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Fredag den 17. Marts 2017:

Frie foredrag: O1 og O2

Postersessioner: P1 og P2

Lørdag den 18. Marts 2017:

Frie foredrag: O3 og O4

Postersessioner: P3 og P4

O1.1-O1.5

Clonal expansion and linear genome evolution through breast cancer progression from pre-invasive stages to asynchronous metastasis

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Introduction. Controversy exists between two fundamental models of malignant progression, addressing the issue of timing of metastasis-enabling genomic alterations and the degree of genomic concordance between primary tumors and its metastases. According to the linear progression model, the malignant cells pass through multiple successive rounds of genetic changes and selection within the primary tumor microenvironment, before tumor cell dissemination successfully results in a metastatic lesion. The parallel progression model proposes parallel, independent progression of metastases arising from early disseminated tumor cells and predicts greater disparity between the primary tumor and metastatic lesions.

Evolution of the breast cancer genome from pre-invasive stages to asynchronous metastasis is complex and mostly unexplored, but highly demanded as it may provide novel markers for and mechanistic insights in cancer progression. The increasing use of personalized therapy of breast cancer necessitates knowledge of the degree of genomic concordance between different steps of malignant progression as primary tumors often are used as surrogates of systemic disease.

Material and methods. Based on exome sequencing we performed copy number profiling and point mutation detection on successive steps of breast cancer progression from one breast cancer patient with invasive ductal carcinoma, including two different regions of Ductal Carcinoma In Situ (DCIS), primary tumor and an asynchronous metastasis. This was followed by targeted deep sequencing for validation of point mutations.

Results. In the copy number analysis we identified a remarkable landscape of somatic mutations, retained throughout breast cancer progression and with new mutational events emerging at each step. Some aberrations, however, were only found in the asynchronous metastasis. Density plots of mutation frequencies of validated somatic nonsynonymous point mutations, not affected by copy number events revealed subclonality within several samples, which was supported by the copy number analysis.

Discussion and conclusion. In this patient, we provide evidence for linear progression of metastatic disease in which dissemination from the primary tumor occurs relatively late in molecular time. Our study reveals common ancestry of the malignant cells and that early acquired copy number

aberrations as well as point mutations are retained as imprints in the cancer genome, but also shows substantial acquisition of additional aberrations in the metastasis. The emergence of new aberrations in the metastasis emphasizes the importance of genomic analysis on not only the primary tumor but also on metastatic tissue at recurrence in order to offer the patients molecularly targeted therapy.

Assessment of the potential use of phosphohistone H3 in mitotic counting on invasive breast cancer needle core biopsies

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Introduction

Mitotic activity is a pivotal prognostic factor in invasive breast carcinoma. Mitosis figure count on hematoxylin and eosin (H&E) stained slides is an integral part of the widely used histological grading system for breast cancer. Mitotic index is one of three constituents of this grading system. In general, histologic grading is highly subjective with moderate interobserver reproducibility, notably mitosis figure count presents great challenges. Furthermore, several studies have demonstrated an overestimation of mitotic count in breast cancer resection specimen (RES) in comparison to biopsy material. Phosphohistone H3 (PHH3) is a specific immunohistochemical marker of mitoses that has been validated in other types of malignancies.

In this study we aimed to evaluate if the use of PHH3 in the manual assessment of mitotic count on needle core biopsies (NCB) from invasive breast carcinomas more reliably will reflect mitotic activity of tumor, evaluated on RES, compared with routine H&E staining.

Material and methods 30 consecutive patients diagnosed with invasive breast carcinoma were retrieved from the archives of the Department of Pathology at Vejle Hospital. Only patients for whom we had NCB material and RES were eligible. They were selected from a period of time (July 2014-December 2014) in which PHH3 immunohistochemistry was performed routinely on NCB and RES with invasive breast carcinomas.

Results In concordance with prior studies, our data showed that H&E mitotic counts in NCB (mean=6.63 mitoses/10 high power fields (HPF)) were underestimated in comparison with their corresponding RES (mean=7.83 mitoses/10 HPF), albeit not reaching statistical significance ($P=0.085$). On the other hand, PHH3 mitotic count was significantly ($P=0.0017$) overestimated in NCB material (mean=26.43 mitoses/10 HPF) compared to the corresponding RES (mean=14.8 mitoses/10 HPF), thus indicating poor agreement of PHH3 between NCB and RES. Correlation between H&E mitotic count in NCB and in the corresponding RES was observed to be higher ($r=0.86$, $P<0.001$) than the correlation between PHH3 mitotic count in NCB and RES ($r=0.72$, $P<0.001$).

Discussion and conclusion The data do not validate PHH3 as a more reliable marker in mitotic counting on NCB. Although potentially identifying more mitotic figures, PHH3 does not show better agreement in mitotic count between NCB and RES. Thus, this study does not lend support to the hypothesis that PHH3 mitotic count in NCB is superior to conventional H&E mitotic count in reflecting mitotic activity of the tumor in RES.

Digital assessment of sentinel nodes in breast cancer by image analysis

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Introduction: Breast cancer (BC) is one of the most common cancer diseases in women with more than 1.67 million cases each year worldwide. In BC, the sentinel lymph node (SLN) pinpoints the first lymph node(s) into which the tumor spreads and is usually located in the ipsilateral axilla. In patients with no clinical signs of metastatic disease in the axilla, a SLN biopsy (SLNB) is performed. Assessment of metastases in the SLNB is done in a conventional microscope by manually observing metastasis and measuring its size and/or counting the number of tumor cells. This is essential in order to categorize the type of metastases as macrometastases, micrometastases or isolated tumor cells, which treatment is optimal. The aim of this study was to evaluate whether digital image analysis can decrease the workload for the examining pathologist without compromising the diagnostic accuracy.

Materials and methods: Consecutive SLNB from 135 patients with localized BC receiving surgery in the period of February to August 2015 were collected and included in this study from Dept. of Pathology Rigshospitalet, Odense and Slagelse. Formalin-fixed and paraffin-embedded tissue sections were analyzed by immunohistochemistry (IHC) using the BenchMark ULTRA Ventana platform. Rigshospitalet used a mixture of cytokeratin CK7 and CK19, Slagelse used pancytokeratin AE1/AE3 and Odense used pancytokeratin CAM5.2 for detection of epithelial tumor cells. SLNB sections were assessed in a conventional microscope according to national guidelines for SLNB in BC patients. Stained sections were scanned by a Hamamatsu NanoZoomer-XR digital whole slide scanner and the images were analyzed by Visiopharm's software using a custom made algorithm for SLNB in BC. The algorithm was optimized to the cytokeratin antibodies, based on staining intensity and background staining.

Results: Conventional microscopy was used as golden standard for assessment of positive tumor cells and compared with digital image analysis (DIA). The algorithm demonstrated a sensitivity of 100% (no false negative slides were observed). By excluding the negative slides the workload could have been decreased by 58.2% by using this SLNB algorithm as a screening tool.

Discussion and conclusion: The SLNB algorithm demonstrated a sensitivity of 100% regardless of the antibody used for IHC and staining protocol. No false negative slides were observed, which proves the SLNB algorithm is an ideal screening tool for selecting those slides not necessary for a pathologist to see. Implementation of automated image analysis of SLNB in BC would decrease the workload for examining pathologists by over 50%.

Transferrin receptor-1 and ferritin heavy and light chains in astrocytic brain tumors: expression and prognostic value

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Background: Astrocytic brain tumors are the most frequent primary brain tumors. Treatment with high-dose radio-therapy and temozolomide has increased survival making prognostic biomarkers increasingly important. The aim of the present study was to use immunohistochemistry to investigate the expression and prognostic value of three key iron-related proteins: transferrin receptor 1 (TfR1), ferritin heavy (FTH) and light (FTL) chain.

Materials and methods: A cohort of 111 astrocytic brain tumors (WHO grade II-IV) was stained immunohistochemically with antibodies against TfR1, FTH and FTL and scored semi-quantitatively. Double-immunofluorescent stainings were established to determine the phenotype of TfR1+, FTL+, and FTH+ cells.

Results: TfR1, FTH, and FTL were expressed by tumor cells in all grades. TfR1 increased with grade ($p < 0.001$), but was not associated with prognosis. FTH and FTL did not increase with malignancy grade, but low levels of tumor cell FTH were associated with shorter survival in anaplastic astrocytomas ($p = 0.01$). FTH and FTL were also expressed by cells with microglial/macrophage morphology. Low FTH expression by microglia/macrophages correlated with shorter survival in anaplastic astrocytomas ($p < 0.05$). FTL+ microglia/macrophages were frequent in glioblastomas, and high FTL levels were correlated with shorter survival in the whole cohort ($p = 0.01$) and in patients with anaplastic astrocytomas ($p = 0.02$). Using double-immunofluorescence, tumor cells were found to co-express the stem cell marker CD133 and TfR1, FTH and FTL. FTH and FTL were also co-expressed by IBA-1+ microglia/macrophages.

Discussion and conclusions: TfR1, FTH, and FTL are present in grade II-IV astrocytomas. TfR1 levels increased with malignancy grade, but had no prognostic value. Low levels of FTH+ tumor cells and microglia/macrophages informed poor survival in anaplastic astrocytomas, while high amounts of FTL+ microglia/macrophages had a negative prognostic value. The results indicate that regulation of the iron metabolism in astrocytic brain tumors is complex involving both autocrine and paracrine signaling.

Impact of primary antibody clone, format, protocol and stainer platform on Ki67 proliferation indices in breast carcinoma

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Introduction In breast carcinoma, Ki67 proliferation index (PI) determined by immunohistochemistry (IHC) is an independent prognostic marker of survival, and is used as a surrogate marker to distinguish the molecular subgroups luminal type A and luminal type B. The St. Gallen International Expert Consensus suggested in 2013 a Ki67 PI cut-off point of 20% for type A vs. B, but changed it in 2015 to 20-29%. However, visual estimation of Ki67 PI is laborious, based on different counting methods, and prone to inter-observer variation. Moreover, the impact of different IHC methods (antibody clones, protocols and platforms) has not been taken into account. Quantitative digital image analysis (qDIA) may increase standardisation but is rarely applied. qDIA may in an objective and standardised way reveal differences in PIs due to different IHC methods. The aim of this study was to assess the impact of Ki67 antibody (Ab) clones, systems, protocols and stainer platforms on the Ki67 PI.

Material and Methods Serial sections of tissue micro arrays from 41 consecutive routinely processed resection specimens of breast carcinoma were immunostained using anti-Ki67 Ab clones Mib1, SP6, MM1 and 30.9 as concentrates (Conc) with protocols optimised for each of the stainer platforms DAKO Autostainer, Leica Bond, and Ventana Benchmark Ultra, and as ready-to-use systems (RTUS) on the appropriate stainer platforms. Neighbouring Ki67 and pancytokeratin stained sections were digitally aligned using the Visiopharm virtual double staining (VDS) algorithm to ensure that only tumour cells were counted.

Results The Ki67 PI obtained by VDS qDIA ranged from 1% to 96% with a median of 27%. The overall mean PIs are shown in the table. Clone Mib1 as Conc gave the most stable results across platforms. With DAKO Mib1 RTUS the PI was 11.3% lower than with Mib1 Conc. With Leica Clone MM1 RTUS the PI was 13.2% lower than with Mib1 Conc. With Ventana SP6 Conc and 30.9 RTUS the PI was 14.4% and 12.9% higher than with Mib1 Conc.

Clone and format	Overall mean PI (%)		
	Dako	Leica	Ventana
Mib1 Conc	35.9	34.0	30.8
SP6 Conc	31.4	34.1	45.2
30.9 RTUS			43.7
Mib1 RTUS	24.6		
MM1 RTUS		20.8	

Discussion and conclusion In breast cancer, the great variation in Ki67 PI results caused by different staining methods must be taken into account. Mib1 Conc in combination with an optimised protocol seems to give the most comparable results across platforms for determining Ki67 PI.

O2.1-O2.6

Immunohistochemical expression of p16, p53 and IMP3 in uterine serous and endometrioid adenocarcinoma

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Introduction

Endometrial carcinoma is the most common gynecological malignancy in Denmark. Primary treatment is based on preoperative risk classification, and tumor subtyping is an essential part of this. According to newer studies, correct subtyping is difficult based on morphology alone, and even with the aid of immunohistochemistry (IHC) inter-observer variation can be substantial. IMP3 has been shown to improve the differentiation between uterine serous (SC) and endometrioid adenocarcinoma (EC). The aim of our study was primarily to determine if IMP3 adds to the diagnostic accuracy when used alone and in combination with p16 and p53 in SC and EC grade 1, 2 and 3, and secondly to determine the inter-observer agreement on IHC results.

Materials and Methods

From the archives of the Department of Pathology, OUH, paraffin embedded tumor tissue from 60 endometrial carcinomas with typical morphology was retrieved (15 SC and 15 EC grade 1, 2, and 3 respectively). Two pathologists with special interest in gynecologic pathology verified correct diagnosis according to WHO 2014 criteria. New whole-slide sections were cut, renumbered in order to achieve blinding, and stained with IMP3, p16, p53 and H&E. Staining was graded semi quantitatively twice by two pathologists.

Results

Positive IMP3 staining (strong cytoplasmatic staining of more than 95% of the tumor cells) was seen in 8 SCs and 1 EC (grade 3). However most ECs and all SCs showed some degree of staining, but with a weaker intensity. A total of 12 SCs and 1 EC (grade 3) were p16 positive (strong, diffuse nuclear and cytoplasmatic staining of more than 95% of the tumor cells) and 14 SCs and 1 EC (grade 3) were p53 aberrant (nuclear staining of more than 75% or 0% of tumor cells). The combination of positive IMP3, positive p16 and aberrant p53 was only seen in SCs (8/15). The sensitivity, specificity, positive and negative predictive values for SC vs. EC were 53%, 98%, 89% and 86% for IMP3, 80%, 98%, 94% and 92% for p16 and 93%, 98%, 93% and 98% for p53. Intra- and inter-observer agreement on p16 and p53 was almost perfect (100%, $\kappa=1$ and 98%, $\kappa=0,96$ respectively). Agreement on IMP3 was substantial (88-98%, $\kappa=0,63-0,94$).

Discussion and Conclusion

The present study shows that IMP3, p16 and p53 are expressed more often in SC than in EC. p16 and p53 may be helpful on their own in differentiating between SC and EC, whereas IMP3 is best used in combination with the other two markers.

Intra- and inter-observer agreement is acceptable for all three markers.

