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PLATFORM and POSTER PRESENTATIONS

Autopsy

1 The Autopsy Findings of Ten Cases with Meckel Gruber Syndrome: A Prospective Study

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Background: Meckel Gruber syndrome (MGS) characterized by the cystic renal dysplasia, occipital encephalocel, and postaxial polydactyly. Due to the recurrence risk of 25%, it deserves an exact diagnosis. Although a confident diagnosis is possible only when the syndrome recurs in subsequent pregnancies, the Meckel Gruber syndrome can usually be suggested on the basis of characteristic pathologic findings and a normal karyotype. This emphasizes the importance of prenatal sonography and a careful postmortem examination to establish the correct diagnosis.

Design: In a 6-year long prospective study, we examined fetal autopsy findings in ten fetuses with MGS out of 118 second-trimester termination of pregnancy cases due to fetal malformation diagnosed by second trimester-ultrasound examination at a tertiary referral center.

Results: Ten fetuses with MGS were analyzed. Nine cases had classical clinic triad. One case had only cystic renal dysplasia and polydactyly. Occipital encephalocel was present in nine cases and in one of them there were combine anomalies with encephalocel and Dandy Walker syndrome. The renal malformations characterized by a bilateral, symmetrical enlargement of the kidneys with abdominal distension and lung compression. The renal parenchyma was diffusely cystic throughout the cortex and the medulla. It contained small and medium-sized, thin-walled cysts that varied a great deal in diameter. Five cases showed ductal plate malformation of the liver with a variable degree of dilatation of the primitive biliary structures. Additional anomalies included cleft plate (n=1), micrognatti (n=1), hypoplastic left ventricule (n=1) , placental hemangioma (n=1), and clubfoot (n=1).

Conclusions: Autopsy provides valuable differential diagnostic information and can be used to validate obstetric management and to evaluate the recurrence risk in future pregnancies in MGS. The differential diagnosis of MGS should include trisomy 13, Zellweger syndrome, Smith Lemli-Opitz Syndrome, Agostino syndrome and Jeune syndrome. Morphological, karyotypic and genetic analyses are necessary for differentiation. If histological examination of the kidneys and liver are performed, the diagnosis of MGS can be made. Cases with MGS, parents should be counseled for their further pregnancy, that they have a risk of 25% recurrence, due to its autosomal recessive transmission nature, and an early targeted sonograph should be advised in future pregnancies.

2 A Pathology Department-Based Decedent Affairs Office Can Contribute to Improved Organ and Tissue Donation Services

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Background: Organ and tissue donation is championed by many stake-holders at Boston Medical Center (BMC). Recent changes in leadership resulted in necessary refocusing of the collaboration between BMC and the New England Organ Bank (NEOB). The pathology department-based decedent affairs office (DAO) was invited to participate as a liaison to the NEOB on behalf of the hospital. The mutually stated goals of BMC and the NEOB were to increase the hospital's donation rate to the national level. The aim of our present study is to report on DAO-supported process improvements for organ and tissue donation.

Design: In a 12 month period we adopted a multifaceted approach to raising awareness of the need to improve tissue and organ donation at BMC and the required steps to accomplish this. The NEOB coordinator provided a dashboard for data collection, continuing education and leadership. The DAO liaison communicated with executive administrators (BMC and NEOB), physician, nursing and support staff. Department and committee minutes were reviewed to determine activity and provide statistics.

Results: Results illustrating DAO contributions to improvements for tissue donation include: a tissue donor conversion rate from 11 to19% (n=246); timeliness of referral (within 1 hr of death) from 31 to 47%; availability of next-of-kin information from 84 to 87%; availability of blood sample for tissue typing from 91 to 94%. Furthermore, in collaboration with nursing leadership, access to operating rooms for tissue recovery was set at 100%. Contributions to improved organ procurement include: establishment of the Steven Laramee Organ and Tissue Donation Committee; quarterly dash-board reporting to nursing, critical care and emergency department executive committees; revision of hospital policies for organ and tissue donation and, continuing education for hospital staff.

Conclusions: The DAO contributed to improved communication between BMC and the NEOB. Tissue donor conversion rates have grown. There exists an opportunity for pathology departments to play an active role in improving organ and tissue procurement. While serving as a liaison to organ and tissue procurement organizations does not normally lie within the purview of a pathology department, an active DAO is uniquely positioned to expand its role beyond the standard boundaries of pathology services, in the context of broader hospital administration.

3 Renal Tubular Dysgenesis, Neonatal Hemachromatosis and Left Coronary Artery Right Ventricular Outflow Track Fistula: The First Case Report

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Background: Renal tubular dysgenesis (RTD) is characterized by absence of proximal renal tubules and late gestational oligohydramniosis leading to the Potter sequence. Neonatal hemachromatosis (NH), a cause of infantile liver failure, is a gestational condition with liver and extrahepatic iron accumulation. To date, only five cases with combined RTD and NH have been reported, none with a coronary artery ventricular fistula.

Design: A male infant was delivered spontaneously at 35 weeks gestation after discovery of late gestational oligohydramniosis. Workup suggested a possible coronary artery to right ventricular outflow tract fistula and liver lesions. The infant's clinical course was characterized by anemia, thrombocytopenia, coagulopathy, anuria, and persistent respiratory distress leading to his death on day four of life.

Results: Autopsy revealed generalized edema, jaundice, and dysmorphic features consistent with the oligohydramniosis sequence. The 40 gm liver was atrophic. Microscopic examination revealed diffuse hepatocellular loss with lobular collapse, cholestasis and early fibrosis. Iron staining revealed abundant iron within hepatocytes and bile duct epithelium, as well as pancreatic acini and thyroid. The reticuloendothelial system was spared. Although grossly unremarkable, the kidneys revealed near complete absence of proximal renal tubules that was further confirmed by lack of PAS staining and a of lack tubules staining for CD10. The presence of distal tubules, loops of Henle, and collecting ducts was confirmed histologically and by the presence of positive staining for EMA, CD15, and A-AT. Step sections confirmed the presence of a left anterior descending coronary artery to right ventricular outflow tract fistula.

Conclusions: We present the sixth case with combined RTD and NH, and the first with an associated left coronary artery-right ventricular fistula. Although a variety of other abnormalities have been associated with either RTD or NH, none have been reported with cardiovascular malformations. While the etiology and pathogenesis of RTD and NH remain unknown, our case does not provide support for the hypothesis of fetal hypoxemia. A mechanism connecting growth factors relating to renal cardiovascular development with disordered fetal iron metabolism is probably more likely.

4 Determination of Gestational Age and Sex by Histology of Fetal Gonads

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Background: The histology of fetal lungs and kidneys has long been used to assess fetal gestational age at autopsy. There are five phases of lung development during gestation and four periods of nephrogenesis that can be used to date the gestational age to within weeks. Up until the 6th week of gestation, the fetal gonads are indifferent. Thereafter, the gonads differentiate into ovaries or testes with distinct, temporally-related histologic changes throughout gestation. However, fine week-to-week details of these changes have not been well documented. It is proposed that the histology of fetal gonads from the autopsy will be equally effective or contributory in determining gestational age, as compared to lung and kidney, and will also allow for sex determination.

Design: Prepared slides of lung, kidney and gonadal tissue from fetal autopsies were reviewed, ranging from gestational age of eight to thirty-nine weeks. A total of 150 cases were obtained from years 1995 to 2008 at our institution. Lungs, kidneys and gonadal tissue were examined blindly and then compared to given gestational age (calculated from last menstrual period). Sex of the fetus was determined from the examined gonadal tissue and then compared to cytogenetics studies performed at the time of autopsy.

Results: The analysis of 150 fetal autopsies demonstrated that gonad development has distinct morphological features during gestation that allow examiners to determine a

gestational age with 99% agreement with clinical gestational age, determined from last menstrual period. Sex determination by gonadal histology correlated with cytogenetic studies with 100% agreement.

Conclusions: Discrete patterns of gonadal development during gestation can be divided into separate stages and subtle differences can be used to date a pregnancy to within weeks. These findings are a valuable resource for pathologists, to be able to correctly determine the age of the fetus and correlate with clinical pathological findings. It was found that fetal gonads are equally useful for gestational dating, as compared to the lungs and kidneys, and can be used for sex determination as well.

5 Autopsy Findings in Hospitalized Renal Transplant Recipients

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Background: Graft and patient survival has greatly improved over the last few years due to immune modulation. Deaths in these patients are commonly related to the severity of immunosuppression. Our aim in this study was to determine the principal cause(s) of death based on post-mortem findings in kidney transplant patients.

Design: Autopsy records of Hahnemann University Hospital were reviewed from 1999 to 2008. 20 renal and simultaneous renal-pancreas transplant autopsies were retrieved. The cases were classified according to cause of death, post-transplant time, and histologic findings in the transplanted kidney at autopsy.

Results: The mean range of the patients ranged from 34-82 years. The most common indications for the transplant were hypertension and diabetes mellitus.

Table 1: Cause of death in hospitalized renal transplant recipients

Cause of death		Time post-transplant (Range)	Number of cases
Sepsis		3 wks-10 yrs	9/20
Hemorrhage	1. S/p renal procedure	1-4 yrs	3/20
	2. Ruptured allograft	5 days	1/20
	3.Hemorrhagic pancreatitis	5 wks	1/20
	4. S/p non-renal procedure	2 yrs	1/20
Sudden cardiac death		48 hrs-4 yrs	2/20
Coagulopathy	1. Disseminated Intravascular Coagulation	24 hrs	1/20
	2. Metastatic pancreatic carcinoma	3 yrs	1/20
	3. Cerebrovascular accident	9 yrs	1/20

Conclusions: In our study, hemorrhagic complications were the second most common cause of death in renal transplant patients, after sepsis. The most common findings in the transplanted kidney were acute tubular injury/acute tubular necrosis, with no evidence of cellular rejection.

