DOTTORATO DI RICERCA IN SCIENZE PNEUMO-CARDIO-TORACICHE
DI INTERESSE MEDICO E CHIRURGICO - XIX CICLO

Sede Amministrativa: Università degli Studi di Bologna

ANALISI CLINICO-PATOLOGICA E MOLECOLARE DELL'ADENOCARCINOMA DELLA GIUNZIONE ESOFAGO-GASTRICA IN PAZIENTI CON ETÁ INFERIORE A 40 ANNI

CLINICOPATHOLOGICAL AND MOLECULAR CHARACTERIZATION OF GASTROESOPHAGEAL JUNCTION (GEJ) ADENOCARCINOMA BEFORE AGE OF 40 YEARS

Tesi di Dottorato di:
ALBERTO RUFFATO

Relatore:
Chiar.mo Prof. SANDRO MATTIOLI

Supervisori:
Dr. VALERIE W. RUSCH
Dr. LAURA H. TANG

Coordinatore:
Chiar.mo Prof. SANDRO MATTIOLI

MED/21 CHIRURGIA TORACICA

Anno Accademico 2005-2006

CONTENTS

SUMMARY

Gastroesophageal junction (GEJ) adenocarcinoma are uncommon before age of 40 years. While certain clinical, pathological, and molecular features of GEJ adenocarcinoma in older patients have been extensively studied, these characteristics in the younger population remain to be determined. In the recent literature, a high sensitivity and specificity for the detection of dysplasia and esophageal adenocarcinoma was demonstrated by using multicolor fluorescence in situ hybridization (FISH) DNA probe set specific for the locus specific regions 9p21 (p16), 20q13.2 and Y chromosome.

We evaluated 663 patients with GEJ adenocarcinoma and further divided them into 2 age-groups of ≤ 40 and ≥ 50 years, respectively. FISH with selected DNA probe for Y chromosome, locus 9p21 (p16), and locus 20q13.2 was investigated with formalin fixed and paraffin embedded tissue from surgical resections of 17 younger and 11 older patients. Signals were counted in > 100 cells with each given histopathological category. The chromosomal aberrations were then compared in the 2 age-groups with the focus on uninvolved squamous and columnar epithelium, intestinal metaplasia (Barrett's mucosa), glandular dysplasia, and adenocarcinoma. Comparisons were performed by the $\chi 2$ test, Fisher's exact test, Student's t-test and Mann-

Whitney U-test as appropriate. Survival was estimated by the Kaplan-Meier method with univariate analysis by the log-rank. Significance was taken at the 5% level.

There was no difference in the surgical technique applied in both age groups and most patients underwent Ivor Lewis esophagectomy. Among clinical variables there was a higher incidence of smoking history in older patient group. We identified a progressive loss of Y chromosome from benign squamous epithelium to Barrett's mucosa and glandular dysplasia, and, ultimately, to a near complete loss in adenocarcinoma in both age groups. The young group revealed significantly more losses of 9p21 in both benign and neoplastic cells when compared to the older patients group. In addition, we demonstrated an increase in the percentage of cells showing gain of locus 20q13.2 with progression from benign epithelium through dysplasia to adenocarcinoma with almost the same trend in both the young and the older patients.

When compared with the older age-group, younger patients with GEJ adenocarcinoma possess similar known demographics, environmental factors, clinical, and pathologic characteristics. The most commonly detected genetic aberrations of progressive Y chromosomal loss, 9p21 locus loss, and 20q13 gains were similar in the younger and older patients. However the rate of loss of 9p21 is significantly higher in

young patients, in both the benign and the neoplastic cells. The loss of 9p21, and possibly, the subsequent inactivation of p16 gene may be one of the molecular mechanisms responsible for the accelerated neoplastic process in young patients.

INTRODUCTION

A rapid increase in the incidence of adenocarcinoma of the distal esophagus and gastroesophageal junction (GEJ) has been observed in Western countries in the last fifteen years. This has reanimated the debate on possible etiologic factors, early diagnosis, and treatment for these tumors (1-4). A number of hypotheses have been proposed with regard to risk factors for the development of GEJ adenocarcinomas that broadly can be divided as environmental and genetic influences (5, 6). It is well recognized that patients with longstanding reflux esophagitis and subsequent intestinal metaplasia (Barrett's esophagus) are at risk for the development of adenocarcinoma that arises within the context of progressive molecular alterations extending through low and high grade dysplastic stages (7-9). However, the incidence of adenocarcinoma in Barrett's esophagus is <5% per year (10) thus clearly Barrett's mucosa cannot be the only criterion to recognize patients who are at risk for acquiring esophageal adenocarcinoma. Therefore, prognostic parameters which can reliably predict malignant progression in Barrett's esophagus are required. It is well documented that GEJ adenocarcinoma is most commonly diagnosed in white male patients in their sixth and seventh decades of life. The median age for patients with adenocarcinoma treated by esophagectomy is 60 to 63

years, and it is extremely rare in patients under 40 years of age (11, 12). It has been reported that the survival of young patients with these tumors is poorer than that of their older patient counterparts (13) although the relationship between the clinicopathologic characteristics and age of patients with esophageal adenocarcinoma is not clear. In addition to our knowledge of known predisposing environmental factors, genetic risk factors may also play a significant role in the development of GEJ adenocarcinoma. Characterization of the underlying molecular mechanisms that promote cancer progression could potentially lead to identification of predictive genetic markers that classify patient's malignant risk. Many studies have identified common genetic alterations associated with a well defined pathological progression from intestinal metaplasia (IM) to low grade dysplasia (LGD), to high grade dysplasia (HGD), and then to carcinoma. Clinical, pathological, and molecular features of GEJ adenocarcinoma in older patients have been extensively studied, but the distribution of these characteristics in the younger population remains unknown. Genes or genetic loci that have been found to be frequently altered include 3p21, 5p15, 5q21-22, EGFR, q36.1, C-myc, p16, p53, Her-2/neu, 20q13.2 and the Y chromosome (14-23). In the recent literature, a high sensitivity and specificity for the detection of dysplasia and esophageal adenocarcinoma was demonstrated by using

multicolor fluorescence in situ hybridization (FISH) DNA probe set specific for the locus specific regions 9p21 (p16), 20q13.2 and Y chromosome. (24).

