

Barrett's Esophagus

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Dr. Romero is Assistant Professor of Medicine at the Mayo College of Medicine in Rochester, Minnesota. She received her MD degree from the University of Nevada School of Medicine. She completed her Internal Medicine residency training at Indiana University Medical Center in Indianapolis, Indiana, under the mentorship of Glen Lehman, M.D. Dr. Romero then traveled to the Mayo Clinic in Scottsdale, Arizona, for her first year of gastroenterology fellowship training, completing the final two years at the Mayo Clinic under the mentorship of Alan Cameron, M.D. Upon graduation, Dr. Romero trained in clinical epidemiology and biostatistics at McMaster University in Hamilton, Ontario, Canada. She returned to the Mayo

Clinic for her advanced fellowship in Diseases of the Esophagus, where she remains on staff. She holds joint appointments in the Department of Otolaryngology and Department of Epidemiology and continues her education in genetic epidemiology and tumor genetics under the mentorship of Gloria M. Petersen, Ph.D. She has many publications in the area of Barrett's esophagus and several funded research trials in the area of the genetics of familial Barrett's esophagus, outcomes of steroid injection for distal esophageal peptic strictures, supraesophageal reflux disease, and validation of symptom questionnaires for use in research.

Good morning everyone. Welcome to the Focused Clinical Update on Barrett's Esophagus.

One of the privileges of presenting the highest ranking abstracts to you is that I am permitted to review the scores of all of the Barrett's abstracts. Those scores guided the selection of the six basic science and six clinical research abstracts we are going to discuss today.

Abstract 224235: "Evidence from linkage analysis for susceptibility genes in familial Barrett's esophagus and esophageal adenocarcinoma"

I would like to disclose that this work was done by my team with the aid of Gloria Petersen, a genetic epidemiologist. Obviously, I feel guilty discussing it at all, but I am presenting it first because it ranked highest among this year's abstracts. Our aim was to map susceptibility loci in familial Barrett's esophagus by performing linkage analysis on families with the following phenotypes: GERD symptoms, hiatal hernia, reflux esophagitis, biopsy-proven Barrett's esophagus and esophageal adenocarcinoma. Families seen at the Mayo Clinic or referred to our group with more than two family members with long segment Barrett's esophagus, with or without cancer, were asked to participate. The index patients, known as probands, provided pedigree and contact information to enable our team to contact their relatives directly. All family members were invited to complete a validated symptom questionnaire and medical release form. Their endoscopy and/or surgical reports, and original pathology slides were reviewed. In families documented to have more than one affected member, blood was sampled to perform genotyping.

Of 466 potential families that have come to our attention so far, 94 have met strict criteria to be considered Barrett's families. The results presented in today's abstract uses 31 of the 94 families. The goal of linkage analysis is to associate a genetic marker with a particular phenotype (e.g., Barrett's esophagus). In linkage analysis, you genotype all affected family members. By performing a linkage analysis, we are assessing the co-transmission of a marker with a particular phenotype.

The results of linkage analyses are expressed as logarithm of the odds scores, or LOD scores. Heterogeneity LOD scores, (or HLOD) scores, take into account variations among different families. Different genes will be more important in different families. If the LOD score is 2, that implies that the

result is due to chance 1 in a 100 times, like a p-value of 0.01. Genetic epidemiologists get very excited if the LOD score is 3. That means the odds that the result is due to chance is about 1 in a 1000. This would be comparable to a p-value of 0.001. A LOD score of 3 is a magic number. In general, the higher the LOD score, the more convinced one is of linkage. Conversely, a LOD score of -2 virtually excludes linkage.

For the phenotype of Barrett's esophagus we found a HLOD score of 3.07 (which, you will recall, suggests that there is only a 1 in a 1000 chance this is a spurious finding) on the short arm of chromosome 2. We found two other remarkable HLOD scores around the same region on the short arm of chromosome 2. There were positive HLOD scores on chromosome 12 and chromosome 19 for Barrett's esophagus as well. For Barrett's with cancer, the same location on the short arm of chromosome 2 was positive.

We performed linkage analyses for other phenotypes and combinations of phenotypes. Is hiatal hernia running through the families? Is it hiatal hernia with GERD symptoms? Are GERD symptoms associated with cancer in these families, as suggested by the Lagergren *New England Journal of Medicine* manuscript published in 1999? That's what we mean by running the analysis by clinically significant phenotypes. For GERD symptoms or the presence of a hiatal hernia, we found a very high HLOD score on chromosome 12. We are almost at a HLOD score of 4 – implying a 1 in 10,000 chance that this is a spurious finding. Other interesting locations for linkage, in regard to the phenotype of hiatal hernia, with or without reflux esophagitis, are on chromosome 7.

Basically we found linkage to the short arm of chromosome 2 for Barrett's esophagus and esophageal cancer. There are interesting regions on chromosome 12 and 19 for Barrett's esophagus as well. For hiatal hernia, there is very impressive linkage on chromosome 12 and an interesting area on chromosome 7. The take home point is that there is strong evidence for major susceptibility genes in familial (not sporadic) Barrett's esophagus and adenocarcinoma, and on separate chromosomes, for familial hiatal hernia and GERD symptoms.

Abstract 218422: “Association of Y-chromosome haplotypes with Barrett's esophagus and esophageal adenocarcinoma in the Dutch Caucasian population”

The loss of the Y chromosome is the most consistent aberration in Barrett's esophagus and is commonly seen in patients with dysplasia. The authors hypothesized that certain Y-chromosome haplotypes are associated with an increased or decreased susceptibility to Barrett's esophagus and esophageal adenocarcinoma. A haplotype is a specific chromosomal configuration. They did a chromosomal analysis to define seven different Y-chromosome haplotypes. These authors assessed the haplotype frequencies on Y-chromosomes in patients with Barrett's and cancer. They had 286 patients with Barrett's, 116 with adenocarcinoma, 109 with esophagitis without Barrett's and 90 controls. They found a single haplotype on the Y-chromosome that was associated with Barrett's compared to controls and a single haplotype that was inversely related (meaning, potentially protective). It was less common in Barrett's esophagus and cancer than in controls. This high-ranking and provocative abstract provides food for thought and future research.

Abstract 225114: “Comparison of digital image analysis (DIA) and fluorescent in situ hybridization (FISH) in the detection of neoplasia Barrett's esophagus”

Biomarkers [e.g., p16, DNA ploidy, loss of heterozygosity (LOH) and p53] have been shown to be of value in indicating long term cancer risk in patients with Barrett's esophagus. The biomarkers have not, however, been used to detect high-grade dysplasia or cancer in biopsies collected at the time of

surveillance. Two methods of assessing biomarkers – DIA and FISH – were compared in this study to assess their ability to detect high-grade dysplasia (HGD) or esophageal adenocarcinoma.

The authors assessed 92 patients; 8 with esophageal adenocarcinoma, 61 with HGD, 13 with low-grade dysplasia (LGD) and 11 without dysplasia. Brush cytology was used to collect specimens for DIA and FISH prior to collecting tissue using a four quadrant biopsy protocol. Patients with cancer or HGD had an abnormal DIA 68% of the time (47/69) and abnormal FISH analysis 82% of the time (57/69). The authors conclude that FISH is better at distinguishing biopsies with HGD and cancer than DIA.

The challenge in this area of research is the known limitation of histology as the gold standard for molecular abnormalities. Previous studies have shown that in 8 of 10 cases, when a pathologist diagnoses HGD or cancer, other pathologists agree. But in 2 of 10 cases, when one pathologist states there is HGD present, another expert pathologist disagrees. So is FISH detecting more true HGD or is it just agreeing with the pathologist more than DIA in this cohort of 92 patients? The hope raised by this abstract is that perhaps some day in the near future, more objective markers of disease progression, beyond pathologist interpretation of a slide, will guide surveillance of patients with Barrett's esophagus.

Abstract 216670: "Cyclin A immunocytology as a risk stratification tool in Barrett's esophagus surveillance"

The authors have previously demonstrated that a marker of proliferation, Mini-chromosome Maintenance Deficiency 2 (Mcm2), is a marker of progression of Barrett's to dysplasia. However, this marker is not specific. In this study, they attempted to determine if cyclin A, which detects proliferating cells in the S and G2 phases of the cell cycle, is a better (early) marker for progression from Barrett's to cancer. To address this question, they have performed three experiments:

The first experiment is a proof of principle study in which they tested the staining qualities of archived material (squamous epithelium N=30, stomach N=12, duodenum N=9, Barrett's N=62, esophageal adenocarcinoma N=16) for cyclin A. Squamous, stomach and duodenal tissue did not demonstrate surface expression of cyclin A. However, expression in Barrett's esophagus samples correlated with degree of dysplasia. HGD specimen stained best with cyclin A, while staining was weakest in LGD specimen.

The second experiment is a case-control study. The authors compare the staining qualities of a cohort of nine patients without cancer at baseline who progressed to esophageal cancer within 3 to 13 years; with 18 Barrett's controls who were matched for age and duration of follow-up, who did not progress to cancer. Patients with positive cyclin A staining at baseline were more likely to progress to cancer. Their odds ratio of progression (OR=10.0, 95% CI 1.5-64.2) was dramatically higher for staining cyclin A compared to the no staining group. We cannot tell if the investigators adjusted for inflammation, aspirin or proton pump inhibitor use, Barrett's length, or same surveillance intervals – as these factors may be potential confounders.

Experiment number three is a prospective cohort study designed to assess the sensitivity and specificity of cyclin A. Tissue was collected from squamous epithelium (N=24), Barrett's with or without dysplasia (N=93) and adenocarcinoma (N=36). The sensitivity (97%) and negative predictive value (98.2%) of cyclin A were similar to Mcm2 in previous studies (100%, 100%). However, the specificity of cyclin A (61.2%) was superior to Mcm2 (35.6%) from previous studies. The authors are implying that cyclin A staining is more specific and hence a potentially better marker for progression of Barrett's to cancer compared to Mcm2. These are exciting early results.

