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Inflammation and oxidative stress markers and esophageal adenocarcinoma incidence in a Barrett's esophagus cohort

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Abstract

BACKGROUND—Persons with Barrett's esophagus experience increased risk of esophageal adenocarcinoma (EA). Prediagnostic inflammation markers predict several cancers, but their role in predicting EA is unknown.

METHODS—We investigated whether biomarkers of inflammation (C-reactive protein (CRP), Interleukin-6 (IL-6), soluble tumor necrosis factor (sTNF) receptors I and II), and of oxidative stress (F2-isoprostanes) predicted progression to EA in a prospective cohort of 397 Barrett's patients, 45 of whom developed EA. Biomarkers were measured in stored plasma samples from two timepoints during follow-up, the mean of which served as the primary predictor. Adjusted hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox regression.

RESULTS—CRP level above the median was associated with 80% increased risk of EA. The HR and 95%CI adjusted for age, gender, and further adjusted for waist-hip ratio and smoking were 1.98 (1.05–3.73) and 1.77 (0.93–3.37), respectively, with p-trend for continuous CRP = 0.04. Persons with IL-6 levels above the median also had almost twofold increased risk (HR and 95% CI adjusted for age and gender, and further adjusted for waist-hip ratio and smoking were 1.95(1.03–3.72) and 1.79(0.93–3.43), respectively but no evidence of a trend was observed. Concentrations of TNF receptors and F2-isoprostanes were not associated with EA risk.

CONCLUSIONS—Further research is needed to evaluate the role of inflammation and associated markers in EA development in persons with Barrett's esophagus.

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IMPACT—This prospective study suggests that inflammation markers, particularly CRP and IL-6, may help identify persons at higher risk of progression to EA.

Keywords

C-Reactive protein; Interleukin-6; soluble tumor necrosis factor receptors; isoprostanes; chronic inflammation; esophageal adenocarcinoma; Barrett's esophagus

INTRODUCTION

Chronic inflammation has been hypothesized to play an important role in the pathogenesis of cancers of the lung, colon and other organs.(1–5) Similarly, oxidative stress has been implicated in cancers of the lung(6), breast(7), and prostate(8). Inflammation may contribute to cancer development through multiple mechanisms, including DNA damage, angiogenesis, promotion of cellular proliferation, and inhibition of apoptosis.(1) Inflammatory processes also lead to generation of reactive oxygen species (ROS) which may cause inactivating mutations in tumor suppressor genes or post-translational modifications in DNA repair proteins, thus promoting carcinogenesis.(1, 5)

In the gastrointestinal tract, several chronic inflammatory conditions have been associated with cancer: inflammatory bowel disease with colorectal cancer(9), hepatitis B & C with liver cancer(10), and chronic *Helicobacter pylori* gastritis with gastric cancer.(11) Similarly, inflammatory conditions of the esophagus, namely reflux esophagitis and Barrett's esophagus (BE), are implicated in the development of esophageal adenocarcinoma (EA), with BE widely considered to be a pre-malignant lesion for EA.(12–14) The inflammatory link with EA is further strengthened by the observation that regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin are associated with decreased risk.(15–17)

The incidence of EA has increased dramatically over the past four decades.(18) Although the relative risk of EA is at least 30 times higher in individuals with BE compared to those without,(19) absolute risk of progression is relatively low (0.12–0.6% per year)(20–22), and it is not yet clear which persons with BE are most likely to develop EA, or whether lifestyle modifications might help prevent EA within this higher-risk population. Previous studies identified obesity, cigarette smoking, gastroesophageal reflux and diet as potential modifiable risk factors for EA.(14, 23) However, the roles of inflammation and oxidative stress as potentially modifiable risk factors or predictors for EA have not been studied directly. In this report, we assess the association between markers of systemic inflammation and oxidative stress and the subsequent risk of EA in a well-characterized BE cohort followed for up to 14.5 years.

MATERIALS AND METHODS

Study population, follow-up & cancer ascertainment

The Seattle Barrett's Esophagus Study (SBES), is a prospective cohort study aimed at understanding the risk factors and mechanisms underlying neoplastic progression to EA among persons with BE. The SBES cohort is one of the largest and longest-running well-characterized cohorts of persons with Barrett's esophagus in the world. Details of the cohort