AIM OF THE STUDY

In an attempt to better understand the causative pathways and to update information on the genetic risk factors related to GEJ adenocarcinoma, we evaluated the clinical features and the role of Y chromosome, the locus 9p21 (p16) loss, and the ploidy of locus 20q13.2 in a spectrum of GEJ mucosa including benign squamous and columnar epithelium, glandular dysplasia, and the invasive carcinoma of patients who underwent surgical treatment under the age of 40 years, and compared their profile with those of more commonly encountered age group (fifth – sixth decades).

METHODS

Patients

We evaluated 663 patients admitted to surgical treatment for GEJ adenocarcinoma and further divided them into 2 age-groups of ≤ 40 and ≥ 50 years, respectively. We identified 29 patients who developed adenocarcinoma under the age of 40 years (mean age of 34.8 ranged from 21–39 years). We retrospectively analyzed and compared the patient's demographics, tobacco exposure and presence of gastroesophageal reflux disease (GERD) between the two age-groups. In addition, we also examined the esophagectomy technique, the neoadjuvant chemoradiation therapy status and post resection pathologic findings. The pathologic stage was assessed with the 2002 American Joint Commission on Cancer staging system.(25)

The disease specific survival was calculated from the date of operation. Patients were followed for the survival through November 30 2006, which constituted the censoring date. The study was performed in accordance with the guidelines of the Institutional Review Board in existence at the time of this analysis.

Operative procedure

Selection of operation was based on identification of the tumor location and depth of tumor invasion and the status of regional lymph node involvement. An Ivor Lewis procedure consisted of a laparotomy and a right thoracotomy with an anastomosis in the chest. A thoracoabdominal procedure consisted of a single left thoracoabdominal incision with an anastomosis in the left chest. A McKeown procedure consisted of a laparotomy, a right thoracotomy, and a left cervical incision, with an anastomosis in the neck. A transhiatal procedure included a laparotomy and a left cervical incision, with an anastomosis in the neck. A transabdominal procedure consisted of a laparotomy only, with an anastomosis above the hiatus and up to the level of the inferior pulmonary vein.

Material

Formalin fixed and paraffin embedded tissue sections from surgically resected specimens were examined by gastrointestinal pathologists to select pertinent sections to include the spectrum of histopathological variant of GEJ mucosa for the subsequent investigation by FISH.

Fluorescence in situ hybridization (FISH)

FISH was performed on serial 4-μm tissue sections. The following commercially available fluorescence-labeled, locus-specific (LSI) and centromere DNA probes (CEP) (Vysis Inc, Downers Grove, IL, USA) were applied according to the manufacturer's instructions: LSI 9p21 SpectrumOrange/CEP9 SpectrumGreen, LSI 20q13.2 SpectrumOrange and CEP Y (α satellite) SpectrumOrange.

More than or less than twice the number of red signals than green centromeres signals were scored as a DNA sequence copy number gain or loss for LSI probe, respectively. More than or less than twice the number of green signals were scored as a DNA sequence copy number gain or loss for CEP Y (α satellite) probe, respectively. Digital images were acquired with a Zeiss Axioplan2 imaging microscope (Carl Zeiss Jena GmbH, Jena, Germany) equipped with a PlanApochromat 100×/NA 1.4 oil objective lens and appropriate filter settings for DAPI, SpectrumGreen (FITC), and SpectrumOrange illumination and detection.

FISH with selected DNA probes were investigated and compared within the groups focusing on uninvolved squamous and columnar epithelium, intestinal metaplasia, glandular dysplasia, and adenocarcinoma. A minimum of one hundred cells were enumerated per hybridization. Nuclei from normal squamous epithelium or

lymphocytes present on the same slide were used as controls of hybridization efficiency and specificity.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package, version 13.0 (Chicago IL) by the χ^2 test and Fisher's exact test for comparison of proportions, and the Mann-Whitney U-test for comparison of non-parametric data. Comparison of means was based on the Student's t-test. Survival was estimated by the Kaplan-Meier method with univariate analysis by the log-rank. Significance was taken at the 5% level.

RESULTS

Clinical and Pathological Features

From the 663 patients admitted to surgery for EGJ adenocarcinoma at our institution there were 539 males and 124 females. The patient's clinical and pathologic characteristics are summarized in Table 1. There was no significant difference in male to female ratio and presence of symptoms of GERD within the two age groups. The smoking history was more frequently reported in the older patients (72% vs. 48%) p=0.001. Most patients underwent Ivor Lewis esophagectomy without a significant difference regarding the age. Approximately half of the patients in both groups received neoadjuvant chemo or chemo-radio therapy due to their locally advance disease. The two age populations presented with similar pathological characteristics regarding presence of Barrett's epithelium, glandular dysplasia, and histological differentiation of their tumors. Localized disease (pTNM stage 0, I, and IIA) was present in 62% young patients and 53% older patients (p=0.79), respectively. Survival rate was also similar in the two groups with a three years disease specific survival 54% in patients ≤ 40 years and a 52% in the older ones (p=0.93), respectively.

Table 1

Clinical and pathological characteristics

Patients	N	Mean age	M:F	Tobacco	GERD history	(Path) Barrett's	P Stage 0, I & IIa	Neo Adjuvant	3-Yr DFS	3-Yr OS
≤ 40 y.o.	29	34.5	23: 6	48.3%	31%	41.4%	62.1%	65.5%	54.1%	64%
≥ 50 y.o.	634	65.7	516: 118	72.1%	35%	45.7%	52.7%	48.7%	52.2%	54%
p value		*<.001*	*0.78*	*<.001*	*0.66*	*0.83*	*0.79*	*0.06*	*0.93*	*0.52*

Chromosomal Aberrations by FISH Analysis

A total of 28 FISH experiments on 17 patients who were ≤ 40 years and on 11 patients who were ≥ 50 years were performed using LSI 9p21 (p16) SpectrumOrange/CEP9 SpectrumGreen and LSI 20q13.2 SpectrumOrange probes. FISH experiment using CEP Y (α satellite) SpectrumOrange was performed on male patients only (12 patients ≤ 40 years and on 7 patients ≥ 50 years. The control group of 11 patients was randomly selected according to reliably match all the clinical and pathological characteristics of the entire group of patients ≥ 50 years that underwent surgery for EGJ adenocarcinoma.

Y Chromosome Loss

Y chromosomal loss has been previously established as a common chromosomal aberration in GEJ adenocarcinoma. In our comparative study between the young and the older patient group, it was apparent that there was a progressive loss of Y chromosome from benign squamous epithelium to Barrett's mucosa and dysplasia and a near complete loss in adenocarcinoma (90% loss in young and 92% loss in older group) (Table 2 and figure 1A, 1B). While there was a slight trend of more losses during this pathologic progression in older patients, they were not statistically significant different from the young group (Figure 2).

