ORAL CONTRIBUTIONS

805 Electron Beam Computed Tomography: Epidemiology and Relation to Plaque Burden

Monday, March 18, 2002, 9:15 a.m.-10:30 a.m.
Georgia World Congress Center, Room 255W

9:15 a.m.

805-1 Ethnicity and Calcified Atherosclerosis: Can Data on Coronary Calcium Be Applied Evenly Across Ethnic Groups? Results From the Prospective Army Coronary Calcium Project

Timothy C. Lee, Patrick G. O'Malley, Irwin Feuerstein, Allen J. Taylor, *Walter Reed Army Medical Center, Washington, Dist. of Columbia.*

Background: Black Americans have more prevalent coronary risk factors and experience greater rates of incident coronary heart disease than white Americans. Coronary artery calcium (CAC), a specific marker for coronary atherosclerosis that is quantifiable with EBCT, is increasingly used to indicate cardiovascular (CV) risk. However, whether existing data should be evenly applied across ethnic groups is unclear because black and white Americans may differ in their prevalence of CAC. This study examines the prevalence of CAC on EBCT across different ethnic groups among asymptomatic, active-duty personnel in the Prospective Army Coronary Calcium (PACC) Project.
Methods: Among 1000 consecutive participants, 989 (mean age 42 ± 2 yrs; range 40-45 yrs) indicated a specific racial affiliation. This included white, nonhispanic in 695 (70.3%) and black, nonhispanic in 194 (19.6%). Univariate associations between race and CV risk variables were entered into a logistic regression model for CAC, controlling for race and socioeconomic status.
Results: CAC was nearly twice as prevalent in white (19.3%) than in black participants (10.3%; p=.003) between the ages of 40 and 45 yrs. Blacks had different CV risk profiles, including a greater prevalence of hypertension, (17.7% vs. 6.6%; p<.001), LVH (13.3% vs. 4.1%; p <.001), ST-T wave abnormalities (18.1% vs. 3.8%; p<.001), and former cigarette smoking (16% vs. 5.2%; p<.001). Black subjects also had significantly greater diastolic BP, HDL, glycosylated hemoglobin, lipoprotein (a) and fibrinogen levels, and lower triglyceride and waist girth than white subjects. After adjustment for these differences, and including socioeconomic adjusters, logistic regression revealed white race, and higher body mass index and triglyceride levels remained statistically significant predictors of CAC.
Conclusions: CAC is less prevalent in black than in white Americans, and this difference is unexplained after adjusting for differences in CV risk factors and socioeconomic status. These differences imply that the use of EBCT as an accurate risk prediction tool in black Americans will require ethnic-specific data on the presence and severity of CAC.

9:30 a.m.

805-2 Sub-Clinical Atherosclerosis in Hypertensive Individuals: The Role of Conditional Risk Factors

Iftikhar J. Kullo, Joseph P. McConnell, Lawrence F. Bielak, Patricia A. Peyser, Sharon L. Kardia, Patrick F. Sheedy, II, Eric Boerwinkle, Stephen T. Turner, *Mayo Clinic, Rochester, Minnesota, University of Michigan, Ann Arbor, Michigan.*

Background: The ability of 'conditional' risk factors [lipoprotein (a), fibrinogen, homocysteine, triglycerides, small-dense LDL, and C-reactive protein] to predict the presence and extent of sub-clinical atherosclerosis is poorly understood.
Methods: We studied the relationship between conditional risk factors and quantity of coronary artery calcification (CAC) as determined non-invasively by electron beam computed tomography among 168 hypertensive siblings recently re-examined in the community-based Genetic Epidemiology Network of Arteriopathy (GENOA) study. The 10-year Framingham risk score was calculated based on conventional risk factors and the CAC score was calculated by the method of Agatston. Population-averaged generalized estimating equations (GEE1) were used to assess the association between the log-transformed CAC score and conditional risk factors while allowing for the familial correlation in these siblings.
Results: Framingham risk score was significantly associated with quantity of CAC ($p < 0.01$). After adjusting for Framingham risk score, homocysteine was the only independent significant predictor of the quantity of CAC among the conditional risk factors ($p < 0.029$). One standard deviation increase in homocysteine was associated with a 1.34 multiplicative increase in the quantity of CAC.
Conclusion: In hypertensive individuals, homocysteine levels are significantly correlated with the extent of sub-clinical atherosclerosis. This suggests a pro-atherogenic role for homocysteine in hypertensive individuals.

