NOVEL BIOMARKERS OF STROKE PROGRESSION IDENTIFIED BY TEMPORAL QUANTITATIVE PROTEOMIC SCREENING

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Purpose of Study: By quantitative proteomic screening of stroke patient plasma over time, we explore novel biomarkers of ischemic infarct progression to predict clinical outcomes and guide treatment decisions. Novel proteomic techniques help to simultaneously identify both known and unknown factors directly at the bedside.

Methods Used: Plasma from acute ischemic stroke patients were isotope tagged and analysed on Orbitrap MS. Patients were co-morbidity matched and blood sampled at 6h & 72h post stroke onset. All patients had worsened clinical outcome with increased infarct size and NIHSS over this time.

Summary of Results: Pathway analysis of quantitative proteomic profiles suggests novel interaction of circulatory factors. Both established markers (S100) -important in cell injury and novel markers (TSP-4), important in cellular response and differentiation to injury are found to increase over time. As infarct size increased over 72 hours, a statistically significant decrease of matrix proteases such as ADAMTS-13 – potentially interactive with elevated vWF was found. ADAMTS-13 deficiency correlate to increase clotting as it inactivates vWF by cleaving the bond between tyrosine and methionine in the A2 domain of vWF – this coagulation pathway is important in acute stroke.

Conclusions: Direct bedside proteomics is feasible as an initial step in understanding ischemic injury progression from the circulation. Quantitative analysis of protein-protein interactions with respect to clinical outcome revealed both an increased expression of relevant injurious markers, and a decrease of novel “protective” factors in disease-relevant pathways. Further validation of these novel biomarkers is ongoing.

SPECKLE TRACKING-DERIVED DIASTOLIC FUNCTION PREDICTS MORTALITY IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Purpose of Study: Myocardial ischemia impairs ventricular relaxation and decreases ventricular compliance resulting in diastolic dysfunction. We sought to determine whether diastolic function, derived from novel speckle tracking echocardiography, could predict mortality in patients with acute coronary syndrome (ACS).
3 THE EFFECT OF RACE ON ALL-CAUSE MORTALITY AFTER CARDIAC SURGERY. A SINGLE INSTITUTIONAL EXPERIENCE IN A VETERAN POPULATION OVER 20 YEARS

Methods Used: 246 patients admitted with ACS were retrospectively studied. Echocardiographic images were analyzed with the sample volume placed on septal, lateral, anterior, and inferior corners of mitral annulus. The software automatically tracked the region of interest and yielded regional mean velocity curves of the mitral annulus. From the curves, peak early- (E) and late-diastolic (A) and systolic (S) velocities of the mitral annulus were measured. Peak early diastolic mitral inflow velocity (E) was also obtained using pulsed wave Doppler. Est/Ast and E/Est ratios were calculated.

Summary of Results: In univariate analysis, E/Est ratio was associated with increased risk of death at 6 months (P = 0.002). The cut-off value of E/Est ratio derived from the receiver operating characteristic analysis for predicting 6-month death was 30 (AUC = 0.66). After adjustment for clinical predictors of mortality and conventional echocardiographic parameters, E/Est ratio >30 remained a significant predictor of 6-month death (adjusted OR, 3.65; CI, 1.21–11.03; P = 0.022). Over the median period of 2 years, overall survival was lower in patients with E/Est ratio > 3.0 (59.7% vs. 81%, log rank, P = 0.0003). In multivariate Cox proportional hazards models, lower Est/Ast (P = 0.003), higher E/Est ratio (P = 0.0003). In multivariate Cox proportional hazards models, lower Est/Ast (P = 0.003), higher E/Est ratio (P = 0.0003), and higher Est/Ast ratio (P = 0.007) were associated with increased risk of death.

Conclusions: Diastolic function derived from measuring mitral annular velocity by speckle tracking echocardiography is predictive of mortality in patients with ACS.

Survival patterns of Caucasians (C) and African Americans (A) undergoing cardiac surgery.

4 OLFATORY PHENOTYPING IN ISOLATED GONADOTROPIN RELEASING HORMONE DEFICIENCY: A SYSTEMATIC ANALYSIS

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Purpose of Study: Gonadotropin Releasing Hormone (GnRH) deficiency presents as either Kallmann syndrome (KS) when associated with anosmia (complete loss of smell) or as normosmic idiopathic Hypogonadotropic Hypogonadism (nHh). Systematic analyses of the true spectrum of olfactory acuity in a large group of GnRH deficient subjects is lacking. Therefore we determined the olfactory phenotype and underlying genetic etiologies in a large cohort of GnRH deficient patients.

Methods Used: Olfactory acuity in 324 GnRH deficient subjects (234M/90F) was studied using the University of Pennsylvania Smell Identification Test (UPSIT), an objective, validated smell test. Using age and gender based normative data, subjects were classified as anosmic, hyposmic (partial loss of smell) or normosmic via UPSIT categorization and olfactory centile scores derived for each subject. Mutualual screening for 8 genes was performed (KAL1, PROK2, PROKR2, FGFR1, FGF8, KISS1R, GNRHR, and NELF).

Summary of Results: A continuous spectrum of olfactory acuity was observed in the cohort (anosmic 31%; hyposmic 33%; normosmic 36%). Compared to normative age and gender matched data, the majority of subjects with isolated GnRH Deficiency tested below the 50th centile (90%). KAL1 and PROK2 gene mutations were seen in anosmic patients only while mutations in PROKR2, FGFR1 and FGF8 genes were seen in both anosmic and hyposmic subjects. Mutations in GNRHR were seen only in normosmic subjects.

Conclusions: Contrary to prior studies, a continuous spectrum of olfactory acuity occurs in a large group of GnRH deficient subjects suggesting a heterogeneous olfactory phenotype in this disorder. The majority of GnRH deficient subjects show impaired olfaction. While some genes produced distinct anosmic or normosmic phenotypes, the genetic basis of the hyposmic group showed a significant overlap with the anosmic group, suggesting a shared molecular basis in these phenotypes.
OUTCOMES AND SURVIVAL WITH AORTIC VALVE REPLACEMENT COMPARED WITH MEDICAL THERAPY IN PATIENTS WITH LOW-, MODERATE-, AND SEVERE-GRADIENT SEVERE AORTIC STENOSIS AND NORMAL LEFT VENTRICULAR EJECTION FRACTION

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Purpose of Study: To evaluate medical versus surgical management of severe aortic stenosis (AS) in patients with a low gradient (< 30 mm Hg mean gradient), a moderate gradient (30–40 mm Hg mean gradient), and a severe gradient (> 40 mm Hg mean gradient) and normal left ventricular ejection fraction (LVEF).

Methods Used: We searched an outpatient faculty cardiology practice database for patients with severe AS (aortic valve area < 1.0cm2) by the continuity equation and normal LVEF (>50%) with no other significant valvular heart disease. Patients were divided into 3 groups: group 1 (low-gradient), group 2 (moderate-gradient), and group 3 (severe-gradient) severe AS. We investigated the incidence of death, aortic valve replacement (AVR), re-admission with death or AVR in the 3 groups. Kaplan-Meier survival curves for medical versus surgical management were constructed for the 3 groups.

Summary of Results: Groups 1, 2 and 3 included 94, 87, and 67 patients, respectively. Follow up was 60 months, 55 months, and 45 months for groups 1, 2, and 3, respectively (p not significant). Death occurred in 52 of 94 group 1 patients (55%), in 34 of 87 group 2 patients (39%), and in 26 of 67 group 3 patients (39%) (p not significant). AVR occurred in 22 of 94 group 1 patients (23%), in 46 of 87 group 2 patients (53%), and in 33 of 67 group 3 patients (49%) (p < 0.001 for group 1 versus 2 and p = 0.0003 for group 1 versus 3). Stepwise Cox regression analysis showed that AVR (hazard ratio = 0.30, p = 0.0001) and mitral annular calcium (hazard ratio = 1.07, p = 0.011) by log-rank test for group 3).

Conclusion: In conclusion, among patients with normal LVEF and severe AS, overall survival did not differ for those with low-gradients, moderate-gradients, or severe-gradients. Survival is significantly improved with AVR, regardless of gradient.

SYNCHRONIZING MITOSIS REDUCES INTRINSIC INFLAMMATION IN ASTHMATIC AIRWAY EPITHELIUM

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Purpose of Study: The standard of care for asthma is based on the paradigm that chronic asthma inflammation is the upstream impetus for long-term airway remodeling. However, this model is called into question by the persistence of airway remodeling despite effective anti-inflammation with glucocorticoids. Therefore, we recently proposed that the target of current asthma treatment regimens, inflammation, is actually downstream of the causal biological defect, remodeling. We showed that human primary differentiated normal (n = 6) and asthmatic epithelial cells (n = 6) secreted more pro-inflammatory cytokines and regenerated less efficiently than normals following wounding.

Methods Used: To evaluate medical versus surgical management of severe AS, we searched an outpatient faculty cardiology practice database for patients with severe AS (aortic valve area < 1.0cm2) by the continuity equation and normal LVEF (>50%) with no other significant valvular heart disease. Patients were divided into 3 groups: group 1 (low-gradient), group 2 (moderate-gradient), and group 3 (severe-gradient) severe AS. We investigated the incidence of death, aortic valve replacement (AVR), re-admission with death or AVR in the 3 groups. Kaplan-Meier survival curves for medical versus surgical management were constructed for the 3 groups.

Summary of Results: Groups 1, 2 and 3 included 94, 87, and 67 patients, respectively. Follow up was 60 months, 55 months, and 45 months for groups 1, 2, and 3, respectively (p not significant). Death occurred in 52 of 94 group 1 patients (55%), in 34 of 87 group 2 patients (39%), and in 26 of 67 group 3 patients (39%) (p not significant). AVR occurred in 22 of 94 group 1 patients (23%), in 46 of 87 group 2 patients (53%), and in 33 of 67 group 3 patients (49%) (p < 0.001 for group 1 versus 2 and p = 0.0003 for group 1 versus 3). Stepwise Cox regression analysis showed that AVR (hazard ratio = 0.30, p = 0.0001) and mitral annular calcium (hazard ratio = 2.3, p = 0.001) were significant independent risk factors for time to mortality. Kaplan-Meier survival curves for patients treated medically versus AVR showed that patients in all 3 groups who underwent AVR had significantly greater survival (p = 0.002 by log-rank test for group 1; p = 0.0002 by log-rank test for group 2; and p = 0.01 by log-rank test for group 3).

Conclusion: In conclusion, among patients with normal LVEF and severe AS, overall survival did not differ for those with low-gradients, moderate-gradients, or severe-gradients. Survival is significantly improved with AVR, regardless of gradient.

The Role of C-Arm Computed Tomography in the Treatment of Vascular Malformations and Prediction of Local Skin Complications

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Purpose of Study: The purpose of this study is to evaluate the efficacy of C-Arm CT for assessment of sclerotherapy agent distribution in venous malformations and for the prediction of skin related complications.

