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Poster Presentations

Autopsy

1 REVERSING THE SLOW DEATH OF THE CLINICAL POST MORTEM EXAMINATION: DEVELOPING THE POST OF THE PATHOLOGY LIAISON NURSE

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Background: By assessing the accuracy of clinical diagnosis and the effects of patient management, post mortem examinations (PMs) provide a valuable clinical audit tool. They also are important in teaching, training and research, and have benefits for surviving family members. Adult clinical (consented) PMs have seriously declined in recent decades, the decline in Norwich being approximately linear between 1996-2003. We wished to reverse this trend, not least to assist in training our medical students and junior histopathologists. We recognised that the burden on clinicians' time and their lack of training in obtaining consent for PM may have been contributing factors in decline of the clinical PM. In order to relieve the demand on doctors' time and to ensure that consent is properly elicited, the Trust established a post of Pathology Liaison Nurse (PLN). Here we describe how we established and developed the post of PLN and the current state of the adult clinical PM in Norwich.

Design: Developing the PLN post: the post was for a one-year trial period. The PLN was to ensure that family wishes about the extent of PM and the retention and disposal of material were properly carried out. The PLN received training in all aspects of the PM service and became confident in eliciting consent herself and in liasing with clinicians, pathologists and the bereavement service. The post was evaluated at the end of the trial year. The opinions of consultants and of some families were sought using questionnaires. These small surveys suggested that the PLN provided a valuable service, and the Trust Board retained the post. Review of the numbers and extent of consented post mortem examinations: The numbers of adult deaths in hospital and of clinical PMs were determined, allowing us to calculate the adult clinical PM rate (number of clinical PMs divided by the number of deaths.

Results: The decline in the number of adult clinical PMs during 1996 – 2003 was reversed during 2004 – 2005. We undertook 167 adult clinical PMs in 1997, but there were only 34 in 2003, a reduction of nearly 80%. The adult clinical PM rate fell from 8.4% in 1997 to 1.4% in 2003. During 2004, 45 adult clinical PMs were undertaken (clinical PM rate = 1.8%) with further improvement in 2005 (58 adult clinical PMs; clinical PM rate = 2.4%). There have also been improvements in the extent of examination permitted and in the retention of material afterwards.

Conclusion: The decline in the adult clinical PM rate in Norwich was reversed in 2004 and 2005, coinciding with the appointment of the PLN and the internal publicity to promote her role and that of the clinical PM. The improvement has been modest, and the adult clinical PM rate remains far short of ideal. However, our achievement shows that the death of the adult clinical PM is not inevitable, and provides a good platform for further improvement. We believe that the profession should recognise its responsibility to improve the status of the clinical PM and act accordingly.

Bone

2 THE EXPERIMENTAL STUDY OF ACETABULAR BONE STRESS IN BIPOLAR HIP PROSTHESIS

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Background: Although considered a revolutionary technique, hip arthroplasty didn't solve the problem of acetabular bone structures degenerative changes at the bone-prosthesis contact. These changes result in changing the bipolar prosthesis with a total hip prosthesis, metal-cup duo representing a temporal solution reserved for young people in order to preserve as much as possible the acetabular bone tissue. The purpose of this study is to identify the acetabular bone areas of maxim stress at the bone-duo prosthetic cup interface using experimental models.

Design: The materials consisted of a hipbone, a bipolar acetabular cup with outer diameter of 48 mm, adequate for the hipbone and a standard neck with a 28 mm diameter prosthetic head. Postero-anterior and lateral radiographs of this joint model were made, illustrating two phases of the walking process. Serial axial CT scans (every 2 mm) of the same articular model were made, from 3 cm above the acetabular dome to 1 cm below the inferior acetabular end. The obtained images were scanned and then a sufficient number of outline points were marked and related to a 3D coordinate system (XYZ). The finite elements model was obtained by computer aided assembling of the points resulted from the radiographic composing and the CT scans and further discretised by adding a number of 30 000 points. Tensional and deformational status were assesed in all considered planes for all prosthesis components. A post-processing software traced the variation curves for the contact pressure at the components interface (hipbone-armour, armour-cup and cup-femoral head) using all the obtained data.

Results: The highest compressive tensions for the femoral prosthesis head were observed in the frontal plane and monopodalic support (ó2 = -176,46 MPa). The compressive tensions for the polyethylene cup are relatively small, the highest value also being in the frontal plane and monopodal support (ó2 = -15,83 MPa). The maximal tensions in the hipbone are observed also in the frontal plane and monopodal support in the proximity of the contact area with the prosthesis metallic armor (ó2 = -70,43 MPa). In bipodal support, ó2 compressive tensions were very small for all the structures analyzed. The pressure variation curves show that maximal value for the contact pressure on the contact area between the armor and the hipbone was also recorded in the frontal plane and monopodal support (pmax = 25 MPa). The maximal pressure in the sagital plane, on the same interface, in monopodal support, is much smaller (pmax = 8 MPa). For the same interface and bipodal support, in both planes, the maximal values of the contact pressure are much smaller (4 MPa and 3 MPa respectively).

