumour was found to be pedunculated and moveable. Several biopsies were taken from the tumour.

Results: Evaluation of the biopsies showed size varying tumour islets of cells with dark polymorphic nuclei with an ample cytoplasm. The immunohistochemical profile shows positivity for Vimentin and Pan-cytokeratin and focal positivity for Synaptophysin and CD56, and negativity for CK7, CK20, TTF, Napsin, Chromogranin, PSA, CK5, P40, P63, CDX2, S100 and Melan-A. The islets are embedded in a cell-rich stroma of abnormal cells with nuclei of varying sizes. Several mitoses are present within the stroma. These cells are surrounded by a small amount of hyaline fibrosis, which shows some blue staining with van Gieson/Alcian blue, which indicates chondroid differentiation.

Discussion and conclusion: The morphology and the immunohistochemical profile show that the tumour is a carcinosarcoma. The main differential diagnoses are other sarcomatoid lung tumours and sarcomas, both primary and metastatic. Differentiation between these tumour types can be aided by identifying areas of non-small cell carcinoma and confirming epithelial differentiation immunohistochemically. The patient was diagnosed with carcinosarcoma and resection of the tumour was scheduled within a week, but the patient died unexpectedly before the surgery was performed.

P-3-11
Biatrial thromboembolus caught in transit through persistent foramen ovale - a case report.
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Introduction: We describe an unexpected autopsy finding in a 53-year-old man with newly diagnosed disseminated colorectal cancer who died from a saddle pulmonary embolism.

Material and method: A 53-year-old man who received chemotherapy for a non-operable T4 colorectal cancer was hospitalized with progressive dyspnea. He previously had a peripheral lung embolus and was undergoing anticoagulation therapy with Fragmin. Blood samples showed impending liver failure with ultrasonographical suspicion of portal vein thrombosis. Echocardiography showed an ejection fraction of 50% and dominating right heart chambers. During subsequent CT scan the patient suffered cardiac arrest and all attempts at resuscitation failed.

Results: At the autopsy we found a large adenocarcinoma in the sigmoid, with spread to the entire peritoneum and pelvis. The pulmonary artery was blocked by a large thromboembolus (saddle embolus), and numerous, smaller peripheral emboli were found. In the heart we found a vermicular thrombus (60 x 8 mm) trapped in a patent foramen ovale. We found no venous or arterial emboli outside the lungs.
The cut surface of the liver was with signs of chronic stasis.

**Discussion and conclusion:** Cancer is one of the most common acquired risk factors for venous thromboembolism (VTE), which can present as deep vein thrombosis, pulmonary embolism or both. The prothrombotic state is exacerbated further by chemotherapy. Patients with active malignancy have a 4-fold to 7-fold higher incidence of symptomatic VTE than the general population.

Foramen ovale shunts blood from the right to the left atrium in fetal life, and remains patent in up to 27% of the adult population. A persistent foramen ovale (PFO) is defined as a valve-like opening between the septum primum and the septum secundum, without evidence of an anatomical septal defect. Among patients with pulmonary embolism (PE), the presence of a PFO is an independent predictor of death with increased risk of ischemic stroke and arterial embolization because of the risk of a paradoxical embolus (PDE). PDE starts with the formation of emboli within the venous system, which traverse an intracardiac communication and enter the systemic circulation.

A PFO is normally closed by the higher left-to-right pressure gradient. PFO is significant in the etiology of PDE if associated right-to-left shunt occurs - in our case increased right atrial pressure caused by high pulmonary vascular resistance.

This case is a classical example of the association between cancer and venous thromboembolism, but with the additional unexpected finding of an impending paradoxical embolus across a patent foramen ovale.
Poster session 4

P-4-01
Sellar germinoma – a rare differential diagnosis to pituitary macroadenoma. A case report.
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Introduction: Germ cell tumors occur in many extragonadal sites, most frequently along the midline of the body, and therefore constitute potential differential diagnoses in many settings. We present a case of a sellar germinoma, mimicking a pituitary macroadenoma.

