249 Frequency of Indeterminate HER-2/Neu Staining and Gene Amplification in Ductal and Lobular Carcinoma: A Comparative Study

L Zhang, H Alatassi, AW Martin, S Sahoo. University of Louisville, Louisville, KY.

Background: Her2/Neu protein overexpression and gene amplification is significantly more frequent in invasive ductal carcinoma compared with invasive lobular carcinoma. We evaluated both immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) on all invasive breast carcinomas to determine the frequency of indeterminate Her2/neu positive staining (2+ staining) and gene amplification by FISH.

Design: Four hundred thirty two consecutive cases of formalin-fixed, paraffin-embedded invasive breast carcinomas were tested for Hercept Test™ (DAKO) and FISH by PathVysion (VYSIS). Immunostained slides were analyzed either manually or quantitatively by the ChromaVision® ACIS® assisted quantitative image analysis system. The ACIS® scored the staining as follows: score 0-1.4, negative; 1.5-2.9, positive requiring confirmation of gene amplification by FISH; and ≥ 3.0 as positive. The authors reviewed all the cases scored between 1.5 to 2.9 by the image analysis system. An Her-2/neu:chromosome 17 signal ratio of > 2.0 indicated amplification of Her-2/neu gene by FISH.

Results: Of the 432 cases analyzed by IHC, 276 were negative (includes 48 cases 1+) (64%), 118 were 2+ (27%) and 38 were 3+ (9%). All the cases with indeterminate score computed by the image analysis were confirmed to be 2+ on review. Of the 118 cases with 2+ staining, 101 were invasive ductal and 17 were invasive lobular carcinomas. Among the 2+ ductal carcinomas, 6 were grade I (modified Bloom-Richardson), 34 were grade II, 55 grade III and 6 were metastases. The overall frequency of Her-2/neu gene amplification in indeterminate IHC cases was 22% (26 of 118); much higher in ductal carcinomas 25% (25 of 101 cases) than lobular 5% (1 of 17 cases) ($p< 0.05$). FISH was positive in 17 cases of grade III and 8 cases of grade II ductal carcinomas. None of the grade I ductal carcinomas showed gene amplification.

Conclusions: In this study, only 22% IHC 2+ positive cases showed gene amplification by FISH. Therefore, IHC is still a very good screening test for the evaluation of Her-2/neu gene amplification status. Although rare, lobular carcinomas with indeterminate IHC (2+ staining) need confirmation by FISH.

250 A Subset of Normal and Hyperplastic Appearing Human Breast Cell Clusters Exhibits Similar Immunohistochemical and Cytological Alterations to Carcinoma Cells

C Zhao, R Barner, T Vinh, C Walker, YG Man. Armed Forces Institute of Pathology, Washington, DC.

Background: Histological evaluation has been universally used as a "golden standard" for clinical diagnosis, while it alone often fails to detect malignant lesions that simulate normal or benign tissues, or to detect the precursor of malignant lesions. This study attempted to assess whether the combination of immunohistochemical and cytological evaluation may assist in the differential diagnosis and early detection of breast tumors.

Design: Consecutive sections from reduction mammoplasties (n=30) and from normal and hyperplastic appearing tissues associated with (n=50) and without (n=50) *in situ* and invasive malignant human breast tumors were immunostained for a panel of malignancy-associated markers, including p53, c-erb-B2, and BP1. Normal and hyperplastic appearing cells with the expression of these markers were subject to examination under the highest magnification of a light microscopy.

Results: None of the reduction mammoplasties showed the expression of p53, c-erb-B2, and BP1. Distinct expressions of these molecules, however, were seen in a subset of normal and hyperplastic appearing cells associated with and without, adjacent to or at a distance from, malignant tumors. These positive cells were generally distributed as clusters with a defined boundary to their adjacent counterparts without the expression of these molecules. The frequency and size of these cell clusters appeared to increase with tumor progression. A majority of these cell clusters showed distinct alterations in the nuclear-cytoplasm ratio, as well as nuclear shape, size, and polarity. Some of these clusters were even arranged as triangle-shaped edges protruding or "puncturing" into the stroma, similar to microinvasive lesions, or had a non-cohesive or "floating" appearance, spreading into tube-like structures that resemble blood vessels. These cell clusters, however, were often morphologically indistinguishable from clear-cut normal and hyperplastic cells on H & E stained sections at low magnification.