Abstract 225932: “The COX-2-765C/-1195A haplotype predisposes for the development of esophageal adenocarcinoma”

In this haplotype study, the authors tested the association between COX-2 haplotypes and the development of cancer. Their study population included 140 patients with esophageal adenocarcinoma, 250 with Barrett’s esophagus and 247 with reflux esophagitis. The authors measured the frequency of three COX-2 haplotypes: CA, GA and GG, and measured their association with particular phenotypes. The haplotype profile was the same for patients with Barrett’s esophagus as it was for patients with esophagitis. In contrast, the CA-haplotype was more common in patients with adenocarcinoma. Patients with adenocarcinoma were also more likely to be homozygous for the CA-haplotype (OR=3.83 95% CI 1.09 – 13.84).

CA-haplotype carriers, meaning those patients with only one copy of the CA-haplotype, were less likely to have cancer (OR=1.94) than patients with two copies (homozygous) of the CA-haplotype (OR=3.83). The authors conclude that there appears to be an association between the COX-2 CA-haplotype and esophageal cancer. Like all of these studies, the hypothesis needs validation in a separate independent group of patients, adjusting for potential confounders such as inflammation and use of particular types of medications.

Abstract 217885: “The Seattle Protocol for high-grade dysplasia in Barrett’s esophagus-It may not be as good as we think”

Does the Seattle Surveillance Protocol identify patients with early cancer better than routine surveillance? The Seattle Protocol was defined as initial biopsy of any mass or bump, followed by four quadrant biopsies every 1 cm along the length of a salmon colored segment in the esophagus. Routine surveillance was defined as four quadrant biopsies at 2 cm intervals. The authors describe 32 patients who underwent esophagectomy for HGD at their institution from 1999 to 2005. All biopsies were reviewed by expert pathologists. Twenty-one patients were surveyed using the Seattle Surveillance Protocol, 33% of whom were found to have intramucosal cancer that was not identified preoperatively. Eleven patients had routine surveillance, three (27.3%) had an intramucosal cancer, (p=0.99). None of the 10 patients with cancer in their specimen had submucosal lesions or nodal metastases. There was no difference in the frequency of hiatal hernia or age; however, patients in the routine surveillance group had longer segments of Barrett’s esophagus. The incidence of cancer in patients with HGD sent to esophagectomy was lower in this cohort than in a series from Johns Hopkins (44%) in the late 1980’s, but higher than in a series reported by the Mayo Clinic in 1991 (11%). These authors conclude that the Seattle Surveillance Protocol is not superior to routine surveillance.

Although this is an important abstract, the authors did not account for 1) the duration or frequency of surveillance prior to the EGD in which the patient was deemed as having high grade dysplasia; 2) compliance with either surveillance protocol; 3) sex, as males have a higher risk of cancer than females, 4) extent of HGD in the specimen, or a number of other potential confounders. The disheartening message of this abstract is that we continue to fall short in our ability to identify patients with cancer at surveillance.

Abstract 218482: “Natural history of high-grade dysplasia in a regional Veterans Administration Barrett’s cohort”

It may be helpful to review prevalence, incidence and lead time bias before we discuss this abstract. Prevalence describes the number of pre-existing and newly diagnosed cases of a certain disease on a certain date. Incidence describes the number of new cases diagnosed during a certain period of time. Lead time bias refers to the situation in which we detect a disease prior to when the patient experiences symptoms. We intervene and believe our intervention actually extended life, when in fact, if we had intervened when they were symptomatic, they would have the same outcome, and die on the same day. Lead time bias can falsely make researchers believe they are having impact when they are not. This is pertinent to patients with HGD. It may be that intervention prior to the diagnosis of cancer does not impact outcome; and that their outcome would be the same if we waited to intervene when they develop symptoms of cancer.

With that as a background let’s discuss the abstract from the VA Medical Center in Los Angeles. The authors reviewed the VA database for everyone diagnosed with Barrett’s esophagus between 1988 and 2002. They compared the risk of progression to cancer and the mortality rates of patients diagnosed with prevalent (endoscoped with symptoms and found to have HGD in their Barrett’s segment) versus incident HGD (HGD identified at surveillance endoscopy). Prevalent high-grade dysplasia progressed to cancer in six people over 70 patient-years. That is a 1 in 12 year cancer rate. Incident HGD patients had a cancer rate of 1 in 45 years. Although these rates appear different, they are not statistically significantly different. When they looked at mortality, patients with prevalent HGD died at a rate of 1 person every seven years. Incident HGD patients died at a rate of 1 in every 27 years ($p=.005$). Only 20% died of cancer.

The authors describe 45 patients diagnosed with prevalent cancer (found when the patient presented with symptoms) and 11 patients with incident cancers (found at surveillance endoscopy regardless of symptoms). Patients with prevalent cancer had early stage disease 16% of the time compared to 45% for those patients diagnosed with cancer at surveillance endoscopy. This was statistically significant ($p=0.03$). Death was due to cancer 96% of the time in prevalent cancer patients and 5% in incident cancer patients ($p=.02$). These data support the premise that cancers discovered at surveillance endoscopy are lower stage, and that surveillance might prolong survival. In their conclusion however, the authors comment that either surveillance really works or there is lead time bias and we just can’t tell the difference. I can’t think of a good way to figure this out either because no one knows when Barrett’s or dysplasia begins.

Question: It is possible that you could actually, by a surveillance program, shorten lifespan?

Answer. Yes. If the mortality from the treatment or from complications of surveillance is high.

Abstract 226987: “The development and validation of an endoscopic grading system for Barrett’s esophagus-The Prague C and M criteria”

This work was done by an international working group who have developed a consensus opinion as to how to measure and report the extent of Barrett’s esophagus at endoscopy. One measurement is the “C” or “circumferential” segment. This is the longitudinal extent of the circumferential (100% around) segment of Barrett’s. The “M” or “maximal” extent is the longitudinal extent of the entire Barrett’s segment. For example, if the circumferential aspect of a patient’s Barrett’s is 6 cm, and they have a one centimeter tongue at the proximal aspect, the endoscopist would describe a C6M7 Barrett’s length. The workgroup has decided that the “maximal” extent will reflect the length of “contiguous” Barrett’s mucosa. So, if a

similar patient has a 6 cm circumferential segment, and there are two salmon-colored islands located 2 cm proximal of the circumferential segment, with normal squamous mucosa in-between, the Prague Criteria would describe the Barrett's extent as C6M6.

With this background in mind, let's discuss the performance of these new measurement guidelines. Twenty-two endoscopists reviewed and scored videos of 29 patients. Reliability coefficients (RC) which are similar to kappa statistics, assess agreement accounting for chance. The RC for localizing the gastroesophageal junction (0.88) and diaphragmatic pinch (0.85) were high as were the overall values for circumference (0.95) and maximal extent (0.94). However, exact agreement was much lower; 53% for the circumferential segment and 38% for the maximal extent. When analyzed in a generous fashion, meaning within 2 cm, the agreement for the C-value rose to 72% and the M value rose to 76%. The RC for Barrett's segments greater than 1 cm in length was 0.72. For segments less than 1 cm in length, the RC was only 0.22.

Only time will tell what impact this grading system will have. On one hand, in this study, agreement was moderate, particularly in regard to measurement of maximal extent among experts in Barrett's esophagus, who under research conditions are doing their best to be precise. On the other hand, if this simple grading system is embraced by the GI Community, with increased familiarity and use, perhaps it will improve communication, diagnosis and ultimately, patient care.

Abstract 226219: "Accuracy of wireless capsule endoscopy for the detection of Barrett's esophagus"

The aim of this abstract is to estimate the sensitivity and specificity of wireless capsule endoscopy, compared to the gold standard of endoscopy, in the detection of Barrett's esophagus. This multi-center study used select patients with known Barrett's esophagus (53%) or GERD symptoms (47%). Patients swallowed the capsule first then underwent routine upper endoscopy (EGD). Images were read blind to endoscopic results. Eighty-four patients participated. Three patients could not swallow the camera and in three other patients, the picture quality was too poor to interpret. Therefore, the first result of the abstract is that among patients sent for capsule endoscopy, the study will fail to yield useful information in 7%.

The results of 78 patients (N=27 short segment Barrett's, N=14 long segment Barrett's) were shown. The authors calculate the sensitivity and specificity of the capsule for short Barrett's to be 67% and 86%, respectively, and 86% and 86% for long segment Barrett's esophagus. Their conclusions were that the capsule is safe and that the sensitivity and specificity were better for long segment Barrett's esophagus compared to short segment Barrett's.

The burning question, forgive the pun, is, "How will the capsule perform in the general population when applied to screening for Barrett's esophagus?" This study, like most done to date, uses an engorged population, in which over half of the subjects have the disease of interest. The major question is how will the capsule perform in society, where the prevalence of long segment Barrett's in patients with long duration frequent GERD symptoms ranges from 3.5 to 7%? Will it be useful in screening asymptomatic patients, in whom the prevalence of long segment Barrett's esophagus is less than 1%?

Abstract 218978: "Squamous overgrowth in a 5-year randomized phase III trial of photodynamic therapy using porfimer sodium in ablation of high-grade dysplasia in Barrett's esophagus"

Overgrowth of squamous tissue after ablation for Barrett's has been a source of concern. Many believe it will result in missed sub-squamous cancer at future surveillance endoscopy. This study asked the question, "What proportion of patients treated with photodynamic therapy with PPI, versus PPI alone develop squamous cell overgrowth?" The authors conducted a randomized controlled trial of patients

with HGD in which patients were randomized to photodynamic therapy with omeprazole twice daily, or omeprazole alone twice a day. Patients were endoscoped and biopsied - four quadrants every 2 cm, along the original length of the Barrett's segment, every three months until HGD was undetectable on four separate occasions for a year. Thereafter, patients were endoscoped and biopsied every six months for up to five years. One hundred thirty eight patients were randomized to photodynamic therapy, and 70 to PPI alone. Squamous overgrowth was seen in equal frequency in both groups and did not affect the detection of neoplasia. No patient had neoplasia or dysplasia beneath the squamous mucosa that was not detected in an area that did not have squamous overgrowth.