9:45 a.m.

805-3 Plasma Homocysteine and Not C-Reactive Protein Predicts Progression of Atherosclerosis

Margaret Leila Rasouli, Matthew J. Budoff, Robert Park, Douglas Aziz, *Harbor-UCLA, Torrance, California.*

Background: Despite the availability of effective preventive therapies, coronary artery disease (CAD) remains the leading cause of morbidity and mortality. Use of traditional cardiovascular risk factors is imprecise and predicts less than one half of future cardiovascular events. Three potential means of identifying subclinical atherosclerosis and predicting future cardiovascular events are electron-beam computed tomography (EBT), homocysteine(HCY), and C-reactive Protein (CRP). Given the evidence that HCY and CRP are involved in atherogenesis, we hypothesized that significant progression of EBT calcium score (a measure of atherosclerotic plaque burden) is associated with higher levels of these markers. **Methods:** We enrolled 65 asymptomatic patients (49 male, 16 female, age 62±9 yr) who underwent previous EBT calcium score testing at Harbor-UCLA 9-36 months previously and whose prior test scores fell within the low to intermediate range (scores between 5-200). Exclusion criteria included those with established symptomatic CAD and chronic renal disease. During enrollment, we measured risk factors, measured serum HCY, lipid panel, ultrasensitive-CRP, elicited personal history such as medication use, family and smoking history, and repeated their EBT calcium scan. Statistical analysis was performed using probable Chi square method, T Test, and multivariate analysis. **Results:** Study subjects with HCY level </= 12 (median) exhibited a mean yearly progression in coronary calcium of 12.6 +/- 19.3% (N=33), compared to 29.9 +/- 26.8% for those subjects (N=32) with HCY value > 12 (p <0.01). Patients with CRP </= 0.07 (N=33) had a mean yearly progression of 17.6 +/- 24.4 %, compared to 24.6 +/ = 26.6% for those with CRP value=0.07-1.1 (p= 0.28). Neither cholesterol values (including LDL and HDL cholesterol), body mass index, gender, age nor presence of individual risk factors predicted progression of coronary calcium. **Conclusion:** Presence of elevated HCY (>12) strongly and independently predicts progression of coronary plaque burden.

10:00 a.m.

805-4 Coronary Calcifications in Young Patients With First, Unheralded Myocardial Infarction: A Risk Factor Matched Analysis by Electron Beam Tomography

Karsten Pohle, Dieter Ropers, Patricia Geitner, Theresa Menendez, Matthias Regenfus, Werner Moshage, Werner G. Daniel, Stephan Achenbach, *University of Erlangen, Erlangen, Germany.*

Electron beam tomography (EBT) is a sensitive tool for the detection of coronary calcifications. The purpose of this study was to assess the presence and extent of coronary calcifications in young patients with first acute myocardial infarction (AMI) in comparison to matched controls without a history of coronary artery disease (CAD).
Methods: In 102 patients below 60 years of age (19-59y., mean 40.1y., 91% male), EBT was performed 5-10 days after unheralded, first AMI before coronary intervention. Coronary calcification was quantified using the "Agatston Score". Age-related calcium percentiles were determined based on the "Epidemiology of Coronary Calcification Study", Mayo Clinic, Rochester, and results were compared to a group of 102 controls without CAD matched for gender, age and risk factors.
Results: Calcifications were present in 96/102 patients with AMI (96%) and 59/102 controls (59%, p≤0.01). The mean calcium score was 529±903 in AMI patients vs. 119±213 in controls (p≤0.001). An Agatston Score above the 50th percentile was present in 90/102 AMI patients and in 47/102 controls (90% vs. 46%, p≤0.01), above the 90th percentile in 62/102 patients and in 6/102 controls (62% vs. 5%), p≤0.001).
Conclusion: In young patients with first, unheralded AMI, presence and extent of coronary calcification is significantly greater than in matched controls. However, the culprit vessel is not calcified in all cases and the overall amount of calcium can be very low while usually, the amount of calcium corresponds to a high age-related percentile.