Materials and methods: A 20-year old man was referred to the Department of Neurology at Odense University Hospital (OUH) under the suspicion of an intracerebral tumor. He had a history of headache, nausea, loss of weight and fatigue for more than one month, and had noted impaired vision. On examination, the most alarming finding was left side hemianopsia and complete loss of light sense on the right side, indicating a severe affection of the optic chiasm. Magnetic resonance imaging (MRI) demonstrated a 34x47x35 mm tumor in the sellar region, suggestive of a pituitary macroadenoma. The patient underwent craniotomy and surgical removal of the tumor, leaving a small remnant along the optic nerve and artery. Fragmented tumor tissue was received and processed in the Department of Pathology, OUH.

Results: Microscopically the tumor consisted of large, pale cells with round, centrally located nuclei and distinct nucleoli. Tumor cells were surrounded by small, mature lymphocytes. Immunohistochemical stains for OCT3/4 and D2-40 were positive and CD30 was negative in tumor cells. Blood tests showed normal levels of AFP, HCG and LDH. The patient underwent further imaging (sonography and PET/CT) but no testicular tumor or other extragonadal tumors were detected, and a diagnosis of extragonadal germinoma was made. At the time of final diagnosis, the tumor remnant had almost grown back to its original size. The patient received four series of chemotherapy followed by radiotherapy (54Gy/30 fractions). The treatment was well tolerated. Following treatment, no tumor remnant could be detected by MRI. The patient was free of initial symptoms and had regained almost normal vision. He will, however, always be panhypopituitary.

Discussion and conclusion: Intracerebral germ cell tumors are rare constituting approximately 0.5% of all primary intracranial neoplasms, and 3% of those encountered in children and adolescents. About 90% occurs in patients younger than 20 years. Germinomas are the most frequent subtype. The most common intracerebral site of involvement is the pineal gland, followed by the suprasellar region. Symptoms vary by location. Histological appearance is identical to the testicular counterpart, and testicular examination should always be done, in order to exclude metastasis. Germinomas are very radiosensitive. Chemotherapy is added in order to reduce radiation doses and thereby minimize adverse effects. The prognosis is good, with 5-year survival rates exceeding 90%.

P-4-02
KRAS/BRAF mutations in colorectal carcinoma by CLART CMA technology. A comparative study between real-time PCR, Sanger Sequencing and Chip-hybridization PCR based technology
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Introduction: KRAS and BRAF mutation testing are indicated for management of mCRC patients. Here we compare the performance of the novel integrated KRAS and BRAF Cancer Mutation Array (CLART® CMA KRAS-BRAF) to COBAS® KRAS and BRAF
Mutation Test and also Therascreen® KRAS Mutation kit.

**Material and methods:** Mutation status for KRAS and BRAF was assessed in 135 human colo-rectal carcinoma tissue samples, using split sample aliquots of tumor DNA tested in parallel at 3 different labs (Hvidovre, Madrid and Barcelona). The performance of the novel CMA assay was compared to COBAS® KRAS Mutation Test (Hvidovre), and Therascreen® KRAS RGQ PCR kit (Barcelona). Furthermore, BRAF status was compared to COBAS® BRAF V600 mutation test on 84 individual samples.

**Results:** The CMA kit detected KRAS and BRAF mutations in 39.2% (53/135) and 32% (27/84) of the samples, respectively. COBAS® KRAS and BRAF detected 38% (50/131) and 31% (26/84) mutated specimens, respectively, whereas Therascreen® detected 41% (46/112) KRAS mutated cases. Overall, concordance between CMA to Therascreen was 98% (113/115) and CMA to COBAS was 94% (123/131).

**Discussion and conclusion:** The novel CLART® CMA KRAS-BRAF kit simultaneously detects the most prevalent mutations in KRAS and BRAF genes with high concordance to established methods, supporting the use of this technology to simplify and integrate multi-gene cancer mutation analysis in mCRC.