Table 2

Average percentage of Y chromosome loss in the different histological categories in the two age groups.

Patients	N	Average percentage of Y chromosome **loss** (mean±sd)			
		Normal Squamous	**Barrett's (if present)**	**Dysplasia**	**Adenocarcinoma**
≤ 40 y.o.	12	13.9±7.6	35.2±12.4	70±18.3	90.1±2.19
≥ 50 y.o.	7	9±2.72	48.7±15.4	86.6±10.1	92.2±5.74
p value		0.273	0.249	0.179	0.360

Figure 1A

Representative examples of squamous cells with biallelic nuclei for Y chromosome (red signal) detected by FISH on formalin fixed and paraffin embedded tissue section.

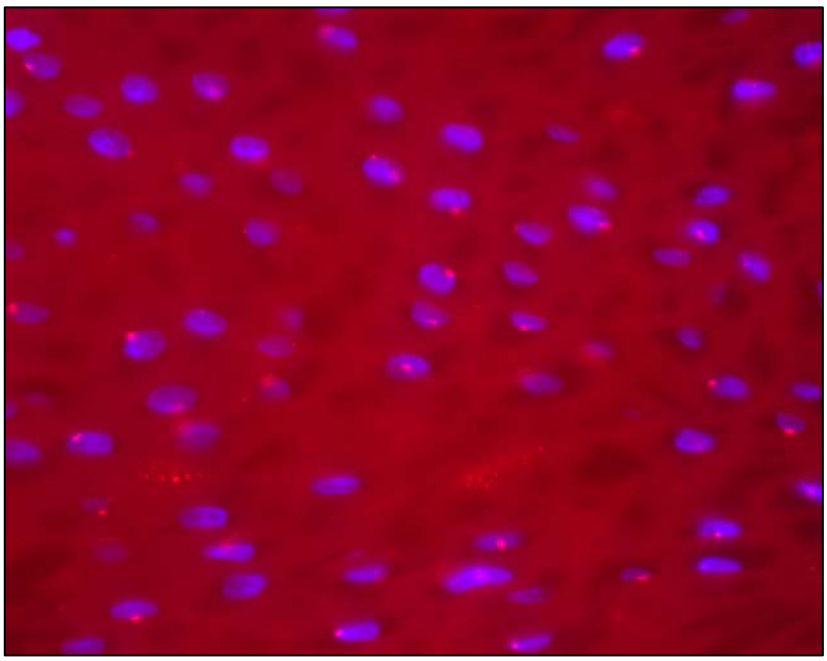


Figure 1B

Representative examples of dysplastic cells with monoallelic nuclei for Y chromosome (red signal) detected by FISH on formalin fixed and paraffin embedded tissue section.

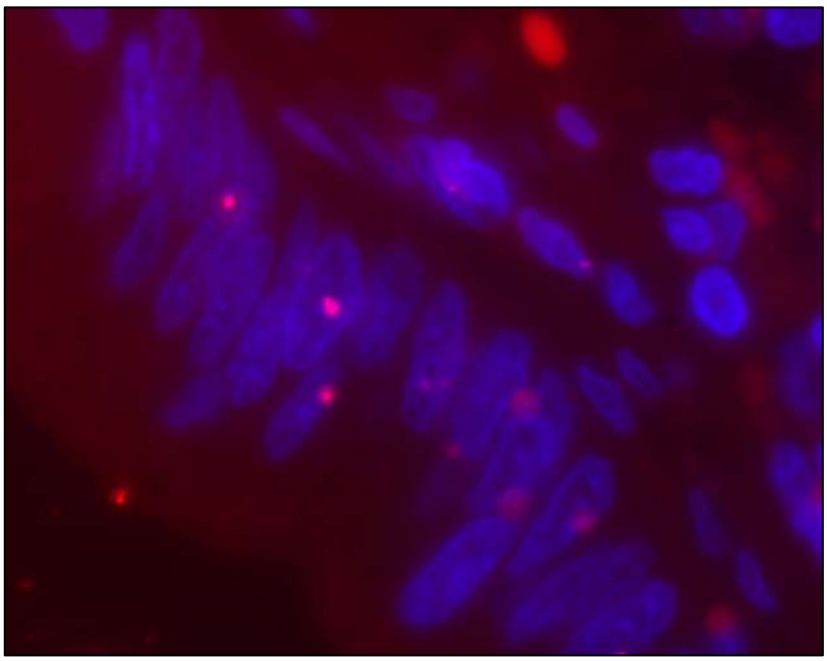
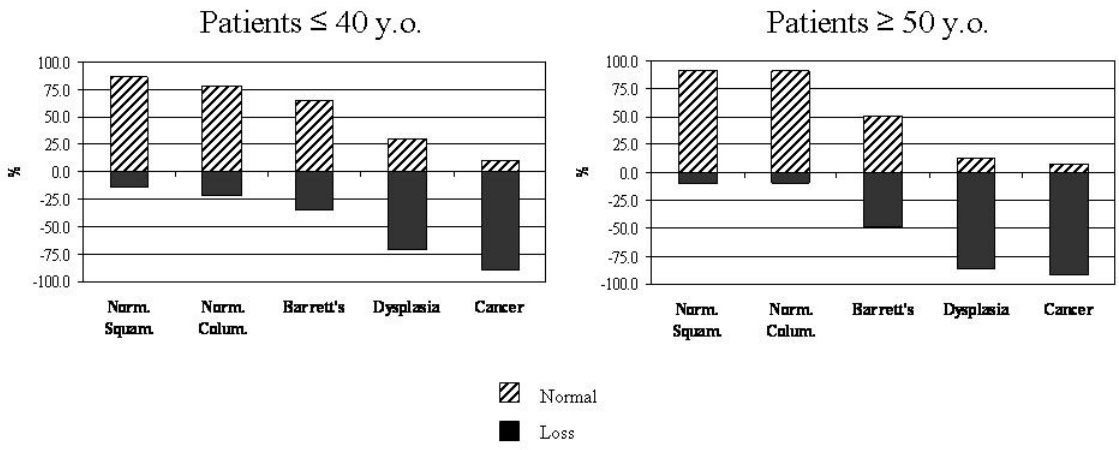


Figure 2

Average percentage of cells in GEJ mucosa with normal ploidy or loss for Y chromosome in the different histological categories in the two age groups.