Methods Used: Over a 2 year period, 19 children (14M, 5F, mean age 14.8) with venous malformations underwent sclerotherapy with additional low dose C-Arm CT imaging. Imaging and clinical records were reviewed for each patient. Pre-treatment imaging with MRI or CT were compared to intra-procedural C-Arm CT imaging in three dimensions by two reader consensus, to assess the volume of lesion treated. 26 C-Arm CT imaging studies were analyzed. When comparing lesion volumes, the percentage of lesion coverage with sclerotherapy agent was calculated.

Summary of Results: The median lesion volume treated was 86.8% (range 21.8%–292.5%). Nine patients’ lesions measured greater than 100% on C-Arm CT when compared to the prior imaging study, attributed to interval lesion growth. Five patients had C-Arm CT measurements less than 50% due to elective partial lesion treatment. Four patients suffered from self limiting skin blistering and two patients with ulceration all noted to have lesion proximity to the epidermis of <3mm with ≤1mm for ulceration. In one patient, C-Arm CT determination of lesion proximity to the airway necessitated a two day intubation post therapy. Based on a phantom study, C-Arm CT doses to cheek/neck, calf and thigh regions were estimated to be 3%, 4%, and 6% respectively of the doses to the same anatomical region using the conventional CT imaging.

Conclusion: Low dose C-Arm CT imaging is a useful intra-procedural adjunct for sclerotherapy treatment of venous malformations. It can predict sclerotherapy agent lesion distribution and potential skin complications.

SEPSIS-RELATED MORTALITY IS REDUCED IN THE ABSENCE OF GRANZYME B

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Purpose of Study: The frequent precursor to mortality from sepsis is Multiple Organ Dysfunction Syndrome (MODS). We recently reported acute sepsis-induced alterations in the megakaryocyte-platelet/leukocyte-transcriptional axis that resulted in strongly cytotoxic platelets expressing potent serine protease granzyme B (GzmB) in mice and humans while knockout of GzmB prevents sepsis-related lung and spleen apoptosis. Importantly, accumulating literature shows platelets amass in many of the commonly affected organ systems (e.g. spleen and lung) in sepsis. Therefore, we hypothesized that mice lacking GzmB will have a lower mortality than wild-type mice.

Methods Used: The celoc ligation and puncture (CLP) model of poly-microbial sepsis was used in C57BL/6J wild-type (WT) mice and B6.129S2-Gzmbtm1tm1J (GzmB KO) mice (lacking granzyme B). Mice were scored post-surgically in 4-hour intervals using a validated murine sepsis severity measure. Mice were euthanized when a critical score (associated with >90% imminent mortality) was reached.

5 OUTCOMES AND SURVIVAL WITH AORTIC VALVE REPLACEMENT COMPARED WITH MEDICAL THERAPY IN PATIENTS WITH LOW-, MODERATE-, AND SEVERE-GRADIENT SEVERE AORTIC STENOSIS AND NORMAL LEFT VENTRICULAR EJECTION FRACTION

Wound regeneration/mitosis was analyzed by microscopy and flow cytometry. Cytokine secretions were analyzed using cytometric bead assays. wounded at 0h and thereafter exposed continuously to bromodeoxyuridine (BrdU).

6 SYNCHRONIZING MITOSIS REDUCES INTRINSIC INFLAMMATION IN ASTHMATIC AIRWAY EPITHELIUM

7 THE ROLE OF C-ARM COMPUTED TOMOGRAPHY IN THE TREATMENT OF VENOUS MALFORMATIONS AND PREDICTION OF LOCAL SKIN COMPLICATIONS

8 SEPSIS-RELATED MORTALITY IS REDUCED IN THE ABSENCE OF GRANZYME B

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Summary of Results: The GzmB KO mice (n=5) had lower sepsis scores at all time points post-CLP than the WT mice (n=4). For example, at 22 hours, the mean sepsis score was 90.8 out of 15 while the GzmB KO score was 6.8/7.0 (P=0.04). At 24 hours post-CLP, the mortality rate of the GzmB KO mice was 0% while the mortality rate of the WT mice 100%. Kaplan-Meier survival analysis showed that GzmB KO mice survived longer following CLP than WT mice (P=0.0019 by Cox Proportional Hazard Regression).

Conclusions: The current data shows improved survival and severity of sepsis in mice lacking GzmB, when compared to WT mice. These findings are likely due to less platelet GzmB-mediated cytotoxicity in end organs and will serve as the foundation for pre-clinical trials of GzmB inhibitors in sepsis.

9 STRESS INDUCED LEFT VENTRICULAR EJECTION FRACTION AUGMENTATION DURING GATED SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY MYOCARDIAL PERFUSION IMAGING HAS NO SURVIVAL ADVANTAGE

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Purpose of Study: The aim of this study was to determine if the presence of post-stress Left ventricular ejection fraction (LVEF) augmentation during Single Photon Emission Computed Tomography (SPECT) myocardial perfusion imaging (MPI) in subjects with baseline normal rest-gated LVEF confers mortality benefit.

Methods Used: Myocardial perfusion as well as mortality data was obtained on patients who underwent SPECT MPI over a 6 year period at the Veteran Affairs Medical Center in Washington, DC. A total of 3520 patients with normal rest-gated LVEF (LVEF=45%) were analyzed. LVEF augmentation was defined as post-stress increment in LVEF of at least 10% during SPECT MPI. Baseline characteristics were compared between subjects with and without LVEF augmentation using chi-square test for categorical variables and independent sample t-test for continuous variables. All-cause mortality was compared between the two study groups using Kaplan-Meier analysis.

Summary of Results: A total of 3340 patients (94.9%) had no LVEF augmentation as compared to 180 (5.1%) who had LVEF augmentation. Mean age between the subjects with and without post-stress LVEF augmentation was similar (62.4 years and 63.4 years respectively, p=0.33). There was no significant difference between the two groups in terms of transient ischemic dilation ratio (0.90 versus 1.64, p=0.63). The group with LVEF augmentation had significantly lower body mass index (BMI) as compared to the group without LVEF augmentation (28.4 versus 29.6, p=0.012). Although the group without LVEF augmentation appeared to have an initial survival edge (Log rank statistic =4.577, p=0.032), there was no significant difference in all-cause mortality between subjects with and without LVEF augmentation beyond 4 years of follow up.

Conclusions: In subjects with normal rest-gated LVEF on SPECT MPI post-stress LVEF augmentation does not confer mortality benefit. Further studies are needed given the small number of patients demonstrating significant augmentation.

10 BENEFICIAL GLUCOCORTICOID EFFECTS IN ASTHMATIC AIRWAY EPITHELIUM ARE NOT DEPENDENT ON RECEPTOR-MEDIATED TRANSCRIPTION

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Purpose of Study: We recently showed that asthmatic airway epithelial inflammatory responses correlate with dysynchronous mitosis upon in vitro mechanical injury while intermittent glucocorticoid (GC) exposure simultaneously decreased these secretions and resynchronized mitosis. GCs have several mechanisms of action including: 1) receptor-mediated transcription that accounts for many side effects, and 2) direct signaling (e.g. via NFκB inhibition) that mediates beneficial anti-inflammation. We sought to separate signaling from transcription in order to define key GC mechanisms of action in asthmatic airway epithelial regeneration. We used a novel delta-9,11 GC-analogue (VBP15) that retains the signaling capabilities of traditional GCs (e.g. inhibits NFκB) but lacks GC-receptor mediated transcription.

Methods Used: Normal and asthmatic primary differentiated airway epithelia were pulsed with dexamethasone (DEX) (n=6), VBP15 (n=3) or vehicle for 2h at +2, -2, +22, and +46h. Cultures were mechanically scrape-wounded at 0h and thereafter exposed continuously to BrdU. Wound regeneration/mitosis was analyzed in BrdU+ cells.

Summary of Results: Both DEX and VBP15 pulses reduced asthmatic epithelial inflammatory cytokine (i.e. TGF-β1, IL-10, IL-13, and IL-1β) secretion for at least one time point (all P<0.05). Further, DEX and VBP15 improved regeneration compared to unexposed asthmatic cultures [wound area reduction: mean(SEM)=50±8% for PBS vs. 79±8% for DEX (P=0.02) and -11±11% for DMSO vs. 53±14% for VBP15 (P=0.046)]. DEX-exposed cultures showed improved mitotic synchrony (G1/G0, S, G2/M: 47±2, 21±2, 33±2% for PBS/DMSO vs. 74±3, 8±3, 18±0% for DEX) although this effect was more modest for VBP15 (59±4, 17±1, 24±3%).

Conclusions: Asthmatic epithelial inflammatory cytokine secretion was reduced by DEX and VBP15, compounds that inhibit NFκB. Meanwhile, wound regeneration and mitotic cell cycle synchrony were improved by both DEX and VBP15 even though VBP15 lacks receptor-mediated transcriptional activity. These data indicate that the regenerative benefits of GCs in asthmatic airway epithelium are not dependent on their receptor-mediated transcriptional activity but are dependent on GC-mediated direct signaling.

11 OXYGEN TENSION AFFECTS RELEASE OF ADENOSINE FROM ASTROCYTES

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Purpose of Study: The coupling mechanisms between cerebral blood flow and brain metabolism are poorly understood. Accumulating evidence supports our hypothesis that Adenosine (Ado) may play an integral role. The cellular source for adenosine in brain is still unclear. Astrocytes appear to be uniquely positioned to influence the cerebral circulation and to be involved in the regulation of CBF. In our current study we hypothesize that astrocytes release Ado dependent on tissue oxygenation.

Methods Used: Primary, mixed cultures were established from the cerebro hemispheres of 1–3 day old male rat pups and purified based on differential adhesion, trypsinized after 11 days in vitro (DIV), attached to microcarrier beads and grown in spinner flasks. After 23 DIV, O2 tension in the airway epithelium are not dependent on their receptor-mediated transcriptional activity but are dependent on GC-mediated direct signaling.

Summary of Results: Extracellular levels of Ado ([Ado]) changed rapidly with changes in pO2. Graded reductions of oxygen tension revealed that [Ado] reached concentrations of 10−4 M to 10−8 M with a pO2 of 30–10 mmHg. These levels correspond well with [Ado] and oxygen levels found in brain tissue during normoxemia. A reduction of pO2 to 3 mmHg resulted in a rapid, more than 1000-fold increase of [Ado] within sec. Under conditions of low pO2 (pO2 <3 mmHg), inhibition of extracellular catabolism of adenosine monophosphate (AMP) prevented an increase of [Ado], and resulted in a rise in [AMP]. The rise in [AMP] exceeded the increase in [Ado]. In the presence of nuclease transporter inhibitors, accumulation of [Ado] persisted. Higher O2 levels were associated with a depression of [Ado]. During all manipulations in pO2; [K+]c, and ph did not change significantly and remained stable.

Conclusions: Based on our studies in culture we conclude that astrocytes are a major source of Ado and release Ado dependent on tissue oxygenation. The changes in [Ado] seen are sufficiently high to affect neuronal activity and vessel diameter, as well as CBF. Astrocytes thus seem to act as O2 sensors in brain.