Conclusions: (1) These p53, c-erb-B2, and BP1 positive cell clusters might belong to a not yet defined malignant cell population or precursors of malignant lesions. (2) An integrated immunohistochemical and cytologic evaluation may have clinical value in the differential diagnosis and early detection.

Cardiovascular

251 Ultrastructural Evidence of Intercalated Disc Remodeling in Arrhythmogenic Right Ventricular Cardiomyopathy: An Electron Microscopy Investigation in Endomyocardial Biopsy

C Basso, M Della Barbera, M Valente, EK Wlodarska, B Bauce, A Rampazzo, A Nava, G Thiene, E Czarnowska. University of Padua Medical School, Padova, Italy; Children Memorial Hospital, Warsaw, Poland.

Background: Despite the advances in the pathology and pathogenesis of arrhythmogenic right ventricular cardiomyopathy (ARVC), the ultrastructural features have been overlooked so far. The recent discovery of gene mutations encoding intercalated disc (ID) proteins in both autosomal recessive and dominant forms prompted us to perform a transmission electron microscopy (TEM) study on endomyocardial biopsies (EMB).

Design: Twenty-one patients (10 M and 11 F, mean age 24,5±14 yrs) with an in vivo diagnosis of ARVC according to the task force criteria underwent right ventricular EMB. Familiarity was present in 8 (38%). Ten EMBs from donor hearts for cardiac transplantation served as controls. EMB samples were fixed in buffered 2.5% glutaraldehyde/osmium tetroxide, embedded in Epon 812 and observed under a Hitachi TEM. Myocyte nuclear, cytoplasmic organelles, contractile apparatus and ID, as well as interstitial abnormalities were assessed. In particular, IDs evaluated in terms of convolution index, D and nexus length (micron), D and nexus percent ID length, and D and nexus number per ID unity length (10 micron). Moreover, D internal and external plaques as well as gap size at the level of D, fascia adherens (FA) and nexus were evaluated.

Results: Extensive fibro-fatty replacement with a mean residual myocardium of 59±23% was found in all EMB samples at histology. TEM did not reveal major differences in terms of ID convolution index in ARVC vs controls. Mean D length and percent D/ID length were higher in ARVC than in controls (0,32±0,17 vs 0,14±0,02 and 10% vs 6%, respectively) whereas the D number/ID unity length was lower (3,38±1.47 vs 5,54±3,06) (all p value <0.01). In ARVC, abnormally located D were detected in 75% and pale internal plaques in 32%. Moreover, widening of both D (29,33±8,95 micron vs 21,68±3,42) and FA gap (41,49±20,36 vs 27,18±10,72) was found.

Conclusions: ARVC shows at ultrastructural level ID abnormalities consisting of decreased D number and increased D length, and D and FA widening in the absence of ID convolution changes. Genotype-phenotype correlation is warranted in order to assess differences between ARVC with and without gene mutations encoding D proteins.

252 Molecular Diagnosis of Acute Myocarditis Causing Sudden Death in Young People

C Basso, E Carturan, F Calabrese, G Thiene. University of Padua Medical School, Padova, Italy.

Background: Although myocarditis usually presents with signs of pump failure and ventricular dilatation as to lead to progressive systolic dysfunction, sudden death (SD) may be the unpredictable fatal clinical presentation in subjects with apparently normal hearts. Aim of our study was to assess the prevalence of viral myocarditis as a cause of SD in the young.

Design: In the time interval 1980-2004, 413 young people (<35 yrs of age, excluding SIDS) who suffered cardiac sudden death (SD) were investigated by a throughout postmortem gross and histologic protocol and 65 (16%) (39 male and 26 female, mean age 21.7±8.7 yrs) were due to acute myocarditis. Since 1998 molecular analysis on paraffin sections (26) or fresh tissue (4) of the myocardium have been also applied in a consecutive series of 30 SDs due to acute myocarditis to search for common cardiotropic DNA and RNA viruses. Sequencing analysis was used to characterize the viral genotype.