have been described previously.(23, 24) The study involves periodic endoscopic surveillance for all participants, with multiple biopsies of the Barrett's segment. The current report includes the 427 SBES participants with BE and no history of esophageal cancer enrolled between February 1995 and September 2009, of whom 411 (96.3%) had at least one follow-up visit. At their baseline visit, participants underwent an extensive personal interview, anthropometric assessment, endoscopy with biopsy and blood draw. At subsequent follow-up visits, baseline information was updated, additional blood was collected and a repeat endoscopy with biopsies was performed.(12, 13, 25) Specimens were fixed, processed and interpreted by a single pathologist blinded to the participants' exposure status.(12) Individuals were classified as having BE, low-grade dysplasia, high-grade dysplasia or EA based on their most severe histological diagnosis. Participants with high grade dysplasia at their initial endoscopy (80/411 participants; 19.46%) were endoscoped twice more within 5 months to detect any occult cancers missed at baseline. Of the 411 SBES participants eligible for analyses, 14 individuals had less than 5 months of follow-up and 11 of these developed cancer. Due to an *a priori* concern that cancers diagnosed during this early period of intensive search for occult malignancies may have been present at baseline, these 14 individuals were excluded from the primary statistical analyses. EA was defined as invasion of neoplastic epithelium beyond the basement membrane of the esophageal mucosa into the surrounding lamina propria, muscularis mucosa or submucosa. (12) This study was approved by the Institutional Review Boards at the University of Washington and Fred Hutchinson Cancer Research Center.

Inflammation marker measurements

Fasting blood samples were collected, processed (most within 2 hours after collection) and stored at −80°C until analysis. Potential inflammation markers were identified from the literature, and selected for use in this study based on the sensitivity and accuracy of available assays, giving consideration to their cost. Plasma levels of CRP, IL-6 and sTNF receptors were measured using samples from the first two available time points (baseline and first follow-up for most participants, mean duration between two samples 1.8 years). F_2-isoprostanes were measured at a single time point (earliest available, baseline for most). Intra- and inter-batch coefficients of variation (CV) were calculated using blinded pooled plasma samples included as quality controls within each batch. Intraclass correlation coefficients (ICC) and 95% confidence intervals (CI) were calculated using the two cytokine measurements per participant, as described previously.(26)

Briefly, CRP concentrations were measured in never-thawed plasma samples with a high-sensitivity assay using immunonephelometry (Dade Behring Inc, Deerfield, IL) [Interbatch CV 2.9%, ICC(95% CI) 54.9% (47.4,62.5)]. The assay detectable limit was 0.2 mg/L; a value of 0.1 mg/L was assigned to all participants with CRP concentrations below the detection limit (< 1% samples). IL-6 was assayed in never-thawed plasma samples using the Quantikine HS human IL-6 Elisa kit (R&D Systems Inc, Minneapolis, MN; HS600B) [Inter-batch CV 4.4%, intra-batch CV 4.1%, ICC(95% CI) 56.4% (49.4,63.4)]. Samples were run in duplicate with a median duplicate CV of 2.7%; samples with CVs greater than 12% were re-run and the repeat measurements used for analysis. sTNFR-I and sTNFR-II were measured on previously thawed and re-frozen plasma samples using the MILLIPLEX MAP

Human Soluble Cytokine Receptor Panel (Millipore, Billerica, MA; HSCR-32K) [sTNFR-I: inter-batch CV 8.9%, intra-batch CV 5.9%, ICC(95% CI) 89.2% (87.2,91.3); sTNFR-II: inter-batch CV 6.1%, intra-batch CV 2.4%, ICC(95% CI) 84.9% (82.1,87.8)]. Samples were run in duplicate with a median duplicate CV of 4.1% and 3.4% for sTNFRI and sTNFRII, respectively. F_2-isoprostanes were estimated in never-thawed plasma samples using gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICI-MS) as described previously (6890N Agilent gas chromatograph, 5973 quadruple mass spectrometer, Santa Clara, CA).(27, 28) This assay had a precision of ±3%, accuracy of 97% and a detection limit of 20 pg/ml.

Other covariates

Detailed information on medical, family, and medication histories, as well as on lifestyle exposures such as tobacco use, were collected in a personal interview conducted by trained staff. Anthropometric measurements including height, weight, and waist, hip, thigh, and abdominal circumferences were determined using an established protocol(23) and were used to calculate waist-hip ratio(WHR) and body mass index (BMI) (weight (kg)/height $(m)^2$), which was categorized as normal (<25 kg/m^2); overweight (≥25 and <30 kg/m^2); and obese (≥30 kg/m^2). Cumulative pack-years of smoking were computed using the number of packs smoked per day and the number of years smoked.