Abstract 222525: "Detection of residual Barrett's mucosa after photodynamic therapy (PDT) using high definition narrow band imaging (HD-NBI)"

This study asks the question, "Is there value-added in high definition narrow band imaging (HD-NBI) to detect residual Barrett's following photodynamic therapy compared to high definition (HD) endoscopy?" Fifty consecutive patients with HGD treated with photodynamic therapy were entered in the study. They were first endoscoped using routine white light, then with HD-white light, followed by HD-NBI to determine the presence of residual Barrett's epithelium. Residual Barrett's was found in 22 patients with standard white light, 25 with HD-white light and 38 with HD-NBI. Their conclusion is that narrow band imaging is twice as good as white light for the detection of residual Barrett's in patients who have undergone photodynamic therapy. The challenge in the study design is that this is a report from a single observer, without blinding or controls. Nevertheless, this may be important preliminary data.

Abstract: "Biomarkers in the therapy of Barrett's esophagus and high-grade dysplasia"

The aim of this study was to identify biomarkers that predict a response to photodynamic therapy (PDT). Patients with Barrett's esophagus with HGD seen from 2002 to 2005 were studied (N=58). Forty-one patients only had HGD (to the best knowledge of the investigators). Seventeen patients had early cancer and HGD. All subjects underwent brush cytology for biomarkers using FISH surveillance using the Seattle Protocol and endoscopic ultrasonography. Patients with a nodule had it resected by endoscopic mucosal resection (EMR). All patients were treated with PDT. Patients were followed every three months and were considered "responders" if at three months they had no dysplasia. Thirty-one of 58 patients responded to PDT (53%). On multivariate analysis, Barrett's length and loss of the P16 locus correlated with response to PDT. This is the first study that hints that biomarkers may help predict response to PDT.

Thank you all for your time, and making the journey at 6 a.m. to discuss the best work in the area of Barrett's esophagus submitted to DDW this year.

Abstracts Discussed

224235: Evidence from Linkage Analysis for Susceptibility Genes In Familial Barrett’s Esophagus and Esophageal Adenocarcinoma. *Yvonne Romero, Joshua P Slusser, Mariza de Andrade, Julie M Cunningham, Teresa G Zais, Debra M Geno, Gloria M Petersen¹ Group Barrett's Esophagus Genomic Study*

Background: The Barrett's Esophagus Genomic Study Group comprises 156 physicians from varying practice environments who collaborate to identify Barrett’s esophagus (BE) families for genomic analysis. Aim: To map susceptibility genes in familial BE pedigrees by genome-wide linkage analysis using BE and esophageal adenocarcinoma (ACA) phenotypes. Methods: Study Group members refer families in which more than two persons are suspected of having long segment BE, with or without ACA. Following informed consent, pathology slides and endoscopy and surgical reports of potential index patients are reviewed. Confirmed index patients provide contact information of living first-degree relatives, or next of kin of deceased relatives. Pathology slides and records on relatives are reviewed to confirm phenotype. To augment phenotype characterization, a separate research project screened family members who had not previously had an upper endoscopy. All available living members of documented BE families were asked to complete a validated symptom questionnaire, and to provide a blood sample. Genotyping of lymphocyte DNA was performed using the ABI PRISM® Linkage Mapping Set version 2.5 using 403 markers equally distributed along the entire autosome at ~10 cM resolution. Two-point and multi-point linkage analyses were performed. Results: 466 families were evaluated for eligibility, and 94 were documented to have multiple affected family members: 2 families had 5 affecteds, 4 families had 4 affecteds, 29 families had 3 affecteds, and 59 had 2 affecteds. Linkage analysis was performed on 278 persons in the 31 most informative families. Under a dominant model with decreased penetrance, the phenotype of BE yielded a multipoint heterogeneity LOD (HLOD) score of 3.07 at 66.9 cM, near D2S2259; 2.51 at 79.43 cM between markers D2S391 and D2S337; and 1.57 at 8.64 cM at D2S2211, all on the short arm of chromosome 2. Other promising HLOD scores include: 1.52 on chromosome 12p at 73.55 cM at D12S368; 1.41 at 43.59 cM near D19S414, and 1.47 at 71.06 cM at D19S902. Linkage remained consistent on chromosomes 2, 12, and 19 when a combined phenotype of BE or ACA was used, with the highest HLOD score being 2.78 at 66.94 cM at D2S2259. Linkage was not identified for either phenotype on chromosome 13 (maximum HLOD= 0.56). Conclusion: These results provide strong evidence for major susceptibility genes for familial Barrett’s esophagus and esophageal adenocarcinoma.

218422: Association of Y Chromosome Haplotypes with Barrett’s Esophagus and Esophageal Adenocarcinoma in the Dutch Caucasian Population. *Agnieszka M Rygiel, Sjoerd Repping, Leon M Moons, Sander Ouburg, Franceska Milano¹ Jantine W van Baal, Jacques G Bergman, Servaas A Morre, Peter Siersema, Cornelis L Mulder, Johannes G Kusters, Maikel P Peppelenbosch, Jan M Hoovers, Kausilia K Krishnadath*

BACKGROUND: Barrett’s esophagus (BE) is a metaplastic condition of the distal esophagus, which predisposes for the highly malignant esophageal adenocarcinoma (EAC). It is known that BE and EAC have a 3-7 times increased incidence in males especially in the Caucasian population. Interestingly the loss of Y chromosome is one of the most consistent aberration in BE and occurs already in the stage of metaplasia and has a high frequency in dysplasia. HYPOTHESIS: We hypothesized that certain Y chromosome haplotypes may be associated with increased or decreased susceptibility for BE and EAC development. MATERIAL and METHODS: To test this hypothesis, we have analyzed a set of 6 Y chromosome linked polymorphisms to define 7 major Y chromosome haplotypes (A,B-C, DE, F(xJ,xK), K(xP), J, P(xR1a), R1a) in the Dutch Caucasian patients presenting with BE (n=286), EAC (n=116) and esophagitis without BE(n=109). Their Y haplotype profile was subsequently compared with matched for age (>50 years old), BE free Control population (n=90). RESULTS: The frequency of Y haplotypes in the investigated populations is shown in the table below. We found significant difference in frequencies of the P(xR1a) and DE haplotypes between the patient groups and the control population. The P(xR1a)haplotype had a significantly higher frequency in BE patients (62%) vs. Control (44%) (OR- 2.0, p<0.001). In contrast the frequency of the DE haplotype was significantly lower in patients with BE (3%) and EAC (1,7%) vs. Control (14,5%)(OR- 0.19, p<0.001 and OR-0.10, p<0.0001, respectively). Also the DE haplotype was underrepresented in esophagitis (7,3%) vs. EAC (1.7%) (OR-0.22, p<0.05). CONCLUSIONS: Our results suggest that the P(xR1a) haplotype is associated with increased susceptibility for BE. In contrast the DE haplotype is associated with decreased predisposition to both BE and EAC. We postulate that haplotypes P(xR1a) and DE might be linked to the variants of genes which predispose and protect against BE /EAC development, respectively. Together with other markers, this may help identify those patients who are at risk for BE or might benefit from surveillance.

Y chromosome haplotypes frequency (%) in BE, Esophagitis, EAC and Control population.

Y hapoltype	Control	Esophagitis	BE	EAC
P(xR1a)	44,4	50,4	62	57,7
F(xJ,xK)	22,2	25,7	28,7	32,7

J	10	8,2	2,8	3,4
A-B or C	0	0	0	0
DE	14,4	7,3	3,1	1,7
K(xP)	3,3	2,7	1,4	2,6
R1a	5,5	2,7	2,1	1,72
Nr of cases	90	109	286	116

225114: Comparison of Digital Image Analysis (DIA) and Fluorescent in Situ Hybridization (FISH) in the Detection of Neoplasia Barrett's Esophagus. *Kenneth K Wang, Ganapathy Prasad, Navtej S Buttar, Michel WongKeeSong, Lynn S Borkenhagen, Sheila Krishnadath, Marlys Anderson, Sarah Papenfuss, Kevin Halling, Thomas Smyrk, Catherine DeMars, Lori Lutzke*

Biomarkers such as LOH of p53, p16, and DNA ploidy have been shown to be of value in determining cancer risk in Barrett's esophagus. However, they have not previously been used as a tool to detect high-grade dysplasia or cancer in Barrett's esophagus. Aim: To compare the ability of DIA and FISH to detect high-grade dysplasia or cancer in Barrett's esophagus. Methods: Patients referred to the Barrett's Esophagus Unit were prospectively assessed with both DIA and FISH performed on brush cytology prior to surveillance biopsies that were taken in 4 quadrants every centimeter and assessed by two expert pathologists. The brush cytology specimens were processed by placing the specimens in PreservCyt solution (Cytoc Corp., Boxborough, MA). These were then collected using a ThinPrep 2000 Processor and stained with Feulgen dye and assessed with a CAS 200 digital imaging system. A DNA index was calculated by comparing the intensity of the nuclear staining of the Barrett's cells obtained by brush cytology to that of morphologically benign appearing epithelial cells within the specimen. A DNA index between 0.90 and 1.10 is considered diploid. Values between 1.1 and 1.9 are considered aneuploid. Values between 1.9 to 2.1 are considered tetraploid. FISH was performed using a twelve probe set including 12-probe set; p15, 5q21-22, CEP7, 7p12, 8q24.12-13, CEP 9, 9p21, CEP 17, 17p13.1, 17q11.2-12, CEP Y, and 20q13. In order to compare results, we dichotomized the results of DIA and FISH as positive or negative based on the presence of any abnormalities (excluding loss of Y on FISH since this is almost ubiquitous in Barrett's mucosa and excluding nuclear morphometry on DIA). Using DIA, abnormalities such as increased tetraploidy or aneuploidy would be considered as a positive DIA test Results: A total of 92 patients were assessed of whom 8 had adenocarcinoma, 61 had high-grade dysplasia, 13 had low-grade dysplasia, and 11 patients had no dysplasia on biopsy. patients with high-grade dysplasia or cancer had DIA abnormalities in 47/69 (68%). This same group of patients had FISH abnormalities in 57/69 (82%) of patients indicating that FISH abnormalities appear to be superior to DIA for predicting the presence of high-grade or cancer (p<0.05) Conclusions: FISH detected genetic abnormalities in cytological specimens correlated significantly better than DIA with the presence of neoplasia in Barrett's esophagus. This test has the potential to improve surveillance in Barrett's esophagus.

