Cardiovascular Pathology

293 Prevention of Perioperative Vascular Infection By a Triple Antimicrobial-Bonded Aortic Graft

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Background: Previously, we investigated a locally developed technique of bonding aortic grafts with 3 antimicrobials to prevent early (within 2 weeks) direct perioperative bacterial contamination related to aortic graft operations. In the current phase, we tested our novel triple-antimicrobial-bonded graft's ability to prevent infection for 8 weeks after implantation.

Design: 9 Sinclair miniature pigs were tested and their data were added and compared with our previous pilot cohort (n=6) that were monitored for 2 weeks postoperative. All pigs received a 6-mm vascular Dacron graft in the infrarenal portion of the abdominal aorta. 5 pigs received grafts chemically bonded with a 60-mg/mL solution of rifampin, minocycline, and chlorhexidine; the other 4 pigs received unbonded grafts. Before implantation, the 5 bonded grafts (Group 3) and 3 (Group 2) of the unbonded grafts were immersed for 15 min in a 2-mL solution containing 1 to 2×10^7 CFU/mL Staphylococcus aureus (ATCC 29213); the 4th unbonded graft served as a control (Group 1).

Results: In the current study, all S. aureus–treated bonded grafts (n=5) showed no bacterial growth on explantation on week 2 and 9, respectively. The unbonded, untreated grafts showed low level bacterial growth ($<1.8\times10^3$, 7.27×10^3/graft); S. cohnii ssp urealyticus, but not S. aureus, was isolated, suggesting accidental direct perioperative contamination. Two pigs that of the group received S. aureus–treated, unbonded grafts (n=3) were euthanized because of severe infection with S. aureus (bacterial counts: $<1.38\times10^7$ CFU/graft). The findings in the 3 groups of the current study were identical to those in the 2 weeks earlier study.

Conclusions: Our results suggest that triple-bonded aortic grafts prevented perioperative aortic graft infection for at least 8 weeks in a porcine model. Its use may be recommended for in situ replacement of infected grafts and possibly for routine primary cases, especially in patients who are immunocompromised, have hostile abdomen, or undergo redo procedures. This proof of concept will provide the first application of a successful bonded graft to combat one of the most devastating complications associated with aortic graft surgeries. Further studies are needed to assess its capabilities to combat entirely the early-onset vascular graft infections for 4 months.

294 Molecular Assessment of Antibody-Mediated Rejection in Formalin-Fixed, Paraffin-Embedded (FFPE) Human Cardiac Allograft Biopsies

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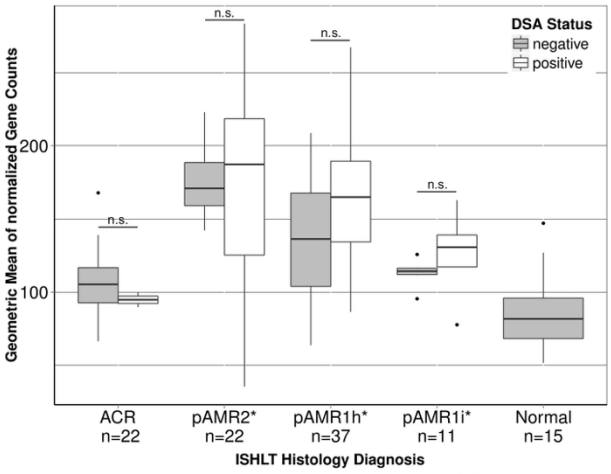
Background: Per consensus Antibody-mediated rejection (AMR) in cardiac allografts is diagnosed by histo- and immunopathological criteria while clinical and serological criteria are excluded from the latest ISHLT classification. Diagnosing AMR remains challenging. Previous studies showed increased expression of endothelial, NK cell and inflammatory genes to be associated with AMR in allograft biopsies. The aim of this study was to assess a literature-derived gene signature for diagnosing AMR in FFPE cardiac allograft biopsies.

Design: RNA was isolated from 107 archival FFPE human cardiac allograft biopsies which were reclassified according to the latest ISHLT classification (22 pAMR2, 37 pAMR1H+, 11 pAMR1I+, 22 ACR, 15 controls). A set of 34 genes, previously shown to be associated with AMR, was quantified with NanoString nCounter system. Results were compared between AMR, ACR and controls by t-test. Receiver operating characteristic (ROC) curves were used to analyze the diagnostic accuracy.

Results: In 70 AMR cases (including 34 concomitant with ACR) gene set expression was significantly higher compared to 22 ACR ($p<0.0001$) and 15 control cases ($p<0.0001$). Detection of Donor-specific antibodies (DSA) overall was associated with higher gene set expression($p=0.001$), whereas restricting to diagnostic groups presence of DSA had no significant effect on gene expression (ACR: $p=0.18$, pAMR2: $p=0.73$, pAMR1H+: $p=0.1$, pAMR1I+: $p=0.54$). pAMR1I+ cases had significantly lower gene set expression than pAMR2 ($p=0.001$) and pAMR1H+ diagnoses ($p=0.003$), while no difference was observed between pAMR1I+ and ACR ($p=0.61$).

Figure 1



* AMR concomitant with ACR: pAMR2 (n=11), pAMR1 H+ (n=26), pAMR1 I+ (n=6)

Diagnosis	Normal	pAMR1 I+	pAMR1 H+	pAMR2
ACR	0.02	0.61	0.0001	0.0003
pAMR2	< 0.0001	0.001	0.17	
pAMR1 H+	< 0.0001	0.003		
pAMR1 I+	0.02			

Comparing Geometric Means of Gene Signature
p-Values of Welch Two Sample t-test

C4d-positivity was borderline associated with increased gene expression ($p=0.052$). ROC Analysis showed higher diagnostic accuracy for discriminating AMR from ACR and Normal through gene set expression (AUC=78.61) compared to DSA (AUC=72.55) or C4d (AUC=70.71).

Conclusions: We demonstrate feasibility of robust gene expression quantification in FFPE cardiac allografts biopsies using the NanoString platform. Molecular quantification of antibody-mediated tissue injury has the potential to enhance diagnostic accuracy in cardiac allograft biopsies with AMR.

295 Primary Cardiac Tumors – 14 Years of Experience in a Portuguese University Hospital

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Background: Primary neoplasms of the heart are rare and occur with lesser frequency than metastatic tumors to the heart. Our objective was to review 14 years of experience in a single University Hospital.

Design: A transversal observational study was conducted on the diagnosed cardiac tumors from 2000 to 2014, at the Department of Pathology in a University Hospital of Lisbon, Portugal. For each case the following clinical and pathological information was retrieved: age, gender, location, size and histological diagnosis.

Results: We received 89 primary heart tumors of which 51 were from female and 38 from male patients. Median age at surgery was 67 years (range 18-82). The main diagnosis were: myxoma (71 cases) and papillary fibroelastoma (10 cases). Other tumors found were (1 of each): lipoma, angiolipoma, hemangioma, paraganglioma, inflammatory myofibroblastic tumor, myxofibrosarcoma, undifferentiated sarcoma and diffuse large B cell lymphoma. The main primary location was the left atrium (63 cases of which 61 were myxomas) followed by the right atrium (7 cases) and aortic valve (4 cases, all papillary fibroelastomas).

Conclusions: Our results are comparable to other larger series. Knowing the frequency, histological type and localization of cardiac tumors can be of assistance not only in patient surgical management, but also in diagnosing difficult cases, such as those presented in intraoperative consultation.