Statistical Analysis

We estimated the associations between markers of inflammation and oxidative stress and the risk of EA by calculating hazard ratios (HR) and 95% CIs using separate Cox models for each biomarker. As we dropped individuals with less than five months of follow-up from our primary statistical analyses, time at risk to development of EA began 5 months post-baseline for all participants. For participants with inflammation marker measurements available at two time points during the follow-up, the mean of the two measures was used as the primary predictor; otherwise the lone measure was used. If the outcome occurred during the same visit as a biomarker measurement, only the first measurement was used for risk prediction. *A priori*, we decided to exclude CRP values over 10 mg/L from analyses due to the possibility that such elevated concentrations may be the result of acute rather than chronic inflammation.(29, 30) Eight participants had both their CRP measures over 10 mg/L and hence were dropped from all CRP-related analyses. Biomarker concentrations were tested for associations with EA risk using three models: unadjusted; age- and gender-adjusted; and adjusted for obesity, smoking and NSAID use in addition to age and gender. These specific confounders were selected based on the previously established risk factors of EA (increasing age, male gender, smoking, higher WHR, use of NSAIDs)(23, 31) determined *a priori*, and their correlations with the biomarkers, based on Pearson's correlation coefficients. Although it is more conventional to adjust for the effects of obesity by adjusting for BMI, we chose to adjust for WHR (a measure of central adiposity) in the current analyses as there is good evidence that WHR is more strongly associated with BE as compared to BMI.(32) Analyses were conducted on quartiles of the various biomarkers, as well as by median level. Analyses were repeated restricted to males (number of females was too small for meaningful results). Tests for trend were based on the likelihood-ratio test associated with addition of the biomarker being evaluated in its continuous form. A two-

sided p-value less than 0.05 was considered statistically significant. The assumption of proportional hazards over time was tested. All analyses were performed using STATA statistical package, release 12 (StataCorp, College Station, TX).

RESULTS

Participant characteristics for the 411 individuals eligible for current analysis are presented in table 1. The majority of the cohort was Caucasian (96.6%) and male (81.3%), with a mean age of 61.2 years. Over 39% of participants were obese, 64% were current or former smokers, 81.5 % reported regular alcohol use in their lifetime and 60.6% had regularly taken NSAIDs at some point in their life. The mean WHR for the entire cohort was 0.95 (males 0.96, females 0.87).

The medians and interquartile ranges for the various biomarkers overall and by gender are presented in table 2 (distributions by gender were based on sex-specific quartiles). Overall, the distributions of the various biomarkers evaluated in this study are comparable to other studies of older populations and obese individuals.(33–35)

The Pearson correlation coefficients between the biomarkers and risk factors for EA are shown in table 3. The correlations between the biomarkers themselves were small to moderate (0.12–0.63), and were statistically significant (except IL-6 and F_2-isoprostanes). Therefore, these biomarkers were evaluated in separate models with respect to their EA risk. Most of the biomarkers were significantly correlated with age, WHR, and cigarette pack-years, suggesting that these factors might confound the biomarker-EA associations.

Table 4 presents the HRs and 95% CIs for developing EA according to biomarker levels among the 397 participants in the primary analysis. They were followed for a median of 6.14 years (31,677 person-months), and 45 developed cancer. Plasma samples were not available for three individuals. For analyses involving CRP, we omitted eight participants for whom all available CRP values were over 10 mg/L (2 out of these 8 developed EA; total 43 cancers in analyses based on CRP). Ultimately, analyses were conducted on 394 participants for IL-6 and sTNF receptors, 386 participants for CRP and 377 participants for F_2-isoprostanes.

Mean CRP levels above the median of 1.9 mg/L were associated with a two-fold increased EA risk compared to those with values below the median, after adjustment for age and gender (HR 1.98; 95% CI 1.05–3.73, p_{trend} for continuous CRP= 0.01). Further adjustment for WHR, smoking and NSAIDs attenuated the association somewhat (HR 1.77; 95% CI 0.93–3.37, p_{trend} for continuous CRP= 0.04). Analyses limited to men revealed slightly stronger associations with CRP.

Participants with average IL-6 levels above the median had a two-fold increased risk for EA (HR 1.95; 95%CI 1.03–3.72) but no evidence of a trend was observed (p_{trend} = 0.94). The increase in risk was more pronounced among males, with an almost three-fold increased risk (HR 2.85, 95% CI 1.38–5.92) after adjustment for age and gender. Overall as well as in the subgroup analysis for males, additional adjustment for obesity, smoking and NSAIDs had little effect. No evidence of an association between sTNF-RI and EA risk was observed. For sTNF-RII, although univariate analyses overall and among males revealed statistically

significant associations, adjustment for confounders attenuated the association substantially such that it was no longer statistically significant. Circulating levels of F_2-isoprostanes were not associated with increased risk of EA in this cohort.

