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Association of serum cytokines with colorectal polyp number and type in adult males

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Abstract

Chronic inflammation contributes to colorectal carcinogenesis. To determine if serum cytokines are associated with colon polyps, 126 asymptomatic men (48–65yr) were recruited at colonoscopy. Serum cytokine concentrations were measured. Odds ratios were determined using polytomous logistic regression for polyp number and type. Males with serum monocyte chemotactic protein-3 (MCP-3) or soluble interleukin-4 receptor (sIL-4R) concentrations in the highest tertile were 0.2 times less likely to have 3 polyps relative to no polyps. For each increase in serum MCP-3 or sIL-4R tertile a man was about 0.4 times less likely to have 3 polyps than no polyps. Males with serum concentrations of interferon- α 2 (IFN- α 2) or interleukin (IL)-7 in the highest tertile were 3 times more likely to have an adenoma relative to no polyps. Those with serum IL-8 concentrations in the highest tertile were 4 times more likely to have an adenoma relative to no polyps. For each increase in serum IFN- α 2, IL-7 or IL-8 tertile an individual was 1.8 times more likely to have an adenoma than no polyp. Serum concentrations of MCP-3, sIL-4R, IFN- α 2, IL-7 and IL-8 may indicate which men are more likely to have colorectal polyps.

Keywords

colon; colorectal; polyp; adenoma; cytokines; colonoscopy; prevention; males; biomarker; human

Introduction

Colonoscopy is known to be highly effective at preventing cases of colorectal cancer (Zauber et al., 2012). However, many individuals are reluctant to be screened in this manner (McLachlan et al., 2012), and the procedure is costly (Rosenthal, 2013; Sinsky and Dugdale, 2013). Methods to identify which individuals would benefit most from colonoscopy are

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needed. One such method may be the use of serum factors to predict individuals at highest risk for the presence of colorectal polyps.

Inflammation is a critical component of cancer initiation and progression (Hanahan and Weinberg, 2011). Initial chronic inflammation can activate cellular functions that induce irreversible DNA damage and increase the risk of cancer (Grivennikov et al., 2010). However, the immune system also responds to already mutated cells, and chronic inflammation may result from an inability of the immune system to kill and clear those cells. Typically it is the local epithelial cells or innate immune cells such as neutrophils, monocytes/macrophages and dendritic cells, which are responsible for these actions. These cell types synthesize cytokines such as monocyte chemoattractant protein (MCP)-3, macrophage derived chemokine (MDC), interferon-alpha2 (IFN- α 2), interleukin(IL)-7 and IL-8. When these proteins are produced in high quantities locally, they can diffuse from the tissue and be detected in the blood.

Because inflammation is commonly linked to cancer initiation as well as progression (Elinav et al., 2013), serum cytokines levels are likely to be useful predictive biomarkers of colorectal polyp formation and risk for future colorectal cancer. In fact, a recent study successfully identified a prognostic classifier for colorectal metastasis using serum concentrations of 17 cytokines (Chen et al., 2014). Another study demonstrated that colorectal dysplasia was positively associated with local intestinal inflammation and inversely associated with immune therapy (Rubin et al., 2013). However, few studies have attempted to identify serum cytokines associated with colorectal polyps. This study investigated potential associations between serum cytokine levels and colorectal polyp number or type in a population of adult males undergoing preventative screening colonoscopy.

Materials and Methods

Study Population

Between August 2009 and February 2011, healthy males ranging from 48-65 years of age were recruited from MSU-affiliated clinics, either Tri-County Gastroenterology Clinic (Macomb, MI) or Michigan State University Clinic (East Lansing, MI), at the time of colonoscopy. These individuals were undergoing routine colonoscopies and were asymptomatic. Exclusion criteria included: 1) cancer within the past two years, 2) surgery within the past two years, 3) inflammatory bowel diseases (i.e. Crohn's, ulcerative colitis), 4) autoimmune disorders (i.e. Rheumatoid arthritis, HIV/AIDS, Lupus), 5) type I or type II diabetes, 6) chronic liver or kidney disease, 7) history of heart failure, 8) current immunosuppressant usage (i.e. Prednisone), 9) asthma, chronic obstructive pulmonary disease or other lung problems, 10) familial adenomatous polyposis, and 11) Lynch syndrome or hereditary non-polyposis colorectal cancer. 126 men (>96% Caucasian) participated in the study (**Table 1**). At the time of enrollment, immediately prior to routine colonoscopy, written informed consent was obtained and clinical metadata on subject comorbidities, current medications, and family history were collected. Anthropometric measures were taken to calculate BMI and to record waist circumference (Comstock et al., 2014a). Also at the time of enrollment, venous blood was drawn, and serum was isolated by