6 Liquefaction ("Caseous Calcification") of Calcified Mitral Annulus: A Great Mimicker. Report of Two Cases

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Background: Calcification of the mitral annulus (CMA) occurs in about 9% of autopsied patients over the age of 50 years, being more common in females and the incidence increases with age. Conditions producing increased LV pressure and mitral valve stress increase the incidence of CMA and so too does abnormal calcium-phosphate metabolism in chronic renal failure.

Design: Case reports: We report two patients with liquefaction of a calcified mitral annulus. Case 1: This 69 year old woman with hypertension and hyper-lipidemia, status post coronary bypass grafting and stent placement was found to have a mitral valve mass that appeared to be growing in size over time. She also suffered from renal failure and had received prolonged peritoneal dialysis prior to undergoing renal transplantation. The patient was operated upon for suspected infective endocarditis and underwent mitral valve replacement. The mitral valve was resected together with a friable tan yellow mass measuring about 1.5 cm in diameter situated behind the posterior leaflet and attached to the mitral annulus. Incision of the mass revealed tooth paste like, caseous looking material. Histology showed amorphous debris with finely dispersed calcific fragments consistent with liquefaction of a previously calcified mitral annulus. The pseudo-vegetation comprised an excrescence of the annular calcification that had hung down behind the posterior mitral leaflet. Case 2: Liquefied calcification of the mitral annulus was an incidental finding at autopsy in a 72 year old woman with end stage renal disease who had received hemodialysis and died of pulmonary infection. Initially, at gross examination of the heart the pathologist was concerned that infection of the CMA had occurred, but histology ruled that out.

Conclusions: Liquefaction of CMA is a rare, under recognized entity that may lead to unnecessary cardiac surgery (as in our case 1 and others in the literature) for misdiagnoses of abscess or tumor. Since the mitral annulus is C-shaped with no ring for the anterior leaflet, a characteristic echocardiographic appearance of a soft, echo-dense mass containing central areas of echolucencies affecting the periannular region of the posterior mitral leaflet should strongly suggest the presence of liquefaction of CMA on 2D echocardiography.

7 Sudden Postpartum Death: An Unexpected Complication of Neurofibromatosis

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Background: Neurofibromatosis type 1 (NF1) is associated with vascular abnormalities including stenosis, aneurysms, and vessel wall dysplasia, sometimes resulting in vessel rupture. NF1 has also been associated with spontaneous hemothorax and sudden death, and spontaneous hemothorax has been reported in pregnant patients with NF1. Furthermore, neurofibromas in patients with NF1 have been noted to increase in size during pregnancy. We present a case of NF1 resulting in maternal mortality secondary to mediastinal vessel rupture and subsequent massive hemothorax in the early post-partum period.

Design: Autopsy was performed with internal gross and microscopic examination of the chest.

Results: A 26-year-old with a known history NF1 delivered a healthy male infant following an uneventful pregnancy and labor course remarkable only for mild persistent elevation of the systolic blood pressure. At 27 hours postpartum, the patient complained of left sided pleuritic chest pain, exhibited signs of shock, and died despite extensive resuscitative efforts. Autopsy revealed left hemothorax, causing marked compression of the left lung. The middle and posterior mediastinum contained hemorrhage, and a ruptured arterial aneurysm was located in the mediastinum. The wall of the affected artery was focally necrotic. Adjacent to and completely surrounding the ruptured artery and nearby vessels and nerves was a diffuse neurofibroma (vascular neurofibromatosis) as confirmed by immunohistochemical studies. Granulation tissue was noted at the site of the rupture.

Conclusions: The patient in this report died from a sudden massive hemothorax and associated repiratory compromise and hemorrhagic shock. The cause of the hemmorhage was rupture of an arterial aneurysm in the mediastinum. The aneurysm apparently formed due to ischemic necrosis of the arterial wall, secondary to encasement of the artery by a diffuse neurofibroma. Granulation tissue at the site of rupture indicates that the initial hemorrhage occured between 1 and 7 days prior to death, and the patient's rapid demise occured after the initial, relatively slow, mediastinal hemorrhage extended into the pleural cavity and rapidly accelerated.

8 Left Ventricular Hypertrophy Is a Common Cause of Sudden Unexpected Death in African-Americans

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Background: The effect of race on the frequency of arrhythmogenic substrates in hypertensive atherosclerotic cardiovascular disease (HASCVD) has not been extensively studied.

Design: All hearts from adult sudden deaths in 2007 were studied if the cause of death after forensic autopsy was cardiac, included features of HASCVD, and excluded specific cardiomyopathy, myocarditis or valve disease. Myocardial scarring was described grossly; cardiomegaly defined by heart weight, body weight and height, and ventricular wall thickness; and severe coronary disease defined as >75% luminal narrowing of at least one epicardial artery. Coronary disease was considered the single mechanism of death if there was an acute thrombus or severe disease in the absence of scars and cardiomegaly.

Results: 406 cases comprising 157 blacks, 249 whites, 119 women, and 287 men were examined. Mean age was 49±13 years in whites and 47±13 in blacks (p=0.2). Coronary atherosclerosis was the single mechanism of death in 27% of whites and 22% of blacks, with acute thrombi present in 48% and 36%, respectively (p=.08). Severe atherosclerosis was present in 58% of whites and 45% of blacks (p=.1), but extent of disease was less in blacks (p=.004). Cardiomegaly was the single mechanism of death in 38% of whites and 50% of blacks (p=.01); a history of hypertension was known in 48% (40% of whites and 57% of blacks). Cardiomegaly was present in 73% of both blacks and whites. Old scar was the primary cause of death in <3% and was present with other substrates in 18% of whites and 11% of blacks. Multiple substrates were present in 32% of whites and 24% of blacks. A history of alcoholism was more frequent in isolated cardiomegaly than coronary disease (11% vs. 1%, p=.001). Deaths were exertional in 2% of isolated cardiomegaly deaths vs. 5% of isolated atherosclerosis (p=0.3). By multivariate analysis, black race (p=.01) was inversely related to extent of coronary disease, independent of age, gender, and risk factors, and positively related to isolated cardiomegaly as a cause of death (p=0.04).

Conclusions: In sudden cardiac death due to HASCVD extent of coronary disease is less in blacks and isolated cardiomegaly more frequent in whites. Cardiomegaly is as important a cause of sudden cardiac death in African-Americans as coronary atherosclerosis.

9 Congenital Stenosing Arteriopathy Is Part of the Spectrum of Williams Syndrome: Case Report and Literature Review

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Background: Congenital stenosing arteriopathy (CSA), (originally termed "macaroni arteries") is a rare form of arteriopathy affecting the aorta and its major branches. All six previously reported cases have been autopsied females and in the pathologic literature the condition has been regarded as uniformly fatal in early life (all died under 30 months of age). Williams syndrome is defined as supravalvular aortic stenosis and mental retardation associated with typical facies in a child aged 1-15 months at time of diagnosis. Peripheral arteriopathy and pulmonary artery stenosis are associated.

Design: We report the autopsy case of an infant with Williams syndrome (confirmed by FISH during life) that showed features of CSA.

Results: The deceased patient was a one month old female with Williams syndrome (FISH positive testing) with clinical aortic valve stenosis, supra-valvular aortic stenosis, narrowed main pulmonary artery and decreased left ventricular function. The mitral valve showed moderate insufficiency. Cardiac catheterization led to cardiac arrest and two successful resuscitations. However, the following morning she died of cardiac electromechanical dissociation. Autopsy revealed diffuse thickening of the aorta throughout its length due to excessive number of lamellar units (112 instead of about 60). The major aortic arch branches were similarly thickened by excessive lamellar units and the coronary arteries showed medial smooth muscle excess. The pulmonary artery shared in the excess of lamellar units and also showed disarray of the outer lamellar units. The features observed in this patient are identical to those described in congenital stenosing arteriopathy.

Conclusions: Autopsy experience with CSA has led to an artificial separation of this subset of patients from other patients with Williams syndrome and has also led

pathologists to erroneously believe that the condition is uniformly fatal in early life. Pediatric cardiologists (JHM, personal communication) have encountered teenage patients with Williams syndrome showing diffuse aortic narrowing. Our present autopsy case with FISH positive confirmation of Williams syndrome indicates that congenital stenosing arteriopathy may form part of the spectrum of Williams syndrome. Prospective cases of congenital stenosing arteriopathy as well as those previously published should be tested for Williams syndrome by FISH.

10 Post-Mortem Diagnosis of Lymphoma: Report of Five Cases with Review of the Literature and Discussion of Specialized Diagnostic Techniques

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Background: Lymphomas are often characterized by non-specific clinical presentations that can lead to misdiagnoses. Rapidly lethal cases of lymphoma may not be discovered during life, leaving only post-mortem examination as a possible source of correct diagnosis. We report a series of cases of primary post-mortem lymphoma, review the previously reported cases and discuss the use of special techniques in post-mortem studies.

Design: A retrospective review of 1942 autopsy cases performed at our institution over a period of 20 years yielded 5 cases with an autopsy diagnosis of lymphoma and a clinical diagnosis other than lymphoma. For these cases, autopsy reports including summary of ante-mortem course, hematoxylin and eosin (H&E) stained sections, immunohistochemical (IHC) stains and results of molecular techniques in one case were reviewed. A literature search was conducted for similar autopsy cases.

Results: The 5 cases included 3 males and 2 females, age ranging from 53 to 81 years. Antemortem clinical diagnoses included portal mass, cardiorespiratory failure, pneumonia, acute pancreatitis and one found dead. One case had an erroneous pre-mortem diagnosis of adenocarcinoma. H&E sections revealed mononuclear cell infiltrates suspicious for lymphoma in all cases. One case had involvement of the head of the pancreas; all other cases (4) had multiple organ involvement. Variable autolysis was seen in all cases that hindered morphologic evaluation. Antigen preservation was acceptable when IHC were performed. Final autopsy diagnoses included 2 peripheral T-cell lymphomas (CD3+, CD20-), 1 anaplastic large cell lymphoma (CD3-, CD20-, CD30+, CD45+/-), 1 diffuse and nodular large cell lymphoma (CD20+, CD3-), and 1 small cell lymphocytic lymphoma (morphology only). In 1 case of peripheral T- cell lymphoma PCR revealed T-cell gene rearrangement confirming the diagnosis.

