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Podium Presentations

Transanal Endoscopic Microsurgery (T.E.M) Treatment Of Rectal Cancer: A Comparision Of Outcomes With And Without Neoadjuvant Radiation Therapy

Elsa B Valsdottir MD, John H Marks MD, Shadi Yarandi MD, David Newman MS, Mohammad Mohiuddin MD, Al DeNittis MD, Gerald Marks MD

Lankenau Hospital and Institute for Medical Research

Acidic Treatment Activates eNOS in Xenografted Melanoma Cells and in Endothelial Cells and Alters Endothelial Cell Permeability by a PKA Dependent Pathway: Implications for Isolated Limb Perfusion Therapy

Dale Han, Giorgos C. Karakousis, Tianyi Du, Mark J.L. Dela Cruz, Jeffrey A. Katz, Indira Prabakaran, Marina A. Guvakova, Douglas L. Fraker

Department of Surgery, Division of Endocrine and Oncologic Surgery, University of Pennsylvania School of Medicine, Philadelphia, PA

Combined Penetrating Iliac Vessel and Hollow Viscous Injury, Does Method of Repair Matter?

Brian P. Smith, MD, Jessica Wobb, BS, John Gaughan, PhD, Heather Kulp, RN, Mark J. Seamon, MD, Paola G. Pieri, MD, Thomas A. Santora, MD, Abhijit S. Pathak, MD, Amy J. Goldberg, MD, Kevin M. Bradley, MD

Department of Surgery, Temple University Hospital, Philadelphia, PA

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Lankenau Hospital and Institute for Medical Research

Introduction: Improved tumor downstaging and complete response rates of rectal cancer treated with neoadjuvant therapy have been observed. There has been concern that the detrimental, local effect of radiation makes this approach unsuitable for excision. We compared 2 groups of patients undergoing comparable TEM surgery, with and without neoadjuvant radiation.

Methods: All patients undergoing TEM for rectal cancer with intent to cure from 11/97 to 06/07 were prospectively entered in a database. Demographics and perioperative information were captured. The neoadjuvant group was treated with a mean dose of 5175 cGy (4000-5580 cGy) and concurrent 5FU based chemo was used preferentially. Surgery was performed at a median of 9 weeks following completion of treatment (4-16 weeks).

Results: 64 patients with rectal cancer were treated with TEM; 43 with neoadjuvant (XRT) therapy and 21 with TEM alone. Patient characteristics for the XRT group were: age 67 years (29-86), 13 women. In the non-XRT group, age was 66 years (49-89) and 9 women. The pre-operative and pathologic T stage was: XRT patients: T0: 0/15, T1: 2/5, T2: 31/18, T3: 10/5. Non-XRT patients: T0: 10/5, T1: 9/9, T2: 2/6, T3: 0/1. There were no mortalities in either group. Overall morbidity rate for the XRT was 30% and 14% for the non-XRT group, this difference was statistically different ($p<0.005$). Wound separation (minor and major) was the most common early morbidity in both groups, 9 (21%) and 2 (9.5%) patient respectively. 6 permanent stomas were identified, 4 (9%) in the XRT group and 2 (9.5%) in the non-XRT group ($p>0.05$). There was one patient with a positive margin in each group, 2.3% from the XRT and 4.8% for non-XRT ($p<0.005$). Average follow up for the XRT group was 35 months, 24 months for the non-XRT. There was no local recurrence or metastasis in the non-XRT, but 2 patients (4.7%) with LR and 2 (4.7%) patients with distant metastasis in the XRT. The KM5YAS was 71% for the non-XRT and 93% for the XRT ($p<0.005$).

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Department of Surgery, Division of Endocrine and Oncologic Surgery, University of Pennsylvania School of Medicine, Philadelphia, PA

Introduction: Acidifying the perfusate during isolated limb perfusion (ILP) improves tumor responses in preclinical models, and in tumor bearing animals, increases levels of nitric oxide (NO). Here, we investigated the effect of acidic treatment on endothelial cells and on xenografted melanoma cells with a focus on the molecular regulators of endothelial cell permeability - protein kinase A (PKA) and endothelial NO synthase (eNOS).