standard procedures and stored at -80°C. The study was approved by the institutional review board of Michigan State University (IRB# 08-786).

Colonoscopy Interpretation

Full colonoscopy was performed on each participant. A gastroenterologist (MSU-affiliated clinics, MI) categorized the location of each polyp during the colonoscopy. Board-certified pathologists assigned a polyp type to each specimen collected during colonoscopy (regional medical center pathology departments).

Serum Biomarker Analysis

Serum concentrations of the soluble interleukin-4 receptor (sIL-4R), soluble CD30 (sCD30), soluble gp130 (sgp130), and the soluble interleukin-2 receptor (sIL-2R α) were determined using a multiplex kit for soluble cytokine receptors (HSCR-32K, Millipore) as per the manufacturer's instructions. Further, IFN- α 2, IL-7, IL-8, MCP-3, eotaxin, *fms*-like tyrosine kinase-3 ligand (FLT-3L), fractalkine, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), GRO1 oncogene (CXCL1/GRO), interferon-gamma (IFN- γ), IL-15, transforming growth factor-alpha (TGF- α), IL-3, IL-4, IL-5, IL-9, IL-13 and MDC serum concentrations were measured using a multiplex human cytokine/chemokine kit (MPXHCYTO-60K, Millipore). Multiplex assays were analyzed on a Bio-Plex 100 using Bio-Plex 4.1 software (Bio-Rad, Hercules, CA).

Statistical Analyses

Frequencies, means, standard deviations and ranges were calculated for descriptive analysis. Because the serum cytokine concentrations were non-normally distributed, Spearman correlations are presented (**Table 3**). All of the cytokine and soluble receptors were categorized into tertiles for polytomous logistic regression analysis. Biological cut off points were used for the outcome variables: polyp number and polyp type. Polyp number was set to four categories: 0, 1, 2, or 3 polyps. Polyp type was set to three categories: no polyps, hyperplastic polyp(s) or tubular adenoma(s). For polyp type, each individual was assigned a single score based on the colorectal polyp with the worst pathology.

Each individual was assigned a smoking status of “never smoked” or “ever smoked”. An individual was classified as “never smoked” if he had smoked fewer than 100 cigarettes over the course of his lifetime. Smoking status was missing for 22 individuals (17.5% missingness). All missing data were considered missing at random.

Multiple imputation (seed = 20121119, imputations = 7) was used to impute all missing smoking data and the one participant's value missing for the factors measured with the multiplex human cytokine/chemokine kit (Yuan, 2011). The factors: age, smoking, polyp type, polyp number and the factor being imputed were used in the imputation algorithm of missing values (Moons et al., 2006).

Odds ratios (OR) were determined using polytomous logistic regression models for categorical outcome data with more than two levels. A generalized logit model (also known as a, “baseline category logit model”) formed the basis of the polytomous logistic regression

model for our analyses. In this model, men having 3 polyps, 2 polyps or 1 polyp were compared to men having no polyps. Results were obtained for each serum factor categorized into tertiles (with lowest tertile as reference) for each category of polyp number or polyp type relative to no polyp. For clarity, only results from the categories featuring the most polyps (3) and the polyp type most likely to progress to colorectal cancer (adenoma) are presented. For dichotomous outcomes odds ratios were determined using logistic regression. All models were adjusted for age and smoking status. Test for trend was carried out across tertiles for the factors of interest. Because imputation was used, multiple imputation analyze (Proc MIANALYZE) was used to determine the results from analysis of the imputed data sets. SAS version 9.3 (SAS Institute Inc.; Cary, NC) was used for all statistical analysis. $p < 0.05$ indicates significance.