216670: Cyclin A Immunocytology as a Risk Stratification Tool in Barrett's Esophagus Surveillance. *Pierre Lao-Sirieix, Laurence Lovat, Rebecca C Fitzgerald*

Background The incidence of esophageal adenocarcinoma (AC) is increasing rapidly. Endoscopic surveillance of patients with Barrett's esophagus (BE) using biopsies for histopathological assessment is prone to sampling bias, is not cost effective and interpretation of dysplasia is subjective. Alternative methods for surveillance are direly needed. We have previously demonstrated that surface expression of a proliferative marker, Mcm2, is a marker of progression. However, the detection of patients at risk of progression through detection of Mcm2 positive cells in cytological specimens lacked specificity. Aims To determine whether cyclin A, which detects proliferating cells in the S and G2 phases of the cell cycle, would be more specific than Mcm2 for the detection of patients at risk of progression to AC. Methods Archival specimens (30 squamous esophagus (SE), 12 gastric antrum (GA) and 9 duodenum (D2), 62 BE +/- dysplasia, 16 AC) were stained for cyclin A. In addition, 9 patients with 3-13 years follow-up who developed AC were compared with 18 controls matched for age and length of follow-up who did not progress. Endoscopic cytological brushings were taken from a prospective cohort (24 SE, 93 BE +/- dysplasia and 36 AC) and scored blind as cyclin A positive or negative. Results There was no surface expression of cyclin A in control samples (NE, GA, D2) and its expression at the surface of BE samples correlated with the degree of dysplasia (p=0.016). In the case-control cohort, patient with biopsies expressing cyclin A at the surface were more likely to progress to AC than those who did not (OR 10.0, 95% CI 1.5-64.2). The sensitivity and specificity of cyclin A expression in brushings for the detection of high-grade dysplasia (HGD) and AC were 97.9% and 61.2% respectively. The associated negative predictive value (NPV) was 98.2%. Comparatively, the sensitivity, specificity and NPV of Mcm2 for detection of AC and HGD patients, in a previous cohort, were 100%, 35.6% and 100%. Using cyclin A nearly doubled the specificity with little impact on the sensitivity or NPV. Conclusions Expression of cyclin A at the luminal surface is a marker of progression to AC. Detection of cyclin A positivity in immunocytology could be used as a first step to stratify BE patients according to their risk of progression. A large clinical prospective study is required to confirm these findings.

225932: The COX-2 -765C/-1195A Haplotype Predisposes for the Development of Esophageal Adenocarcinoma. *L.M.G. Moons, J. G Kusters, A. M Rygiel, Z.M.A. Groothuismink, W.A. Bode, H. Geldof, K.K. Krishnadath, J.G.H.M. Bergman, A.H.M. van Vliet, P.D. Siersema, E.J. Kuipers*

Introduction: The expression level of COX-2 is associated with an increased tendency for neoplastic progression in the esophagus, since COX-2 expression increases the metaplasia-dysplasia-adenocarcinoma sequence. High expression levels are also associated with poor 5-year survival rates of esophageal adenocarcinoma. COX-2 is the rate-limiting enzyme for the production of prostaglandin E2 (PGE2), a compound associated with carcinogenesis in many inflammatory diseases, and recently, it was suggested that PGE2 levels were COX-2 promoter haplotype dependent. Aim: To determine the association between COX-2 haplotypes and the development of esophageal carcinogenesis. Methods: DNA was obtained from 140 Caucasian patients with an adenocarcinoma of the esophagus (mean age 63±11; 91% male), 250 Caucasian patients with histologically confirmed Barrett's esophagus (mean age 62±13; 68% male) with a mean Barrett-segment length of 4.2 ± 2.3 cm, and 247 Caucasian patients with endoscopically confirmed reflux esophagitis (mean age 56±14; 53% male). COX-2 haplotypes were determined by amplification of the promoter region by PCR, followed by determination of the polymorphisms at -765C/G and -1195A/G by RFLP with restriction enzymes *AciI* and *PvuII* respectively. COX-2 haplotype dependent PGE2 levels were determined by EIA. Results: The tested population contained 171 (14%) CA (-765C & -1195A), 817 (66%) GA, and 258 (20%) GG haplotypes, and none of the relatively rare GC-haplotype. The haplotype distribution in patients with reflux esophagitis and Barrett's esophagus was similar (CA 12%, GA, 68%, GG 21%), but differed significantly from patients with esophageal adenocarcinoma (CA 21%, GA 58%, GG 20%), in whom the CA-haplotype was significantly more common ($p < 0.0001$). Furthermore, homozygosity of CA was only found in patients with Barrett's esophagus patients (1.6%) and esophageal adenocarcinoma (5.0%) ($p = 0.002$). CA-carriership was associated with an increased risk for esophageal adenocarcinoma (OR 1.94; 95%CI 1.23-3.08; $p = 0.005$), and homozygosity for the CA-allele was associated with an even greater risk (OR 3.83; 95%CI 1.09- 13.84; $p = 0.036$). Conclusion: The COX-2 CA-haplotype is associated with an increased risk for the development of esophageal adenocarcinoma in Barrett's esophagus and reflux esophagitis patients. As the COX-2 CA-haplotype is associated with increased levels of PGE2, PGE2 seems to promote esophageal carcinogenesis in patients with a Barrett's esophagus and reflux esophagitis.

217885: The Seattle Protocol for High-Grade Dysplasia In Barrett's Esophagus-It May Not Be As Good As We Think. *Revital Kariv, Thomas P Plesec, Mary P Bronner, John R Goldblum, Mary Oldenburgh, Thomas W Rice, Gary W Falk*

Background: The optimal management of high-grade dysplasia (HGD) in Barrett's esophagus (BE) remains controversial. A biopsy protocol consisting of 4 quadrant jumbo biopsies at 1 cm intervals plus biopsies of any mucosal abnormalities no matter how trivial (Seattle protocol) is thought to be a reliable method to detect early cancers in HGD patients. However, this protocol has never been validated outside of Seattle. Aim: To validate the Seattle protocol in a cohort of BE patients who underwent esophagectomy for a diagnosis of HGD. Patients and Methods: This is a single center study of 32 consecutive BE patients with a biopsy diagnosis of HGD who underwent esophagectomy between 1999-2005. None had mass lesions suggestive of obvious malignancy at the time of preoperative endoscopy. All biopsies were confirmed by expert GI pathologists prior to surgery. Patients were divided into 2 groups: Group 1 had preoperative surveillance biopsies done according to the Seattle protocol as described above; Group 2 had 4 quadrant biopsies every 2 cm. Postoperative pathology findings confirmed by expert GI pathologists were compared to preoperative findings in both groups. Results: There were 21 patients in Group 1 and 11 patients in Group 2. Age and hiatal hernia size were not significantly different between the 2 groups. Median [IQR]BE length was greater in Group 2 (10.0 cm [4.0,10.0] vs 4.0 cm [2.0,5.0] ($P = 0.01$)). Postoperatively, unsuspected intramucosal cancer was found in 7/21(33.3%) Group 1 vs 3/11(27.3%) in Group 2 ($P = 0.99$). No patients in either group had a postoperative diagnosis of submucosal cancer or lymph node metastases. Conclusions: Intensive preoperative surveillance biopsies using the Seattle protocol does not reliably predict the presence of intramucosal carcinoma at the time of esophagectomy any better than a less intensive surveillance biopsy protocol. This calls into question the concept that the Seattle protocol consistently detects early cancer arising in BE patients with HGD.

218482: Natural History of High Grade Dysplasia in a Regional Veterans Administration Barrett's Cohort. *Gareth S Dulai, Dennis M Jensen, Fasiha Kanwal, Brennan M Speigel, Ian M Gralnek, Paul G Shekelle*

OBJECTIVES: Published data on the natural history of high grade dysplasia (HGD) in Barrett's esophagus (BE) give widely varying estimates of risk for esophageal cancer. The risk of cancer and related mortality may be greater in prevalent than incident cases due to detection of early stage disease in surveillance or bias. Our primary aim was to describe the natural history of prevalent and incident HGD in a large cohort of BE patients. A secondary aim was to compare outcomes in those with prevalent vs. incident cancer. METHODS: Consecutive BE cases from 1988-2002 were identified via pathology databases in a regional VA healthcare system and medical record data were abstracted. The risk of progression to cancer as well as mortality

was measured and compared in cases with prevalent vs. incident HGD/cancer using survival analysis. RESULTS: There were 30 cases of prevalent HGD, six of whom developed cancer over 70 patient-years of follow-up. Three of thirteen cases with incident HGD developed cancer over 136 years of follow-up. The crude rate of cancer was 1 in 12 years for those with prevalent vs. 1 in 45 years with incident HGD ($p=0.085$). Ten cases with prevalent and five cases with incident HGD died, but only 20% were due to cancer. Mortality was 1 in 7 years for those with prevalent vs. 1 in 27 years with incident HGD ($p=0.005$). There were 45 prevalent and 11 incident cancers. Cancer was early stage in 16% of prevalent vs. 45% of incident cases ($p=0.03$). Twenty-eight cases with prevalent and six with incident cancer died. Death was due to cancer in 96% of prevalent vs. 50% of incident cases ($p=0.01$). Mortality rates were 1 in 4 years for those with prevalent vs. 1 in 16 years for incident cancers ($p=0.02$). CONCLUSIONS: In a large cohort study of Barrett's, high-grade dysplasia was associated with a high rate of progression to cancer. The risk of overall mortality was significantly higher in those with prevalent vs. incident high-grade dysplasia and cancer. The reduction in risk may be an effect of surveillance and/or other factors including bias.