296 Amyloidosis in Atrial Appendages Removed During the Cox-Maze Procedure: A Study of 349 Consecutive Cases (2010-2014) With Clinical Implications

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Background: Histomorphologic parameters of atrial appendages removed during the Cox-Maze procedure have been shown to correlate with recurrence of atrial fibrillation. While amyloid deposition has been noted within the atrial appendages of this population, the incidence, distribution and proteomic spectra of such are not understood.

Design: Tissue registry archives of Mayo Clinic (Rochester, MN) were queried for atrial appendages removed during the Cox-Maze procedure (2010-2014). Patient demographics, imaging features, and salient clinical findings (including recurrence of atrial fibrillation and valvular heart disease) were recorded. Paraffin blocks were procured from each case, cut at 3 µm, and stained with sulfated alcian blue by established protocol. Evaluation for amyloid deposition was performed by a cardiovascular pathologist, blinded to the clinical information. Pattern and extent of deposition were recorded. Typing of the amyloid protein, when present, was performed on a subset of cases by laser capture microdissection with mass spectrometry-based proteomic analysis.
Results: 384 atrial appendages from 349 consecutive patients that underwent the Cox-Maze procedure were included in the study (mean age 70.8 years, range 26-95). 141 (40.4%) cases were women. Amyloid deposition was present in 139 (39.8%) cases. Of these, only a lace-like deposition was identified in 124 (89.2%), only vascular deposition in 6 (4.3%), only nodular deposition in 1 (0.7%), and mixed in 8 (5.8%). Atrial amyloidosis was more prevalent in individuals > 75-years-old ($p<0.05$). Women tended to have a greater tendency for atrial amyloidosis than men ($p<0.05$). Typing of a sub-cohort of cases showed the lace-like pattern to consistently be composed of AANF (atrial natriuretic factor)-type amyloid whereas the nodular pattern exhibited a peptide profile compatible with ATTR (transthyretin)-type amyloid.
Conclusions: Similar to ventricular amyloidosis, atrial amyloid deposition appears to be associated with increasing age. Routine screening of atrial appendages with a special stain for amyloid resulted in a surprisingly high prevalence of atrial amyloidosis in this population. Stratification by type has important clinical implications. Interestingly, the pattern of deposition appears to be a reliable indicator of what amyloid type is ultimately identified (by mass spectrometry-based proteomics). The clinical significance of the finding of AANF-type amyloidosis is the subject of ongoing study.

297 MicroRNA Modification of AMPK Signaling Axis in HCM Hearts

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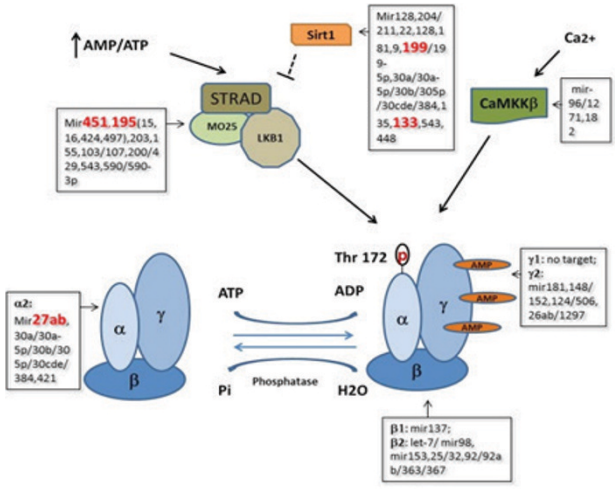
Background: In industrial countries, the prevalance of congestive heart failure (CHF) is increasing steadily and has become one of the leading causes of morbidity and even mortality. Recently, MicroRNAs (miR) and AMP-kinase (AMPK) have emerged as prominent players in the development of cardiac hypertrophy and heart failure. Our previous study indicates that differential AMPK regulation through one miR species (miR195) plays a role in the development of hypertrophic cardiomyopathy (HCM) in mouse.
Design: Using bioinformatic algorithms (TargetScanMouse 5.2), we applied the messenger RNA sequences of the components of AMPK pathway to predict miR candidates that potentially target AMPK axis. Using real-time PCR, a candidate miR that included miR species implicated in human pathological cardiac disease and/or metabolic dysregulation was performed on angiotensin II induced hypertrophic neonatal rat cardiomyocyte (NRCM). The miR species that shows similar expression pattern in human, and cell culture model was chosen to determine whether the conserved sites in AMPK 3'UTR acted as functional targets using pmirGLO Dual-Luciferase Expression Vector system.
Results: 1. The miRs species that are predicted to potentially target AMPK pathway and their targets are listed in [figure1].



2. Altered expression of miRs that target AMPK axis in angiotensin II induced hypertrophic NRCM, as shown in [table1].

	miR species
Up-regulated	15, 27a, 19, 25, 26, 30, 32, 96, 98, 99, 100, 101, 105, 107, 124, 128, 135, 138, 195, 370, 374, 451, 1192
Down-regulated	22, 92, 93, 103, 106, 129, 133, 152, 155, 183, 192, 194, 196, 199, 200, 204, 211, 215, 294, 295, 301a, 410, 421, 429, 488, 497
Unchanged	9, 33, 130, 141, 142, 144, 146, 153, 495, 1907
Undetected	16, 17, 20, 126, 137, 148, 291, 375, 377, 384, 433, 504, 539, 543, 590, 721

3. 3' UTR of AMPK axis components are functional targets of miRs, such as miR-27 targeting AMPKa2.

Conclusions: Thus, Our findings indicate that components of the AMPK axis are targeted by miRs which in turn altering AMPK signaling in the developement of HCM.

298 Anatomic Barriers Within the Coronary Sinus: Surgical Implications

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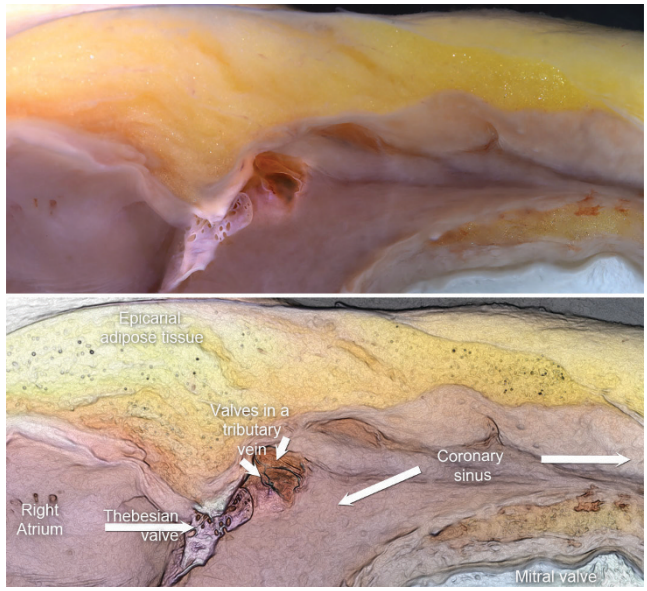
Background: The coronary sinus (CS) is the principal conduit for venous drainage of the ventricular myocardium, coursing behind the left atrioventricular junction and draining into the right atrium. This anatomical feature allows use of the CS as a delivery route for retrograde perfusion of the heart with cardioplegia solution during both standard and minimally invasive cardiac surgery procedures. There is scarce data about the frequency of venous valves within the CS and its tributaries and their potential impact on delivery of retrograde venous perfusion.
Design: We examined 126 formalin fixed hearts of patients that underwent autopsy from 2012 to 2014. The CS was opened longitudinally and the number of venous valves and their position away from the ostium were recorded.