P-4-03

**DNA methylation in osteosarcoma**

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**Introduction:** The main objective of the presented study is to obtain a better understanding of the epigenetic deregulation in osteosarcoma (OS) tumorigenesis. The focus has been genome-wide analysis of DNA methylation patterns.

Osteosarcoma is the most common primary malignant bone tumor with a strong predilection for occurrence in children and young adults. The first indication of OS is pain, which is often misjudged with “growing pains,” resulting in a late diagnosis. OS is characterized by a complex array of cytogenetic abnormalities with the consequence that only very few predictive markers exist to help predict the prognosis and determine the best treatment of the patient. Therefore, there is a great necessity in understanding the biology of OS progression and metastasis. It is now well accepted that the required molecular alterations for neoplastic initiation and progression can, in addition to genetic changes, be acquired through epigenetic mechanisms. These are mitotically heritable changes in gene expression not caused by underlying changes in the primary DNA sequence.

**Material and methods:** To characterize the DNA methylation pattern of osteosarcoma a methylation specific array screening for the identification of differential DNA methylation patterns between osteosarcoma and non-malignant bone tissue was performed. The Infinium 450k Methylation BeadChip (Illumina) was used to obtain genome-wide coverage as it allows the interrogation of >480.000 methylation sites per sample.

**Results:** The genome-wide epigenetic changes of OS have been established and eight genes with a possible biological effect in OS development and metastasizing have been found altered in the DNA methylation patterns of the promoter regions.

**Discussion and conclusion:** We have substantiated the epigenetic features of OS, with focus on DNA methylation changes. This contributes to a better understanding of the tumorigenesis and progression of this disease.
Can ERG protein expression predict time to castration-resistance in patients with hormone-naive advanced or metastatic prostate cancer managed with primary androgen deprivation therapy?

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Introduction: Androgen deprivation therapy (ADT) is the standard treatment for patients with metastatic prostate cancer (PCa), but biomarkers predicting response to primary ADT and time to castration-resistant PCa (CRPC) is lacking. We assessed ERG protein expression in diagnostic biopsies from advanced and/or metastatic PCa patients to study the association between the androgen regulated TMPRSS2:ERG gene rearrangement and risk of CRPC.

Material and methods: In total, 194 PCa patients managed with first-line ADT were included. Forty-one patients (21%) had no sign of dissemination at treatment start, 38 (20%) had dissemination to lymph nodes only and 115 (59%) had bone metastasis. Immunohistochemical staining (anti-ERG, clone: EPR3864; Roche/Ventana) was performed on formalin-fixed, paraffin-embedded biopsies collected prior to ADT. Patients were labelled ‘ERG-positive’ if minimum one focus demonstrated ERG expression and ‘ERG-negative’ if all foci were negative. CRPC was defined according to EAU guidelines. Multiple cause-specific Cox regression was applied with risk of CRPC as the endpoint and included age, PSA, Gleason score, clinical tumour stage and dissemination stage as explanatory variables. Predictions for CRPC obtained with a model stratified on ERG-status were compared to those obtained with a second model omitted ERG. Time-dependent area under the ROC curve was used to assess the effect of ERG on the discriminative ability.

Results: In total, 105 patients (54%) were ERG-positive and 89 (46%) were ERG-negative. Median follow-up was 5.6 years. ERG groups did not differ in age, alkaline phosphatase, cT category, dissemination stage, but ERG-positive patients had lower PSA-values ($p=0.009$) and Gleason scores ($p=0.012$). The proportional hazard assumption was rejected for ERG-status, thus a stratified multiple Cox regression model was applied. Only PSA at treatment start (HR: 1.33 for 2-fold differences [95%CI: 1.18-1.49], $p$)

Discussion and conclusion: Within the limitations of our study ERG expression was not associated with time to CRPC suggesting that ERG is not a candidate biomarker for predicting response to primary ADT in patients with advanced PCa.

Teratoid hepatoblastoma mimicking neuroblastoma: A case report.

