LABORATORY INVESTIGATION
USCAP AN OFFICIAL JOURNAL OF THE UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY

Volume 94, Issue S1 (February 2014)

Title: HER2 M-Scores in Gastric/Gastroesophageal Junction Adenocarcinomas: Comparison of Digital Image Analysis and Pathologist Interpretation

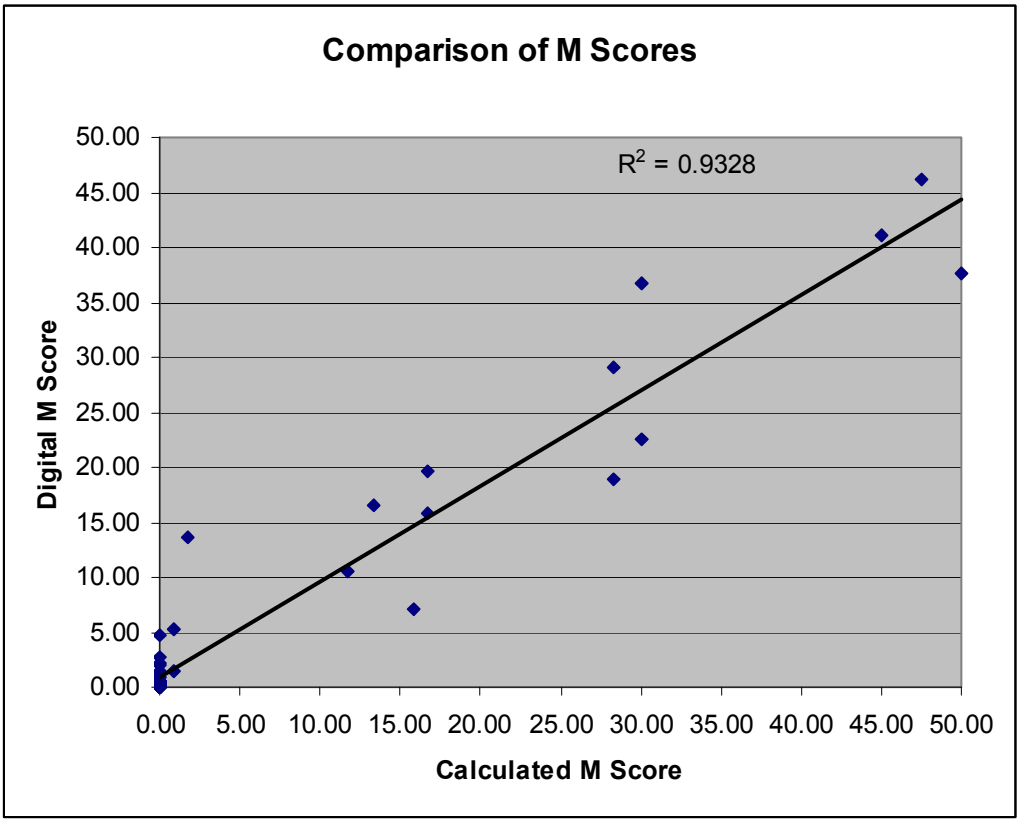
Authors: Christa L. Whitney-Miller, Brooke R. Koltz, Chanan Sluszny, David G. Hicks, Aaron R. Huber

Background: Approximately 20% of gastric and gastroesophageal junction (GEJ) adenocarcinomas are HER2 positive (3+ overexpression by immunohistochemistry [IHC] or gene amplification by in situ hybridization [ISH]). In the ToGA trial, the addition of trastuzumab was shown to prolong survival in patients with advanced gastric and GEJ carcinomas. Prior studies have examined HER2 IHC scoring using digital image analysis (DIA) and some have implemented H-scores in their evaluation. The M-score is a quantitative measure of membrane staining with a calculated range of 0 to 50. We investigated the potential utility of a commercially available image analysis platform (IAP) in determining M-scores for HER2 expression in gastric/GEJ adenocarcinomas.

Design: 46 cases (29 biopsies and 17 resections) were reviewed from which 65 blocks were selected for IHC staining using the commercially available Hercep Test (DAKO). One pathologist estimated the M-score. Targeted DIA was then performed on fields selected by a second pathologist using Go-Path GenASIs (Applied Spectral Imaging) which generated a separate M-score. DIA was performed by an independent third party familiar with the instrument and software; the pathologists were uninvolved and blinded during DIA. When the pathologist derived and IAP derived M-scores were compared there were 9 very disparate cases. Review of those slides showed non-specific staining within tumor or crush artifact. These 9 cases were removed from the analysis. The M-scores were compared using linear regression analysis.