Methods: Human microdermal endothelial cells (HMDEC) were treated with control medium or acidic medium (pH 6.1-6.2). Western blot (WB) was used to detect activated eNOS, phosphorylated at serine 1177 (pSer1177eNOS), and activated vasodilator-stimulated phosphoprotein, phosphorylated at serine 157 (pSer157VASP), a known marker for activated PKA. Permeability across an HMDEC monolayer was measured using transwell units and fluorescein-labeled dextran. Immunofluorescent staining was used to detect eNOS and pSer1177eNOS in tumor sections obtained after ILP treatments of nude rats xenografted with NIH1286 human melanoma cells. Two-step quantitative real time PCR (qRT-RCR) and WB were used to detect eNOS mRNA and protein in melanoma xenografts.

Results: *In vitro*, when compared with control treatments, treatment of HMDEC with acidic medium led to a 5.1 fold increase in the expression of pSer157VASP that was reversed by the PKA inhibitor H89. In addition, permeability across an HMDEC monolayer was significantly ($P<0.05$) decreased in a PKA dependent manner after 30 minutes of acidic treatment. *In vivo*, expression of eNOS was detected within xenografted melanoma cells as demonstrated by immunofluorescent staining. qRT-RCR using human specific primers and WB confirmed expression of human eNOS mRNA and protein in all melanoma xenografts. Furthermore, staining for pSer1177eNOS was markedly increased in the endothelium of blood vessels and within xenografted melanoma cells in tumors exposed to acidified perfusate compared with tumors treated with control perfusate.

Conclusion: Acidic treatment of endothelial cells activates PKA *in vitro* and causes a PKA dependent decrease in endothelial monolayer permeability. However, *in vivo* acidic treatment activates eNOS in blood vessels and in xenografted human melanoma cells, and the generated NO could increase endothelial cell permeability. The balance of these regulatory mechanisms on endothelial barrier function *in vivo* may have implications for the improved tumor responses observed after ILP treatment with acidified perfusate.

Combined Penetrating Iliac Vessel and Hollow Viscous Injury, Does Method of Repair Matter?

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Department of Surgery, Temple University Hospital, Philadelphia, PA

Introduction: Penetrating iliac injuries are frequently encountered in urban trauma centers. These patients often have additional intra-abdominal injuries, particularly to hollow viscera. To date, there is little information regarding the combination of an associated hollow visceral organ and iliac injury on outcome. We hypothesized that the method of hollow viscous management, in the setting of penetrating iliac injury, contributed to abscess formation and subsequent hemorrhage.

Methods: Fifty-seven patients between ages of 18 and 65, with iliac vessel gunshot injuries from 2001 through 2006, were reviewed yielding 30 patients who survived greater than 24 hours. Demographics, Injury Severity Scores (ISS), hemodynamics, resuscitative measures, perioperative antibiotics, operative time and temperatures, and operative data regarding vascular repair and hollow viscera management were gathered. These variables were analyzed in relation to postoperative hemorrhage and abscess. Univariate analysis was utilized to define significance, and odds ratio estimates.