Conclusions: In none of our cases was lymphoma clinically suspected prior to death. At least 3 published cases were similar to ours; additional published cases were clinically suspected but diagnosed at autopsy. Our series represents successful diagnosis and accurate classification of lymphoma at autopsy based on morphology (cytology and architecture) and ancillary techniques. While accurate post-mortem diagnosis may not benefit the patient, it can be invaluable for relatives wishing to know their own risk or for recipients of transplanted organs.

11 Catastrophic Antiphospholipid Syndrome (CAPS) Presenting in a Young Child with Trisomy 21

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Background: Antiphopholipid syndrome (APS) is an acquired thrombophilic disorder involving autoantibodies against phospholipids (PL) and PL-binding proteins. APS is usually associated with autoimmune disorders, and its frequency is increased in patients with trisomy 21. Catastrophic APS (CAPS) is a rare accelerated form of APS that leads to acute multi-system organ failure, with extensive microscopic thrombosis in multiple organs. First described in 1992, CAPS represents less than 1% of APS cases, but has a mortality rate of 50%. The mean age at presentation is 37 years, with the youngest reported case occurring in a 7 year old child.

Design: We report the autopsy findings of a unique case of CAPS in a 2 year old child with trisomy 21, presenting after an insidious onset of rash. The case met all major criteria for CAPS including: involvement of 3 or more organs systems, manifestations occurring within a week, positive serology for anti-PL antibodies, and microscopic evidence of thrombosis in at least 1 organ.

Results: A two year old girl with trisomy 21 and a past medical history including surgically treated congenital heart disease presented with a skin rash that was initially thought to be a drug hypersensitivity reaction, but with subsequent rapid development of abdominal distention and livedo reticularis. A skin biopsy revealed an ischemic epidermis, with dermal hemorrhage and microthrombi. Pneumatosis intestinalis and frank air within the portal vein accompanied the bowel distention and two subsequent intestinal resections revealed fulminant ischemic enterocolitis with micothrombi in mesenteric vessels. An extensive rheumatologic work-up revealed lupus anticoagulant, anticardiolipin antibodies, and hypocomplementemia. Despite aggressive therapy, the patient expired and an autopsy was permitted. Grossly, there were extensive areas of skin breakdown simulating Steven-Johnson's reaction. The remaining intestines and spleen had multiple infarcts without grossly evident thrombi. Microscopically, several organs including intestines, skin, heart, and spleen, had marked congestion and microthrombi. Immunohistochemistry for C4d revealed extensive complement deposition within the blood vessels.

Conclusions: CAPS is a rare entity with only a few published cases. The pathogenesis is thought to involve massive activation of complement, as demonstrated in this case. This is the first reported case of CAPS occurring in a young child and associated with trisomy 21. The relationship between them still remains to be established.

12 Community-Acquired Methicillin-Resistant *Staphylococcus aureus* (MRSA) Sepsis in a 29 Week Neonate

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Background: Community-acquired Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections are becoming an increasing cause of significant morbidity and mortality among neonatal intensive care unit (NICU) patients. We present a case of disseminated MRSA sepsis resulting in the death of a 29 week premature infant.

Results: This 29 week infant, born to a 28 year old gravida 2 para 2 with necrotizing MRSA pneumonia and active skin lesions, was found to have disseminated MRSA sepsis at autopsy, following a sudden clinical decline. The mother was a poorly compliant insulin-dependent diabetic and a polysubstance abuser, including intravenous drugs. The infant's initial NICU course was relatively unremarkable. Her initial blood cultures were negative. On day of life (DOL) 4, she developed erythematous papulo-vesicular lesions on her right hand, arm, and leg, possibly reflecting herpes simplex virus (HSV) infection. Acyclovir was begun intravenously and administered for two days until HSV polymerase chain reaction (PCR) of the lesions returned negative. On DOL 9, the infant began having significant desaturations, tachycardia followed by bradycardia, and a metabolic acidosis resistant to bicarbonate. The infant died shortly after being taken off of life support. Blood, respiratory, and cerebrospinal fluid cultures taken before death were positive for MRSA. Gross and microscopic findings at autopsy confirmed disseminated sepsis with multiorgan abscess formation. The gross findings included multiple eroded papules over the chest, abdomen, limbs, and vulva. Examination of internal organs revealed abscess formation throughout the lungs and liver. Significant findings microscopically included abscess formation in the dermis, lungs, liver, spleen, heart, right kidney, and central nervous system. Thymic involution was present, as well as, a transverse growth arrest line of the costochondral junction, both signs of systemic stress. Cultures of the liver and spleen taken at autopsy were positive for MRSA.

Conclusions: In summary, this case represents a premature infant with MRSA sepsis who had an unremarkable initial newborn course and negative initial blood cultures. The mother was found to have multiple skin lesions and necrotizing MRSA pneumonia at the time of delivery. The rapid progression to death indicates the significant morbidity MRSA poses to premature infants.

13 Pathology of Hypertrophic Cardiomyopathy Leading to Congestive Heart Failure

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Background: Hypertrophic cardiomyopathy (HCM) is a recognized cause of sudden death in some patients with HCM. Recent studies indicate a better long term prognosis. Its association with left ventricular dilation and congestive heart failure (CHF) in 3.5-15% of longer surviving subjects with HCM is less widely appreciated. The mechanism of production of CHF in HCM is unknown.

Design: We studied the explanted hearts of two patients with HCM who received cardiac transplants for CHF. The patients' medical records as well as the hearts and glass slides and reports were reviewed. Note was made of the duration of symptoms of CHF, left ventricular ejection fraction (LVEF), heart weight, presence of asymmetric septal hypertrophy (ASH) (ratio of VS to LVFW), presence of mirror image plaque in LV outflow tract, mean diameter of mid-portion of LV cavity (two measurements at right angles to each other: antero-posterior and lateral), presence and severity of LV replacement fibrosis (0 = no fibrosis; + = mild fibrosis, affecting < 10% of the myocardium, ++ = moderate, 10-20% and +++ = severe fibrosis, >20%); and the presence of small coronary artery disease (SCAD). The pathology was reviewed with reference to establishing a cause for the onset of CHF. Two randomly selected autopsy patients with HCM without CHF (aged 61 and 70 years) served as histological controls.

Results: Both hearts (Table) showed a severe degree of replacement fibrosis of the LV myocardium as well as SCAD. Patient 1 (24 mths symptoms of CHF) showed fibrosis of about 50% of the left ventricular myocardium and patient 2 (1 mth symptoms of CHF) showed 15% fibrosis. The fibrosis evolves via from ischemia induced myocyte vacuolation and myocytolysis. The controls both showed grade 1+ replacement fibrosis with heart weights of 820 gm and 460 gm, respectively. One out of two (1/2) controls showed SCAD; neither control showed ASH.

Table

Age (year), Sex	LVEF	Heart Weight (gm)	Asymmetric Septal Hypertrophy (ratio)	Mirror Image Plaque	LV Cavity Diameter (mean, cm)	LV Fibrosis	Small Coronary Disease
Patient 1, 20, F	15%	441	Yes (1.5)	No	2.1	+++	Yes
Patient 2, 28, F	35%	500	Yes (1.7)	No	2.5	++	Yes

Conclusions: Replacement fibrosis of hypertrophied myocardium associated with dysplastic SCAD appears to be the basis for the onset of ventricular systolic dysfunction that is imperfectly compensated for by ventricular dilatation. The young age of both of the patients is noteworthy and indicates that heart failure may occur at a relatively early stage of the course of HCM and without severe LV dilatation.

14 Death Following Endoscopic Retrograde Cholangiopancreatography: Findings at Autopsy

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Background: Over half a million endoscopic retrograde cholangiopancreatography (ERCP) procedures are performed annually in the US. The risk of severe complications is less than 1%. Autopsy pathologists see a select group of patients having fatal complications of ERCP. This presents a unique opportunity to investigate pathologic findings and possible risk factors in these patients.

Design: Thirty-five autopsies were performed after ERCP over a 13-year period. Detailed procedure notes were available for 27 patients and 38 ERCPs. Clinical history, indication for ERCP, details of the procedures, ERCP complications, and autopsy findings were

recorded. A standardized scale was used to rate technical complexity of ERCP from 1 (simple diagnostic) to 5 (very advanced).
Results: Fourteen of 35 patients died of complications related to ERCP. Fatal complications included acute pancreatitis (7), sepsis (5), gastrointestinal perforation (3), bleeding (2), and myocardial infarction (2). Non-ERCP-related deaths were most often attributed to complications of cancer (14) or chronic pancreatitis (4). Mean times to death after ERCP in ERCP versus non-ERCP-related deaths were 15 and 70 days. The most common indications for ERCP in ERCP-related deaths were suspected choledocholithiasis (50%) and jaundice/biliary obstruction (21%); in non-ERCP-related deaths, jaundice/biliary obstruction (57%) and chronic pancreatitis (33%) were more common. Patients with ERCP-related deaths had more cannulations reported as "difficult" (69% vs 20%, p = 0.003), less commonly had pancreatic stents placed (18% vs 50%, p = 0.09), had fewer ERCPs (% with single ERCP 79% vs 48%, p = 0.07), and more often had gallbladders (79% vs 52%, p = 0.12). Technical complexity was similar between groups.
Conclusions: Death is a rare complication of ERCP and occurs most commonly due to pancreatitis or sepsis. Autopsied ERCP-related deaths tend to occur in patients undergoing first ERCP for suspected choledocholithiasis who have difficult common bile duct cannulation and do not have pancreatic stents placed during the procedure. Patients dying of causes unrelated to ERCP tend to have had multiple ERCPs for mass related biliary obstruction or chronic pancreatitis and die of complications of cancer. These results lend support to previous studies suggesting that healthy acinar tissue is more susceptible to ERCP-related complications, especially in the setting of difficult cannulation.