9p21 (p16) Loss

Loss of 9p21 and mutation of the CDKN2/p16 gene have been reported to occur in early lesions during neoplastic progression in Barrett's esophagus. In this study, we demonstrated a significant increase in the percentage of cells showing loss of locus 9p21 during the progression from benign squamous epithelium through dysplasia to adenocarcinoma in both groups (Table 3 and figure 3). The young group revealed significantly more losses of 9p21 in both benign and neoplastic cells when compared to the older group, whereas the percentage of loss was not significantly different in Barrett's and in the dysplastic epithelium between the two age group (figure 4). The significance in loss 9p21 was not altered regardless the presence of absence of Barrett's status in each group (Table 4 and figure 5).

In addition, when the young patient group was further stratified into those with the condition of Barrett's and those without, the average percent of 9p21 loss was significantly higher in benign epithelium (19.7 ± 8.1 vs. 12 ± 2.5, p=0.0158) in the former; and the losses of 9p21 in dysplastic epithelium (51.2 ± 7.2 vs. 53.4 ± 9.4, p=0.6163) and in adenocarcinoma (84.7 ± 14.4 vs. 79.6 ± 3.8, p=0.3208) were similar regardless the Barrett's status (Table 5 and figure 6).

Table 3

Average percentage of locus 9p21 (p16) loss in the different histological categories in the two age groups.

Patients	N	Average percentage of 9p21 **loss** (mean±sd)				
		Normal Squamous	**Normal Columnar**	**Barrett's (if present)**	**Dysplasia**	**Adenocarcinoma**
≤ 40 y.o.	17	13.9 ± 5.1	25 ± 6.9	45 ± 6.4	48.5 ± 7.9	81 ± 7.6
≥ 50 y.o.	11	6.6 ± 4.3	14.6 ± 5.8	55.3 ± 11	46 ± 18.2	39.2 ± 10.5
p value		**0.0006**	**0.0003**	**0.0042**	0.6201	**< 0.0001**

Figure 3

Representative examples of tumor cells with aneuploidy nuclei for locus 20q13.2 (red signal) detected by FISH on formalin fixed and paraffin embedded tissue section.

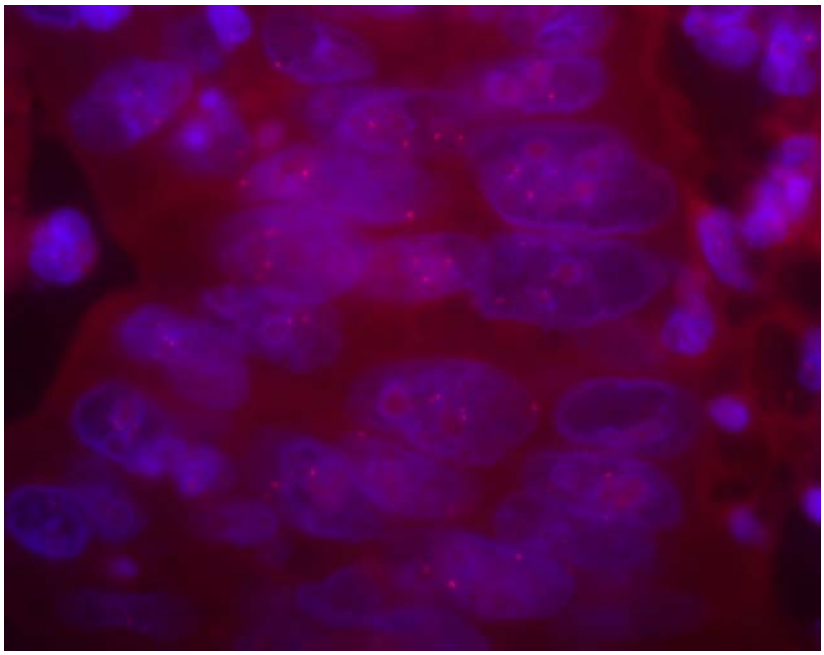


Figure 4

Average percentage of cells in GEJ mucosa with normal ploidy or loss for locus 9p21 (p16) within the different histological categories in the two age groups. (*= p<0.05)

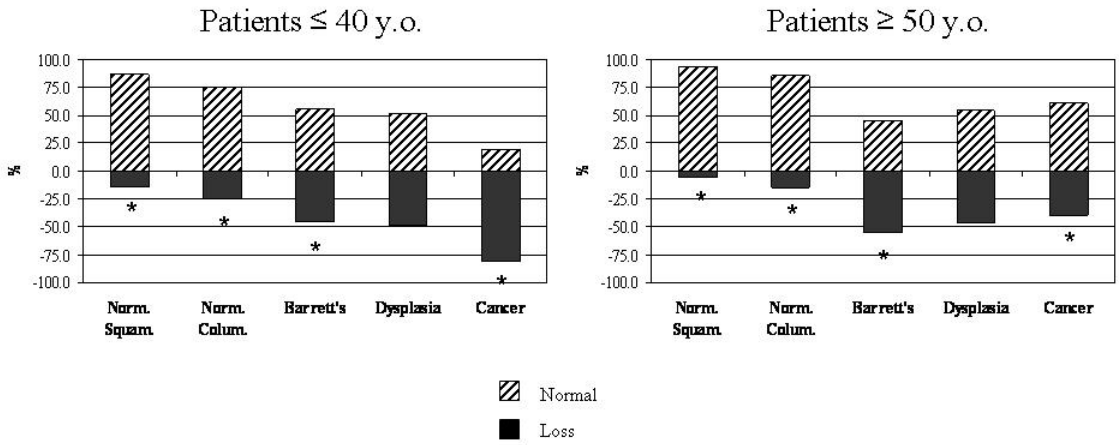


Table 4

Average percentage of locus 9p21 (p16) losses within the different histologic categories in the two age groups in patients with Barrett's metaplasia only.