Results: Twenty-nine males and 1 female were included. Mean age = 27.7 ± 8.2 years. Mean ISS = 19.6 ± 9.1. Of the 30 patients with iliac injuries, the associated hollow visceral injuries were as follows: 13 isolated small bowel (SB), 7 isolated colon, 10 combined SB and colon injuries, and zero gastric wounds.
Ten patients with iliac injuries developed abdominal abscesses of which 2 had isolated colon, 3 had SB, and 5 had combined colon and SB wounds. Colon injury was predictive of abscess, p=0.0351 (O.R.=30.0, C.I. 2.7-328.6); however, method of colon injury management was not significant. The presence of an associated SB injury in the setting of iliac injury was not predictive of abscess, p=0.081 (O.R. = 4.7, C.I. 0.8-27.1). Likewise, combined colon and SB injuries were not predictive of abscess, p=0.5228 (O.R.=2.6, C.I. 0.144-453.7). Iliac vessel ligation, direct repair, or interposition grafting did not predict abscess, nor did perioperative antibiotics, pelvic closed suction drains, intraoperative temperature or transfusion requirements. Six patients with iliac artery injuries developed delayed postoperative hemorrhage from their repair site, and 5 of 6 had an abscess. Three patients developed iliac artery fistulae, and 3 patients bled from iliac pseudoaneursyms. The presence of abdominal abscess predicted delayed hemorrhage, p=0.0054 (O.R.=30.0, C.I. 2.7-328.6).

Discussion: The presence of an associated colon injury increases the risk of abdominal abscess in patients with penetrating iliac injuries. Patients with abdominal abscess following hollow viscous and iliac repair were 30 times as likely to hemorrhage from the repaired iliac arteries. Our study suggests that additional measures should be undertaken to prevent abdominal abscess formation in this patient population. Consideration towards exclusion of the iliac vascular repair from the hollow viscous injury should be made. Further investigation is warranted to determine which method may be most effective.

Poster Presentations

An Evaluation of Invasive Breast Cancer Presenting with Microcalcifications on Initial Mammogram: is this a prognostic indicator of tumor aggressiveness?

Brown, A, Thanasoulis L, Frazier T.

Bryn Mawr Hospital, Bryn Mawr, PA

Trading Cases For Better Academic Performance? The 80-Hour Work Week In A Non-University Program

Sree Suryadevara, MD; Christopher Pezzi, MD; Thomas Leibrandt, MA; Diane Opatt, MD; John Kukora, MD

Department of Surgery, Abington Memorial Hospital, Abington, Pennsylvania

Targeting of MYC-oncogene Inhibits Breast Cancer Molecular Markers and Growth Parameters *In Vitro and In Vivo* in a Chick Embryo Model for Tumor Growth

Beth A. Rymeski[1,2], Deni S. Galileo[3], Valerie B. Sampson[1], Nancy H. Rong[1], Virginie Aris[4], Patricia Soteropoulos[4], Nicholas J. Petrelli[5], Stephen P. Dunn[1], and Leslie J. Krueger[1]

[1]Department of Molecular Genetics, Cellular and Tissue Transplantation, Alfred I. duPont Hospital for Children, Wilmington, DE. 19803; [2]Department of Surgery, Christiana Care Health System, Newark, DE 19713; [3]Department of Biological Sciences, University of Delaware, Newark, DE 19716, [4]Center for Applied Genomics, Public Health Research Institute, of UMDNJ-New Jersey Medical School, International Center for Public Health, Newark, NJ 07103. [5]Helen F. Graham Cancer Center, Christiana Care Health System, Newark, DE. 19713

Manipulation of Angiogenesis and Mucosal Cell Proliferation: A Novel Approach to Altering the Pathophysiology and Possible Treatment of Inflammatory Bowel Disease(IBD)

Edward Y. Yoo, M.D.*, Duane Duke M.D.$^{\infty}$, Marian Haber M.D.*, Marshall Z. Schwartz M.D.*$^{\blacktriangle}$

*Drexel University College of Medicine, $^{\infty}$Walter Reed Medical Center, $^{\blacktriangle}$St. Christopher's Hospital for Children

Single Surgeon Surgery: 3 Trocar Technique For Colon Resection

Elsa B Valsdottir MD, Gabriel del Corral MD, John H Marks MD, Shadi Yarandi MD, David Newman MS, Gerald Marks MD,

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A Comparison of 3hr and 6 hr Chest Radiographs for Earlier Identification of Complications from Penetrating Chest Trauma in Asymptomatic Patients".

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Brown, A, Thanasoulis L, Frazier T.