12 FGF-2: A NOVEL URINARY BIOMARKER FOR ACUTE KIDNEY INJURY IN CHILDREN WITH CRITICAL ILLNESSES

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Purpose of Study: Acute kidney injury (AKI) causes significant morbidity in pediatric intensive care units (ICU). New urinary biomarkers are needed to identify children with AKI in a timely manner. Neutrophil Gelatinase-associated Lipocalin (NGAL) is considered a highly sensitive predictor of AKI in children with septic shock. Previous studies show that the urinary levels of Fibroblast Growth Factor-2 (FGF) and Epidermal growth factor (EGF) might be promising biomarkers to identify children undergoing renal injury. The goal of this study is to identify if a biomarker profile corresponding to urinary levels of NGAL, FGF-2, and EGF will identify children undergoing AKI secondary to sepsis or shock, and be useful to follow their renal outcome.

Methods Used: In a prospectively observational pilot study in a large urban children's hospital, urine samples were collected from 27 children at risk of AKI admitted to PICU with sepsis, shock, or after cardiopulmonary bypass (ECMO). AKI was defined by the p-RIFLE criteria. The urinary levels of NGAL, FGF-2, and EGF were measured by ELISA. All values were expressed as a ratio of the urinary creatinine (UCr), and adjusted to the normal values for age. ROC analysis was performed to generate cut-off values for each biomarker and define their sensitivity and specificity. Differences between two or more groups were compared using the Student-Newman-Keuls test, ANOVA, Chi-square for trend, or Fisher's exact test.

Summary of Results: Five out of 17 septic children and 6 out of 11 ECMO children developed AKI. A urinary cut-off value of NGAL above 150 mg/g was highly sensitive (100%) but not specific (0.25) to identify children with AKI. The urinary cut-off values for FGF-2 adjusted for age were less sensitive (0.91) but more specific (0.62) than NGAL. In contrast urinary EGF was less sensitive and specific when compared to NGAL or FGF-2 respectively.

Conclusions: Our findings suggest that the urinary levels of NGAL in combination with FGF-2, an angiogenic growth factor released by injured endothelial and tubular epithelial cells, may provide a highly sensitive and more specific biomarker tool to identify critically ill children undergoing AKI secondary to sepsis and other forms of shock.

13 EXPRESSION OF THE LECTIN LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR-1 (LOX-1) IN ENDOTHELIAL CELLS MEDITATED BY ADENOSINE A2A RECEPTOR

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Purpose of Study: The lectin like oxidized low-density lipoprotein receptor-1 (LOX-1) is a membrane bound receptor that is found at high concentrations in human atherosclerotic lesions and considered as a novel marker of the atherogenic process. The LOX-1 receptor, by promoting oxidized low density lipoprotein (oxLDL) influx, induces endothelial dysfunction and mediates numerous pro-atherogenic effects such as induction of adhesion molecules, monocyte chemotactic protein-1, and growth factors and activation of transcription factor nuclear factor (NF)-κB. Recently, LOX-1 has been considered a possible target for drug therapy. We previously showed potent anti-atherosclerotic effects of A2AR stimulation including inhibition of macrophage foam cell transformation and upregulation of the reverse cholesterol transport proteins. Now we report the effect of A2AR stimulation on the oxLDL influx through the LOX-1.

Methods Used: Primary human coronary arterial endothelial cells (HAECs) were incubated in the presence of oxLDL (20 μg/ml) for 18h. After incubation cells were washed with PBS and incubated for another 18h in the presence of oxLDL and a) HAEC media only b) DMSO c) A2AR agonist CGS-21680 (1 μmol) d) A2AR antagonist ZM-241386 (10 μmol/l) e) oxLDL/resveratrol (10 μmol/l) f) A2AR agonist ZM-241386 (10 μmol/l, 1h) / CGS-21680 (1 μmol/l) (n=5). Gene expression was evaluated by real-time PCR. Studies were done in triplicate.

Summary of Results: Our studies have shown that oxLDL stimulated expression of LOX-1 in primary human coronary arterial endothelial cells (HAECs) could be downregulated to 49.00±2.55% by treatment with resveratrol, respectively versus untreated HAECs, set as 100% (n=5, P<0.001). Moreover, this effect completely diminishes by pretreatment of HAECs with A2AR antagonist - ZM-241386. A2AR blockade upregulates LOX-1 expression by 72.10±39.69% versus control (n=6, P<0.001).

Conclusions: A2AR activation could be a novel therapeutic approach that prevents lipid overload via effect on LOX-1 expression. Our report could lead to discovery of the new compounds, which may prevent atherosclerotic lesion formation and decrease cardiovascular events.

14 PLATELET-INDUCED SPLENOCYTE APOPTOSIS DURING SEPSIS IS INHIBITED BY GPⅡB/ⅢA BLOCKADE

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Purpose of Study: To determine if known anti-platelet aggregation agents inhibit platelet-induced splenocyte apoptosis ex vivo. End-organ apoptosis is considered a diagnostic hallmark of progressive sepsis and multi-organ dysfunction syndrome (MODS). Platelets have been shown to accumulate in many commonly affected end-organs (i.e. spleen and lung) during sepsis. We previously reported an increase in platelet granzyme B (GzMB) expression during experimental sepsis that co-localized with platelet aggregates and induced apoptosis in both splenic and lung tissue. Our subsequent work confirmed that this platelet-induced splenocyte apoptosis is dependent upon direct platelet target cell contact.

Methods Used: We used a recalcification and puncture (CLP) model of murine sepsis and our previously published platelet:platelet co-incubation assay. Anti-platelet agents tested included a GPⅡB/Ⅲa inhibitor (i.e. eptifibatide) and an antibody with blocking activity against the platelet cell adhesion molecule P-Selectin (i.e. CD62P). Splenocyte apoptosis was measured using TUNEL-based assays and flow cytometry. Summary of Results: Ex vivo co-incubation of septic platelets and healthy splenocytes in the presence of eptifibatide significantly decreased apoptosis (i.e. TUNEL staining) in splenocytes (overall and among CD4+ populations) as compared to co-incubation with non-treated septic platelets (meanSEMoverall = 66.510.6% reduction, P=0.008; meanSEMCD4+ = 8520.7% reduction, P=0.026). When septic platelets were co-incubated in the presence of blocking anti-Cd62P antibody, there was no statistical difference from baseline levels of apoptosis.

Conclusions: Inhibition of aggregation/contact between platelets and splenocytes with eptifibatide decreased levels of splenocyte apoptosis ex vivo. If eptifibatide inhibition of apoptosis is translatable to in vivo studies, it may represent a novel therapeutic option in the treatment of sepsis and MODS.

15 Atherosclerotic Properties of Coxibs in Human Monocytes/Macrophages

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Purpose of Study: Coxibs, a cyclooxygenase (COX)-2 inhibitors, are anagelse, anti-inflammatory agents. However, some of them possess cardiovascular toxicity. We demonstrated that coxibs suppress expression of cholesterol 27-hydroxylase (27-OHase) and AATP binding cassette transporter A1 (ABCA1), involved in reverse cholesterol transport (RCT). RCT proteins prevent lipid overload and macrophage foam cell formation (FCF). Here we report that exposure THP-1 macrophages to COX-2 specific inhibitors leads to cholesterol overload via disruption of cholesterol efflux and influx balance.

Methods Used: THP-1 macrophages were incubated for 18h in the presence or absence of a) celecoxib (5 μM); b) rofecoxib (5 μM); c) naproxen (5 μM); d) acetaminophen (1 mM). 1 μg of total RNA was used per condition for QRT PCR. 27-OHase and ABCA1 were measured to assess cholesterol efflux. CD36 and LOX1 scavenger receptors were detected for analysis of cholesterol influx. THP-1 macrophages (phorbol dibutyrate, 100nM, 48 h) were exposed to oxLDL (25 μg/ml, 48h) at conditions described above. FCF was quantified as percentage red stainable cells. Studies were performed in triplicate.

Summary of Results: Incubation of THP-1 macrophages with specific coxibs decreased 27-OHase and ABCA1 message by (30.2±1.7% and 24.6±6.5% of control, P=0.01 for coxib and by 35.6±10.7% and 26.0±7.1% of control, P=0.01 for rofecoxib). Nonspecific coxibs had no significant effect on these proteins. Specific and nonspecific coxibs, had a great impact on the expression of CD36 (150.0±20.4% for coxib; 167.4±38.7% for rofecoxib; 271.8±30.0% for naproxen; 171.7±18.9% for acetaminophen,
The study included 69 patients that were referred to our institution for nuclear stress testing. The patient population consisted of 45 men and 24 women (mean age 62 +/-12 years). Thirty three patients were past smokers, 28 non-smokers and 8 were current smokers. Forty had a diagnosis of hypertension (33 currently treated with antihypertensives), 17 had a history of diabetes mellitus, 28 patients had a family history of coronary artery disease (CAD) and 28 patients carried a diagnosis of CAD. Prior to nuclear stress testing all patients underwent a 2 minute arm cuff reactive hyperemia test that measured fingertip temperature rebound; VR was then calculated using Endothelix Inc. software. Patients were divided into 2 groups based on normal VR (VR$>$2, n=11) or abnormal VR (VR$<$2, n=58), and were compared based on nuclear test results. A positive nuclear test indicated the presence of a reversible defect on scanning. Continuous variables were compared with the unpaired t-test and dichotomous variables with the Fisher's exact test.

**Summary of Results:** There was no statistical difference in age, tobacco use, hypertension, family hx of CAD, dx of CAD, or DM between the groups with VR$>$2 and VR$<$2 (p=0.08). Among patients with a positive nuclear test, 9/10 had VR$>$2; among patients with a negative nuclear test, 49/59 had VR$<$2. No significant relationship was revealed between the vasoreactivity group and nuclear results (p=0.96).

**Conclusions:** DTM was highly sensitive (90%) but poorly specific (17%) for reversible defects in myocardial perfusion scans.

**P3**

**COMPARISONS OF SPECKLE TRACKING ECHOCARDIOGRAPHY, TISSUE DOPPLER IMAGING AND TRANSMITRAL FLOW VELOCITY FOR ESTIMATION OF DIASTOLIC FUNCTION IN PATIENTS WITH ACUTE CORONARY SYNDROME**

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P1

**A NOVEL METHOD TO PREDICT INCREASED LEFT VENTRICAL MASS INDEX**

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**Purpose of Study:** Increased left ventricular mass index (LVMI) is independently associated with increased cardiovascular mortality and morbidity. The estimation of LVMI involves calculation of the BMI and application of a formula incorporating LV wall thickness, diameter and numerical constants. This study was performed in order to determine if a single measurement in the formula could be applied to predict increased LVMI in large cohorts.

**Methods Used:** The initial 749 consecutive echocardiograms performed in subjects over 2 yrs at a hospital laboratory were analyzed. After BMI was calculated, the modified Devereux formula was applied to estimate left ventricular mass. Of these 749 subjects, 398 (53%) had an increased LVMI. The sensitivity, specificity, positive and a negative likelihood ratios (LR) and ROC areas were compared for: left ventricular dimension at end-diastole (LVID-D) and an increased 1 or 2 wall thickness.

**Summary of Results:** When compared with the other modified Devereux formula measurements, a LVID-D exceeding 4.8cm was the strongest independent predictor of increased LVMI with a sensitivity of 85%, specificity of 84%, positive likelihood ratio of 4.8, negative likelihood ratio of .16 and a ROC area of .90.