Results

Some details about this study population have recently been published (Comstock et al., 2014a; Comstock et al., 2014b; Comstock et al., 2014c). Briefly (**Table 1**), 57 (45%) of the 126 participants had at least one polyp. Remaining participants had no polyps. 37 (29.4%) of the participants had a tubular adenoma. Furthermore, 17 of the 23 (18%) participants with 3 polyps had at least one tubular adenoma.

No associations between polyp number or polyp type were found for the following factors: eotaxin, FLT-3L, fractalkine, G-CSF, GM-CSF, GRO, IFN- γ , IL-15, TGF- α , sCD30, sgp130, sIL-2R α , IL-3, IL-4, IL-5, IL-9, or IL-13. Only serum concentrations of MDC were associated with the presence of hyperplastic polyps. There were no individual effects among MDC tertiles, but there was a 2.2 fold increase in odds (CI, 1.1-4.4) of having a hyperplastic polyp compared to having no polyp for each increase in the tertile of MDC serum concentration ($p=0.0227$).

Concentrations of cytokines and soluble receptors significantly associated with either polyp number or type are presented in **Table 2**. These serum factors correlated with each other as well as with participant characteristics (**Table 3**). Only one factor was significantly correlated with age. The sIL-4R was inversely correlated with age. Multiple cytokines including IFN- $\alpha 2$, IL-7, IL-8, IL-10 and MDC positively correlated with BMI. All of these serum cytokines, except IL-10, also positively correlated with waist circumference. Neither BMI nor waist circumference correlated with serum sIL-4R concentrations. With few exceptions, serum cytokine levels were positively correlated with each other. Notably, serum MDC concentration was not correlated with serum IL-10 or MCP-3 concentrations and tended to be negatively correlated with serum sIL-4R concentrations. Neither IFN- $\alpha 2$ nor IL-8 correlated with sIL-4R serum concentrations.

High serum concentrations of MCP-3 and sIL-4R reduced the odds that a participant would have 3 polyps. Compared to males with a serum MCP-3 concentration 7.16 pg/ml, males with a serum MCP-3 concentration > 15.98 pg/ml were 0.21 times less likely to have 3 polyps ($p=0.0196$) relative to no polyps (**Figure 1A**). In addition for each increase in serum MCP-3 tertile, a man was 0.44 times less likely to have 3 polyps than no polyps ($p=0.0126$). When compared to males with a serum sIL-4R concentration 1360.0 pg/ml,

males with a serum sIL-4R concentration > 1575.0 pg/ml were 0.22 times less likely to have 3 polyps ($p=0.0246$) relative to no polyps (**Figure 1B**). For each increase in serum sIL-4R tertile, a man was 0.45 times less likely to have 3 polyps than no polyps ($p=0.0180$). Conversely high serum levels of MDC tended to increase the odds that a participant would have 3 polyps compared to no polyps (**Table 4**). Serum concentrations of IFN- α 2, IL-7, IL-8 and IL-10 were not associated with polyp number.

Concentrations of several serum cytokines were associated with the presence of a colorectal adenoma (**Table 5**). Males with a serum IFN- α 2 concentration > 33.12 pg/ml were 2.8 times more likely than males with a serum IFN- α 2 concentration 14.98 pg/ml to have an adenoma ($p=0.0468$) relative to no polyps (**Figure 2A**). As the tertile of serum IFN- α 2 increased an individual was 1.75 times more likely to have an adenoma than no polyp ($p=0.0391$). When compared to males with a serum IL-7 concentration 3.54 pg/ml, males with a serum IL-7 concentration > 12.06 pg/ml were 3.3 times more likely to have an adenoma ($p=0.0277$) relative to no polyps (**Figure 2B**). As the serum IL-7 tertile increased an individual was 1.82 times more likely to have an adenoma than no polyp ($p=0.0288$). Compared to males with a serum IL-8 concentration 6.76 pg/ml, males with a serum IL-8 concentration > 10.74 pg/ml were 4.0 times more likely to have an adenoma ($p=0.0158$) relative to no polyps (**Figure 2C**). Further, for each increase in serum IL-8 tertile, an individual was 1.85 times more likely to have an adenoma than no polyp ($p=0.0213$). High serum concentrations of IL-10 tended to be positively associated with the presence of an adenoma (**Table 5**). However, high serum concentrations of sIL-4R tended to be negatively associated with the presence of an adenoma. The serum concentrations of MCP-3 and MDC were not associated with the presence of an adenoma.