Bryn Mawr Hospital, Bryn Mawr, PA

Introduction: To determine if microcalcifications on mammograpm were prognostic of tumor aggressiveness or an indicator of slower tumors possibly evolving from an in-situ component.

Methods: 93 consecutive patients from 2005-2006 with a diagnosis of invasive breast cancer were divided according to presence or absence of microcalcifications on their presenting mammogram.

Results: 28 patients (30.1%) had microcalcifications (Ca++) and 65 patients (69.9%) had no microcalcifications (no Ca++). 1 pt with Ca++ had multifocal disease, accounting for 29 cancers. 7 pts with no Ca++ had multifocal disease, with 1 pt having bilateral breast cancer, accounting for 73 breast cancers. Patients were then evaluated according to: tumor size/ node status/ stage/ estrogen receptor (ER)/ progesterone receptor (PR)/ HER-2/ Lymphovascular invasion (LVI), as well as, tumor grade with invasive component. In the Ca++ group, 7 pts (25.0%) had invasive lobular CA and 21 pts (75.0%) had invasive ductal CA; 8 (28.6%) well differentiated, 7 (25.0%) moderately differentiated, and 6 (21.4%) poorly differentiated CA. In the no Ca++ group, 10 pts (15.2%) had lobular CA, while 56 (84.8%) had ductal CA; 32 (48.4%) well differentiated, 12 (18.2%) moderately differentiated, and 12 (18.2%) poorly differentiated CA. Node status: in Ca++ group, 11 (39.3%) were positive and 17 (60.7%) were negative; the no Ca++ group, 18 (27.3%) were positive and 48 (72.7%) were negative. Stage at diagnosis: in Ca++ group, 14 (50.0%) were Stage I, 11 (39.3%) were Stage II, 3 (10.7%) were Stage III; in no Ca++ group, 37 (56.1%) were Stage I, 25 (37.9%) were Stage II, and 4 (6.0%) were Stage III. Tumor grade at diagnosis: in Ca++ group, 10 (34.5%) were Grade I, 10 (34.5%) were Grade II, and 9 (31.0%) were Grade III; in no Ca++ group, 34 (46.6%) were Grade I, 25 (34.6%) were Grade II, and 14 (19.2%) were Grade III. Tumor marker analysis: in Ca++ group, ER+- 19 (65.5%), PR+ - 12 (41.4%), HER-2 – 12 (41.4%), and LVI – 7 (24.1%); in no Ca++ group, ER+ - 60 (82.2%), PR+ - 36 (49.3%), HER-2 -4 (5.5%), and LVI- 14 (19.2%).

Conclusion: We conclude that invasive breast CA that presents with microcalcifications on mammogram may be diagnosed at a later stage, higher grade, likely to be node +, less likely to be hormone +, and likely to be HER-2 amplified. As such, microcalcifications on mammogram may require prompt diagnostic evaluation for definitive diagnosis, preferably with a noninvasive technique (i.e. stereotactic biopsy).

Trading Cases For Better Academic Performance? The 80-Hour Work Week In A Non-University Program

Sree Suryadevara, MD; Christopher Pezzi, MD; Thomas Leibrandt, MA; Diane Opatt, MD; John Kukora, MD

Department of Surgery, Abington Memorial Hospital, Abington, Pennsylvania

Purpose: To assess the impact of implementation of work hours restrictions by the Accreditation Council for Graduate Medical Education (ACGME) on resident operative experience, as represented by resident operative logs, and on educational experience, as represented by the American Board of Surgery In-Training Examination (ABSITE). Board passage rates were also analyzed pre- and post-work hour restrictions to determine if there were any significant changes.

Methods: Data were collected retrospectively from ABSITE scores, ACGME operative logs, and American Board of Surgery first-time taker board passage rates for graduating chief residents of our non-university program from 2001 to 2006. Data for chief residents who graduated in 2001, 2002, and 2003 (prior to implementation of work hour restrictions) were compared with data for residents who graduated in 2004, 2005, and 2006. In each of these years, three chief residents graduated, except for 2001 when two chief residents graduated. Data were analyzed using the un-paired t-test to determine statistical significance.