226987: The Development and Validation of an Endoscopic Grading System for Barrett's Esophagus-The Prague C and M criteria. Prateek Sharma, David Armstrong, Jacques JGHM Bergman, John Dent, Liebwint Gossner, Yoshio Hoshihara, Janusz A Jankowski, Ola Junghard, Lars Lundell, Guido NJ Tytgat, Michael Veith

Background and Aims Barrett's esophagus (BE) is a pre-malignant condition for esophageal adenocarcinoma, and its diagnosis relies initially on the endoscopic recognition of a columnar lined distal esophagus (i.e. suspected or endoscopic BE). The accuracy of this endoscopic assessment is hampered by a lack of consensus-developed, explicit criteria for the endoscopic diagnosis and grading of its extent. In this study we have developed and validated such criteria. Methods Over several meetings, an international working group agreed on endoscopic diagnosis and grading criteria and developed materials for their formal evaluation by use of video-endoscopic recordings (of the distal esophagus, the gastroesophageal junction (GEJ) and proximal stomach) gathered in a standardized manner in 29 patients. The criteria included assessment of the Circumferential (C) and Maximum (M) extent of the endoscopically visualized BE segment as well as landmarks such as the diaphragmatic hiatus, the GEJ and the proximally displaced squamo-columnar junction. The recordings were scored for multiple criteria by an international panel of 29 endoscopists especially interested in BE (22 of those endoscopists scored C and M for all 29 patients). Results The criteria, now named the Prague C&M Criteria, give explicit guidance on the endoscopic recognition of BE and grading of its extent. Among the 29 patients, the means of the values for 'C' ranged from 0 to 12 cm and for 'M', the range was 0 to 14 cm. The overall reliability coefficients (RC) were excellent for the assessments by the 22 endoscopists of both Circumferential and Maximum extent of the endoscopic BE segment above the GEJ (RC for C value 0.95 and for M value 0.94). For C and M measures, the rates of exact agreement for pair-wise comparisons of individual patient values were 53% and 38% respectively whereas the agreement within a 2 cm interval was 72% and 76% respectively. The overall RC for endoscopic recognition of BE > 1 cm was 0.72, but for < 1 cm, the RC was poor at 0.22. The RC for recognizing the location of GEJ and the diaphragmatic hiatus was 0.88 and 0.85 respectively. Conclusions The Prague C&M criteria have high overall validity for the endoscopic assessment of visualized BE lengths, when used by endoscopists especially interested in BE. For less than 1 cm however, use of the criteria did not result in acceptable levels of diagnostic reliability and the causes for this need further research. The reliability of recognizing endoscopic landmarks is excellent. The criteria represent a useful advance in the endoscopic assessment of BE for clinical practice and research.

226219: Accuracy of Wireless Capsule Endoscopy for the Detection of Barrett's Esophagus. Prateek Sharma, Amit Rastogi, Romeo Esquivel, Krishna Gurram, Sachin Wani, Ajay Bansal, Srinivas R Puli, April Higbee, Lisa Camargo, Richard Sampliner

Introduction: The initial step in the diagnosis of Barrett's esophagus (BE) requires an upper endoscopy to document the presence of a columnar lined distal esophagus (suspected BE), followed by biopsies from the columnar segment. The availability of a wireless esophageal capsule (Pillcam ESO) allows the recording of images from the esophagus and can be potentially used as an office based screening tool in patients with GERD. Aim: To compare esophageal capsule to standard upper endoscopy for the detection of endoscopic Barrett's esophagus. Methods: This study was conducted at 2 sites utilizing standardized methodology, data collection and analysis. Patients with chronic GERD and BE were prospectively evaluated; all patients initially underwent capsule endoscopy followed by standard upper endoscopy. The esophageal capsule is similar to the small bowel capsule, but acquires images from both ends (2 cameras) of the device at a rate of 7 frames/sec/camera. The quality of the images were graded from 1-5 (not scoreable-excellent). Capsule images were analyzed by investigators blinded to the upper endoscopy findings; sensitivity and specificity of these findings were then analyzed using the standard endoscopy findings as the gold standard. Results: Eighty-four patients were initially enrolled in the study, 3 were unable to swallow the capsule, whereas images from 3 patients could not be evaluated. Data from 78 patients were available for analysis; mean age of 56.9±12.3 years; 71 males. By standard endoscopy, BE was suspected in 41 patients; 27 with short BE (<3 cm) and 14 with long BE (>3 cm). The mean BE length was 3.17 cm. The sensitivity and specificity of esophageal capsule endoscopy for the detection of suspected BE were 73% and 86% respectively; sensitivity and specificity for the detection of short BE were 67% and 86% and for long BE were 86% and 86% respectively. The quality of the images did not contribute to the false positive/negative results. No adverse events were noted using capsule endoscopy. Conclusions: Esophageal capsule endoscopy

can be safely performed in the majority of GERD patients undergoing screening upper endoscopy. The sensitivity and specificity of capsule endoscopy for the diagnosis of suspected BE are high especially for long BE. Future studies should test inter observer variability and the learning curve in the reading of images and steps to further improve the diagnostic accuracy capsule endoscopy.

218978: Squamous Overgrowth in a 5-year Randomized Phase III Trial of Photodynamic Therapy using Porfimer Sodium in Ablation of High-Grade Dysplasia in Barrett's Esophagus. *Mary Bronner, Shari Taylor, Bergein Overholt, Kenneth Wang, Steven Burdick, Charles Lightdale, Michael Kimmey, Hector Nava, Michael Sivak, Norman Nishioka, Hugh Barr, Chad Davis, Norman Marcon, Marcos Pedrosa, Michelle Depot*

PURPOSE: Squamous overgrowth may obscure the endoscopic extent of Barrett's epithelium. Although squamous overgrowth has never been rigorously studied, it has been speculated that photodynamic therapy (PDT) increased the risk of subsquamous metaplastic glands. Consequently, its true diagnostic significance was assessed following PDT with porfimer sodium plus 20 mg omeprazole BID [PORPDT] versus 20 mg omeprazole BID [O alone] in patients with high-grade dysplasia (HGD) in Barrett's esophagus (BE) in a large randomized trial. **METHODS:** Patients were randomized (2:1) to PORPDT or to O alone. Patients on PDT received 2 mg/kg i.v. of POR followed by endoscopic laser light exposure of Barrett's mucosa at a wavelength of 630 nm within 40-50 hours up to a maximum of 3 courses administered at least 90 days apart. Starting at baseline, every 3 months patients underwent 4-quadrant jumbo biopsies of their pre-treatment Barrett's length until four consecutive quarterly follow-up results were negative for HGD and then biannually up to 5 years or treatment failure. Biopsies were taken at 2-cm intervals and centrally processed for standardized histologic interpretation by GI pathologists blinded to treatment assignment and patient identity. **RESULTS:** There were 138 patients and 23,473 total biopsies in PORPDT and 70 patients with 10,160 total biopsies in O alone. Occurrence of squamous overgrowth was similar for PORPDT relative to O alone both per patient (31% vs. 33%) and per biopsy (1.2% vs. 2.2%). In no patient was the highest grade of neoplasia per endoscopy found exclusively beneath squamous mucosa. **CONCLUSIONS:** This 5-year large randomized trial with a rigorous surveillance biopsy program documents that squamous overgrowth occurrence is similar following either PORPDT or O alone. This trial confirms that squamous overgrowth is not detrimental to the longer-term safety of PORPDT.

222525: Detection of Residual Barrett's Mucosa after Photodynamic Therapy (PDT) using High Definition Narrow Band Imaging (HD-NBI). *Herbert Wolfsen, Lois L Hemminger, Michael B Wallace*

Endoscopic ablation using porfimer sodium PDT significantly decreases the risk for progression to invasive carcinoma in Barrett's high grade dysplasia (BE+HGD) patients. After PDT, Barrett's mucosa persists in nearly 50% of patients and must be detected and destroyed to prevent metachronous carcinoma. Use of chromoendoscopy with Lugol's dilute iodine for residual disease detection is cumbersome and associated with risk of toxicity. The aim of this study was to compare detection of residual Barrett's mucosa after PDT with standard endoscopy, high definition (HD) endoscopy and HD-NBI. **Methods:** After IRB approval, consecutive patients undergoing treatment with Ps-PDT for BE+HGD were studied using standard definition white light endoscopy (Olympus GIF-Q180), high definition white light endoscopy (Olympus XGIF-H160Y2) and HD-NBI (Olympus XGIF-H160Y2, 415 and 540 nm). Endoscopic imaging was performed at a single session under identical conditions with digital imaging documentation. Detection rates of residual areas of glandular mucosa were compared. **Results:** After Ps-PDT, 50 consecutive BE+HGD patients were studied (42 men, 8 women; mean age 68 yrs, range 51-83 yrs). The pre-Ps-PDT mean BE segment length was 4 cm; range 1-12 cm. The mean time interval after PDT was 20 mos; range 3-66 mos. Areas of persistent glandular mucosa (peninsulas or islands of glandular epithelium) were found in 22 pts using standard endoscopy, 25 pts using HD white light endoscopy and 38 pts using HD-NBI imaging. **Conclusions:** 1) The use of Narrow Band, high definition endoscopic imaging in patients treated with PDT for Barrett's high grade dysplasia detected additional areas of glandular dysplasia in more than 50% of cases compared with standard conventional and high definition video endoscopy. 2) The use of NBI obviated the need for Lugol's chromoendoscopy. 3) Long term studies are underway to determine the impact of NBI on clinical outcomes in patients who have undergone endoscopic ablation therapy.

Biomarkers in the therapy of Barrett's esophagus with high-grade dysplasia. Ganapathy A Prasad, Kenneth K. Wang, Halling C. Kevin, Shannon Brankley, Navteg S. Buttar, Louis M. Wongkeesong, Lori S. Lutzke, Sarah M Papenfuss, K. K. Krishanadath.