Results: The semiquantitative M-score obtained by the pathologist showed good agreement with the M-score determined by digital image analysis (CC 0.9328).

Conclusion: The M-score calculated by the pathologist is comparable to that done by digital image analysis. Areas of crush artifact and non-specific staining contributed to disparate results between image analysis and the pathologist interpretation and therefore careful selection of fields for analysis is important to help ensure the accuracy of the computer-assisted scoring.


Comparison of M Scores
R^2 = 0.9328
Digital M Score
Calculated M Score
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00

Conclusions: A precursor non-dysplastic serrated polyp was present in 22% of TSAs in which background mucosa was present in the polypectomy sample. 92% of TSAs showed concordant *BRAF/KRAS* mutation genotype in both components, which suggests that a significant subset of TSA arise from precursor non-dysplastic serrated polyps.

851 Reduced Membranous Expression of EpCAM-ICD Correlates with Poor Patient Outcome in Primary Colorectal Adenocarcinoma

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Background: Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein involved in cell adhesion, signaling, migration, proliferation and differentiation. It is known to be expressed in normal epithelium and epithelial neoplasms. Altered EpCAM expression correlates with aggressive biological behavior in gastric, breast, renal and thyroid carcinomas. Recent studies have proposed the proteolytic cleavage of the intracellular domain of EpCAM (EpCAM-ICD) triggers a signalling cascade leading to the activation of the Wnt/beta-catenin pathway and aggressive tumour behavior. The expression profile and prognostic value of EpCAM-ICD have not been elucidated for primary colorectal carcinoma. In this study, we examined EpCAM-ICD expression in a large cohort of primary colorectal adenocarcinoma, and assessed its role as a potential prognostic marker and therapeutic target.
Design: EpCAM-ICD immunohistochemical expression was assessed in 137 primary colorectal adenocarcinoma resected in our institution between 2007 and 2008 using tissue microarrays. The presence and intensity of EpCAM-ICD membranous staining was independently scored by 3 pathologists. Patient chart review was performed for a wide range of clinicopathological parameters and correlated with the average staining score by the Pearson correlation coefficients, Mann-Whitney U-tests and two-tailed T-test.
Results: The membranous EpCAM-ICD staining was calculated as a weighted average, based on results from three core samples per tumour. EpCAM-ICD expression levels were positively associated with well (versus poorly) differentiated tumours (n=18; p=0.05), low preoperative serum carcinoembryonic antigen (n=76; p=0.0002), and 5 year survival (n=128; p=0.01). The presence of perineural invasion and macroperforation were associated with lower EpCAM-ICD staining scores, but small sample numbers precluded statistical analysis of these results.
Conclusions: Our study findings demonstrate that reduced EpCAM-ICD membranous expression may be a useful marker to identify tumours with aggressive clinical behavior and poor prognosis. Also, EpCAM-ICD expression in primary colorectal carcinoma may serve as a useful tool for defining a subgroup of patients who could benefit from targeted immunotherapy against the EpCAM antigen.

852 NIK- and IKK2-Binding Protein: A Novel Neuroendocrine Marker That Labels Cells with Unique Pattern of Distribution in Gastrointestinal Tract and Is Useful in Diagnosis of Neuroendocrine Tumor of the Rectum