15 Incidence and Characteristics of Myocarditis and Parvovirus B19 Viral DNA at Autopsy – A Single Institution Study of 21 Cases

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Background: Multiple reports have described Parvovirus B19 (PVB) DNA in association with myocarditis. Despite this association, limited information exists to establish whether PVB is a contributor or bystander in the pathogenesis of myocarditis. We evaluated the prevalence of PVB DNA in cases of myocarditis at autopsy in order to investigate and correlate the clinicopathologic findings associated with PVB positive cases of myocarditis.
Design: All postmortem cases of non-fungal myocarditis at UNMC from 2002 through October 2008 (n = 21, females = 12, age range=22-78 years; mean = 46) were tested for PVB DNA using PCR on paraffin-embedded cardiac tissue with appropriate positive and negative controls. The gross and histologic pathologic characteristics and clinical data were assessed.
Results: The incidence of non-fungal myocarditis at autopsy was 2%. Of the 21 patients with myocarditis, PVB DNA was present in 13 cases, absent in 1 case, and indeterminate in the remaining 7 cases due to inadequate genomic DNA. Clinically, five of the PVB-positive patients had compromised immune function from either advanced malignancy or transplantation. Cardiomyopathic features were present in all 13 patients and included hypertrophy in 12 patients (5 males, 7 females), biventricular dilatation in 8 patients (4 moderate, 4 severe) and interstitial fibrosis in 10 patients (5 mild, 5 moderate). Histologically, the PVB DNA positive-myocarditis tended to be characterized by a mild lymphohistiocytic, patchy infiltration with minimal myocyte damage. The maximum CD45-positive cell count per high-power field averaged 37.4 in immunocompetent patients and 10.6 in immunocompromised patients (p=0.03). Two cases showed significant myocardial eosinophils and/or giant cells in addition to the patchy, mild lymphocytic infiltration.
Conclusions: Our findings indicate that PVB DNA is present in at least 62% of the cases of postmortem myocarditis at our institution. The typical histologic pattern of PVB-associated myocarditis consisted of a mild, patchy lymphocytic infiltrate with minimal myocyte damage. All PVB-positive cases also had associated features of a cardiomyopathy. These results indicate that PVB infection in the myocardium may contribute to a mild, insidious myocarditis. Further studies to localize the virus in cardiac tissue and to assess for viral activity should further elucidate the role of PVB in myocarditis and dilated cardiomyopathy.

16 Study of Two Cases of Fatal Necrotizing Pneumonia in Healthy Adults, Due to Staphylococcus Aureus Infection. Is It Always Penton-Velentine Leukocidin?

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Background: Necrotizing pneumonia due to community acquired methicillin –resistant Staphylococcus aureus (MRSA) is reported with increasing frequency in healthy individuals. Most of the cases in the USA are attributed to USA 300 MRSA-clone which carries genes, encoding Panton-Valentine leukocidin (PVL) and are often associated with influenza or influenza-like illnesses. PVL is a leukotoxin which acts on human neutrophils, monocytes, and macrophages by inducing massive release of inflammatory mediators. It has been suggested that PVL has a major role in the inflammation and necrosis of the respiratory epithelium and parenchyma in the course of MRSA-associated necrotizing pneumonia. We report two cases of fatal necrotizing pneumonia with nearly identical clinical presentations and autopsy findings, but different etiologies.
Design: We reviewed the clinical and pathologic results from two autopsy cases of necrotizing pneumonia that occurred at OHSU. Fresh lung tissue was sent to a regional laboratory for routine microbiology and viral cultures and to the Oregon Public Health Division (OPHD) for toxin studies, including PVL and toxic shock syndrome toxin 1 (TSST-1).
Results: Previously healthy 18 year old male and 54 year old female presented to local hospitals with histories of sore throat, chills, and fever. Chest radiographs were unremarkable. Both patients were thought to have viral respiratory illness and were discharged home. Within 24 hours, both patients deteriorated and were admitted with hemoptysis, acute respiratory failure, severe metabolic acidosis, hypotension, and leukopenia. Despite resuscitation efforts, both patients expired within 12 hours after admission. Autopsy showed necrotizing pneumonia, associated with left sided cardiac hypertrophy. Microbiology studies demonstrated MRSA and Influenza A in the younger patient and methicillin-sensitive Staphylococcus aureus (MSSA) in the older patient. Toxin studies showed USA 300 producing PVL, negative for TSST-1 in the younger patient and USA 200 producing TSST-1, negative for PVL in the older patient.
Conclusions: We report two cases of fatal necrotizing pneumonia, associated with PVL producing MRSA and TSST-1 producing MSSA. PVL-associated necrotizing pneumonia has been increasingly reported in the literature in the last few years. To our best knowledge, cases of necrotizing pneumonia, associated with TSST-1 producing MSSA have been rarely reported in the literature.

17 Incidence of Brain Malformations. A Study of 395 Perinatal Autopsies

JC Mantilla, JA Diaz, LP Herrera. Universidad Industrial de Santander, Bucaramanga, Santander, Colombia; University of Miami, Miami, FL.
Background: Brain malformations are common congenital malformations, with diverse morphological and etiopathogenic features. The aim of this study is to determine the frequency and clinicopathological features of brain malformations in a tertiary care center.
Design: A prospective study was conducted from March 2004 to February 2008 of patients that underwent perinatal autopsy performed at the Hospital Universitario de Santander, Colombia.
Results: During the length of the study 395 perinatal autopsies were performed. The average gestational age was 29.4 weeks, with a range between 12 to 42 weeks. The male: female ratio was 1.2:1. Congenital malformations or dysmorphic features were present in 64 (16%) of cases.Twenty seven (7%) patients had brain malformations, of these 9 had hydrocephalus (2.2%), 7 holoprosencephaly (1.8%), 6 anencephaly (1.5%), 2 agenesis of the corpus callosum (0.5%), 1 agenesis of brain stem (0.2%), 1 acrania (0.2%) and 1 microphthalmia (0.2%).
Conclusions: Central nervous system malformations are common amongst patients with congenital malformations. Hydrocephaly and holoprosencephaly are the most common types observed.

18 Autopsy Findings in Patients with AIDS/Tuberculosis Coinfection

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Background: Disseminated Tuberculosis (TB) has been associated to advanced stages of AIDS.These patients present with atypical clinical presentations and often a definitive diagnosis is only obtained after autopsy examination. The aim of this study is to describe the autopsy findings of a series of patients with AIDS/TB confection.
Design: Medical records, autopsy files and slides from 17 patients with AIDS/TB confection were reviewed. Patient's demographics, AIDS stage, laboratory findings and pathogical features of TB were analysed.
Results: Patient's age ranged from 4 to 52 years, and had a male to female ratio of 1.8:1. All patients were stage 3C AIDS. A premortem diagnosis was obtained in 4 patients (23%). Disseminated TB was found in 12 patients (70%) whereas 5 (29%) had localized pulmonary disease. In consecutive order the organs most frequently involved were lung, lymph nodes, spleen, liver, bone marrow, testicles, adrenal, meninges, brain, stomach and colon. Comorbid conditions such as Pneumocystis jiroveci pneumonia was seen in 3 patients (17 %), toxoplasmosis in 2 (11%), appendicitis, severe malnutrition and Chagas' disease in 1 (6%) respectively. Macroscopically, three morphological patterns were observed: Micronodular pattern (generalized 0.5 to 2 cm lesions) seen in 70%, a miliary pattern (18%) and a cavitary fibrocaseous pattern (12%). Histopathological findings demonstrated a chronic inflammatory process with ill defined granulomas, abundant neutrophils, scant multinucleated giant cells, epitheliod histiocytes and necrosis. Ziehl-Nielsen stain revealed numerous acid fast bacilli.
Conclusions: TB coinfection in advanced stages of AIDS leads to unusual clinical presentations further complicated by the presence of comorbidities. The present study shows the pattern and frequency of organ involvement seen in a series of patients with this coinfection.

19 Limb-Body Wall Complex and Amniotic Deformity, Adhesions, and Mutilations Sequence: A Rare Fetal Polymalformation

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Background: Limb-body wall complex (LBWC) is a rare, sporadic fetal polymalformation with an incidence of 0.33/1000 live births. It is diagnosed by the presence of two out of three defects: exencephaly; facial clefts or thoraco-/abdominoschisis and limb defects. There are two distinct phenotypes, one showing craniofacial defects and amniotic bands or adhesions and the second with urogenital anomalies, anal atresia, placental adhesion and an extraembryonic coelom. Amniotic deformity, adhesions, and mutilations sequence (ADAMS) is a disorder caused by rupture of the amnion leading to formation of multiple amniotic/chorionic bands and subsequent fetal anomalies.
Design: The following is a case report of an autopsy performed on an infant with congenital anomalies on prenatal ultrasound. A routine pediatric autopsy was performed with careful examination of the body and placenta. Genetic karyotyping was done to rule out chromosomal anomalies.
Results: The patient (46, XX) was born to a 24 year old G2P0A1 woman at 24 5/7 weeks gestational age. Delivery was induced vaginally due to congenital malformations

considered incompatible with life on prenatal ultrasound. APGARS were 1 and 1 at 5 and 10 minutes respectively, and the infant survived for 1 hour 44 minutes. The autopsy examination demonstrated wide cranial sutures, edematous facies, webbed neck and scoliosis. A large abdominal defect with exstrophy of multiple organs, including ectopia cordis, was present. A thin membrane covered the defect and fused with skin, pericardium and many amniotic bands. There were multiple limb anomalies including abnormal rotation of the limbs, fusion of the extremities and digits and absence/amputation of digits. Genitalia were indistinguishable grossly. Internal examination demonstrated hypoplastic kidneys, heart, and lungs with a bilobed right lung and single lobed left lung. There was a bilateral superior and inferior vena cavae and double cervix. The placenta showed acute chorioamnionitis, chorangiosis and placental abruption.

