of malignant appearing calcifications was the highest among women diagnosed with ER+ /PR+/HER2+ (55%) followed by women diagnosed with ER-/PR-/HER2- (18%) (p=.032).

Conclusions: Among premenopausal women, extremely dense breast may be a risk factor for ER-/PR-/HER2+ subtype but not ER-/PR-/HER2- subtype. The prevalence of "malignant appearing calcifications" was the highest among ER+/PR+/HER2+ subtype followed by ER-/PR-/HER2- subtype.

273 Immunohistochemical Characterization of Hormone Receptor Negative (Triple Negative and HER2+) Breast Carcinomas

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Background: Hormone receptor negative breast cancers are focus of attention due to poor outcome. The histologic features overlap between ER-/PR-/HER2- tumors (triple negative, TNT) and ER-/PR-/HER2+ tumors (HER2+), with poorly differentiated grade as hallmark for both. Majority, but not all, TNT are regarded as basal-like based on HMWCK expression. It is not well known if some ER-/PR- tumors express androgen receptor (AR). We compared the immunohistochemical profile of TNT and HER2+ tumors for basal-like differentiation, AR expression, proliferative activity, and biologic behavior.

Design: 181 ER-/PR- breast cancers from 2001 to 2005 were grouped as TNT and HER2+ based on HER2 status (IHC +/-FISH). Immunostains performed on tissue microarray blocks were: CK5/6, CK8, AR, MIB-1, BCL-2, p53, C-KIT, Cyclin D1, and Vimentin. Cytoplasmic stain in >10% of the tumor cells was scored as + for CK 5/6, Vimentin, CK8, BCL-2, and C-KIT. Nuclear stain in >10% of tumor cells was scored as + for AR, p53, Cyclin D1, and MIB-1. Follow up data was collected for all cases.

Results: Among 181 tumors, 142 were TNT (78.5%, 67 node+, 22 mets, 14 deaths, 2 recurrences); 39 were HER2+ (21.5%, 18 node+, 5 mets, no death). CK 5/6 positivity was more frequent in TNT, 34 (23.9%, 12 node+, 9 mets) vs 1 (2.6%, 1 node+, no met) in HER2+ ($p<0.05$). Vimentin was more frequently positive in TNT, 54 (38.0%) vs 5 (12.8%) in HER2+. AR was rarely positive in either group, 1 (0.7%) and 2 (5.1%) respectively in TNT and HER2+. CK8 positivity was similar between TNT (46, 32.4%) and HER2+ (20, 51.3%). Increased p53 and MIB-1 expression was seen in both groups, 60 (42.2%, 21 node+, 19 mets) and 97 (68.3%, 25 node+, 20 mets) respectively in TNT, 16 (41.0%, 6 node+, 1 met) and 30 (76.9%, 13 node+, 5 mets) respectively in HER2+. BCL-2 positivity was seen in 21 (14.8%) TNT and 1 (2.6%) HER2+ ($p<0.05$). Cyclin D1 was detected in 8 (20.5%) HER2+, higher than those in TNT (6, 4.2%). HER2+ tumors were negative for C-KIT (0%), in contrast to TNT (20, 14.1%) ($p<0.05$).

Conclusions: 1). CK5/6 positive tumors were typically seen in TNT (23.9%) with exception of 1 in HER2+ group and were associated with adverse outcome. 2). AR was rarely positive in ER-/PR- tumors. 3). CK8 was positive in about half of tumors in both groups. 4). Increased proliferative activity (MIB1) and p53 mutations were seen in both groups and were associated with adverse outcome. 5). C-KIT, BCL-2, and cyclin D1 expression differences between two groups were not associated with adverse outcome.

Cardiovascular

274 Migration of Human Umbilical Cord Blood Monomuclear Cells for the Treatment of Acute Myocardial Infarction

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Background: Previous studies indicate that Human umbilical cord blood mononuclear progenitor cells (HUCBC) injected into infarcted myocardium of rats within 2 h or at 24 hours after left anterior descending coronary artery (LAD) occlusion resulted in significantly smaller infarction sizes 1 month later than the control injected saline group of rats. Experiments showed not only limitation to the infarct size at 2 and 12 hour post ligation. Herein we explore the both the localization of stem cells with in the infarcted myocardium as well as their tropism for specific tissues.

Design: Source of HUCBC: Cryopreserved (-196°) mononuclear fractions of HUCBC were given by Saneron CCEL Therapeutics, Inc. The LAD was permanently ligated in 4 rats, with 10 x 6 HUCBC in 0.5 ml of saline directly injected at the edge of the infarction zone at the apex as soon as the infarcted area could be seen. In two of the rats 0.5 ml of saline was injected at the edge of the infarction zone as soon as it was seen after ligation (roughly around 30-45 minutes). All the rats were evaluated for the presence of stem cells in the spleen, thymus and liver in both the control and treated lines along with any histological abnormalities. Stem cells were enumerated at 60x with the average whole number reported from 10 consecutive non-overlapping fields. Human stem cells were enumerated with the aid of the following immunohistochemical antibodies: CD117, CD-34 and HLA-A All slides were evaluated with adequate negative and positive controls by a single board certified pathologist.

Results: HLA-A (+), CD-117(+), CD-34 (+) HUCBC were seen within and adjacent to infarcted myocardium 4/HPF, as well as the spleen 6/HPF, thymus 12/HPF, and liver 2/HPF. The lung, brain, thyroid, pancreas, and soft tissues were essentially devoid of HUCBC. The spleen demonstrated stem cells at the red/white pulp interface, and the thymus showed preferential location at the cortical/medullary interface. Stem cells within the liver were present uniformly within the portal regions.

Conclusions: Stem cell homing to infarcted myocardium was demonstrating confirming previous studies. Significant numbers of stem cells were unexpectantly observed within the reticuloendothelial organs including the thymus, spleen, and liver. Interestingly, the stem cells were seen preferentially within the latter organs at the interfaces between B and T-cell lymphocytes zones. The mechanism(s) that underlie these observations are unknown yet could involve dendritic cell interaction of cytokine/surface receptor homing.

275 Pathologic Features of Hypertrophic Cardiomyopathy in Exertional and Non-Exertional Sudden Deaths

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Background: The pathologic features that characterize hypertrophic cardiomyopathy (HCM) in exertional vs. non-exertional sudden deaths have not been extensively studied.

Design: We prospectively performed gross measurements and histologic analysis on 107 autopsy cases of HCM and correlated them with clinical findings.

Results: There were 107 cases, separated into four groups: exertional sudden death (n=38), non-exertional sudden death (n=36), non-sudden deaths in patients with known HCM (n=14), and incidental HCM in patients dying of non-cardiac cases (n=19). Pathologic features of the 74 sudden deaths were compared between exertional and non-exertional deaths. Age at death was significantly lower in exertional (26.6 ± 13.6 years) vs. non-exertional sudden deaths (42.7 ± 15.0 years, $p<.0001$). There was no significant difference in the incidence of syncope in the exertional sudden deaths (27%) compared to the non-exertional sudden deaths (26%, p=0.8), or in the rate of a prior diagnosis of HCM (13% vs. 17%, respectively). The proportion of women was significantly less in the exertional sudden death group (7.9%) vs. the non-exertional sudden death group (36%, p=.01). The mean heart weight in men was significantly less in the exertional sudden deaths (521 ± 169 g) vs. the non-exertional sudden deaths (698 ± 190 g, $p<.001$). There was no difference in the proportion of hearts with septal: free wall ratios >1.3 (43%) in exertional vs. non-exertional (43%) sudden deaths, in macroscopic septal scarring (15% vs. 15%), or intramural coronary dysplasia (37% vs. 42%). There was a non-significant increase in myocardial bridging >3 mm (21 vs. 13%, p = 0.6) and left ventricular outflow tract plaque (58 vs. 38%, p=.06), respectively. By multivariate analysis, including all categories of HCM, only age (p=.002) and heart weight (p=.02) were significantly associated with exertional sudden death, both in an inverse relationship.