To evaluate if the significant associations observed with CRP and IL-6 were more pronounced within the first few years of follow-up, we conducted subanalyses restricting the follow-up time to 3 and 5 years from baseline (Figure 1). The associations with CRP were stronger and the statistical trends more pronounced (p_{trend} for continuous CRP = 0.02 in a fully adjusted model) with the restricted 5 year follow-up. The associations with IL-6 did not alter much after restricting follow-up to 3 or 5 years. We repeated our main analyses after adding the 14 individuals with less than 5 months of follow-up that we had initially excluded, with no important differences found (data not shown). To examine whether prolonged storage of biological samples affected our results, we also conducted a sensitivity analysis restricted to only those individuals whose biological samples had been stored for 10 years or less (mean storage time for the earlier of the two samples from an individual was 12 years). We found that while the results for CRP were slightly stronger and those for IL-6 were weaker as compared to the main statistical analysis, the overall conclusions remained the same (data not shown). As IL-6 may be produced by the metaplastic epithelium(36), we also investigated the effect of adjustment for BE segment length in models involving IL-6 and found that the point estimate was reduced by a small amount - from 1.79 to 1.60.

We also computed an “inflammation score” based on the quartile categories for various biomarkers such that individuals in the lowest quartile for a biomarker received a score of 0 and those in the highest quartile received a score of 3. Using the summed combined score as the primary predictor, we found that EA risk non-significantly increased by 2% per unit increase in the score in adjusted models (data not shown).

DISCUSSION

In this prospective study, we observed that elevated pre-diagnostic blood concentrations of CRP and possibly IL-6 are associated with subsequent increased incidence of EA among persons with BE. Plasma levels of soluble TNF receptors or isoprostanes were not statistically significantly associated with EA risk.

The role of inflammation and resulting oxidative stress in the development of cancer has been the focus of extensive research.(3, 37, 38) More recently, inflammation markers, particularly CRP and IL-6, have been reported to be associated with all cancers in prospective and nested case-control studies. In a prospective Danish cohort of 10,000 individuals, the risk for any cancer associated with CRP levels over 3 mg/L was 1.3 (95%CI 1.0–1.6)(39); a similar 1.2-fold (95% CI, 1.10–1.32) increased risk for any cancer with increasing CRP was observed in a Greek cohort.(40) In another prospective study involving the Health Aging and Body Composition cohort, risk for all cancers increased with higher levels of CRP, IL-6 and TNF-α.(41) In studies looking at individual cancer sites, the evidence for a possible role of chronic inflammation is the strongest for colon cancer.(39, 41–44) Elevated levels of inflammatory markers have also been shown to be associated with cancers of the lung(39–41, 45, 46), breast(40, 41, 46) and ovary.(47) Oxidative stress

markers such as F_2-isoprostanes and oxodeoxyguanosine have been shown to be associated with cancers of colon, lung, prostate and breast.(6–8, 48, 49).

Our study adds to the accumulating evidence for a key role of inflammation in EA development, even among persons already diagnosed with BE. Exposure of the esophageal epithelium to bile salts and acid resulting from gastroesophageal reflux can cause chronic inflammation of the lower esophagus and result in increased release of pro-inflammatory mediators(50–52), which may in turn cause DNA damage and promote progression.(1, 50) In support of this hypothesis, an experimental study showed that BE tissue secretes significant amounts of IL-6 resulting in an increased expression of STAT3 transcription factor that may lead to neoplastic conversion.(36) In another study, the levels of ROS were higher in biopsy specimens from BE patients than those from controls suggesting that they play a role in the tissue injury associated with BE.(53) In our own cohort, we have previously shown that leukocyte telomere length, a measure of person's long-term inflammation level and oxidative damage(54) was associated with more than a three-fold increased risk of EA (p-trend 0.009)(55), and that anti-inflammatory drugs such as NSAIDs reduce the risk of EA even among those with dysplastic changes in their Barrett's segment.(15) Here, we show a link between pre-diagnostic concentrations of inflammation markers and EA among BE patients, suggesting that pathways involving inflammatory biomarkers present prevention targets.

Blood-based markers of chronic systemic inflammation may also reflect systemic response to other exposures that predispose to esophageal cancer, such as obesity. Inflammatory cytokines including IL-6 and TNF-α have been observed to be systematically elevated in obesity,(56, 57) and dietary intervention studies have shown that weight loss reduces CRP levels among obese individuals.(58) We have previously reported a modest increase in EA risk associated with measures of central adiposity.(23) Results from the present study indicate that CRP and IL-6 may be predictive in EA development even after adjustment for confounding effects of obesity in BE patients. Taken together, these results suggest that inflammation markers may mediate the association of obesity with EA, but they also have some independent effect on EA development beyond their effect through obesity.