Rationale: Photodynamic therapy (PDT) has been shown to be effective in the treatment of high-grade dysplasia (HGD) in Barrett's esophagus (BE), however a substantial proportion of patients do not respond. **Aims:** To determine if biomarkers known to be important in Barrett's neoplasia can predict response to PDT in BE and HDG/mucosal cancer. **Methods:** Patients with BE and HGD/mucosal cancer referred for endoscopy therapy to the Mayo Clinic were prospective studied from 2002-2005. All patients underwent a protocol assessment with EGD, 4 quadrant biopsies every cm, EMR of visible nodules and EUS. Biomarkers were assessed using FISH for region specific and centromeric probes. Biomarkers were assessed included: loss of 9p21 (site of p16 gene) and 17p13.1 (site of p53 gene) loci; gains of 8q24 (c-myc), 17q (HER2-neu) and 20q13

loci. Respective centromeric probes were used to assess chromosomes 9, 17, 20, and 8. Cells were also assessed for loss or gain of chromosomes (aneusomy and polysomy). Patients were treated with PDT 48 hours following administration of sodium porfimer. Demographic and clinical variables were prospectively collected. Logistic regression was performed to determine predictors of response to PDT (defined as the absence of dysplasia at surveillance biopsies 3 months following PDT). Results: 58 patients have been entered with a mean age 67.6 years (SEM1.38). 54 (93%) were males. 41 (82%) had HGD and the remainder had cancer. The mean BE segment length was 5.5 cm (SEM 0.144). 46 (80%) patients had EMR done before PDT. 31 (53%) of patients were responders at 3 months following PDT. On univariate analysis, BE segment length (OR 0.67 [0.53, 0.85] p=0.001), 9p21 (p16) loss (OR 0.15 [0.05, 0.52] p=0.003), were significant predictors of lack of response to PDT. Loss of the 17p13.1 (p 53) locus (OR 0.25 [0.06, 1.15] p=0.08) was a marginally significant predictor. Age, gender, EMR, and other biomarkers were not significant predictors on univariate analysis. On multivariate analysis, BE segment length and loss of p16 locus were independent predictors of response to PDT. The presence of polysomy and/or gain at 8q24/17q/20q13 loci was a marginally significant predictor of response to PDT. (see table) Conclusion: This is the first prospective study which identifies a biomarker, p16, as predicting response to PDT in BE and HGD/cancer. Identification of biomarkers may help in the selection of appropriate therapy for patients and improve treatment outcomes.

Additional Reading: Barrett's Esophagus

211874: Corn Derived Carbohydrate Inositol Hexaphosphate (IP6) inhibits Barrett's Adenocarcinoma Growth by Pro-Apoptotic Mechanisms. *David W McFadden, Dale R Riggs, Barbara J Jackson, Cynthia Cunningham*

INTRODUCTION: Inositol Hexaphosphate (IP6) is a naturally occurring polyphosphorylated carbohydrate found in food sources high in fiber content. IP6 has been reported to have significant inhibitory effects against a variety of primary tumors including breast and colon. The effects of IP6 have not been evaluated in Barrett's adenocarcinoma. We hypothesized that IP6 would significantly inhibit cell growth rate of Barrett's adenocarcinoma in vitro. **METHODS:** Two Barrett's-associated adenocarcinoma cell lines, SEG-1 (wild type p53) and BIC-1 (mutant p53), were treated with corn derived IP6 at 0.5, 1.0, and 5.0mMolar concentrations. After 72 hours, cell viability was measured by MTT assay. Apoptosis and necrosis was evaluated by the Annexin V FITC assay. Statistical analysis was performed by ANOVA. **RESULTS:** Reductions (p<0.01) in cellular proliferation was observed in both cell lines. In the BIC cells, 5mM (80.0%) and 1.0mM (65.4%) of IP6 decreased cell growth (P<0.001). In the SEG1 cells, 0.5mM (55.8%), 1.0mM (93.1%) and 5mM (86.2%) of IP6 decreased cell growth (P<0.001). IP6 increased late apoptotic activity (p=0.046) in the BIC cells. In the SEG-1 cells, early apoptosis (p=0.038), late apoptosis (p=0.002) and necrosis (p=0.036) were all increased by IP6 treatment **CONCLUSIONS:** Treatment of Barrett's adenocarcinoma with the common dietary polyphosphorylated carbohydrate IP6 significantly decreased cellular growth by pro-apoptotic mechanisms. Our findings suggest that IP6 has the potential to become an effective adjunct for Barrett's adenocarcinoma. Further in vivo and human studies are needed to evaluate safety and clinical utility of this agent in patients with Barrett's adenocarcinoma.

214592: Pathologic Distinction Of Barrett s Esophagus From Carditis With Intestinal Metaplasia: A Blinded Interobserver Variability Study. *Amitabh Srivastava, Mark Redston, Gregory Y Lauwers, Donald A Antonioli,Robert D Odze, Jonathan N Glickman*

Background: Barrett s esophagus (BE) and carditis with intestinal metaplasia (CIM) have different etiologies, natural history and risk of malignancy. We have previously identified several morphological parameters, listed below, that are useful in distinguishing these two conditions in mucosal biopsies from the gastro-esophageal junction (GEJ) region. The purpose of this study was to evaluate the level of interobserver agreement among expert GI pathologists in recognizing the various morphological parameters and in distinguishing BE from CIM in GEJ biopsies. **Design:** Routinely processed H&E stained biopsies from the GEJ region, representing 10 of distal esophagus in BE and 10 of proximal stomach in CIM, were evaluated by four GI pathologists in a blinded manner without knowledge of the clinical or endoscopic findings. Each pathologist was asked to categorize cases as either BE or CIM, and to score the presence or absence of the following previously reported BE-associated features: crypt atrophy, crypt disarray, incomplete or complete IM, extensive incomplete IM (>50% crypts), squamous epithelium over metaplastic crypts (Sq/IM), hybrid glands, multilayered epithelium (ME) and esophageal gland/ducts (EG/ED). Interobserver agreement was calculated for each morphological parameter and for the overall diagnosis, using kappa (k) statistics for concordance between multiple raters. Interobserver agreement was rated as excellent (k > 0.80), good (0.6-0.8), fair (0.4-0.6) and poor (<0.4). **Result:** Interobserver agreement was good for the overall diagnosis of BE vs. CIM (k=0.6). Three or more observers correctly diagnosed BE or CIM in 15/20 (75%) cases. The interobserver agreement for each individual morphological parameter ranged from excellent to good for some features (EG/ED: k= 0.83, Sq/IM: 0.65, incomplete IM: 0.56, crypt atrophy: 0.53), fair for extensive IM (0.47), and poor for the others (complete IM: 0.25, crypt disarray: 0.21, and ME: 0.17). Interestingly, when none of the BE-associated features were present (5/20 cases), agreement for a diagnosis of CIM was excellent (3 or more observers agreed in all five cases). Similarly, when four or more BE-associated parameters were present in the same biopsy specimen (10/20 cases), three or more observers agreed on the diagnosis of BE in all cases. **Conclusion:** There

is good agreement among expert GI pathologists for distinguishing BE from CIM in GEJ biopsies when using the morphological features outlined above. In fact, a correct diagnosis of BE is more often reproducible when a combination of these features (>4) are present together in the same biopsy specimen.

219581: DNA Methylation In The Esophageal Mucosa Of Patients 5 Or More Years After A Fundoplication For Barrett's Esophagus. *John J Kelly, Eric Smith, Paul A Drew, Stuart R Phillis, Andrew R Ruszkiewicz Glyn G Jamieson*

Introduction: Barrett's esophagus results from reflux and is a major risk factor for adenocarcinoma of the distal esophagus. Aberrant DNA methylation is frequent in Barrett's esophagus and adenocarcinoma and is thought to contribute to the disease progression. Fundoplication protects the oesophagus from reflux and may result in regression of the Barrett's mucosa, but it is not known what effect it has on methylation in the esophagus. Aim: To test the hypothesis that reflux control by fundoplication in patients with Barrett's esophagus will reduce DNA methylation in the esophageal mucosa. Method: We studied 23 patients with histologically proven Barrett's esophagus preoperatively who had a laparoscopic fundoplication more than 5 years ago. Reflux was measured by 24 hour pH monitoring. Biopsies at 2 cm intervals from any columnar epithelium and squamous epithelium were obtained at endoscopy. Methylation was measured in the promoter region of 9 genes - APC, CDKN2A, ID4, TEMMF2, MGMT, RBP1, RUNX3, SFRP1 and TIMP3. Results: The median follow up was 8 years (60 - 127 months). Of the 18 patients with no reflux, 17 had an apparent regression of the columnar mucosa. This regression was complete in 9 patients (5 long segment and 4 short segment) and partial in 8 patients. Five patients had reflux and persistent Barrett's esophagus. There was significantly less methylation in the columnar epithelium of patients without reflux compared to those with reflux ($p < 0.0001$). The squamous biopsies from patients without reflux but continuing Barrett's had more methylation than either squamous epithelium proximal to the Barrett's in patients with reflux ($p = 0.005$), or from patients with complete regression ($p < 0.001$). This may reflect the squamous biopsies coming from areas in which metaplastic changes are reversing. Conclusions: We observed complete regression to squamous mucosa in 50% of patients with a fundoplication and no acid reflux. In patients with complete regression the regenerated squamous had minimal methylation which did not differ from the squamous proximal to Barrett's. In patients with partial regression there was significantly less methylation in the columnar lining compared to the patients with continuing reflux, but more methylation in the squamous regenerating region than in the normal esophagus. The significance of the methylation in the partially regressed esophagus warrants further investigation.

225227: Correlation of DNA Ploidy Analysis by Digital Image Analysis and Flow Cytometry in Barrett's Esophagus. *Kenneth K Wang Ganapathy Prasad, Navtej S Buttar, Michel WongKeeSong, Peter Rabinovitch, Thomas Smyrk Lynn Borkenhagen, Sarah Papenfuss, Catherine DeMars, Lori Lutzke*

Flow cytometry has been shown to be an important biomarker for cancer risk in Barrett's esophagus. Digital image analysis (DIA) is another method to obtain similar information that requires a much smaller sample of cells for analysis. Aim: To compare the results of DIA and flow cytometry in a consecutive series of patients with Barrett's esophagus and high-grade dysplasia. Methods: Patients referred to the Barrett's Esophagus Unit with high-grade dysplasia were prospectively studied with DIA and flow cytometry. All patients underwent four quadrant biopsies taken every centimeter with histological results reviewed by two expert pathologists. DIA was performed on cytological specimens that were Feulgen stained and then assessed with a CAS 200 digital imaging microscope. A minimum of 50 cells were assessed for DIA. Flow cytometry was performed on histological specimens at the University of Washington. A minimum of 20,000 cells were assessed for flow cytometry. In cases with dichotomous results between DIA and flow cytometry, fluorescent in situ hybridization was performed to determine if genetic abnormalities did exist in p16, p53, c-myc, 20q, and Her-2. Results: 18 patients were assessed, 15 with high-grade dysplasia and 3 with carcinoma found on biopsy. In one case, there was inadequate specimens available for flow analysis. Of the remaining 17 cases, DIA and flow cytometry made concordant assessments of DNA ploidy in 82% of the cases, ($p > 0.3$). These included 5 cases that were diploid and 9 that were aneuploid. There were three discordant pairs that included one that was near diploid on DIA and aneuploid on flow, this case was completely normal on FISH. Two cases were tetraploid on DIA and diploid on flow, Both cases were abnormal by FISH, with loss of heterozygosity of both p53 and p16. Conclusions: DNA ploidy by DIA or flow cytometry were reasonably concordant. In the discordant cases, examination of other biomarkers suggest that DIA may have correlated better with genetic abnormalities. This study suggests that DNA ploidy determined by DIA on cytology may be as useful as flow cytometry in determining cancer risk.