Results: A history of flu-like illness in the previous days was documented in 11 (37%). None of them had cardiac symptoms or signs either in the past or in the preceding days. At postmortem, the heart was grossly normal in all, the inflammatory infiltrate was either diffuse (11, 37%) or focal (19, 63%), and at immunohistochemistry was lymphocytic in 19 (67%) and polymorphous in 11 (33%). Clear-cut evidence of myocyte necrosis was present in 15 (50%). Nucleic acids extraction was adequate in 26 (87%) and 17 (65%) had evidence of viral infection: enterovirus in 13 (either isolated -9- or associated with cytomegalovirus in 2, and Epstein Barr virus or Epstein Barr virus and cytomegalovirus one each), parvovirus B19 in 2 (associated with herpes and Epstein Barr virus, respectively), cytomegalovirus and adenovirus one each. No difference was found in terms of myocyte necrosis and inflammatory infiltrate type and extent when comparing viral vs non viral myocarditis (all p=NS).

Conclusions: Acute myocarditis is a no so rare cause of arrhythmic SD in previously healthy young people with grossly normal heart and it is viral in origin in the majority of cases. Double/multiviral infection is common, a feature in keeping with the aggressive pattern of arrhythmogenic myocarditis. Although enterovirus are the most frequent (two-thirds of cases), other virus are detected highlighting the need for a comprehensive molecular pathology screening.

253 Distinctive Peripheral Blood Gene Expression Profiles in Patients Forming Nodular Endocardial Infiltrates (Quilty Lesions) Following Cardiac Transplantation

KE Chu, PG Lal, J Wohlgemuth, G Berry, M Billingham, CC Marboe. Columbia University, New York, NY; Expression Diagnostics, South San Francisco, CA; Stanford University, Stanford, CA.

Background: The origin and significance of Quilty lesions forming after cardiac allografting are unknown. The Cardiac Allograft Gene Expression Observational (CARGO) Study has studied peripheral leukocyte expression profiles after heart transplantation; expression profiles for patients forming Quilty lesions are compared with those of patients not forming Quilty lesions.

Design: From 8 centers, adult cardiac transplant recipients were consented and enrolled in an IRB approved study. Patients were followed at post-transplant visits with endomyocardial biopsy and collection of peripheral blood. Biopsies were graded by a panel of expert cardiac pathologists and the presence of Quilty lesions noted. Using RNA isolated from 145 peripheral blood samples representing 107 patients, real time PCR (QPCR) for 240 genes were performed. These genes were chosen either because they showed differential expression in microarray experiments or are known to be related to alloimmune mechanisms. The gene expression pattern from the Quilty formers was compared to non-Quilty formers within Not Rejecting (ISHLT biopsy grade <2 (NR)) and Rejecting (ISHLT biopsy grade >3A (R)) classes.

Results: 109 samples from 86 patients were classified as NR of which 23 (21%) had formed Quilty lesions in the first year post-transplantation (Q). 36 samples from 28 patients were classified as R of which 15 (41%) were Q. We found the expression of

24 genes to be significantly different between Q and NQ (not forming Quilty) in Non-Rejectors by t-test at ($p < 0.05$) and 10 genes were found to be significantly different between Q and NQ in Rejection. NQ were significantly more likely than Q to over express genes from the S-100 family, integrin (CD11B), and plasminogen activator urokinase receptor in both NR and R classes, while genes for tumor necrosis factor (CD134 and CD134L) and platelet factor 4 are over expressed in Quilty patients in NR.

Conclusions: Cardiac allograft recipients who form Quilty lesions have a distinctive profile of peripheral blood gene expression which differs from non-Quilty formers and may also differ between rejection and non-rejection status of the samples. The pattern of differentially regulated gene expression suggests that monocyte migration and adhesion to inflammatory sites may play an important role in Quilty lesion formation.

254 Juvenile Sudden Cardiac Death: An Autoptic Analysis

G d'Amati, CRT di Gioia, C Autore, MD Romeo, A Lopez, C Ciallella, P Gallo. La Sapienza University, Rome, Italy.