Conclusion: The obtained data suggestes that the main stress in the case of the prosthesis femoral head is in the frontal plane. The very high contact pressure recorded at the armor-hipbone interface suggests a risk for bone tissue crush in certain areas of the contact surface. These destructions can lead, in time, to armor detachment from the bone cavity and thus requiring another surgical procedure.

3 MALIGNANCY IN GIANT CELL TUMORS OF BONE: IS THERE A REPRODUCIBLE HISTOLOGIC THRESHOLD? A STUDY OF THREE GIANT CELL TUMORS WITH WORRISOME FEATURES

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Background: Giant cell tumor of bone is well known to recur and metastasize while preserving non atypical histology. Malignancy (MGCT), however, is a very rare event that has great implications for treatment. It occurs in 1% of all cases, typically following multiple recurrences and rarely de novo in a primary tumor. In the literature, the proposed criteria adopt the dedifferentiation theory and describe an undifferentiated sarcomatous overgrowth devoid of giant cells juxtaposed to a typical GCT, or in a previously diagnosed GCT. Atypia is generally accepted in GCT. However, cytologic and architectural atypia beyond the permissible level of benign GCT and short of malignancy can cause serious diagnostic dilemma as to its benign degenerative or malignant significance. Herein, we describe the histological features of three cases of GCT with such atypical foci in which the diagnosis of malignancy was controversial.

Design: Three cases of GCT with atypical foci derived from the Pathology files of the McGill University Health Center, Montreal, and Saint Vincent Comprehensive Cancer Centers, New York, were reviewed. The histologic features examined included: Stromal spindle cell overgrowth and hypercellularity, cytoatypia, hyperchromasia, presence/absence of atypical mitotic figures, presence/absence of intermixed giant cells, and prominent nucleoli. Additional outside experts' opinions was sought given the complexity of the cases.

Results: One case presented with a solitary distal femoral mass. Two presented with lung metastases, 3 and 30 years following the initial diagnosis of GCT, respectively. The primary tumor in all three cases was treated by surgical curettage alone. The patient with the longer disease-free interval experienced two recurrences prior to metastases. All the three cases had somewhat similar histologic features: Typical GCT within which were foci of increased stromal cellularity, mild to moderate pleomorphism, rare atypical mitoses, and variably prominent nucleoli. In addition, one case showed a minute focus of stromal overgrowth of mildly atypical spindle cells with decreased number of giant cells. There was no hyperchromasia or frank high grade sarcomatous growth in any of the cases. Outside experts' opinions ranged from GCT, to GCT with areas suspicious for malignancy, to MGCT. The final diagnosis for which treatment was based upon was GCT (1 case) and

MGCT (2 cases). Of the two cases diagnosed as malignant, one patient underwent limb salvage surgery after the initial curettage with no evidence of residual tumor.
Conclusion: The traditional histologic criteria of MGCT do not include a subset of GCT that exhibits focal architectural and cytologic atypia beyond the degenerative changes sometimes seen in this tumor. Yet, some of these tumors are variably called malignant by experts and surgically treated accordingly. The behaviour and prognosis of such tumors need to be studied in order to correctly classify them. Moreover, if they are to be considered malignant, reproducible criteria for MGCT that include this group should be established. Whether the creation of a "borderline" or "atypical" GCT category is necessary is yet to be determined.

4 OSTEARTHRITIS IN HUMANIZED MICE

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Background: Osteoarthritis causes considerable morbidity and cost. An animal model to study this condition would be useful. A mouse that has human type cartilage might fill this need.
Design: We had previously generated gene knockout mice for collagen type II gene. However, the homozygous mice for the gene knockout, die soon after birth from achondrogenesis. In the current study the lethal phenotype in collagen type II null mice was rescued by introducing a normal human collagen type II gene in the genome of these mice. Joints from these mice were studied by histology at day 7 as well as 1, 3, 6, 12 and 15 months and compared with wild types as controls.
Results: Humanized mice were completely normal with regards to their weight, reproduction capabilities and life span (2 years). The mice were initially indistinguishable from wild type, however, by 12 months, the humanized mice displayed extreme slowness. By 15 months these symptoms were obvious with the marked distortion of joints. Histology of weight bearing joints of day 7, as well as 1 and 3 months showed normal growth plate and knee joints in both groups. However, the knee joints from 9 month-old animals showed deterioration of articular cartilage. At 15 months - the growth plate had almost disappeared and there was complete loss of articular cartilage (a stage at which they showed very slow movements). These symptoms were more prominent in female mice.
Conclusion: Humanized mice may be useful model to study osteoarthritis, its biomarkers and the effect of treatment.