THE QUALITY OF IMMUNOHISTOCHEMICAL p57 STAINING IS IMPORTANT IN DIAGNOSING HYDATIDIFORM MOLES

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Introduction Routine histopathological examination of hydatidiform moles has become more difficult in the recent years, since evacuation occurs at an earlier gestational age due to the introduction of early routine ultrasound examination. A significant inter- and intraobserver variability in the diagnosis of hydatidiform mole has been reported. The value of immunohistochemical staining for p57 as an ancillary technique in the diagnostic process is generally accepted. The quality of the p57 staining is therefore essential.

Material and Methods The Nordic Immunohistochemical Quality Control (NordiQC) external quality assessment scheme evaluates the performance of immunohistochemical tests from pathology laboratories¹. We present the results of the p57 challenge from NordiQC run 41/2014². Serial sections from a multi-tissue block containing tonsil, placenta, two partial and two complete hydatidiform moles were sent to 121 laboratories in 22 countries, which returned the stained slides for central assessment in NordiQC.

Results The laboratories used six different p57 antibodies as concentrates and four different in a ready to use format. Ninety-five laboratories (79%) achieved a sufficient mark (optimal or good), while 26 laboratories (21%) achieved an insufficient mark (borderline or poor).

The best results were obtained with clone KP10. Prevalent features of insufficient stains were a too weak staining reaction (73%) or a poor signal-to-noise ratio (27%). The most frequent causes of insufficient stains were use of clone 25B2, too low concentration of the primary antibody and/or insufficient heat-induced epitope retrieval.

Discussion and Conclusion Insufficient staining may lead to misclassification of hydatidiform moles causing inappropriate treatment and follow up. A false negative staining reaction may imply that a partial mole is interpreted as a complete mole. Selection of primary antibody, careful calibration of antibody dilution and efficient heat-induced epitope retrieval are the main prerequisites for optimal staining results.

¹www.nordiqc.org

²www.nordiqc.org/Run-41/Assessment/Run41_p57.pdf

VERY LONG TERM FOLLOW-UP (9½ YEARS) OF APTIMA mRNA ASSAY IN TRIAGE OF LSIL.

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Introduction

Long term follow up data on HPV mRNA testing are warranted to establish the use and safety of the test. We have previously reported high sensitivity and negative predictive value (NPV) and relative good specificity of the Aptima HPV assay in triage of women with LSIL (Waldstrøm et al; Arch Pathol Lab Med, 2011). We now aim to report register-based follow-up data after 5 and 9½ years.

Material and Methods

Follow-up data on the 442 women with LSIL included in the original study was retrieved from “Patobank”; a national register containing all diagnosis of cytological and histological samples from Denmark. The baseline Aptima testing in 2009 was performed on the residual material from 3 years old Thin-Prep samples.

Results

Follow up data was available on 421 (5 years) and 431 of the women (9½years).

76 and 102 of the women were diagnosed with CIN2+ after 5 and 9½ years respectively ,and 38 and 59 with CIN3+.

The sensitivity and specificity after 5 years of follow-up was 90.8% and 38.8% for CIN2+ and 94.7% and 36.3% for CIN3+ with an NPV of 95.0% and 98.6%, respectively.

After 9½ year the sensitivity for CIN2+ and CIN3+ dropped to 88.2% and 89.8% with NPV of 91.6% and 95.8% respectively.

3 carcinomas were detected and they were all positive by the baseline Aptima HPV test.

Discussion and Conclusion

Our data shows high sensitivity and NPV after 5 years, similar to the previous reported follow-up data after 3 years. The sensitivity and NPV slightly drops after 9½ years.

Digital assessment of mitosis activity in leiomyosarcomas by automated counting of PHH3 expression

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Introduction: The diagnosis of leiomyosarcoma (LMS) in corpus uteri is based on three criteria: cytological atypia, tumour cell necrosis and mitotic count. The latter is a time-consuming process that comes with a high inter-observer variability. This study aimed at comparing digital and automated phospho-histone H3 (PHH3) count with mitotic count in this tumor type.

Material and Methods: Twenty-five cases of LMS and 19 cases of smooth muscle tumours of uncertain malignant potential (STUMP) were included. From each tumour, two consecutive slices were cut and mounted on glass slides. One slide was stained with haematoxylin and eosin (HE) and the other slide stained with polyclonal PHH3 antibodies (CellMarque; Rocklin, CA, USA). HE sections were assessed by either manual assessment of the mitotic figures globally and in hotspots (10 HPF) or manual PHH3 count in hotspots (10 HPF). The PHH3 sections were in addition assessed by digitalised image analysis (DIA) counting the number of PHH3 positive nuclei globally and in hotspots, respectively.

Results: The study showed a significant correlation between manually assessed counts for mitotic cells and manually assessed counts for PHH3-positive nuclei. Correlation were also found between DIA and manually assessed global counts for PHH3-positive nuclei and mitoses, respectively ($p < 0.00001$), between DIA assessed PHH3 counts in hotspots and manually assessed PHH3 counts in hotspots ($p < 0.00001$) and between DIA-assessed PHH3 counts in hotspots and manually assessed mitotic counts in hotspots ($p = 0.01$). With a cut-off at 10 mitoses or PHH3 positive nuclei per 10 HPF, a sensitivity of 94% and a negative predictive value of 93% were obtained.

Discussion and conclusion: The significant correlation found between PHH3-positive nuclei and mitotic cells in this study supports the use of PHH3 as a surrogate marker for mitosis count in malignant stromal tumors. However, when patient safety is taken into account the use of DIA needs minor adjustments before taken into clinical use.

Detection of EGFR mutation in plasma from advanced non-small cell lung cancer patients.

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Introduction: Next-generation EGFR tyrosine kinase inhibitors (TKIs) have been developed to overcome resistance to earlier generations of such drugs mediated by a secondary T790M mutation in EGFR. Obtaining a second tumor biopsy to assess the T790M mutation status can be problematic depending on the size and location of the tumor. Alternatively, liquid biopsy, a non-invasive means to detect cancer cell DNA in blood, has the potential to allow detection of cancer, measurement of tumour burden, and evaluation of drug sensitivity or resistance.

In the present study, we retrospectively examined whether PCR or NGS based analysis of cell free DNA (cfDNA) might allow assessment of TKI-sensitizing and the T790M resistance mutation of EGFR in patients with advanced NSCLC initially and after resistance to EGFR-TKI therapy.

Materials and Methods: Plasma and FFPE biopsy material from patients diagnosed with histopathological confirmed stage IV NSCLC from a retrospective biomarker study were included in this study. Using a PCR based method (COBAS, Roche) and NGS (Oncomine Lung cfDNA assay) we tested 20 patients, 10 EGFR mutated and 10 EGFR wild-type. From the EGFR mutated patients an initial sample and a sample at the time of progression were tested. Using both methods, we have initiated analyzing plasma from patients with resistance to EGFR-TKI therapy when a tissue biopsy is impossible to obtain. So far 3 patients have been tested.

Results: In the initial sample, after diagnosis, we found concordance between EGFR mutation status of the FFPE sample and the plasma sample. At time of progression we found the T790M mutation in 2 out of 10 plasma samples from the retrospective project and in 2 out of 3 patients tested in the daily clinical practice.

Discussion. In this limited study, we found concordance between the EGFR mutation results from plasma and FFPE material. We detected the T790M mutation in 4 out of 13 patients with resistance to anti EGFR treatment indicating a T790M based resistance to tyrosine kinase inhibitors. This is in concordance with the literature.

Using the experience from the retrospective study we have started to test plasma from patients with resistance to EGFR-TKI if a tissue biopsy is impossible to obtain. In two of the patients we found the T790M EGFR mutation enabling the oncologist to treat the patients with Next-generation TKI.

Conclusion: In our view cfDNA is a valuable addition to mutation analysis on tissue biopsies, especially in the diagnosis of second-mutation based EGFR-TKI resistance.

Multiplexing: next-generation immunohistochemistry

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Introduction: Immunohistochemistry (IHC) is used in almost every cancer case to determine its type and treatment. Usually, in routine IHC, only one biomarker is visualized at a time and each stain is reviewed manually by microscopy. Accordingly, a more cost- and time-efficient method that also preserves tissue seems desirable. Moreover, only little attention is directed at co-expression in IHC single stains. For instance, key markers such as Ki67 and PD-L1 are often expressed on both tumor and inflammatory cells, which may have profound consequences for the accuracy of their quantification. Instead of conventional IHC with chromogenes, fluorescent dyes enable multiplexing of numerous biomarkers. Because cells more easily are distinguished, accuracy of potential image analysis also increases. A profound drawback of fluorescence is, however, the loss of morphology and contextual tissue. The method is thus rarely used in routine pathology. To address this, we propose a digital alignment that adds the information of an H&E stain. The study aimed to develop a simple multiplexing technique with three fluorescent markers with the possibility of creating a virtual stain that conveys fluorescent signals on an H&E stain.

Material and methods: Two sections from a formalin-fixed, paraffin-embedded melanoma were cut. An indirect, sequential IHC procedure was utilized where primary antibodies were visualized with fluorescent dyes instead of, e.g., DAB or Fast Red. The antibodies Ki67, CD163, and CD4 were applied on the first section and Ki67, CD163, and Melan-A on the second section. They were dyed by CY5, FITC, and Rhodamin. Nuclei were counterstained with DAPI. One of the sections may be re-stained with H&E and their whole slide images may be aligned as a virtual multiple stain.

Results: The three biomarkers of each slide were successfully multiplexed on a fully commercial platform for routine IHC. By visual inspection, all signals were very distinct, and they displayed sufficient sensitivity and specificity. It seemed practicable to align multiple multiplexed stains or add an H&E stain.

Discussion and conclusion: A virtual combination of fluorescent signals on H&E stains seemed very feasible to perform and assess. In time, they are potential alternatives to conventional IHC in both routine pathology and research studies. Of great importance, multiplex imaging that includes morphologic and contextual information may accurately define the suppressive mechanisms within the tumor microenvironment, which may guide immunotherapy towards an increased tumor-specific immune response.

O3.1-O3.7

Detection of mRNA of telomerase protein (hTERT) in benign naevi and malignant melanomas using RNAscope

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Introduction: Telomerase is reactivated in approximately 90 % of all cancers, including malignant melanoma. It is a revers transcriptase consisting of human telomerase RNA (hTR) and human telomerase reverse transcriptase (hTERT) with the ability of elongating the telomeres to achieve cell immortality. The possibility of hTERT mRNA being a diagnostic or prognostic biomarker of malignant melanoma is investigated in this study where a novel in situ hybridization technique is examined. Furthermore, the hTERT mRNA expression is analysed according to the association with the Ki-67 proliferation index and Breslows thickness.

Material and Methods: Archival formalin-fixed, paraffin-embedded (FFPE) tissue of 17 malignant melanomas and 13 benign naevi were used. The tissue was cut in a series of three and detection of hTERT mRNA by RNAscope, immunohistochemical staining for melan-A and melan-A/Ki67 double staining was performed. After a virtual double stain of the RNAscope and melan-A slide, the amount of hTERT mRNA expressed in the tissue was manually quantitated using counting frames. The Ki-67 proliferation index was calculated using digital image analysis.

Results: There is a statistical significant ($p < 0.001$) difference in the hTERT mRNA expression between malignant melanomas and benign naevi. There is a correlation between the hTERT mRNA expression and the Ki67 proliferation index (Spearmans rho = 0.72) and between hTERT mRNA expression and Breslows thickness (Spearmans rho = 0.55).

Discussion and conclusion: RNAscope is a reliable method for the detection of hTERT mRNA in FFPE tissue. Because of an overlap in the hTERT mRNA expression between malignant melanoma and benign naevi, hTERT can not be used as a diagnostic marker. Furthermore, because of the association between hTERT mRNA expression and Breslows thickness and the Ki67 index, hTERT mRNA could be a possible prognostic marker of malignant melanoma.

Characterization of the Inflammatory Subtype in Metastatic Melanoma

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Introduction

Recent results suggest that innate immune cells are associated with ulceration, tumor progression, and poor survival in melanoma. However, if the pattern of innate immune cells, number and type, is similar in primary tumors and corresponding metastases (synchronous and metachronous) is yet to be elucidated. Thus, we aimed to characterize key factors of an inflammatory microenvironment in primary melanomas and their corresponding metastases. Also, we aimed to evaluate the immune cell infiltration in BRAF^{V600E} positive melanomas compared with BRAF^{V600E} negative tumors. Our results may elucidate the infiltrative patterns of innate immune cells in metastatic melanoma, which may lead to novel therapeutic options.

Materials and Methods

Our cohort consisted of 385 primary melanomas and 96 cutaneous and lymph node metastases. The tumor specimens were stained with immunohistochemistry for BRAF^{V600E}, CD163+ macrophages, CD123+ plasmacytoid dendritic cells (pDCs), and CD66b+ neutrophils. The tumor sections were visualized and analyzed in Visiopharm Denmark that estimated an objective area fraction of the stained markers. BRAF^{V600E} status was scored as either present or absent.

Results

We found a distinct inflammatory subtype in primary cutaneous melanoma associated with denser macrophage (p=0.03) and pDC (p=0.02) infiltration in BRAF^{V600E} positive tumors compared with BRAF^{V600E} negative tumors. In the metastases, we detected massive macrophage infiltration (p<0.0001), and neutrophils (p=0.38) and pDCs (p=0.15) expressed at the same extent as in primary tumors. Primary melanomas in men exhibited 2.8 (95% CI: 1.42; 5.49, p=0.003) times increased neutrophil influx compared to melanomas in women and this was associated with worse melanoma-specific survival HR=1.52 (95% CI: 1.04-2.21, p=0.03).

Discussion and Conclusion

Our results may suggest an essential role for innate immune cells in the metastatic process. Also, our results highlight the importance of recognizing the functions of innate immune cells as they may be used to improve the effects of immunotherapy and BRAF^{V600E} targeted therapy.

Anaplastic Lymphoma Kinase protein: Promising marker for the differential diagnosis of Merkel Cell Carcinoma and Small Cell Lung Carcinoma.

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Introduction: Merkel Cell Carcinoma (MCC) is a rare neuroendocrine, highly malignant, aggressive cutaneous neoplasm of elderly patients. Important differential diagnosis is metastatic Small Cell Lung Carcinoma (SCLC). Varying immunohistochemical markers are used to differentiate between these two neoplasms. This study, investigated the potential of Anaplastic Lymphoma Kinase protein (ALK) for discriminating MCC from SCLC.