Results: The mean number of cases for graduating chief residents prior to July 2003 was 1098.5 and decreased to 932.2 after July 2003 ($p=0.0098$). The mean percentile on the ABSITE increased from 38.0 prior to the 80-hour work week to 62.8 after implementation of work hour restrictions ($p=0.0773$). The mean percentage of correct answers on the ABSITE also increased from 73.4 to 74.9 ($p=0.5855$). The mean board passage rate for the qualifying examination increased from 83.3% prior to July 2003 to 100% after July 2003 ($p=0.3739$). The mean board passage rate for the certifying examination increased from 78.0% before July 2003 to 100% after July 2003 ($p=0.1161$). The only failures for first-time examinees, on both the qualifying and certifying examinations, occurred before implementation of the 80-hour work week.

Conclusion: The residents' operative experience was reduced by 15% after implementation of the 80-hour work week, which was significant. A trend toward increased academic performance as reflected by higher ABSITE scores and first-time taker board passage rates did not reach statistical significance. Reduced work hours clearly decreased the number of operative cases, but may have a positive impact on resident academic performance.

Targeting of MYC-oncogene Inhibits Breast Cancer Molecular Markers and Growth Parameters *In Vitro and In Vivo* in a Chick Embryo Model for Tumor Growth

Beth A. Rymeski[1,2], Deni S. Galileo[3], Valerie B. Sampson[1], Nancy H. Rong[1], Virginie Aris[4], Patricia Soteropoulos[4], Nicholas J. Petrelli[5], Stephen P. Dunn[1], and Leslie J. Krueger[1]

[1]Department of Molecular Genetics, Cellular and Tissue Transplantation, Alfred I. duPont Hospital for Children, Wilmington, DE. 19803; [2]Department of Surgery, Christiana Care Health System, Newark, DE 19713; [3]Department of Biological Sciences, University of Delaware, Newark, DE 19716, [4]Center for Applied Genomics, Public Health Research Institute, of UMDNJ-New Jersey Medical School, International Center for Public Health, Newark, NJ 07103. [5]Helen F. Graham Cancer Center, Christiana Care Health System, Newark, DE. 19713

Introduction: Dysregulation of MYC oncogene is commonly found in breast cancer patients resulting in more frequent relapse and poorer survival. Using 10058-F4, a small molecular weight drug that inhibits MYC-MAX transcription, we studied two-breast cancer lines differing in metastatic potential.

Methods: MDA-MB-231 and -468 was studied using a MTT assay in drug-exposed cells. In silico analysis of gene expression results on Affymetrix microarrays was compared in these 2 lines. Real time Taqman-MGB technology PCR evaluated gene specific expression in exposed cells and extent of invasive and metastatic breast cancer cell colonization in our chick embryo *in vivo* model. Tumor localization in the chick brain, liver and lung was determined by histochemical and lacZ staining. G418 selection determined explanted surviving cancer colonies.

Results: 12,000 gene expression levels were compared using robust multi-array analysis (RMA) and only 6 genes showed either a 2-fold increase - decrease (231/468). i.e., LDH-B, PRG1, AXL, unknown; (up) and FOXA1 and RARRES1; (down). These changes may identify key pathways involved in the divergent phenotypes (Table I). Triplicate PCR analysis of MYC showed a 3.5-fold relative increase in 468 over 231 cells. Cells exposed to drug showed decreased cell growth (range 62-93 uM compound, $p \leq 0.05$) by MTT in replicates e.g., control absorbance (562 nm) of 2.5+/-0.25 and 1.13+/- 0.08 and exposed (93 uM) of 1.43 +/-0.12 and 0.87+/-0.05, respectively, for 468 and 231 cells. 468 cells were 2.4-fold more sensitive to drug at 93 uM. This confirms results in Burkitt lymphoma where higher MYC lines showed a paradoxical greater response to MYC inhibition. Effects on MYC-MAX downstream targets were shown by PCR and independently confirmed in MYC-targeted siRNA results. Baseline colonization by breast cancer cells also was determined in the chick embryo model for comparison to ongoing experiments on the injection of drug treated breast cancer cells.