Patients	N	Average percentage of 9p21 **losses** (mean±sd)				
		Normal Squamous	**Normal Columnar**	**Barrett's Esophagus**	**Dysplasia**	**Adenocarcinoma**
Young	8	19.7 ± 8.1	27.9 ± 11.1	45 ± 6.4	51.2 ± 7.2	84.7± 14.4
Old	11	6.6 ± 4.3	14.6 ± 5.8	55.3 ± 11	46 ± 18.2	39.2 ± 10.5
p value		**0.0003**	**0.0033**	**0.0042**	0.4570	< **0.0001**

Figure 5

Average percentage of cells in the surgical specimens with normal ploidy or loss for locus 9p21 (p16) within the different histologic categories in the two age groups in patients with in patients with Barrett's metaplasia only. (*= $p<0.05$)

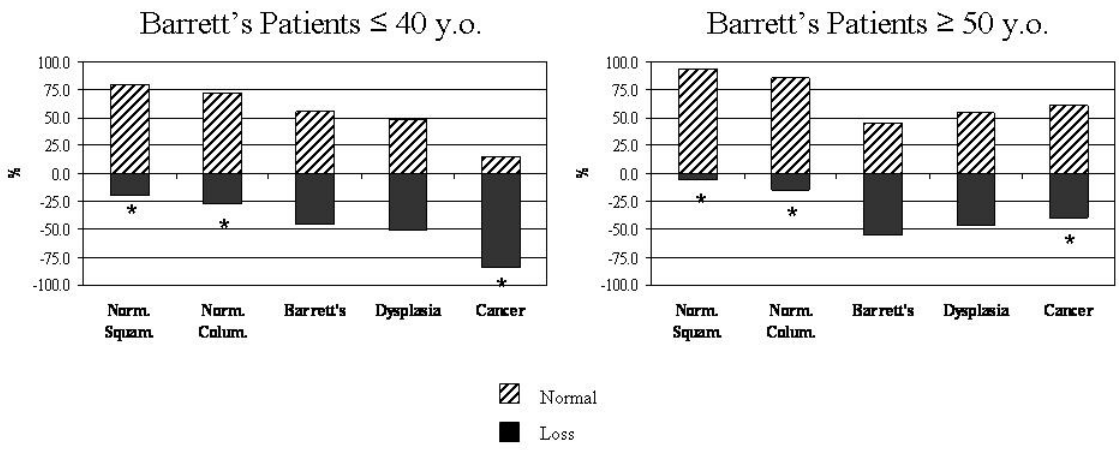


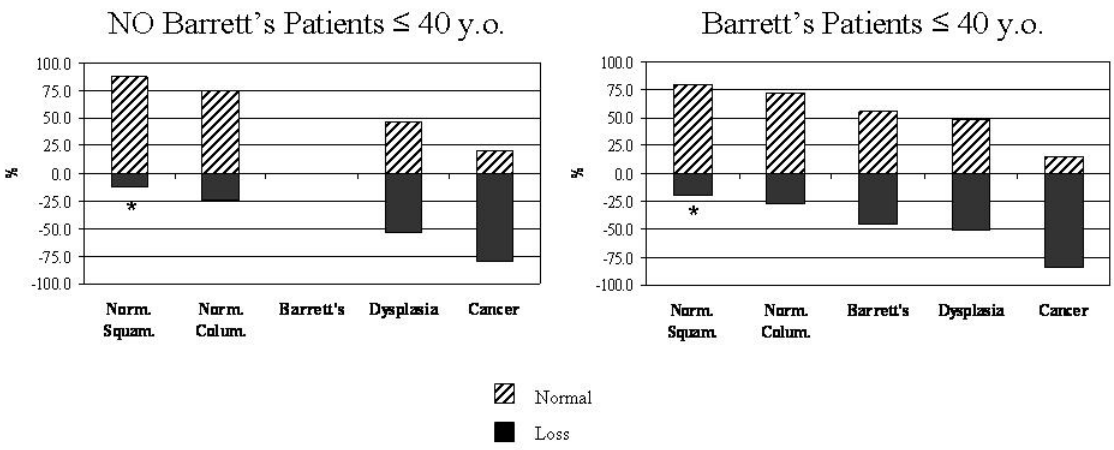
Table 5

Average percentage of locus 9p21 (p16) losses within the different histologic categories in patients ≤ 40years with and without Barrett's metaplasia only.

Patients	N	Average percentage of 9p21 **losses** (mean±sd)				
		Normal Squamous	**Normal Columnar**	**Barrett's Esophagus**	**Dysplasia**	**Adenocarcinoma**
No Barrett	9	12± 2.5	24.1 ± 7.3	-	53.3 ± 9.4	79.6 ± 3.8
Barrett	8	19.7 ± 8.1	27.9 ± 11.1	45 ± 6.4	51.2 ± 7.2	84.7± 14.4
p value		**0.0158**	0.4121	-	0.6163	0.3208

Figure 6

Average percentage of cells in the surgical specimens with normal ploidy or loss for locus 9p21 (p16) within the different histologic categories in patients ≤ 40years with and without Barrett's metaplasia only. (*= p<0.05)



20q13.2 Gain

In contrast to the loss of Y chromosome and 9p21, the gains of locus 20q13.2 was less significant with progression from benign epithelium through dysplasia to adenocarcinoma (Table 6 and figure 7). A significant difference between the two age group was only detected in the Barrett's epithelium (young 14.4 ± 4.1 vs. older 11.1 ± 1, p= 0.015) and in a marginal difference in the adenocarcinoma (young 25.0 ± 3.5 vs. older 27.6 ± 2.7, p= 0.0466). Nevertheless both groups presented almost the same trend of progressive gain of 20q13.2 during the dysplastic and neoplastic transformation to carcinoma (Figure 8).

Table 6

Average percentage of locus 20q13.2 gain within the different histologic categories in the two age groups.

Patients	N	Average percentage of 20q13.2 **gain** (mean±sd)				
		Normal Squamous	**Normal Columnar**	**Barrett's (if present)**	**Dysplasia**	**Adenocarcinoma**
≤ 40 y.o.	17	0.3 ± 0.4	1 ± 1.2	14.4 ± 4.1	16.6 ± 6.9	25.0 ± 3.5
≥ 50 y.o.	11	0	0.5 ± 1	11.1 ± 1	12.2 ± 6.8	27.6 ± 2.7
p value		-	0.2621	**0.0150**	0.1095	**0.0466**

Figure 7

Representative examples of tumor cells with monoallelic nuclei for p16 (red signal) retaining two copies of chromosome 9 (green signal) detected by FISH on formalin fixed and paraffin embedded tissue section.