5 CO-OCCURRING GOUTY ARTHRITIS AND PIGMENTED VILLONODULAR SYNOVITIS IN A KNEE: FIRST CASE REPORT

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Background: Co-occurrence of gouty arthritis and intraarticular pigmented villonodular synovitis (PVNS) has never been reported in the literature. Here we reported such case in a knee.
Design: A 38 yr-old Thai male who had been suffered from swelling, pain, and limited range of motion of right knee for 1 year was referred to Institute of Orthopaedics after failed medical treatment. Physical examinations of other organs were within normal limit. Preoperative MRI revealed an extensive, infiltrative, irregular synovial mass involving entire right knee joint and suprapatellar bursa with multifocal bone erosion and large amount of joint effusion, radiographically consistent with PVNS. CBC, blood chemistry, and right knee joint aspiration were taken. Histopathological examination of synovium was performed after arthroscopic biopsy and debridement.
Results: Hyperuricemia (uric acid = 9.0 mg/dl), consistent with presence of monosodium urate crystal in joint fluid, was found. Arthroscopy revealed thick, milky white joint effusion, extensive synovial tophi, and multifocal erosion of articular cartilages. Histological examination showed synovial hyperplasia with extensive deposition of urate crystal associated with foreign-body granuloma. Interestingly, a synovium villus with typical features of PVNS was also observed. The lesion was covered by synoviocytes with underlying fibrovascular tissue containing variable number of mononuclear histiocytes, interspersed osteoclast-like multinucleated giant cells and multifocal hemosiderin deposits. Postoperatively, marked improvement of joint motion was observed. Allopurinol and colchicine were prescribed as home medications.
Conclusion: We reported the first case of co-occurring gouty arthritis and intraarticular PVNS in a knee. The diagnosis was confirmed by serum uric acid level, microscopic examination of joint effusion, and histopathology of synovium.

6 CARTILAGE TRANSPLANTATION PATHOLOGY: FEATURES OF GRAFT FAILURE AND SURVIVAL

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Background: Fresh cadaver osteochondral shell allografts are used for young active patients to replace knee joint tissue affected by trauma or focal degenerative arthritis. To optimize this procedure, it is important to understand mechanisms of graft failure and survival. 290 fresh cartilage osteochondral transplants were performed at Mount Sinai Hospital, Toronto, Canada from 1971 to 2006, with 69 known graft failures. Clinically, cartilage graft survival was 95% at five years, 31% at 10 years and 66% at 20 years. Our objective was to assess the cartilage graft histopathology with a view to determining the histologic features of early and late graft failure, as well as prolonged graft survival.
Design: We reviewed histopathologic features of cartilage, bone and synovium in 44 cases in which remnants of graft were removed at time of subsequent arthroplasty. Grafts examined were functioning in place from one year to greater than 25 years. All grafts were examined by the same protocol, which utilized specimen radiography, preparation of decalcified blocks, 5 ìm H&E and Toluidine blue, Safranin O stained sections, as well as transmission electron microscopy of graft cartilage samples.
Results: Graft failure was classified as early failure and late failure. Histologic features contributing to early failure were: lack of graft chondrocyte viability, loss of matrix cationic staining (glycosaminoglycan loss), mechanical instability and presence of systemic disease such as inflammatory arthritis. Histologic features contributing to late graft failure included: fracture through the graft, active and incomplete remodelling of graft bone by host bone, resorption of cartilage graft tissue by synovial cell inflammatory activity at the edges of the graft. Histologic features contributing to cartilage graft survival included: prolonged viability of graft chondrocytes, functional preservation of matrix (cationic staining of graft cartilage matrix). Graft chondrocyte viability as seen by electron microscopy was demonstrated up to 25 years posttransplantation. Additionally, complete replacement of graft bone with host bone and integration of the host bone subjacent to graft cartilage, as well as clinical mechanical stability of the graft were all features associated with prolonged graft survival.
Conclusion: Cartilage osteochondral allografts can survive in human knee joints greater than 25 years provided that mechanical stability and graft chondrocyte viability are preserved.

7 CLINICOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND CD-34 IN HUMAN OSTEOSARCOMAS

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Background: Osteosarcoma is a relatively rare malignant neoplasm and a little information has been reported on its angiogenesis.
Design: Pretherapeutic paraffin- embedded biopsies from 35 patients newly diagnosed high grade central osteosarcoma at our institution with available clinicopathological data were studied by immunohistochemical technique for VEGF and microvessel density (MVD) determined by CD 34 expression. VEGF expression and MVD were correlated with survival and demographic and tumor related variables. Tumors were considered VEGF positive if immunoexpression was strongly positive in greater than 10% of the tumor cells. MVD was obtained by microvessels counting 10 high power fields (X400 field) in each biopsy sample and than the MVD was defined as the mean count of microvessels. The median MVD of the entire group was predetermined to classify patients into two groups with high (> median) and low (¡Ü median) .Differences in mean vessels counts were analyzed by Student t test.The correlation between clinicopathological variables and the expression of VEGF was statistically analyzed using the ¦Ö2 test for comparation of two groups. Curves for overall survival and disease free survival were drawn according to the Kaplan-Meier method and differences were analyzed by applying the log-rank test. Statistical significance was defined as $P < 0.05$.
Results: The mean age was 17 years (median: 15,34). There was male gender predominance (65,71%). VEGF staining was positive in 30 tumors (85,7%) in which MVD was higher of than that in 5 VEGF negative tumors (14,3%). Non-significant correlations were observed in metastatic and non-metastatic patients when variables as histological subtypes, surgery, tumor site, death and relapse were compared with tumors VEGF positive and negative. Patients with VEGF positive tumors were poorer in survival compared those with a VEGF ¨Cnegative tumor ($P = 0.003$) .
Conclusion: These finds strongly suggest that VEGF expression is predictive of poor prognosis in patients with osteosarcoma and provides basis for a therapeutic strategy targeting angiogenesis.