Conclusions: We demonstrate down regulation of MYC results in an altered phenotype of metastatic and non-metastatic breast cancer lines *in vitro* and *in vivo*.

Manipulation of Angiogenesis and Mucosal Cell Proliferation: A Novel Approach to Altering the Pathophysiology and Possible Treatment of Inflammatory Bowel Disease(IBD)

Edward Y. Yoo, M.D.*, Duane Duke M.D.[∝], Marian Haber M.D.*, Marshall Z. Schwartz M.D.*[♠]

*Drexel University College of Medicine, [∝]Walter Reed Medical Center, [♠]St. Christopher's Hospital for Children

Purpose: Alteration of gastrointestinal microvasculature angiogenesis and/or epithelial cell proliferation (by intestinal growth factors) could provide unique insights into the role that angiogenesis and epithelial cell proliferation play in IBD. This study was designed to assess the role of these different physiologic processes on IBD.

Methods: Female transgenetic HLA-B27 Fisher rats (a rat immunologic model of IBD) were divided into three groups: Group1; saline(control), N=6; Group 2; vascular endothelial growth factor($VEGF_{165}$), 50μg/kg/day, N=10; and Group 3; epidermal growth factor(EGF), 250 μg/kg/day, N=10. The saline, VEGF, and EGF were delivered via a catheter in the jugular vein connected to an osmotic minipump for 14 days and the rats were euthanized. Intestinal microvascular density (MVD), weight, histologic assessment of bowel inflammation and intestinal mucosal TNF-α were evaluated. The Student's *t* test (p-values<0.05) was used to determine statistical significance. IACUC approval #15978.

Results: Both the EGF and VEGF groups had a significant increase in MVD over the control group (50% and 40% respectively, p-value<0.05). Animals given VEGF showed increased inflammatory cell infiltration in the colon (8.1 cells/HPF ±0.97 SEM) compared to control animals (4 cells/HPF ± 1.34 SEM). Colon and ileum histology and TNF-α expression in the VEGF group revealed no improvement to inflammation/injury compared with the control group. The EGF group showed a decreased histological score (p=0.08) and colonic TNF-α expression (p=0.07). The EGF group had less weight loss than the control group but it was not significant.

Conclusion: Although VEGF and EGF enhanced intestinal angiogenesis (increased MVD), the histologic and cytokine responses to inflammation appear to be different. The response to direct angiogenesis stimulation (VEGF) was adverse whereas the response to the intestinal growth factor (EGF) appears favorable. Additional studies focusing on the physiologic pathways and systemic effects of these agents may provide insight into the pathophysiology and treatment of IBD.

Single Surgeon Surgery: 3 Trocar Technique For Colon Resection

Elsa B Valsdottir MD, Gabriel del Corral MD, John H Marks MD, Shadi Yarandi MD, David Newman MS, Gerald Marks MD,

Lankenau Hospital and Institute for Medical Research

Introduction: Laparoscopic colon resection has been demonstrated to be safe and feasible. Despite proven patient benefits, adoption of the technique has been slow. A significant hurdle for adoption has been the technical challenge and need for 1 or 2 (highly) skilled assistants. We present our experience with a single surgeon, 3 trocar technique for colon resection to evaluate its feasibility and safety.