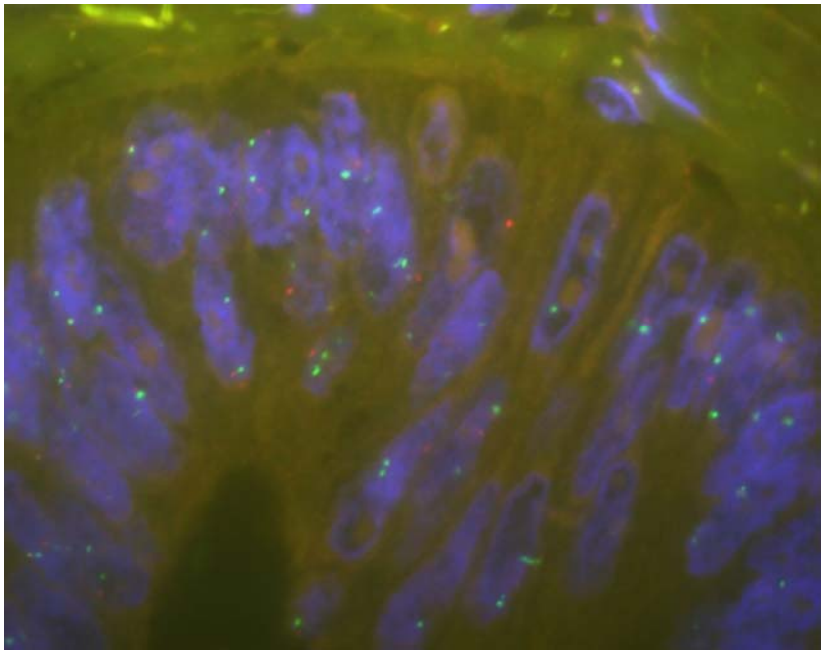
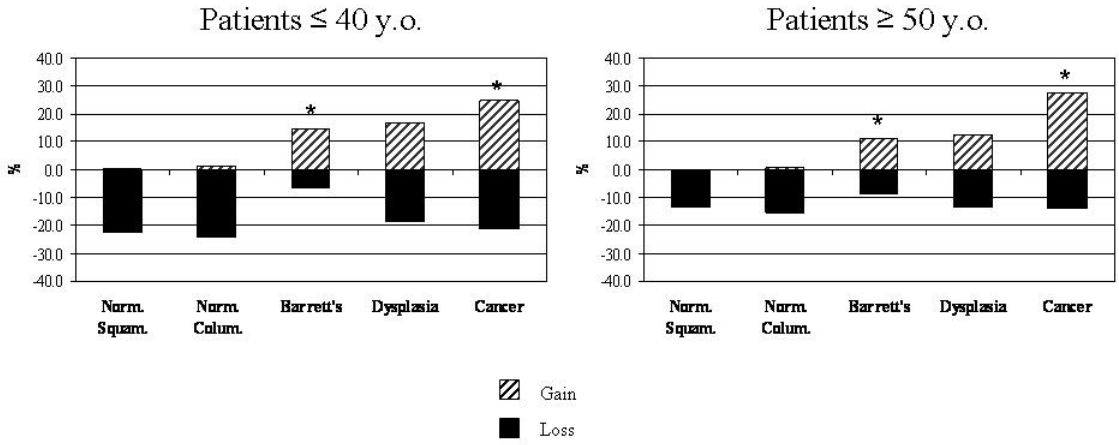


Figure 8

Average percentage of cells in GEJ mucosa with gain or loss of locus 20q13.2 within the different histological categories in the two age groups. (*= $p<0.05$)



DISCUSSION

The cause of the increase in the incidence of adenocarcinoma of the esophagus and gastroesophageal junction in all age groups over the past two decades is unclear. Parallel to the increase in overall incidence, an increase in the number of patients <50 years old as well as those with early intramucosal cancer has been recorded (11, 26). In young patients, the cancer is often diagnosed in a more advanced stage, possibly because of a delay in diagnosis or because of a more biologically aggressive (13).

Our data suggest that, when compared with the older age-group, young patients with GEJ adenocarcinoma possess similar known demographics, environmental factors, clinical and pathologic characteristics. Patients in both groups are predominantly males with a history of tobacco exposure, gastroesophageal reflux disease and Barrett's metaplasia. There was no significant difference in prevalence of early stage disease or disease specific survival when matched for tumor stage. An equal group of patients in each group received neoadjuvant chemoradiation therapy. Thus, our findings do not support the results reported suggesting an higher prevalence of late-stage disease in younger patients at the time of initial diagnosis (11).

FISH and comparative genomic hybridization (CGH) studies have found both gains and losses of chromosomes in the dysplastic and malignant GEJ tissue (16, 23, 24, 27-29).

The most consistent numerical chromosomal aberration found in karyotyping and in similar studies is the loss of the Y chromosome (30, 31). In GEJ adenocarcinoma, Y chromosome loss was found in 31% to 93% of the tumors (32). In one study, the frequency of Y chromosome loss in Barrett's esophagus increased along with the grade of dysplasia (33). Although Barrett's associated adenocarcinoma occurs more commonly in men, no specific oncogene or tumor suppressor genes have been assigned to the Y chromosome. It has been proposed that as genetic instability increases during the malignant transformation of Barrett's mucosa, Y chromosome loss occurs in a random fashion rather than through a specific pathogenic mechanism (32). Evaluation of Y chromosome status by FISH in the sequence normal mucosa – metaplasia – dysplasia – adenocarcinoma has been previously reported by authors (24, 27). Our results confirm the progressive Y chromosome loss in a similar rate in both the young and the older patient populations. Thus the loss of Y chromosome is associated with the development of GEJ adenocarcinoma of male patients in general, but is not restricted to the older age group in particular.

Frequent allelic loss of locus 9p12 and the deletion of corresponding p16 tumor suppressor gene have been reported to occur early in GEJ adenocarcinoma pathway (15, 19), and increase progressively in the metaplasia-dysplasia-carcinoma sequence. The p16 gene encodes a 16-kD protein that forms complexes with the cyclin-dependent kinases CDK4 and CDK6, and subsequently inhibits their ability to phosphorylate the retinoblastoma protein. Unphosphorylated retinoblastoma protein prevents the cell from entering the S phase of the cell cycle. Thus, inactivation of p16 gene may lead to uncontrolled cell growth. Barrett et al. reported a higher prevalence (23%) of p16 gene mutations in GEJ adenocarcinoma with loss of heterozygosity of 9p21 (20). It has been proposed that inactivation of p16 may occur via a number of different molecular mechanisms and p16 inactivation could be a useful biomarker to stratify the risk of progression of Barrett's metaplasia to dysplasia and neoplasia (34).