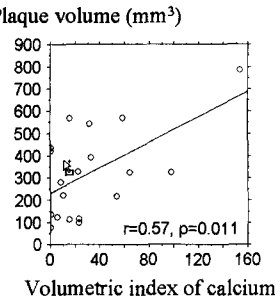
10:15 a.m.

805-5 What Is the Relationship Between Extent of Calcification and True Atherosclerotic Burden In Vivo? A Volumetric Intravascular Ultrasound Analysis

Adrienne M. Tinana, Gary S. Mintz, Neil J. Weissman, *Washington Hospital Center, Washington, Dist. of Columbia, Cardiovascular Research Foundation, New York, New York.*

Pathology studies have suggested that the extent of coronary calcium correlates with the atherosclerotic burden. EBCT is used to screen for coronary calcium as a surrogate for atherosclerosis but prior in-vivo validations used angiography, which can only assess stenosis severity and not overall atherosclerosis burden. Using volumetric intravascular ultrasound (IVUS) in 19 patients with a focal RCA stenosis, we assessed the true correlation between the degree of calcification and overall plaque burden. IVUS measurements of the external elastic membrane (EEM) cross sectional area (CSA), lumen CSA, and plaque&media (EEM minus lumen) CSA, and arcs of calcium were obtained using computerized planimetry every mm throughout the RCA from crux to ostia and volumes were calculated. Results: There was volumetrically more calcium in the nonstenotic segments than in the stenosis (28±28 mm^3 vs 9±14 mm^3,p=0/0055). There was a significant correlation of atherosclerotic volume with a volumetric measure of calcium: p=0.011 in all segments, p=0.0091 in the stenoses, and p=0.096 in the nonstenotic segments. Conclusions: Although the maximum arc of calcium may be greater in the stenotic lesion

than the non-stenotic segments, there is volumetrically more calcium contained in the non-stenotic arterial segments. Plaque volume correlated strongly with a volumetric index of arterial calcification .



ORAL CONTRIBUTIONS

816 New Applications of Microbubbles

Monday, March 18, 2002, 11:00 a.m.-12:15 p.m.
Georgia World Congress Center, Room 255W

11:00 a.m.

816-1 Development of an Angiogenesis-Targeted Microbubble Ultrasound Contrast Agent

Howard Leong-Poi, Alexander L. Klibanov, Jonathan P. Christiansen, Yuqing Huo, Jonathan R. Lindner, *University of Virginia, Charlottesville, Virginia.*