Materials and methods: 34 well-characterized, formalin fixed and paraffin embedded blocks of MCC and 69 blocks of SCLC were retrieved. After confirming the diagnosis, immunohistochemistry for ALK D5F3 was performed. The staining results were quantified as *H*-score by multiplying the percentage of positive tumor cells (0% to 100% with interval of 10) by the immunohistochemical staining intensity.

Results: A positive reaction for ALK(D5F3) was observed in 32(94.11%) cases of MCC and in 5(7.24%) cases of SCLC. The difference of *H*-score in ALK (D5F3) expression between these two entities was statistically significant ($P < .001$). The overall median *H*-score (240) for ALK (D5F3), in MCC was significantly higher than the overall median *H*-score (0) obtained in the group of SCLC ($P < 0.0001$). All 25 cases of MCC with the typical IHC pattern (CK20_{pos} TTF1_{neg}) were positive for ALK (D5F3) with median *H*-score of 270. In the sub-types of atypical MCCs – all 4 cases with the IHC pattern (CK20_{pos} TTF1_{pos}) were positive for ALK (D5F3) with a median *H*-score of 195. In the 2 cases of MCC with the atypical IHC pattern of (CK20_{neg} TTF1_{neg}) and in the 3 cases with the atypical IHC pattern of (CK20_{neg} TTF1_{pos}), 1 (50%) and 2(66.66%) were positive for ALK (D5F3) with a median *H*-score of 160 and 165, respectively. Five (7.24%) out of 69 cases of SCLC were positive for ALK (D5F3) and were all related to the sub-type of SCLC with the typical IHC pattern of (TTF1_{pos} CK20_{neg}). The median *H*-score in this group was 60. The number of ALK (D5F3) positive SCLCs was low and 64 (93%) out of 69 SCLCs analyzed were completely negative.

Discussion: The most common markers used to differentiate MCC from SCLC are CK7, CK20 and TTF1. A significant proportion of both the types neither express CK7 nor CK 20. Nowadays, TTF1 is being used as a sensitive as well as a specific marker for SCLC in which 80 to 90% of all cases have been reported positive. This means that 10-20% of the SCLC's are TTF1 negative.

Our results showed that a high frequency of MCC(32 cases; 94.11%) is positive for ALK(D5F3) whereas only a minor fraction of SCLC(5 cases; 7.24%) revealed any positivity. Harumi Nakamura et al studied the aberrant ALK expression in high-grade pulmonary neuroendocrine carcinoma where 69 cases were of SCLC. We also analyzed 69 cases of SCLC in addition to 34 cases of MCC. In their study immuno-positive cases comprised 2 (2.9%) of 69 SCLCs. In comparison we found 5 cases (7.24%) of SCLCs showing expression with ALK(D5F3).

Conclusion:

ALK is very useful to differentiate MCCs from SCLCs. We suggest immunostaining with ALK as an additional marker in the panel of TTF1, CK7 and CK20.

Study of the local dissection guideline for re-excision specimens of nonmelanoma skin cancer

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Introduction:

In the case of incompletely excised basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) of the skin the department of pathology will often receive a re-excision specimen. There are no national guidelines for the optimal dissection procedure of these specimens. At the department of pathology at Sjællands Universitetshospital, Roskilde, the dissection procedure is to sample the ends of the specimen and every second slice or slices for each centimeter depending on the location on the body. The dissection is carried out by a biomedical laboratory scientist. It is important to examine these specimens thoroughly in order to find any residual tumor and to evaluate the margins properly. The purpose of this study was to evaluate if the local dissection procedure is sufficient to identify residual tumor.

Material and Methods:

We collected samples from March to November 2016 and included re-excision specimens of BCC or SCC where tumor was found at the margins of the index specimen, which included biopsy (punch, shave or excisional) and excision. In total 95 samples were examined microscopically and those containing residual tumor were not examined further. In specimens with no residual tumor all the remaining tissue was consequently sampled and examined microscopically. As the current dissection procedure was used initially, this served as an internal control when comparing to the result from the subsequent sampling of the specimen in full.

Results:

In 35 samples we did not find any residual tumor in the tissue blocks sampled using the current dissection procedure. When the remaining tissue was subsequently sampled, we found residual BCC in 3 samples (9%), all of which had negative margins. In the rest of the samples (n=32, 91%) no residual BCC or SCC was found in the remaining tissue. We also checked if the correct procedure was used according to local guidelines. In 1 of the 3 samples where residual BCC was found by the second sampling, too little tissue was sampled initially compared to the guideline.

Discussion and conclusion:

When considering the best dissection procedure it is a question of cost-efficiency and finding the balance between sampling sufficiently and unnecessarily. Another challenge is making the guideline explicit and easy to follow. This is especially important when biomedical laboratory scientists perform the dissection. Regardless of the dissection procedure there might be individual cases where the pathologist find it necessary to extend the sampling. This was a small study, however we conclude that the current cutting procedure, if followed, is sufficient, also when taken into consideration that all the re-excisions with residual tumor in the secondly embedded blocks had negative margins.

Immunohistochemical detection of the BRAF V600E mutation in melanomas

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Introduction: Oncogenic BRAF mutations are found in approximately 50% of malignant melanomas, and the majority (90%) of these mutations occur in exon 15 (V600E). Targeted therapies have improved clinical outcome in patients with BRAF-mutant metastatic melanoma, and therefore detection of BRAF V600E mutation in melanomas have become a mandatory requirement before treatment with BRAF-inhibitors. Several methods to detect the BRAF-genotype on formalin-fixed, paraffin-embedded tissue are usable, such as PCR-based methods. However, these methods are not available at every Pathology laboratory. Immunohistochemistry (IHC) using anti-BRAF V600E monoclonal antibody VE1 has shown high sensitivity and specificity in recent studies. The purpose of this study was to examine the sensitivity and specificity of the monoclonal antibody VE1 in comparison to the PCR-based technic on tissue from primary melanomas and metastatic melanoma.

Materials and methods: 54 cases of primary malignant melanoma (n=21) and metastatic melanomas (n=33) were included in the study. All 54 samples had been examined by PCR-based technique, by which 23 samples were found to harbor the BRAF V600E mutation and 31 samples didn't. IHC staining for the BRAF-V600E mutated protein was performed using the monoclonal antibody BRAF (V600E), clone VE1. All 54 BRAF-V600E immunostained slides were evaluated by 2 independent observers, who were blinded to the BRAF-mutational status by PCR-technique. The IHC-staining was labeled as positive, if > 70% of the tumor cells showed cytoplasmatic staining. The staining intensity was graded, 0 (absent), 1 (weak), 2 (moderat) and 3 (strong).

Results: The two independent observers were concordant in IHC staining in all cases. Interpretation due to melanin pigmentation and unspecific antibody/chromogen binding in histiocytes was challenging in a few cases. Staining was homogenous in all cases. We found 18 cases were positive with IHC anti-BRAF VE1, 3 cases were inconclusive and graded as negative, while 33 cases were completely negative. The sensitivity of the VE1 antibody was 78% and specificity was 100%.

Discussion and conclusion: This study finds that detection of BRAF V600E by IHC in formalin-fixed and paraffin-embedded tissue-samples is a highly sensitive and specific method. The sensitivity in this study is, however, quite low compared to results in previously published studies. This is due to the five cases of false-negative results for IHC in the study, and adjustments to our IHC-protocol could probably increase the sensitivity. This study supports previous studies, that recommends IHC BRAF-V600E as a first step in searching for BRAF-V600E mutation, and that all negative and inconclusive IHC results also are assessed by molecular methods.

CD271⁺ stem-cell expression correlates with melanoma development and relapse in a case-control study

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Introduction: Cutaneous melanoma is often curable with surgery in its early stages, but high metastatic potential and resistance to adjuvant therapy challenges clinicians. Recent studies on mice indicate existence of melanoma stem cells (MSC) that may self-renew indefinitely, differentiate into heterogeneous progenies with limited proliferative potential, and survive chemotherapy.

Conceivably, eradication of MSC instead of its offspring will improve treatment responses. The study aimed to investigate the correlation between proliferative MSC and the occurrence of metastases in human melanoma, and track stem-cell existence in nevi, melanomas, and metastases.

Material and methods: In patients diagnosed from 1992-1996, 30 with recurrent disease (cases) and 30 without (controls) were matched for tumor thickness, ulceration, Clark level, subtype, site, gender, and age. One paraffin-embedded tissue block from each primary tumor ($N=60$), metastasis ($N=21$), and possible nevus ($N=17$) were immunohistochemically stained for Ki67/MART1 and stem-cell markers: CD271, CD166, and CD20. Their whole slide images were aligned as virtual quadruple stains. The proliferative potential of each stem-cell marker was automatically quantified (e.g., no. of CD271⁺Ki67⁺MART1⁺ cells divided by area of CD271⁺MART1⁺ cells) in addition to the common Ki67 index (no. of Ki67⁺MART1⁺ cells divided by area of MART1⁺ cells). Percentage levels of each marker were calculated within either epidermis or MART1⁺ cells of dermis or metastases. Median values were used in all paired analyses.

Results: In cases vs controls, proliferation indices (no./mm²) were 211 vs 103 ($P=0.04$) for CD271, 512 vs 227 ($P=0.3$) for CD166, 184 vs 97 ($P=0.3$) for CD20, and 95 vs 103 ($P=0.6$) for common Ki67. For percentage levels, all differences were insignificant ($P>0.11$). Yet, marked differences were observed between nevi and melanomas. Epidermal CD271⁺ cells added up to 8.8% in nevi and 0.98% in melanomas ($P=0.0007$). For CD166, levels were 0.33% for nevi and 4.6% for melanomas ($P=0.002$) in epidermis and 0.69% for nevi and 3.1% for melanomas ($P=0.006$) in dermis. CD20⁺ stem-cell expression was absent in all nevi and metastases. Twelve metastases contained CD166⁺MART1⁺ cells and all included CD271⁺MART1⁺ cells. In melanomas, 4.4% of MART1⁺ cells were CD271⁺ whereas 5.8% were CD271⁺ in metastases ($P=0.07$).

Discussion and conclusion: In contrast to common Ki67 indices, high levels of CD271⁺Ki67⁺MART1⁺ cells was linked to the occurrence of metastases. With further investigation, they may be potential targets of therapy. Especially, loss of epidermal CD271⁺ cells seemed necessary for melanoma development adhering to their key role in epidermal homeostasis. Identification may serve as a diagnostic tool with additional research.

Automated, digital assessment of sentinel node biopsies in malignant melanoma

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Introduction: In malignant melanoma, analysis of Melan-A in sentinel lymph node biopsies (SLNB) is pivotal for finding lymph node metastases and therefore crucial for the treatment plan. This study aimed at investigating whether digital automated image analysis (DIA) of Melan-A in sentinel lymph node biopsies (SLNB) can be used as a screening tool of SLNB assessment, which would ultimately decrease the workload of the pathologists.

Material and methods: Samples from 18 patients were selected from the archives of the Dept. of Pathology at Rigshospitalet, including 36 individual SLNB and 243 individual sections. The nodes were embedded according to national guidelines, and sections were cut in four levels. For each level, one immunostaining were made for the cytoplasmic melanocytic differentiation antigen, Melan-A, whereas the rest were stained with HE, for S-100 or left unstained. Immunohistochemical staining (IHC) of Melan-A was performed with a ready-to-use monoclonal antibody (clone A103) from DAKO (Rødovre, Denmark) on a Benchmark ULTRA platform (Roche/Ventana; Tucson, AZ, USA). SLNB whole slide images were acquired by the digital whole slide scanner NanoZoomer XR (Hamamatsu; Shizuoka, Japan) and DIA was performed by using an algorithm by Visiopharm (Hørsholm, Denmark). Half of the slides were scanned using a x20 lens, the other half by a x40 lens. The results were compared to the manual assessment by an expert melanoma pathologist.

Results: DIA selected 20% of the stained slides as correct negative with a sensitivity of 95.6 % and a specificity of 32.7%. Four of the 243 slides (two patients, two SLNB) were wrongly read by DIA as negative, but only in one patient this would have influence on the overall assessment of the patient. The kappa value was only 0.23, but this includes both negative and positive readings and is therefore of minor interest for screening purposes. Interestingly, using the x20 lens, provided a higher kappa value than using the x40 lens, although the sensitivity and the positive predictive value both were 100% using the x40 lens. The data indicate that while DIA cannot be used as a diagnostic tool for malignant melanoma, it is applicable for screening purposes, selecting those slides that the pathologist does not need to view specifically.

Conclusion: This study found that application of DIA for Melan-A expression as a screening tool was able to decrease the workload of pathologists by 20% without compromising with patient safety or diagnostic correctness.

Solar elastosis and TERT promoter mutations in cutaneous melanoma

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Introduction: Solar elastosis is a degenerative change seen in the dermis in sun damaged skin and is a surrogate marker of chronic sun exposure. TERT promoter mutations are typical C>T and CC>TT UVB signature mutations giving rise to a reactivation of the telomerase. With reactivation of the telomerase the tumour cells are able to divide or proliferate infinite.

In this large retrospective study of cutaneous melanoma samples, the aim was to evaluate the degree of solar elastosis and TERT promoter mutations and to assess their impact on melanoma prognosis as well as their interrelationships.

Material and methods: Solar elastosis in dermis was evaluated in HE stained whole slides from 486 malignant melanomas. Pyrosequencing was used to detect TERT promoter mutations in 189 of the samples.

Results: Solar elastosis and TERT promoter mutations were not associated ($p=0.3$). Severe elastosis was mostly seen in older patients ($p<0.0001$), in ulcerated melanomas ($p=0.03$), and in melanomas originating in the head/neck region ($p<0.0001$). Absence of elastosis was mostly seen in younger patients ($p<0.0001$), in melanomas with benign nevus remnants ($p=0.001$), and in melanomas with BRAF V600E oncoprotein expression ($p<0.0001$). Severe elastosis was associated with a worse overall relapse free survival than no/mild elastosis (HR:2.18; 95%CI:1.30-3.64; $p=0.003$). However, this adverse effect of elastosis was not independent of age. TERT promoter mutations were not associated with any adverse prognostic or clinicopathological outcome.

Discussion and conclusion: Severe solar elastosis can be used as a surrogate marker of chronic sun damaged skin in which melanomas may develop de novo. Absence of solar elastosis is associated with melanomas harbouring BRAF mutations and nevus remnants. TERT promoter mutations are not associated with severe elastosis and may thus be triggered by both chronic and acute intermittent sun exposure. TERT promoter mutations do not predict an adverse outcome in melanoma.