8 PROGNOSTIC SIGNIFICANCE OF VEGFR-2 EXPRESSION IN OSTEOSARCOMA

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Background: Osteosarcoma is a rare disease with the peak incidence in adolescence. Usually arises in long tubular bones, mainly around the knee. During physiological bone formation there is a strength relation between osteoblasts and vasculoendothelial system. VEGFR2 expression is highly augmented during osteoblast differentiation and mineralization. VEGFR2 is expressed in several tumor cells, and in many tumors has demonstrated prognostic significance.
Design: We retrospectively analyzed all patients treated in our institution with any anatomopathological specimen available. Samples were classified as B1: diagnostic biopsy, B2: post-neoadjuvant chemotherapy specimen and B3: relapsed or metastases resection. Immunohistochemical staining of VEGFR-2 (Santa Cruz, Biotechnology) was analyzed on all samples. In B1, anti-CD31 antibody (DAKO) was employed to estimate microvessel density (MVD), using a Chalkley eye-piece graticule. Immunohistochemical reactions were evaluated by 2 pathologists (FM-T and EC). Cut-off values were decided based on published studies.
Results: Between June 1977 and March 2003 forty three patients with any anatomopathological specimen available were treated of osteosarcoma at our institution. Median age was 14 years, 66% were male, and 42 (98%) were in the extremity. Five patients (11%) had distant metastases at diagnosis and 2 patients (4.5%) had skip metastases. VEGFR2 was expressed in 54% of osteosarcoma cells in B1: 15 (45%) negative, 7 (21%) < 10%, 6 (18%) 10-50%, 5 (15%) > 50%. VEGFR2 was expressed in 69% of osteosarcoma cells in B2: 8 (30%) negative, 6 (23%) <10%, 6 (23%) 10-50%, 6 (23%) > 50%. Anti-VEGFR2 stained 86% of B3 samples: 1 (14%) negative, 3 (43%) < 10% and 3 (43%) > 50%. MVD was available in 30 B1 samples: <3: 13 (43%), 3-5: 6 (20%), 5-7: 4 (13%), >7: 7(23%). With a median follow up of 185 months, 48% have progressed and died. VEGFR2 expression in B1 was associated with higher MVD (p=0.03), a better disease free survival (DFS) RR 1.7 (CI95%:1.26-4.16, p=0.03) and a better overall survival (OS) RR 2 (CI95%:1.38-5.26, p=0.02). VEGFR2 expression was also associated with a strong statistical tendency toward better response to chemotherapy RR 6.4 (CI95%: 0.9-43, p=0.057). VEGFR2 expression in B2 was also correlated with a better DFS RR 1.6 (CI:

1.03-4, p=0.07) and OS RR 2 (CI95%:1.25-4.76, p=0.05) in univariate analysis. VEGFR2 expression in relapsed or metastasic specimens (B3) and MVD was not associated with prognosis.
Conclusion: VEGFR2 may be a novel prognostic factor aiding to identify patients with osteosarcomas with better prognosis at diagnosis. In our study, MVD does not have prognostic value in osteosarcomas, although MVD was correlated with VEGFR2 expression.

9 PATHOLOGY OF VASCULARISED FIBULAR IMPLANTS IN TREATMENT OF AVASCULAR NECROSIS OF THE FEMORAL HEAD
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Background: Vascularised fibular implants have been used in the management of avascular necrosis of the femoral head as a way to support the articular surface and prevent/delay collapse and secondary arthritis.
Design: Review of femoral heads with grafts removed at revision to total hip arthroplasty.
Results: Early collapse and clinical failure in 28 resected cases from a clinical surgical experience of over 280 operated cases was observed. Vascular pedicle of fibular graft showed good retention of blood supply to graft. Portion of graft in epiphyseal necrotic zone was subject of extensive resorption and loss of ability to provide support for joint surface. In the metaphyseal region where femoral head was viable and outside of zone of necrosis there was excellent osseointegration of graft.
Conclusion: Rate and duration of success is less than expected. Some time delay to total hip arthroplasty is gained for younger patients.