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Introduction: Hepatoblastoma is the most common type of pediatric liver tumour, constituting 1 % of all pediatric malignancies in children. Most cases are sporadic. Distant metastases are present in 20 % of patients at the time of diagnosis. Histologically, the tumour is classified as either purely epithelial or mixed epithelial and mesenchymal type, which can be with or without teratoid features. We here report a case, which presented a diagnostic challenge.

Material and methods: In January 2013, a 5 year old girl was admitted to hospital with dyspnea and enlarged cervical lymph nodes. A large hard mass was palpable beneath the right costal margin. A CT scan of the thorax and abdomen showed an 11 cm large tumour in the right lobe of the liver and a 9 cm large mediastinal tumour compressing the left main bronchus. There were multiple, enlarged abdominal lymph nodes.

Results: A biopsy from the cervical lymph nodes revealed tumour tissue with neuroblastic differentiation. It was composed of sheets and nests of small, round blue cells with fine
nuclear chromatin and scanty cytoplasm. The cells were arranged in a jigsaw puzzle-like pattern separated by narrow fibrous septae. The Ki67 index was almost 100%. There was no ganglion cell differentiation and no neurofibrillary stroma. Immunohistochemical staining showed immunoreactivity for CD56 and synaptophysin, but not for alpha-fetoprotein (AFP). Measurement of urinary catecholamine excretion showed normal levels, while a MIBG (iodine-123-meta-iodobenzylguanidine) scan was negative. Laboratory results revealed a serum AFP level of 112.000µg/l. Since alternate modalities were unable to confirm the diagnosis of neuroblastoma, new biopsies were taken. The liver biopsy was composed of cords and sheets of a mixture of granular and clear fetal-type liver cells. Tumour cells expressed AFP and glypican, but not CD56 or synaptophysin. The mediastinal tumour biopsy was composed of a myxoid stroma with osteoid-like material with small, ovoid tumour cells with scanty cytoplasm arranged in trabeculae and as single cells. Tumour cells expressed AFP, CD56, synaptophysin and glypican. It was concluded that the girl had a mixed epithelial-mesenchymal type hepatoblastoma with teratoid features with metastases to cervical lymph nodes and mediastinum.

Discussion and conclusion: Initial chemotherapy containing carboplatin resulted in partial tumour regression and a decrease in AFP to 18.800µg/l. After several cycles of chemotherapy, the tumours were deemed resectable. The resection margins of the liver were free, while the resection margins of the mediastinal tumour were not. After further chemotherapy, a CT scan of the thorax and abdomen in March 2014 showed no residual tumour, while AFP levels had fallen to 3µg/l.

Discussion and conclusion: Oral ulceration is a frequently encountered clinical diagnosis in surgical pathology. Diagnosing the cause of ulceration requires a combination of clinical information and pathological examination.

Material and methods: A case of oral ulcer of the palate is illustrated. Microscopy shows mixed inflammatory reaction with a relatively high number of plasma cell. Discussion of the case with referring clinician resulted in supplementary clinical information.

Results: Serological testing was positive, both by non-treponemal (WR, RPR) and by treponemal methods. Warthin-Starry staining demonstrates thin, thredlike “cork-screw” organisms in inflamed tissue. Secondary syphilis was diagnosed.

Discussion and conclusion: Syphilis is a rare infection I Denmark with approx. 400 cases reported to SSI annually, but the number has increased in recent years.

P-4-07
Mucinous cystadenocarcinoma of the breast - a case report
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Introduction: Mucinous cystadenocarcinoma (MCACA) of the breast is an extremely rare variant of primary breast carcinoma with only 15 cases reported worldwide. It bears a striking resemblance to MCACA of the ovary and pancreas. Here we report a case of mucinous cystadenocarcinoma of the breast in an 83-year-old woman.

Material and methods: A 83-year-old woman presented with a 13 mm tumor in the left breast. The patient had previously had invasive lobular carcinoma in her right breast in 1986, which was treated with lumpectomy. Radiologic evaluations now demonstrated a hypo-echoic mass in the left breast. A needle biopsy was performed followed by a lumpectomy including sentinel node procedure.