Conclusions: There are no pathologic features which would identify patients with HCM at risk for exertional death. Because young age and relatively low heart weight are strongly associated with exertional death, and because a high proportion of exertional sudden deaths with HCM are not associated with significant asymmetry, cardiologists should be careful in excluding the diagnosis of HCM in athletes with even mild degrees of cardiomegaly, especially young males.

276 Myocarditis in Arrhythmogenic Right Ventricular Cardiomyopathy Due to Desmosomal Gene Mutations: Is There an Infective Etiopathogenesis?

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Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited disease characterized by a gradual loss of myocytes and fibro-fatty replacement. Recently, mutations of gene encoding desmosomal proteins have been demonstrated in up to 50% of probands. Inflammatory infiltrates are identified in two-thirds of cases as to support an infective etiopathogenesis. The aim of this study was to assess the presence of viral genomes in the myocardium by molecular pathology investigation on hearts of genotyped ARVC patients.

Design: Ten ARVC hearts (8 male, 2 female, mean age 28 yrs), coming from either sudden death (7) or cardiac transplantation (3) were investigated. Genetic screening identified pathogenetic mutations in plakophilin-2 (5 cases), desmoplakin (3), desmoglein-2 (1) and plakoglobin (1). After gross examination, extensive sampling of both ventricles and septum was performed for histology and immunohistochemistry. Paraffin-embedded or formalin fixed myocardial samples were analysed by polymerase chain reaction for the presence of cardiotropic viruses, including adenovirus, herpes virus, influenza virus A and B, hepatitis C, enterovirus and parvovirus.

Results: At macroscopic examination, there was biventricular involvement in all (predominantly right in 3 and left in 1). At histology, fibro-fatty replacement with inflammatory infiltration were evident in all (100%). The latter was either diffuse (3, 30%) or focal (7, 70%), and mostly consisted of T-lymphocytes in 8 (80%) and was polymorphous in 2 (20%). Clear-cut evidence of myocyte necrosis was present in 3 (33%). Nucleic acids extraction was adequate in 9 (90%). Molecular investigation was negative in all but 1 case in which HCV was identified (10%)

Conclusions: Myocarditis is a usual feature in genotyped ARVC hearts, which are characterized also by biventricular involvement and fibrofatty replacement. On the opposite, viral genome is a exceptionally detected in the myocardium as to question a causative role of viruses and to support the view of myocarditis as a reactive phenomenon accompanying the injury and repair process of ARVC.

277 Morphologic Findings of Coronary Culprit Lesions in Premature Familial Sudden Coronary Death

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Background: The morphologic features of premature familial coronary artery disease are not known. The presence and type of coronary thrombus may have important implications in the genetic basis for familial heart disease.

Design: Autopsies of sudden coronary death (SCD) victims over a 5-year period from a statewide medical examiners office were studied. Coronary arteries were sectioned at 3-5 mm and every segment with narrowing of >50% was submitted for histologic evaluation. Familial disease was defined as sudden death at ≤50 years in women and ≤45 years in men, with premature SCD or acute coronary syndrome in a first-degree relative. Culprit lesion was defined as acute plaque rupture, plaque erosion, and severe narrowing without thrombus (stable plaque).

Results: There were a total of 441 hearts with sudden coronary death (SCD). There were 174 acute plaque ruptures (age 50 ± 10 years, 8% women), 40 plaque erosions (age 41 ± 9 years, 40% women), and 226 stable plaques (age 56 ± 13 years, 26% women). There were 8 men with plaque rupture and a family history (age 39 ± 6 years). There were 7 plaque erosions with family history (5 men and 2 women, age 35 ± 10 years). There were 7 stable plaques with family history (6 men and 1 women, age 35 ± 5 years). The related family member in plaque ruptures was the father (n=6), brother (n=1), and both parents (n=1); in plaque erosions, the father (n=4), mother (n=2), and grandfather (n=1); in stable plaques, the father (n=4), brother (n=2), and mother (n=1). One man dying with acute plaque rupture had a history of familial hypercholesterolemia. The rate of familial history in premature coronary disease was 17.5% in erosions, 4.6% in ruptures (p=.01 vs. erosion), and 3% in stable plaque (p=.002 vs. erosion).
Conclusions: The frequency of family history of premature sudden death due to CAD may be higher in plaque erosion as compared to patients dying with acute plaque rupture or without acute epicardial thrombi, and shows diverse hereditary patterns.

278 Cardiac Sarcoidosis and Sudden Death

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Background: There are few pathologic studies of sudden cardiac death (SCD) in patients with sarcoidosis.
Design: We retrospectively reviewed autopsy reports and pathologic sections from a statewide medical examiner's cases of SCD spanning 5 years. Any case with a clinical history of sarcoid or cardiac sarcoid at autopsy was included. Gross and histologic findings were compared in which the cause of death was attributed to sarcoid (group 1), cardiac sarcoid contributed to death (group 2), and cardiac sarcoid was incidental (group 3). Microscopic lesions were classified as acute (lymphocytic infiltrates with scattered giant cells, without well-formed granulomas), active (well-formed granulomas with multiple giant cells and minimal fibrosis), and healed (predominantly scars with occasional giant cells).
Results: 38 SCD victims with a history of sarcoidosis were identified. 12 patients had a history of sarcoidosis and 26 patients had sarcoidosis diagnosed at autopsy. Of the 12 with history, 7 (58%) died from cardiac sarcoidosis. Of the 33 patients with histologically documented cardiac sarcoid, the cause of death was attributed to sarcoid in 19 (58%, group 1), contributing in 7 (21%, group 2), and incidental in 7 (21%, group 3). In group 1, mean age at death was 42 years, 74% were men, 21% had a history of sarcoid, mean heart weight was 565 g, and mean left ventricular (LV) cavity diameter was 42 mm. These findings did not differ from all groups combined (mean age 43, 73% men, 21% with a history of sarcoid, 548 g mean heart weight, 42 mm mean LV cavity). Gross lesions secondary to sarcoid were found in 15 (45%). The incidence of gross involvement was significantly higher in group 1 (69%), as compared to groups 2 (29%) and 3 (0%, p=.001). Epicardial involvement was noted grossly in 58% and was significantly higher in group 1 (79%) as compared to groups 2 (29%) and 3 (29%, p=.0003). Microscopically, there was typically a coexistence of acute, active, and healed lesions, without any significant differences by group.
Conclusions: We conclude that SCD in patients with a history of sarcoidosis is caused by cardiac involvement in slightly over 50% of cases. In cases of SCD due to sarcoid, sarcoid is found at autopsy in 80% of cases. Cardiac sarcoid that results in SCD is more likely to be grossly visible than incidental cardiac sarcoid and more likely to demonstrate epicardial involvement. There are no significant differences in the degree of healed lesions or LV dilatation between incidental and fatal cardiac sarcoid.

279 WT1 Expression in Benign, Borderline and Malignant Primary Cardiac Vascular Tumors: Its Diagnostic Significance

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Background: Although primary cardiac vascular tumors are rare, angiosarcoma is the most common malignant neoplasm of the heart in adults. WT1 protein was recently suggested to be an important regulator in endothelial proliferation. We analyzed the immunohistochemical staining patterns of WT1 in 13 cases of benign, borderline and malignant primary cardiac vascular tumors that were seen and treated at one institution over the past eight years.
Design: Tissue microarray slides were prepared from representative tissue blocks of cardiac vascular tumors including angiosarcomas (10), unclassified vascular neoplasm of uncertain malignant potential (borderline) (1) and hemangiomas (2). Nine cases of normal heart tissue (9) were also included in the study for comparison. Routine hematoxylin and eosin and immunohistochemical (IHC) stains including WT1, CD31, CD34, FLI-1 and D2-40 were analyzed.
Results: All tumors were positive for CD31, CD34, FLI-1 and negative for D2-40. WT1 expression was consistently observed in all cases of angiosarcomas (10/10) with a diffuse cytoplasmic staining pattern. However, WT1 staining was exclusively negative in hemangiomas (0/2), unclassified vascular neoplasm of uncertain malignant potential (0/1) and benign blood vessels in normal heart tissue (0/9).
Conclusions: WT1 was invariably expressed in all cases of primary cardiac angiosarcomas and stained negatively in benign and borderline cardiac vascular tumors as well as normal blood vessels of the heart. The data suggest WT1 may play an important role in malignant transformation of endothelial cells in cardiac angiosarcoma. The consistent and strong WT-1 staining in angiosarcomas can be useful in helping to distinguish them from benign and borderline vascular tumors and other poorly differentiated neoplasms. However, a precaution is required because of the limited cases of benign and borderline cardiac vascular tumors available for the study. A careful light microscopic evaluation in conjunction with WT1 in addition to ordinary vascular markers will enhance our diagnostic strategy.