Background. An accurate non-invasive technique for assessing angiogenesis in patients is not available. We hypothesized that microbubbles targeted to α_v-integrins, which are expressed by endothelial cells in response to growth factors, would be retained in angiogenic vessels.
Methods. Microbubbles targeted to α_v-integrins were prepared by conjugating either monoclonal antibodies against murine α_v-integrins (MB_α), or the disintegrin echistatin (MB_E), to the shell surface. Control microbubbles bearing an isotype control antibody (MB_{iso}) were also prepared. Flow cytometry was performed to assess microbubble attachment to cultured murine endothelial cells (MEC) that express α_v-integrins when activated by IL-1β. The microvascular behavior of these microbubbles was assessed by intravital microscopy of 5 mice following intrascrotal placement of heparin-alginate pellets for time release of 1.5 μg of basic fibroblast growth factor (bFGF) over 5 days, and in 3 sham-treated control mice. Observations were made following venous injections of 1×10^7 targeted or control microbubbles. Vascular density was assessed following intravenous injection of FITC-dextran.
Results. Flow cytometry demonstrated that MB_α and MB_E, but not MB_{iso}, adhered to activated MEC that express α_v-integrins. On intravital microscopy, bFGF-treated mice demonstrated increased cremasteric vascular density compared to controls. Microbubble retention in control mice was uncommon (4 ± 2 mm^{-3}) for all microbubbles, and was mostly due to venular leukocyte attachment. In bFGF treated animals, microbubble retention was greater ($p<0.05$) for MB_α (13 ± 9 mm^{-3}), and MB_E (13 ± 8 mm^{-3}), compared to MB_{iso} (1 ± 1 mm^{-3}). Enhanced retention of MB_α and MB_E was due to direct endothelial attachment predominantly in arterioles, with some attachment in capillaries and venules.
Conclusions. Microbubbles targeted to endothelial α_v-integrins are retained in vessels stimulated by bFGF. Microbubbles targeted to endothelial α_v-integrins may provide a means to non-invasively assess angiogenesis using contrast-enhanced ultrasound imaging.

11:15 a.m.

816-2 Myocardial Contrast Echocardiography Can Be Used to Detect Acute Heart Transplant Rejection

Erxiong Lu, Melissa M. Black, David Fischer, William R. Wagner, Flordeliza S. Villanueva, *University of Pittsburgh, Pittsburgh, Pennsylvania.*

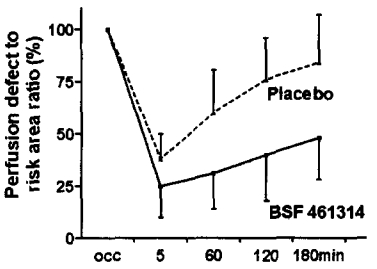
Background: Albumin microbubble (mbub) adhesion to activated leukocytes provides an approach to ultrasonic identification of inflamed endothelium. Because acute heart transplant rejection (REJ) generates a strong myocardial inflammatory response, we hypothesized that myocardial contrast echocardiography (MCE) can detect REJ.
Methods: Heterotopic heart transplant was performed to the abdominal aorta of Lewis rats. Rat donor strains were Brown Norway (REJ group, n=18) or Lewis (Control group, n=11). On post-op day 5, rats were given i.v. 0.05ml albumin mbub. Mbub retention was detected by intermittent transthoracic high mechanical index harmonic MCE imaging of the transplant to capture 4 frames spaced 300msec apart at both 2.5 and 3 minutes after injection. Rats were killed after 3 injections and post-mortem H&E staining was performed for histologic REJ grade. MCE background-subtracted videointensity (VI) was measured in the entire left ventricular (LV) myocardium, LV areas with greatest histologic lymphoid infiltration, and the right ventricle. Data are expressed as VI difference between the first 2.5-minute frame (adhered+circulating mbub) and 3-minute frame (circulating mbub), equivalent to the VI attributable to adhered mbub.
Results: Three rats died; 8 were excluded due to segmental infarction, attenuated images, or inflammation in a Control. All remaining rats in the REJ group (n=12) showed histologic REJ, compared to none in Controls (n=6). LV VI was higher in the REJ vs Controls, both for the entire myocardium (11±3 vs 5±1, $p<0.04$), as well as those regions paralleling severe inflammation (12±2 vs 4±1). There was a trend towards higher right ventricular VI in the REJ rats, and higher VI in rats with severe compared to mild REJ.
Conclusions: In this heart transplant model, persistent myocardial enhancement during MCE is a marker of acute REJ. These data are consistent with mbub retention in the microcirculation of REJ heart, and are the first demonstration of MCE imaging of myocardial inflammation. The use of mbub specifically targeted to inflammatory endothelial markers might augment such imaging. MCE may thus permit diagnosis of heart disease associated with endothelial activation.

11:30 a.m.