Results: The needle biopsy showed fibrotic breast tissue focally with carcinoma with mucin and tumor cells with an eosinophilic cytoplasm and atypical nuclei. Microscopic findings(lumpectomy): The tumor consisted of numerous irregular mucin filled...
cysts lined with single layer of tall columnar cells, some of them containing mucin. Some of these cells had intracytoplasmic mucin. No tubular structures were identified. The nuclei were highly pleomorphic and mitoses were seen.

Immunohistochemical staining of the tumor cells showed positive reaction for GATA3, GCDFP15 and CK7 and no reaction for estrogen receptor, CK20, Cdx2, and PAX8. The HER2 score was 3+, only seen in few of the prior cases. Ki67 index was 15 %
The sentinel node was without any micro- or macrometastases.

Discussion and conclusion: Most of the cases of primary MCACA of the breast were reported in postmenopausal women. Only 2 of 15 cases had axillary lymph node metastasis. Although no patient has died of this disease so far, longer follow-up is required to determine the exact course of the disease.

P-4-08
Granular Cell Tumor of the Breast
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Introduction: Granular cell tumors, GCT, are rare mesenchymal tumors of neuroectodermal origin and are derived from the Schwann cells of peripheral nerves. GCT accounts for 0.5% of all soft tissue tumors and just 6% of these are found in breast tissue.
The tumor can found throughout the body but occur most frequently in the oral cavity, typically the tongue.
A challenge in the optimum management of GCT of the breast arises from the clinical and radiological presentation which often mimics invasive carcinomas.
We report a case of GCT of the breast in which the patient underwent repeat core needle biopsies due to strongly suspicious imaging.

Material and methods: A 57 year old woman presented with a palpable mass in the left breast situated in the upper, medial quadrant.
Physical examination revealed a rather small, firm mass. No lymphnode enlargement.

Subsequent mammography showed a 1,2 cm mass strongly suggestive of malignancy. Ultrasoundography were equally suggestive of malignancy.
Core needle biopsy was performed and the diagnosis of GCT was made.
With imaging being so convincingly malign it was decided to repeat the core needle biopsy which came to the same conclusion and diagnosis of GCT.
Final tumorectomy confirmed the diagnosis of GCT.

Results: HE-stain showed an ill circumscribed tumor consisting of large, polygonal cells with abundant, highly granular, eosinophilic cytoplasm. The nuclei were centrally located, uniformly small and inconspicuous. The predominant arrangement of the cells were in sheets and nests and showed an infiltrative growth pattern. No features of malignancy were present.
Additional staining with PAS + diastase highlighted the granules in the cells.

Immunohistochemistry was strongly positive for S100 and weakly positive for CD68 (KP1-clone). Cytokeratin, estrogen-receptor and CEA were all negative.

Discussion and conclusion: GCT rarely poses difficulties in histopathological diagnosing but the radiological findings are often indistinguishable from carcinoma and can lead to additional, and perhaps unnecessary, biopsies.
With only 1/1000 of tumors found in the breast being GCT it is naturally rarely encountered and the pathological diagnosis can be drawn into question.
ICH is essential for making a correct diagnosis of GCT with positive S100 and negative ER and cytokeratin getting the pathologist a long way in excluding other possible diagnoses.
GCT has a favourable prognosis with only <2% being malignant. That said, the natural history for malignant GCT is not unlike what is seen for high-grade sarcomas with a high rate of metastasis and short survival.
Perception and interpretation of the CanMEDS roles among pathologists in Denmark

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Introduction: In 2003 Denmark’s current postgraduate medical training program was implemented, introducing competency based education (CBME), applying the CanMEDS framework of competence related to the seven medical roles: Medical expert, Communicator, Collaborator, Manager, Health Advocate, Scholar and Professional. In 2012 the report “Postgraduate medical training in Denmark – status and future perspectives” was published by the National Board of Health. Concerning the CanMEDS roles, the report advised each specialty to develop a more nuanced view on the roles and to adapt the roles to specific work tasks of each specialty. This initiated the implementation of a study on pathologists’ view on CBME and the CanMEDS roles.