**Conclusions:** In this study group, a LVID-D exceeding 4.8cm was the strongest independent predictor of an increased LVMI compared to all the other variables employed in the modified Devereux formula. This easily obtained echocardiographic measurement may prove useful in large cohort evaluations of LVMI.

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P2

**DIGITAL THERMAL MONITORING AND OUTCOMES IN NUCLEAR STRESS TESTING**

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**Purpose of Study:** Previous studies have shown that vascular dysfunction measured by digital thermal monitoring (DTM) is associated with the extent of myocardial perfusion defects measured by summed stress score. This study evaluated the relationship between vasoreactivity (VR) measured by DTM and nuclear stress test results.

**Methods Used:** The study included 69 patients that were referred to our institution for nuclear stress testing. The patient population consisted of 45 men and 24 women (mean age 62 +/-12 years). Thirty three patients were past smokers, 28 non-smokers and 8 were current smokers. Forty had a diagnosis of hypertension (33 currently treated with antihypertensives), 17 had a history of diabetes mellitus, 28 patients had a family history of coronary artery disease (CAD) and 28 patients carried a diagnosis of CAD. Prior to nuclear stress testing all patients underwent a 2 minute arm cuff reactive hyperemia test that measured fingertip temperature rebound; VR was then calculated using Endothelix Inc. software. Patients were divided into 2 groups based on normal VR (VR$>$2, n=11) or abnormal VR (VR$<$2, n=58), and were compared based on nuclear test results. A positive nuclear test indicated the presence of a reversible defect on scanning. Continuous variables were compared with the unpaired t-test and dichotomous variables with the Fisher's exact test.

**Summary of Results:** There was no statistical difference in age, tobacco use, hypertension, family hx of CAD, dx of CAD, or DM between the groups with VR$>$2 and VR$<$2 (p=0.08). Among patients with a positive nuclear test, 9/10 had VR$>$2; among patients with a negative nuclear test, 49/59 had VR$<$2. No significant relationship was revealed between the vasoreactivity group and nuclear results (p=0.96).

**Conclusions:** DTM was highly sensitive (90%) but poorly specific (17%) for reversible defects in myocardial perfusion scans.

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*Linear correlation analysis between the methods.*
Purpose of Study: Speckle tracking echocardiography has recently been used in clinical practice for estimating diastolic function. However, its correlations with the traditional methods are conflicting, and their prognostic values have not been compared.

Methods Used: Echocardiographic studies of 30 patients admitted with acute coronary syndrome were reviewed. Diastolic function was assessed by three different techniques. Peak early and late diastolic mitral inflow velocities (E and A) were measured from conventional Doppler echocardiography. Speckle tracking method is a relatively new technique which provides a faster, easier, and more accurate estimation of diastolic function. In age-adjusted Cox proportional hazards models, E/E' ratio (P = 0.009), E/A ratio (P = 0.05), and E/A ratio (P = 0.007) were associated with increased mortality risk over the mean period of 11.5 months. However, when all three methods were incorporated into the same multivariable model, only E/E' ratio remained significantly predictive of mortality.

Conclusions: Diastolic functions derived from speckle tracking and tissue Doppler imaging are well correlated. However, speckle tracking method is more promising as a prognosticator in patients with acute coronary syndrome.

P5 PARADOXICAL EMBOLUS ACROSS A PATENT FORAMEN OVALE AS A POSSIBLE ETIOLOGY OF ACUTE ST ELEVATION MYOCARDIAL INFARCTION

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Purpose of Study: Paradoxical embolus across a patent foramen ovale (PFO) is a known etiology for cerebral ischemic events. However, embolus across a PFO resulting in myocardial infarction has not been well-described in the literature.

Methods Used: Case Review.

Summary of Results: Patient #1 is a 47 year old female without prior medical history who presented to the emergency department (ED) after syncope while on the toilet and was found to have an anterior wall STEMI. Emergent coronary angiography revealed extensive thrombus in the proximal LAD and LCX and otherwise normal coronary arteries. Transthoracic echocardiogram (TTE) revealed a PFO. Her risk factors for deep venous thrombosis (DVT) was oral contraceptive use. Patient #2 is a 44 year old male without prior medical history who developed subternal chest pain while jogging and was found to have an inferior wall STEMI on presentation to the ED. Emergent coronary angiography revealed thrombus in the right PDA and otherwise normal coronary arteries. TEE revealed a PFO. Patient #3 is a 28 year old male without prior medical history who was awakened from sleep with the sudden onset of substernal chest pain and was found to have an inferior wall STEMI on presentation to the ED. Emergent coronary angiography revealed thrombus in the mid RCA and otherwise normal coronary arteries. Th patient returned to the ED several days after discharge with acute pain and swelling of his left lower extremity. Ultrasound revealed extensive left lower extremity DVT involving the common femoral vein, the superficial femoral vein, the popliteal vein, the gastrocnemius vein and the perforating vein. Workup revealed the he was positive for lupus anticoagulant. TEE revealed PFO.

Conclusions: Among 59 patients taken to our catheterization lab for PCI of STEMI between January-May 2010, we identified 3 patients in whom acute coronary occlusion appeared to be embolic related to a paradoxical embolus across a PFO. These patients were younger, less likely to have atherosclerotic coronary disease, less likely to have CAD risk factors, and more likely to have evidence of venous thromboembolism and/or hypercoagulable states. Paradoxical embolus across a PFO may be an under-recognized cause of acute myocardial infarction.

P6 LEFT BUNDLE BRACH BLOCK SHOULD NOT BE USED AS AN ST SEGMENT ELEVATION EQUIVALENT IN THE MANAGEMENT OF ACUTE ST ELEVATION MYOCARDIAL INFARCTION

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Purpose of Study: To assess the sensitivity and specificity of LBBB in identifying acute coronary artery occlusion, using cardiac catheterization and/or cardiac biomarker data.

Methods Used: Cardiac catheterization results, cardiac biomarkers and ECG data were reviewed for all patients for whom STEMI protocol was activated between January 2009 and May 2010.

Summary of Results: STEMI protocol was activated for 274 patients, including 14 patients with new or unknown LBBB. None of the LBBB had associated ST segment changes that have been suggested to indicate acute MI (Sgarbossa, NEJM 1996 334:481-7) Emerging cardiac catheterization was cancelled by the admitting cardiologist in 4/14 patients (29%) because the LBBB was determined to be pre-existent, while 1 patient with old LBBB...
underwent emergent cardiac catheterization and revascularization. 9 patients (64%) had LBBB that was new or of unknown duration. 2 did not undergo emergent cardiac catheterization, 1 due to patient refusal, and 1 was cancelled by the attending cardiologist. 8 patients underwent cardiac catheterization emergently. 1/8 had evidence of acute coronary artery occlusion in contrast to 204/236 patients who presented with STEMI. Only 3/14 patients with LBBB had positive cardiac biomarkers during hospitalization (median value was Tnl 6.22, with range 3.95–67), 2/3 had this following PCI. 0% patients for whom STEMI protocol was cancelled developed positive biomarkers.

Conclusions: Thus, patients with new or unknown LBBB without Sgarbossa 2011 The American Federation for Medical Research 1

1.2

DEVIATION OF THE REDUCTION OF VENTRICULAR ARRHYTHMIAS BY ANGIOTENSIN II CONVERTING ENZYME INHIBITORS AND POSSIBLE ROLE OF ANGIOTENSIN II RECEPTOR BLOCKERS
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Purpose of Study: An increase in left ventricular mass (L.V.M.) is a pathological consequence of prolonged hypertension and ischemic heart disease. Reduction of this abnormality is very important in the reduction of ventricular arrhythmias (V.A.). We want to discuss the importance of this observation and other mechanisms involved in lowering V.A. in hypertensive cardiovascular disease and ischemic heart disease.

Methods Used: 1. 85 patients (p.) with an increase in L.V.M. were given 20mg of Enalapril for a year. There was a reduction of L.V.M. of 40%, this was accompanied by a reduction of V.A. of 88%. At the same time there was an improvement of the E.F. of 45 % to 60 0.001. This is related to a reduction of angiotensin II (All) by E. 2. Junction Gaps (J-G) are physiologic structures extremely important in the function of the myocardium. Uncoupling of J.G. may produce heart failure and arrhythmias. Using paired myocytes, infusion of E. intracel lularly showed an increase of J.G. conductivity of 106 ± 3% m/sec. increasing J.G. coupling, reducing heart failure and arrhythmias. Infusion of All reduces J.G. by 80% decreasing cell coupling, increasing the possibility of heart failure and arrhythmias. 3. Infusion I.V. of E. reduced QRS duration 113 ± 10 to 107 ± 7 msec. P<0.05; also a reduction of QTC and QT dispersion. 20% showed disappearance of late potentials.

4. E. produced by hyperpolarization, an increase in the amplitude of action potentials and an increase of refractory period (displacing the strength curves to the right). The minimal current intensity required to induce a propagated response was clearly increased. These experiments were done using isolated paired myocytes and myocardial trabeculae.

Summary of Results: All these mechanisms produced by E. has the potential of reducing V.A.; All will produce the opposite.

Conclusions: E. and All receptor blockers are useful in reducing V.A. in hypertensive cardiovascular disease and ischemic heart disease.

P7

P8

LONG TERM RESULTS OF ISOLATED VALVE OPERATIONS IN PATIENTS WITH DIABETES
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Purpose of Study: The influence of diabetes on events after isolated valve operation is not well defined. We analyzed the effect of diabetes on short and long term outcomes in patients undergoing valvular operations.

Methods Used: A total of 2,200 patients had cardiac surgery at a single Veterans Affairs Medical Center between 1990 and 2008. 355 patients had undergone either isolated valve replacement or repair. Data documenting the presence of diabetes was collected prospectively and captured into the Veterans Affairs electronic medical record. All cause mortality was assessed utilizing both the VA Continuous Improvement in Cardiac Surgery Project and the death data field in the VA CPRS-VISTA electronic medical record.

The long term survival was compared in patients with and without diabetes.

Summary of Results: Of the total of 355 patients who had an isolated valvular operation, 19% (n=69) had diabetes. Patients with DM were significantly older 67 ± 8.7 years vs. 63 ± 12 years (p=0.006) and larger with a BSA of 1.97 ± 0.19 vs. 1.96 ± 0.19 (p=0.03). The serum creatinine and LVEDP were lower between groups. During a total follow up of 18 years, 42 (60%) patients with diabetes and 186 (65%) patients without diabetes were alive (p=0.118). At 1, 5, 10, 15 years survival in patients with vs. without diabetes were 91% vs. 87%; 71% vs. 74%; 40% vs. 56%; 23% vs. 48% (p=NS).

Conclusions: The presence of diabetes does not appear to adversely affect long term survival in patients undergoing isolated valve operations.
P10 RESIDENT EDUCATION IN AN EARLY HEAD START (EHS) PROGRAM: A NEW MODEL TO ENHANCE MEDICAL EDUCATION AND COMMUNITY INVOLVEMENT

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Purpose of Study: It has been shown that pediatricians have effectively implemented educational programs in Head Start centers, with families and Head Start advocates welcoming pediatricians as educators. It also has been shown that pediatricians may lack basic knowledge of early intervention services. Our pilot study seeks to increase residents’ knowledge of EHS programs, to enhance residents’ understanding of the pediatrician’s role in the community, and to improve residents’ ability to communicate non-medical individuals in an effective manner. Further, our study will enhance health knowledge of EHS parents through resident-led didactic sessions for the parents.