Conclusions: This case is consistent with the diagnosis of LBWC and contains features of both known phenotypes: amniotic bands, facial abnormalities and genital anomalies. Additionally, there were the unusual findings of a double inferior and superior vena cavae and double cervix. We believe the unsupported amnion ruptured leading to multiple amniotic bands consistent with ADAMS and secondary to LBWC.

20 Postmortem Analysis of Synovial Fluid: An Alternative Method for Determining the Presence of Ethanol

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Background: Postmortem toxicological analysis is often an important tool in autopsy in determining the cause of death. There are circumstances however when traditional samples such as blood and vitreous fluid are unobtainable. In such instances, alternative body fluids such as synovial fluid may be useful adjuncts. However, synovial fluid has not been accepted as a widely used alternative. Earlier studies (Winek 1993, Ohshima 1997) demonstrated the potential for synovial fluid use in toxicological studies. The purpose of this study was to validate and determine the strength of correlation between blood ethanol content and synovial fluid ethanol content.

Design: Synovial fluid was obtained from 98 cases presenting to the Office of the Medical Investigator in Albuquerque, New Mexico over a 4 month period. Of these, 20 cases had measurable blood ethanol content at the time of demise, and were selected for use in the present pilot study to determine the viability of using synovial fluid as an alternative specimen for postmortem toxicology screening. Analysis of synovial fluid ethanol content (SEC) was performed using gas chromatography and compared with similarly obtained blood ethanol content (BEC).

Results: Blood ethanol content (BEC) and Synovial fluid ethanol content (SEC) were found to have a strongly positive linear relationship, with a Pearson correlation coefficient of 0.96 and a p-value of <0.0001.

Conclusions: The present study confirms prior research which indicates synovial fluid is a suitable substitute for the analysis of blood ethanol content particularly when traditional toxicology samples are unavailable, and synovial fluid can be adequately analyzed with current gas chromatograph protocols for volatile alcohols.

21 Concentrations of Opiates and Psychotropic Agents in Polydrug Overdoses: A Surprising Correlation between Morphine and Antidepressants

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Background: Heroin related deaths remain prevalent nationwide. Detection of heroin (or morphine) as the sole drug in postmortem (PM) toxicologic analyses is uncommon. Psychoactive agents, particularly central nervous system depressants and antidepressants, are often identified along with morphine in drug fatalities. Few studies have investigated trends in concentrations of each drug in combined overdoses. This study attempts to determine if a relationship exists between PM concentrations of morphine and other CNS agents in fatal polydrug overdoses.

Design: Autopsies performed by the MA OCME were included if the term "opiate" appeared in the cause of death. In 2006, 161 cases met inclusion criteria that morphine and at least one other drug were detected. The cases were then divided into subsets based on drug class, including "opioids", "antidepressants", "ethanol", "benzodiazepines", and "other". Each subset was then split into high or low concentration groups based on median concentrations of each drug within the subset. For each psychotropic drug subset, morphine concentrations of the [high] and [low] groups were compared.

Results: There was no significant difference in concentrations of morphine in the [high] versus [low] groups for the opioid, ethanol, or benzodiazepine subsets. Morphine concentrations did show a significant direct relationship ($p = 0.01$) with antidepressants, namely increased concentrations of antidepressant drugs are associated with an increased concentration of morphine. This trend remains even after excluding cocaine positive cases.

Conclusions: The unsuspected finding that PM concentrations of antidepressants positively correlate with morphine levels may be important in the treatment of depression in drug addicts. Research suggests that chronic antidepressant use preferentially decreases opiate receptors in the cerebral cortex but not the corpus striatum or hippocampus. Possibly addicts on antidepressants increase their heroin doses to achieve cortex-mediated euphoria, while unwanted analgesic effects in the hippocampus may cause failure to trigger respiration. High volume of distribution of antidepressants and postmortem redistribution cannot be ignored, though use of the median in this study to differentiate high and low concentrations provides an internal control. Equally surprising, trends in morphine versus CNS depressant drug concentrations were not detected.

22 Spectrum of Liver Pathology in an Irish Coroner's Autopsy Population – A Retrospective Study

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Background: Fatty liver disease, both alcoholic and non alcoholic is an increasing public health issue. Ireland has one of the highest rates of alcohol consumption world wide.The aim of this study was to evaluate the spectrum of liver histopathology findings in an Irish medicolegal autopsy population and to determine if fatty liver disease was a risk factor for accidental or other unnatural cause of death.

Design: All medicolegal (Coroners) autopsies in Cork and Kerry region (population approx 500,000) for a period of one year were evaluated for liver histopathology (Total 702 cases). The following parameters were assessed and graded according to standard histologic criteria; steatosis, steatohepatitis, fibrosis, hepatitis, other. All autopsy reports were reviewed for demographic details, cause of death and toxicology results. The causes of death were grouped into natural and accidental/ other unnatural causes. Unnatural causes included drowning, fire, hanging, road traffic accidents and falls.

Results: Histologic sections from 702 consecutive autopsies were reviewed. 488 males, 214 females (ratio 2.5:1), age range 3 weeks to 92 years (mean age 46years). Breakdown by cause of death was natural 517; unnatural 185. Mean age for natural and unnatural deaths was 63years and 45years respectively. Of the total cases (n=702), 336 (48%) showed evidence of steatosis,122 were graded as moderate or severe.162/702 (23%) had fibrosis, 7.3% had pericellular fibrosis and 4.5% had cirrhosis. Of the patients with steatosis (336), 21(6%) had coexisting cirrhosis, 36(10%) had coexisting pericellular fibrosis and 60 (18%) had coexisting steatohepatitis. Other pathologic findings included hepatitis 10/702 (1.4%), granulomas 5/702 (0.7%) and metastatic carcinoma 3/702 (0.4%). Of 185 unnatural deaths, 80 (43%) had histologic evidence of steatosis with 18% having blood alcohol levels in excess of 80mg%. In the natural group 50% had steatosis.

Conclusions: Liver pathology is a common finding in the medicolegal autopsy service in Ireland. Fatty liver disease with or without associated fibrosis is the dominant histologic pattern in this population. This spectrum is found in equal percentages in both the natural and unnatural cause of death groups. However, the age adjusted prevalence of fatty liver disease is higher in unnatural deaths which raises the question of a possible association between unnatural cause of death and fatty liver disease.

23 Diagnostic Approach to Osteochondral Dysplasias by Perinatal Autopsy: A Series of 8 Cases

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Background: Osteochondrodysplasias (OCDs) are rare, heterogeneous group of disorders that result from an abnormal maturation and an impaired growth of cartilage and bones. Lethal OCDs frequently include achondroplasia [thanatophoric dysplasia (TPD)], osteogenesis imperfecta (OGI), achondrogenesis, and short-rib (with or without polydactyly) dysplasias type I-IV. Accurate diagnosis is critical for counseling the parents for subsequent pregnancies; however, the diagnosis of a fetal OCD is often difficult at autopsy.

Design: Eight fetal autopsies of OCD were assessed in terms of postmortem findings and radiological evaluation to determine the contributory value of autopsy to the final diagnosis. Clinical information included previous obstetric history and prenatal USG findings. All 8 cases had skeletal radiographs and extensive photographs.

Results: The pregnancy was terminated at 18-33 weeks of gestation following detection of skeletal anomalies by prenatal USG in all 8 cases. In 6 cases a definitive diagnosis could be rendered on basis of the autopsy findings and clinicoradiologic correlation; in additional 2 cases, differential diagnoses were proffered with a suggestion for confirmation by genetic studies. Amongst the 8 cases, the diagnoses were made or altered by autopsy findings in 6 cases; whereas autopsy confirmed the diagnoses given by prenatal USG in the remainder.

The diagnoses of perinatal autopsies with OCD

Case no	Age of Fetus (week)	Clinical Diagnosis	Final Diagnosis at Autopsy
Case no I	33	Hypophosphatasia	OGI
Case no II	29	OCD	OGI
Case no III	21	OCD	TPD
Case no IV	18	OCD	Differential diagnoses: Achondrogenesis IA or severe form of achondrogenesis II
Case no V	26	Ellis-van Creveld Syndrome	Ellis-van Creveld Syndrome
Case no VI	18	OGI	OGI type II
Case no VII	18	Achondroplasia	Differential diagnoses: Achondrogenesis II or type I (required genetic information)
Case no VIII	28	Fetal anomaly	Short rib-polydactyly syndrome type II (Majewski)

Conclusions: Autopsy plays an important role in establishing the diagnoses of OCD detected prenatally by USG. Accurate post mortem diagnosis of OCD requires a correlation of gross and microscopic findings with clinical history, postmortem radiological examination. A definitive diagnosis of OCD is crucial as it guides counselling of the parents for subsequent pregnancies.

24 Bile Casts in the Kidney: A Clinicopathologic Study of 18 Autopsies

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Background: Acute kidney injury associated with liver dysfunction in jaundiced patients (historically termed cholemic nephrosis) is not well studied. Tubular injury by direct toxicity of bile salts and bilirubin has been proposed. To determine the pathologic spectrum of renal pathology that may be encountered in this clinical setting, we reviewed the autopsy findings of patients with severe liver dysfunction.