This study has several strengths. Its prospective design allowed for measurement of multiple markers of inflammation and oxidative stress prior to the development of cancer, minimizing the possibility of reverse causality. For the majority of the biomarkers, we also were able to assess plasma levels at two time points during follow-up, thus reducing random measurement error and the potential for regression dilution bias.(59, 60) Additionally, measuring the biomarkers at two time points enabled us to improve on the ICCs reported earlier (methods section) by capturing some of the intraperson variation. For example, the ICC of 0.55 for CRP that we reported earlier is actually improved to 0.71 just by averaging over two CRP measurements.(61) We also blinded the laboratory personnel to participants' disease status. Comprehensive measurement of covariates such as WHR and pack-years of smoking enabled us to limit confounding. We also carried out analyses restricting to the first 3 and 5 years of follow-up, so as to minimize the misclassification of inflammation marker status due to prolonged duration between measurement of inflammation markers and occurrence of EA events, and observed stronger associations and more pronounced trends.

Our study is limited by the relatively small number of incident EA cases, despite being one of the largest, well-characterized cohorts of BE patients reported in the literature. Although we controlled for potential confounding effects of smoking, obesity and NSAID use in multivariable analyses, we cannot exclude the possibility of residual confounding by measured and unmeasured risk factors. In particular, we did not collect data on *Helicobacter pylori* status, which has been shown to be inversely related to EA risk(62, 63), while at the same time being associated with systemic inflammation(64) such that H.pylori eradication therapies reduce the blood levels of pro-inflammatory cytokines including CRP.(65) We did not adjust for confounding by the length of Barrett's segment in our analysis. Although a recent study showed that presence of long-segment BE carried a sevenfold increased risk of progression to EA(66), BE segment length is only modestly associated with the risk of EA in our cohort.(67) We attempted to limit measurement error by using high sensitivity and reliable assays for biomarker measurement. However, we cannot exclude the possibility of errors in biomarker measurement resulting from degradation of biological samples during storage. Finally, as the study cohort represents a specialty clinic, results presented in the current report should be cautiously interpreted in terms of their generalizability to the general population.

In conclusion, our results indicate that systemic levels of CRP and to some extent IL-6 are associated with progression to EA in persons with BE. Soluble TNF receptors and F_2-isoprostanes were not found to be associated with increased EA risk. Our findings with CRP and IL-6 are consistent with the literature that supports the role of chronic inflammation in the development of cancer, and EA in particular. Additional analyses involving further follow-up of this and other cohorts are needed to confirm these findings, as well as to evaluate the utility of biomarker assessment in clinical prediction and risk stratification of EA.

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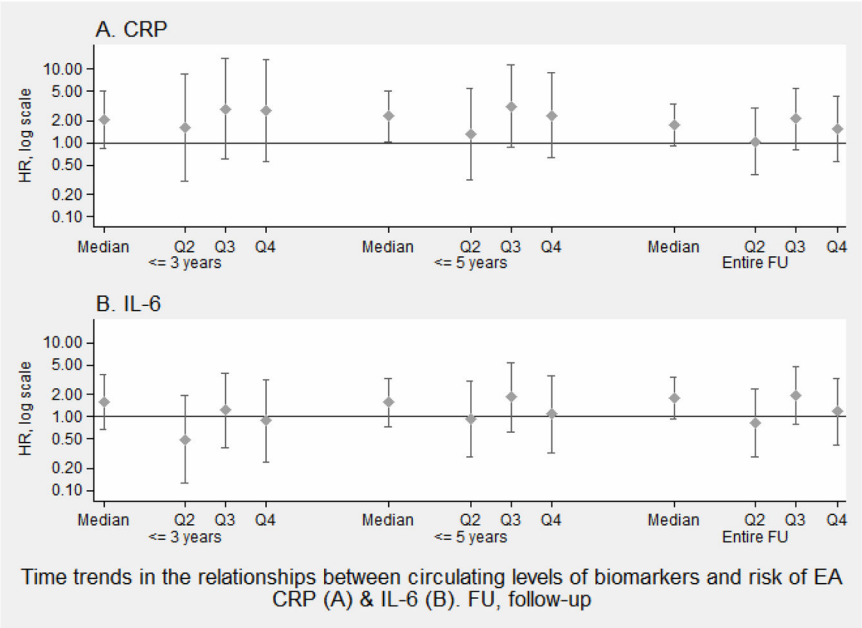

Figure 1.