10 THE EXPRESSION OF INSULIN-LIKE GROWTH FACTOR-1(IGF-1), IGF-1 RECEPTOR AND TRANSFORMING GROWTH FACTOR-β IN CHORDOMA
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Background: Chordoma is a unique and low to intermediate grade malignant tumor that recapitulates notochord. Chordomas account for 1-4% of all primary malignant tumors, and little is known about the etiologic factors that predispose to them. The effect of IGF-1 on bone tissue and its role in bone development have been extensively studied, and in addition, IGF system has been implicated in tumor development and progression in various neoplasms including osteogenic and soft tissue sarcomas. In bone, the synthesis of IGFs is down-regulated by many locally produced growth factors, particularly transforming growth factor β (TGF-β) and cortisol. On the other hand, it has been shown that mitogenic and matrix synthetic actions of IGF-1 and TGF-β are synergistic in chondrosarcoma chondrocytes. However, the expressions of IGF-1, IGF-1R and TGF-β in chordomas have not been studied so far.
Design: Formalin-fixed, paraffin-embedded tissues from three patients with chordomas who underwent surgical resection were used in this study. All three patients were in the 8th decades. Histologically, all tumors are characterized by lobules and fibrous septa, and consisted of vacuolated round to oval cells with physaliphorous cells proliferated in the myxoid stroma. Immunohistochemical studies for IGF-1, IGF-1R and TGF-β on these tumors were performed with or without antigen retrieval. Appropriate positive and negative controls were run together with the cases.
Results: All three cases were positive for IGF-1 (cytoplasmic) and strongly positive for IGF-1R (cytoplasmic and membranous). TGF-β was expressed in two cases (cytoplasmic), and was not in one case.
Conclusion: These data may support the involvement of IGF-1 and IGF-1R in the growth of chordoma. The treatment of chordomas is difficult and wide surgical excision is desirable, but rarely feasible based on the anatomic location of the tumor. This is the first report showing IGF-1 and IGF-1R expression in chordomas, and may offer new targets for therapeutic intervention in the management of this tumor.

11 COMPUTERASSISTED MORPHOMETRY FOR THE GRADATION OF SYNOVITIS ACCORDING TO THE SYNOVITIS-SCORE
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Background: The so-called synovitis-score has recently been established as a histopathologic standard for the gradation of chronic synovitis of any origin. It is based on the semiquantiative evaluation of the three alterations that occur in any kind of synovitis: enlargement of the lining cell layer, lymphocytic inflammatory infiltrate and enhancement of the synovial stroma. We wanted to find out if computerassisted morphometry leads to the same inflammatory grades as examination by a pathologist.
Design: 70 synovial samples covering degenerative and rheumatic joint diseases (controls n=8, osteoarthritis n=26, psoriatic arthritis n=4, rheumatoid arthritis n=32) were evaluted by three pathologists and by morphometry according to the synovitis-score. The slide images were processed with a DXC-930P videocamera (Sony) and the Quantimet Q600 (Leica) software. The lining cell layer was measured interactively, the density of the inflammatory infiltrate and of the resident cells were counted in the R/G/B mode with a self-written program.
Results: When compared to manually counted lymphocytes and stroma cells, the morphometrically counted cell density was slightly overestimated (5%). In contrast, the mean inflammatory grades according to the morphometry were lower when compared to the pathologists' semiquantitative evaluation. Still, the computer assisted scoring discriminated the clinical diagnoses (higher values for rheumatic diseases, lower values for degenerative diseases), and it correlated with the pathologists' grading.
Conclusion: Computer assisted morphometry turned out to be a feasible tool for the quantification of cells. However, the differentiation between lymphocytes and stroma cells needs to be improved. The synovitis-score was validated by computer assisted morphometry, justifying its application in routine histopathology for the gradation of chronic synovitis.

12 FGF/FGFR2 SIGNALING MAY STIMULATE OSTEOBLAST DIFFERENTIATION BY INCREASING BMP2 EXPRESSION
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Background: Activating (gain of function) mutations of Fibroblast Growth Factor Receptor 2 (FGFR2) are associated with various types of craniosynostosis syndromes and limb deformities indicating that it plays a role in skeletal development and bone development.
Design: We generated transgenic mouse lines expressing activating or dominant negative (loss of function) mutations. The transgenes were driven with a bone-specific COL1A1 promoter so that the expression was limited to bone forming cells. Bone was examined by histology and micro-CT scanning. We also examined osteoblast marker gene expression using Northern blot analysis.
Results: Loss of FGFR2 affects bone formation by delaying osteoblast differentiation, producing a severe form of osteoporosis and defective vertebral development. Gain of FGFR2 function in osteoblasts causes premature ossification of cranial sutures by stimulating osteoblast differentiation. FGFR2 activation induces type II Runx2 (an essential transcription factor for osteoblast differentiation) and reduces the expression of Msx2 (a transcription factor known to inhibit osteoblast differentiation). FGFR2 activation enhances BMP signaling by increasing BMP2 expression and decreasing noggin expression.
Conclusion: This indicates that FGF/FGFR2 signaling has a critical role in osteoblast differentiation that may require activation of the BMP signaling pathway.