Results: The average patient's age was 63.5 ± 15.5, with 55/126 being female. The average diameter of the CS was 6.5±1.5 mm. A single valve is present within the coronary sinus at the opening of the tributary veins in 55 hearts (43.7%; 95% confidence intervals 30.5%- 56.8%), while in 20 cases (15.9%; 95% confidence interval 0%-31.9%) there were two or more valves present. No valves were seen in 51 hearts (40.5%; 95% confidence intervals 27%- 54%).
The first valve occurred at 22.3±15.4 mm (Mode 30 mm) from the CS opening (Thebesian valve), while in patients with more than one valve the last valve occurred at 39±6.3 mm. These valves were present within the coronary sinus and/or at the opening of major tributaries into the coronary sinus. They were always found in the territories that drain venous blood from the posterior right ventricle and posterior left ventricle.
Conclusions: In this study, there were venous valves within the CS in 59.5% of patients. Thus, we show that potential impediment to effective retrograde cardioplegia delivery to the posterior right ventricular wall and potentially part of the posterior interventricular septum occurs in more than half of the patients. This may explain the clinical correlates of right ventricular dysfunction or tricuspid regurgitation after open heart or minimally invasive cardiac surgery.

299 Evaluation of Cellular Ingrowth Within Porcine Extracellular Matrix Scaffolding in Congenital Heart Disease Surgery

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Background: The search for an ideal material for cardiac tissue repair has led to utilization of porcine small intestinal submucosa extracellular matrix (ECM). Although thousands of patients have received ECM grafts worldwide, little information exists on the histopathology of implanted scaffolds in humans. Here, we examine the histologic features of ECM and the associated cellular growth at a variety of time intervals.
Design: ECM from eight patients (4male, 4 female) with age ranging from 2 weeks to 2 years, and implant duration ranging from 1 week to 2 years were included in this study. Samples for analysis were collected at autopsy (n=5) or following surgical revision (n=3). Surgical repair sites included aortic arch (n=4), mitral valve (n=2), pulmonary artery (n=1), pulmonary valve(n=1), and a right ventricle to pulmonary artery conduit (n=1). All tissue samples were formalin fixed, paraffin embedded and stained with H&E, VVG elastin, Movat pentachrome, CD31, CD3, CD68, vimentin, and smooth muscle actin (SMA).
Results: In all specimens, the ECM was composed of dense laminated regions of collagen, without appreciable elastin staining. Interestingly, in most ECM grafts, especially those implanted for extended periods of time, a 'neointima' and 'neoadventia' had formed and covered the intimal and adventitial surfaces of the ECM graft. This neointima consisted of spindled myofibroblasts (SMA) and occasional mononuclear cells in a matrix composed of collagen, glycosaminoglycans, and rarely elastin. These

features were readily identified in patients as early as one month after ECM implantation. Moreover, the matrix comprising the ECM itself remained largely acellular, despite implantation times up to 2 years.
Conclusions: We provide a basic framework for histologic expectations when evaluating explanted ECM grafts. In this regard, the ECM matrix is likely to remain acellular without significant elastin deposition, whereas the intimal and adventitial surfaces become coated by proliferating cells in a novel matrix of collagen and glycosaminoglycans.

300 Optimization of Serum Free Light Chain Analysis for Rapid and Reliable Subclassification of Cardiac Amyloidosis

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Background: Accurate classification of cardiac amyloidosis, between transthyretin and light chain kappa or lambda, is paramount for optimal patient management. However, direct subtyping of amyloid deposits has significant drawbacks, such as long turnaround time, high cost, and in some cases, the need to send the material to an outside reference laboratory. Potential differences in serum free light chain kappa/lambda ratios may provide a method to differentiate types of cardiac amyloidosis. However, the standard limits of the assay are not optimized for cardiac amyloidosis classification. Therefore we investigated optimization of the kappa/lambda serum free light ratio values as a novel method to accurately and rapidly subclassify cardiac amyloidosis.
Design: We investigated 78 cases of tissue proven cardiac amyloidosis (endomyocardial biopsy: n=65, ventricular septal myectomy: n=1, ventricular apical core: n=1, explanted heart: n=9, and autopsy heart: n=2) at two separate medical centers. All patients had serum free light chain analysis obtained prior to plasma cell neoplasm treatment in conjunction with routine classification of cardiac amyloidosis (mass spectrometry n=51, or immunofluorescence n=27). The cases were then stratified based on the ratio of kappa to lambda serum free light chains and that value was correlated to their type of cardiac amyloidosis.
Results: The serum free light chain kappa/lambda ratios were non-overlapping for the three types of amyloid identified: AL-lambda (0.01-0.41, n=29), ATTR (0.63-2.7, n=38), and AL-kappa (6.7-970, n=11). A kappa to lambda ratio value between 0.5 and 5.0 had 100% sensitivity and 100% specificity for distinguishing transthyretin from immunoglobulin light-chain amyloidosis (n=78, 95% confidence interval: 89%-100% for both sensitivity and specificity). The capacity of this test to distinguish between forms of amyloidosis was equivalent for cases subtyped by either mass spectrometry or immunofluorescence.
Conclusions: Optimized ranges for serum light chain kappa/lambda ratio can provide extremely robust classification of cardiac amyloidosis. The assay is widely available, relatively inexpensive and can deliver accurate, rapid results. Cases of cardiac amyloidosis in which the kappa/lambda free light chain ratio falls close to these new cut-off values may benefit most from direct amyloid subtyping.

301 Pathology of the SynCardia Total Artificial Heart: A Case Series

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Background: The SynCardia Total Artificial Heart (TAH, SynCardia Systems, Inc.; Tucson, Ariz) has been implanted worldwide in more than 1,000 patients with biventricular heart disease. The device is an implantable, pneumatically-driven pulsatile pair of pumps consisting of polyurethane ventricles, whose inflows are anastomosed to the left and right atria and whose outflows are anastomosed to the ascending aorta and pulmonary artery after complete removal of the native cardiac ventricles and all four valves. Medtronic-Hall mechanical valves on the inflow and outflow aspects of the pumps ensure unidirectional flow. Systemic infection and thromboembolic or hemorrhagic events have been reported as the most common complications that prevent successful bridge to transplant. There are no published data on the pathological findings associated with TAH explants to correlate with potential clinical complications.
Design: We reviewed the gross and microscopic findings in five (5) TAHs at the time of explant or autopsy along with clinical information.
Results: The mean age of the patients (all male) was 52.8 years (range 39 to 68), with a median support time of 247 days (range: 127-341 days) on the TAH. Two patients had amyloid heart disease, while one each had valvular heart disease, repaired congenital heart disease and allograft vasculopathy; all had biventricular heart failure as the indication for TAH. Four patients (80%) survived to transplantation. One patient with a TAH died from Klebsiella device infection, pneumonia and sepsis. In four of the five devices, thrombosis was present at the junction of the right and left atrium with the atrial sewing cuff of the TAH on the luminal surface, ranging from 0.6cm to 6.5cm in size along the circumference of the anastomosis. A small laminated thrombus was present in the subpulmonic area in the autopsy case. One patient had a radiologically confirmed cerebrovascular accident on the TAH and one patient had a pulmonary embolism confirmed at autopsy. All atrial cuffs showed near transmural necrosis and mummification, with some viable myocardium in the subendocardial region. There was no evidence of dehiscence in any device.
Conclusions: Thrombosis at the atrial-device interface and devitalization of the atrial myocardium were the dominant findings in the TAHs. This is the first study to report pathologic findings in SynCardia TAHs, and may serve as a guideline for surveillance and management for those awaiting a heart transplant on these devices.