Methods Used: A single group pretest-posttest design was used to evaluate residents (N=3) and parents of EHS enrollees (N=18) in Washington, DC. Educational modules were created for residents on EHS, communications skills, and community pediatrics. After completing the modules, residents taught health information sessions for parents of EHS enrollees with a Spanish interpreter. Parents of EHS enrollees and residents completed pre- and post-intervention surveys available in English and Spanish. One-tailed, paired t-test analysis was used to analyze pre- and post-intervention surveys.

Summary of Results: Analysis of parental health knowledge showed a significant increase in health knowledge after sessions (p=0.02). All parents thought that sessions with residents were ‘useful’ or ‘very useful,’ with 94% of parents stating they would return to another session. Analysis of residents’ EHS knowledge displayed a significant increase in EHS knowledge (p=0.017). All residents expressed having an overall positive learning experience, although residents’ self-assessments on being a community pediatrician and of their communication skills did not significantly improve (p>0.05).

Conclusions: In general, our pilot study has shown to significantly improve health knowledge of parents and EHS knowledge of pediatric residents. Both parents and residents felt the experience was a positive and useful learning experience. Resident self-assessments of their communication skills and of being a community pediatrician did not significantly change, which may be due to the limitations of the questionnaire/study design or the small sample size.

P11 HOME FIRE SAFETY PRACTICES IN AN URBAN PEDIATRIC EMERGENCY DEPARTMENT POPULATION

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Purpose of Study: Children less than 5 years old, individuals with low socioeconomic status and minorities are high risk groups for residential fire death. These risk factors are prevalent among urban pediatric emergency department (ED) patients. Emergency medical services (EMS) resources are available in the Washington, DC area to provide free home fire safety inspection and smoke detector installation; however, it is unknown if these resources are utilized by this vulnerable population. This study will describe home fire safety practices and awareness of community-based fire safety resources in an urban pediatric ED population.

Methods Used: In this cross-sectional study, we surveyed a convenience sample of caregivers accompanying patients in an urban pediatric ED in Washington, DC. Survey contents focus on risk factors associated with residential fire injury and participant knowledge/interest in available community-based EMS home fire safety resources. Descriptive epidemiologic analysis of responses was conducted.

Summary of Results: This analysis included 305 caregivers reflecting a 64% participation rate. Patients accompanying the caregivers were 49% male, 77% African American and had a mean age of 6.8 (±5.9) years. Of study participants, 190 (62.3%) had children <5 years of age living in their homes. The presence of a home smoke detector was reported by 301 respondents (98.7%); however, 223 (73.1%) reported testing these less than the recommended rate of every month. 190 (62.3%) did not have a carbon monoxide detector and 155 (50.8%) had no fire escape plan. 51 (17%) reported smoking inside the home, and 66 (21.6%) reported space heater use. When asked about available EMS programs to provide home fire safety inspections and install free smoke detectors, 184 (60.3%) did not know these programs existed, 239 (78.4%) were interested in participating, and 165 (54.1%) enrolled immediately.

Conclusions: While self-reported smoke detector prevalence rates are high in our study population, other home fire safety practices are suboptimal. Prevention strategies should focus on home smoke detector maintenance and carbon monoxide detector use. Our results also show limited awareness of free fire safety community-based resources yet a high level of interest in these programs.

P12 IMPLEMENTATION AND IMPACT OF A CONSENSUS DIAGNOSTIC AND MANAGEMENT ALGORITHM FOR COMPLICATED PNEUMONIA IN CHILDREN

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Purpose of Study: Variable treatment exists for children with bacterial pneumonia complications such as pleural effusion and empyema. At a tertiary children’s hospital, a literature-based diagnosis and management algorithm for complicated pneumonia in children was created. We proposed that algorithm implementation would reduce use of computed tomography (CT) for diagnosis of pleural infection, thereby decreasing radiation exposure, without increased adverse outcomes.

Methods Used: A cross-sectional study was undertaken in children (3 months to 20 years old) with principal or secondary diagnoses codes for empyema and/or pleural effusion in conjunction with bacterial pneumonia. Study cohorts consisted of subjects admitted 15 months before (Cohort 1, n=91) and after (Cohort 2, n=98) algorithm implementation. Data was collected using clinical and financial data systems. Imaging studies and procedures were identified using Current Procedural Terminology® codes. Vancomycin use was identified by hospital charge codes. Statistical analysis included χ2 test, linear and ordinal regression and analysis of variance.

Summary of Results: Age (P=0.65), gender (P=0.56), diagnoses (P=0.20), and severity level (P=0.93) were similar between cohorts. There was a significant decrease in CT use (20.9% vs. 57.2%; P=0.001) and reduction in readmission rate (0% vs. 7.2%) in Cohort 2 without concomitant increases in vancomycin use (P=0.59) or hospital length of stay (P=0.66).

Conclusions: Implementation of an institutional complicated pneumonia management algorithm reduced CT scan use and radiation exposure in children diagnosed with pleural infection without associated increases in LOS or vancomycin use. This algorithm provides the framework for future prospective quality improvement studies in pediatric patients with complicated pneumonia.

P13 RECOGNIZING OPIOID AND BENZODIAZEPINE RELATED ADVERSE DRUG EVENTS IN CHILDREN THROUGH AN AUTOMATED DETECTION SYSTEM

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Purpose of Study: Excessive sedation and/or respiratory depression associated with the use of opioid or benzodiazepine can cause significant

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morbidity in children. Identifying correctable patterns can improve care. Adverse drug event (ADE) detection utilizing triggers derived from the electronic health record (EHR) has been shown to be more effective and efficient than voluntary incident reporting. The goal of this study is to determine if an EHR-driven automated detection system is effective in identifying opioid and benzodiazapine ADEs utilizing their respective antagonists, and if it can efficiently targets areas for improvement in children.

Methods Used: A retrospective observational study of all automated naloxone/flumazenil triggers generated by an EHR-driven surveillance system was conducted at a large urban children’s hospital over 30 months. All triggers were investigated to determine if an ADE occurred, the degree of patient harm if the ADE was preventable and to identify populations at risk for ADEs.

Summary of Results: Sixty naloxone triggers were identified, of which 48 were ADEs (PPV = 0.8) and 54% were preventable. Three out of 6 flumazenil triggers were determined to be ADEs (PPV= 0.5) and 66% were preventable. Of 51 total ADEs identified via this system, only 2 (3.7%) were also identified via voluntary incident reporting. Seventy percent of ADEs from opioid/benzodiazapine use occurred during procedural sedation, pain control and following tracheal extubation. Of the preventable ADEs, 25% occurred when the analgesic regimen was escalated concurrent with respiratory distress, 20% from extubation following recent use of sedation infusions, and 12% during in-hospital transfers. Eighty percent of preventable ADEs caused temporary harm while 15% lead to prolonged hospital stays, of which 50% occurred from extubation following recent sedation.

Conclusions: Automated antagonist triggers are much more effective in detecting opioid and benzodiazapine ADEs than incident reporting. Our data suggest that most ADEs can be prevented by early recognition of respiratory distress, diligent assessment of pre-extubation sedation status and improved communication during patient hand-offs.

P14

PEDIATRIC MALARIA TREATMENT DELAYS ACROSS SOCIOECONOMIC LEVELS IN SUBURBAN ACCRA, GHANA

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Purpose of Study: The study reported here examines how the socioeconomic status (SES) of Ghanaian families affects the delay-time before children with Plasmodium falciparum malaria are brought to a government hospital. It was hypothesized that delays of appropriate clinical treatment result from families’ apprehension of inability to pay hospital fees.

Methods Used: In this ethnographic study, parents of pediatric patients with blood smear-confirmed malaria (N=15) were interviewed through both quantitative questionnaires and narratives at the Legon Hospital Pediatrics Ward. The questionnaire ascertained the dependent variable of time lag during which the child languished at home, as well as the independent variable of SES measured by: 1) monthly income range, 2) health insurance, and 3) years of household education. Over the course of four months, 170 hours of dialogue and observation supplemented the numerical data.

Summary of Results: There was actually no statistical association between delay-time and family financial standing, nor a correlation between delay-time and enrollment in Ghana’s recently-established National Health Insurance Scheme. Out of practical reasons, parents of all backgrounds postpone clinical treatment even after the onset of clear malaria symptoms; waiting lines at hospitals often stretch several hours and multiple bouts of malaria each year are common for a child in West Africa. Yet, this study does confirm that lower-SES households less-effectively prevent malaria. Only half of families in the lowest income group used insecticide-treated mosquito nets, while all six families in the highest income group utilized the preschool.

Conclusions: In this township, the study suggests that low-SES families do not disproportionately delay clinical treatment. Especially when raising multiple children, parents of all SES groups delay at home and self-treat with herbal teas or street-bought (and often counterfeit) chloroquine and artemisinin anti-malarials. This has risks. Delayed clinical care and the rapid deterioration of originally-uncomplicated P. falciparum cases contribute to 20,000 annual pediatric malaria fatalities in Ghana.
0.27 (0.03–0.38), 0.97 (0.83–2.81), 1.12 (1.03–3.47), 3.57 (2.44–4.55), for periods 1, 2, 3, and 4, respectively, with highest use in period 4. Compared to patients who enrolled, those with delayed/failed engraftment had significantly higher RBC use (p > 0.0058) in period 4. PLT support was almost exclusively seen in period 3 but not significantly different between either group. PLT refractoriness was noted in 3 (33%) patients and required either HLA or crossmatched compatible PLTs. Extensively antigen matched RBC units were required for 3 (33%) and limited antigen matching for 6 (67%) of patients.

Conclusions: SCD patients undergoing NMSCT have significantly higher RBC and PLT use post transplant. Appropriate planning for specialized blood product support of SCD patients during NMSCT is advised.

P17 FAMILIAL POLYCYTHEMIA VERA IN A PATIENT WITH IRON DEFICIENCY ANEMIA
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Purpose of Study: Familial and congenital Polycythemia Vera (PV) cases are rare and some ethnic groups are more prone to this disease - Jews, Western Europe and in the Chuvash Republic. Familial clustering and often encountered genetic mutations have led to the suggestion that this disease may be inherited.

Methods Used: A 66 year old Italian male with a history of hypertension was referred for an elevated platelet count. At presentation, the patient had no complaints. He denied headaches, dizziness, skin redness, titching after a warm bath, chest pain, dyspnea or abdominal fullness. He had no history of numbness, tingling or weakness of the extremities, nor of smoking, alcohol or drug abuse. His father had PV diagnosed at a young age. Examination was unremarkable. Laboratory findings: WBC 9.8, Hb 13.6, Hct 44.8, platelets 717, MCV 67.7, RDW 15.5, ferritin 8.59, iron 25, TIBC 443, transferrin saturation 5.6, B12 level 1331. Peripheral smear exam showed microcytosis, elliptocytes, hypochromia and large platelet clumps. No hemoglobinopathy was detected on hemoglobin electrophoresis. JAK2 V617F mutation was not present. He was started on hydroxy and aspirin.