Material and methods: By means of four focus group interviews, two in Aarhus and two in Copenhagen with a total of 25 participants, and a national questionnaire, pathologists in Denmark were asked: How do pathologists perceive and interpret the content of the CanMEDS roles? How do they view the roles in relation the work tasks of the specialty? This initiated the implementation of a study on pathologists’ view on CBME and the CanMEDS roles.

Results: The challenges of the perception of CBME and the CanMEDS roles may be summarized in this way
1. a lack of cohesion between the CanMEDS roles and the written curriculum of postgraduate pathology
2. a gap between theory and practice of the CanMEDS roles
3. a need for incorporating the CanMEDS roles into daily practice

Discussion and conclusion: To meet these challenges, firstly, it is suggested to give residents a better understanding of the CanMEDS roles through Knud Illeris’ three dimensions of learning; this socio-culturally oriented learning theory may motivate residents to “learn about learning” and to look at postgraduate medical training in the context of lifelong education. According to international educational literature, socio-cultural learning theories may provide a more profound understanding of the CANMEDS roles, in theory and praxis, and foster a holistic approach to CBME. Secondly, implementation of entrustable professional activities (EPAs), translating the CanMEDS roles into daily practice by defining specialty specific core activities, is recommended. In this way the initial purpose of CBME may be reached: not only training residents to get ready for employment, but also educating them for practice in a complex health system.

More details on pathologists’ view on CBME and the CanMEDS roles are available in the Master thesis "Learning in postgraduate Pathology" acquired at University of Dundee, Scotland in November 2014.

Kompetencevurderingsmetoder i speciallægeuddannelsen i patologi.

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Introduktion: Sundhedsstyrelsen anbefaler i rapporten om Kompetencevurderingsmetoder 2013 (1), at et givet speciale anvender ensartede metoder til at bedømme de uddannelsessøgende kompetencer. DPAS har i foråret 2014 derfor nedsat en arbejdsgruppe for at a) identificere de bedst egnede kompetencevurderingsmetoder indenfor speciallægeuddannelsen i patologisk anatomi og cytologi, b) at udarbejde en enseretning af de identificerede metoder i alle tre uddannelsesregioner.

Materiale og metoder: Arbejdsgruppen har på baggrund af den gældende målbeskrivel-
se, rapporten om kompetencevurderingsmetoder og ved hjælp af den aktuelle medicinsk-pædagogiske litteratur udarbejdet forslag til hvordan de udannelsessøgende bedst kan vurderes. Der er i metodevalg lagt vægt på at opnå både formativ vurdering af den uddannelsessøgendes kompetencer, og en summativ vurdering af, om der er opnået et tilfredsstillende fagligt niveau ved uddannelsens slutning.

**Resultater:** I målbeskrivelsen anføres fem metoder til kompetencevurdering: struktureret observation og samtale, audit af beskrivelser, 360 graders evaluering og kursusgodkendelse. Arbejdsgruppen foreslår at disse fem metoder suppleres med EPA (Entrustable Professional Activities) (2). EPAs kan beskrives som en metode til at oversætte de syv lægeroller til arbejdsopgaver der er essentielle for det pågældende speciale, og de kan bruges til dels at vurdere den uddannelsessøgendes kompetencer, dels til at følge den uddannelsessøgendes udvikling.


**Diskussion og konklusion:** Med EPAs knyttes lægeroller og læringsmål til arbejdsopgaver der er essentielle for specialet, de integreres herved i det daglige arbejde og bliver dermed meningfulde. I en travl hverdag er EPA en hjælp til at vurdere de uddannelsessøgende mhp. hvornår arbejdsopgaverne kan udføres på egen hånd. Med indførelse af EPAs opnås en mere ensartet kompetencevurdering af den enkelte uddannelsessøgende i alle tre uddannelsesregioner.


http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3613304/