302 Pulmonary Vascular Changes After the Fontan Operation: A Series of 84 Cases (1978-2012)

Joseph Kennedy, Ahmed Fayyaz, Sarah Jenkins, William Edwards, Joseph J Maleszewski. Mayo Clinic, Rochester, MN.
Background: Patients that have undergone a Fontan atriopulmonary anastomotic operation for repair of congenital single functional ventricle have anecdotally been observed to have changes in pulmonary vessels and parenchyma. To date, no study has systematically evaluated the histopathological features of the lungs in a large cohort of these patients.
Design: Tissue registry archives were queried for all autopsy cases of individuals that had undergone Fontan repairs. Eligibility criteria included having lung tissue available for review as well as corresponding clinical and hemodynamic data. Demography and interval times between establishment of Fontan circulation and death were recorded. Paraffin blocks from bilateral upper and lower lobes in each case were cut at 4 μm and stained with hematoxylin-eosin and Verhoeff-van Gieson. Medial hypertrophy of muscular pulmonary arteries (200-600 μm diameter) was graded relative to overall vascular diameter (<10% normal, 10-20% mild, 20-25% moderate, and >25% severe). Histomorphologic features of elastic arteries, small vessels (<100 μm diameter), capillaries, lymphatics, alveoli, interlobular septa, and veins were also recorded. Findings were compared with Fisher's exact tests.
Results: 84 cases met inclusion criteria (mean age 11 years, 60% men). 31(37%) cases exhibited moderate to severe hypertrophy of muscular pulmonary arteries in at least one lobe. Among these, only 5(16%) cases had these changes reflected in all lobes. The severity of arterial medial hypertrophy was discordant between the two lungs in 17 cases, such that one lung had moderate to severe disease and the other lung was normal or had only mild disease. 27(32%) patients had no muscular arterial medial hypertrophy in any lobe. When compared with patients that died within 1 day of their operation, those that lived >2 years after surgery more frequently displayed arteriolar hyalinosis (43% vs 5.0%, p=0.009) and fibrosis of the interlobular septa (57% vs 5.0%, p=0.0005).
Conclusions: Among patients surviving a Fontan operation, pulmonary vascular changes vary considerably between lobes and between patients, suggesting that the findings may not be due to the operation alone or that arterial kinking/anastomotic narrowing may contribute to the regional differences. Furthermore, hyalinosis of small vessels and marked fibrosis of interlobular septa represent relatively late manifestations of pulmonary vascular and interstitial remodeling that have not been previously reported. Additional study is needed to determine why such changes occur and how they affect long-term patient outcome.

303 Recurrence of Amyloid in Endomyocardial Biopsies Following Orthotopic Heart Transplantation

Priyadarshini Kumar, Daniel Luthringer. Cedars-Sinai Medical Center, Los Angeles, CA.
Background: Orthotropic heart transplantation (OHT) is an effective treatment modality for cardiac amyloidosis. Amyloid recurrence in the graft remains a problem and its detection is critical to therapy and prognosis. However, the rate and timing of amyloid recurrence in the donor heart are not widely known due to the few cases treated by this modality. Furthermore, histologic detection of amyloid can be challenging, especially when present in small amounts on H&E stained sections. The premise of our study was to analyze sequential endomyocardial biopsies (EMBs) from a cohort of patients transplanted for amyloidosis, to assess overall rate and timing of histologic amyloid recurrence, and determine if routine use of Congo red (CR) stain enhances detection of recurrent disease in EMBs.
Design: Under IRB approval, clinical and pathological records were reviewed to identify patients who underwent OHT for cardiac amyloidosis at Cedars-Sinai Medical Center between September 2006 and January 2013. EMBs done as part of routine protocol transplant rejection surveillance were selected and the pertinent data recorded. As amyloid recurrence is considered highly unlikely in the immediate post-transplant period, only those EMBs performed beyond 6 months of OHT were utilized for the study. All H&E slides were carefully reviewed by two pathologists for deposits of recurrent amyloid. Furthermore, a CR stain was performed on each of the study EMBs and reviewed under polarization microscopy for any evidence of recurrent disease.
Results: Twelve OHT patients performed for cardiac amyloidosis were identified with a follow up longer than 6 months. Amyloid subtypes included 6 AL, 3 hereditary and 3 senile types. Ten patients were male, 2 female, with an age range of 53-79 (mean age 68.4 years). The duration of follow up post - OHT ranged from 8.6 to 23.3 months (mean = 13.02 months). Of a total of 150 EMBs, 43 were performed beyond 6 months of OHT; these composed the study cases. These EMBs ranged from approximately 6 months to 23.3 months post OHT (mean = 11.23 months). Careful histologic review of these EMBs showed no morphologic evidence of amyloid. Congo red showed no positive staining in any case, and polarization microscopy showed no areas of "apple-green" birefringence.
Conclusions: Histologic recurrence of amyloidosis in the cardiac graft following OHT does not occur in the first year post orthotopic heart transplantation. Furthermore, Congo red stain is not useful in detecting amyloid deposits not seen by H&E staining.

304 CAV and Vascular Inflammation as Predictors of Cardiac Related Death in Cardiac Allograft Recipients: An Autopsy Series

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Background: With improved early outcomes in cardiac transplantation, coronary artery vasculopathy (CAV) is now a major limiting factor in survival. No definitive criteria exist for evaluation and early diagnosis of CAV. Development of histopathologic criteria to recognize CAV at an early stage is urgently needed to further prolong allograft survival.

In this autopsy study we reviewed allograft hearts as well as pre-mortem clinical data from an autopsy series with the aim to identify possible histologic or immunologic criteria that may aid in the early diagnosis of CAV.

Design: Autopsy archives were searched for cardiac transplant patients who died between 2000-2013. A total of 22 cases with complete clinical history were identified. In these patients, all episodes of clinically significant (2R or 3R) acute cellular rejection (ACR), antibody mediated rejection (AMR), and donor specific antibodies (DSA) were tallied. Autopsy slides were examined for the presence of CAV and inflammation in epicardial and intramural blood vessels. CAV was graded based on the degree of vascular occlusion by concentric non-atherosclerotic intimal hyperplasia and inflammation was semi-quantitatively graded as mild, moderate, or severe.

Results: Of 22 patients, 15 died of cardiac causes and 7 of non-cardiac causes. There was no difference in the incidence of ACR or DSA positivity between the two groups. Patients with cardiac causes tended to have a higher incidence of AMR (table 1). Patients who died of cardiac causes had (a) a higher incidence of moderate to severe inflammation or CAV in epicardial blood vessels and (b) a higher incidence of mild to severe CAV in intramural vessels. While a trend towards increased intramural blood vessel inflammation was also noted in this group, it did not reach statistical significance.

	Cardiac Death	Non-Cardiac Death	p value
ACR	60%	57%	1.000
AMR	40%	0%	0.1206
DSA	13%	14%	1.000
Epicardial Vessel Inflammation ≥ Moderate	53%	0%	0.0225
Epicardial Vessel CAV ≥ Moderate	93%	29%	0.0043
Intramural Vessel Inflammation	40%	0%	0.1206
Intramural Vessel CAV	60%	0%	0.0167

Conclusions: 1. Cardiac death in heart transplant patients is associated an increased incidence of CAV in intramural vessels.

2. Evaluation of intramural blood vessels for intimal injury in biopsy specimens may predict future risk of cardiac death.