Relationship between association of EA with plasma CRP(A) and IL-6(B) with follow-up restricted to 3 and 5 years from baseline[a]

[a]All models adjusted for confounding effects of age, gender, smoking (pack-years) and obesity (waist-hip ratio)

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Table 1

Baseline characteristics of all participants, males and females in the Seattle Barrett's Esophagus Study (SBES) cohort (n = 411)

Variable	All participants (n = 411)		Males (n = 334)		Females (n = 77)	
	N	% or Median(IQR)	N	% or Median(IQR)	N	% or Median(IQR)
Age[a] (yrs)						
30–44.9	30	7.3	25	7.5	5	6.5
45–54.9	97	23.6	83	24.9	14	18.2
55–64.9	110	26.8	84	25.2	26	33.8
65–74.9	114	27.7	93	27.8	21	27.3
≥ 75	60	14.6	49	14.7	11	14.3
Race[a]						
White	397	96.6	324	97.0	73	94.8
Non-white	14	3.4	10	3.0	4	5.2
BMI[a] (kg/m^2)						
≤ 25	56	13.6	41	12.3	15	19.5
25.1–≤ 30	194	47.2	167	50.0	27	35.1
30.1 – ≤ 35	125	30.4	102	30.5	23	29.9
>35	36	8.8	24	7.2	12	15.6
Cigarette smoking[a,c]						
Current	40	9.7	28	8.4	12	15.6
Former	223	54.3	192	57.5	31	40.3
Never	148	36.0	114	34.1	34	44.1
NSAID use[a,d]						
Current	169	41.1	145	43.4	24	31.2
Former	79	19.2	58	17.4	21	27.3
Never	162	39.4	130	38.9	32	41.6
Waist-hip ratio[b,e]	409	0.95(0.91–0.99)	332	0.96 (0.93–1.00)	77	0.87(0.81–0.91)

[a] Frequency (%) of categorical variables

[b] Median (Interquartile range) for continuous variables

[c] Cigarette smoking was based on whether participants smoked 1 cigarette/day for 6 months or longer currently or in the past or never.

[d] NSAID use was defined as having taken NSAID at least once a week for six months or longer, NSAID use history for one male participant was missing.

[e] Two male participants had missing information on waist-hip ratio.

N = Number, BMI = Body mass index, NSAID = Non-steroidal anti-inflammatory drugs

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Table 2

Distribution of mean biomarker levels overall and by gender in the SBES cohort. (n= 411)

	All participants (n = 411)			Males (n = 334)			Females (n = 77)		
	N	Median	IQR	N	Median	IQR	N	Median	IQR
CRP[a] (mg/L)	398	1.90	0.95–3.60	329	1.80	0.90–3.35	69	2.95	1.7–4.35
IL-6[b] (pg/ml)	407	1.88	1.34–3.06	332	1.77	1.31–2.87	75	2.62	1.48–3.79
sTNF- Receptor I[b] (ng/ml)	407	1.39	1.14–1.65	332	1.42	1.15–1.66	75	1.31	1.08–1.61
sTNF- Receptor II[b] (ng/ml)	407	5.35	4.71–6.44	332	5.34	4.68–6.40	75	5.49	4.74–6.62
Isoprostanes[c](pg/ml)	390	53	41–74	320	51	40–66	70	74	47–107

[a] CRP was not measured in 4 people due to exhausted baseline plasma. CRP levels over 10 mg/L were excluded as per an apriori hypothesis. 4 people had plasma measured only at one time point and it was greater than 10 mg/L, 5 people had both CRP measurements greater than 10 mg/L.

[b] IL-6 and TNF receptors were not measured in 4 people due to exhausted baseline plasma samples

[c] Isoprostanes were not measured in 16 individuals due to exhausted baseline plasma samples, 5 samples were not successfully measured by the laboratory.

N = Number/frequency, IQR = Inter-quartile range, CRP = C-reactive protein, IL6 = Interleukin-6, sTNF-RI & II = soluble tumor necrotic factor receptor I & II

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Table 3

Relationship between the biomarkers and correlates of esophageal adenocarcinoma (n = 411)

	CRP	IL6	sTNF-RI	sTNF-RII	Isoprostanes
Age[a]	0.08	0.18*	0.37*	0.43*	−0.03
Waist Hip Ratio[a]	0.10*	0.08	0.13*	0.08	−0.12*
Cigarette pack years[b]	0.24*	0.22*	0.22*	0.12*	0.07
NSAID use[c]	−0.15	0.21	0.01	0.04	−0.06
CRP[a]	-	0.44*	0.18*	0.20*	0.22*
IL-6[a]	-	-	0.21*	0.26*	0.08
sTNF-RI[a]	-	-	-	0.63*	0.12*
sTNF-RII[a]	-	-	-	-	0.14*
Isoprostanes[a]	-	-	-	-	-

*p-value < 0.05

[a]Pearson's correlation coefficient

[b]Spearman correlation coefficient

[c]Difference in biomarker (β coefficient) between current users and non-current users of NSAIDs

Non-smokers coded as have smoked zero pack-years

NSAID = Non-steroidal anti-inflammatory drugs, NSAIDs coded Non-current vs. current

CRP = C-reactive protein, IL6 = Interleukin 6, sTNF-RI & II = soluble tumor necrosis factor receptor I & I