In our investigation, the young group reveals significantly more losses of 9p21 in both benign and neoplastic cells when compared to the older group. In addition, the loss was significantly higher in patients with Barrett's esophagus than those without the condition within the young age group. A significant loss of 9p21 in benign squamous and columnar epithelial cells has not been previously reported. The finding of locus 9p21 genomic loss in majority of dysplasia/adenocarcinoma

cases in this study was consistent with the previous observation that the deletion of p16 gene locus constitutes a major alteration accompanying the progression from Barrett's esophagus related dysplasia to adenocarcinoma (20, 28, 35). However, the observation of a >80% loss of 9p21 in carcinoma cells in young patients has exceeded the previously reported loss in adenocarcinoma of GEJ in general patient populations (35% - 70%) (24, 27, 36). It is thus plausible that the loss of 9p21 and the subsequent inactivation of p16 in benign appearing and pre-metaplastic epithelial cells constitutes one of the molecular mechanisms that are responsible for the accelerated and early development on adenocarcinoma in young patients.

Chromosome arm 20q was a frequent target of DNA copy gain, usually consisting of gain of the entire chromosome, with a few tumors showing gain of the long arm alone (37).

In particular, 20q13 has been shown to be a frequent site of amplification in breast cancer and amplification of this locus has been associated with immortalization of cells in tissue culture (38). Walch et al. described amplification of locus 20q13.2 by FISH in a subset of patients with esophageal adenocarcinoma (29), and this feature has been confirmed by other studies (39, 40). The most frequently upregulated genes associate with this gain are ZNF217, BCAS1 and CYP24 (41).

Similar to previously reported data, our studies have demonstrated an increase in the percentage of cells showing locus 20q13.2 gain with progression from benign epithelium through dysplasia to adenocarcinoma with almost the same trend in the young and the older group. While a marginal statistical difference are detected in the percentage of nuclei with abnormalities in Barrett's epithelium and in adenocarcinoma between the two age groups, it is unclear that this difference is responsible for the cancerogenetic process in these patients.

CONCLUSIONS

Patients with GEJ adenocarcinoma who are ≤ 40 years old share similar clinical and molecular findings with those reported in older patients. Thus GEJ adenocarcinomas in younger are likely to evolve from similar mechanisms of tumor pathogenesis. Possible additional molecular alterations responsible for an eventual accelerated neoplastic process in young patients may include an early and a significant loss and inactivation of p16 gene among other molecular and genetic mechanisms.

Current recommendation regarding indications for endoscopy surveillance in patients with symptomatic reflux have focused on patients over 50-60 years of age (42). Despite advances in endoscopic technology, screening and surveillance strategies for early detection of these tumors have had limited efficacy in preventing these deadly tumors (43). Advances in technologies and molecular diagnosis offer the promise to understand the pathogenesis of this type of adenocarcinoma; among those, FISH may offer a more practical and accurate surveillance tool for patients with GEJ adenocarcinoma. It is clear that young patients with Barrett's esophagus are not immune to the development of adenocarcinoma and that a liberal use of a molecularly-tailored diagnostic approach may be an appropriate

measure to increase the rate of disease detected at an early curable stage.

REFERENCES

1. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 1998;83: 2049-53.

2. Pera M, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. Gastroenterology 1993;104: 510-3.

3. Heitmiller RF, Sharma RR. Comparison of prevalence and resection rates in patients with esophageal squamous cell carcinoma and adenocarcinoma. J Thorac Cardiovasc Surg 1996;112: 130-6.

4. Sharma P. Cancer of the esophagogastric junction: epidemiology and pathogenesis. J Gastrointest Surg 2002;6: 516-7.

5. DeMeester SR. Adenocarcinoma of the esophagus and cardia: a review of the disease and its treatment. Ann Surg Oncol 2006;13: 12-30.

6. Marsman WA, Tytgat GN, ten Kate FJ, van Lanschot JJ. Differences and similarities of adenocarcinomas of the esophagus and esophagogastric junction. J Surg Oncol 2005;92: 160-8.

7. Iravani S, Zhang HQ, Yuan ZQ, *et al.* Modification of insulin-like growth factor 1 receptor, c-Src, and Bcl-XL protein expression

during the progression of Barrett's neoplasia. Human pathology 2003;34: 975-82.

8. Krishnadath KK, Reid BJ, Wang KK. Biomarkers in Barrett esophagus. Mayo Clinic proceedings 2001;76: 438-46.

9. Sarbia M, Geddert H, Klump B, Kiel S, Iskender E, Gabbert HE. Hypermethylation of tumor suppressor genes (p16INK4A, p14ARF and APC) in adenocarcinomas of the upper gastrointestinal tract. International journal of cancer 2004;111: 224-8.

10. Sharma P, Falk GW, Weston AP, Reker D, Johnston M, Sampliner RE. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2006;4: 566-72.

11. Portale G, Peters JH, Hsieh CC, *et al.* Esophageal adenocarcinoma in patients < or = 50 years old: delayed diagnosis and advanced disease at presentation. The American surgeon 2004;70: 954-8.

12. Scott Bolton J, Wu TT, Yeo CJ, Cameron JL, Heitmiller RF. Esophagectomy for adenocarcinoma in patients 45 years of age and younger. J Gastrointest Surg 2001;5: 620-5.

13. Bowrey DJ, Clark GW, Rees BI, Williams GT, Carey PD. Outcome of oesophagogastric carcinoma in young patients. Postgraduate medical journal 1999;75: 22-6.

14. Riegman PH, Burgart LJ, Wang KK, *et al.* Allelic imbalance of 7q32.3-q36.1 during tumorigenesis in Barrett's esophagus. Cancer research 2002;62: 1531-3.

15. Sanz-Ortega J, Hernandez S, Saez MC, *et al.* 3p21, 5q21, 9p21 and 17p13.1 allelic deletions are potential markers of individuals with a high risk of developing adenocarcinoma in Barrett's epithelium without dysplasia. Hepato-gastroenterology 2003;50: 404-7.

16. van Dekken H, Vissers CJ, Tilanus HW, Tanke HJ, Rosenberg C. Clonal analysis of a case of multifocal oesophageal (Barrett's) adenocarcinoma by comparative genomic hybridization. The Journal of pathology 1999;188: 263-6.

17. Varis A, Puolakkainen P, Savolainen H, *et al.* DNA copy number profiling in esophageal Barrett adenocarcinoma: comparison with gastric adenocarcinoma and esophageal squamous cell carcinoma. Cancer genetics and cytogenetics 2001;127: 53-8.