These findings should be further validated in a larger autopsy series with concurrent review of sequential pre-mortem biopsies over the life of the graft.

305 Clinico-Pathological Assessment of Left Ventricular Non-Compaction Cardiomyopathy in End Stage Heart Failure Patients Undergoing Orthotopic Heart Transplantation

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Background: Previous studies reported that left ventricular non-compaction (LVNC) is a cardiomyopathy (CMP), familial or sporadic, arising from arrest of the normal process of trabecular remodeling during embryonic life. The diagnosis is usually made by echocardiography, but to date, there has been little research on the occurrence and clinico-pathological features of LVNC in the explanted hearts of orthotopic heart transplant (OHT) recipients.

Design: The clinical, echocardiographic and pathologic findings were reviewed for evidence of LVNC in 57 patients with end stage heart failure (HF) undergoing OHT. Histologic studies graded semi-quantitatively remodeling parameters of fibrosis (F) (reactive and replacement), myocyte hypertrophy (H) and myocytolysis (M) in left ventricle (LV), right ventricle (RV), interventricular septum (S) and atria (A), Grades 0, negative; 1, mild/occasional foci; 2, moderate/multiple foci; 3, severe/extensive, and total sum (*Segura AM et al. Cardiovasc Pathol 2011; 20:139-45*). Absolute measurements of non-compacted (NC) and compacted (C) portions of the LV wall and NC/C ratios were calculated.

Results: LVNC was observed in 0 of 29 ischemic CMP (ICMP) and in 3 of 28 (10.7%) non ischemic CMP (NICMP) patients- 2 men, 1 woman, mean age ±SEM, 38±8.1 years. The echocardiogram disclosed marked LV dilatation, prominent trabeculations without hypertrophy, positive for LVNC by Stöllberger criteria (*Stollberger C et al. J Am Soc Echocardiogr 2004; 17:91–100*) and LV ejection fraction (EF) <20%. Mural thrombus was seen in 2/3 patients (66.7%). The heart weight mean ± SEM was 510.7±49.8 (range, 428-600 gm), NC was 25.7±6.4 mm, C was 16+3 mm, NC/C ratio was 1.6/1.0±0.4. The H, M, F total scores were LV 7.5±0.2, S 6.5±0.5, RV 6±0.6, A 6.5±0.4.

Conclusions: LVNC is an unusual form of NICMP in patients suffering from end stage HF undergoing OHT. Quantification of the extent and severity of fibrosis, hypertrophy, and myocytolysis, using a semi quantitative grading scale helps determine histopathologic features in these patients. Further studies in larger series, correlating the anatomo-clinical variables would improve our understanding of LVNC as a cause of advanced HF leading to OHT.

306 Left Ventricular Scar and Sudden Death in Competitive Athletes: The Diagnostic Role of Molecular Autopsy and Cascade Family Screening

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Background: Arrhythmogenic cardiomyopathy (AC) is a major cause of sudden cardiac death (SCD) in young people. In the recent years a variant mostly involving the left ventricle (LV AC) has been increasingly described. In this setting, differential diagnosis between LV AC and chronic myocarditis is mandatory. However, the lack of gold-standard diagnostic criteria both in vivo and at post-mortem explains the failure of timely identification at pre-participation screening and the difficult recognition even at autopsy.

Design: Among 75 competitive athletes dying suddenly, 15 athletes had a non-ischemic LV fibro-fatty scar, with a subepicardial/midmural distribution, in the absence of wall thinning/aneurysm. JUP and N-cadherin immunohistochemistry (dilution 1:50000, 1:1000 respectively) was performed. According to the recent guidelines for SCD, molecular investigation by PCR analysis on samples of myocardium and spleen, to exclude viral infection, and genetic screening for all major disease-causative genes (*desmoplakin-DSP, plakophilin2-PKP2, desmoglein2-DSG2, desmocollin2-DSC2, plakoglobin-JUP, desmin-DES, aT-catenin-CTNNA3*) were carried out. Cascade family screening was performed in genotype positive athletes.

Results: The 15 athletes with LV scarhad inverted T wave in V5-V6 in 47%, low QRS complex in 27%, left/right bundle branch block ventricular arrhythmias in 15% of cases. Molecular investigation was feasible in 10 due to appropriate autoptic material for DNA isolation. PCR and RT-PCR for cardiotropic viruses genomes were negative in all. Genetic screening identified 7 pathogenic nucleotide variants (2 in *PKP2*, 2 in *DSP*, 1 in *DSG2*, 1 in *JUP* and 1 in *DSC2*) in 5 of the 10 athletes (50%), two of whom resulted to be compound heterozygote carriers. Reduced immune-reactivity of JUP was observed at the intercalated discs in 5 of the 10 athletes (50%), 3 of which were positive and 2 negative at genetic screening. Cascade family screening identified 17 asymptomatic carriers; contrast enhanced CMR was performed in 8 and evidenced LV involvement in all.

Conclusions: Non ischemic LV scar is a no-so rare finding in young athletes dying suddenly. Viral genome search and genetic test are mandatory for differential diagnosis with chronic myocarditis. A final diagnosis of LV AC is achieved through molecular autopsy and cascade family screening. The identification of pathogenic mutations in half of cases reproduces the diagnostic yield of genetic screening in AC and allows the early recognition of asymptomatic carriers for SCD prevention.

307 CD123 (IL3-Receptor) Identifies Antigen Presenting Cells in Cardiac Sarcoidosis and Eosinophilic Myocarditis But Not in Idiopathic Giant Cell Myocarditis

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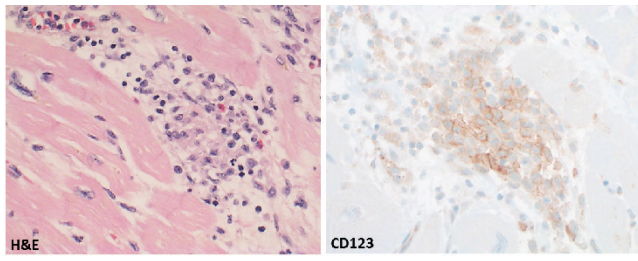
Background: Antigen presenting cells (APCs) are key immune regulators. The main classes of APCs are macrophages and dendritic cells. Macrophages are a known component of inflammatory infiltrate in idiopathic giant cell myocarditis (IGCM) and sacoidosis with other APCs (plasmacytoid (PDCs) and myeloid dendritic cells (MDCs)). To date, the role of APCs in inflammatory disease of myocardium has not been studied.

Design: Archives (2000-2014) of Tufts Medical Center were searched for cases of inflammatory cardiomyopathy and sarcoidosis. We identified 7 idiopathic giant cell myocarditis (IGCM), 9 cardiac sarcoidosis (CS), 3 eosinophilic myocarditis (EM) and 1 myocarditis NOS (M) (14 men, 9 women; mean age 52, range 25-73); 15 cases were biopsies, 7 heart explants and 1 autopsy. The inflammatory process was evaluated by morphology and immunohistochemistry (IHC) for CD123 (for PDCs), CD1c (for MDCs), CD68 (for macrophages), CD3, CD4, CD8, Ki67, CD117 and CD34. Evaluation of IHC was semi-quantitative using a 0-3 scale (0 = weak staining and/or <5% cells; +1 = scattered single cells and <5% cells; +2 = moderate staining in loose aggregates and >5% cells; +3 = strong staining in tight clusters and >5% cells). 8 cases of extra-cardiac sarcoidosis were controls.

Results: All cases of CS were positive for CD123 (+2-3 in 7, +1 in 2 cases). Staining was present in the granulomas and in scattered cells within the inflammatory infiltrate.