280 FOXP3-Expressing T-Regulatory Cells Increase with the Severity of Inflammation and Allograft Rejection in Heart Transplants

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Background: FOXP3-expressing $CD4^{+}CD25^{+}$ T cells (T-regs) are a subset of T lymphocytes that inhibit immune responsiveness and, thereby, control alloreactivity. In experimental models, tolerance of cardiac allografts can be both induced and maintained by intragraft T-regs. In order to assess the association between T-regs and acute cellular rejection in human cardiac allografts, we evaluated in biopsies of heart transplants the relationship between the grade of rejection, the degree of inflammation, and the density of T-regs.
Design: 73 biopsies (1A or higher) from 20 heart transplants were stained for FOXP3 (eBioscience cat#14-4777-82). Inflammation was quantified by a 3-point scale, 1=<25% of 20X field; 2=25-50%, and 3=>50%. T-regs were quantitated by a 4-point scale, 0=no cells per 20X field; 1=<5; 2=5-10 cells, and 3=>10. Data were analysed with Spearman's rank coefficient and Fisher's exact test using SAS 9.1.
Results: Table 1 shows the association between FOXP3 T-regs and inflammation.

Table 1

		INFLAMMATION			
		1	2	3	Total
	0	10	0	2	12
FOXP3	1	24	6	0	30
	2	3	16	3	22
	3	0	1	8	9
	Total	37	23	13	73

T-regs and inflammation were positively correlated (spearman correlation coefficient 0.68; p=0.001). among biopsies with low T-regs, the majority showed low inflammation: 77% (10/13) of those with FOXP3 score of 0 had an inflammation score of 1, as did 80% of those with FOXP3 score of 1. By contrast, biopsies with more T-regs showed greater inflammation. In biopsies with a T-regs score of 2, 74% (16/22) had an inflammation score of 2, and wthose with a score of 3. Table 2 shows the association between inflammation and the grade of rejection.

Table 2

		INFLAMMATION		
		1	2	3
	0	3 (8%)		
REJECTION	1A	19 (51%)	13 (56%)	2 (14%)
	2	12 (32%)	4 (17%)	5 (38%)
	3A	3 (8%)	6 (26%)	6 (46%)
	Total	37	23	13

Inflammation correlated with the grade of rejection. Among biopsies with a high degree of inflammation (3), 46% (6/13) had grade 3A rejection, and 38% (5/13) had grade 2. By contrast, with less inflammation (1), 51% (19/37) had grade 1A.
Conclusions: In heart transplants, T-regs increased with greater inflammation. Higher inflammation scores correlated with higher grades of rejection. Thus, T-regs increased with higher grades of rejection and reflected anti-allograft reactivity.

281 Eosinophilic Myocarditis in Native and Donor Hearts

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Background: Eosinophilic myocarditis may be associated with a variety of clinical presentations, however, it is often diagnosed incidentally on biopsy or on examination of an explanted heart. It is likely the result of a drug hypersensitivity reaction, which may be caused by a variety of drugs, including dobutamine and furosemide. Although there is adequate literature on eosinophilic myocarditis in native or explanted hearts, there is essentially no literature discussing this process in donor transplanted hearts. We studied the profile of the inflammatory infiltrate in both native/explanted hearts and donor/transplanted hearts to determine whether these processes may be immunologically similar.
Design: With institutional IRB approval, immunohistochemistry for B-cells (anti-CD20), T-cells (anti-CD3), macrophages (anti-CD68), mast cells (anti-mast cell tryptase), and endothelial cells (anti-CD34) was performed on formalin-fixed paraffin embedded tissue from five explanted native hearts, two biopsied donor hearts, and one autopsied donor heart with a diagnosis of eosinophilic infiltrates or eosinophilic myocarditis. Clinical symptoms and drug regimen were correlated with the findings. Of note, one native heart specimen and one donor heart biopsy were from the same patient.
Results: The transplanted donor hearts with eosinophilic myocarditis had a greater proportion of B-cells in the inflammatory infiltrate than the native/explanted hearts (see Table).

Inflammatory cells in eosinophilic myocarditis

Heart specimen	CD20	CD3	CD68	MCT
Native/explant	+	++++	++++	+
Donor/transplant	+++	++++	++++	+

MCT: mast cell tryptase

In both groups, the inflammatory infiltrate consisted mostly of CD68 positive macrophages and CD3 positive T-cells, with a similar amount of scattered mast cells. The inflammatory infiltrates were not perivascular in distribution.
Conclusions: This is the first reported study of eosinophilic myocarditis in donor transplanted hearts. The majority of non-eosinophil inflammatory cells in eosinophilic myocarditis from both native/explanted hearts and donor hearts are T-cells and macrophages. In donor hearts, however, there are more B-cells in the inflammatory infiltrate than in native/explanted hearts. B-cells are an expected component of eosinophilic myocarditis, due to production of IL-4 and IL-5 by T_H2 cells. Differences in the inflammatory infiltrates among native/explanted hearts and donor/transplanted hearts with eosinophilic myocarditis may be due to different drugs, and immunosuppression may play a role.

282 Intravascular Papillary Endothelial Hyperplasia as a Main Cause of Obstruction in Arteriovenous Fistula for Hemodialysis

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Background: Arteriovenous fistula (AVF) is a common procedure to maintain hemodialysis for the patients with ESRD (End Stage Renal Disease). But many patients who need continuous hemodialysis experience a crisis because the AVF may be obstructed and eventually has to be removed. We reviewed 6 cases of AVF patients that could not use AVF to maintain hemodialysis due to obstruction at infusion site and investigated most common cause of AVF obstruction.

Design: We reviewed 6 cases of AVF obstruction patients and performed immunohistochemical staining for CD-31, SMA, Desmin, GLUT-1, Elastic fiber, Masson Trichrome staining.

Results: In 3 of 7cases (43%), we could observe intravascular papillary endothelial hyperplasia (IVPEH) at obstruction site. we found diffuse strong positivity of endothelium of the thrombus at CD-31 staining.

Conclusions: IVPEH, which was reported by Masson at 1923, has papillary structure of a single endothelial cell layer containing fibrohyalinized tissue. We found that 43% of the AVF obstruction at infusion site exhibited IVPEH and then described IVPEH as a main cause of AVF obstruction with a review of the literature.

283 Bradycardia and Syncope as a Presentation of Cardiac Allograft Rejection Involving the Conducting System

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Background: After cardiac transplantation, multiple factors may contribute to bradycardia. Beyond the first two weeks post-transplant, bradyarrhythmias should prompt evaluation for causes such as sinus node dysfunction, ischemia, rejection, allograft vasculopathy or drug effects. Rejection preferentially involving the conducting system is a potential cause which is not detected by biopsy.

Design: We identified 5 patients (4 males, mean age 52 years) from our institution who presented between January 2000 and December 2005 with unexplained bradycardic syncope 34 ± 19 months after transplantation.

Results: Three patients had prior cellular rejection 18-30 months prior to the event, but none had history of hemodynamically significant rejection. Left ventricular function was normal by echocardiography in all patients at presentation. Four patients underwent permanent pacemaker placement. Four patients had endomyocardial biopsies, and none showed more than mild rejection (International Society for Heart and Lung Transplantation grade 0 to IB). Two patients were treated for rejection and survived the event. The remaining three patients died, and two underwent autopsy. One patient's post-mortem examination revealed mild acute allograft rejection in routine myocardial sections; the conducting system was not examined histologically. In the last patient (who did not receive a pacemaker), autopsy revealed little inflammation in the ventricular myocardium. Sections from the cardiac conducting system, however, showed severe inflammation and myocyte damage in the sinus and AV nodes, including the AV nodal artery, consistent with more severe rejection than seen elsewhere in the myocardium. This unexpected finding of rejection preferentially involving the conducting system may represent a previously unrecognized mechanism of post-transplant bradycardic syncope and sudden death.