Summary of Results: Familial PV refers to rare form of polycythemia in which other family members have the same phenotypic and/or genotypic presentations. It is thought that most cases of familial PV are inherited in autosomal dominant fashion. Others concluded that the disease is not directly inherited, and that a genetic component must be at best a precondition rather than a major factor in pathogenesis. Chuvash polycythemia, a secondary form of familial polycythemia, seen not only in the Chuvash Republic but also in Campania (South Italy) is inherited in autosomal recessive fashion. There is increased incidence of thrombotic complications and markedly decreased longevity in homozygotes for the Chuvash polycythemia. These complications do not correlate with the absolute level of elevated hematocrit.

Conclusions: This is a rare case of familial polycythemia in a patient with Italian ancestry. His presentation was unusual; with only thrombocytosis. Further work up revealed iron deficiency with no elevated hemoglobin. We need to be aware of this unusual presentation of PV and obtain good family history in patients suspected of having PV.

P18 A RARE INITIAL PRESENTATION OF UNCOMMON DISORDER
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Purpose of Study: A case report and discussion of unusual presentation of sarcoidosis.

Methods Used: Case study and review of literature.

Summary of Results: Case presentation: 30 year old healthy male was evaluated in office for urinary frequency and intentional weight loss of 60 pounds within 6 months. Physical examination was significant for palpable spleen. Initial blood chemistry was normal except elevated urea (25mg/dl), creatinine (1.8mg/dl) and calcium (11.4mg/dl). After 4 weeks, blood chemistry revealed worsening renal failure (Urea: 47mg/dl, Creatinine: 4.8mg/dl) and hypercalccemia (13.2mg/dl). He was admitted to the hospital. CBC revealed anemia (Hb: 12.8mg/dl) and leukopenia (3,900/summ). Urine analysis revealed trace protein and few WBCs. IV normal saline and SC calcitonin were started. Parathyroid hormone was 11pg/ml (Normal: 8.5–72 pg/ml). 24-hour urinary calcium was 419mg (Normal: 100–300). CT-Abdomen revealed retroperitoneal lymphadenopathy and spleenomegaly. Multiple myeloma and lymphoma were excluded by normal serum and urine electrophoretic strip, normal bone scan, and bone marrow exam. An autopsiobty screen (ANA, RF, ANCA) was negative. Serum 1.25(OH)2D3 level was elevated at 90pg/ml (Normal: 18–64pg/ml). Serum angiotensin-converting enzyme activity was 133U/L (Normal: 8–53 U/L). Retropertoneal lymph node and transbrachial lung biopsies revealed non-caseating granulomas. Fungal and acid fast cultures were negative. Sarcoïdosis was diagnosed. He was started on 40mg prednisone daily and sent home. After 4 weeks, serum creatinine and calcium level became normal. Prednisone treatment is ongoing.

Conclusions: Discussion: Spectrum of sarcoidosis related renal disease includes hypercalcemia, hyperpericaria, granulomatous interstitial nephritis (GIN), glomerular disease, renal tubular dysfunction, renovascular disease, and obstructive uropathy. Hypercalcemia occurs in 10–15% of patients and is the most common cause of renal dysfunction. Both hypercalcemia and GIN respond well to glucocorticoids. Hyperpericaria patients who cannot tolerate or fail to respond to steroids can be treated with chloroquine. Supportive care for hyperpericaria patients includes adequate oral hydration, minimization of dietary calcium, oxalate and vitamin-D intake, and avoidance of UV light exposure.

P19 CHARACTERISTICS OF NECROTIC ACRAL ERYTHEMA IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS
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Purpose of Study: To determine the prevalence and characteristics of NAЕ in patients with chronic HCV.

Methods Used: We performed a multicenter prevalence study among chronic HCV patients. Subjects with quantifiable HCV RNA in serum were recruited from viral hepatitis clinics of 3 Philadelphia hospitals. Patients underwent a dermatologic examination and collected a questionnaire collecting demographic and clinical data. Laboratory data were collected from medical records. Subjects with skin findings consistent with NAЕ, skin biopsies were performed and the diagnosis confirmed by a dermatopathologist.

Summary of Results: Among 226 patients with chronic HCV infection (median age, 55 years; 74% male; 71% HCV genotype 1), 3 (1.3%; 95% CI, 0.3–3.8%) had NAЕ confirmed by skin biopsy. Among all three subjects, skin biopsies demonstrated variable psoriasiform hyperplasia, mild papillary dermal edema, spongiosis, parakeratosis, and necrotic keratinocytes in the epidermis. All 3 patients were >50 years old, African American, infected with HCV genotype 1 and had a high viral load (>800,000 IU/mL). Patient one had diabetes mellitus and was infected with human immunodeficiency virus infection (HIV; CD4 cell count = 901 cells/mm3). Patient two was also HIV-infected (CD4 cell count = 270 cells/mm3). Patient three was identified with hepato-cellular carcinoma (HCC).

Conclusions: The prevalence of NAЕ among chronic HCV patients in this sample was very low. It is possible that this finding is attributed to the specific HCV genotype seen in distinct geographic areas. Further research is needed to identify the etiology of NAЕ.

P20 THE USE OF SEDATIVES DRUGS AMONG THE IN-HOSPITAL ELDERLY PATIENTS, INDICATIONS AND COMPLICATIONS
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Purpose of Study: To understand the use of sedatives in an in hospital setting among the elderly with the hope of reducing the use of sedation and its related complications by educating the physicians treating the elderly. As the population ages more and more elderly patient are being treated in hospitals. Due to the age and multiple comorbid factors these patients tend to stay in hospital for a longer time leading to anxiety, agitation and confusion because of the unfamiliar surroundings.

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Methods Used: Retrospective study was done using medical records from a community hospital for a period of two years. Patients age, gender, sedation used, indication for sedation and complications associated with sedation was documented for a period of two years.

Summary of Results: A total of 1771 patients were included in this study and the average age of the patient was 75 years. 59 % of the patients were female and the rest were males. The most common sedatives used were Lorazepam which accounted for 52%, followed by Haloperidol 34%, Morphine 10%. The most common indication was Agitation/anxiety 78% followed by insomnia 13% and pain in 7%. The most common complication associated was altered mental status/over sedation in 68%; fall in 30% and dizziness in 2% of the patients.

Conclusions: It was noted that longer the patients stayed in hospital more frequent was the use of sedation. There was only a minimal adjustment of the doses to the change of the type of sedative used, based on the age and weight of the patient. The physicians prescribing sedatives to the elderly should be aware of the side effects, complications as well as the use of alternative methods in treating patients with anxiety/agitation.

P21 ETIOLOGIES AND RISK FACTORS FOR REHOSPITALIZATION FOR SYCONE AND FOR LONG-TERM MORTALITY IN 325 CONSECUTIVE PATIENTS HOSPITALIZED FOR SYCONE

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Purpose of Study: Investigate etiologies and risk factors for rehospitalization in syncpe. Determine long-term mortality in patients hospitalized for syncpe.

Methods Used: We investigated the etiologies of syncpe and risk factors for rehospitalization in 325 consecutive patients (59% men and 41% women) mean age 66 ± 17 years. Mean follow-up was 27 ± 8 months.

Summary of Results: In 325 consecutive patients hospitalized for syncpe, the cause was diagnosed in 241 patients. Of 325 patients, 13 (4%) were rehospitalized for syncpe and 38 (12%) died in 27 ± 8 months. The causes of syncpe were vasovagal syncpe in 58 patients, volume depletion 39, orthostatic hypotension and aortic stenosis in 8 each, bradyarrhythmias in 28, tachyarrhythmias in 36, acute coronary syndromes in 23, hypertrophic obstructive cardiomyopathy in 4, cardiomyopathy requiring insertion of a de-fibrillator and hypersensitive carotid sinus in 7 each, intra cerebral causes and drug overdose in 5 each, hypoglycemia in 3, pulmonary embolism, ana-phylaxis, primary pulmonary hypertension and micturition syncpe in 2 each, heat stroke and cardiac tamponade in 1 each, and undetermined etiology in 84 patients.

Stepwise logistic regression analysis showed significant independent prognostic factors for rehospitalization for syncpe were low glomerular filtration rate (GFR) (odds ratio (OR) 0.97; 95% CI 0.95-0.99) diabetes (OR 5.7; 95% CI 1.6-20.4), tachyarrhythmias (OR 4.0; 95% CI 1.0-15.6), and smoking (OR 4.6; 95% CI 1.3-16.8). Stepwise Cox regression analysis showed significant independent prognostic factors for mortality were low GFR (hazard ratio (HR) 0.98 (95% CI, 0.97-0.99), diabetes (HR 2.7; 95% CI 1.4-5.2), coronary artery surgery (HR 2.9; 95% CI 1.3-6.5), malignancy history (HR 2.5; 95% CI 1.2-5.2), drug overdose (HR 4.0; 95% CI 1.7-9.8), smoking (HR 2.8; 95% CI, 1.4-5.5), and tachyarrhythmias (HR 2.4; 95% CI 1.0-5.4).

Conclusions: In patients hospitalized for syncpe, significant independent prognostic factors for rehospitalization for syncpe were low GFR, diabetes, tachyarrhythmias, and smoking. Significant independent prognostic factors for mortality were low GFR, diabetes, coronary artery surgery, malignancy history, drug overdose, smoking and tachyarrhythmias.

P22 RENAL CELL CARCINOMA AND HEMATOLOGIC MALIGNANCY IN THE SAME PATIENTS

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Purpose of Study: We observed the clinical association of renal cell cancer (RCC) and hematologic malignancy (HM) in several RCC patients (pts), and sought to characterize the frequency, clinical features, and characteristics of this association.

Methods Used: Our data base of 250 pts with RCC was accessed to identify 16 pts with RCC who have a personal history of HM. WE characterized the type of HM, and course and treatment in both RCC and HM.

Summary of Results: There are 11 males and 6 females. In 12 pts, HM preceded RCC, was simultaneous in one pt, and occurred after the diagnosis of RCC in 3 pts. HM diagnosis was Hodgkin’s disease (HD) in 4 pts, non-Hodgkin lymphoma (NHL) in 7 pts, 3 small and 4 large B-cell lymphoma, chronic lymphocytic leukemia (CLL) in 2 pts, hairy cell leukemia (HCL), monoclonal gammopathy of undetermined significance (MGUS) and myelodysplasia (MDS) in one pt each. Thus, all but one pt had a B-cell malignancy. Clinical outcomes: All 4 pts developed HD prior to RCC, and were cured of HD. Two died of RCC; and two have ongoing active RCC. Of the two pts with CLL, both developed RCC after more than a decade of CLL; one died of RCC and one is alive, with no evidence of RCC. The pt with HCL was cured of HCL 2 decades prior to the diagnosis of RCC and has ongoing metastatic RCC. MGUS preceded RCC by at least 5 years and remains stable, and this pt is alive with oligometastatic RCC. The pt with MDS which preceded RCC has no evidence of RCC after nephrectomy. Of the 7 pts with NHL, 6 were treated and responded to initial therapy for NHL, but two developed recurrent lymphoma, requiring subsequent therapy. One remains on observation, never treated. No pt died of HM. 6 pts have died of RCC, and 10 pts remain alive, 6 with active RCC.