Table 4

Hazard ratios (HRs) and 95% confidence intervals (CIs) for esophageal adenocarcinoma (EA) associated with markers of systemic inflammation (CRP, IL-6, sTNF-RI & sTNF –RII) and markers of oxidative stress (F2-isoprostanes) in the SBES cohort (n = 397)

Biomarker	EA cases/total	Unadjusted HR(95% CI)	Adjusted for age[a] and gender HR(95% CI)	Adjusted for age, gender, WHR[b], smoking[c] and NSAID use[d] HR(95% CI)
CRP (mg/L)[e]				
All participants				
Median				
Below	15/192	REF	REF	REF
Above	28/194	1.83 (0.98–3.43)	**1.98 (1.05–3.73)**	1.77 (0.93–3.37)
Quartiles				
Q1 (0.1-)	6/95	REF	REF	REF
Q2 (0.9-)	9/97	1.29 (0.46–3.63)	1.18 (0.42–3.32)	1.05 (0.37–2.99)
Q3 (1.9-)	15/95	2.35 (0.91–6.06)	2.29 (0.89–5.92)	2.12 (0.81–5.56)
Q4 (3.6-)	13/99	1.90 (0.72–5.01)	2.06 (0.78–5.44)	1.55 (0.56–4.24)
Continuous p-trend[*]		***0.03***	***0.01***	***0.04***
Males				
Median				
Below	12/155	REF	REF	REF
Above	28/165	**2.31 (1.18–4.55)**	**2.21 (1.12–4.36)**	1.93 (0.96–3.89)
Quartiles				
Q1 (0.1-)	5/79	REF	REF	REF
Q2 (0.9-)	7/76	1.20 (0.38–3.77)	1.20 (0.38–3.79)	1.09 (0.34–3.49)
Q3 (1.8-)	15/84	2.74 (0.99–7.55)	2.55 (0.92–7.02)	2.33 (0.83–6.51)
Q4 (3.4-)	13/81	2.37 (0.85–6.66)	2.36 (0.84–6.62)	1.76 (0.60–5.15)
Continuous p-trend[*]		***0.01***	***0.01***	***0.05***
IL-6 (pg/ml)				
All participants				
Median				
Below	15/197	REF	REF	REF
Above	30/197	**2.06 (1.11–3.82)**	**1.95 (1.03–3.72)**	1.79 (0.93–3.43)

Biomarker	EA cases/total	Unadjusted HR(95% CI)	Adjusted for age[a] and gender HR(95% CI)	Adjusted for age, gender, WHR[b], smoking[c] and NSAID use[d] HR(95% CI)
Quartiles				
Q1 (0.4-)	7/98	REF	REF	REF
Q2 (1.3-)	8/99	1.14 (0.41–3.15)	0.95 (0.34–2.66)	0.82 (0.29–2.34)
Q3 (1.9-)	19/99	**2.74 (1.15–6.52)**	2.35 (0.96–5.77)	1.93 (0.78–4.79)
Q4 (3.1-)	11/98	1.65 (0.64–4.24)	1.40 (0.52–3.78)	1.17 (0.42–3.26)
Continuous p-trend[*]		*0.64*	*0.94*	*0.87*
Males				
Median				
Below	10/161	REF	REF	REF
Above	31/161	**3.19 (1.57–6.52)**	**2.85 (1.38–5.92)**	**2.52 (1.19–5.33)**
Quartiles				
Q1 (0.4-)	6/81	REF	REF	REF
Q2 (1.3-)	4/80	0.67 (0.19–2.36)	0.56 (0.16–2.01)	0.53 (0.15–1.89)
Q3 (1.8-)	18/80	**3.06 (1.21–7.71)**	2.51 (0.96–6.51)	2.04 (0.77–5.41)
Q4 (2.9-)	13/81	2.25 (0.86–5.93)	1.77 (0.64–4.86)	1.56 (0.55–4.43)
Continuous p-trend[*]		*0.66*	*0.99*	*0.81*
sTNF-I (ng/ml)				
All participants				
Median				
Below	18/197	REF	REF	REF
Above	27/197	1.62 (0.89–2.93)	1.29 (0.69–2.42)	0.99 (0.51–1.92)
Quartiles				
Q1 (0.3-)	11/98	REF	REF	REF
Q2 (1.1-)	7/99	0.64 (0.25–1.64)	0.56 (0.22–1.45)	0.60 (0.23–1.56)
Q3 (1.4-)	13/98	1.24 (0.55–2.76)	0.94 (0.41–2.15)	0.87 (0.38–1.98)
Q4 (1.7-)	14/99	1.41 (0.64–3.10)	1.02 (0.44–2.37)	0.68 (0.27–1.68)
Continuous p-trend[*]		*0.18*	*0.68*	*0.69*
Males				
Median				
Below	17/161	REF	REF	REF