Background: Aim.To assess the causes of juvenile cardiac sudden death in the population of the Lazio region, in central Italy.

Methods. From January 2001 to June 2004, 112 cases of juvenile sudden death (SD) (age ≥ 1 year ≤ 40 years) were consecutively referred to our Department from the Forensic Institutes and Hospitals of our region. A complete autopsy was performed in all cases, and the circumstances of death were recorded. Toxicological tests were available in 40 cases. According to our protocol, we examined the whole hearts when non-cardiac causes of death were excluded at autopsy. Hearts were carefully inspected and multiple samples from both ventricles, the coronary tree and cardiac valves were obtained for histology.

Results: Of 112 cases of juvenile SD, 19 (17%) were natural deaths due to non-cardiac causes. Of the remaining 93 cases, 68 (63%) were male and 25 (37%) female subjects. The mean age was 29.7 years. Death occurred at rest in 71 cases (73%), under physical or emotional stress in 18 (19%) and was unwitnessed in 4 (8%). The whole hearts of 82 subjects (88%) were examined. In 11 cases, the diagnosis was referred. Ischemic heart disease (IHD) due to coronary atherosclerosis (CAD) accounted for 14 (15%) deaths; arrhythmogenic right ventricular cardiomiopathy (ARVC) was diagnosed in 12 cases (13%), congenital heart disease (CHD) in 9 (9.6%), dilated cardiomyopathy (DCM) in 5 (5%), hypertrophic cardiomyopathy (HCM) in 3 (3.2%), hemopericardium due to aortic dissection in 3 (3.2%), mitral valve prolapse in 3, acute myocarditis in one (1%). In 3 cases the only cardiac finding was left ventricular hypertrophy. In 32 cases (34%) the heart was normal or showed only mild fatty infiltration of the right ventricular anterior wall; toxicological tests were negative and death was attributed to an arrhythmic event. When available, clinical records of subjects with cardiomyopathies (n = 20) or sudden arrhythmic death syndrome (n = 32) were reviewed and first-degree relatives underwent cardiological assessment. Six of 22 families were diagnosed with inherited cardiac disease.

Conclusions: A significant proportion of juvenile cardiac SD is due to familial cardiomyopathies or inherited arrhythmogenic syndromes without an anatomical substrate.Sudden death is often the first symptom of the underlying disease. Thus, a careful autopsy becomes the only diagnostic tool in these cases, providing important information to guide clinical and genetic assessment of the families.

255 Juvenile Sudden Death in a Family with Polymorphic Ventricular Arrhythmias Due to a Novel RyR2 Gene Mutation: Evidence of Specific Morphologic Substrates

G d'Amati, A Bagattin, C Basso, B Bauce, A Rampazzo, P Gallo, A Nava, C Autore, GA Danieli, G Thiene. "La Sapienza" University, Rome, Italy; University of Padua, Italy.

Background: A significant proportion of juvenile sudden cardiac deaths is due to inherited cardiovascular disorders. The fatal outcome is often the first symptom in apparently healthy subjects with an underlying concealed disease.Thus, a careful autopsy frequently becomes the sole diagnostic opportunity, providing important information to guide clinical and genetic assessment of the families.

Design: A family is reported with history of sudden death and effort-induced polymorphic ventricular arrhythmias.

Results: The index case was a 17 year old boy who died suddenly and at postmortem had evidence of trasmural fibro-fatty replacement in the right ventricular free wall in keeping with arrhythmogenic right ventricular cardiomyopathy; calcium phosphate deposits within still viable cardiac myocytes were also observed in the right ventricle, in absence of histologic signs of either ischemia or inflammation. Molecular genetics investigation, carried out in paraffin embedded myocardium of the index case and in blood samples of family members, disclosed a missense mutation in exon 3 (230 C>T; A77V) of cardiac ryanodine receptor type 2 gene (ARVD2). The carriers showed effort-induced polymorphic ventricular tachycardia in the setting of normal resting ECG and trivial echocardiographic abnormalities, in keeping with cathecolaminergic polymorphic ventricular tachycardia (CPTV). The observation of both ARVD2 and CPTV in the same family suggest that the two entities might correspond to different degrees of phenotypic expression of the same disease. The presence of intracellular calcium deposits might be specifically related to the underlying molecular defect. The preferential location of such deposits in cardiomyocytes seems to support the hypothesis that RyR2 mutation might *per se* determine structural myocardial damage and death, eventually leading to fibro-fatty tissue substitution.