O4.1-O4.8

Gene expression of autophagy related genes in CLL

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Introduction: Chronic lymphocytic leukemia (CLL) is the most common leukemia among adults in the Western world. It has a heterogeneous course with an average survival after diagnosis of seven years. The prognosis is dependent on a number of clinical and biological factors. A variety of different prognostic models has been published including the most recent CLL-IPI which merges classical clinical factors and most important biological factors.

Autophagy is a highly conserved process in eukaryotic cells. It was originally described as a cell strategy to ensure nutrition supply under stressed conditions, but it may ultimately lead to programmed cell death. In CLL, autophagy is known to be involved in mediating the effect of chemotherapy but its potential role in CLL pathogenesis remains unknown.

Material and Methods: In the present study we included 149 samples of fresh frozen lysed peripheral blood from patients with CLL from the time of diagnosis. The samples were stored in the biobank at the Department of Pathology, Odense University Hospital. We used nine healthy donors as normal controls. RNA was extracted using MagNA Pure LC, converted into cDNA and finally PCR amplified using real-time RT-PCR to analyze expression of 41 autophagy related genes.

Results: Compared to normal controls the gene expression level in autophagy related genes were generally the same or slightly decreased. Of the 41 autophagy related genes 10 were significantly associated with time to treatment (TTT) and nine with overall survival (OS) in univariate analysis. In multivariate analysis, seven genes were independently associated with TTT and OS. Especially three genes - the *PIK3C3*, *PIK3R4*, and *BECN1* genes - were found to have possible prognostic impact. These genes encode the components of the PI3K core complex, which is central to initiation of autophagy. All three genes were observed to be independent markers of prognosis in CLL with high expression being associated with more aggressive disease.

Discussion and Conclusion: The uniform prognostic association of the genes included in the autophagy initiation complex suggests that this complex could be of importance in CLL pathogenesis. At the moment not all gene expression data are explored in depth and other potentially interesting findings may emerge from the data. Furthermore, an analysis of the gene expression data compared to the protein expression level as well as functional studies is warranted. This may reveal important insight to the autophagy flux in CLL.

Inter-departmental differences in adenomas diagnosed with histological risk factors in the Danish National Bowel Cancer Screening Programme

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Introduction: In 2014 a bowel cancer screening programme, using immunochemical fecal occult blood testing (iFOBT), was launched in Denmark. People screened positive, are offered colonoscopy, where polyps are removed and histologically examined. Depending on the outcome, patients are risk stratified to surveillance re-colonoscopy or scheduled screening. Histological risk factors (HRF) are adenomas with high grade neoplasia (HGN) or a tubulovillous or villous architecture (DPAS/DKS guidelines 2013). In the screening programme the nationwide frequency of HGN is 6.7% and within the expected range, however, the inter-departmental variability ranges from 2.2% to 17% (annual report 2015).

Objective: The primary objective was to compare frequencies of adenomas, diagnosed with histological risk factors at Lillebælt Hospital (LH) and Herlev Hospital (HH). The secondary objective was to examine the extent to which adenoma size may be associated with HRF and contribute to inter-departmental variability.

Methods: We searched the local pathology databases using Cyres for size and histological diagnosis of specimens, examined as part of the screening program at LH and HH within the period from the 1st of March 2014 to the 31st of October 2016. Screening specimens were identified by the SNOMED-code P01601.

Results: LH examined 3167 and HH examined 6236 specimens, whereof both centers classified 72% as adenomas. Architecturally LH classified 68.1% as tubular, 30.8 % as tubulovillous and 1.1% as villous adenomas, whereas HH classified 95% as tubular, 4.9% as tubulovillous and 0.1% as villous adenomas. The percentage of adenomas, diagnosed with HGN, was 18.1% at LH and 2.4% at HH, while the percentage of adenomas, diagnosed with HRF, was 36.5% at LH and 6.7% at HH. Overall, frequencies of HRF increased with size of the adenomas. Compared to HH, frequencies of adenomas, diagnosed with HRF at LH, were 12.5-fold higher in 5-9 mm adenomas, 6.2-fold higher in 10-14 mm adenomas, 4.1-fold higher in 15-19 mm adenomas and 3-fold higher in ≥ 20 mm adenomas. However, differences in polyp-size distribution at LH and HH were negligible.

Discussion and conclusion: We find that adenomas of any size, diagnosed with HRF, are more frequent at LH than at HH, and that HRF are more likely to occur in large than in small adenomas. Inter-departmental differences are relatively higher in small than in large adenomas, however, sample size distribution was similar. To optimize cost-benefit, safety and efficacy of the screening programme, patients across regions should be diagnosed, risk stratified and treated equally. We suggest studies, that examine inter-departmental variability of the histological evaluation.

Introductory to a functional *in vitro* tumoroid model for individualized colorectal cancer chemotherapy

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Introduction: There is an emergent need for valid *in vitro* models to investigate the effect of individualized chemotherapy regimen for cancer patients. A valid testing assay will assist the oncologists in selecting the most effective chemotherapy for each patient. A functional *in vitro* 3-D model showing tumor growth and angiogenesis is being established. The 3-D cultures of cancer tissue originated from the patient's own primary or metastatic tumor (i.e. tumoroids). Such a system needs to be validated with pathological methods before clinical introduction. This study investigated 3D cultures for their morphological characteristics and demonstrated a 3D reconstruction method for further investigations.

Material and Methods: Following resection of the primary colon adenocarcinoma, tumoroids of the malignant tumor were established and grown in hydrogel matrix for 15 days prior fixation and embedding. Sections were PAS stained and immunohistochemically stained for Ki67, active caspase 3 and CK20. Furthermore, tumoroids were serially cut in 2 μm sections, stained with hematoxylin and scanned by a digital whole slide scanner. 3D figures were constructed by aligning the segments and executing the 3D viewer plug-in of the ImageJ software.

Results: In this experiment the tumoroids increased their size more than 10-fold when cultured for 15 days resulting in tumoroids exceeding 500 μm in diameter. CK20 and PAS stain confirmed the epithelial and glandular nature of the tumoroids, and Ki67 and active caspase 3 demonstrated that the tumoroids were made from proliferating cells with minimal apoptosis. The 3D reconstruction demonstrated a non-symmetric anatomy of the tumoroids.

Discussion and conclusion: Immunohistological investigations showed that cancer cells maintain their original characteristics after culturing into miniature tumors. Tumoroids thus appears to be good candidates for chemosensitivity assays in relation to personalized anti-cancer treatment, including anti-angiogenetic drugs.

Quantitative Assessment of Apoptosis by Cleaved Caspase 3

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Introduction: In a selected population of patients with colorectal cancer the clinical outcome has been related to the ratio between apoptosis and proliferation in the tumor. This was done using Cleaved Caspase 3 (CC3) as marker of apoptosis, assessing the apoptotic count in focal areas without regarding intratumoral heterogeneity. The corresponding Cohens kappa value was 0.6 [1]. We wanted to evaluate the intratumoral heterogeneity of apoptosis and the interobserver agreement of the quantitative assessment.

Material and methods: From 25 cases of colorectal cancer a standard section from the invasive front and the transition zone was stained with CC3. On the scanned slides an area comprising the deepest invasion or most lateral part of the transition zone was selected. In these areas the total number of tumor cells was counted by computer and the CC3-positive cells were counted by two pathologists in order to calculate Cohen's kappa. Only darkly stained cells, at the size of neighboring tumor cells was considered positive, while shedded, luminal tumor cells were excluded.

Results: Intratumoral heterogeneity: Each area contained on average 2583 tumor cells (606-5595). In 44 of the 50 areas, the percentage of apoptotic tumor cells was < 1 %. In 6 cases the apoptotic cells exceeded 1 % at either the invasive front or at the transition zone, but not both at once, corresponding to intratumoral heterogeneity. When reviewing the patient data, no consistent differences was noted in these 6 cases.

- 4 of the 6 were left sided cancers.
- 4 of the 6 were stadium T3, and 2 were T2.
- None of them had loss of MMR proteins, were low differentiated or had lymph node metastasis.

Cohens Kappa

In > 90 % of the cases the total count of CC3-positive cells was < 25. When these cases were grouped in five intervals (0-4, 5-9, 10-14, 15-19 and 20-24) Cohens kappa was 0.21, corresponding to an interobserver agreement well below moderate. Subjectively it was difficult to determine which cells to include and whether larger stained areas consisted of more than one positive cell.

Discussion and conclusion: Unpredictable intratumoral heterogeneity was shown in 6/25 cases. This aspect should be taken into consideration, when assessing apoptotic rates in future studies. With a Cohen's kappa value of 0.21, we cannot recommend CC3 as viable method of quantitative apoptosis-assessment.

References

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Interobserver agreement on histological grading of 70 colorectal adenomas within the Danish colorectal cancer screening programme

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Introduction:

Histopathological examinations take up a key position in risk assessment and patient allocation within the Danish iFOBT (immunochemical fecal occult blood test)-based colorectal cancer screening programme. In order to increase the reproducibility of the reporting, the use of a revised, simplified Vienna Classification on histological grading of colorectal adenomas is recommended, dividing the specimens into either low or high grade neoplasia (HGN). According to the set quality indicators, not more than 10 % of the lesions within a FOBT (fecal occult blood test) screening population should be characterized as HGN. In an iFOBT-based screening programme, the share of HGN adenomas is believed to account for less, presumably about 5 %. The annual report of the Danish colorectal screening database, 2014, showed large interregional differences in number of reported HGN adenomas with Herlev Hospital (2.3 %) and Sygehus Vendsyssel (24.4 %) representing the two extremes of the scale. Different factors, such as case-mix and poor interobserver agreement, can explain this, including a too restrictive approach to HGN diagnosis amongst the pathologists at Herlev Hospital.

The aim of the study was to evaluate the interobserver agreement among 8 pathologists at Herlev Hospital on histological grading of colorectal adenomas using the two-tiered grading system.

Material and method:

The study was set up as an interrater reliability study on histological grading of 70 colorectal adenomas with a diameter of 15 mm or more, resected consecutively within a randomly selected period of time. Glass slides of the specimens were retrieved from our archive, anonymized and converted into digital slides using the NanoZoomer scanning system. The adenomas were digitally re-evaluated by 8 of the Department's pathologists, and the level of agreement was assessed using Fleiss' and Cohen's kappa statistics.

Results:

10.0 – 17.1 % of the specimens were graded as HGN. The overall interobserver agreement on degree of neoplasia was moderate ($\kappa = 0.57$). Between pairs of raters the value of kappa ranged from 0.26 to 0.80. 21 adenomas were by one or more of the observers characterized as HGN, and complete consensus between the observers was reached in three of these cases.

Discussion and conclusion:

The finding of a moderate, suboptimal interobserver agreement on grading of colorectal adenomas stresses the need for ongoing knowledge sharing and consultation between pathologist colleagues and for the implementation of a national quality assurance programme.

Histopathological tumor regression grading of gastroesophageal adenocarcinomas after neoadjuvant Chemotherapy. - A study focusing on interobserver variability and practical usability.

Kiran Sheikh, patologiafdelingen, Region Sjælland, afsnit Roskilde Jane Preuss Hasselby, patologiafdelingen, Rigshospitalet Gro Linno Willemoe, patologiafdelingen, Rigshospitalet

Introduction: Since 2009 standard treatment for locally advanced adenocarcinomas of the gastroesophageal junction in DK has been neoadjuvant Chemotherapy followed by surgery. Not all tumors show regressive changes in a similar manner. The effects of preoperative treatment can be determined by histology based on either therapy induced fibrosis in relation to residual tumor or the estimated percentage of residual tumor in relation to former tumor site. Currently many TRG systems are in use and there is still a lack of standardization. The aim of the study is to identify a practical usefull TRG system with high interobserver agreement.

Materials and Methods: This study included patients with gastroesophageal adenocarcinomas- all treated with neoadjuvant chemotherapy followed by surgery. The hematoxylin and eosin-stained tumor slides from 100 consecutively selected patients treated between 2009-2013 at Rigshospitalet, Copenhagen University Hospital were assessed. The slides were reviewed independently by 3 pathologists, 1 trainee and 2 experienced consultants. TRG was assessed by using the Becker model and by use of the 5-tiered and a modified 3-tiered Mandard model. Estimation of agreement was evaluated by Fleiss Kappa values. The applicability of each TRG system was evaluated by usage of a questionnaire.

Results: The 3-tiered Mandard and the Becker model showed excellent Fleiss kappa values of agreement (0,83 and 0,82), whereas the agreement was substantial in the 5-tiered Mandard (0,67). All three models were applicable in a daily routine setting, but considering factors as consumption of time and certainty of categorization the 3-tiered Mandard was found to be superior compared to 5-tiered Mandard and Becker.

Discussion and conclusion: In our hands tumor regression grade (TRG) can be estimated using different models with a substantial to excellent agreement. However several issues including the role of regression in metastatic lymph nodes needs to be clarified and there is some conflicting evidence of the prognostic value of TRG in the literature.

The clinical usage of TRG in order to adjust the neoadjuvant treatment in the postoperative setting is still awaiting and ongoing research into other modalities such as PET/MR, molecular subtyping and detection of circulating tumor DNA might add more value to the monitoring and treatment tailoring than the TRG.

In conclusion, all the TRG systems tested in this study provides substantial to excellent agreement. A simple 3-tiered Mandard system maintains the highest rate of reproducibility and is preferred in the daily routine.

Computer-assisted stereology and automated image analysis for quantification of tumor infiltrating lymphocytes in colon cancer.

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Introduction: In stage II colon cancer (CC), there is a need for more precise prognostic and predictive variables, which will allow improved post-operative treatment stratification. In this context, tumor infiltrating lymphocytes (TILs) have been analyzed in various settings, and several studies have shown promising results. However, the lack of a standardized analytic technique is a major concern, and so far none of the proposed immunohistochemically classifications has been incorporated into routine clinical practice. Manual stereological counting is considered the gold standard, but digital pathology with image analysis is preferred due to time efficiency. The purpose of this study was to compare manual stereological counts of TILs with automatic counts obtained by image analysis.

Material and Methods: Three, paraffin embedded, tumor containing tissue blocks from 43 patients treated for stage II CC in 2002, one of which representing the deepest invasive tumor front, were selected. Serial sections from each of the 129 blocks were immunohistochemically stained for CD3 and CD8, and the slides were scanned with a NanoZoomer XR scanner (Hamatsuma, Japan).