13 EXPRESSION OF RUNX2 AND INDIAN HEDGEHOG IN CARTILAGINOUS TUMORS
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Background: Runx2-Cbfa1, a Runt transcription factor, plays important roles during skeletal development. In its absence, chondrocyte hypertrophy is severely impaired and there is no vascularization of cartilage templates during skeletal development. Also, Indian hedgehog (Ihh) signaling molecules control the space and timing of chondrocyte differentiation.
Design: In order to gain a better understanding of the molecular process underlying development of chondrosarcoma, and to investigate whether there is a biological difference among variable types of chondrosarcomas, a series of 10 enchondromas and 57 chondrosarcomas (conventional, n = 17; mesenchymal, n = 20; clear cell, n = 20) was collected. We investigated the expression of Runx2 and Ihh in these cartilaginous tumors by immunohistochemistry. Cellular and matrix-rich areas were evaluated separately.
Results: Runx2 was expressed in 100% of conventional, mesenchymal, and clear cell chondrosarcomas compared to complete absence in enchondromas. Higher levels of expression were found in cellular areas than in matrix-rich areas. Expression levels increase with increasing histological grade in conventional chondrosarcoma, suggesting involvement in tumor progression. Ihh was expressed in 100% of conventional and clear cell chondrosarcomas, especially in matrix-rich areas. Mesenchymal chondrosarcomas revealed only focal expression of Ihh in matrix-rich areas. Small cell areas were negative. Ihh was absent or focally expressed in enchondromas.
Conclusion: These findings demonstrate that Runx2 expression is active in variable chondrosarcomas compared to enchondromas, confirming its importance in growth and differentiation of neoplastic cartilage. Ihh expression is considered as the hypertrophic stage of differentiation in these tumor cells.

14 PRIMARY OSSEOUS, TFE3 POSITIVE ALVEOLAR SOFT PART SARCOMA (ASPS) OF THE ILIUM: A CASE REPORT OF AN EXCEEDINGLY RARE PRESENTATION
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Background: ASPS is a very rare sarcoma of uncertain histogenesis, and it accounts for only 0.5 -1% of all soft tissue sarcomas. It most commonly affects the lower extremities and the head and neck region. While bony erosion at the primary site or bone involvement as a metastatic target is not uncommon, primary osseous ASPS is exceedingly rare with only 13 cases reported in the literature with the ilium described in only 2 occasions.
Material: We present a case of an immunohistochemically confirmed, TFE3 positive primary osseous alveolar soft part sarcoma in a 23-year-old female patient who presented with a one month history of back pain. Subsequent MRI investigation revealed an 11.0 cm, destructive, lobulated lytic mass without matrix production, epicentered in the right iliac crest. A total body PET scan did not show any other sites of increased tracer activity.
Results: An intraoperative open biopsy with a demand for a frozen section was performed with a clinical differential diagnosis of Ewing`s Sarcoma versus Lymphoma. Microscopically, the biopsy showed a hypervascular tumor arranged in nests, and alveolae lined by large granular to clear cells exhibiting round nuclei with well dispersed chromatin and macronucleoli. Mitotic activity and tumor necrosis were not present. The cytoplasm of the cells uniformly contained coarse, crystalline shaped, diastase resistant PAS granules. Immunohistochemically, the cells were non-reactive to Vimentin, CD34, CAM5.2, AE1/3, Myogenin, MSA, SMA, S100, Fli-1, Desmin and HMB45. TFE3 immunostain has recently been recognized to be highly sensitive and specific for ASPS. In our case, it tested strongly/diffusely positive with its characteristic nuclear labeling confirming the diagnosis. The patient was considered inoperable, and palliative chemotherapy was offered. Presently, she is well with no evidence of metastatic disease after a follow-up of 6 months.
Discussion: Although extremely rare and usually unexpected in a differential diagnosis of a soft tissue tumor and to a much lesser degree in the bone, the morphologic recognition of Alveolar Soft Part Sarcoma is usually immediate due to its unique histologic appearance. An immunohistochemical proof of histogenesis has not been successful due to the ongoing dilemma of its origin. Of all the attempted studies, the most celebrated but short lived theory was the myogenic differentiation attributed to the occasional expression of MyoD1.

Despite the unresolved histogenetic origin of ASPS, the molecular translocation has been successfully recognized resulting in der17t(X;17)(p11.2;q25) fusing TFE3 gene on the chromosome X with ASPL on chromosome 17. This fusion leads to the overexpression of TFE3 which can be detected with a commercially available antibody and its positive staining is considered diagnostic of ASPS. Our case not only demonstrates that ASPS can arise as a primary bone sarcoma, but also that TFE3 immunostain can be of extreme utility in confirming this diagnosis in a location where metastatic renal clear cell carcinoma or other mimics are more favored possibilities.

15 PULMONARY EMBOLISM IN LIMB SALVAGE PROCEDURE FOR OSTEOSARCOMA

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Background: Sarcomas rarely embolize to the lung. To date, twelve case reports of fatal pulmonary embolism from osteosarcoma have appeared in the literature. Five of these cases were chondroblastic type. We present an additional case involving a chondroblastic osteosarcoma occuring in a 15 year old male with embolism of the tumor occurring during a limb salvage procedure.