Conclusions: This experience underscores the importance of a precise autopsy diagnosis in case of sudden cardiac death, including molecular genetics, and the pathologist's mission to guide further clinical investigation in family members.

256 Proteomic Footprints of Rheumatic Heart Disease: Comparison of Normal and Rheumatic Mitral Valvular Interstitial Cells

SD Dhaliwal, BM Cheyne, RP Cruz, DJ Granville, A Lee, D Smith, R Olafson, BM McManus. University of British Columbia, Vancouver, BC, Canada; Genome British Columbia Proteomics Centre, Victoria, BC, Canada.

Background: Rheumatic fever (RF) is a systemic inflammatory disease, occurring after an episode of group A streptococcal pharyngitis. The acute phase of carditis associated with RF can progress into chronic rheumatic heart disease (RHD), a condition wherein the mitral valve is most often affected. RHD is characterized by valve thickening, neovascularization of the spongiosa, and inflammation, resulting in mitral stenosis and/or regurgitation. Valvular interstitial cells (VIC) play a key role in the valvulopathic process by virtue of their proliferation, movement, and connective tissue production. We *hypothesize* that the abnormal phenotype of rheumatic heart valves, particularly the mitral valve, is reflected in the protein expression of the VIC resident to diseased valves as compared to those from normal valves.

Design: Ten normal and nine rheumatic mitral valves were examined grossly and microscopically. VIC from representative normal and rheumatic mitral valves were cultured and then underwent protein extraction and analysis via iTRAQ™. In each iTRAQ™ experiment, two normal and two rheumatic protein samples were proteolysed with trypsin and labeled with one of four different iTRAQ™ tags. Labeled samples were mixed and analyzed by 2D LC-MS/MS. The MS/MS results provided protein expression profiles for normal and rheumatic VIC and the relative quantities of each protein between normal and rheumatic samples were calculated.

Results: iTRAQ™ analysis of rheumatic and normal mitral valves shows several key proteins with altered expression of relevance to valve structure. In particular, myristoylated alanine-rich C-kinase substrate, alpha-B-crystallin, collagen 1A1 and PDGFB fusion transcript, and calmodulin 2, showed at least a two-fold decrease in expression in diseased VIC as compared to non-diseased VIC ($p<0.01$). In addition, the rheumatic valve VIC had a two-fold increase in expression of ubiquitin carboxyl-terminal hydrolase, UDP-glucose ceramide glucosyltransferase, and procollagen-lysine ($p<0.01$).

Conclusions: Our unique study, using highly sensitive iTRAQ™ technology, demonstrates that the proteome of rheumatic mitral valve VIC is distinctively altered as compared to normal mitral VIC. Such differences in protein expression provide new insights into the molecular mechanisms which lead to RHD.

257 Is Segmental Mediolytic Arteriopathy (SMA) an Elastase Induced Arterial Disease? Report of 6 Patients and Review of 26 Published Cases

AG Rose, LJ Martin, PV Kaye, S Tawfic, BM Segal. University of Minnesota, Minneapolis, MN; University of Cape Town, Cape Town, W.Cape, South Africa.

Background: SMA is a rare, idiopathic, noninflammatory lesion of muscular arteries characterized by focal lysis of medial smooth muscle cells (SMCs) leading to hemorrhage, thrombosis, dissection or aneurysm and resulting in ischemia in organs. Only 26 patients with SMA have been reported.

Design: SMA arterial lesions in 6 patients were studied by light microscopy of sections stained by H&E, EvG, trichrome and CD31 stains. Staining for immunoglobulins IgG, IgA and IgM was done on cases 3 and 5; case 3 was also stained for kappa and lambda light chains.

Results: Table 1 gives clinicopathologic features of our 6 patients. Table 2 lists features of 26 published cases. A striking feature of the arterial ruptures was focal destruction of the elastic laminae of affected arteries that may reflect primary weakening of elastin rather than resulting from lysis of SMCs. There is experimental evidence for elastase induced aortic and arterial aneurysms.