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Background: NIK- and IKK2-binding protein (NIBP) is a key member of trafficking protein particle (TRAPP) complex II involved in trans-Golgi networking, and the maturation of neuroendocrine cells (NECs). Its overexpression has been associated with poor prognosis in patients with gastrointestinal (GI) malignancies. However, the distribution of NIBP expressing cells has not been studied in human GI tract and the value of NIBP in assisting the diagnosis of GI neuroendocrine tumors (NETs) is unknown.
Design: Immunohistochemistry (IHC) for NIBP was performed on normal tissues of excisional specimens from 10 esophagus, 10 stomach, 10 small intestine, 10 colon, 5 pancreas, and 25 cases of rectal NETs using one or two anti-NIBP antibodies (NP417 and TPPC9). The NETs were also stained with synaptophysin.
Results: NECs with NIBP expression were identified by NP417 in stomach, small intestine and colon with their numbers increased gradually from stomach to colon. These cells were predominantly located at the deep crypt of mucosal epithelium with a granular cytoplasmic staining pattern. Neurons and NECs in the muscularis propria failed to demonstrate NIBP immunoreactivity; and no NIBP expression was identified in the NECs of esophagus. Islet cells of pancreas were strongly positive for NIBP. Besides the NECs, plasmas cells, neutrophils and some mucinous cells in the GI tract were also stained weakly. 17 of 25 (68%) rectal NETs displayed cytoplasmic staining of NP417, and 19 of 25 (76%) were positive for TPPC9. Synaptophysin was positive in 21 of 25 NETs (84%). It was noticed that 4 synaptophysin-negative NETs demonstrated moderate to strong positivity for TPPC9, and 2 of which were also positive for NP417. By a combination of NIBP and synaptophysin IHC, the NETs were properly identified with a sensitivity of 100%.
Conclusions: NIBP is selectively expressed in a subset of NECs of GI tract and may serve as a novel neuroendocrine marker in identifying the NETs that fail to express synaptophysin. Additional investigation with a larger sample size is warranted to validate the findings and to further explore the clinical utility of NIBP immunostaining. The function of the subset of NIBP positive NECs also deserves further study.

853 Clinicopathologic Features and Frequency of *KRAS* Mutation in Colorectal Adenocarcinoma in Patients ≤ 40 Years of Age

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Background: Colorectal cancer (CRC) in patients ≤ 40 years (yr) of age was thought to be rare, and frequently arising in predisposing conditions such as Lynch syndrome, hereditary polyposis syndromes, or inflammatory bowel disease, and associated with poor outcome. Recent studies have reported complicated and contradictory epidemiology, histology, genetics, and outcomes in these patients. The purpose of this study is to characterize the epidemiological, pathological, and molecular features of CRC in patients ≤ 40yr of age in our institution.
Design: Clinical and pathological records from 100 consecutive CRC patients ≤ 40yr of age at the time of diagnosis between 2006 and 2012 were reviewed. Clinical information obtained included age, gender, family history, predisposing factors, anatomic tumor site, stage, treatment, and outcome. Histological review included tumor grade, morphological features, extent of invasion, lymph node and distant metastases. Mismatch protein repair and *KRAS* mutation status were also obtained where available.
Results: In our cohort, the mean age at diagnosis was 33.9yr (range 18-40); 51% were male, 80% Caucasian and 17% African-American. Twelve percent had a first degree relative with CRC, while 40% had a family history of CRC in any relative. Only 16% of patients had a predisposing condition. Most cases presented in the left colon (63%), with 56% located in the rectosigmoid colon. Majority (69%) of the cases presented with stage III or IV disease, with 34% showing distant metastasis at presentation. Histologically, all tumors were adenocarcinomas, of which 22% had a mucinous component and 5% showed signet ring morphology. *KRAS* mutational analysis was performed in 45 patients, and among which, 57.8% harbored a *KRAS* mutation. MSI testing was performed in 46 patients; only 6 tumors (13%) showed high microsatellite instability (MSI). The median overall and recurrence-free survival was 27.9 and 16.9 months, respectively, with a mean duration of follow up of 35 months.
Conclusions: Our study showed that the majority of early onset CRC cases were not associated with predisposing conditions. We confirmed that most of these patients presented with tumors in the left colon and with late stage disease, and were rarely MSI-high. However, in contrast to recent studies where only a minority of these patients were shown to harbor activating *KRAS* mutations, the majority of patients in our cohort were positive for *KRAS* mutation. This finding is important for therapeutic and prognostic considerations, and emphasizes the importance of *KRAS* testing in younger patients with CRC.