219581: Cancer Risk in Barrett's Oesophagus: A Meta-Analysis. *Titus Thomas, Keith R Abrams, John de Caestecker, Richard J Robinson*

Background: The risk of cancer in Barrett's oesophagus (BO) is uncertain with studies showing a variable annual incidence (from 1/52 to 1/694). Recent reports have suggested regional variations in cancer incidence in the West. However no formal meta-analysis has been performed. Aims and Methods: We aimed to determine by meta-analysis the incidence of oesophageal cancer in patients undergoing surveillance for BO and to examine geographical variation. A MEDLINE, EMBASE and Pub Med search of all English articles from 1966 to 2004, using the key words "Barrett's oesophagus", "Oesophageal cancer",

“surveillance”, “short segment Barrett’s” (SSBO) was done. References in retrieved papers and relevant review articles were scrutinised to identify papers missed on the initial search. Studies with patients who had histological confirmation of BO on surveillance, documented follow up data and cancer as the outcome measure were included. Heterogeneity between studies was calculated using the homogeneity statistic (Q value) and if significant ($p < 0.05$) a random effects model of meta-analysis was used. Short Segment BO was defined as length of < 3 cm. Results: 42 articles were included in the analysis for conventional BO and an additional 7 articles were included for SSBO. The overall cancer prevalence was 11.4% (95% CI 7.6-17.3%). The overall incidence rate for cancer was 8/1000 person-years duration of follow up [pyd] (95% CI, 6-10). There was minimal geographical variation, with the incidence rate in UK being 7/1000 pyd (95%CI 4-12), USA, 8/1000 pyd (95% CI 5-13) and Europe, 8/1000 pyd (95% CI 5-12) The overall cancer incidence in SSBO was 5/1000 pyd (95% CI 2-12). There was a non-significant increase in cancer risk in conventional BO compared to SSBO (OR 1.6, 95% CI 0.56-4.91, $p = 0.30$). Similarly there was a non-significant increase in cancer risk in patients who had medical therapy compared to surgical treatment (OR 1.2, 95% CI 0.29-5.0, $p = 0.78$). There was a non-significant reduction of Barrett’s cancer incidence over time ($p = 0.11$) Conclusion: We found less geographical variation BO cancer risk than previously suggested between US and UK and a non-significant increase in the risk of cancer in conventional BO vs SSBO. The cancer incidence in Barrett’s oesophagus has decreased with time although this is not significant.

224473: HER-2/neu in Barrett’s Esophagus: A Comparative Study Between Histology, Immunohistochemistry and Molecular Technique (FISH). *Elisa Rossi, Domenico Della Casa, Vincenzo Villanacci, Guido Missale, Michele Ghedi GianPaolo Cengia, Renzo Cestari*

Introduction: HER-2/neu is a protooncogene frequently overexpressed in breast cancer and recently found to be overexpressed in other carcinomas and in Barrett’s esophagus (BE). Immunohistochemistry and Fluorescence In Situ Hybridization (FISH) are conventionally used for HER-2 testing in carcinomas, but a single assay is not yet accepted as a “gold standard” in BE. The aim of our study was to evaluate the correlation between histopathologic characteristics and gene expression/amplification along the sequence BE, low grade dysplasia (LGD), high grade dysplasia (HGD) and adenocarcinoma. Methods: we collected 50 endoscopic esophageal biopsies in 42 men and in 8 women with a previous diagnosis of BE. Histopathologic evaluation was carried out using Hematoxylin & Eosin staining. Paraffin embedded tissue was studied using the HercepTest kit for immunohistochemistry (HercepTest DAKO) and PathVysion kit (PathVysion HER-2 DNA Probe Kit, Vysis) for FISH. HercepTest was scored 0, 1+, 2+ and 3+ depending on % membrane staining (cutoff 10%), and assessment of gene amplification was based on the ratio between the copy number of HER2/neu and 17 chromosome. The number of chromosome 17 and HER2/neu signals was scored based on 60 non overlapping nuclei, and HER-2/neu was judged as amplified when the ratio was > 2 . Results: 21 BE, 4 LGD, 12 HGD and 13 cancer of the esophagogastric junction were observed. There was a positive correlation (correlation coefficient 0.88) between gene amplification and protein overexpression. In no cases with HercepTest scoring 0 or 1+ was gene amplification detected, but this was present in 20% of cases scoring 2+ and in all cases scoring 3+. Her-2/neu amplification or overexpression was never observed in BE. Coefficient of correlation between the Her-2/neu gene and protein was lower for LGD and HGD when compared to histology (0.51 and 0.68 respectively). Gene amplification and overexpression was observed in 7 out of 13 adenocarcinomas. Conclusion: Our results suggest that HER-2/neu amplification/overexpression may be considered as a marker of progression from BE to dysplasia. The FISH technique may represent an important and useful diagnostic tool to integrate the result of HercepTest for selecting patients for immunotherapy.

212218: Patients With Barretts Esophagus Experience Less Reflux Complaints. *Ahmed Madisch, Stephan Miehleke, Susanne Sell, Bernd Wigglinghaus, Manfred Stolte*

Background: Barrett’s epithelium is assumed to be more resistant to aggressive stomach contents. Thus, it can be postulated, that sensory perception may be less in patients with Barrett’s oesophagus (BE). Aim: to assess the severity of reflux complaints in consecutive patients presenting with Barrett’s esophagus and reflux esophagitis. Methods: Prospectively consecutive patients with BE (diagnosed by histology) were interviewed by standardized questionnaires comprising both current and history of reflux complaints (heartburn, acid regurgitation). Patients with a reflux esophagitis diagnosed by endoscopy served as a control group. Results: 181 patients with BE (60 female, mean age 58.4 years) and 85 patients with reflux esophagitis (36 female, mean age 50.6 years) were included in the analysis. Severity and frequency of current reflux complaints were significantly more pronounced in patients with reflux esophagitis than in patients with BE ($p < 0.05$). In both groups younger patients (< 60 years) suffer from more severe and frequent reflux complaints than the older patients ($p < 0.05$). Endoscopy in the past was performed in 76.1% of reflux esophagitis patients due to reflux complaints compared to 47.9% in BE patients ($p < 0.0001$). The history of reflux complaints were comparable in both groups ($p > 0.05$). Conclusion: Patients with BE suffer less from reflux complaints than patients with reflux esophagitis. Thus, reflux complaints do not seem to be suitable for symptom-based screening of patients with BE.

226336: Does Degree of Acid Exposure Differ between Distal versus Proximal Esophagus in Patients with Barrett’s Esophagus (BE)? Prateek Sharma, Ajay Bansal, Sachin Wani, Srinivas R Puli, April Higbee, Amit Rastog

Introduction:BE pts have been shown to have abnormal esophageal acid exposure by catheter pH systems with probe placed 5 cm above LES. However, it is not known if acid exposure is higher in distal vs. proximal esophagus of BE pts using a wireless pH system. Aim:In BE pts, compare acid exposure in very distal esophagus (1cm above anatomic GEJ) to conventional location (6 cm above GEJ) and to a group of controls. Methods:Pts with confirmed BE, off PPI therapy for 7 days were enrolled. Under endoscopic vision, 2 pH capsules were placed 1 and 6 cm above GEJ. Controls [subjects without GERD symptoms on 2 validated GERD questionnaires (GERQ and RDQ)] were also studied. All subjects were encouraged to continue usual activities and diet. pH tracings were reviewed. Only tracings consistent with esophageal and not gastric cardia placement and with 16 hrs of data were used for analysis. Proximal and distal pH values in BE pts were compared using paired t-test and Wilcoxon summed ranks test. Correlation between pH (at both locations) and BE length was performed using Pearson’s test and scatter plot. Mean pH values of BE pts were also compared to controls with unpaired t-test. Results: 23 BE pts were enrolled (all Caucasian males) - mean age 65.9 yrs (range:40-81) and mean BE length 3.68±2.7 cm. Data from 19 pts could be analyzed. Within BE group, % time pH<4 in 24 hrs was significantly higher in distal vs conventional position, Table 1. This was independent to BE length.Also, esophageal acid exposure in BE pts was significantly higher at both locations compared to controls, Table 2. Conclusions:BE pts have significantly higher acid exposure in distal compared to proximal esophagus, independent of BE length. Its implications on preferential location of dysplasia/cancer in distal vs. proximal esophagus needs to be evaluated. This issue has special relevance given the significant rise in GEJ adenocarcinoma.