Design: The autopsy archives were reviewed between 2004 and 2008. 18 patients with a history of jaundice and liver failure were identified. Relevant clinical data, including liver and renal function tests and autopsy findings were reviewed with particular attention to the kidneys. A Hall's histochemical stain was performed on all cases to confirm the presence of intratubular bile. Immunohistochemistry (IHC) for Epithelial Membrane Antigen (EMA) which identifies distal nephron segments was used in a subset of cases to localize the presence of the intratubular bile casts. Iron stains and myoglobin IHC were used to exclude the possibility of other types of pigmented tubular casts in selected cases.
Results: Ages ranged from 5 weeks to 89 years. 10 patients had hepatorenal syndrome. Gross examination of the kidneys after formalin fixation showed green discoloration of the cortex in 6 cases. Bile pigment casts were focal and localized to the lumen of distal nephron segments in most instances. The extent of bile cast deposition correlated significantly with the serum conjugated bilirubin (R=0.8) and alkaline phosphatase (R= 0.7). (p<0.001 for each).
Conclusions: Bile casts were common in this series being present in the distal nephron segments of jaundiced patients at autopsy. The extent of intrarenal bile cast accumulation correlates with the severity of liver injury as measured by serum bilirubin and alkaline phosphatase. Intratubular bile casts may contribute to acute kidney injury by direct toxicity, nephron obstruction, or both.

25 Multiorgan Distribution of Gadolinium Deposits at Autopsy in Nephrogenic Systemic Fibrosis (NSF)

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Background: Initially recognized as a fibrosing dermopathy, the multitude of organs involved in NSF was first identified in 2003. Epidemiologic studies implicated Gadolinium (Gd) from MRI contrast agents as a trigger for the development of NSF, and scanning electron microscopy (SEM) with energy dispersive x-ray analysis (EDS) demonstrated insoluble Gd deposition in lesional skin. Until now, there have been only limited studies examining the distribution of Gd in tissues apart from skin.
Design: To evaluate the tissue distribution, correlation with histopathology and relative amounts of Gd in various organs in a case of NSF, freshly cut paraffin block surfaces of autopsy tissues were examined under standardized conditions using the variable pressure mode of the SEM. This allows detection and multi-elemental EDS analysis of Gd-containing deposits *in situ* in tissues, and also semi-quantitative morphometric analysis with a spatial resolution of less than 1 µm.
Results: Gd was present in skin, skeletal muscle, lung, kidney, lymph node, liver, intestine, dura mater and the cerebellum. It was present in the form of insoluble hydroxyapatite-like deposits. These deposits had Ca, P, Na and sometimes Fe. In the skeletal muscle these deposits were associated with Zn and in the cerebellum, many of these deposits additionally contained K. Although the location of Gd was predominantly in the vascular walls of all organs, these deposits were also found in the parenchyma of the organs, in between muscle bundles, around fat compartments, in the alveolar septa, in the basement membrane of renal tubules and in and around hepatocytes. Gadolinium deposits could not be demonstrated in heart, major blood vessels (aorta, superior vena cava), bone, bone marrow, pons, corpus striatum, thalamus. Highest amounts of Gd were found in affected skin, skeletal muscle, lymph node and dura mater. Lungs, kidney and liver had moderate amounts and unaffected skin, intestine and cerebellum had relatively low quantities of Gadolinium.
Conclusions: The widespread deposition of Gd in tissues and organs supports the systemic nature of NSF. Gd undergoes transmetallation and deposits as hydroxyapatite-like particles. The composition of these particles can vary according to the local milieu of the tissue including the inflammatory state. We could not demonstrate Gd in decalcified bone, from which Gd-containing deposits may have been dissolved.

26 Subinvolution of the Placental Site, Pneumonia and Pyelonephritis, and Splenic Sequestration Related to Hemoglobinopathy – A Lethal Combination

CM Schneider, J Wurzel. Temple University Hospital, Philadelphia, PA.
Background: Subinvolution of the placental site, in which there is delayed or subnormal occlusion and sloughing of the superficial spiral arteries at the site of attachment, is a cause of secondary postpartum hemorrhage. This condition occurs at least one day after delivery and can cause life-threatening hemorrhage. We report a case of fatal hemorrhage related to subinvolution of the placental site. Other factors, including multiorgan infection, possible sepsis-related coagulopathy, and sickle hemoglobinopathy likely contributed to the outcome.
Design: A 31-year-old African American female presented with heavy vaginal bleeding and shortness of breath for an unspecified time. She had spontaneously delivered a healthy infant nine days previously. The only complication of the pregnancy was gestational diabetes mellitus. History also included hemoglobin S/beta thalassemia and obesity. The patient presented with brisk vaginal bleeding and a hemoglobin of 2.8 gm/dL. Treatment included administration of multiple blood products and coil embolization of the internal iliac arteries, but she died approximately seven hours after the initial presentation. A complete autopsy was performed.
Results: The uterus weighed 700 grams with extensive blood clot in the endometrial canal. Microscopy showed groups of dilated, widely patent superficial myometrial vessels with little intervening tissue. Immunohistochemistry showed rare keratin positive, presumed trophoblasts in and around vessels; incomplete to absent lining of the vessels by CD31(+) endothelium; rare bcl-2(+) apoptotic cells within the walls. These findings indicated subinvolution of the placental site. Other causes of bleeding such as retained placenta, gestational trophoblastic tumors, and endometritis were excluded. Findings of sepsis included bilateral pneumonia; septic infarctions of the right lung lower lobe; acute left pyelonephritis with numerous small cortical abscesses and an absence of acute cystitis. Supporting the diagnosis of sickle hemoglobinopathy was the presence of sickled erythrocytes in numerous tissues and hepatic extramedullary erythropoiesis. The spleen weighed 1190 grams, consistent with sequestration.
Conclusions: Although not well known to pathologists, subinvolution of the placental site is an important cause of secondary postpartum hemorrhage that can be life-threatening. In this unusual case, sepsis-related coagulopathy and splenic sequestration due to hemoglobinopathy could have contributed to the fatal outcome.

27 Eosinophilic Endomyocardial Disease: A Clinicopathological Study of Autopsy and Surgical Heart Specimens

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Background: Eosinophilic endomyocardial disease (EoEMD), also known as Löeffler's or tropical endomyocardial disease, is a rare cardiac disorder associated with prolonged eosinophilia of any cause. In the temperate zones, the most common association is with hypereosinophilic syndrome, whereas in tropical locations, helminth-induced hypereosinophilia is usually the etiology. Clinically, most patients develop restrictive cardiomyopathy or atrioventricular valve insufficiency. Ventricular mural thrombus may also be present. Endomyocardial biopsy is regarded as the gold standard for diagnosis. The aim of this study was to characterize more fully the clinical and pathologic spectrum of changes found in EoEMD from autopsy and larger surgical specimens, rather than from endomyocardial biopsy tissues.
Design: We searched the pathology files of the Mayo Clinic Tissue Registry for cases of EoEMD (1980-2008). Of the 79 cases, 2 were from autopsies, 1 from surgical explantation, and 1 from surgical decortication/thrombectomy. Clinical, gross, and microscopic findings of each case were systemically reviewed.
Results: Clinical and pathologic data are presented in Tables 1 and 2, respectively.

Table 1. Clinical Data

Patient	Source	Age, Sex at Examination	Type of Cardio-myopathy	Peripheral Blood Eosinophilia	History of Peripheral Thrombus	Relevant Medical History
1	Autopsy	42M	Restrictive	Yes	Portal vein thrombosis, pulmonary emboli	Hypereosinophilic syndrome
2	Surgical explant	46F	Dilated	Yes	Pulmonary emboli	Severe asthma
3	Autopsy	74F	Restrictive	No	Pulmonary emboli	Rheumatoid arthritis
4	Surgical decortication	37M	Restrictive	Yes	None	Thrombocytopenia

Table 2. Pathologic Features

Patient	Mural thrombus	Endocardial fibrosis	Active myocarditis	Myocardial ischemia	Eosinophil degranulation	CAD
1	Yes	Yes	Marked	Yes	Yes	Absent
2	No	Yes	Marked	No	Yes*	Mild-moderate
3	Yes	Yes	Moderate	Yes	Yes	Mild
4	Yes	Yes	Absent	No	No	Absent

"*" included numerous Charcot-Leyden crystals; CAD=coronary artery disease

Conclusions: While generally congruent with recognized features of EoEMD, this study highlights the variability both in the clinical manifestations (dilated cardiomyopathy; late-phase disease without peripheral eosinophilia), and in the histopathologic findings (2 cases with patchy ischemic change likely due to global hypoperfusion or microthrombi; another case with massive Charcot-Leyden crystals) seen in this rare disorder.

28 Natural, Unexpected Deaths: Reliability of a Presumptive Diagnosis

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Background: Despite declining hospital autopsy rates, medical examiner and coroner offices receive numerous inquiries about autopsies each year from the next of kin. Many of these family inquiries involve deaths that normally would undergo postmortem examination due to the decedent's medical history and circumstances of death. A small number of them, however, involve deaths in which an autopsy is performed because of suspicions and concerns expressed by the family.
Design: We retrospectively analyzed 100 of these deaths and compared the pre-autopsy proximate cause of death, as determined by a thorough review of the clinical data and circumstances, to the autopsy-derived cause of death determined at the New York City Office of Chief Medical Examiner.
Results: In the majority (91/100), the pre-autopsy and post-autopsy proximate causes of death were in agreement. In 9% (9/100), the autopsy provided information that resulted in a proximate cause of death different than anticipated. In 4 instances, the manner of death also was incorrect and was determined to be an accident rather than the originally presumed natural. No homicides or suicides were misclassified. In another 9 instances, where the premortem and postmortem proximate causes of death were in agreement, the autopsy provided a specific mechanism of death.
Conclusions: With a quality initial medicolegal death investigation, a subset of sudden deaths in adults may be reliably certified without an autopsy.

29 Disseminated *Scedosporium prolificans*: A Case Report and Diagnostic Review

TP Seybt, DG Falls. Medical College of Georgia, Augusta, GA.
Background: Opportunistic fungal infections in the immunocompromised host are not uncommon; however, many fungi are morphologically similar presenting a diagnostic challenge with treatment implications. We report an unusual case of disseminated *Scedosporium prolificans* in a myelofibrosis patient presenting with a left lower lobe pneumonia and effusion. The comparative morphology and alternative methods used to identify *Scedosporium* spp., *Pseudallescheria* spp., *Aspergillus* spp., and *Candida* spp. are reviewed.