816-3 Dynamics of Ischemia-Reperfusion Injury Evaluated by Real-Time Myocardial Contrast Echocardiography: Protective Effects of a Novel Endothelin A Antagonist BSF 461314

Alexander E. Hansen, Raffi Bekeredjian, Arthur Filusch, Marie-Luise Gross, Klaus Muenter, Marie Gebhardt, Anne Broillet, Helmut Kuecherer, *Cardiology, Heidelberg, Germany.*

Recanalization of the infarct-related artery does not guarantee myocardial salvage due to reperfusion injury of microvessels. Real-time myocardial contrast echocardiography (MCE) allows visualisation of spatial and temporal dynamics of infarct expansion.
Methods: 12 open-chest pigs underwent 45 min of mechanical LAD occlusion followed by 180 min of reperfusion. A new highly selective endothelin A antagonist BSF 461314 (20 mg) or placebo was given intravenously 20 min before releasing LAD occlusion. MCE was performed using Power Pulse Inversion imaging (PPI) at MI=0.09 during steady state intravenous infusion of Sonovue (90ml/h). Perfusion-defect-to-risk-area-ratio and contrast replenishment kinetics (A=peak intensity as % of baseline intensity, β=rate of intensity rise) were measured at baseline, during occlusion (occ) and following reperfusion and compared to histology.
Results: During LAD occlusion signal intensities were reduced in anterior regions (A=4.2±3%, β=0.05±0.02) defining risk areas and approached baseline levels 5 min post recanalization (A=102±40%, β=0.58±0.16) but gradually decreased during reperfusion (A=37±12%, β=0.18±0.13; $p<0.005$). Accordingly, the defect-to-risk-area-ratio progressively increased during reperfusion. This increase however was markedly reduced in the BSF treated group (see fig., p=0.004).
Conclusions: Real-time MCE can evaluate dynamics of myocardial reperfusion injury and monitor cardioprotective effects of endothelin A antagonism.



11:45 a.m.

816-4 Albumin Microbubbles Can Be Targeted to Activated Neutrophils In Vivo: Application to Assessment of the Neutrophils Infiltration Pattern Produced by Ischemia/Reperfusion

Isao Kondo, Koji Ohmori, Akira Oshita, Hiroto Takeuchi, Kaori Shinomiya, Yang Yu, Yuichiro Takagi, Kazushi Yukiiri, Yoshihiro Wada, Katsufumi Mizushige, Masakazu Kohno, *kagawa Medical University, Kagawa, Japan.*

Background: Contrast enhancement at the site of inflammation has been attributed to the interactions between activated neutrophils (NP) and microbubbles (MB) including adhesion and phagocytosis. Intensity of signal from MB with lipid shell was correlated with the degree of inflammation in reperfused whole kidneys in mice. However, a direct comparison of spatial pattern of NP infiltration with that of contrast enhancement has not been reported. The ability of MB with albumin shell to detect and locate the activated NP in the site of inflammation remains unknown. Therefore, in an ischemia/reperfusion model, we correlated the regional signal intensities from albumin shell MB with the distribution of activated NP in the tissue, assessed with immunohistochemistry. Methods: In seven SD rats unilateral total renal ischemia (20 min) and reperfusion (4 hours) were implemented. Long axis harmonic (1.8/3.6MHz) images of the reperfused and control kidneys were obtained by an S4 transducer of SONOS5500 with MI of 1.1 before, during, and 10 min after the intravenous infusion of 10% Optison (1mL/5min). Ultrasound emission was limited only to obtain two frames each time. Then, immunohistochemical staining of NP elastase was performed to assess the distribution of the activated NP on the tissue slices corresponding to the ultrasound images. Results: On the first frame of the image during MB infusion, dense opacification was observed both in reperfused and control kidneys in all animals. On the first frame at 10 min after the infusion, CI was significantly reduced both in medulla (2±1) and cortex (2±1) in control kidneys. In contrast, significant signal enhancement was observed in reperfused kidneys, in which CI was significantly higher in medulla (23±13) than in cortex (10±8, $p<0.05$ vs. medulla). Quantitative histological