Conclusions: Unexplained bradycardic syncope due to damage to the cardiac conducting system may be a manifestation of allograft rejection. This presentation heralds a poor prognosis, and aggressive treatment similar to that for hemodynamically significant rejection should be strongly considered even if not indicated by standard right ventricular biopsy. To our knowledge, this is the first report correlating bradycardia with preferential rejection of the conduction system.

284 Significance of B-Cells in Heart Biopsies for Allograft Rejection, and Incidence of Non-Endocardial "Quilty-Like" Lesions

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Background: Although it is well known that endocardial-based infiltrates in allografts (Quilty effect) are composed in part of B-lymphocytes, the frequency and extent of B-cells in cardiac allograft rejection and its mimickers has not been studied in detail.

Design: 748 heart biopsies from 95 patients (46% women) were prospectively studied for the presence of B cells. Quilty was assessed by histologic criteria and immunohistochemical stains performed for B-cells (CD20), T-cells (CD3, CD4, and CD8).

Results: Of the 95 patients (mean 7.8 biopsies each), 66 showed ≥ 1 episode of rejection. Of the total 748 biopsies, 191 shows mild rejection (1R)(152 grade 1A, 37 grade 1B), and 21 moderate rejection (19 grade 3A, and 2 grade 3B). B-cell infiltrates (assessed as at least 5% of infiltrate) occurred in 15% of grade 1A, 19% of grade 1B, 67% of grade 3A, and 100% of grade 3B rejection, and occurred at least once in 38 of 66 patients with rejection. Among 73 patients with ≥ 4 metachronous biopsies, Quilty lesions were more frequent in any biopsy with rejection (40/58 vs. 8/15, mean 1.5 positive biopsies vs. 0.7, p=.2) and significantly more frequent in patients with B-cell positive infiltrates (28/35 vs. 19/38, p=.01, mean 2.2 vs. 0.7, p=.0004). In 5 patients with ≥ 1 episode of rejection there were nodules of B-cells mixed with CD4 and CD8 positive T-lymphocytes, which in 2 cases extended into the epicardial fat. In 273 biopsies with prior biopsy site change, 14% showed adjacent B-cells; in 27 biopsies with healing ischemic lesions, 7% showed adjacent B-cells. The proportion of CD4 and CD8 positive T-lymphocytes did not differ in infiltrates with and without B-cells and were present in similar proportions in Quilty lesions as infiltrates.

Conclusions: We conclude that B-cells are not infrequent in rejection, especially moderate rejection, and that they are associated with Quilty lesions. A subset of patients with cellular rejection (approximately 4%) will demonstrate extensive B cell myocardial or epicardial infiltrates and numerous Quilty lesions. Because of the association with Quilty and prevalence in rejection, B-cells within an infiltrate are not helpful in the differential diagnosis with tangential Quilty lesions. Because of the strong association between myocardial infiltrates with abundant B-cells and Quilties, we propose the concept of intramyocardial and epicardial lesions akin to endocardial Quilty effects.

285 Foreign Material in Endomyocardial Biopsies

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Background: Current uses for the endomyocardial biopsy (EB) include evaluation of cardiac allograft rejection, cardiomyopathy, anthracycline cardiotoxicity and myocarditis. This study describes 28 cases in which foreign material (FM) was observed in EBs, and the importance of differentiating this phenomenon from other cardiac processes, especially those asscociated with giant cell reaction.

Design: Retrospective search of all EBs performed at Cedars-Sinai Medical Center over an eleven year (1996-2007) period was undertaken to identify cases with FM. For each case identified, slides was reviewed (which included microscopic evaluation on three hematoxylin and eosin stained slides containing two to four sections/slide). Microscopic characteristics of the FM and associated tissue reaction were assessed, including the features of the FM, distribution of FM, the presence of giant cell reaction, granulomas, quality of cellular infiltrate, fibrosis, myocardial necrosis and relationship to foci of acute cellular rejection and Quilty lesions.

Results: Review of 5,521 consecutive EBs revealed 28 cases which contained FM (male: female= 23:5; age:17-74), representing 0.5% of all biopsies. All FM cases were from heart transplant patients; 3 cases demonstrated grade 1a cellular rejection, 10 had Quilty lesions. FM was found in the endocardium (n=24), epicardial/fatty tissue (n=4) and myocardium (n=1). Tissue reaction to FM included histiocytes (n=25), fibrosis/scarring (n=22), multinucleated foreign body-type giant cells (n=21) and lymphocytes (n=4). No granulomas or myocardial necrosis were found. FM appearance was heterogeneous in nature including filamentous-crystalline (n=5), suture-like material (n=4), oil-like droplets (n=2), clear vacuolar devoid of contents (n=10), vacuolar with refractile contents (n=2), granular crystalline (4) and talc-like (n=1). FM was not associated with foci of cellular rejection or Quilty lesions.

Conclusions: Identification of FM in the EB is rare, involving 0.5% of all cases. FM is heterogeneous in nature, is typically found in the endocardium, and is associated with scarring and foreign body-type giant cell reaction. Tissue reaction to FM should be considered in the differential of cardiac lesions, especially when endocardial-based and when associated with fibrosis and giant cell reaction. The origin of FM is likely the result of previous cardiac instrumentation.

286 Histopathologic Findings in Surgically Resected Aortas from Individuals with Loeys-Dietz Syndrome (LDS)

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Background: Loeys-Dietz syndrome (LDS) is an autosomal dominant connective tissue disorder resulting from mutations in the type II transforming growth factor beta receptor (TGFBR2) gene. The syndrome is characterized phenotypically by hypertelorism, bifid uvula and/or cleft palate, and arterial tortuosity that can result in aneurysms and dissections. LDS has a much more rapid clinical course than Marfan syndrome and thus LDS afflicted individuals are currently being recommended for elective aortic root replacement at a young age. To date there have been no case series to investigate the histopathologic finding of LDS in the ascending aorta.

Design: Aortic root tissue was examined from thirteen patients with confirmed LDS. Ascending aorta samples from two nondiseased aortas and from two Marfan syndrome individuals were also analyzed. Standard hematoxylin & eosin (H&E), Movat, alcian blue, and Verhoeff-Van Gieson (VVG) stains were performed for each sample. Additionally, an immunohistochemical (IHC) stain for pSmad-2 was obtained for each sample.

Results: By H&E, LDS samples were fairly unremarkable showing little cystic medial degeneration (10 none or minimal, 3 moderate, 0 severe) and no laminar medial necrosis. Movat staining revealed that aortic roots obtained from LDS patients had more collagen within the tunica media of the vessel compared to the controls. VVG showed significant elastic fiber fragmentation. IHC staining for pSmad-2, a marker of TGF-beta activity, was markedly elevated relative to the controls (2.3 vs 0 on a 0-3 scale).

Conclusions: The histologic findings of LDS are best appreciated with special stains to evaluate fibrosis and elastic fiber fragmentation. LDS is fairly unremarkable by H&E alone and does not display prominent cystic medial degeneration or laminar medial necrosis as has been described in Marfan syndrome. By special stains (VVG and Movat) LDS has similar findings to Marfan syndrome. A limitation of this study is that it is comprised almost entirely of specimens removed prophylactically and extensive pathologic changes may not have yet occurred. This study expands the range of connective tissue disorders that have histopathologic descriptions.

287 Distal Coronary Artery Disease Has Less Calcification, Macrophage Infiltration and Necrotic Core Than Proximal Coronary Artery Disease, Independent of Percent Stenosis

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Background: It is not known if the coronary artery plaque composition in distal vessels differs from that of proximal vessels. The extent of calcification, inflammation and necrotic core formation may affect optimal stent design.