Table 1: Clinicopathologic Features of 6 Patients with SMA

Case/ Age,Sex	Associated disease	Pregnant	Mediolysis	Dissection	Rupture	Tears	Saccular aneurysm
1/ 30 F	Nil	Yes	Iliacs	Iliac, splenic, renal	Splenic	Iliac (acute, healed)	Nil
1/ 35 F	Nil	No	Renal	Renal	Aortic branch	Nil	Nil
3/ 27 F	Nil	Yes	Coronary, renal	Abd aorta, coronary, iliac	Abd aorta	Nil	Nil
4/ 54 M	idiopathic lung fibrosis (familial)	N/A	Renal	Renal	Nil	Nil	Nil
5/ 23 M	Ulcerative colitis	N/A	Tibials	Posterior tibial	Posterior tibial	Posterior tibial	Nil
6/ 48 M	AATD, emphysema	N/A	Nil	Nil	Intercostal	Nil	Intercostal

AATD = alpha-1-antitrypsin deficiency

Table 2: Clinicopathologic features of 26 Published Cases of SMA. (Mean age= 55 years, range 3-87, SD=20.2. Sex: 17 females, 9 males).

Arteries Affected	Associated Conditions
Splenic (6)	Systemic hypertension (6)
Internal carotid (6)	Emphysema (2) (AATD in 1)
Pancreatic-duodenal (5)	Crohn's disease (1)
Hepatic (4)	SLE (1)
Mesenteric (3)	Thrombophlebitis (1)
Renal (4)	Urinary infection (1)
Vertebral (4)	Heart valve disease (1)
Omental (2)	Pulmonary embolism
Colic (3)	Brain infarct (1)
Basilar (3)	Meningioma (1)
Circle of Willis (3)	
Other (13)	

SLE = systemic lupus erythematosus. AATD = alpha-1-antitrypsin defiency.

Conclusions: Since arteritis is not a feature of SMA, elastase overactivity is a possible etiology that warrants further study. This final common pathway may explain the development of SMA in a number of apparently disparate conditions including autoimmune disease, alpha-1-antitrypsin deficiency and pregnancy.

258 The Value of C4d Detection in the Diagnosis of Humoral Cardiac Allograft Rejection

AM Safley, S Fedson, P Pytel, S Meehan, A Hussain. University of Chicago, Chicago, IL.

Background: Detection of C4d in endomyocardial biopsies (EMBs) has previously been correlated with the presence of detectable alloantibodies, early and late allograft complications, and cardiac graft loss. However, the clinical value of this marker for diagnosing cardiac humoral rejection has not been well-established.

Design: We evaluated C4d immunohistochemistry (IHC) in 64 archival paraffin-embedded EMBs from 26 cardiac transplant patients. Biopsies were graded as negative or positive for C4d capillary and interstitial staining. Positive biopsies were further graded as focal, diffuse, weak, or strong staining. Allograft survival and clinical status was obtained by chart review.

Results: Strong diffuse (5) and focal (10) capillary C4d staining was observed in 14 biopsies from 7 patients. C4d staining for all other patients showed either negative or focal weak vascular and interstitial reactivity. Among 4 patients with biopsies showing strong positivity, surveillance biopsies at additional time points, post-transplant, 2 months, and later, mean 7.2 months, showed persistent, strongly positive C4d staining. However, clinical cardiac function for these patients remained stable. In contrast, detection of strong C4d positivity for 2 patients appeared to be isolated to episodes of clinical rejection. Overall, negative and weak focal capillary and interstitial C4d staining was observed with similar frequency in normal surveillance biopsies and biopsies performed during episodes of clinical rejection.

Conclusions: Weak capillary and interstitial C4d staining in this study appears to be a nonspecific finding with little or no predictive value or correlation with humoral rejection. Moreover, our results suggest that the clinical correlation between strong C4d IHC positivity in EMBs and acute humoral rejection is unclear. The value of C4d in the diagnosis of humoral rejection remains unclear at this time.