Conclusions: RCC and HM observed in the same pt occurs significantly more frequently than expected, based on the SEER database. There is a preponderance of B-cell malignancy associated with RCC (15/16). HM preceded RCC (12/16) and was cured (8/16) or stable (6/16) in the majority of patients. The etiology of this association is unknown but needs exploration. We will further explore the association of RCC and B-cell malignancy in RCC families.

P23 USING QUANTITY OF PUBIC GRAY HAIRS AS A PREDICTOR OF DIMINISHED OVARIAN RESERVE

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Purpose of Study: To determine the predictive value of the presence of pubic gray hairs to ovarian reserve.

Methods Used: (IRB approval, patient consent) Five hundred Private Practice subjects from 2007-2010 were enrolled. Subjects underwent a routine physical examination as part of the basic infertility evaluation prior to implementation of treatment modalities. During the physical examination, the entire mons pubis was examined for presence and number of gray pubic hairs.

Summary of Results: (Baseline data on the 500 subjects). 99% (495/500) subjects with 20 or ‘too numerous to count’ (TNTC) gray pubic hairs were found to have either elevated cycle day 3 FSH levels (>13 mIU/ml) or were poor responders to gonadotropin treatment. None (0/500) of these subjects achieved a viable pregnancy with their own eggs. P value of <.001. 98% (490/500) of all patients undergoing donor egg cycles had from 10 to TNTC gray pubic hairs. P value of <.001. 87% of subjects with elevated day 3 FSH had from 5 to (TNTC) gray pubic hairs. P value of <.005. There were both negative controls and positive controls.

Conclusions: The presence of pubic gray hair may be a reflection of the biochemical processes occurring in the hypothalamus that determine the onset of ovarian failure. The presence of gray pubic hair alone has a significant predictive value for decreased ovarian reserve.

P24 PREDICTORS OF FOREARM FRACTURE RISK IN AFRICAN AMERICAN CHILDREN

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Purpose of Study: Pediatric forearm fractures result in substantial morbidity and costs and are increasing in incidence. Modifiable risk factors, including overweight status and/or deficient bone health, may contribute to injury risk. High body mass index (BMI) and risk factors for poor bone health (low dietary calcium intake and hypovitaminosis D) are prevalent in African American (AA) children. Our objective is to determine the association of forearm fractures with weight status and bone health in AA children.

Methods Used: This case-control study is enrolling AA children, ages 5-9 years, with and without forearm fracture. Evaluation includes measurement
of BMI, calcium intake, serum 25-hydroxy vitamin D level (25OHD) and bone mineral density (BMD). Bivariate and multivariable analyses are used to test the associations of the independent variables reflecting weight and bone health with fracture status.

Summary of Results: To date, we have enrolled 65 cases and 72 controls. The mean age and the proportion who were male did not differ comparing cases to controls. The mean BMI percentile for cases [73.1 (±25.8)] was significantly higher than controls [59.0 (±30.0)] (p=0.008). More cases (30/58, 52%) than controls (22/68, 32%) had a BMI ≥ 85th percentile (OR=2.2, 95% CI=1.1-4.6). There was no difference in the proportion of cases (24/52, 46%) and controls (19/63, 30%) meeting the recommended daily dietary calcium intake for age (OR=1.1, 95% CI=0.9-1.3). The mean 25OHD level for cases [21.7 (±6.9) ng/mL] was similar to that of controls [22.6 (±7.3) ng/mL] (p=0.45). The proportion of cases and controls with 25OHD insufficiency [29/59, (49%) vs 27/69, (39%), OR=1.5, 95% CI=0.7-3.0] and the BMD for cases and controls [mean total body z-scores 0.7 (±1.0)] vs 1.0 (±1.1), p=0.10] were not statistically different.

Conclusions: These data support an association between overweight status and risk for childhood forearm fracture and provide additional rationale for aggressively addressing obesity. Further study is needed to determine how increased weight status may increase forearm fracture risk in this population. Our findings also suggest that bone health (25OHD status and BMD) may play a role in the increasing rates of childhood forearm fracture.

P25 FACTORS ASSOCIATED WITH ORTHOPEDIC INTERVENTION FOR PEDIATRIC FOREARM FRACTURES

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Purpose of Study: Pediatric forearm fractures are increasing in incidence and result in substantial costs. Orthopedic intervention rates for these fractures are also rising, which may suggest an increase in injury severity. Because overweight children fall with greater force, increased weight status may increase risk for forearm fracture displacement. This study will evaluate the hypothesis that fractures treated with orthopedic intervention are associated with major trauma mechanisms and increased weight status (weight for age/gender percentile ≥ 95th).

Methods Used: Our study population included Washington DC children, ages 0–17 years, with an isolated forearm fracture treated in a large urban pediatric emergency department between 2003–2006. Demographic and clinical data were obtained from medical record review. Orthopedic intervention included closed or open reduction. Major trauma mechanisms included motor vehicle collision, pedestrian/bicyclist struck by a moving vehicle and/or fall greater than patient height. Minor trauma mechanisms included other mechanisms not meeting criteria for major trauma. Descriptive epidemiologic and bivariate analyses were conducted.

Summary of Results: Of 929 forearm fractures, there were 333 cases (35.9%) treated with orthopedic intervention and 596 (64.1%) non-intervention controls. The overall cohort was 64% male, 80.1% African-American, and had a mean age of 8.4 (3.9) years. Orthopedic intervention cases were more likely to be male [OR 1.6 (95% CI 1.2–2.1)] but did not differ in mean age or race/ethnicity. Orthopedic intervention cases were more likely to have a major trauma mechanism [OR 1.9 (95% CI 1.1–3.4)]. The proportion of orthopedic intervention cases (75/329, 22.8%) and non-intervention controls (140/563, 24.9%) having a weight for age/gender percentile ≥95th percentile were not different [OR 0.9 (95% CI 0.6–1.2)].

Conclusions: A large proportion of forearm fractures in our study population require reduction. Orthopedic intervention is significantly associated with male gender and more severe mechanisms of injury but is not associated with increased weight status. Preventive strategies to reduce displaced forearm fractures should target major trauma mechanisms.

P26 LATERAL MEDULLARY INFARCT PRESENTING AS VOMITING AND VERTIGO

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Purpose of Study: This is a case report of a 48-year-old Hispanic male who presented to our ED with unremitting vomiting and vertigo and was ultimately diagnosed with a lateral medullary infarct (Wallenberg Syndrome). Wallenberg Syndrome is a rare cause of vertigo that should be considered in high risk patients with vertigo and concomitant neurological symptoms.

Methods Used: This is a single case report of a patient seen in the Emergency Department.

Summary of Results: A 48 year old Hispanic male with a history of hypertension, Type II DM, and coronary artery disease presented with three days of constant vertigo and nausea. His physical exam was remarkable for a blood pressure of 219/108 and nystagmus with right sided facial numbness. The patient’s vertigo was resistant to metoclopramide 10mg IV, diazepam 2mg IV, diphenhydramine 50mg IV, ondansetron 4mg IV, and 500cc normal saline IV. The patient’s head CT without contrast was negative for any acute pathology. The patient was admitted to the neurological service for further diagnostic studies and symptomatic treatment. He underwent MRI of his brain and MRA of his head and neck under stroke protocol, which showed an acute right dorsal medullary infarct consistent with Wallenberg syndrome. The major vessel supplying the infarct was patent, therefore the patient was followed with permisive hypertension, given daily aspirin, and kept flat for 24 hours to maximize cerebral perfusion. His vertigo improved with clonazepam 0.5mg twice daily and he was discharged to acute rehabilitation with follow-up in the stroke clinic.

Conclusions: ED physicians should raise their suspicion of a vascular accident as the cause of a patient’s vertigo when the symptoms are resistant to conventional therapies and when other predisposing risk factors and neurological symptoms are present. Risk factors for CVA (male sex, hypertension, diabetes, coronary artery disease, or atrial fibrillation), and neurologic symptoms should increase suspicion of a stroke despite a normal head CT, which does not accurately image the posterior fossa. MRI of the brain and neck should be considered in such patients. Recent studies have shown that a stroke or TIA is often missed in the ED if vertigo is the predominating symptom. An increased suspicion of infarct in those patients with risk factors and vertigo will raise the likelihood of correct diagnosis.

P27 EFFECT OF CARDIAC PFO PHYSIOLOGY ON CIRCULATORY PROTEOMIC PROFILE IN STROKE PATIENTS

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Purpose of Study: Patent foramen ovale (PFO), a congenital cardiac abnormality allowing venous clots to travel directly to the brain, is an independent stroke risk factor associated with more than 150,000 strokes per year. Since the best treatment for PFO related stroke is unclear, a better understanding of the molecular landscape of this multi-organ disease involving the brain, heart and blood is direly needed. We conduct discovery plasma proteomic and transcriptomic analysis to identify proteins and RNAs that are associated with stroke in the presence and absence of a PFO.

Summary of Results: To date, we have enrolled 65 cases and 72 controls. Our study population included Washington DC children, ages 0–17 years, with an isolated forearm fracture treated in a large urban pediatric emergency department between 2003–2006. Demographic and clinical data were obtained from medical record review. Orthopedic intervention included closed or open reduction. Major trauma mechanisms included motor vehicle collision, pedestrian/bicyclist struck by a moving vehicle and/or fall greater than patient height. Minor trauma mechanisms included other mechanisms not meeting criteria for major trauma. Descriptive epidemiologic and bivariate analyses were conducted.

Summary of Results: Of 929 forearm fractures, there were 333 cases (35.9%) treated with orthopedic intervention and 596 (64.1%) non-intervention controls. The overall cohort was 64% male, 80.1% African-American, and had a mean age of 8.4 (3.9) years. Orthopedic intervention cases were more likely to be male [OR 1.6 (95% CI 1.2–2.1)] but did not differ in mean age or race/ethnicity. Orthopedic intervention cases were more likely to have a major trauma mechanism [OR 1.9 (95% CI 1.1–3.4)]. The proportion of orthopedic intervention cases (75/329, 22.8%) and non-intervention controls (140/563, 24.9%) having a weight for age/gender percentile ≥95th percentile were not different [OR 0.9 (95% CI 0.6–1.2)].

Conclusions: A large proportion of forearm fractures in our study population require reduction. Orthopedic intervention is significantly associated with male gender and more severe mechanisms of injury but is not associated with increased weight status. Preventive strategies to reduce displaced forearm fractures should target major trauma mechanisms.
proteomic profiling in PFO patients undergoing clinical endovascular PFO closure -a good bedside model to study the direct effects of PFO on circulatory protein signaling in stroke patients.

**Methods Used:** Plasma was collected from PFO-related stroke patients at baseline, pre and post PFO closure. Samples were analyzed on MS and intra-patient pre- vs post-closure proteomic profiles were compared, with each patient serving as their own control to reduce potential confounders.