Design: We selected 36 hearts at autopsy (24 men, 60±14 years and 12 women, 70±12 years) based on the presence of moderate coronary calcification on post-mortem radiograph and ≥ 1 epicardial artery with ≥ 75% cross sectional area luminal narrowing. All hearts were perfusion fixed at 100 mm Hg. Epicardial arteries were radiographed,

decalcified, and sectioned at 3 mm and submitted for histologic morphometric evaluation of internal elastic lamina, lumen area, % necrotic core, % calcified matrix and % CD68 staining. The lumen diameter was used to compare two groups: mean diameter > 3 mm (proximal) and mean diameter < 2.5 mm (distal). These were further grouped as % area stenosis 50-75% (moderate) and % area stenosis >75% (severe).

Results: In 18 cases the cause of death was coronary artery disease (69 ± 12 years, culprit plaque 6 acute ruptures, 3 acute erosions, and 9 with severe disease without acute thrombus). The cause of death in the remaining 18 cases (58 ± 15 years) was cardiac non-coronary in 12 and noncardiac in 6. There were a total of 1317 sections with coronary artery lesions; of these 571 had at least moderate disease > 3 and < 2.5 mm: 275 moderate proximal lesions, 118 severe proximal lesions, 89 moderate distal lesions, and 89 severe distal lesions. The % necrotic core was 10.4 in proximal moderate lesions vs. 6.7 in distal moderate lesions (p=.05), and 14.7 in proximal severe lesions vs. 4.0 in distal severe lesions (p=.0001). The % calcification was 15.0 in proximal moderate lesions vs. 10.3 in distal moderate lesions (p=.05), and 14.5 in proximal severe lesions vs. 4.9 in distal severe lesions (p<.0001). The % macrophage content was 2.0 in proximal moderate lesions vs. 1.3 in distal moderate lesions (p<.0001 using log-normalized data), and was 2.5 in proximal severe lesions vs. 1.2 in distal severe lesions (p<.0001). % necrotic core (p=.03), percent calcification (p=.03), and macrophage content (p=.0001) was greater in proximal vessels independent of patient age, gender, percent stenosis, and mechanism of death (coronary vs. non-coronary).

Conclusions: Distal coronary artery disease has less calcification, macrophage infiltration and necrotic core than proximal coronary artery disease, independent of percent stenosis.

288 Inflammatory Aortic Aneurysm May Be a Reactive Form of Inflammatory Myofibroblastic Tumor: An Immunohistochemical Study on 23 Cases

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Background: Inflammatory aortic aneurysms (IAA) are characterized by outer wall thickening of abdominal aorta forming a mass characterized by fibroblastic proliferation and a heavy lymphoplasmacytic infiltrate. The histologic features are similar to those observed in inflammatory myofibroblastic tumor (IMT), a distinctive entity in which the ALK-1 gene has been shown to be rearranged.

Design: We obtained 23 cases of IAA from the surgical pathology files of the Department of Pathology at The Methodist Hospital in Houston, Texas from 1995 to 2007. We evaluated the expression of C-Kit, CD21, CD34, S-100 protein, SMA, vimentin, p53, beta-catenin, and ALK-1. EBV-LMP1 was also performed by in situ hybridization. The grade and the distribution of the signal were scored semiquantitatively from absent (-) to strong (+++) and from focal to diffuse.

Results: Of the 23 patients, 20 were males and 3 were females (M:F ratio 6:1); age ranged from 43 to 81 years (average 64.3 years). Clinically, an abdominal mass was detected in 19 patients and abdominal pain was present in 4 patients at presentation. All had a history of arterial hypertension or coronary artery disease, and 6 also had Diabetes mellitus. Histologically, all 23 cases formed a mass that displayed IMT features. All lesions stained strongly and diffusely for vimentin and SMA (100%); 17 stained strongly and focally for CD34 (74%); and all were negative for C-Kit, CD21, S-100 protein, p53, beta-catenin, EBV-LMP1, and ALK-1.

Conclusions: To our knowledge, this study is the first to describe the immunohistochemical profile in IAA. Our findings indicate that IAA lacks ALK-1 which has been reported positive in neoplastic form of IMT. The expression of CD34 suggests that dendritic-type interstitial fibroblastic cell proliferation, in addition to myofibroblasts, is an important cellular component for the pathogenesis of IAA Thus, IAA appears to be a reactive form of IMT.

289 Analysis of Clinical Transcatheter Pulmonary Valves: Novel Methods, Findings and Clinicopathologic Correlations Advance Device Development and Use

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Background: Conduits to repair congenital heart defects involving the right ventricular outflow tract (RVOT) often become stenotic. The Medtronic Melody™ Transcatheter Pulmonary Valve (TPV, a glutaraldehyde-fixed bovine jugular vein containing its venous valve mounted within a platinum-iridium stent that is deployed percutaneously) was designed as a transcatheter device to treat RVOT conduit dysfunction.

Design: Six (6) explanted TPVs that had been deployed for 327 +/- 109 days (range 160-459 days) within stenotic RVOT conduits in patients 15 +/- 4 years old (range 10-26 years old), and were explanted for residual stenosis (5 devices) or endocarditis (1 device) were analyzed. Gross, microscopic and radiographic evaluations were performed.

Results: The devices explanted for residual stenosis suffered from incomplete and asymmetric stent expansion that did not allow the bovine vein to achieve its intended geometry. Additional pathologic findings included punctures of the bovine vein wall (3/6 devices) secondary to stent fractures, bovine vein wall calcification (3/6), pannus formation (6/6), adventitial chronic inflammation (4/6), small holes in the valve cusps (4/6), thrombosis of the outflow aspect of the valve cusps (2/6), fusion of the valve cusps to the vein wall (1/6), stent fractures (5/6) and bacterial endocarditis (1/6).

Conclusions: Pathologic considerations with TPVs or any percutaneously implanted device are inherently different than those of surgically implanted valves due to their structure and the nature of the device-patient interactions. Therefore, careful consideration of the dissection technique and detailed clinicopathologic correlations are needed to accurately demonstrate the findings with these novel devices and elucidate their etiologies and significance. The pathologic findings, correlated with pre-implant angiographic, echocardiographic and clinical data, have directly enabled improvements in patient selection criteria, pre-deployment strategies and device design.

290 *UGT1A1* Promoter Genotype Is Not Associated with Coronary Artery Disease

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Background: Atherosclerosis is a leading cause of morbidity and mortality. Oxidation of lipoproteins contributes to atherosclerosis while antioxidants are protective. Bilirubin is an intrinsic antioxidant that is mildly elevated in people with Gilbert syndrome. Homozygosity for a $(TA)_7TAA$ (seven-repeat) variant of the *UGT1A1* promoter is necessary for expression of the Gilbert phenotype whereas $(TA)_6TAA$ (six-repeat) is wildtype. This study investigates the relationship between coronary artery disease (CAD) and the Gilbert genotype. We hypothesize that patients with mild CAD have a greater frequency of the Gilbert genotype compared to those with severe CAD.

Design: Decedents who underwent autopsy at UNC Hospitals from 2004 thru 2005 were categorized into *none/mild*, *moderate*, and *severe* CAD groups based on autopsy findings. Known CAD risk factors were evaluated for each decedent in the *severe* CAD group and for an age, race, and gender-matched control group with *none/mild* CAD. FFPE tissue was evaluated for mutations in the *UGT1A1* promoter region by PCR and capillary electrophoresis. The frequency of the Gilbert genotype was compared between the *none/mild* cohort and the *severe* cohort.

Results: Chart review identified 35 subjects with *severe* CAD and 45 subjects with *none/mild* CAD. The only significantly different CAD risk factor between the *none/mild* and *severe* cohorts was tobacco use.

CAD Risk Factors

	None/Mild	Severe
Age (yrs)	64.5	67.6
Male	40%	59%
White	53%	62%
Black	38%	32%
Diabetes	40%	42%
Tobacco*	36%	71%
Lipid Disorder	18%	42%
Hypertension	58%	77%

*p=0.03

UGT1A1 promoter genotype data were obtained for 73/80 subjects, of whom 42 were in the *none/mild* group and 31 were in the *severe* group. The method allowed differentiation among five-, six-, seven-, and eight-repeat *UGT1A1* promoter variants. The overall frequency of the Gilbert genotype was 15%, with a frequency of 17% in the *none/mild* group and 13% in the *severe* group.