854 Immunohistochemical and Molecular Evaluation of *BRAF* Mutations in Tumors of the Serrated Neoplastic Pathway

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Background: Sessile serrated polyps (SSPs) frequently harbor *BRAF* mutations and are potential precursors to colonic adenocarcinomas with MSI-H phenotype. Our previous sequencing analysis of SSPs revealed that *BRAF*^{V600E} mutations often involved a minority of cells within mutation-positive polyps, but the distribution of cells harboring these mutations within the lesion is unknown. In this study, we evaluated *BRAF*^{V600E} mutational status by sequencing and immunohistochemistry in invasive adenocarcinomas and SSPs with and without cytologic dysplasia in order to determine the correlation between molecular and immunohistochemical testing for *BRAF* ^{V600E} mutations and assess the distribution of *BRAF*^{V600E} mutated cells in preinvasive lesions.
Design: Sequencing assays for *BRAF*^{V600E} mutations were performed on 26 selected invasive adenocarcinomas, 39 SSPs, and 7 SSPs with dysplasia. All cases were stained with an antibody against mutated BRAF (Spring Bioscience, clone VE1) and reviewed in a blinded fashion. Cytoplasmic staining of neoplastic cells was considered a positive result. Staining was scored as either focal (≤ 50% of cells) or diffuse (>50% of cells) and intensity was scored from 0 to 4+.
Results: There were 16 *BRAF* mutated (100% MSI-H) and 10 *BRAF* wild-type (80% MSS, 20% MSI-H) invasive adenocarcinomas in the study group. Immunostains for BRAF^{V600E} were positive in 15 (94%) *BRAF* mutated carcinomas. Of these, 14 showed a 2+/4+ staining reaction in a diffuse (n=13) or focal (n=1) distribution and one displayed diffuse 1+/4+ staining. All *BRAF* wild-type tumors were negative for this marker (sensitivity: 94%, specificity: 100%). Sequencing assays detected *BRAF* mutations in 37 (95%) SSPs and 6 (86%) SSPs with dysplasia. Immunohistochemistry revealed 1+/4+ focal cytoplasmic staining for BRAF^{V600E} in only 3 of 43 cases, all without dysplasia. The staining was too focal in these cases to assess the distribution of positive cells in these lesions.
Conclusions: Immunohistochemical analysis of *BRAF*^{V600E} has a high correlation with sequencing assays in invasive colonic adenocarcinomas. However, the level of mutated BRAF protein in SSPs was below the detection threshold of this BRAF^{V600E} antibody in our analysis. Immunostaining with the currently available antibody is not a sensitive marker of *BRAF*^{V600E} mutations in serrated colonic polyps.

855 HER2 M-Scores in Gastric/Gastroesophageal Junction Adenocarcinomas: Comparison of Digital Image Analysis and Pathologist Interpretation

CL Whitney-Miller, BR Koltz, C Sluszny, DG Hicks, AR Huber. University of Rochester Medical Center, Rochester, NY; Rochester General Hospital, Rochester, NY; Applied Spectral Imaging, Ltd, Carlsbad, CA.

Background: Approximately 20% of gastric and gastroesophageal junction (GEJ) adenocarcinomas are HER2 positive (3+ overexpression by immunohistochemistry [IHC] or gene amplification by in situ hybridization [ISH]). In the ToGA trial, the addition of trastuzumab was shown to prolong survival in patients with advanced gastric and GEJ carcinomas. Prior studies have examined HER2 IHC scoring using digital image analysis (DIA) and some have implemented H-scores in their evaluation. The M-score is a quantitative measure of membrane staining with a calculated range of 0 to 50. We investigated the potential utility of a commercially available image analysis platform (IAP) in determining M-scores for HER2 expression in gastric/GEJ adenocarcinomas.
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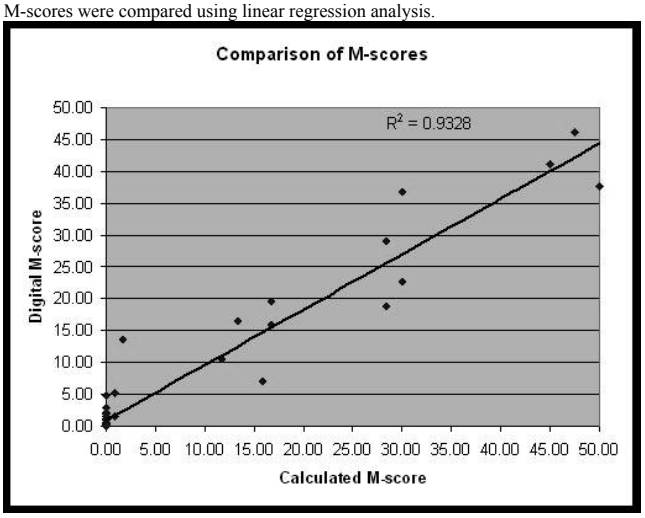
which generated a separate M-score. DIA was performed by an independent third party familiar with the instrument and software; the pathologists were uninvolved and blinded during DIA. When the pathologist derived and IAP derived M-scores were compared there were 9 very disparate cases. Review of those slides showed non-specific staining within tumor or crush artifact. These 9 cases were removed from the analysis. The M-scores were compared using linear regression analysis.