18. Weiss MM, Kuipers EJ, Hermsen MA, *et al.* Barrett's adenocarcinomas resemble adenocarcinomas of the gastric cardia in terms of chromosomal copy number changes, but relate to squamous cell carcinomas of the distal oesophagus with respect to the presence of high-level amplifications. The Journal of pathology 2003;199: 157-65.

19. Wong DJ, Paulson TG, Prevo LJ, *et al.* p16(INK4a) lesions are common, early abnormalities that undergo clonal expansion in Barrett's metaplastic epithelium. Cancer research 2001;61: 8284-9.

20. Barrett MT, Sanchez CA, Galipeau PC, Neshat K, Emond M, Reid BJ. Allelic loss of 9p21 and mutation of the CDKN2/p16 gene develop as early lesions during neoplastic progression in Barrett's esophagus. Oncogene 1996;13: 1867-73.

21. Blount PL, Ramel S, Raskind WH, *et al.* 17p allelic deletions and p53 protein overexpression in Barrett's adenocarcinoma. Cancer research 1991;51: 5482-6.

22. Croft J, Parry EM, Jenkins GJ, *et al.* Analysis of the premalignant stages of Barrett's oesophagus through to adenocarcinoma by comparative genomic hybridization. European journal of gastroenterology & hepatology 2002;14: 1179-86.

23. Riegman PH, Vissers KJ, Alers JC, *et al.* Genomic alterations in malignant transformation of Barrett's esophagus. Cancer research 2001;61: 3164-70.

24. Brankley SM, Wang KK, Harwood AR, *et al.* The development of a fluorescence in situ hybridization assay for the detection of dysplasia and adenocarcinoma in Barrett's esophagus. J Mol Diagn 2006;8: 260-7.

25. Greene FL, American Joint Committee on Cancer., American Cancer Society. AJCC cancer staging manual. 6th ed. New York: Springer-Verlag; 2002.

26. Eloubeidi MA, Mason AC, Desmond RA, El-Serag HB. Temporal trends (1973-1997) in survival of patients with esophageal adenocarcinoma in the United States: a glimmer of hope? The American journal of gastroenterology 2003;98: 1627-33.

27. Doak SH, Jenkins GJ, Parry EM, *et al.* Chromosome 4 hyperploidy represents an early genetic aberration in premalignant Barrett's oesophagus. Gut 2003;52: 623-8.

28. Fahmy M, Skacel M, Gramlich TL, *et al.* Chromosomal gains and genomic loss of p53 and p16 genes in Barrett's esophagus detected by fluorescence in situ hybridization of cytology specimens. Mod Pathol 2004;17: 588-96.

29. Walch AK, Zitzelsberger HF, Bruch J, *et al.* Chromosomal imbalances in Barrett's adenocarcinoma and the metaplasia-dysplasia-carcinoma sequence. The American journal of pathology 2000;156: 555-66.

30. Krishnadath KK, Tilanus HW, Alers JC, Mulder AH, van Dekken H. Detection of genetic changes in Barrett's adenocarcinoma and Barrett's esophagus by DNA in situ hybridization and immunohistochemistry. Cytometry 1994;15: 176-84.

31. Menke-Pluymers MB, van Drunen E, Vissers KJ, Mulder AH, Tilanus HW, Hagemeijer A. Cytogenetic analysis of Barrett's mucosa and adenocarcinoma of the distal esophagus and cardia. Cancer genetics and cytogenetics 1996;90: 109-17.

32. Wijnhoven BP, Tilanus HW, Dinjens WN. Molecular biology of Barrett's adenocarcinoma. Annals of surgery 2001;233: 322-37.

33. Krishnadath KK, Tilanus HW, van Blankenstein M, *et al.* Accumulation of genetic abnormalities during neoplastic progression in Barrett's esophagus. Cancer research 1995;55: 1971-6.

34. Galipeau PC, Prevo LJ, Sanchez CA, Longton GM, Reid BJ. Clonal expansion and loss of heterozygosity at chromosomes 9p and 17p in premalignant esophageal (Barrett's) tissue. Journal of the National Cancer Institute 1999;91: 2087-95.

35. Falk GW, Skacel M, Gramlich TL, Casey G, Goldblum JR, Tubbs RR. Fluorescence in situ hybridization of cytologic specimens from Barrett's esophagus: a pilot feasibility study. Gastrointestinal endoscopy 2004;60: 280-4.

36. Walch AK, Zitzelsberger HF, Bink K, *et al.* Molecular genetic changes in metastatic primary Barrett's adenocarcinoma and related lymph node metastases: comparison with nonmetastatic Barrett's adenocarcinoma. Mod Pathol 2000;13: 814-24.

37. Moskaluk CA, Hu J, Perlman EJ. Comparative genomic hybridization of esophageal and gastroesophageal adenocarcinomas shows consensus areas of DNA gain and loss. Genes, chromosomes & cancer 1998;22: 305-11.

38. Savelieva E, Belair CD, Newton MA, *et al.* 20q gain associates with immortalization: 20q13.2 amplification correlates with genome instability in human papillomavirus 16 E7 transformed human uroepithelial cells. Oncogene 1997;14: 551-60.

39. Rosenberg C, Geelen E, MJ IJ, *et al.* Spectrum of genetic changes in gastro-esophageal cancer cell lines determined by an integrated molecular cytogenetic approach. Cancer genetics and cytogenetics 2002;135: 35-41.

40. Albrecht B, Hausmann M, Zitzelsberger H, *et al.* Array-based comparative genomic hybridization for the detection of DNA sequence copy number changes in Barrett's adenocarcinoma. The Journal of pathology 2004;203: 780-8.

41. van Dekken H, Vissers K, Tilanus HW, *et al.* Genomic array and expression analysis of frequent high-level amplifications in adenocarcinomas of the gastro-esophageal junction. Cancer genetics and cytogenetics 2006;166: 157-62.

42. Souza RF, Spechler SJ. Concepts in the prevention of adenocarcinoma of the distal esophagus and proximal stomach. CA: a cancer journal for clinicians 2005;55: 334-51.

43. Sampliner RE. Should patients with GERD be screened once at least for Barrett's epithelium? Pro: The need to screen GERD patients for Barrett's esophagus--a greater yield than surveillance. The American journal of gastroenterology 2004;99: 2291-3.