Stereology: The stereological analysis was performed using the computer assisted stereology system NewCAST. We used an integrated test system consisting of a 2D unbiased counting frame for cell density estimation and a point grid for area fraction estimation.

Image Analysis: An App-based algorithm was developed, using Visiopharm Integrator System (VIS) software. The algorithm included two processing steps:

1. Automatic exclusion of artifacts, including folds, fatty tissue and necrosis.
2. Automatic classification of CD3+ and CD8+ cells using a Bayesian classifier.

For both methods we counted in two regions: a central and an invasive area delineated manually by the observer.

Results: We found an excellent correlation between the stereological method and the App-based image analysis in both the central and invasive areas and for both CD3 and CD8 stained sections. For the density count, Spearman's correlation coefficient varied from 0.9457 through 0.9638 ($p < 0.0001$). Area fraction estimates showed similar results with Spearman's correlation coefficient varying from 0.9400 through 0.9603 ($P < 0.0001$).

Discussion and Conclusion: For histopathological quantification stereology is considered as the gold standard. In this study we found a high correlation between stereology and image analysis, and we conclude that computer-assisted image analysis provide both exact, objective and time efficient quantification of TILs. However, in using the algorithm it is important to take staining variability into consideration.

Early detection strategies have reduced the effect of curative surgery in patients with low risk localized prostate cancer – A nationwide analysis.

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Introduction: Population-based prostate specific antigen (PSA) testing is not recommended in Denmark. Nevertheless, increased use of PSA has introduced both an increase in prostate cancer (PCa) incidence and a lead time- and stage migration at diagnosis, altering the natural history of PCa. Contemporary PCa patients are likely younger and have smaller tumor burden at diagnosis. Through analysis of a nationwide cohort, we wished to investigate whether changes in the PCa landscape have altered the course of low-risk localized PCa.

Material and Methods: The Danish Prostate Cancer Registry (DaPCaR) is based on histopathological information obtained from the Danish Pathology Register (Patobank). In DaPCaR, all patients diagnosed in Denmark from 1995 to 2011 with localized (T1-2, N0/X, M0) PCa with Gleason score (GS)≤6 were identified. Patients were stratified into three periods of diagnosis; 1995-2000 (period 1), 2001-2005 (period 2) and 2006-2011 (period 3). Competing risk analysis treating PCa specific and other-cause death as competing events was performed.

Results: A total of 5,660 patients were identified and of these 2,030 (35.9%) had undergone radical prostatectomy (RP). From period 1 to period 3, the median age at diagnosis decreased from 72.2 years (IQR: 65.7-78.2) to 66.0 years (IQR: 61.3-70.8) and the median PSA decreased almost 50% from 16.2 ng/mL (8.0-32.3) to 8.6 ng/mL (IQR: 6.0-13.0). The overall 5-year risk of PCa-death decreased from 14.3% (95%CI: 12.1-16.4%) to 1.3% (95%CI: 0.83-1.7%), $p<0.0001$, when comparing patients diagnosed in period 3 to those diagnosed in period 1. Other cause mortality decreased from 18.1% (95%CI: 15.8-20.5%) to 7.2% (95%CI: 6.2-8.2), $p=0.0001$.

In patients undergoing RP, the 5-year risk of PCa-death decreased from 0.67% (95%CI: 0.67-2.0%) for patients diagnosed in period 1 to 0.45% (95%CI: 0.0055-0.84), ($p=0.92$) for patients diagnosed in period 3. For patients not undergoing RP, the 5-year risk of PCa death decreased from 16.6% (95%CI: 14.1-19.1) to 2.0% (95%CI: 1.2-2.7%), $p<0.0001$.

Discussion and Conclusion: In a nationwide cohort of patients with low risk localized PCa, the 5-year risk of PCa-specific mortality significantly decreased when comparing patients diagnosed during 2006-2011 to those diagnosed during 1995-2000. This was mainly driven by patients not undergoing RP. In the most recently diagnosed group, the difference in 5-year PCa-death between patients undergoing RP and patients not undergoing RP was very small (0.45% vs. 2.0%). Our data demonstrate that the impact of PSA-induced lead-time and stage migration has diminished the absolute effect of RP on the risk of 5-year PCa- mortality because contemporary low-risk localized patients have a significantly better prognosis.

P1.1-P1.6

Good Role models make a difference

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Introduction: The Danish Health Authority tells us in “The 7 roles of Physicians” (7RP) to work with social competences, and now The Medical Association of Senior Hospital Physicians has issued a codex of ten messages (10M) about the importance of a good work environment and how to obtain it. How does this new codex fit into 7RP and how can physicians benefit from those new messages.

Material and methods: A group of seven physicians decided to focus for 7 weeks on those of 10M, which concern the psychological work environment: Message (m) 1 about seeing themselves as a role model - the superior message - was supported by m2, m3 and m4 describing the good role model as one who contributes to a good atmosphere, creates thriving and a sense of community and acknowledges colleagues and their work. Tools chosen for obtaining results were self-reflection in daily life and group-reflection during three semistructured group interviews.

Results: Comparing 7RP and 10M made clear, that seeing oneself as a role model is not in opposition to the social competences in 7RP fitting well into the professional role that tells physicians to know themselves, to act with integrity, to be aware of the role they play in creating the culture and to optimize the work and teaching environment.

Results from the first group interview regarding the understanding of the word role model described a good role model as friendly with a positive attitude, acknowledging colleagues and having an open door for questions. A good role model shows confidence in young doctors, which makes them want to choose exactly this medical specialty. These results were guiding the group, while practising the messages in their daily work. The second group interview dealt with possible changes in the atmosphere. The group agreed that the good atmosphere was maintained showing an improved understanding of the use of social competences - also in potential conflicts. The group also agreed that the project had encouraged a sense of community and brought acknowledgement of colleagues in focus as well as acceptance of differing ways of working. In the third group interview group members were telling that they were inspired by each other in preventing conflicts, and that they recognized very good and improved skills for dialogue in themselves and each other as well as awareness of the contagious use of acknowledging each other.

Discussion and conclusion: The study showed that when it comes to social competences 10M complements the 7RP fitting well into the professional role. We recommend that physicians work with 10M in groups to benefit from group reflections and sharing of knowledge and good atmosphere while becoming good role models.

***PIGT* mutation and leukodystrophy – a case rapport**

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Introduction: Here we report the first case of leukodystrophy diagnosed by a post mortem neuropathological examination in a male, aged eleven months, where an exome sequencing revealed a phosphatidylinositol-glycan biosynthesis class T (*PIGT*) mutation.

Leukodystrophies are rare heritable myelin disorders affecting the white matter of the central nervous system with myelin sheath abnormalities. Among the clinical manifestations are hypotonia, spasticity, seizures and delay in cognitive development. A large group of leukodystrophies, varying from 30-50% in different studies, remains without a known underlying disease mechanism.

Material and methods: Postmortem autopsy with neuropathological examination including microscopic evaluation with special stains and immunohistochemistry of brain sections.

Furthermore, an exome sequencing was performed.

Results: The clinical manifestations of the male in our case were neuro-ophthalmologic with hypotonia, intractable seizures, cortical visual impairment, nystagmus and strabismus.

The most striking features of the neuropathological post mortem examination were a substantial reduction of myelination and a pronounced astro- and microgliosis in the white matter.

Furthermore, there were macrophages in the white matter containing lipid deposits that became visible when stained with OilRedO and Sudanblack.

In conclusion, the pathological findings in the brain were suitable with an orthochromatic (sudanophilic) leukodystrophy. The general post mortem examination revealed no other organ malformations. A subsequent exome sequencing revealed a mutation in *PIGT*.

Discussion and conclusion:

PIGT encodes phosphatidylinositol-glycan biosynthesis class T, a crucial subunit of the glycosylphosphatidylinositol (GPI) transamidase that facilitates both the transfer of GPI to proteins in the endoplasmic reticulum and the subsequent allocation of the GPI anchored proteins to the outer layer of the cell membrane. The GPI anchored cell-surface proteins are involved in several processes such as for example signal transduction, immune response, and myelination

This is to our knowledge the first time to link neuropathological findings revealed in a post mortem examination to a congenital disorder of glycosylation (CDG) and certainly to a CDG *PIGT*. The mutation in the *PIGT* can explain the disease mechanism in at least some orthochromatic (sudanophilic) leukodystrophies.

Kufs Disease, an Adult-onset Neuronal Ceroid Lipofuscinosis – a Case Report.

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Introduction: The neuronal ceroid lipofuscinoses (NCLs) are a heterogeneous group of inherited neurodegenerative disorders with accumulation of auto fluorescent storage material in neural and peripheral tissues and neurodegeneration. The adult-onset of NCLs (ANCL), also known as Kufs disease, is rare and challenging to diagnose. The age of onset varies between 25 and 45, but the symptoms often appear around the age of 30. The common characteristics of affected individuals includes generalized seizures, movement disorders, cognitive deterioration and progressive dementia. All childhood forms of NCLs are recessive diseases, but ANCL is inherited as both a recessive and a dominant form.

Material and methods: A woman in her mid-thirties developed progressive dementia, dysarthria and ataxia. Later on, she developed epilepsy and died at the age of 44. She had a congested family history with her father, uncle, grandmother and grandmother's sister who each passed away in their early forties. A brain MRI at age 35 showed diffuse cortical atrophy and a frontal lobe brain biopsy revealed few neurons containing periodic acid-Schiff positive granules. Electron microscopic examination showed several neurons with granular osmophilic deposits. There were no fingerprint- or curvilinear profiles found. The neuropathological findings in vivo did not verify - but supported the diagnosis Kufs disease.

Results: Autopsy was performed and macroscopic examination of the brain revealed atrophy, especially in the frontal- and parietal lobe, -and minor dilation of the ventricular system. The brain weighed 968 g after fixation. Microscopic examination showed widespread neuronal storage of a yellowish pigment in both the brain and spinal cord. The storage material was strongly periodic acid-Schiff positive. In frozen sections, the storage material was positive in Oil Red O and Sudan-black. The brain tissue was, unfortunately, too autolyzed for electron microscopic examination.

Discussion and conclusion: In the present case, clinical history and pathological examination are compatible with ANCL. However, an exact diagnose of ANCL is challenging due to the relative rarity of the condition, the heterogeneity, and genetic characterization of ANCL is at an early stage. Differential diagnoses are many, including aged related accumulation of lipofuscin. The degree of genetic heterogeneity of ANCL remains unclear, but molecular genetics studies have begun to unravel the underlying genetic defects in ANCL, where mutations in the gene CLN6, CTSF and DNAJC5 have been identified. The efficacy of genetic testing will improve as further ANCL genes are discovered. At this time, pathologic diagnosis remains the gold standard.

Spindle cell conundrums in the bladder

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Introduction: Reactive pseudosarcomatous myofibroblastic proliferation in the bladder (PMP) can provide a diagnostic challenge, as they can resemble some sarcomas, inflammatory myofibroblastic tumor (IMT) and sarcomatoid urothelial carcinoma.

Sarcomatoid carcinoma can have a prominent component of spindle cell proliferation and mimic a myofibroblastic proliferation.

Material and Methods: Case 1: a 70 year old male present with massive hematuria. Cystoscopy reveals a tumor like lesion. Case 2: 77 year old male present with irritative bladder symptoms. Cystoscopy reveals a papillary and solid tumor.

Results: Case 1 was a polypoid lesion with ulceration. In lamina propria there were spindle cells, stellate cells and ganglion-like cells in an edematous background with sprinkling of erythrocytes and lymphocytes.

Immunohistochemically the tumor cells were positive for vimentin, CK, smooth muscle cell actin (SMA) and ALK. There was negative reaction against: desmin, CD34, S100, p63 and GATA3.

ALK FISH showed gene rearrangement

Case 2 was a tumor with biphasic morphology. The surface was with a papillary low grade urothelial lesion. In the underlying lamina propria there was presence of spindle cells. In this component there were also two growth patterns. In some area the spindle cells were reminiscent of IMT-PMP and in other areas there were a storiform-histiocytoma like growth pattern. No area of conventional urothelial carcinoma (UC). The mitotic activity was high (30/ 10HPF) and the atypia was moderate. It was impossible by morphology to make the distinction between urothelial tumor, pTa with PMP or on the other hand a sarcomatoid carcinoma. Immunohistochemically the spindle cells were positive for vimentin, SMA, CK (focal), EMA (focal), p63 (strong diffuse) and GATA3 (strong diffuse). Negative against: CD34, desmin and ALK.

ALK FISH was normal. When all the above factors were considered then it was assumed that the tumor represented a sarcomatoid UC

Discussion and conclusion: These two cases showed a morphological and immunohistochemically overlap, which can be a serious diagnostic pitfall with therapeutic consequences. In case 1 the ages of the patient was unusual, but the diagnosis of IMT was made confidently by ALK IHC/FISH and lack of p63 expression. In case 2 the morphology was peculiar in that the surface was with a low grade lesion and the underlying spindle cells phenotypically expressing CK, EMA, p63 and GATA3 with lack of ALK. Morphology was inseparable from a reactive lesion. The diagnosis of sarcomatoid UC was confirmed by high mitotic index, cellular atypia in combination with p63 and GATA3 expression. These cases show a diagnostic pitfall in diagnosing spindle cell lesions in the bladder.

A rare case of myelomatosis with onset in the pituitary gland

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Introduction: Myelomatosis (bone marrow) and solitary plasmacytomas (osseous or extra-osseous) are diseases characterised by clonal proliferations of neoplastic plasma cells. In myelomatosis the disease is widespread and associated with high levels of monoclonal paraproteins in serum (M-protein). Myelomatosis is a common malignancy, representing approximately 1% of all cancers in Denmark. Disease onset is often in bone marrow giving rise to symptoms caused by a varying degree of hematopoietic depression, but bone pain or malignant fractures can be seen as first symptoms. Only a few cases of myelomatosis or solitary plasmacytomas, presenting as a sellar mass, has been described in the literature.

Material and methods: A 62-year-old man was admitted to the department of neurosurgery for a pituitary mass biopsy. The mass was discovered on a CT scan during an investigation of recurrent sinusitis symptoms and frontal headaches. Besides the recurrent headaches, coughs, a throat mucus sensation, and fatigue, the patient had also developed a blurred vision on one eye and diplopia within a few weeks. He had only mild intermittent fever sensations but no night sweats. The mass was considered benign, probably a pituitary macro adenoma.