259 Sustained Progression and Loss of Gender-Related Difference of the Atherosclerosis in the Very Old: A Pathological Study of 1,074 Consecutive Autopsy Cases

M Sawabe, T Arai, A Hamamatsu, K Chida, N Tanaka. Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan; Tokyo University, Tokyo, Japan.

Background: Recent epidemiological surveys show high cardiovascular mortality in females over 85 years, but it is unknown whether the atherosclerosis continues to worsen, or the gender-related difference exists in the very old. To address these issues, we have performed a pathological study of elderly autopsy cases.

Design: The subjects were 1,074 consecutive autopsy cases of in-hospital death. No medicolegal cases were included. The male to female ratio was 1.1:1 and the average age was 80 years. The autopsy rate was 40% and the brain was available in 85% of the cases. Macroscopical evaluation was performed on the atherosclerotic degrees of 10 large arteries including intracranial arteries, common carotid artery, aorta, coronary artery, superior mesenteric artery, and femoral artery.

Results: The severity of atherosclerosis differed greatly among arteries. The age-related increase of atherosclerotic degrees was evident, even after 80 years of age. The atherosclerosis was severer in males than in females in the 60's, but this male predominance decreased with ageing and finally disappeared in the 90's. The changing rate of age-related increase was especially high in the femoral arteries. The correlations of the atherosclerotic degrees among the arteries were generally good with simple correlation coefficients more than 0.4, except for splenic, superior mesenteric and intracranial arteries. The multiple regression analysis of the severity of systemic atherosclerosis showed significant contributions of traditional risk factors such as age (standardized regression coefficient = 0.459, $P < 0.0001$), smoking history (0.278, $P < 0.0001$), hypertension (0.210, $P < 0.0001$), and diabetes mellitus (0.089, $P < 0.005$). Parkinson's disease, chronic hepatitis/cirrhosis and some malignancy (lung cancer, gastric cancer and hematopoietic malignancy) lowered the risk of atherosclerosis. The multiple regression analysis showed the adopted risk factors accounted for 37.7% of the total variation in the systemic atherosclerosis.

Conclusions: The atherosclerosis continued to proceed in the very old. The gender-related difference of atherosclerosis was lost in the 90's. These data accounts for the high cardiovascular mortality in the female very old.

260 Proteomic Profiling of Human Vascular Intimal Proteoglycans

JR Stone, P Talusan, T Kattapuram. Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Background: The proteoglycans are important components of the extracellular matrix and play key roles in vascular diseases. The proteoglycans are upregulated in thickened vascular intima, and are believed to contribute to atherosclerosis by binding and retaining lipoproteins in the vessel wall, and by promoting cell growth. Our current understanding of the complete extracellular proteoglycan composition of human intima is limited, as is our understanding of how this intimal proteoglycan composition varies between arteries with different susceptibilities to the development of atherosclerosis.

Design: Atherosclerosis-prone internal carotid arteries, at the level of the carotid bifurcation, and atherosclerosis-resistant internal thoracic arteries, at the level of the bifurcation of the intercostal arteries below the second rib, were obtained from autopsies. The vessels were first examined histologically; those displaying intimal hyperplasia, but not atheroma formation, were utilized for the proteomic analysis. The thickened intima was dissected from the vessels, and the proteoglycans extracted and isolated using micro-scale anion exchange chromatography. The proteoglycan core proteins present were then identified using liquid chromatography tandem mass spectrometry.

Results: The extracellular proteoglycan profile of human vascular intima was readily obtained with this technique. This profile was found to be substantially more complex than previously realized with up to ten distinct proteoglycan core proteins present. Importantly, there was a significant difference in the intimal proteoglycan profile of the atherosclerosis-prone internal carotid artery compared with that of the atherosclerosis-resistant internal thoracic artery.

Conclusions: Proteomic techniques can be utilized to profile vascular intimal proteoglycan core proteins. There are significant variations in the intimal proteoglycan composition between different anatomical sites, and these variations may be responsible for the marked differences in susceptibility to atherosclerosis at these sites. Intimal proteoglycan profiling may be a useful approach for the subclassification of vascular diseases.