EM was variably positive (+1 and +3 in 2 and 1 case respectively) and M-NOS showed +3 staining. IGCM did not show significant CD123 positive cells, only rare scattered +1 positive cells in 2 cases. CD1a positive MDCs were seen in a single case of EM. CD68 identified macrophages in the IGC; all cases showed presence of variable CD4 and CD8 positive T-cells. No CD34 or CD117 positive cells were present. All pulmonary and cutaneous sarcoid cases were strongly (+3) positive for CD123.

Conclusions: PDCs and CD123 positive cells are components of granulomas in CS but are not significant in IGCM. Presence of CD123+ cells and PDCs in clusters and tight aggregates supports hypersensitivity related process in cardiac biopsies and can be used to differentiate from IGCM.

308 Transvenous Laser-Assisted Lead Extraction: A Pathologic Study of 287 Cases

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Background: Placement of cardiovascular implantable electronic devices has grown over the years. This is accompanied by increasing numbers of lead extractions, using techniques such as laser-assisted transvenous removal. The most common reasons for removal are infection, malfunction, and device upgrade. The use of laser sheath has improved the efficacy of extraction with major complication rates between 1- 3%. Major complications include bleeding and death from vascular perforations and tears.

The clinicopathologic findings in consecutive lead extraction cases over 18 months in a high-volume tertiary referral center are evaluated to document the presence of vein tissue and its correlation with adverse events.
Design: Consecutive patients who had undergone laser-assisted lead extraction with specimens submitted for pathologic evaluation were included. All tissue present on the lead underwent histologic evaluation with a Movat stain. The electronic medical record was reviewed to identify any adverse event associated with the procedure.
Results: Between March 2013 and August 2014, 287 consecutive cases of transvenous lead extraction submitted for pathologic evaluation were identified. Mean patient age was 62.9 years old with a male-to-female ratio of 3.9:1. Tissue cuffs were present on the leads and were evaluated histologically in 184 patients (64%). Vein tissue was identified in 45 cases (16%). In cases with vein tissue present, the most common indications for the procedure were infection/bacteremia, device upgrade, and malfunction. Adverse events associated with extraction in cases with vein tissue were identified in 16 of 45 cases (36%). The most common events were bleeding requiring transfusion (n=4), severe pain (n=4), hematoma at surgical site (n=4), hypotension (n=3), and small pericardial effusion not requiring pericardiocentesis (n=1). Major complications requiring emergent surgical intervention or procedural deaths were not identified in this series.
Conclusions: Transvenous laser sheath-assisted lead extraction is a technically challenging procedure with potentially fatal complications. Vein tissue was identified on histologic evaluation of peri-lead tissue cuffs in 16% of cases. The presence of vein tissue was associated with minor complications in 36% of cases but not with mortality in this series.

309 Osseous and Chondroid Metaplasia in Tri- and Bicuspid Aortic Valves Resected for Calcific Stenosis

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Background: Aortic valve (AV) replacement for calcific aortic valve stenosis (CAVS) is one of the most common cardiac procedures. However, the underlying pathophysiology of CAVS is unclear and controversial. We correlated the pathologic and clinical characteristics of AVs excised for CAVS that also demonstrate osseous metaplasia (OM) and chondroid metaplasia (CM).
Design: A retrospective analysis was performed for 6688 native AVs excised for CAVS at a single institution from 1987 to 2013. Excluded from analysis were cases with active endocarditis or with fragmented valves where cuspal number could not be evaluated. Patient demographics were correlated with histologic features diagnosed on paraffin embedded, formalin-fixed, decalcified H&E stained sections.
Results: Of the analyzed AVs, 1473 (22%) were bicuspid (BC), 5200 (77.8%) were tricuspid (TC), 13 (0.2%) were unicuspid, and 2 were quadricuspid. The M:F ratio was 1.4:1. The overall incidence of OM and/or CM in valves with CAVS was 15.6%. Compared to TC valves, BC valves had a higher incidence of OM and/or CM (30.1% vs 11.5%, Z=17) and had an earlier mean age of excision (60.2 vs 75.1, p<0.001). Additionally, the frequency of OM and/or CM increased with age at time of excision of BC valves (19.7% age <50, 27.7% age 50-59, 33.5% age 60-69, 34.2% age 70-79, 39.3% age 80-89), while TC valves showed the same incidence regardless of patient age (12.7% age <50, 12.5% age 50-59, 11.4% age 60-69, 10.9% age 70-79, 12.1% age 80-89, 10.7% age 90+). Male gender was associated with increased incidence of OM an/or CM in both BC (33.5% vs 22.3%, Z=4.26) and TC valves (13.8% vs 8.6%, Z=5.84) and with earlier age of excision (BC: 59.4 vs 62.3 years, p<0.0001; TC: 74.3 vs 76.1 years, p<0.0001). OM and/or CM was also more common in males with BC valves with concurrent chronic kidney disease (CKD) or atherosclerosis than in those without CKD or atherosclerosis (37.8% vs 30.9%, Z=2.29).
Conclusions: OM and CM are common findings in native AVs excised for CAVS, but are more than two-fold more frequent in BC valves. Furthermore, there is a higher incidence of OM and/or CM at earlier age in BC compared to TC valves, suggesting that BC valves have an inherent susceptibility (mechanical effects versus intrinsic molecular phenotype of the valve cells) to metaplasia. This is supported by the increase in incidence of OM and CM in BC valves with increasing age. This metaplastic process might be moderated by sex hormones and presence of concurrent CKD or atherosclerotic disease.

310 Attenuation of Neointima Hyperplasia Without Thrombosis By a Small Molecule Inhibitor of the Store-Operated Channels

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Background: Stromal interaction molecule 1 (STIM1) is associated with neointima hyperplasia by mediating store operated Ca^{2+} entry (SOCE) in multiple cell types. We tested whether a small molecule SOCE inhibitor could prevent neointima formation and vascular stenosis using 2-aminoethyl diphenylborate (2-APB).
Design: Vascular smooth muscle cells (VSMCs) were isolated from rat aortas or rabbit common carotid arteries. VSMC proliferation was induced by 5% fetal bovine serum and determined using the propidium iodine (PI)/5-bromodeoxyuridine (BrdU) double-staining method. Neointimal hyperplasia and vascular stenosis were induced in rabbit carotid arteries by air-dry injury and 2-APB was applied to the extracellular surface of the injured vessels.
Results: SOCE in the VSMCs was inhibited by 2-APB accompanied by cell cycle arrest in the G1 phase. In the air dry artery injury model, 2-APB effectively blunted neointima formation with a potency comparable to rapamycin. Intriguingly, 2-APB attenuated leukocyte infiltration by perturbing the degranulation process, preserving integrity of the vascular internal and external elastic lamina. Likewise, the SOCE inhibitor prevented platelet activation and fibrin accumulation by preventing leukocyte activation. More importantly, 2-APB did not seem to induce thrombosis, a known fatal complication of public concern caused by sirolimus-eluting stents.
Conclusions: Small molecule SOCE inhibitors like 2-APB may have protective effects in intimal thickening.

311 Does the Type of Mechanical Circulatory Support System Have a Predictive Value for the Outcome of the Cardiac Allograft With Regard To Antibody-Mediated Rejection?