Table 1: Acid Exposure in the Proximal vs. Distal esophagus in BE patients

	Percent time pH <4		paired t test	Wilcoxon summed ranks test	unpaired t test
	Proximal	Distal			
Total	9.89	18.64	p<0.02	p<0.09	p<0.05
Upright	12.15	21.69	p<0.02	p<0.09	p<0.05
Supine	6.31	11.93	p<0.16	p<0.14	p<0.13

Table 2: Comparison of Percent times pH<4 in BE vs. Controls at both locations

Location		BE	Controls	p(unpaired t test)
Esophageal	Total	14.56	4.21	0.01
	Upright	16.74	4.94	0.005
	Supine	11.57	0.74	0.03
GEJ	Total	29.16	10.4	0.01
	Upright	30.88	10.26	0.003
	Supine	26.2	8.54	0.07

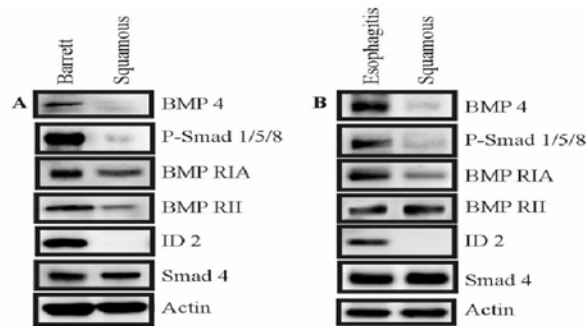
214502: The Burden of Upper Gastrointestinal Endoscopy. Michelle E Kruijshaar, Peter D Siersema Marjon Kerkhof, Ewout W Steyerberg, Marie-Louise Essink-Bot

INTRODUCTION: Upper gastrointestinal (GI) endoscopy is an invasive diagnostic procedure that causes pain, discomfort and psychological distress in patients. Regular endoscopic surveillance, as recommended for patients with Barrett’s esophagus (BE), may therefore be experienced as burdensome by patients. To what extent it is experienced as burdensome and whether the burden decreases when patients get used to it is unknown. AIM: We prospectively assessed the burden of upper GI endoscopy in BE patients under regular surveillance and in a control group of patients with non-specific upper GI symptoms, and investigated whether these groups perceived the burden of endoscopy differently. METHODS: A total of 394 patients (180 BE patients and 214 patients with non-specific GI symptoms) filled out questionnaires one week before, on the day of, one week after and one month after upper GI endoscopy. Inclusion criteria were a BE segment of at least 2 cm for the BE group, and dyspepsia without alarm symptoms for the control group. Four variables were assessed: 1) pain and burden experienced during endoscopy, 2) symptoms, 3) psychological distress levels (Hospital Anxiety and Depression scale and Impact of Event Scale), and 4) perceived risk of developing a malignancy. RESULTS: Patients with non-specific GI symptoms were diagnosed with a hiatal hernia (45%), non-specific gastritis (25%), reflux esophagitis (20%), and other (10%). Of all patients, only 16% experienced pain from the endoscopy. However, 87% reported it to be burdensome. Apart from an increase in sore throat (47% after endoscopy versus 22% before) the procedure did not cause symptoms. Patients’ distress levels were increased in the week

prior to the endoscopy compared to the week after. BE patients experienced significantly more burden from the endoscopy (e.g. 7% reported no burden versus 20% of the control group) but reported lower distress levels compared to the control group. Patients with a higher risk perception did not report a higher burden or more psychological distress. Having had more prior endoscopies was associated with lower distress levels as measured by the impact of event scale. **CONCLUSION** Upper GI endoscopy is burdensome for many patients and causes moderate psychological distress. Getting used to the procedure may reduce distress but increases the burden experienced from the procedure in BE patients. Recommendations for endoscopic surveillance should take into account the burden and distress of upper GI endoscopy for patients.

218095: Bone Morphogenetic Protein (BMP)-4-Mediated Transformation of Inflamed Squamous Esophageal Mucosa into Barrett's Esophagus. *Jantine van Baal, Francesca Milano, Navtej S Buttar, Agnieszka M Rygiel, Floor de Kort, Jacques J Bergman, Kenneth K Wang, Maikel P Peppelenbosch, Kausilia K Krishnadath*

BACKGROUND: To identify genes specifically involved in the transformation of squamous epithelium into Barrett's esophagus (BE), we previously used SAGE to compare the expression profile of BE with normal squamous esophagus (SQ) and gastric cardia mucosa. BMP4 was found to be uniquely expressed in BE. The aim of this study was to investigate the potential role of BMP4 in the metaplastic development of BE. **MATERIAL AND METHOD:** Immunoblotting was performed on patient biopsies of BE, SQ and inflamed SQ mucosa for the presence of BMP4 and protein members of the BMP pathway i.e. BMP Receptor IA and II, Smad 4, P-Smad 1/5/8 and ID2. Furthermore, primary cell cultures of biopsy specimens of SQ and BE were established. The primary cell cultures of SQ cells were treated with rec. h. BMP4 and the BMP4 pathway was investigated by immunoblotting. Immunohistochemistry was performed for analysis of the Cytokeratin (CK) expression pattern and microarray analysis was done to analyze and compare the gene expression profiles. **RESULTS:** Immunoblotting revealed BMP4 expression and activation of associated signaling in BE and inflamed SQ mucosa but not in normal SQ (see Fig). Upon treatment of SQ cells with BMP4 the level of P-Smad 1/5/8 was increased, this was blocked through addition of the BMP antagonist Noggin. Phenotypically, a shift of the CK expression pattern, of the BMP4 treated SQ cells towards that of columnar cell type was found with upregulation of CK7 and CK20 and downregulation of CK10/13. Finally analysis by microarrays demonstrated a shift of the gene expression profile of the BMP4 treated SQ cells towards that of BE cells. **CONCLUSION:** BMP4 is a keyplayer in the transformation of inflamed esophageal mucosa into BE. We propose that the premalignant metaplastic development of BE is mediated by BMP4 and that inhibition of BMP4 signaling offers a novel avenue for the therapeutic management of this disease.



217468: Origin of Barrett's epithelium: Development of columnar characteristics and increased expression of anti-apoptotic proteins in Het-1A squamous esophageal cells made resistant to low pH and bile acids. *Katerina Dvorak, Melissa Chavarria, Claire M Payne, Harris Bernstein, Carol Bernstein, Harinder Garewal*

Background: Barrett's esophagus (BE) is characterized by the replacement of squamous epithelial cells by metaplastic intestinal-like columnar epithelium. The cell of BE origin remains unclear. We favor the hypothesis that BE originates from esophageal stem cells as a result of reflux with gastric and bile acids, two components of refluxate potentially important to the pathogenesis of BE. To test this hypothesis we developed esophageal squamous cells lines resistant to low pH and/or bile acids as in vitro model systems and evaluated them for expression of columnar characteristics and anti-apoptotic proteins. **Methods:** Normal squamous Het-1A cells were gradually exposed to low pH (pH 6 or 5) and/or 0.5mM bile acid cocktail (BA), consisting of equimolar concentrations of glycodeoxycholic, glycocholic, taurocholic, glycochenodeoxycholic and deoxycholic acid. This BA incorporates known bile acids present in the refluxate. Our goal was to develop cell lines able to survive exposure to pH 5 or 6 and/or BA for 3 hours. The resistant cells have thus far been evaluated (1) morphologically using electron microscopy and Giemsa staining in conjunction with brightfield microscopy, (2) for expression of columnar markers, Cdx-2 and villin, by western blot, and (3) for expression of anti-apoptotic proteins, Mcl-1, Bcl-xL, avn and survivin, using western blot and fluorescence microscopy. **Results:** Over 85 weeks, we have gradually developed several cell lines resistant to different conditions for up to 3 hours, including low pH alone (pH 6, 5), 0.5mM BA, and 0.5 mM BA plus low pH (pH 6, 5). An untreated long-passage sensitive cell line was also maintained, as a control, to compare with the resistant cells. The resistant

lines displayed changes with the most significant altered pattern observed in the cells resistant to pH 5 and pH 5 and BA. These cells were morphologically different in appearance and showed enlarged nuclei, condensed mitochondria and lipid droplets by EM. Increased expression relative to control cells, was observed for columnar differentiation markers, villin and Cdx-2, as well as anti-apoptotic proteins, including Mcl-1, survivin, bcl-2 and Bcl-xL. Conclusion: Chronic, repeated reflux with gastric acids and bile acids is the major clinical factor for the development of BE. Our studies show for the first time that squamous cells may show characteristics of transdifferentiation after repeated exposures to low pH and bile acids. These cells also express markers of columnar differentiation and increased levels of antiapoptotic proteins.

224480: PGE2 Regulation by Cytosolic PLA2 α , a Potential Non-COX-2 Chemopreventive Target, During Carcinogenesis in Barrett's Mucosa. Malini Madhavan, Cathrine DeMars, Sarah Papenfuss, Ganapathy Prasad, Lori Lutzke, Marlys Anderson, Louis Wong Kee Song, Kenneth Wang, Navtej Buttar

INTRODUCTION Reflux of gastroduodenal contents into the esophagus increases PGE2 production, which is closely associated with esophageal adenocarcinoma. Cytosolic phospholipase A 2 α (cPLA2 α) catalyzes the release of arachidonic acid (AA), the substrate for PGE2 production, from peri-nuclear membrane phospholipids. Pharmaceutical inhibitors of cPLA2 α are under development to be used as an anti-inflammatory agent in patients. The AIM of this study was to examine the expression of cPLA2 α during carcinogenesis in Barrett's esophagus and its effect on Barrett's epithelial (BE) cell survival. **METHODS** Endoscopic mucosal resections from 10 patients that contained squamous, non-dysplastic and dysplastic Barrett's mucosa as well as esophageal adenocarcinoma were selected and stained with anti-cPLA2 α antibody. Semi-quantitative immunohistochemistry was used to assess cPLA2 α expression in the mucosa. BE cell lines were treated with 20 or 40 μ M of the selective cPLA2 α inhibitor, AACOCF3, with and without AA (30 μ M) and PGE2 (20 ng/ml). Total cell count, apoptotic cells (Caspase 3 and 7 Immunoassay) and potentially proliferating cells (Ki-67) were counted. **RESULTS** Dysplastic Barrett's epithelium had higher expression of cPLA2 α than non-dysplastic Barrett's and squamous epithelium. Adenocarcinoma arising in a background of Barrett's mucosa had the highest expression of cPLA2 α . The expression was both epithelial and stromal. Compared with control- treated cells, treatment with 40 μ M AACOCF3 decreased the proliferation of Barrett's epithelial cells by 34 ± 6.6 % ($p < 0.05$). Treatment with 40 μ M AACOCF3 also increased the percentage of apoptotic Barrett's cells by 69 ± 4.4 % ($p < 0.05$). Exogenous AA, as well as PGE2 treatment, reversed the effect of inhibition of cPLA2 α by AACOCF3 on BE cell proliferation and apoptosis. **CONCLUSION** Expression of cPLA2 α increases progressively and appears to be functionally relevant to the process of carcinogenesis in BE. Inhibition of cytosolic PLA2 α appears to be an important chemopreventive approach as it decreases arachidonic acid, which is the substrate on which the COX-2 enzyme acts to generate PGE2. The inhibition of PGE2 synthesis by decreasing the release of arachidonic acid rather than inhibiting COX-2 may avoid the thrombo-embolic effects of COX-2 inhibition. With this approach, the supply of arachidonic acid will be decreased to both pro and anticoagulation cascades.