Design: A 49-year-old Caucasian female with a history of cyclic neutropenia and a prior bone marrow biopsy revealing myelofibrosis of uncertain etiology was admitted to our institution for sepsis and left lower lobe pneumonia. The patient subsequently developed respiratory distress. Sepsis workup revealed stool cultures positive for *Clostridium difficile* as well as blood and sputum cultures positive for an uncommon mold (*Scedosporium prolificans*), which was verified by the Mayo Clinic in Rochester, MN. The patient was treated with metronidazole for her Clostridium infection; however, her condition continued to worsen requiring vasopressors for cardiovascular support. Upon further deterioration of the patient's health, the family opted to withdraw care and the patient subsequently died. Permission for unrestricted autopsy was granted.

Results: Gross examination at autopsy revealed a bilateral hemorrhagic lobular pneumonia. Multiple small ulcerations were noted involving the mucosa of the stomach, small bowel, and bladder. Microscopic examination revealed evidence of a fungal organism within the tissues of the thyroid and parathyroid glands, right and left lungs, heart, stomach, small bowel, bladder, bone marrow, and brain. Morphology was identified with Hematoxylin and Eosin (H&E), Gomori methenamine silver (GMS), and Periodic Acid Schiff (PAS) staining.

Conclusions: The isolated fungus displayed hyphae measuring 4-6 microns in thickness with dichotomous branching at 45 degrees in a haphazard manner. Multiple conidia were also identified budding from the hyphae. The sputum specimen was sent to the Mayo Clinic where microscopy and colony morphology were performed confirming the presence of *Scedosporium prolificans*. While this is an uncommon organism, it does possess the ability to grow in an angioinvasive pattern as does *Pseudallescheria* spp., *Aspergillus* spp., and some *Candida* spp. Fungal morphology can appear strikingly similar in the above species; therefore, additional testing may be warranted to correctly identify the causative organism in order to tailor pharmacotherapy.

30 Survey of Coronary Artery Atherosclerosis among 30-45 and 60-75 Year Old Patients: An Autopsy Based Study

AJ Wilhorn, DV Miller. Mayo Clinic, Rochester, MN.

Background: Ischemic heart disease and coronary atherosclerosis remain the leading cause of death in the United States. The literature is replete with studies comparing the severity and distribution of coronary artery stenosis as assessed at autopsy and by other in-vivo methods (angiography, coronary CT, and other imaging modalities). However, the vast majority of autopsy based studies were conducted more than 2 decades ago. Since that time, education and prevention programs have helped increase patient awareness of coronary disease and ostensibly made an impact on the overall coronary disease burden in our population. Given the potential impact of these initiatives over the past 2 decades, it is of interest to assess the severity and distribution of coronary artery disease in a more recent cohort of patients in a large autopsy series.

Design: This study included a retrospective review of autopsy reports to abstract manner and cause of death, degree of coronary atherosclerosis in each coronary vessel, and other significant clinical and pathological parameters. Autopsy reports from 2003-2008 were used for the purpose of this study to identify patients between the ages of 30-45 and 60-75 years of age. Coronary obstructions were graded as 1: <25%, 2: 26-50%, 3: 51-75%, and 4: >75% luminal occlusion.

Results:

30-45 yr. Old Patients

n=	211
Average Age:	39.2
Average BMI:	29.9
Average LAD Disease Grade:	1.87*
Average LCX Disease Grade:	1.51*
Average RCA Disease Grade:	1.73*
% with Severe 3-vessel Disease:	6%
% with at least 1-vessel Severe Disease:	18%
Cardiac Related Cause of Death	20%

*p<0.001

60-75 yr. Old Patients

n=	586
Average Age:	67.7
Average BMI:	30.4
Average LAD Disease Grade:	3.11*
Average LCX Disease Grade:	2.77*
Average RCA Disease Grade:	2.94*
% with Severe 3-vessel Disease:	29%
% with at least 1-vessel Severe Disease:	58%
Cardiac Related Cause of Death	30%

*p<0.001

Conclusions: Like the majority of older studies, lesions of the LAD were slightly more common in both groups. The incidence of severe single vessel disease is significant (58%) in the age 60-75 group, even among patients not dying from cardiac disease. A surprisingly high rate of single vessel disease was also seen in the 30-45 year age group as well.

31 Survey of Coronary Artery Atherosclerosis among 40-54 Year Old Normal Weight and Obese Patients: An Autopsy Based Study

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Background: Ischemic heart disease and coronary atherosclerosis remains the leading cause of death in the United States. Obesity is a recognized independent risk factor for atherosclerosis in general, but there are few recent data on associations between obesity and coronary artery atherosclerosis specifically.

Design: This study included a retrospective review of autopsy reports from 2003-2008 to abstract manner and cause of death, body weight and height, age, and degree of coronary atherosclerosis in each coronary vessel. Coronary atherosclerosis was graded as 1: <25%, 2: 26-50%, 3: 51-75% and 4: >75% luminal occlusion. Patients between the ages of 40-54 to focus on a population segment at risk for coronary disease and likely to be of stable body weight (without age-related loss of muscle mass).

Results:

Normal Weight Patients (BMI= 18.5-24.9)

n=	94
Average Age:	47.2
Average BMI:	22.4
Average LAD Disease Grade:	2.13*
Average LCX Disease Grade:	1.73*
Average RCA Disease Grade:	2.03*
% with Severe 3-vessel Disease:	7%
% with at least 1-vessel Severe Disease:	32%
Cardiac Related Cause of Death:	19%

*p<0.001

Obese Patients (BMI= 30.0-39.9)

n=	150
Average Age:	49.0
Average BMI:	33.6
Average LAD Disease Grade:	2.62*
Average LCX Disease Grade:	2.11*
Average RCA Disease Grade:	2.32*
% Severe 3-vessel Disease:	12%
% with at least 1-vessel Severe Disease:	35%
Cardiac Related Cause of Death	27%

*p<0.001

Conclusions: While overall the average difference in coronary atherosclerosis severity in a given artery as well as the overall incidence of severe single vessel disease showed only minor (but statistically significant) differences among the obese and normal body weight groups, there was a substantial increase in severe 3 vessel disease with obesity. Cardiac related causes of death were also more common in the obese group.

32 The Diagnostic Significance of Post-Mortem Troponin I Levels in Cadavers with Risk of Myocardial Injury

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Background: This study investigated the diagnostic significance of serum post-mortem troponin I (TnI) levels in cadavers with a high risk of myocardial injury versus cadavers with a low risk of myocardial injury. The presence of coexisting, antemortem, cardiopulmonary resuscitation and its relationship to post-mortem TnI elevation was also evaluated, to determine if serum TnI can still be a reliable diagnostic tool in this setting. To distinguish elevation of cardiac enzymes due to myocardial injury from elevation secondary to autolysis, total creatine kinase (CK) and the percent creatine kinase MB (% CK-MB) were also evaluated.

Design: Cardiac blood samples were obtained from forensic and non-forensic autopsies and analyzed for TnI levels, CK, CK-MB, and % CK-MB. Cadavers less than 18 years of age or without a known time of death were excluded. The cases were divided into three groups: high risk of myocardial injury and CPR, low risk of myocardial injury and CPR, and low risk of myocardial injury and no CPR. Risk factors for myocardial injury included patient age greater than 50, hypertensive heart disease, cardiomegaly and coronary atherosclerosis identified at autopsy.

Results: Of the ten samples obtained, 4 were in the high risk group (3 with CPR) and 6 were in the low risk group (4 with CPR). Serum TnI was significantly more elevated in the high risk group when compared to the low risk group, even when compared at the same post-mortem sampling interval. There was no clear relationship between the presence of ante-mortem CPR and the elevation of serum TnI. The total CK generally showed a mild and gradual post-mortem time-dependent elevation, regardless of risk for myocardial injury. This elevation was not sufficient to account for the marked elevation in TnI seen in the high risk group.

Conclusions: These findings suggest that the elevation in total CK was due to autolysis, rather than true ante-mortem myocardial injury. In addition, the CK-MB and %CK-MB showed no time-dependent elevation and remained relatively stable over time, suggesting that autolysis rates of cardiac and skeletal muscle are equal. Although our study group is small, the findings suggest that post-mortem TnI may be a useful diagnostic aid in determining the presence of myocardial injury in post-mortem examinations. The reliability and accuracy of post-mortem TnI levels will give the pathologist an additional tool when investigating the presence of myocardial injury at autopsy, and may prove especially helpful in the absence of histopathologic evidence of myocardial infarction.

33 The Impact of Placental Examination in the Autopsy of the Structurally Normal Stillborn

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Background: Stillbirth of an apparently normal fetus is an emotionally devastating event for both patients and clinicians. Identifying the cause of death (COD) in these cases not only brings emotional closure but may also help with future pregnancy planning. Placental examination is recognized as an important component of the fetal autopsy. We wished to compare the rates of determination of COD in structurally normal stillborns at our institution prior to and after the placental examination was made a requisite component of the perinatal autopsy protocol.

Design: Prior to 1992, fetal autopsies at our institution did not routinely include the placenta. In 1992, a departmental policy was instituted strongly recommending that the placenta be submitted on all perinatal cases. We reviewed our autopsy records from 01/01/1987 through 12/31/2007 to assess the impact of this policy on the ability of postmortem examination to determine the COD in cases of stillbirth and to evaluate the contribution of the placental examination in these cases. Cases with congenital abnormalities were excluded from the study to minimize confounding factors. The autopsies with placental examinations were categorized by whether the evaluation

of the placenta provided confirmatory evidence concerning the COD; provided new information and COD; or did not provide additional information regarding the COD.
Results: During the 21-year period examined, 458 stillborn autopsies were performed; 388 of these autopsies revealed structurally normal fetuses (84.7%). Of the structurally normal fetal autopsies, 94 cases (24%) were performed prior to the policy institution and 294 after. Comparing the frequency of placental examination and COD determination before and after the policy (1992), we demonstrated that the percentage of placental examinations increased from 42.2±5.3 % (1987-1991) to 92.1±2.1 % (1992-2007), and the frequency of COD determination increased from 38.5±7 % to 79.5±3.2 % (P=<0.0001). The placental examination provided confirmatory evidence in 24.6 %, new diagnostic information and COD in 44.7 % and no additional information in 30.7 %. However, in those that did not yield any additional information, COD was identified from the clinical history in 27 % and in the remainder, several common causes of intrauterine demise, such as intrauterine infection or villitis of unknown etiology, were excluded.
Conclusions: The cause of death in structurally normal intrauterine fetal demise cases is more likely to be identified if routine pathologic examination of the placenta is performed.