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Background: As the use of mechanical circulatory support (MCS) is constantly increasing due to the decreasing number of available donor organs, its possible effects in terms of allosensitation are of growing importance in the routine diagnostic workup for antibody-mediated rejection in cardiac allografts. We investigated the hypothesis that the type of MCS device used may influence the outcome of the cardiac allograft in terms of intramyocardial capillary C4d depositions.
Design: We evaluated right ventricular endomyocardial biopsies (EMBs) of two consecutive years (2011-2012) taken from 82 patients with MCS treatment prior to heart transplantation (MCS group) and 138 patients without MCS treatment (non-MCS group). The MCS patients were subdivided into groups with a minimum of five devices per group (HeartMate II, HeartWare, Novacor, Incor, Excor).
The diagnostic workup included conventional histology and immunohistochemistry (C4d) on formalin-fixed paraffin-embedded tissue sections. The differences in C4d depositions between groups were analyzed with Fisher's exact test. The variance of interference between groups was validated by generalized Cochran-Mantel-Haenzsel test.
Results: While there was a significant difference in C4d deposition between the overall MCS group (n=82) and the non-MCS group (p=0.021, odds ratio=0.524), the Excor patients (39/82) did not show an increase in C4d depositions (p=0.838, odds ratio=1.11). Patients with the remaining MCS types (43/82) showed signs of antibody-mediated rejection (AMR) in the form of C4d depositions in significantly more cases than the non-MCS group (p=0.003, odds ratio=2.97).
Conclusions: Our results suggest that not the MCS treatment as such, but the type of device used, may influence the outcome of cardiac allografts with regard to AMR, which may be due to allosensitation to the different device materials.

312 Myocardial Metal Levels in the Setting of Total Joint Arthroplasty: A Study of 94 Cases With Establishment of Normative Range

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Background: Orthopedic joint implants commonly contain elemental metal such as cobalt (Co), chromium (Cr), and vanadium (V) that may undergo wear-corrosion release. Although periprosthetic reaction to metallic debris is well described, systemic distribution and adverse effects remain poorly understood. Recently, cases of implant-associated myocardial injury have been reported; however, no study has systematically measured metal levels in heart tissue or examined their relationship with joint replacement.
Design: Tissue registry archives of Mayo Clinic (Rochester, MN) were queried for autopsies of individuals that had undergone hip, knee or shoulder replacement (1990-2011) from which formalin-fixed paraffin-embedded (FFPE) myocardium was available. 80 non-arthroplasty, age- and sex-matched controls were also obtained. Demography, implant type, and the presence of heart disease were abstracted from the medical/autopsy record. Samples were acid-digested using closed vessel microwave digestion, diluted with standards, and analyzed for Co, Cr, and V by inductively coupled plasma mass spectroscopy. Wilcoxon rank-sum and chi-squared tests were used to assess differences between cohorts.
Results: 94 arthroplasty cases met inclusion criteria (mean age 77.4 years; 53.2% men). 77 (81.9%) cases had at least one hip replacement at the time of death, while 13 cases had knee-only and 4 had shoulder-only replacements. All hip implants had a Co/Cr femoral head component, but none were metal-on-metal. Significantly higher concentrations of Co were observed in individuals with implants compared to controls (median 0.105 vs. 0.0771 μg/g, p=0.0032), while Cr and V were similar. Significant difference between concentrations of Cr or V was not observed. Median Co concentrations were was 60% higher in patients with hip revision vs no revision (p=0.008). In general, the highest Co levels were observed in those with multiple replaced joints. Cardiomegaly and interstitial fibrosis were observed more frequently in those with implants (p=0.0008 and p=0.015, respectively).
Conclusions: This is the first study to quantify metal levels in cardiac tissue in patients with and without joint replacement. The elevated Co levels, in concert with cardiomegaly and increased interstitial fibrosis in the arthroplasty cohort are novel, important findings. Although Co levels were significantly elevated above controls, they were below that seen in cases of clinical cobalt cardiotoxicity associated with metal-on-metal prosthetics. Additional study is needed to more fully characterize the clinical implications of these findings.

313 Evaluation of the Cardiac Conduction System in a Blended (Medical-Forensic) Autopsy Practice: A Review of 215 Cases (1994-2013)

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Background: Evaluation of the cardiac conduction system (CCS) may be performed in the setting of sudden death or to confirm known or suspected disease involvement. The former is usually done in the context of out-of-hospital cardiac arrest or unwitnessed death (forensic autopsy), while the latter within the context of a medical autopsy.

There is, however, significant practice variability, with such examination becoming increasingly uncommon in routine practice. This has been attributed to a lack of consensus on when to evaluate the CCS, a lack of data on pathogenicity of findings, and a perception of low diagnostic yield. In retrospective review, we sought to examine the rationale for CCS examination in a large blended autopsy practice and evaluate the overall diagnostic yield.
Design: Archival databases of the Mayo Clinic Tissue Registry (Rochester, MN) were searched for cases in which the cardiac conduction system was evaluated. Reasons for evaluation, demography and histopathologic findings were recorded. Whether or not the findings were deemed contributory to cause of death was also noted.
Results: Of the 9290 complete autopsies performed, 215 cases (2.3%) had CCS examination. Mean age was 53.9 yrs (range 0.2-98 yrs) and 127(59.1%) were men. There were 43(0.5%) cases of sudden death without an anatomic substrate, of which 22 were infants and 9 were epileptic deaths. Of the remaining 12 cases of undetermined death, 9(75%) had CCS examination, 2 of which had pathology believed to contribute to death. There were 79 cases where the CCS was submitted because of clinical history of arrhythmia or systemic disease that may involve the CCS. For this cohort, 37(46.8%) cases had CCS pathology believed to contribute to death. In the remaining 125 cases, the reason for submission was primarily educational. In this last cohort, 8(6.4%) cases had pathology that contributed to death. The most frequently encountered incidental finding was fibromuscular dysplasia of the AV nodal artery.
Conclusions: This represents the largest retrospective analyses of examination of the CCS to date. Determining whether histopathology of the CCS is likely to help in determination of cause of death can be guided by the clinical circumstances, with the highest yield in sudden unexpected adult deaths. Interestingly and unexpectedly, however, incidental findings within the CCS that likely contribute to the cause of death can be encountered with some frequency. More routine and widespread examination of the conduction system is necessary to help determine significance of findings and their overall bioepidemiology.

Cytopathology

314 Multi-Institutional Study of Fine Needle Aspiration for Thyroid Lymphoma

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Background: Primary thyroid lymphoma is a rare neoplasm accounting for 1-5% of thyroid malignancies. Although thyroid fine needle aspiration (FNA) biopsy is currently an extremely common procedure, little information exists on the accuracy and morphology of FNA cytopathology in thyroid lymphoma.
Design: Pathology databases at Washington University in St. Louis, Wexner Medical Center, and the Cleveland Clinic Foundation were searched for thyroid FNA biopsies having a diagnosis of lymphoma or atypical lymphoproliferative cells, or a corresponding tissue diagnosis of thyroid lymphoma having a prior FNA biopsy.
Results: Sixty-eight cases were retrieved from 64 patients from three institutions; 67 cases with histologic confirmation. Forty-six specimens were from women (68%), ages 21 - 87 years (mean= 60). A great majority of aspirates were surgically confirmed diffuse large B-cell lymphoma (DLBL) (n=43), followed by classical Hodgkin lymphoma (5), chronic lymphocytic leukemia (5), high grade non-Hodgkin lymphoma (3), follicular lymphoma (2) and single cases each of mantle cell lymphoma, Burkitt lymphoma, 'double-hit' lymphoma, mucosa-associated marginal zone B-cell lymphoma, low grade non-Hodgkin lymphoma, and a plasma cell neoplasm. Fifty-one cases were in patients with no prior history of lymphoma. Light chain restriction was detected in 34 specimens (by FCM in 32 cases or PCR, 2 cases). FCM was polyclonal (n=7) or inconclusive (2) with a 25 cases not having FCM performed or not having enough viable cells for evaluation. Four cases showed lymphocytic thyroiditis on surgical follow-up with 2 of these cases having a small monoclonal lymphoid population detected by flow cytometry. Forty-seven aspirates were diagnosed as lymphoma (n=29) or suspicious (n=18) for lymphoma (sensitivity 73%), 11 atypical, 7 benign, 2 unsatisfactory, and 1 suspicious for carcinoma.
Conclusions: An FNA diagnosis of lymphoma or suspicious for lymphoma was most often encountered in women, and was possible in over half of our cases. DLBL was the most common form of thyroidal lymphoma in this series (63%). 7% of aspirates were mistakenly interpreted as lymphocytic thyroiditis.