Design: Case report, discussion of pathophysiology and review of the literature.

Results: As in our case, diagnosis of pulmonary embolism in cases of osteosarcoma is usually made on post mortem examination. Our patient presented with a 10.5 x 10.5 x 7.5 mucoid lesion in the right distal femur. A biopsy showed a myxoid spindle cell tumor and a limb salvage procedure was performed. The patient had progressive respiratory distress, with onset shortly after surgery, eventually dying of right-sided heart failure. Pathologic examination of the resected distal femur revealed chondroblastic osteosarcoma diffusely infiltrating the marrow cavity. At autopsy, complete occlusion of the main as well as branches of the left and right pulmonary arteries by tumor emboli was found. The mechanism of seeding the lungs by tumor appears to be similar to that of fat and bone marrow embolism in trauma patients. Fat embolism syndrome (FES) is a known complication of long bone trauma and orthopedic surgery with intramedullary manipulation. Long bones of the lower extremities are responsible for 95% of the cases of FES. Factors deemed essential for the development of FES are: liquefaction of the fat; vascular injury; and a suitable pressure difference to force the liquefied material into the veins. In chondroblastic osteosarcoma, the tumor likely gains access to the pulmonary vasculature by the same mechanism. Microscopic tumor emboli become lodged in small pulmonary vessels, causing symptoms and signs of subacute cor pulmonale. Only 12 cases of osteosarcoma causing pulmonary embolism have been reported. In all cases, an intravascular growth of tumor in the lung was demonstrated. Cases have been reported in which, as in our case, the tumor produced complete obliteration of large and small pulmonary arteries. The majority of the cases have been females with the primary site being the right femur. Half of the cases, now, have been chondroblastic osteosarcoma. In a series of 222 autopsies, pulmonary tumor embolism was detected in 19 cases (8.5%). In another autopsy series, pulmonary tumor embolism was detected in 26% of cases, being a significant contributing factor in the death in 8.3% of the cases. Fatal pulmonary tumor embolism is, thus, an unusual phenomenon. Early diagnosis requires a high index of suspicion.

Conclusion: Limb salvage surgery for osteosarcoma carries a high risk of tumor embolism with potentially fatal consequences.

16 THE EXPRESSION OF MATRIX METALLOPROTEINASE-9 AND TUMOR ANGIOGENESIS IN HUMAN OSTEOSARCOMA

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Background: Matrix metalloproteinase-9 (MMP-9) is a matrix-degrading enzyme that's believed to play a crucial role not only for tumor invasion and metastasis, but also for a variety of stromal reactions, including neovascularization. The aim of this study was to investigate the expression of MMP-9 and to compare its expression with the angiogenesis activity in human osteosarcoma.

Design: Archival tumor tissue samples from 20 patients with osteosarcoma were analyzed by performing immunohistochemistry for the expression of MMP-9 and CD34. The vascularity was measured as the average microvascular density (MVD) of the CD34-positive vessels. The clinical information was obtained through searching the computerized retrospective database from the tumor registry.

Results: MMP-9 was expressed in 90% (18/20) of the tumors we examined. The MVD ranged from 10.5 to 179.7 with a mean of 64.9. There was no significant correlation between the MMP-9 expression and the MVD (p=.613). The MMP-9 expression was not associated with any of the clinicopathologic variables, whereas the MVD showed an increasing tendency according to the metastasis status (p=.073).

Conclusion: We demonstrated that MMP-9 activation is likely to occur in human osteosarcoma. However, there was no direct involvement of MMP-9 with tumor angiogenesis. It is noteworthy that MVD may aid physicians to predict the presence of distant metastasis in osteosarcoma patients.

Breast

17 DIFFERENTIAL DIAGNOSIS OF SPINDLE CELL TUMORS OF THE BREAST: REPORT OF EIGHT CASES

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Background: Spindle cell breast tumours, although rare, represent a heterogeneous group with different treatment modalities. The aim of this study was to evaluate the utility of immunohistochemistry in differentiating breast spindle cell lesions.

Design: Fine needle aspiration cytology (FNAC) of eight breast masses diagnosed cytologically as benign, suspicious or malignant spindle cell tumours was followed by excision biopsy and subjected to immunostaining using a panel of antibodies including pan cytokeratin, smooth muscle actin, desmin, CD34, s100 protein, and CD10 to define their nature.

Results: Out of the eight cases, five were benign and three malignant. Of the benign tumors, one turned out to be leiomyoma, one fibromatosis, one spindle cell variant of adenomyoepithelioma, one myofibroblastoma and one spindle cell lipoma. The malignant breast spindle cell tumors were leiomyosarcoma, spindle cell carcinoma and malignant myoepithelioma.

Conclusion: Three important conclusions are drawn from this study: First, FNAC in spindle cell breast lesions is not sufficient and should be followed by wide excision or wide biopsy. Second, inadequate sampling and processing of the specimen is insufficient to differentiate benign from malignant spindle cell lesions. And, lastly, immunostaining is of value in benign lesions and is mandatory in the malignant ones to result in optimal treatment modalities.