**Summary of Results:** Post PFO closure, the plasma protein profile changes dramatically – showing a decrease of coagulation markers such as tissue factor (TF) and fibrinogen, synthesis of various fibrinogen chains (FGA, FGG), and differential expression of protease inhibitors such as A2M and SERPINA 1, important in vascular disease. Cell differentiation factors such as RLF, and factors that exert negative control of cell growth, such as PPI2R2A, are upregulated – suggesting a pattern of repair focused on cell differentiation rather than division. (See Figure).

**Conclusions:** As an initial step in understanding the physiology in PFO-related stroke, our results highlight important changes in coagulation, cell-signaling and differentiation induced by PFO repair - suggesting PFO may have important roles in stroke circulatory signaling.

**P28 NEUROTOXICITY OF CHLORINATED Dopamine**

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**Purpose of Study:** To identify the products of the reaction of hypochlorous acid and dopamine, and to assess the toxicity of these reaction products.

**Methods Used:** The chlorodopamine species were identified by a combination of spectrometry, electron microscopy, HPLC coupled with visible and electrochemical detection, GC-MS, and NMR. The cytotoxicity of soluble chlorodopamine to retinoic acid-differentiated SH SY5Y cells was assessed by the reduction of MTT, the release of LDH, and α-ketoglutarate dehydrogenase activity measurements. Apoptosis due to chlorodopamine precipitates with THP-1 cells was quantified using the Tunel assay.

**Summary of Results:** The reaction of HOCl and dopamine produces at least four products, as distinguished by thin-layer chromatography. One of these is a black precipitate, which over time, becomes the major reaction product. Pigmented aggregates are evident in most neurodegenerative diseases. The chlorodopamine precipitate is composed of spheres with diameters in the micron range. When absorbed by macrophage-like cells, these aggregates cause these cells to die by apoptosis. These observations suggest that the formation or pinocytosis of chlorodopamine precipitates by neurons or their supporting cells could contribute to the neurodegenerative process. The soluble reaction products of the reaction of HOCl and dopamine cause retinoic acid-differentiated SH SY5Y cells to die by necroptosis. This death is associated with loss of cell attachment and α-ketoglutarate dehydrogenase activity, as well as, ultra-structural changes in mitochondria. In contrast, HOCl forms no precipitates, is less toxic to retinoic acid-differentiated SH SY5Y cells, leaves cell attachment unaffected, and has a lesser effect on α-ketoglutarate dehydrogenase activity than chlorodopamine. The formation and toxicity of chlorodopamine are mitigated by sulfur compounds, in particular, hydrogen sulfide.

**Conclusions:** The reaction of dopamine and hypochlorous acid is facile and results in products that are cytotoxic. The formation of chlorodopamine represents a novel pathway for the loss of neurons in diseases like Alzheimer Disease, Parkinson Disease, Huntington Disease, and Amyotrophic Lateral Sclerosis. Sulfur compounds prevent the formation and toxicity of chlorodopamine in vitro and may mitigate its toxicity in vivo.

**P29 PLACENTAL HYPERVASCULARITY DOES NOT CAUSE PERINATAL BRAIN INJURY**

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**Purpose of Study:** To refute the assertion that ‘chorangiosis’ is a causal factor in perinatal hypoxic brain injury.

**Methods Used:** Critical analysis of an index case and review of pertinent archival autopsy, placental, clinical, and epidemiological data.

**Summary of Results:** Dizygotic twins at 38 weeks with separate placenta: twin A, a 2479 gram female, was healthy after vaginal delivery. Five minutes later when the amnion of twin B was ruptured artificially, the cord prolapsed and could not be repositioned. Some 25 minutes later a 2791 gram male was delivered by section. Brain injury was noted soon afterward and subsequent development was marked by severe cerebral palsy and mental retardation. Initial diagnosis of twin B’s placenta was ‘chorangiosis,’ overlooking fresh thrombus blocking the umbilical vein and one umbilical artery. Subsequent assessment revealed the same change in twin A’s placenta. Archival records had 18/500 (3.6%) stillborns and 17/418 (4.07%) newborns with placental hyppervascularity. Of 125 recent consult placentas there were 17/100 singleton and 11/25 (44%) twin placentas displaying this change. Of 229 section deliveries there were 0/42 stillborns and 5/187 newborns with this vascular pattern. Another set of 625 autopsies revealed none with both hypoxic encephalopathy and cerebral palsy. This is the same often seen in placentas from high altitude such as in Denver. Cerebral palsy occurs less often in Colorado than in other American states, per epidemiological data.

**Conclusions:** Central villous hypervascularity is an adaptation of placental development prior to midgestation through exaggerated growth of the paracapillary network and is not a pathogenetic agent or process.

**P30 OSTEOPOINTIN IS SIGNIFICANT IN PEDIATRIC ASTHMA**

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**Purpose of Study:** Osteopontin (OPN) is a multifunctional cytokine secreted by neutrophils, eosinophils, and epithelial cells. It is increasingly implicated in numerous TH-2 mediated diseases, including asthma where it is important in inflammation, cell migration, and remodeling. Recent studies in adults with asthma have demonstrated increased levels of OPN are associated with asthma severity. We sought to determine the association of plasma OPN with asthma and biological and functional markers of asthma severity in pediatric patients.

**Methods Used:** We studied pediatric patients aged 6 to 20 years, inclusive, with physician-diagnosed asthma for greater than one year who had extensive asthma phenotyping including blood analyses (IgE, eosinophil and neutrophil levels), and assessment of asthma severity and control using the NAEPP 2007 criteria for asthma severity, asthma control test (ACT), and Integrated Therapeutics Group (ITG) Short Form for quality of life measurement. 111 pediatric subject samples were available for analysis. Plasma OPN levels from 56 healthy subjects over 18 years served as non-asthmatic controls. Osteopontin levels were determined via a flow cytometry-based bead assay or ELISA.

**Summary of Results:** The mean±SD ages of cases and controls were 11±4 and 27±6 years, respectively. OPN levels were significantly higher in asthmatic cases than controls (86±48 vs. 14±9 ng/mL (p=0.001) controlling for age, race, gender. Comparing age and OPN levels, we found an increase in one year of age decreased the OPN by 3.56 [95% 8240; CI: 1.48, 5.63] (p=0.001). Within cases, OPN was not associated with IgE, eosinophil and neutrophil levels. Additionally, no association was found between OPN and with NAEPP level, ACT, or ITG scores, number of hospital or unscheduled doctors visits in the preceding 12 months. However, a one point of the FEV1 change after bronchodilator was associated with a 0.07 increase in OPN (p=0.046).

**Conclusions:** OPN is significantly higher in children with asthma compared to healthy adults. Likely limited by sample size, OPN is promising as a measure for asthma severity in pediatric patients and additional studies with a larger cohort of pediatric asthma patients are warranted.
Purpose of Study: Asthmatic inflammation is thought to contribute to airway remodeling via immune cell interactions with airway tissues. However, we previously showed that in vitro airway epithelium, lacking immune cells, contributes to asthmatic inflammation via direct secretion of inflammatory cytokines in association with dysynchronous mitotic regeneration. We hypothesize these asthmatic secretions alter regenerating airway epithelial morphology (i.e., goblet cell hyperplasia); they do so without influencing mitotic characteristics, which are intrinsic to the cell.

Methods Used: Normal airway epithelia were grown to air liquid interface on collagen membranes. Cultures were mechanically-injured at 0 hours and exposed continuously to Bromodeoxyuridine (BrdU) and secretions from injured or non-injured, normal or asthmatic airway epithelia for 48 hours. Wound regeneration and mitosis were analyzed by microscopy and flow cytometry.

Summary of Results: When exposed to secretions from non-injured normal (n=3) and asthmatic (n=3) epithelia, there was no difference in quantity or quality of normal epithelial mitosis. However, we found decreased mitosis in epithelia exposed to secretions from injured asthmatic compared to injured normal epithelia (mean±SEM=0.033±0.001% vs. 0.045±0.001%; p=0.02). Correspondingly, epithelial wounds healed less when exposed to secretions from injured asthmatic compared to injured normal epithelia (48 hour wound area reduction: 57.5% vs. 76.8%; although p=0.19). Mitotic synchrony was similar in all conditions.

Conclusions: We found that secretions from injured asthmatic airway epithelia (we previously showed to contain TGFβ1, IL-1, IL-10, and IL-13) reduced mitotic regeneration in normal airway epithelium in vitro. However, mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic synchrony was not affected; supporting the concept that synchrony is intrinsic to the cells. If ongoing analyses of cytokine secretions and immune mitotic

Summary of Results: In our cases (n=38), we found that an increase of 1 point in the PRISCA II score was associated with a 0.63 decrease in 25(OH)D level (P=0.01). There were no associations between 25(OH)D level and length of stay (P=0.36) or risk for ICU admission (OR 3.63,P=0.16). Among our cases, admission diagnoses included asthma(n=9), pneumonia(10), asthma with pneumonia(7), bronchiolitis(4), respiratory distress/failure(3), empyema(1), and other illnesses(4). 25(OH)D levels did not differ between cases and controls (n=84) (26.8±11.5 vs 25.8±10.9, respectively, P=0.44). In both groups, an increase of one year in age was associated with a 0.21±0.04 decrease in 25(OH)D (P < 0.001).

Conclusions: 25(OH)D levels did not differ between young children hospitalized with and without respiratory illnesses. We found a significant association between 25(OH)D level and PRISCA II score within our cases. This is a novel significant finding that suggests 25(OH)D is associated with severity of illness in children hospitalized with respiratory illnesses.

P33 MOLD AND ASTHMA IN AN URBAN INNER-CITY PEDIATRIC POPULATION

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Purpose of Study: Over the past 20 years, asthma has steadily increased in prevalence; this is attributable in part to social risk factors inherent in inner-city living and poverty. Environmental allergens have long since thought to be a major culprit in asthma severity, but there is little information on how environmental allergens such as mold directly impact the child with asthma. Delineating the direct effects of mold on asthma control and quality of life measures could potentially drive in-home interventions. We sought to determine if self-reported mold in the home environment was associated with asthma severity phenotypes.

Methods Used: We conducted a cross-sectional study of 154 6-20 year old AA children with physician diagnosed asthma for greater than 1 year. These children were extensively phenotypeing, including NAEPP 2007 criteria for asthma severity, the asthma control test (ACT), and the Integrated Therapeutics Group Short form(ITG), a score of quality of life. We compared these dependent variables in patients with and without mold exposure, using multiple linear regression to control for age, gender, race, and body mass index (BMI) percentile.

Summary of Results: Self-reported exposure to mold or dampness in the home was reported by 29.9% of our cases. Exposure to mold was associated with a worse ITG daytime score (scores decreased by −12.46 +/− 4.1 [P=0.003]) and composite score (decreased by −8.32 +/− 3.69 [P=0.026]). Additionally, mold exposure was also associated with worse ACT scores (decreased by −1.77 +/− 0.74 [P=0.17]). Mold exposure was also associated with 2.25 +/− 0.73 (P = 0.002) more days with asthma symptoms in the preceding 2 weeks of the study visit than in children whose families reported no mold exposure.

Conclusions: Mold exposure is associated with worse asthma control and worse quality of life scores, and increased symptomatic days in children with asthma. Identifying mold as a modifiable risk factor for asthma can lead to focused interventions in our population that could reduce asthma morbidity.