315 Lower Endoscopic Ultrasound-Guided Fine-Needle Aspiration: A Useful Diagnostic Tool for Perirectal and Rectal Lesions – A Large Series in a Single Tertiary Referral Hospital

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Background: Lower Endoscopic Ultrasound-Guided Fine-Needle Aspiration (LEUS-FNA) of rectal /perirectal lesions is a safe, minimally invasive, and well tolerated procedure that provides valuable information which affects patient management. EUS-FNA is useful to demonstrate or rule out cancer recurrence in patient with perirectal lesions and a history of cancer (evidence level 2+). Herein, we presented our experience of LEUS-FNA to evaluate pelvic (rectal/perirectal) lesions.
Design: LEUS-FNAs were retrieved from the archives of our University Hospital, from 2001- 2014. The cytopathology findings, corresponding histology, immunohistochemistry, and clinical data were collected. The sensitivity and specificity of LEUS-FNA were calculated in a subset of patients with available surgical pathology.
Results: 114 specimens were retrieved. Histopathology materiel obtained after LEUS-FNA were available in 37 patients. Masses measured 5–100 mm (mean: 27.5 mm) in diameter. Recurrent cancer was clinically suspected in 46% of cases (n=53). The aspirated material showed malignant (n=48), benign (n=41), atypical/suspicious (n=6) and nondiagnostic cytology (n=19). Malignant cases were adenocarcinoma (n=24), neuroendocrine tumor (n=3), squamous cell carcinoma (n=5), urothelial carcinoma (n=2), positive for malignant cells NOS (n=12), gastrointestinal stromal tumor (n=1) and non Hodgkin lymphoma (n=1). The benign cytology cases were negative for malignant cells (n=32), schwannoma (n=1), and 8 non neoplastic lesions including: abscess (n=3), endometriosis (n=2), hematoma (n=1), malacoplakia (n=1) and mucinous cyst (n=1). Histology confirmed 11/12 negative cytology; one false negative cytology of lymph node. Compared to surgical pathology, LEUS-FNA showed 91% sensitivity, 100% specificity, with diagnostic accuracy of 95%, a positive predictive value of 100% and a negative predictive value of 88%. Discrepancies were likely due to cytology sampling errors.
Conclusions: Lower EUS-FNA has an excellent diagnostic accuracy for lesions in the gut wall and surrounding tissues; LEUS-FNA has a high diagnostic accuracy for preoperative staging for rectal cancer and for early detection of recurrent local disease. Further more, LEUS-FNA allows cytological examination and ancillary studies (immunohistochemistry, flow cytometry), that are helpful for accurate determination of nature of pelvic masses in patient without a history of cancer.

316 Combining the Most Commonly Used Immunomarkers—TTF-1, Napsin A, CK7, CK5/6, and P63—in the Subclassification of Primary and Metastatic Non-Small Cell Lung Carcinoma (NSCLC): A Retrospective Study of 246 Fine Needle Aspiration Cases

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Background: Fine needle aspiration (FNA) biopsy plays a critical role in the diagnosis and staging of non-small cell lung carcinomas (NSCLC). The accurate subclassification of NSCLC into adenocarcinoma (ADC) and squamous cell carcinoma (SqCC) is crucial for targeted therapy. FNA specimens, which are often small and contain artifacts, pose a diagnostic challenge in distinguishing between ADC and SqCC. Although prior studies have addressed the performance of individual diagnostic markers, the evidence-based utility of these markers in combination has yet to be determined. Therefore, a restrospective study was performed to evaluate the utility of TTF-1, Napsin A, CK7, P63, and CK5/6, individually and in combination to subclassify primary and metastatic NSCLCs by FNA.
Design: A total of 246 FNA cases comprised of 102 primary NSCLC and 144 primary NSCLC metastases were identified by a medical record search over a two year period. The immunostaining patterns of TTF-1, Napsin A, CK7, P63 and CK5/6 were correlated with the morphological diagnosis of the tumor. The Bootstrap re-sampling approach was used to analyze the performance of individual markers and the combination of individual markers. A P value less than 0.05 was considered statistically significant.
Results: In 72 primary ADCs, TTF-1, Napsin A, and CK7 showed a sensitivity/specificity of 84.5%/96.4%, 92.0%/100%, and 93.8%/50.0%, respectively. In 30 primary SqCCs, P63 and CK5/6 showed a sensitivity/specificity of 91.7%/78.3% and 100%/77.8%, respectively. In 131 metastatic ADCs, Napsin A showed a significantly higher specificity (100%) than TTF-1 (87.5%) and CK7 (25%). In 13 metastatic SqCCs, CK5/6 showed a significantly higher specificty (84.6%) than P63 (68.4%). The combination of markers demonstrated an improved sensitivity and specificity in certain cases; however, TTF-1 and Napsin A in ADCs and CK5/6 in SqCCs performed acceptably individually.
Conclusions: This study demonstrates that TTF-1, Napsin A, CK7, P63 and CK5/6 have variable sensitivity and specificity in the subclassification of NSCLCs. Further, both sensitivity and specificity were increased by combining the interpretation of these individual markers in certain cases. Based on these results, it is recommended to use an algorithmic approach combining TTF-1, Napsin A, and CK7 alongside p63 and/or CK5/6 to subclassify NSCLCs by FNA.

317 Poor Cell Block Adequacy Rate for Molecular Testing Improved With the Addition of Diff Quik Stained Smears: Need for Better Cell Block Processing

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Background: As molecular assays improve and the number of targeted therapeutic agents increases, the clinical demand for ancillary molecular testing on small samples, including cytologic specimens, will continue to grow. Fine needle aspiration provides a minimally invasive source of tumor cells for molecular analysis, and this testing is typically performed on the paraffin-embedded cell block. However, there is currently a lack of standardization in cytologic specimen preservation as well as cell block preparation, and this may account for the fact that a substantial number of cell blocks contain a quantity of tumor cells insufficient for molecular analysis. The objective of this study is to assess the frequency and type of samples, including both surgical biopsies and cytologic material, deemed unsatisfactory for molecular testing in a large academic institution.
Design: A retrospective search of the laboratory information system was performed for all cases submitted for EGFR, KRAS, or ROS-1 molecular testing from Sep 2013 to Aug 2014. These cases included both primary and metastatic lesions of pulmonary adenocarcinoma, poorly differentiated non-small cell carcinoma, and large cell neuroendocrine carcinoma. The specimens consisted of surgical biopsies as well as cytologic material. The number of specimens deemed unsatisfactory for analysis was compared across four specimen categories: large biopsy, small biopsy, cell block alone, and cell block with air-dried Diff Quik stained (DQ) smears.