UGT1A1 Promoter Genotype versus Severity of CAD

	None/Mild	Severe
$(TA)_7TAA$ Allele Frequency	36%	44%
Gilbert Genotype	17%	13%

Conclusions: The overall frequency of the Gilbert genotype compares well with previously reported frequencies. The *none/mild* CAD group exhibits a slightly greater frequency of the Gilbert genotype compared to the *severe* CAD group; this difference is not statistically significant. The data suggest that *UGT1A1* promoter genotype is not a major factor contributing to risk of coronary artery disease. To our knowledge, this is the first study to successfully apply *UGT1A1* promoter genotyping to paraffin embedded tissue.

291 Endomyocardial Biopsy – Experience with Amyloid Typing

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Background: Diagnosis of amyloidosis involves the generic detection of deposits with Congo red (CR) stain and typing of the amyloid protein. While, among systemic amyloidoses, deposits derived from the immunoglobulin light chain (AL) are the most prevalent type, other types of amyloidosis (in particular hereditary) are emerging as important differential diagnoses. While AL is treated with aggressive chemotherapy, hereditary amyloidoses are managed differently (including by liver transplantation).

Design: CR stain was done per protocol on all native endomyocardial biopsies (EMB). EMB positive for amyloid by CR stain (n=14) were subsequently analysed for amyloid type. In 2 cases, amyloid typing was done on paraffin sections (PS) by immunoperoxidase (IP) and in 12 cases on frozen sections (FS) by immunofluorescence (IF). A panel of antibodies was used in each case: anti κ and λ light chains, amyloid A protein and transthyretin (TTR); stain for amyloid P component served as a built-in control. Immunoelectron microscopy (IMEM) was performed in 2 cases and biochemical characterization in 1 case. Cases typed as amyloid derived from transthyretin (ATTR) were subsequently tested for mutation. Clinico-pathologic correlation was performed in all cases.

Results: There were 8 males and 6 females, age range 48-75. One patient had a pre-biopsy diagnosis of multiple myeloma and another of MGUS; no patient had a family history of amyloidosis. In 8 cases with light chain restriction (λ x6, κ x2) AL was diagnosed in all cases by IF on FS. In 5 cases with stain limited to TTR (PS x2, FS x3), ATTR was diagnosed. In one patient, there was positivity for TTR and λ light chain. Subsequent IMEM and molecular studies of the extracted deposits confirmed the presence of both proteins, with a predominance of TTR. All patients with AL had evidence of plasma cell dyscrasia or multiple myeloma (x1). Mutation in TTR was subsequently demonstrated in 4/6 patients and hereditary ATTR was diagnosed; in 2 patients ATTR was derived from the wild-type protein ("senile type"). Detection of 2 proteins in amyloid deposits suggests that the presence of ATTR may have facilitated fibrillogenesis of the monoclonal light chain derived from MGUS.

Conclusions: While AL is the most prevalent type of cardiac amyloidosis, ATTR is the second most common type. Hereditary ATTR was detected in 4 patients without a positive family history. In 2 patients, wild type ("senile") ATTR was diagnosed. While clinico-pathologic correlation is mandatory in all cases, it is in itself not a substitute for amyloid protein typing. On rare occasions, more than one protein can be detected.

292 Circulating Anti-HLA Antibodies Are Associated with Antibody Mediated Cardiac Allograft Rejection

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Background: Pre-sensitization, as detected by circulating anti-HLA antibodies, is a predictor of reduced survival in cardiac transplant patients, but its association with histopathologic and immunopathologic features of antibody mediated rejection (AMR) in post-transplant endomyocardial biopsy (EMB) has not been well defined.

Design: Paraffin-embedded EMB tissue from pre-sensitized patients undergoing cardiac transplantation at the Mayo Clinic (2004 - 2006) was obtained. EMB tissue from a control group of cardiac transplant recipients without evidence of HLA-antibodies was also obtained. EMBs taken at 1, 3 to 7, and 12 mos post-transplantation (and yearly thereafter when available) were stained with H&E and antibodies to C4d (Alpco Diagnostics, Salem, NH) and CD68. Histologic features of AMR were evaluated (endothelial swelling, intravascular macrophages). A semiquantitative (0 – 3+) assessment of capillary endothelial C4d staining intensity and intravascular CD68 positive cells was made. Only diffuse staining of capillaries for C4d was considered significant.

Results: 7 pre-sensitized patients (33 EMBs) and 10 controls (45 EMBs) were studied. The data are summarized in the table.

Immunopathologic features and histopathologic findings of post-transplant EMBs of study population

Pt#	Method / HLA Class	# EMBs reviewed / with AMR	Features of AMR on H&E	C4d	CD68	ISHLT Grade	Time to first EMB with AMR
1	CDC Pos I	6 / 4	Y	3+	3+	1R; AMR1	5 wks
2	Lum Pos II	6 / 1	N	3+	1+	1R; AMR1	6 mos
	CDC Neg						
3	Lum Pos I	5 / 4	Y	2+	2+	1R; AMR1	3 wks
	CDC Neg						
4	Lum Pos I	5 / 4	Y	3+	3+	1R; AMR1	3 wks
	CDC Neg						
5	Lum Pos I&II	5 / 4	Y	3+	2+	1R; AMR1	2 wks
	CDC Neg						
6	Lum Pos I	1 / -	N	0	2+	0R; AMR0	1 d
	CDC Neg						
7	Lum Pos I&II	9 / 9	Y	3+	3+	1R; AMR1	2 wks
	CDC Pos I&II						

Lum: Luminex screen, CDC: Complement dependent cytotoxicity, Y: yes, N: no, neg: negative, pos: positive

In the majority of pre-sensitized patients (6 of 7), at least 1 post-transplant EMB was positive (histological features suggestive of AMR and/or ≥2+ C4d and CD68). One patient (#6) died the day after transplantation, but at autopsy there were no histologic features of AMR and C4d staining was negative. In the control group, only 1 biopsy from 1 patient (tested by CDC) had AMR in an EMB taken 1 year post transplant (C4d 2+, CD68 3+, AMR1).

Conclusions: Histopathologic and immunopathologic features of AMR are common in EMBs from pre-sensitized patients and can be detected within 1 month after transplant in most.

293 Array Based Comparative Genomic Hybridization and Expression Profiling of Aortic Aneurysms with and without Bicuspid Aortic Valves

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Background: In the United States, an average of 43199 patients die per year from diseases of the aorta, excluding carotid and coronary disease. Aneurysms occurring in thoracic aorta are associated with the presence of bicuspid aortic valves in at least 13.4% of these patients. Thus the association of bicuspid aortic valve (BAV) and thoracic aortic aneurysms may have a common genetic component.

Design: Segments of aorta were collected from unrelated patients at the time of surgical repair of their aortic aneurysm (23 males and 4 females, ages 32 - 73 with an average age of 52). Sixteen of 27 patients had concomitant BAV, the remaining 11 patients had tri-leaflet aortic valves (TAV). DNA and RNA were extracted for array-based comparative genomic hybridization (aCGH) spanning throughout all the chromosomes (Agilent) and gene expression arrays (GX) (Agilent). All arrays (aCGH and expression) were run in duplicates. Analysis was performed with CGH-Analytics and Genespring GX software packages (Agilent).

Results: aCGH showed imbalance in known loci for thoracic aortic aneurysms (3p24-25 and 5q13-14). It also showed an imbalance in chromosome 7q11.22 in this group of unrelated patients. However, this new imbalance did not distinguish between patients with BAV or TAV. Gene expression profiling of elastin, emilins and fibrillins did not distinguish between BAV and TAV. However fibulin 2 showed slightly higher expression levels in patients with BAVs .

Conclusions: In a population of unrelated patients with aortic aneurysms, aCGH is a useful tool to detect new chromosomal abnormalities. Gene expression profiling can distinguish differences in gene expression of extracellular matrix proteins ubiquitous in the aorta which may represent an adaptive response of the aneurysmal aortic wall.