Biomarker	EA cases/total	Unadjusted HR(95% CI)	Adjusted for age[a] and gender HR(95% CI)	Adjusted for age, gender, WHR[b], smoking[c] and NSAID use[d] HR(95% CI)
Above	24/161	1.56 (0.84–2.90)	1.23 (0.63–2.41)	0.95 (0.47–1.94)
Quartiles				
Q1 (0.3-)	11/80	REF	REF	REF
Q2 (1.1-)	6/81	0.51 (0.19–1.37)	0.47 (0.17–1.26)	0.51 (0.19–1.38)
Q3 (1.4-)	12/80	1.10 (0.48–2.49)	0.85 (0.36–2.00)	0.73 (0.31–1.74)
Q4 (1.7-)	12/81	1.23 (0.54–2.80)	0.88 (0.36–2.14)	0.66 (0.26–1.71)
Continuous p-trend[*]		*0.30*	*0.83*	*0.57*
sTNF-II (ng/ml)				
All participants				
Median				
Below	15/197	REF	REF	REF
Above	30/197	**2.23 (1.20–4.15)**	1.90 (0.98–3.67)	1.78 (0.90–3.52)
Quartiles				
Q1 (1.6-)	8/98	REF	REF	REF
Q2 (4.7-)	7/99	0.83 (0.30–2.29)	0.78 (0.28–2.17)	0.81 (0.29–2.25)
Q3 (5.4-)	13/98	1.68 (0.70–4.05)	1.46 (0.59–3.61)	1.47 (0.59–3.70)
Q4 (6.4-)	17/99	2.44 (1.05–5.66)	1.95 (0.76–4.95)	1.75 (0.68–4.49)
Continuous p-trend[*]		*0.22*	*0.75*	*0.86*
Males				
Median				
Below	14/161	REF	REF	REF
Above	27/161	**2.18 (1.14–4.16)**	1.81 (0.91–3.62)	1.63 (0.80–3.34)
Quartiles				
Q1 (1.6-)	8/80	REF	REF	REF
Q2 (4.7-)	6/81	0.69 (0.24–1.98)	0.62 (0.21–1.80)	0.69 (0.24–2.00)
Q3 (5.3-)	12/80	1.52 (0.62–3.72)	1.27 (0.50–3.23)	1.28 (0.50–3.30)
Q4 (6.4-)	15/81	2.17 (0.92–5.14)	1.59 (0.60–4.19)	1.44 (0.54–3.83)
Continuous p-trend[*]		*0.20*	*0.78*	*0.78*
Isoprostanes (pg/ml)				
All participants				

Biomarker	EA cases/total	Unadjusted HR(95% CI)	Adjusted for age[a] and gender HR(95% CI)	Adjusted for age, gender, WHR[b], smoking[c] and NSAID use[d] HR(95% CI)
Median				
Below	27/186	REF	REF	REF
Above	18/191	0.60 (0.33–1.09)	0.69 (0.38–1.26)	0.56 (0.30–1.03)
Quartiles				
Q1 (14-)	13/90	REF	REF	REF
Q2 (41-)	14/96	0.98 (0.46–2.09)	1.18 (0.55–2.53)	0.93 (0.43–2.02)
Q3 (53-)	10/98	0.68 (0.30–1.54)	0.76 (0.33–1.74)	0.61 (0.26–1.41)
Q4 (74-)	8/93	0.52 (0.22–1.26)	0.74 (0.30–1.85)	0.46 (0.18–1.20)
Continuous p-trend[*]		*0.34*	*0.92*	*0.41*
Males				
Median				
Below	23/143	REF	REF	REF
Above	18/157	0.74 (0.40–1.36)	0.79 (0.42–1.46)	0.64 (0.34–1.20)
Quartiles				
Q1 (14-)	11/74	REF	REF	REF
Q2 (40-)	12/79	1.03 (0.45–2.33)	1.18 (0.52–2.69)	0.98 (0.43–2.27)
Q3 (51-)	11/80	0.96 (0.42–2.21)	1.04 (0.45–2.41)	0.80 (0.34–1.88)
Q4 (66-)	7/77	0.55 (0.21–1.43)	0.66 (0.25–1.72)	0.47 (0.18–1.25)
Continuous p-trend[*]		*0.88*	*0.79*	*0.54*

[a] Age was modeled as a continuous variable.

[b] Waist to Hip Ratio was modeled as a continuous variable.

[c] Cigarette smoking was modeled as pack-years smoked, never smokers were assigned a pack-year value of 0.

[d] NSAID use was modeled as current vs. non-current users of NSAIDs at the baseline visit.

[e] CRP measurements over 10 mg/L were excluded from the analysis.

[*] Test for trend was based on the likelihood-ratio test associated with addition of the variable under consideration in its continuous form