Results: A transsphenoidal resection of the sellar mass was intended. The mass showed excessive bleeding during the operation and it was attached to and infiltrating the bone in the sellar region, thus not allowing a complete removal. Microscopy of frozen sections was inconclusive, but a preliminary diagnosis of a benign pituitary adenoma was given based on the homogeneity of the tumour cells, the approximately glandular pattern and the tumour cell appearance with lots of cytoplasm and a peripheral nucleus. However, in the defrosted material, tumour had numerous mitotic figures and atypical, pleomorphic nuclei. The tumour cells were immunohistochemically positive for plasma cell markers (vimentin, MUM1, and CD138) and negative for chromogranin, synaptophysin, melan-A, GFAP, and cytokeratins. There was a monoclonal expression of kappa chains.

Discussion and conclusion: We here describe a rare case of myelomatosis presenting as a sellar mass disguised radiologically and morfologically as a benign pituitary adenoma.

P2.1-P2.6

Abnormalities of the vermiform appendix

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Introduction: Anatomic abnormalities of appendix, generally diagnosed in children or young adults, rarely come to our attention. However, we recently documented two such examples. One of the cases represented an appendix duplication. The other case was interpreted as a septate appendix. Here, we call attention to these uncommonly observed lesions, which triggered some considerations regarding histological details and clinical significance.

Material and methods: Relevant clinical and morphological details of the two lesions were recorded. Informed consent from the patients was obtained.

Results: Case 1: A 30-year-old male, in 2009 appendectomized for appendicitis (the specimen measured 40 X 6 mm), was hospitalized with pain in right lower abdominal quadrant. Because of the previous appendectomy, a CT-scan was performed, which indicated a new case of appendicitis or a stump appendicitis. The location of the appendix was not specified intraoperatively. The resected specimen measured 53 X 6-11 mm. Case 2: A 46-year-old female presented clinically with appendicitis. The appendix measured 90 X 26 mm. Lumen of the central portion was subdivided by several projections, the remaining portions orally and anally were expanded by mucinous material. Histologically, complete and incomplete septae of none-atrophic mucosa with prominent lymphoid tissue and submucosa corresponded to the grossly observed projections. Mucosa of the remaining portions orally and anally was similarly none-atrophic, focally lining diverticles. Submucosal fibrosis was not a feature. A diagnosis of septate appendix was considered most likely.

Discussion: The significance and challenge of case 1 featuring a duplicated appendix is largely clinically. The condition is rare with an incidence of 0.004% [1]. Thus, the duplicate nature with a sizeable “second” appendix was a surprise. Duplicated appendix may have medico-legal consequences for the surgeon if the second appendix is not identified with a potential risk of surgical delay (ie inappropriate postponement of surgical intervention until after radiologic examination). Case 2 is on the other hand primarily a morphological issue. We considered a septate appendix most likely, though some atypical features (patient’s age and the none-atrophic mucosa) triggered alternative considerations. Specifically, acquired NSAID-induced damage (most commonly seen in the small bowel, but also reported in the cecal region), resulting in Diaphragm Disease (DD), grossly mimicked the macroscopy of the present case. However, the patient’s drug history included only minimal NSAID-intake, nor was submucosal fibrosis, a key finding of DD, observed. Ref: 1. Collins. A study of 50,000 specimens of the human vermiform appendix. Surg Gynecol Obstet 1955;101:437.

PAX8 expression in Gastrointestinal Stromal Tumours (GIST tumours)

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Introduction: PAX8 is a transcription factor that is crucial in the development of several organs such as urinary, reproductive, eye and thyroid organs. PAX8 has an association with tumours of the upper urinary tract, reproductive system and thyroid. Thus it has many uses for pathologists but to our knowledge, there isn't a known association with GIST tumours. Nevertheless we had a case of a female patient with a pelvic GIST tumor that was PAX8 positive which gave rise to differential diagnostic considerations. The aim of the study was therefore to evaluate PAX8 expression in known GIST tumours

Material and methods: The study included material collected at the Department of Pathology, Herlev Hospital, Denmark. Information regarding tumor type was retrieved from PATOBANK. Formalin fixed paraffin embedded samples from 15 GIST tumours, from February 2013 – June 2016, were immunohistochemically stained for PAX8. PAX8 expression was subsequently evaluated by two pathologists.

Results: Complete absence of PAX8 expression was observed in 14 of 15 GIST tumour samples (93.3%)

Discussion and conclusion: There is very little correlation between PAX8 expression and GIST tumours. The results of this pilot study warrant validation in a larger series of GIST tumours.

Hepatic perivascular epithelioid cell tumor - an accidental finding.

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Introduction:

Perivascular epithelioid cell tumors, PEComas are a family of mesenchymal tumor composed of histologically and immunohistochemically distinctive perivascular epithelioid cells. PEComas include angiomyolipoma, lymphangiomyomatosis, clear cell 'sugar' tumor of the lung, and a group of rare, morphologically and immunophenotypically similar lesions arising at a variety of visceral and soft tissue sites. They most often affect young female patients with one third of the cases arising in the pediatric population. PEComas occurs over a wide range of sites. The primary sites are the uterus, vulva, rectum, heart, urinary bladder, abdominal wall, pancreas, retroperitoneum and liver - the falciform ligament. Some of tumors are seen frequently in tumorous sclerosis complex.

Material:

16 years old female was admitted to hospital due to an acceleration-decelerations trauma. She had unstable blood pressure and underwent acute surgery. The site of the left triangular ligament of the liver was torn and the part was removed.

When she was 8 years old she underwent surgery due to an ependymom, grad II between the spinal cord and the brainstem.

Results:

We found a tumor with pushing borders without infiltrating pattern, nested clear cell appearance and partial bleeding. There was no prominent cytologic atypia and sparse mitosis.

Immunohistochemistry, the tumor was positive for Vimentin, Melan-A and HMB45. We found negative markers excluding malignant melanoma, clear cell sarcoma, ectopic adrenal glands and adrenal cortex tumor.

Discussion and conclusion:

The tumor was diagnosed as a PEComa, uncertain benign or malignant. There were no malignant features such as infiltrating appearance, cytologic atypia and high mitotic index. The tumor was not definitely associated to the falciform ligament. Accordingly, our patient needs to undergo further investigations to exclude disseminated disease since PEComas can display characteristics of both benign and malignant behavior. The primary treatment is resection. There may be benefits of chemo-radiation therapy.

PEComas and especially hepatic PEComas are rare. The etiology of PEComas remains uncertain and the behavior of the tumor is difficult to predict.

Malignant melanoma in vagina and vulva

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Introduction: Malignant melanoma is a rare type of cancer in the vagina and in the vulva with a poor prognosis due to late diagnosis and early dissemination. Only a limited amount of literature exists. This study will elucidate the effect of the current treatment.

Methods: All patients diagnosed with malignant melanoma in the vagina or vulva at Aarhus University Hospital, Skejby in the period from 1996 to 2013 are included.

Data from the electronic patient's file and from the Danish Pathology Register has been collected.

Results: Seventeen patients were included and the average age at the time of diagnosis was 77 years and the median overall survival time was 21,9 month. The 5-year survival in this study was 17,7%.

Most of the melanomas were nodular and all of the superficially spreading melanomas were only found in the vulva. Malignant melanoma in the vagina has a worse prognosis than in the vulva as it is diagnosed at later point.

Discussion and Conclusion: Older women with vaginal discharge should always have a gynecological examination.

The primary treatment is resection of the tumor, but future treatment might be a combination with immunotherapy.

Endometriosis and pregnancy complications: a Danish cohort study.

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Objective: To study the association between endometriosis and risk of pre-eclampsia, cesarean section, postpartum hemorrhage, preterm birth, and small for gestational age (SGA), in a large Danish birth cohort, while taking fertility treatment into account.

Design: Population-based study.

Setting: Not applicable.

Patients: A total population of 82,793 singleton pregnancies from the Aarhus Birth Cohort (1989 through 2013); 1,213 women had a diagnosis of endometriosis, found in the Danish Pathology Register, affecting 1,719 pregnancies.

Intervention(s): None.

Main Outcome Measures: Pre-eclampsia, cesarean section, postpartum hemorrhage, preterm birth, and SGA.

Results: Endometriosis was associated with an increased risk of preterm birth (adjusted odds ratio (AOR) 1.67, 95% confidence interval (CI) 1.37-2.05), with the risk being highest for very preterm birth (AOR 1.91, 95% CI 1.16-3.15). Compared with unaffected women, women with endometriosis also had an increased risk of pre-eclampsia (AOR 1.37, 95% CI 1.06-1.77) and cesarean section (AOR 1.83, 95% CI 1.60-2.09). Assisted reproductive technology did not explain the findings. No association was found between endometriosis and postpartum hemorrhage or SGA.

Discussion and Conclusions: Women with endometriosis were at increased risk of pre-eclampsia, preterm birth, and cesarean section, irrespective of use of assisted reproductive technology.

Ovarian metastasis following minimal invasive endocervical adenocarcinoma. A case study.

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Introduction: Endocervical adenocarcinomas uncommonly metastasize to the ovaries. Moreover, the metastases may be difficult to distinguish from primary ovarian tumors, because they often simulate primary ovarian mucinous and endometrioid tumors. Grossly, these metastatic tumors are often large, unilateral, multicystic tumors lacking surface involvement in contrast to what is expected for ovarian metastasis in general. Moreover, the histological features include “borderline-like” or confluent, glandular growth patterns and lack of destructive stromal invasion and therefore cannot be readily recognized as metastatic.

Material and methods: A 48-year old woman originally presented with postcoital bleeding. Cervical biopsies showed adenocarcinoma measuring 3 x 1.5 mm and no lymphovascular space invasion. The following cone showed adenocarcinoma in situ (AIS) with no residual tumor, hence FIGO IA1 cervical cancer. Posttreatment cervical cytology showed AIS with HPV 18. A hysterectomy was performed and widespread AIS was found in the transformation zone but the lesion was radically removed.

18 months following the primary cancer diagnosis the patient was admitted to the hospital with abdominal pain and a cystic right ovary with torsion was surgically removed.

Results: Grossly, the right ovary measured 9.5 cm and was multicystic with a smooth surface. Morphologically the tumor simulated endometrioid adenocarcinoma, but immunohistochemistry showed no expression of PAX8 and staining for p16 was diffuse and intense, WT1 was negative and p53 was wildtype. Cervical cancer metastasis was considered, thus a HPV test was performed and HPV18 was detected.

It was concluded that the tumor represented a metastasis from the patient’s former endocervical adenocarcinoma.

Discussion and conclusion: Metastatic ovarian adenocarcinomas can be difficult to identify since the metastasis often simulate the morphology of primary ovarian neoplasms and stromal invasion is often subtle. Gross features that normally favour metastasis include small size (often < 10-12 cm), bilaterality, a nodular growth and surface involvement. However, metastases from endocervical adenocarcinomas are often unilateral and multicystic with no surface involvement and a growth patterns that imitate borderline or well differentiated adenocarcinoma.

Diffuse intense immunohistochemical expression of p16 without concomitant aberrant p53 and the presence of identical HPV types in the paired ovarian and endocervical tumors support interpretation of the ovarian tumor as metastatic.

5 YEARS FOLLOW-UP OF HPV mRNA TRIAGE OF ASCUS AND LSIL SAMPLES TESTED IN A ROUTINE LABORATORY SETTING

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Introduction

According to guidelines HPV mRNA testing has been used as triage of women with ASCUS and LSIL, but long-time follow-up data on mRNA testing has been quite sparse. Accordingly, the aim of this study was to record 5 years follow-up data.

Material and Methods

5 years follow-up data from a total of 841 ASCUS and LSIL samples, tested with Aptima HPV mRNA at baseline, was retrieved from “Patobank”; a national register containing all diagnosis of cytological and histological samples from Denmark. Follow-up data were available from 305 women with ASCUS and 522 with LSIL.

Results

827 women had available follow-up, with 264 being diagnosed with CIN2+ and 166 with CIN3+. Sensitivity, specificity and predictive values are shown in table 1.

Table 1		Sensitivity	Specificity	PPV	NPV
CIN2+	Overall	94.70	39.25	42.23	94.04
	ASCUS	94.90	49.76	47.21	95.37
	LSIL	94.58	33.15	39.75	92.91
CIN3+	Overall	96.39	34.64	27.03	97.45
	ASCUS	95.65	44.49	33.50	97.22
	LSIL	96.91	29.18	23.80	97.64

Discussion and Conclusion

The overall 5 years follow-up data shows a good sensitivity and high NPV. However, divided in groups of ASCUS and LSIL, the specificity for ASCUS is significantly higher than LSIL both at CIN2+ and CIN3+. Likewise, a better PPV is seen for ASCUS compared to LSIL.

P3.1-P3.6

Case report: Idiopathic Giant-Cell Myocarditis in a 53-year-old woman

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Introduction: Idiopathic Giant-Cell Myocarditis is a rare and often fatal cardiac disease of unknown origin generally affecting otherwise healthy young and middle-aged adults.

It is characterized microscopically by myocytic necrosis, a mixed infiltrate with multinucleated giant cells and fibrosis. There is a strong association with autoimmune disorders, with 20% of the patients suffering from such a disease. In this report we describe a fatal case of this disease. A 53-year-old woman died in a state of cardiac arrest after having no cardiac symptoms or history of cardiac disease prior to the event. She was known with diabetes, hypertension, depression and positive quantiferon test without tuberculosis ever being confirmed. There were no known hereditary diseases in the family.

Before her death she was investigated for unspecific symptoms. The only pathologic alteration found was a no-hormone producing myelolipoma in the right adrenal gland, which was removed.

Material and methods: An autopsy following the normal protocol was made. The heart was removed and fixed in formalin. Representative sections of changes in the myocardium were taken out, embedded in paraffin and stained with hematoxylin and eosin. Two sections were stained with Ziehl-Neelsen, Auramin/Rhodamin and PAS. A PCR test was made for mycobacterium tuberculosis.

Results: The cut surface of the heart was found with several small, pale, non-palpable foci in the endocardium and myocardium of both ventricular walls. The coronary arteries all showed a moderate degree of atherosclerosis. There were no signs of myocardial infarction.

Microscopically the changes found at gross inspection were consisting of vital but damaged myocytes in a widespread fibrotic infiltrate. Focally, areas were found consisting of centrally located necrotic myocytes surrounded by an inflammatory infiltrate of histiocytic cells including few multinucleated giant cells. The vessels in the tissue were normal.