Cytopathology

261 Spermatogenesis Status in Azoospermic Patients, Correlation of Fine Needle Aspiration Results with Testicular Size and Hormonal Status

HA Abu-Farsakh. King Hussein Cancer Center & First Medical Lab, Amman, Jordan.

Background: The new advances in infertility by injecting sperms into ova, made finding rare sperms in the testis by fine needle aspiration (FNA) is of paramount importance. Azoospermic patients have variable degree of sperm production in the testis (varies from none to abundant).

Design: 720 azoospermic patients studied from the period (1/2000-12/2003). 10 fine needle aspirations from the testis (5 from the right and 5 from the left) were performed on each patient under local anesthesia. Spermatogenesis status reported as normal spermatogenesis (NS), hypospermatogenesis (HS), spermatogenesis arrest at spermatid stage (SATS), spermatogenesis arrest at spermatocyte stage (SACS), or sertoli cell only (SCO). Testicular size recorded as follow : normal testicular size, intermediate size: ¾-1/2 of the normal size and small size: less than ½ of the normal size. FSH hormone records available in 292 patients.

Results: sperms seen in 41% of all cases, regardless of their testicular size. Testicular size in azoospermic patients was noted to be as follows: 37% normal size, 32% intermediate size, 31% small size. Sperms seen at almost the same rate in normal testis compared with smaller type testis 39% vs. 37%. Patients with normal testicular size and normal hormones (total of 145 cases) may have the following spermatogenesis status: NS 17%, HS 32%, SATS 6%, SACS 26%, SCO 19%. Obstructive azoospermia was seen only in only 6% of all azoospermic patients, 15% of all patient containing sperms, and 17% of patient with normal testicular size. Of all patient with normal hormones, 49% have sperms, while sperms are much less seen in patient with abnormal hormones (in only 30%). Patient with less than normal testicular size and with abnormal hormones have 32% chance of having sperms. The number of sites sperms seen is much higher in normal size testis than in smaller testis, as sperms seen in 5-10 sites in normal size testis (27%), compared with 13% of smaller size testis.

Conclusions: Azoospermic patient have all the different cytopathological patterns in the testis (NS, HS, SATS, SACS, and SCO). Testicular size and abnormal hormones alone are not good predictors of finding sperms in the testis. Testis is a heterogenous organ in sperm production. The more sites we sample the testis, the better chance of having sperms. FNA is an excellent procedure for multiple testicular samples with very high sensitivity in detecting spermatogenesis status in azoospermic patients.

262 Spectral and Spatial Analysis of Thyroid Fine Needle Aspiration Specimens Using the Hybrid Genetic Algorithm "GENIE"

C Angeletti, NR Harvey, V Bossuyt, RM Levenson, DL Rimm. Yale University School of Medicine, New Haven, CT; Los Alamos National Laboratory, Los Alamos, NM; CRI,Inc, Woburn, MA.

Background: Fine needle aspiration (FNA) of the thyroid is widely accepted as the most cost-effective diagnostic test. However, ambiguous diagnostic terms, such as suspicious or indeterminate, are a major limitation of this technique. GENetic Imaginery Exploitation (GENIE) is a recently developed artificial intelligence system that generates mathematical algorithms for the classification of images using spatial and spectral (color) analysis.

Design: Multispectral images from Papanicolaou stained cytology ThinPrep® slides from ten thyroid FNA specimens with surgical follow up were used as training data to distinguish benign follicular cells from papillary carcinoma. The images were imported into the GENIE platform through a Java tool graphical interface. The algorithms generated were tested on a separate validation set containing 46 FNA specimens (one high power image per slide).

Results: Through the use of selected training features, GENIE generated various algorithms that detect papillary carcinoma with sensitivities and specificities of up to 92 and 86 %, respectively. Because these algorithms are generally feature-specific, we designed a new experiment to test if they could be integrated by means of a second higher order algorithm in an analogous way to abstractive thinking. We performed four independent training sessions using the exact same training set, which generated four different algorithms, suggesting that each time GENIE randomly concentrated on particular classificatory features. The areas under ROC curve for each algorithm ranged between 0.771 and 0.828. Using the same platform, the result images of each algorithm in the training set were re-imported to generate a new second order algorithm capable of analyzing the information from the other four (first order) algorithms. On the validation