Methods: Since 1996 all patients undergoing laparoscopic colon resections have had their data prospectively entered into a database. The database was queried for all patients undergoing colon resection from the beginning of adoption of this technique in 03/99 to 07/07. Data regarding patient demographics, perioperative course, laparoscopic completion rates, early and late morbidity and mortality are reported. Results: 742 laparoscopic colon resections (LCR) were performed. The use of 3 trocar technique became prevalent in 07/00. Prior to that time, 7 of 114 laparoscopic colon resections were done with 3 trocars. After that time, 336 of 628 LCR were done with 3 trocars (54%). Of the 343 patients treated with 3 trocars, 185 were women, average age was 63 years (19-92 y.o.). 186 patients (54%) had BMI >25. Surgeries included left colectomies (62%), right colectomies (33%), APRs (2%), total colectomies (2%) and segmental resection (2%). Indications were cancer (36%), diverticulitis (30%) and polyps (20%). 3% were converted to an open procedure, most commonly due to inflammatory process or tumor involvement. Return of bowel function (flatus) was 2.1 days (1-25 days) and length of stay was 5.9 days (1-27 days). There was one mortality, from a postop MI. Major morbidity rate was 8% with overall morbidity rate of 17%. Anastomotic leaks were 1.5%.

Conclusion: Single surgeon laparoscopic colon resection using a 3 trocar technique is a safe and reliable approach for a variety of pathology. It can be used with good outcome and low conversion rates in various anatomical location and indication. This technique can be performed by only a skilled laparoscopic surgeon and a camera operator and holds promise for rural surgeons or those in single practice interested in adopting laparoscopic colon resection.

A Comparison of 3hr and 6 hr Chest Radiographs for Earlier Identification of Complications from Penetrating Chest Trauma in Asymptomatic Patients".

Carlos R. Medina, et. al.

Department of Surgery, Temple University

Background: Asymptomatic, penetrating thoracic injury is relatively common in urban trauma centers. Despite hospital presentation without signs or symptoms of intrathoracic injury, delayed, potentially life-threatening injuries such as pneumothorax (PTX) may present several hours after initial trauma assessments in up to 12% of patients. Furthermore, the majority of these delayed injuries will require timely intervention such as tube thoracostomy.

Currently, patients with asymptomatic penetrating chest trauma are evaluated for delayed pneumothorax with serial chest radiographs (CXR) upon arrival and then 6 hours later. We hypothesized that delayed pneumothorax or hemothorax after asymptomatic thoracic injury would be detectable after only 3 hours of observation.

Methods: A prospective evaluation of all patients with asymptomatic penetrating thoracic injuries over a 29-month period in our urban, level-1 trauma center enrolled 127 patients. Asymptomatic patients without pneumothorax or hemothorax on the initial CXR (supine AP) underwent observation and serial CXR at 3 and 6 hours (erect PA/lateral). Complications during the observation period were treated as clinically indicated, and the asymptomatic patients were discharged home after all three CXR (0 hour, 3 hour and 6 hour). Findings from 3-hour and 6-hour CXRs were compared. Assuming a delayed PTX or HTX rate of 3%, the exact binomial probability of detecting *at least one* delayed event between 3 and 6 hours in 100 patients is 95.25%.

Results: Of 648 patients with penetrating thoracic injuries, 100 patients met inclusion criteria and completed all facets of the study. Patients were predominantly young (32.5±13.3 years [mean±SD]) males (75% male) with stab wounds (75% stab wounds, 25% gunshot wounds). In total, the 100 patients had 148 thoracic wounds. Of these, 48% were chest wounds and 52% were back wounds. Thirty-three patients had multiple thoracic wounds. Nineteen of 100 patients were admitted for various injuries and 81 patients were discharged from the ED. The mean length of stay for patients discharged from the ED was 8.8±2.6 hours.

While two patients developed a PTX between arrival and 3 hours, no patient developed a PTX between 3 and 6 hours. Both patients with delayed PTX were admitted and treated by tube thoracostomy. No patient in this series developed a delayed HTX.

Conclusion: No patient in our study population developed a delayed PTX or HTX after 3 hours. Our results suggest that shortening the observation period after asymptomatic penetrating thoracic injuries to 3 hours is safe, cost-effective, allows earlier therapeutic intervention when needed, and may help relieve congested urban emergency departments.