There was no sign of fungi seen in the PAS stain, no positive acid-fast bacteria in Ziehl-Neelsen or Auramin/Rhodamin stains. The PCR test was negative for mycobacterium tuberculosis.

Discussion and conclusion: The microscopic findings in the heart suggest that the woman died of giant cell myocarditis. The granulomatous changes found did not morphologically support the diagnosis of sarcoidosis, which normally will be without central necrosis and with centrally located giant cells too. The presence of myocyte necrosis and granulomatous changes did not support lymphocytic myocarditis, and the negative stainings and PCR test for fungi and mycobacterium tuberculosis exclude these as the cause.

In conclusion, the findings suggest that we here have described a case of fatal Idiopathic Giant-cell Myocarditis.

Next Generation Sequencing based mutation analysis in Non-Small Cell Lung Cancer

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Introduction:

During recent years Next Generation Sequencing (NGS) has become beneficial in prediction of treatment of Non-Small Cell Lung Cancer (NSCLC) due to specifically designed panels, low amount of required in-put material, lowered analysis time and reduced costs.

We have implemented NGS as the routine test in the molecular profiling of lung cancer for prediction of an individual's response to targeted therapies.

Here we have assessed the routine samples from 2016 in order to evaluate the feasibility/robustness of the method.

Material and Methods:

NGS was performed using the Ion Ampliseq Colon and Lung Cancer panel, v2 (Life Technologies). The samples were NSCLC biopsies (FFPE) or cytology material. 10 ng of DNA was used for library preparation, and sequencing was done using the PGM instrument, Life Technologies. A coverage of 1000x for EGFR amplicons was required for an accepted sequencing result.

The Cobas EGFR mutation test (Roche) was used, when NGS was not possible.

Results:

A total of 217 NSCLC samples were investigated using NGS (129 samples were FFPE lung biopsies and 88 were cytology samples). 196 samples (90.3%) were EGFR wildtype, 21 samples (9.7%) were EGFR mutated and 1 sample was not suitable for sequencing due to preparation in formic acid.

For 30 samples (13.8%) a NGS result was not obtained due to either, low gDNA concentration (3 samples), low library concentration (16 samples), poor sequencing data (3 samples) or instrument failure (8 samples).

Discussion and Conclusion:

For 86% of the tested NSCLC samples a satisfactory NGS result was obtained, and the method was suitable for both biopsy and cytology material.

Furthermore, additional and potentially useful knowledge was achieved regarding gene mutation status of the genes BRAF, MET, PIK3CA and KRAS among others.

In conclusion, NGS is a useful and beneficial method for diagnostic of NSCLC, however thorough evaluation of the in-put material and library is important in order to lower the number of samples to be re-tested.

Comparison of 2 decalcification methods influence on immunohistochemical analysis

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Introduction:

A group of Biomedical Laboratory Scientist students did their bachelor project about decalcification and DNA extraction on bone marrow biopsies. Their results made the department change decalcification method from approx. 7 hours decalcification at room temperature in formic acid to 5 hours decalcification in a microwave (KOS, Milestone, Italy) at 50°C in MolDecal from Milestone, Italy. However the immunohistochemical analysis seemed to be effected, and we returned to formic acid. This project aimed at testing several immunohistochemical analysis commonly used on bone marrow biopsies (CD34, CD117, CD61, Glycophorin A, MPO, CD3, CD20, CD19, CD5, CD23, Ki67, PAX5, TDT, CD138, Kappa and Lambda), by simultaneously staining a section decalcified by formic acid and a section decalcified by MolDecal.

Materials and Method:

We chose 15 bone marrow biopsies decalcified in MolDecal for 5 hours in a KOS microwave at 50°C and paired them with 15 bone marrow biopsies with a similar diagnosis decalcified in formic acid for 7 hours at room temperature. The analysis was assessed and marked by all participants in the project according to the assessment marks used by NordiQC.org. 2 of the participants also marked the morphology of the sections as normal or poor.

Results and Discussion:

CD34 and CD 117 showed all tissue decalcified in formic acid get score 3 or 2. The same analysis decalcified in MolDecal show a wider spread over the scoring system with 3,08% scoring 1 and 7,49% scoring 0.

When we add score 2 and 3 for CD20, CD19, PAX5, TDT and CD138 it shows more tissue decalcified in formic acid as acceptable for diagnostic use than sections decalcified in MolDecal with a rather large difference. For Glycophorin A, CD5 and MPO the highest score is for tissue decalcified in formic acid, but when we add score 2 and 3 both methods are equally acceptable. CD61 and Lambda has most score 3 in tissue decalcified with formic acid, but when we add score 2 and 3 MolDecal has most tissue acceptable for diagnostic use. CD3 shows the opposite results with most tissue decalcified in MolDecal scoring 3, but when we add score 2 and 3 most tissue decalcified in formic acid are acceptable for diagnostic use. For Ki67 and Kappa the difference between the two methods was too small to matter. In CD23 the acceptable score for both methods was very low and over 50% could not be scored on account of too few positive or reliably positive cells, even though more tissue decalcified in formic acid is acceptable for diagnostic use. Overall most tissue decalcified with formic acid scores 2 or 3, whereas tissue decalcified with MolDecal shows more tissue with scores of 0 or 1.

Conclusion:

Based on this project we cannot recommend decalcification in a microwave with MolDecal at 50°C for 5 hours.

Comparison of scanning quality on slides covered with film or glass

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Introduction:

The pathology department at Herlev and Gentofte Hospital have bought a couple of slide scanners (NanoZoomer, Hamamatsu), in an attempt to minimize physical archives of glassslides. According to the producer and supplier the scanner gives the best scanning results when slides are covered with glass instead of film. Since we use film routinely, and the film coverslipper from Sakura can cover 660 slides an hour compared to the glass coverslipper also from Sakura, which only covers 360 slides an hour, we decided to test this claim on 200 randomly chosen samples.

Materials and Method:

Samples: 400 slides was sectioned from 200 randomly chosen blocks of several different tissue types, 2 serial sections was mounted on 2 slides. 1 slide was covered with film on Tissue-Tek® Film® Coverslipper and the other was covered with glass on Tissue-Tek® Glas™ g2.

Stains: All sections was stained H & E on The Tissue-Tek® Prisma® (Sakura) and scanned on Nanozoomer 2.0-HT without prior cleaning of the slides.

Evaluation: All scans was evaluated by the author on a resolution of x20 and given a score (0 = scan cannot be evaluated, 1 = scan is poor, 2 = scan is acceptable and 3 = scan is optimal) according to quality of scan, how much of the section was clear and in focus.

Results and Discussion:

The results are given as a percentage of how many slides covered with either film or glass scored 0 – 3. 18 slides had to be excluded do to missing slides of one of the cover methods, another 28 slides had to be excluded do to a possible oversight by the scanning personnel.

Of the remaining 354 slides, 74% covered with film scores 3 where only 65% of slides covered with glass get the optimal score. In the acceptable category the percentage was 26% for film and 32 for glass. Only glass covered slides scored 1 = poor, but only 2%, and one of the glass covered slides had not been scanned.

The artifacts that affected the scanning quality were air bubbles, folds in the section, but also the size of the section affected the scanning quality. Air bubbles was most frequent in slides covered with glass which might be prevented by adjusting the amount of adhesive, but since the glass coverslipper only covers half the amount of slides an hour compared to the film coverslipper, it will not be tested.

Conclusion:

Based on the results of this study we can conclude that slides covered with film by Tissue-Tek® Film® Coverslipper gives better results compared to slides covered with glass by Tissue-Tek® Glas™ g2.

Tissue preparation: Does one method fit all?

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Introduction:

Molecular pathology techniques for detection of genetic and chromosomal abnormalities have become important clinical tools. This study aim to establish a fixation and decalcification protocol for FFPE tissue that leaves the tissue applicable for molecular assays, FISH and immunohistochemistry (IHC).

Material and methods:

Thirteen fresh tissues (tonsil, uterus, placenta, lung, mamma, adnexa, colon, kidney) were each split in sections, which underwent combinations of fixation and decalcification (agent (EDTA or formic acid) and time (6h, 24h, 72h) leading to 18 different pre-analytical procedures plus 1 fresh frozen sample. These 247 tissue samples were paraffin embedded, sectioned and split for IHC-(MLH1, MSH2 staining), FISH - (HER2/CEN17) and RQ-PCR analysis (Quantimize, Qiagen).

Results:

Evaluating quality of analyses, fixation time *per se* does not significantly influence the percentage of samples that could be analysed with IHC, FISH or RQ-PCR. The decalcification agent, on the other hand, had a significant impact on the analysis quality. In general, decalcifying tissue with microwaves impaired downstream applications - up to 80% of the samples were insufficient for FISH analysis, up to 30 % were insufficient for IHC and the DNA output was very low. The effect of formic acid as decalcifying agent varied between methods. At all fixation times 7-15% of the samples were insufficient for analysis with IHC. However, FISH analysis suffered more severely from formic acid, reflecting the duration of the prior fixation; 25% insufficient samples with fixation in 6h, 58% were insufficient with 24h fixation and 66% of the samples could not be evaluated with fixation in 72h. In terms of DNA output, exposure to formic acid resulted in amplifiable DNA below 10 ng/ul whereas EDTA as decalcifying agent gave rise to 29-53 ng/ul amplifiable DNA. Treatment with EDTA gave the overall best results as all IHC results were sufficient, FISH analysis could be performed on all samples (although longer fixation might be needed) and the amplifiable DNA output was high.

Discussion and conclusion:

We demonstrate that the best overall downstream analysis results are obtained when tissues fixate for 24 -72 hours in formalin followed by decalcification with EDTA for 24 hours. Tissues treated according to this protocol are available for high quality evaluation by both immunohistochemistry, FISH and RQ-PCR. This study has been performed on soft tissue due to availability and should be repeated on calcified tissue in order to evaluate if the tissue can be sectioned after the decalcification steps applied herein.

Optimization of a co-culture system for *in vitro* chemosensitivity screening assay

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Introduction: *In vitro* co-culture of murine embryonic stem cells (embryoid bodies, EB) into 3D-micro-tumors developed from patients' own cancer tissue is a potential candidate for a patient-specific angiogenesis assay. Such co-cultures could be used in a chemosensitivity screening assay in order to individualize the anti-cancer treatment, as they would illustrate the effect of anti-angiogenic drugs. Furthermore, EB are good candidates for stem cell therapy of damaged tissues. The culture conditions affects embryonic cells to differentiate into the desired cells. The aim of the present study was to optimize growth and differentiation of EBs into endothelial cells.

Material and Methods: Green fluorescent protein expressing mouse embryonic stem cell line D3 (D3-GFP) were cultured into EBs in spinner flask (SF) (3, 6 and 10 days culture) or a Low Attachment (LA) round bottom microtiter plate (10 days culture and 125, 250, 500, 1000 and 2000 cells/well). EBs were cultured in Dulbeccos's Modified Eagle's Medium (DMEM) for the entire culture period, with the exception of one group where EBs were transferred to Round Bottom (RB) microtiter plates (13 days total culture) after 6 days of culture in SF and grown in either DMEM or Matrigel matrix. Cultures were established with 7 and 14 days gap between subsequent passages A and B, respectively. EB were stained immunohistochemically for Ki67 and CD31. All experiments were investigated in duplicate.

Results: Comparison of EB cultures in RB, SF and LA showed that the optimal proliferation and differentiation of EBs were reached in RB plates. Differentiation to endothelial cells occurred significantly slower in passage B and C. Comparison of different groups showed more structured CD31 positive cells in DMEM than in Matrigel. We were able to detect CD31 positive cells in EB that were cultured for six days (200-300 μ m). Furthermore, cultures of EB in LA microtiter plates showed more CD31 positive cells and less necrosis when fewer cells were inoculated per well.

Discussion and conclusion: The study showed that the culture settings of stem cell cultures are critical not only for the physical configuration but also for the differentiation into endothelial cells

P4.1-P4.7

Characterizations of megalin expression in healthy mammary gland epithelium and in invasive ductal carcinomas reveal frequent loss of megalin expression in HER2 and triple negative tumors

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Introduction: Invasive ductal carcinomas can be divided into four subtypes (originally based on gene expression profiles); luminal A, luminal B, Human Epidermal growth factor Receptor 2 (HER2), and triple negative (TN) tumors. HER2 and TN tumors are the most aggressive, and patients with TN tumors hold the poorest prognosis. We hypothesized that expression of essential proteins in healthy mammary gland epithelium compared with the expression of these proteins in breast cancer tumors might identify novel biomarkers or point towards new therapeutic targets; specifically in the TN tumors. We therefore conducted a systematic investigation to identify the expression pattern of the transmembrane receptor megalin (encoded by the *LRP2* gene), previously shown to be expressed in both the healthy mammary gland and in breast cancer-derived cell lines.

Material and methods: By immunohistochemical labeling using two different anti-human megalin antibodies (a monoclonal as well as a polyclonal antibody) the megalin expression was characterized in formalin fixed, paraffin embedded (FFPE) healthy mammary glands from four healthy women and in FFPE invasive ductal carcinomas from 42 cancer patients across the four subtypes; luminal A, luminal B, HER2, and TN. The specificity of the anti-human megalin antibodies was validated by immunoblotting. Furthermore, the *LRP2* expression was investigated in some of the invasive ductal carcinomas by quantitative PCR (qPCR).

Results: Megalin is predominantly expressed at the apical plasma membrane in epithelial cells in the healthy mammary gland. Our analyses of megalin expression in invasive ductal carcinomas demonstrate a large degree of variation across the subtypes. Megalin expression is sustained in luminal A and luminal B tumors, whereas megalin expression is frequently lost in HER2 tumors. In some TN tumors megalin appears to be expressed intensely, while others have lost megalin expression completely. The qPCR analyses of *LRP2* support the immunohistochemical analyses.

Discussion and conclusion: Our results reveal that the expression level of the megalin is modulated in breast cancer. Megalin expression is maintained in the less aggressive luminal A and luminal B tumors, whereas megalin expression is frequently lost or varies in the more aggressive HER2 and TN tumors.

Case Report: Rare finding of Synchronous Nipple Adenoma and Breast Carcinoma

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Introduction: Adenoma of the nipple (Florid papillomatosis) is a benign uncommon lesion arising in the lactiferous duct. The clinical presentation mimics Paget's disease of the nipple, skin lesion, erythema, bloody discharge and pain. Synchronous existence of nipple adenoma and ipsilateral breast carcinoma has been described previously and is considered extremely rare. We report a case of a retropapillary invasive ductal carcinoma presenting with clinical involvement of the nipple where the pathological examination revealed a coexisting nipple adenoma.