18 THE INVERSE LEVELS OF EXPRESSION OF CD44S AND HYALURONAN ACROSS THE SPECTRUM OF NORMAL-HYPERPLASIA-CARCINOMA IN BREAST

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Background: The interaction between epithelial tumor cells and their surrounding stroma, mediated via transmembrane receptors such as CD44 and the extracellular matrix hyaluronan (HA), is important in tumor progression and metastasis. The purpose of this study was to evaluate the expression of HA and CD44s in normal, benign, hyperplastic and malignant breast epithelium.

Design: Archival paraffin-embedded cellblocks from normal breast tissue (10 cases), ductal hyperplasia (DH, 13), ductal carcinoma in-situ (DCIS, 12), infiltrating ductal carcinoma (IDCa, 8 stage I and 9 stage II) and their corresponding positive lymph nodes (9), and infiltrating lobular carcinoma (ILCa, 3) specimens were retrieved from the surgical pathology files. All cases were stained for CD44s (1:1000, Bender MedSystems, CA) and HA using a highly specific HA binding peptide (1:2000, Seikagaku Corp, MI). Positive staining was defined as uniform membranous staining for CD44 and droplet to diffuse intracytoplasmic or extracellular staining for HA. Staining was classified according to its distribution (rare, focal, and diffuse) and intensity (1+, 2+, 3+).

Results: The distribution and intensity of stromal HA staining were greatest in infiltrating carcinomas (diffuse and 2+ to 3+ in 88% of the cases) and were particularly pronounced surrounding the metastatic deposits in lymph nodes compared to normal and benign lesions (focal and 0 to 1+ in 92% of cases). Stromal cells contain HA in 20% (2/10) of normal, 29% (4/14) of DH, 50% (6/12) of DCIS, 88% (7/8) of IDCa Stage I, 89% (8/9) of IDCa Stage II, and 0% (0/3) of ILCa. The epithelial component was devoid of HA in all cases. In contrast, the expression of CD44s in breast epithelium progressively decreased with increasing deviation from normal histology. Epithelial cells expressed CD44s in 100% (10/10) of normal, 100% (13/13) of DH, 83% (10/12) of DCIS, 75% (6/8) of IDCa Stage I, 33% (3/9) of IDCa Stage II, and 0% (0/3) of ILCa. Stromal cells expressed CD44s only in normal and DH but not in any other lesions.

Conclusion: This study demonstrates an inverse relation in the expression of CD44s and hyaluronan as the histology progresses along the spectrum of normal-hyperplasia-carcinoma. Thus, HA and CD44 are both involved in the development and progression of breast cancer.

19 HISTOPATHOLOGICAL FEATURES AND TISSUE MICROARRAY EXPRESSION PROFILE OF BREAST CARCINOMA IN YOUNG WOMEN

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Background: Breast cancer is relatively uncommon in young women. It has been suggested to have more virulent form of disease compared to that seen in older women. The aim of this study is to compare the histopathological features, immunohistochemical expression profile, and clustering analysis by using tissue microarry of breast cancer in these two subsets of women.

Design: The study population consisted of 80 breast cancer's patients in which 13 patients were 35 year-old or younger and 67 patients older than 35 years at the time of diagnosis. The patients were treated at our institutions either by lumpectomy or modified radical mastectomy. Slides were reviewed and the diagnosis confirmed, tumors graded according to Scarff-Bloom-Richardson grading system, and tumor histologic type assessed. Tumor size and lymph nodes status were recorded. A tissue microarray consisting of duplicate 0.6 mm cores of tumor was constructed and serial sections were immunostained with a panel of six antibodies (ER, PR, Her2/neu, p53, E-Cadherin, and CK5/6). The immunostain scoring results were recorded and analysed using Hierarchal clustering analysis. The histopathological features, immunohistochemical expression profile, and clustering results of the two subsets of patients were statistically analysed. Correlation between clustering result and pathological features was examined by either Chi-square or Fisher's exact test. Multivariate analysis was performed using Cox's proportional hazards method.

Results: The two subsets of patients correlated significantly with the tumor grade (p=0.004), ER (p=0.04), PR (p=0.04), Her2/Neu (p=0.018), and p53 (p=0.01) immunoexpresssion. There were no statistical significance of the two subsets seen for other histopathological features (tumor type, tumor size, lymph node status, and lymphovascular invasion) or the immunostain of E-Cadherin, and CK5/6. The clustering analysis divided the tumors into 3 clusters based on the relatedness of their expression profile. A small cluster group consisted predominantly of young patients (62%) has significantly high-grade tumor (p=0.04), more likely larger tumor (p=0.06), and positive nodes (p=0.07).

Conclusion: Breast carcinomas in young women are more likely to have aggressive morphology, ER negative, PR negative, Her2/Neu positive, and p53 positive immunostains, as compared to older women. Furthermore, two thirds of young women fall in the small