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856 Traditional Serrated Adenomas: A Morphologic, Immunohistochemical, and Molecular Assessment

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Background: Traditional serrated adenomas (TSAs) are rare colorectal polyps with malignant potential. Previous studies have suggested that TSAs may arise via a non-dysplastic serrated precursor lesion, and can harbor either BRAF or KRAS mutations.
Design: We studied 56 left-sided TSAs diagnosed at a single institution between 2009-2013. Five subspecialty GI pathologists assessed each polyp for the following features: cytoplasmic eosinophilia, ectopic crypt foci, a non-dysplastic serrated precursor, and conventional dysplasia. Interobserver agreement was measured using kappa statistics. A subset of polyps were assessed for BRAF/KRAS mutations, CpG island methylation (CIMP), and annexin A10 (ANXA10) expression (a protein highly expressed in sessile serrated polyps (SSPs)).
Results: Upon review, 55/56 (98%) polyps had a consensus diagnosis of TSA. All polyps had cytoplasmic eosinophilia while 34/56 (61%) had ectopic crypts. Non-dysplastic serrated precursors were identified in 13 polyps (21%; 6 HP, 3 SSP, 4 unclassified) with a moderate level of interobserver agreement (kappa: 0.47). Conventional dysplasia was identified in 17 polyps (30%; 15 low grade, 1 carcinoma, 1 indeterminate grade) with a moderate level of interobserver agreement (kappa: 0.46). Only 2 polyps had ANXA10 expression in greater than 50% of serrated crypts. Twenty-five (25/53; 47%) polyps harbored a BRAF mutation, 23/53 (43%) harbored a KRAS mutation, and 5/53 (9%) polyps were wild type for both. CIMP was tested in 33 polyps and 7 (21%) were CIMP-high, 13 (39%) were CIMP-low, and 13 (39%) were CIMP negative. Upon multivariate analysis, there were no significant associations between clinical and morphologic features of TSAs and their molecular features. However, all 3 TSAs which harbored a precursor SSP demonstrated a BRAF mutation. Additionally, BRAF mutated TSAs and KRAS mutated TSAs showed different methylation patterns at the RUNX3 locus upon CIMP analysis (p=0.018).
Conclusions: TSAs appear to arise in the left colon via a non-dysplastic serrated precursor, and have potential to progress to conventional dysplasia and carcinoma. We found a moderate level of agreement in the recognition of precursor lesions and conventional dysplasia in TSAs. Although TSAs may harbor BRAF or KRAS mutations, these mutations are not associated with clinical or morphologic features; however, patterns of CpG methylation are different.

857 Expression and Clinical Significance of EGFR, IGF-1R and HER-2 in Patients with Ampullary Adenocarcinoma

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Background: EGFR, IGF-1R and HER2 have been shown to play an important role in the pathogenesis of human malignancies and have been used as markers for targeted therapies for cancers. However their expression and role in ampullary adenocarcinoma (AA) has not been examined in detail.
Design: We retrospectively reviewed 106 cases of AA at our institution. Tissue microarrays were constructed using the formalin fixed paraffin embedded tissue with three 1.0 mm cores from representative areas of each tumor. Immunohistochemical stains for EGFR, IGF-1R and HER-2 were performed on 4.0 μm unstained slides from tissue microarrays. The staining results were evaluated for membranous staining using the modified HercepTest for gastrointestinal cancers independently by two pathologists. The results were correlated with the clinicopathologic parameters and survival.
Results: Strong (3+) expression of EGFR, IGF1R and Her2 was detected in 18 (17%), 26 (25%) and 0 (0%) AAs respectively. Overexpression of EGFR correlated with poorer overall survival (mean survival: 109.8 ± 22.3 months in EGFR high group vs 164.2 ± 10.6 months in EGFR low group, P=0.04). In multivariate analysis, EGFR overexpression is an independent prognostic factor for overall survival (P=0.03). However, no significant correlation between EGFR expression and other clinicopathologic factors were identified (P>0.05). No correlation between IGF-1R or Her-2 expression and survival or other clinicopathologic factors were observed in our patient population.
Conclusions: Our study showed that EGFR and IGF1R, but not Her2 are overexpressed in a subset of AAs. Strong membranous expression of EGFR is an independent predictor for overall survival in patients with AA.