Material and Methods: Case: 69-year old woman selected from breast cancer screening mammography, which showed a spiculated dense tumor of 23 mm, centrally and retropapillary in the right breast. Ultrasound guided core needle biopsy from the tumor showed invasive ductal carcinoma. There was no lymphnode involvement. Subsequently, the patient was referred to mastectomy and sentinel node biopsy.

Results: Macroscopic histopathological examination of the mastectomy specimen revealed an uncommon anatomy with the nipple placed in the inframammary fold. The nipple was edematous and the skin over the nipple was intact, and not characteristic of Paget's disease. On gross cross section, a distinct border between tumor and epidermis was observed. There was an underlying 35x35 mm solid malignant tumor. Microscopic examination on mega slide revealed two distinct morphologies. In the nipple adenomatous proliferations lined with hyperplastic 2 layered epithelium and microglandular extensions. Both adenosis pattern and papillomatosis patterns were present. Adjacent to the adenoma we found an invasive ductal carcinoma grade II, estrogen receptor positive and borderline HER2 protein expression. FISH analysis showed normal HER2 geneexpression (HER2/CEN17 showed a ratio of 1.45). Sentinel node biopsy was negative.

Discussion and Conclusion: In the literature review by Jones et al. (*modern pathol.* 1995) synchronous nipple adenoma with ipsilateral breast carcinoma was found in 1.2% of 967 patients. Furthermore, when they were coexisting they were not immediate adjacent like in the present case. Generally, the nipple adenoma is believed to be an incidental finding rather than related to the carcinoma.

Fatal outcome of lung transplantation due to an undiagnosed malignant vascular tumor: A case report

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Introduction: Pulmonary vascular tumors are usually asymptomatic and therefore incidental findings. Most diagnosed vascular tumors, such as hemangiomas, are benign (about 90%). In a very small subset of cases low grade malignant vascular tumors have to be considered in the differential diagnoses. Diagnosis can be challenging, as both clinico-radiological features of low grade malignant vascular tumors such as size and multifocality, as well as immunohistochemical features are often similar and seldom helpful. We report a case wherein a 37-years-old female who presented with history of microprolactinoma and hyperprolactinemia for which she was treated with dopamine-agonists. The patient was referred with dyspnea, severe pulmonary arterial hypertension, and dilated right ventricle. A drug-induced mediastinal fibrosis was clinically suspected. Mediastinal biopsy was inconclusive. The patient's pulmonary hypertension-induced lung insufficiency was treated with double lung transplantation. Massive uncontrolled bleeding from right pulmonary artery and intercostal arteries during lung transplantation resulted in *exitus in tabula*.

Materials and methods: The explanted lungs were carefully sectioned and examined. There was focal vessel-centered consolidation of lung parenchyma in close proximity to the hilus of the right explanted lung. All macroscopic features were photodocumented. Representative tissue sections of the lesion and unaffected lung parenchyma were submitted for histological examination. Formalin fixed paraffin embedded tissue sections were examined on conventional histology and supplemented with immunohistochemistry for endothelial markers (CD31, CD34, CD10, Factor VIII, HHV8, and PHH3).

Results: Histologically, plexogenic lesions and fibrointimal proliferation as morphological correlate for the clinically diagnosed pulmonary hypertension were relatively prominent. The consolidated lung parenchyma did correspond to an angiocentric and abnormally branched angioinvasive vascular low grade spindle cell neoplasm, with aberrant lumen formation. The tumor infiltrates bronchioles and extends along pulmonary arteries causing occlusion and expansion. The lesional cells show bland cytological morphology with indistinct cytoplasmic margins. The neoplastic cells were set in a fibrous stroma. There was focal tumor necrosis demarcated from the surrounding lung parenchyma by palisading histiocytic population imparting morphology of granulomatous inflammation. Tumor cells were positive for CD31, CD34, CD10 and Factor VIII and were negative for HHV8. The mitoses-index (PHH3) shows up to 2 positive cells per 10HPF. The bronchial and vascular resection margins were free from tumor. In addition, there was constrictive bronchiolitis in both lungs.

Discussion: Constrictive bronchiolitis is often a complication of an ongoing chronic inflammatory disease, which might be drug-induced, as reported for dopamine-agonists. The vascular tumor explains the clinically diagnosed occlusion of right sided pulmonary artery and represents probably the underlying cause of severe pulmonary hypertension. Histomorphology and immunohistochemistry of the vascular lesion strongly favored a low grade angiosarcoma, which explains the unfavorable outcome at the surgical intervention. Whether mediastinal fibrosis is tumor related remains unresolved as autopsy was not performed.

Concomitant Nodal HHV8 Associated Multicentric Castleman Disease and Kaposi Sarcoma in a HIV-Negative Patient

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Introduction: Multicentric Castleman disease (MCD) is a rare systemic lymphoproliferative disorder with poor prognosis, characterized by mantle zone proliferation of polyclonal plasmablasts. MCD is strongly associated with both HIV-associated immunosuppression and human herpesvirus-8 (HHV8) infection. Patients with HIV-associated MCD often develop a second HHV8-associated disease, Kaposi sarcoma (KS). HIV-positive patients may develop concomitant HHV8-positive MCD and KS in the same tissues. Rarely, MCD may be seen in HIV-negative patients, usually associated with other forms of immunosuppression, fewer than half of these cases containing HHV8. We present a case of concomitant HHV8-positive MCD and KS developing in the same lymph node of an HIV-negative man without overt immunodeficiency.

Material: The patient was a 51-year old HIV-negative Somali man living in Denmark, whose only medical history of note was type 2 diabetes. Three months after a visit to Somalia, he presented with fever, fatigue, night sweats, weight loss, hepatosplenomegaly, ascites, pleural effusions, and generalized lymphadenopathy. He was admitted to the Department of Infectious Medicine on **suspicion of tuberculosis. Excision biopsy of cervical lymph nodes was performed.**

Results: Several enlarged lymph nodes were found, with preserved architecture, containing numerous B-cell follicles with varying degrees of germinal centre atrophy and hyalinization, surrounded by expanded mantle zones with an 'onion skin' pattern of concentric small lymphocytes. Hyalinized vessels were prominent. Plasmacytosis was seen in and around the mantle zones and in the interfollicular areas, the infiltrate including variable numbers of plasmablasts. On immunohistochemistry (IHC), the many para- and perifollicular plasmacytoid cells expressed plasma cell markers (CD38, CD138, MUM-1) and HHV8 (LANA1). A diagnosis of MCD (mixed hyaline-vascular and plasma cell type) was made. The same lymph nodes also contained prominent areas of vascular spindle cell proliferation, positive on IHC for CD31, CD34, D2-40 and HHV8 (LANA1), confirming the diagnosis of KS. The patient is currently showing a positive response to Rituximab (anti-CD20) combined with anti-KS therapy.

Discussion and conclusion: HHV8, first isolated from KS in 1994, is an oncogenic virus associated with KS, MCD and several rare lymphoma types. Diagnosis of MCD is challenging, although HHV8-positive cases show characteristic IHC LANA1-positivity. Concomitant nodal occurrence of MCD and KS is rare, but well documented in an HIV-setting. It is exceptionally rare in HIV-negative patients. Our case, occurring in an apparently immunocompetent individual may be unique.

Plasmablastic myeloma with aberrant expression of CD3

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Introduction: It is well known that expression of antigens in hematological neoplasms are often altered and deviating from the pattern seen in their normal counterparts. This can be seen as either *loss* of antigen expression or *gain* of certain antigens which are usually absent in their normal counterparts, i.e., aberrant expression.

Aberrant co-expression of CD3, a T-cell lineage specific antigen, is very rare in mature B-cell neoplasms. We present a case of plasmablastic transformation of multiple myeloma with aberrant expression of CD3.

Material and Methods: The patient, a 71-year old man, was diagnosed with multiple myeloma, april 2012, with a bone lesion of Th3. Two years later he suffered a pathological fracture of the left collum femoris and acetabulum. The bone marrow biopsies had shown an infiltration between 5-20% during the course of his disease. All samples were with kappa light chain restriction and none expressed CD3. FISH studies were not performed. March 2016, he presented with several processes in the liver from which a needle biopsy was taken.

Results: The biopsy showed an abrupt transition from near-normal liver tissue to a neoplasm consisting of sheets of somewhat poorly differentiated plasma cells with a high nuclear-to-cytoplasmic ratio, containing nuclei with dispersed chromatin and many with small nucleoli. The neoplastic cells expressed a plasma cell phenotype including positivity for MUM1, CD38, CD138 and with kappa light chain restriction. Furthermore aberrant expression for CD3, CD117 and CD56 (partially) was seen. The Ki-67 proliferation index was around 90%. There was a negative reaction for CD5, CD20, cyclinD1, CD10, BCL-2 and BCL-6. CD79 was non-evaluable. Markers for Epstein Bar Virus (EBV) and other T-cell markers could not be evaluated due to lack of tissue.

Discussion and conclusion: Aberrant expression of antigenic markers is important to appreciate, as it may cause diagnostic confusion since the classification of lymphoid neoplasms is largely based on immunophenotyping to determine the cell lineage.

CD3 is identified in T-cells at all developmental stages, and in virtually no other hematopoietic cells except for NK-cells. Due to its high specificity, CD3 has been considered a specific antigen marker to define T-cell lineage in hematolymphoid neoplasms. However aberrant expression of CD3 has recently been reported in rare cases of mature B-cell neoplasms. It has been suggested that EBV may promote T-cell antigen expression in B-lineage neoplasms but additional studies are necessary to determine the mechanisms responsible for the aberrant expression. The biological significance of expression of CD3 is at present unknown.

Multiple miliary osteomas of the face induced by a dermaroller - A case report

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Osteoma of the skin is a rare, benign disorder of the skin, characterised the formation of bone in the skin. It can occur as a primary or as a secondary phenomenon. Secondary osteoma cutis can be a sequela to different disorders of the skin including nevi, scleroderma, pilomatricoma, dermatomyositis, basal cell carcinoma, scars, inflammation and trauma. Most commonly it is demonstrated in patients with a long history of severe acne and scarring. Primary osteoma cutis develops earlier in life and is less common. It is not related to preexisting skin disease and it may represent an independent phenomenon or may be part of a syndrome associated with phenotypic or metabolic characteristics. The pathogenesis of primary osteoma is not fully understood. One, likely theory interprets the presence of bone in the skin as a result of osteoblastic metaplasia of fibroblastic mesenchymal cells. Another theory assumes a disordered embryologic proces, whereby primitive mesenchymal cells differentiate normally into osteoblasts but migrate to the wrong location. Skin lesions are typically asymptomatic and appear as skin-coloured to pigmented, very firm nodules. Skin biopsy, blood pressure measurement, laboratory exams and imaging by MR is recommend in the diagnostic process. Different treatment options exist including: Treatment of the base disease and concomitantly excision, curettage, dermoabrasio, topical and laser treatment of the osteomas are suggested.

We here present a case of multiple osteomas in the face of a 47 years old Philippine woman who in 2013 undervent a single treatment with a derma roller on her cheeks as a treatment of scarring caused by acne. The treatment was provided by the patients sister in law. The primary skin condition with acne had started 3 to 4 years earlier and had previously been treated with Accutin. The development of the osteomas on the cheeks and the chin did not develop until after derma roller treatment. She has had punch biopsies taken two times from the cheeks as well as from the chin demonstrating an orthokeratotic epidermis, a reticular dermis with perivascular and perifollicular chronic inflammation and a cyst like formation lined by cells undergone osteoblastic metaplasia and without any sign of epithelial lining. Blood samples revealed low vitamin D and secondary hyperparathyreoidism. MR scan was normal.

In conclusion, we here describe a rare cause of multiple osteoma of the face in a patient who was treated with a derma roller for scarring caused by a previous acne.

The first Danish family reported with an AQP5 mutation presenting diffuse non-epidermolytic palmoplantar keratoderma of Bothnian type, hyperhidrosis and frequent *Corynebacterium* infections

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Introduction: Palmoplantar keratodermas (PPKs) comprise a clinically and genetically heterogeneous group of hereditary disorders of the skin characterized by thickening of the stratum corneum of the palms and soles. Based on the clinical presentation, palmoplantar keratodermas are divided into four subtypes, including diffuse, punctate, focal and striate PPK. Diffuse PPK can be further subdivided histopathologically into epidermolytic and non-epidermolytic forms depending on the presence or absence of cytolysis in the upper spinous and granular layers of the epidermis. An autosomal dominant form of diffuse non-epidermolytic palmoplantar keratoderma, palmoplantar keratoderma of Bothnian type, is caused by mutations in the *AQP5* gene encoding the cell-membrane water channel protein aquaporin 5, which leads to defective epidermal-water-barrier function in the epidermis of the palms and soles.

Material and methods: We report the first Danish family diagnosed with diffuse non-epidermolytic palmoplantar keratoderma of Bothnian type in which fourteen individuals are potentially affected.

Results: The proband, a 36-year-old male had since childhood been affected by palmoplantar keratoderma and pronounced hyperhidrosis of the palms and soles. The palmar and plantar skin was affected by yellow tinted keratoderma, pitted keratolysis and erythrokeratotic plaques with a clear demarcation to normal skin on the dorsum of the hands and feet.

A skin biopsy showed a markedly thickened stratum corneum with a prominent stratum granulosum and moderate acanthosis. The acrosyringial ducts were remarkably dilated in the epidermis and in the stratum corneum. In dermis, however, the sweat ducts and the secretory coil appeared normal without recognizable dilatation.

Following informed consent, genomic DNA was analyzed using bidirectional Sanger sequencing of the *AQP5* gene. The proband was heterozygous for a missense mutation in the *AQP5* gene, c.562C>T, (p.Arg188Cys) and his eight year old son was found to be heterozygous for the same *AQP5* mutation. Water immersion test revealed aquagenic wrinkling, also known as “hand-in-the-bucket-sign”, seen as pronounced maceration of the skin with translucent white papules and a whitish spongy appearance due to swelling of the stratum corneum following three minutes exposure to water.

The patient presented recurrent fungal infections and periodic worsening with pitted keratolysis and malodour due to bacterial infections.

Discussion and conclusions: Palmoplantar keratoderma of Bothnian type is a rare, hereditary skin disorder characterized by diffuse palmoplantar hyperkeratosis, acral hyperhidrosis and the peculiar phenomenon aquagenic wrinkling. The condition is most likely caused by aberrant expression of aquaporin 5.