Bone & Soft Tissue

34 Novel Kinase Mutations in *KDR/VEGFR2, TIE1*, and *SNRK* in Angiosarcoma (AS) Patients

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Background: The pathogenesis of AS is not well understood and anecdotal evidence suggests that at least a subset of patients respond to VEGFR targeted therapy. However no potential molecular candidates have been identified so far to guide a more specific therapeutic intervention.
Design: Forty-two samples from 39 AS patients were included in the analysis. A HG U133A Affymetrix platform (22,000 transcripts) was used to mine the gene expression of 22 AS, compared to a control group of a well-characterized set of 45 soft tissue sarcomas. Candidate kinase genes overexpressed in AS compared to other sarcoma types were selected for full-length sequencing using a high throughput mutational profiling. Identified mutations were then validated by direct sequencing in matched tumor/normal samples. Selected genes were also validated at the protein level by IHC in an AS tissue microarray.
Results: Unsupervised clustering showed that AS formed a tight genomic group distinct from all other sarcoma types. Its distinctive expression profile included overexpression of a number of kinase genes, such as *TIE1*, *KDR*/*VEGFR2*, *SNRK*, *TEK*, and *FLT1*/*VEGFR1*. Full-sequencing of these 5 genes identified mutations in 10 (25%) patients, including *KDR* (6/10), *TIE1* (2/10) and *SNRK* (2/10). *KDR* mutations were identified only in AS located in the breast/chest wall, with or without radiation exposure. However, overall kinase mutations were seen more often in radiation-associated tumors (40% vs 18%). The 2 patients with lymphedema-associated tumors lacked mutations. The presence of *KDR* mutations correlated with the protein expression by IHC in the AS TMA, being 3+ positive in all 6 KDR-mutated tumors, while negative in the *TIE1* or *SNRK*-mutated AS.
Conclusions: We identified novel mutations in *KDR, TIE1* and *SNRK* in 25% of AS patients. These findings open new ground for understanding the AS pathogenesis and will be instrumental in identifying the subset of patients responding to VEGFR specific targeting. Furthermore, KDR immunoreactivity, which was seen in 60% of AS, may be useful as a screening method for potential kinase mutations.

35 RT-PCR Analysis for FGF23 Using Paraffin Sections in the Diagnosis of Phosphaturic Mesenchymal Tumors with and without Known Tumor Induced Osteomalacia: A Study of 27 Cases

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Background: Phosphaturic mesenchymal tumors of the mixed connective tissue type (PMTMCT) are extremely rare, histologically distinctive neoplasms which cause tumor-induced osteomalacia (TIO) in most cases through elaboration of a phosphaturic hormone, fibroblast growth factor-23 (FGF-23). Rarely, identical tumors without known TIO may be seen. We studied a large group of PMTMCT for expression of FGF-23, utilizing a novel RT-PCR assay for FGF-23 in formalin-fixed, paraffin-embedded (FFPE) tissues.
Design: 27 PMTMCT (14 with, 13 w/o TIO) and 11 non-PMTMCT (6 chondromyxoid fibromas (CMF), 2 chondroblastomas, 1 SFT, 1 osteosarcoma, 1 paraganglioma) were retrieved. Total RNA was extracted from FFPE sections for RT-PCR analysis. FGF23 was amplified using three sets of primers that spanned the intron/exon boundaries to amplify the three exons of FGF23 gene (140 bp, 125bp, and 175 bp). The housekeeping gene phosphoglycerokinase (PGK, 189bp) was co-amplified to check RNA quality. Products were visualized by Agilent 2100 Bioanalyzer, Agilent DNA 1000 Kit and DNA chips. 2 PMTMCT with TIO, 3 PMTMCT without TIO and 5 non-PMTMCT lacked a positive PGK control and were excluded.
Results: 11 of 12 (92%) PMTMCT with known TIO were FGF23-positive. 9 of 13 (69%) PMTMCT without known TIO were FGF23-positive. One CMF was positive; all other non-PMTMCT were negative.
Conclusions: We conclude that RT-PCR for FGF-23 is a sensitive and specific means of confirming the diagnosis of PMTMCT both in patients with and without TIO. FGF-23 gene expression was present in >90% of PMTMCT with known TIO, confirming the role of FGF-23 in this syndrome. Rare FGF-23-negative PMTMCT with known TIO likely express other phosphaturic hormones (e.g., frizzled related protein 4). Our finding of expression of FGF-23 in 69% of histologically identical tumors without known TIO confirms the reproducibility of the diagnosis of PMTMCT, even in the absence of known phosphaturia. FGF-23-positive PMTMCT without known TIO were likely excised prior to becoming symptomatic. The exact nature of FGF-23-negative putative PMTMCT without TIO is unclear, although histological re-review did not suggest alternative diagnoses. Ongoing study of additional non-PMTMCT should further establish the frequency of FGF-23 expression in other tumor types.

36 Characterization of CXCR4 Expression in Chondrosarcoma of Bone

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Background: The CXCR4/SDF-1 system has been found to strongly correlate with neoplastic progression leading to metastases in a number of tumors including those of prostate, skin, breast, muscle, and bone. Increased CXCR4 mRNA expression in osteosarcoma tumor samples has previously been shown to correlate with reduced overall survival and with the presence of metastases at diagnosis. Excluding hematologic malignancies, chondrosarcoma (CS) of bone is the most common primary malignant tumor of bone, in adults in the U.S. Whether CXCR4 is detectable in CS and whether CXCR4 expression level correlates with CS grade, progression, and prognosis was the subject of this study.
Design: Archived materials from 22 CS samples banked between 2001 and 2006 were retrieved. All the slides were reviewed microscopically to confirm the initial diagnosis. The pathological features of the tumors were evaluated. Immunohistochemistry using monoclonal anti-CXCR4 antibody (R&D Systems) was performed on formalin-fixed, paraffin-embedded tissue sections and analyzed by histomorphometric techniques. Invasive ductal carcinoma of the breast was used as the positive control. All controls reacted appropriately.
Results: There were 14 high-grade (Grade II-III) CSs and 8 low-grade (Grade I) CSs. The 14 high-grade CS samples were derived from 12 patients. A single sample was provided by 18 patients and 2 samples were available from the remaining 2 patients. Of the study cohort, 10 were males and 10 females, ranging in age from 24 to 80 years. Follow-up ranged from 3 to 84 months. Essentially, all CS cells stained for CXCR4. However, the percentage of the positive area in the selected tumor fields between these two groups was significantly different, with significantly greater positivity in the high-grade tumors ($p<0.001$). More interestingly, the staining intensity of the CXCR4 between the two groups was also significantly different. There was a higher staining intensity in high grade CS cells ($p<0.001$). Neither recurrences nor metastasis were seen in the low grade group. 5 patients suffered a local recurrence and 2 had remote metastases in the high grade group. The CXCR4 expression levels increased in the recurrence/metastases samples for the 2 patients with more than one sample, indicating with progression of the tumor, the expression level of CXCR4 rises.
Conclusions: CXCR4 expression exists in all CS cells with its quantity and intensity increasing with progression which, in turn correlates with a poorer prognosis as assessed by recurrence or metastases.

37 Chemo-Resistant Ewing Sarcoma Is Susceptible to Natural Killer Cell-Mediated Cytotoxicity: Implications for Immunotherapy

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Background: Despite multimodal therapy, patients with refractory or relapsed Ewing sarcoma (EWS) have poor prognoses. To explore the feasibility of natural killer (NK) cell-mediated immunotherapy for patients with advanced-stage EWS, we investigated whether susceptibility of EWS to NK cell-mediated cytolysis is affected by chemo-sensitivity and identified pivotal molecular mechanisms involved in NK cell-mediated cytolysis.
Design: Expression of ligands for inhibitory and activating NK cell receptors was evaluated in chemo-resistant and -sensitive EWS cell lines (n=10) by flow cytometry. Cytotoxicity was determined in chromium release assays, using freshly isolated (resting) and interleukin (IL)-15 activated NK cells obtained from healthy donors. Blocking antibodies against specific ligands/receptors were used to study contribution of these molecules.
Results: All EWS cell lines were lysed by resting NK cells, regardless of chemo-sensitivity, except for one chemo-resistant cell line (CADO-ES). Ligands for the activating NK cell receptors DNAM-1 and NKG2D were expressed by all cell lines, though in heterogeneous patterns. Cytotoxicity depended on these receptors, since blocking either of these receptors abrogated cytolysis by resting NK cells. IL-15 activation of NK cells increased efficacy of lysis in all cell lines, including CADO-ES, and resulted in more efficient recognition of EWS cells, since only combined DNAM-1/NKG2D-blockade inhibited lysis. CADO-ES, resistant to lysis by resting NK cells, is characterized by high levels of HLA class I expression compared to other EWS cell lines; in addition, the HLA class I alleles expressed by CADO-ES are ligands for all inhibitory NK cell receptors (KIR). In this cell line, cytolysis by resting NK cells depended on loss of inhibition, since blocking antibodies against HLA class I reversed resistance. Induction or blockade of HLA class I did not significantly affect lysis in all other cell lines.
Conclusions: The observed susceptibility of chemo-resistant Ewing sarcoma to cytolysis by cytokine-activated NK cells may provide patients with advanced-stage Ewing sarcoma with an additional treatment modality.