858 Clinical Significance of Beta-Catenin and E-Cadherin Expression in a Large Cohort of Patients with Gastric Cancer

MD Xia, Y Xie, S Lee, D Tan. University of Texas Medical Branch, Galveston, TX; University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Gastric cancer is a major health issue and is the second leading cause of death worldwide. Both beta-catenin and e-cadherin are adhesion molecules that have been shown to promote metastatic potential through epithelial-mesenchymal transition (EMT). The goal of this study is to explore relationship between the immunohistochemical expressions of these EMT markers with clinicopathologic parameters in patients with gastric cancer.
Design: We retrospectively reviewed 205 cases of gastric cancer that were previously diagnosed at University of Texas MD Anderson Cancer Center. Tissue microarrays were constructed using the formalin fixed paraffin embedded tissue with three 1.0 mm cores from representative areas of each tumor. The slides were stained with beta-catenin and e-cadherin and evaluated for membranous staining by H-score (intensity multiplied by percentage of tumor cells stained), modified HercepTest for gastric cancer and percentage of loss of membranous (LOM) staining. The results were analyzed using Chi-square, general linear model, and logistic regression model.
Results: Decreased membranous expression of e-cadherin and beta-catenin is correlated with worse overall survival ($p < 0.05$). In addition, loss of membranous staining of beta-catenin is also correlated with poorer overall survival ($p = 0.04$). Loss or decreased membranous staining for both e-cadherin and beta-catenin is significantly correlated with poorly differentiated tumors ($p<0.05$). Loss of membranous staining in beta-catenin was associated with a diffuse Lauren-type (versus intestinal, p=0.016) whereas a strong membranous staining (3+) with beta-catenin had a significant lower rate of lymphovascular invasion (p= 0.011). Moreover, strong (3+) membranous staining of both e-cadherin and beta-catenin is associated with H.pylori infection ($p<0.05$).
Conclusions: Loss or decreased membranous expression of both e-cadherin and beta-catenin show poorer overall survival and also correlate with clinicopathologic parameters that indicate a more aggressive clinical behavior. Beta-catenin appears to be a better marker than e-cadherin since it shows significant correlation with more clinical parameters and may be a useful immunohistochemical prognostic marker for patients with gastric cancer.

859 Ethnic Differences in the Prevalence of *Helicobacter pylori* Infection and Gastric Pre-Neoplastic Lesions in US Veterans

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Background: In any community, differences in prevalence and manifestations of *H. pylori* infection are believed to be more related to socioeconomic status than to ethnic characteristics. We hypothesized that in US Veterans, a population of relatively uniform socioeconomic status, the prevalence of both *H. pylori* infection and intestinal metaplasia (IM) would show only minor variations amongst White, African-American, and Hispanic subjects.
Design: Topographically defined gastric biopsy specimens (2 each from corpus and antrum and 1 from the *incisura angularis*) were obtained from patients recruited into a trial designed to test a novel therapy for *H. pylori.* Two blinded pathologists used the Updated Sydney System to evaluate the biopsies. For this study arm, endpoints included: presence of *H. pylori* (detected by hematoxylin and eosin and anti-*Helicobacter* immunohistochemical staining, as needed); and presence and location of IM.
Results: We recruited 310 Veterans: 185 White (median age 63 years; 92% men), 97 African-American (median age 58 years; 86% men) and 28 Hispanic (median age 57 years; 82% men). The prevalence of *H. pylori* gastritis and IM in each group are summarized in Table 1. Intestinal metaplasia was found in the corpus of 2 (15.4%) of 13 Whites who had this lesion, in contrast to 9 (42.9%) of 21 African Americans (OR 4.12 95%CI 0.72 – 23.4).

Table 1. The prevalence of H. pylori gastritis and of intestinal metaplasia in the three ethnic groups

Ethnic group	Hp (+) (%)	IM (%)	IM in corpus (%)
White (n = 185)	64 (34.6)	13 (7.0)	2 (15.4) - 1 in Hp (+)
African American (n = 97)	67 (69.1)	21 (21.6)	9 (42.9) - 6 in Hp (+)
Hispanic (n = 28)	18 (64.3)	5 (17.9)	0

Conclusions: In spite of broadly similar socioeconomic backgrounds, both African-American and Hispanic Veterans had a much greater prevalence of *H. pylori* infection (combined OR 4.02; 95%CI 2.48 – 6.51; $p<.0001$) and IM (combined OR 3.47; 95%CI 1.71 – 7.07; $p<.001$) than White patients. Neither age nor sex differences amongst the