294 Correlation between Expression of Regulators of Complement Activation, Deposition of Complement Markers C4d and C3d and Cardiac Allograft Dysfunction in Multiparous Heart Transplant Recipients

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Background: Antibody mediated rejection (AMR) in heart transplantation is a type of rejection with low incidence but high morbidity and if unrecognized high mortality. Unregulated activation of the complement cascade with deposition of complement in capillaries occurs in AMR and is noxious to the allograft. In some patients activation and deposition of complement occurs but it is not associated with clinical dysfunction of the allograft. A protective mechanism may be the local activation of regulators of complement activation. Multiparity is thought to be an important factor that increases sensitization and the incidence of AMR.

Design: Endomyocardial biopsies from 25 multiparous heart transplant recipients were evaluated at 1, 6 and 12 months after transplant with a mean follow up of 4 years for the presence of complement split-products C4d and C3d in capillaries by immunofluorescence. The presence of regulators of complement activation in endothelial cells was assessed by immunofluorescence for: complement receptor 1 (CD35), membrane cofactor protein (MCP or CD46), decay accelerating factor (CD55) and protectin (CD59).

Results: Expression of CD35 and CD46 in capillary endothelial cells was absent or weak in most patients. Expression of CD55 and CD59 in granular pattern in capillaries was commonly found. Eighteen of 25 patients (72%) had expression of CD55 in capillaries and 16 of 25 (64%) showed expression of CD59. Only 13 of 25 patients (52%) expressed CD55 and CD59 concomitantly. The expression of regulators of complement activation was variable over time. Three of 25 patients (12%) had C4d and C3d deposits with concomitant dysfunction of their allograft and one died of it. Two of these 3 patients showed minimal expression of the regulators of complement CD55 and CD59.

Conclusions: The presence of regulators of complement in the endothelial cells correlates with the absence of complement deposition and dysfunction of the allograft. Either CD55 or CD59 seem to be sufficient for protection against the deleterious effects of complement activation in cardiac allografts. The absence or low local expression of regulators of complement activation was associated with deposition of C4d and C3d in capillaries and concomitant allograft dysfunction (AMR). The overal incidence of AMR in this cohort of multiparous heart transplant recipients was 12%.

295 More FoxP3+ Lymphocytes Are Present in Quilty Lesions Than in Acute Cellular Rejection

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Background: Nodular endocardial infiltrates or Quilty lesions (QL) have been recognized in transplant endomyocardial biopsies for more than 25 years, but their biology and significance remain enigmatic. QL commonly appear in the first year post transplant and are more common in younger patients. They were initially considered an effect of cyclosporine therapy. Recent studies have shown the cellular constituents in QL recapitulate developing lymphoid tissue. Theories about their function include the orchestration or resolution of acute cellular rejection (ACR), a similar role in vascular rejection, and viral reaction or incipient lymphoproliferative disorder. QL appear to be associated with significant ACR in the first year, but curiously have not been shown to correlate adversely with survival or graft failure. Thus this early temporal association might suggest an immune regulatory role for QL. A recently characterized antigen, FoxP3, shows specific nuclear expression in "regulatory" T-cells (CD4+/CD25+) by paraffin immunohistochemistry. The aim of this study was to compare FoxP3 expression in lymphocytes of QL and ACR.

Design: Transplant endomyocardial biopsies with prominent QL or cellular rejection lesions (ISHLT grade 1R-2R) providing ample lymphocytes to study were selected from biopsies taken at our institution between 2003-2006. Formalin-fixed paraffin-embedded sections were immunoperoxidase stained using antibodies against FoxP3. Cells within the lesions of interest showing nuclear FoxP3 expression as well as the total number of lymophocytes in these fields were counted. The percent of FoxP3+ cells per total lymphocytes was calculated and compared.

Results: 10 biopsies showing large QL, 10 showing significant areas of ACR, and 5 biopsies showing both QL and ACR were identified. FoxP3+ cells comprised 21.1% (1.1% - 33.6%) of the lymphocytes in the QL in the 1st group and only 4.2% (1.5% - 7.0%) of the ACR lymphocytes in the 2nd group ($p<0.0001$). In last group, the QL again showed more FOX-P3+ cells than were seen in the ACR lesions ($p=0.001$), but the numbers were not as high as the QL in the first group (11.6 % (7.5% – 17.2%) vs 3.9% (2.6% - 5.9%)).

Conclusions: QL contain substantially more FoxP3+ regulatory T-cells compared to lymphocyte populations seen in ACR. Though statistically significant, this difference is modest and not likely to be of practical diagnostic value in differentiating QL from ACR. This observation does provide important insights into the biology of QL.

296 The Left Atrial Endoskeleton: Analysis by Light and Electron Microscopy

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Background: The cardiac atria and ventricles are structurally and functionally different. The subendothelial layers of the cardiac chambers contain extracellular matrix composed of collagen and elastic fibers, providing support and elasticity. With aging and in disease states (left atrial fibrillation), increased amounts of collagen may be observed. This study compared subendocardial thickness and collagen fibril diameter in the atria and left ventricle in a cohort of adult and pediatric human hearts.

Design: Histologic samples from 7 adult and 8 pediatric (4 infant, 4 adolescent) hearts were obtained at autopsy from specific sites (in adults: left ventricle LV, right atrium RA, left atrium LA; in children:atrial septum AS). The tissue was formalin fixed, processed in routine fashion and stained with H&E, VVG and trichrome. Under light microscopy, the subendothelial layers of the atria, left ventricle (in adults) and myocardium were measured using an ocular micrometer. Transmission electron microscopy was performed on the subendothelial layers of the atria in adult, adolescent and infant hearts and collagen fibril diameters were measured at multiple sites.

Results:

Subendothelial Layer Thickness

Case	RA mm	LA mm	LV mm	PV mm	EM LA μ	EM RAμ
Adult n=7	0.3	0.9*	0.04	0.5*	0.0512*	0.0348*
Adol n=4	0.2	0.5	-	-	0.0435*	0.0409
Infant n=4	0.2	0.2	-	-	0.0432*	0.0374

*Significant differences are noted between average intimal thicknesses in the adult LA and pulmonary vein (PV) and in collagen fibril diameters, respectively, in the adult LA and RA; adolescent (Adol) LA and adult LA, and the infant LA and adult LA; $p<0.001$.

Conclusions: The subendocardial thickness is greatest in the adult left atrium and increases with age; however, the right atrial intimal thickness does not. In adults, left atrial collagen fibers are thicker than right atrial collagen fibers and are approximately the same diameter in the pediatric age group. Adult left atrial collagen fibers are thicker than those of pediatric left atria and adult right atrial collagen fiber thicknesses are similar to those of the pediatric age group. The increased thickness of the adult left atrial subendocardial layer may represent a "cardiac endoskeleton" in response to shear stresses and correlate with increased stiffness of the left atrium with age.

297 Endomyocardial Biopsy Guided by Electroanatomic Voltage Map in Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC): Histologic and Histomorphometric Findings

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Background: Endomyocardial biopsy (EMB) is used to show RV fibrofatty replacement in ARVC, but its sensitivity is low. Recent data support CARTO system as a new approach to identify low-voltage regions of fibrofatty myocardial replacement.

Design: To improve EMB accuracy for ARVC diagnosis, we hypothesized a RV sampling focused on pathological areas identified by voltage mapping. Twenty-two consecutive patients (10 M,12 F; mean age: 34 ± 10 years) were divided in two groups: Group A with evidence of ARVC (fulfilment of standardised noninvasive diagnostic criteria: 11 pts); Group B with suspicion for ARVC (ventricular arrhythmias, with LBBB morphology, but inadequate number of ARVC Task Force diagnostic criteria: 11 pts). An electroanatomic (EA) reconstruction of RV was performed in all pts. In patients with RV pathological segments (all pts from group A, three refused consent for EMB, and 8 Group B pts) an EMB focused on low-voltage areas was attempted. A disposable bioptome was inserted in the RV and positioned, under fluoroscopic guidance, as close as possible to the catheter tip targeting low-voltage areasUp to 6 specimens were obtained from low-voltage regions. Multiple histologic sections (15-30) were obtained from each sample and stained with H&E, Masson Trichrome and antibodies for CD3, CD20 and CD68. Computerized histomorphometry was also performed on all trichrome-stained sections, to measure the extent of fibro-fatty replacement

Results: Biopsies focused on low-voltage RV segments revealed myocardial fibrofatty replacement consistent with a histologic diagnosis of ARVC in 6/8 Group A patients (75%) and in 7/8 Group B patients (87%). Histomorphometric analysis documented a 27 ± 17% mean amount of fibrofatty myocardial substitution, with no significant differences between group A and group B. Also, in the whole study population there was no significant difference in the amount of fibrofatty replacement in the different biopsy sites (RV efflux: 22%; inferior wall: 30.5%; anterior wall: 33%; apex: 29%).

Conclusions: Our results show that: a) EMB targeting low-amplitude areas is a safe and highly sensitive technique for ARVC diagnosis confirmation; b) there is a high prevalence of RV low-voltage segments also in patients with clinical suspicion for ARVC; c) the amount of fibrofatty replacement in this selected population is similar between patients with clinical evidence and suspicion of ARVC, and does not vary in the different biopsy sites.

298 Pathology of Viridans Streptococcal Endocarditis Revisited

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Background: The viridans group of Streptococci accounts for 40-60% of community-acquired subacute infective endocarditis of native heart valves. These are slow-growing bacteria that produce glycocalyx (exopolysaccharides). The glycocalyx can induce platelet aggregation important in the formation of vegetations and appears to protect the bacteria from host immune defense mechanisms and from the action of antibiotics.

Design: From July, 2005 to June, 2007, 83 cases of infective endocarditis were retrieved from the surgical pathology files. Eighteen cases were confirmed to be caused by viridans streptococci by history of positive blood cultures. The histologic features of streptococcal endocarditis were reviewed. Special staining with Gram and periodic acid Schiff (PAS) were routinely performed. Fluorescent in situ hybridization (FISH) using a FITC-labeled genus-specific 16S rRNA probe for Streptococcus spp. was performed on 5 of the 18 cases and 5 negative controls.

Results: Streptococcal endocarditis involved 11 native and 7 bioprosthetic valves. The aortic valve alone was affected in 10 cases, mitral valve alone in 1 case, aortic and mitral valves in 5 cases and mitral and tricuspid valves in 2 cases. The vegetations showed Gram-positive cocci that were also intensely stained with PAS. The following histologic features on H&E were consistently observed: 1. exuberant fibrin-rich vegetations with evidence of granulation tissue; 2. readily identifiable pale foamy macrophages which contained microorganisms that stained PAS-positive and Gram-positive; and 3. presence of multinucleated giant cells in the vegetations but not in the valve stroma. Tissue cultures were obtained at the time of excision in 13 cases and were negative in 9. FISH demonstrated intense staining of clusters of cocci in the vegetations and faint staining of intracellular microorganisms.

Conclusions: Viridans streptococcal endocarditis shows distinct histologic features that include pale foam cells identifiable on H&E, Gram and PAS-positive cocci and intracellular organisms in macrophages and giant cells. The production of glycocalyx by viridans streptococci accounts for the tinctorial properties that react with the Schiff reagent. The presence of pale foamy macrophages and giant cells in vegetations should prompt pathologists to perform PAS staining which is very helpful in the diagnosis of viridans streptococcal endocarditis. Molecular detection of these organisms can confirm the diagnosis of infection by viridans streptococcus in culture-negative endocarditis.

299 Patterns of C4d and C3d Immunofluorescence Staining in the Evaluation of Antibody-Mediated Rejection in Heart Transplants

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Background: The diagnosis of acute antibody-mediated rejection (AMR) in cardiac transplantation is based on a set of clinical, serologic and histopathologic findings. The immunopathological evaluation of endomyocardial biopsies for AMR needs further standardization and correlation with clinical findings.

Design: Frozen sections of all heart transplant endomyocardial biopsies (EMBs) from October 2006 to August 2007 were routinely evaluated for AMR with C4d and C3d immunofluorescence staining. Biopsies were scored semi-quantitatively from 0 to 4+. Clinical information was obtained by a retrospective review of electronic medical records.

Results: A total of 1223 consecutive EMBs from 316 adult heart transplant patients were evaluated. The number of EMBs per patient ranged from 1 to 23. Fifty one out of 316 patients showed evidence of complement deposition in at least one occasion. Based on the presence of complement deposits, patients were divided into 4 groups depending on the pattern of immunofluorescence staining. Group A (n=15) had diffuse linear capillary staining of 2+ or higher with C4d and C3d. Group B (n=15) had diffuse linear capillary staining of C4d only. Group C (n=11) had focal capillary staining of C4d only. Group D (n=10) had linear perimyocytic staining of C4d with or without C3d. Hemodynamic compromise (decreased cardiac output/index, decreased ejection fraction, rise in pulmonary capillary wedge pressure) was noted in 87% (13/15) of group A patients, 6.7% (1/15) of group B patients and none in groups C and D. There were 6 deaths that occurred, 5 group A patients died of cardiac-related causes and 1 group D patient died of sepsis.

Conclusions: The majority of patients with concurrent C4d and C3d capillary deposition had cardiac allograft dysfunction. C4d capillary staining alone does not discriminate between those who have hemodynamic compromise and those who are asymptomatic. Focal and perimyocytic complement deposition can be found in asymptomatic patients. Long-term follow-up is needed to correlate immunofluorescence staining pattern with overall prognosis in heart transplant recipients.

300 Spontaneous Angiogenesis in Human Ischemic Hearts Is Not Clearly Correlated with VEGF Gene Expression

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Background: VEGF is a critical growth factor for angiogenesis. However, relationship between VEGF level and endothelial proliferative activity in end-stage human heart is not well-established.

Design: We have studied 11 human hearts explanted at the time of transplantation which displayed severe coronary artery disease (ischemic hearts). As controls, we also studied 8 explanted hearts from patients with idiopathic dilated cardiomyopathy. Endothelial proliferative indices were measured using a double immunostaining method for PCNA and Ulex Europaeus lectin. Proliferating endothelial cells were detected in capillaries and in a few arteries.

Results: The endothelial proliferative indices were low in both groups of hearts, but the proliferation was significantly elevated in the ischemic hearts compared to the cardiomyopathic hearts (mean ± SD = 0.12 ± 0.16% vs. 0.009% ± 0.009%, respectively). Within the ischemic hearts, there was also an increased endothelial proliferative index in the region of scars (myocardium = 0.12 ± 0.16%, myocardium adjacent to scar = 1.41 ± 0.88%, scar tissue = 1.45 ± 1.34%). Immunostaining for VEGF protein revealed no detectable VEGF in the myocardium, although focal VEGF positivity was seen in the atherosclerotic plaques of the ischemic hearts. Based on ELISA determinations, low-to-absent levels of VEGF protein were also seen in myocardial samples from both groups without significant differences. Semi-quantitative RT-PCR detected VEGF receptor flk-1 mRNA expression in ischemic heart myocardium, but not in cardiomyopathic myocardium.

Conclusions: These data suggest that the low but elevated level of endothelial proliferative activity is spontaneously present in end-stage ischemic human hearts and is not clearly associated with VEGF expression. However the significant expression of flk-1 in these ischemic hearts is consistent with these tissues being possibly responsive to administered VEGF.

Cytopathology

301 Imuncytochemical Study of the Urine Cytological Preparations of the Secondary Prostatic Adenocarcinoma of the Urinary Bladder

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Background: Involvement of the urinary bladder by prostatic adenocarcinoma (PAC) occasionally occurs and is usually associated with high grade and high stage PAC. In this study, we analyzed urine cytological findings in patients with secondary involvement of the urinary bladder by PAC with the help of the immunocytochemistry.

Design: Urine specimens from 15 patients with history of PAC with suspected secondary involvement of the urinary bladder and adequate urine cytospin specimens were included in the study group. The cases were divided into two groups: 1) prospective study group: 3 cases and 2) retrospective study group: 12 cases which were retrieved from the Cytopathological files. The urine cytology specimens (cytospins) from all the cases in the study group were submitted for PSA immunocytochemistry. Additional immunostaining