Discussion

Recent studies have evaluated the relationship between serum cytokines and colorectal cancer, yet few have comprehensively analyzed the relationship between serum cytokine concentrations and colorectal polyps (Bunger et al., 2012; Kang et al., 2013). In this study, serum concentrations of the cytokines MCP-3, sIL-4R, IFN- α 2, IL-7 and IL-8 were associated with polyp number or type in asymptomatic adult males. Specifically, high levels of MCP-3 or sIL-4R were associated with decreased odds of having 3 polyps. Whereas, high levels of IFN- α 2, IL-7 and IL-8 were associated with increased odds of having a colorectal adenoma. Many other serum cytokines, including eotaxin, G-CSF, GM-CSF, GRO1, IFN- γ and IL-5, were not associated with either polyp number or the presence of a colorectal adenoma.

Most studies of colorectal polyps focused on select markers of inflammation, most commonly IL-6, TNF- α and c-reactive protein (CRP) (Hopkins et al., 2012; Il'yasova et al., 2005; Kim et al., 2008; Wild et al., 2010). These studies found IL-6, TNF- α , and CRP as well as non-cytokine markers, such as carcinoembryonic antigen and ferritin, are positively associated with the presence of colorectal adenomas as well as with general cancer incidence. As we have previously published, although IL-6 was not associated, TNF- α was significantly associated with the presence of colorectal adenomas in our study population (Comstock et al., 2014a). We did not measure CRP concentrations. Notably, some of the

serum expression patterns identified herein are consistent with findings in studies of colorectal polyps and colorectal carcinomas. Of the studies evaluating large sets of cytokines, TNF- α and IL-8 were associated with polyps (Bunger et al., 2012; Kang et al., 2013). We also identified associations between TNF- α (Comstock et al., 2014a) and IL-8 with polyps. In a report by Chen et al (Chen et al., 2014), IFN- α 2, IL-10, MCP-3, MDC, IL-7, IL-8, tumor necrosis factor (TNF)- α , interferon-gamma-induced protein (IP)-10 and vascular endothelial growth factor (VEGF) were associated with metastatic colorectal cancer. IL-8, IP-10 and TNF- α were also positively associated with the presence of colorectal carcinoma in a separate population (Choi et al., 2013). Our study identified associations of IFN- α 2, IL-10, MCP-3, MDC, IL-7 and IL-8 with colorectal polyps. Additionally, TNF- α , IP-10 and VEGF have been previously reported to be associated with colorectal polyps in this population (Comstock et al., 2014a; Comstock et al., 2014c). Therefore, data from the current study population supports that of other studies analyzing serum cytokines in cases of colorectal neoplasia. Furthermore, it expands previous knowledge by demonstrating that these serum cytokine levels are modified even in the early stages of colon cancer initiation.

Cytokines are important for cancer progression. They affect apoptosis, proliferation, angiogenesis and mutagenesis. Most frequently these functions are accomplished by signaling through molecules such as nuclear factor-kappa B (NF- κ B), cyclooxygenase-2 (COX-2) or signal transducer and activator of transcription 3 (STAT3) (Dreesen and Brivanlou, 2007; Grivennikov and Karin, 2010; Parsons et al., 2005). For instance, STAT3 activation is pivotal in tumorigenesis (reviewed in (Coussens et al., 2013; Fan et al., 2013)). Several of the cytokines positively associated with increased numbers of polyps or the presence of an adenoma including IFN- α 2 (Raz et al., 1994), IL-7 (Qin et al., 2001), and IL-10 (Finbloom and Winestock, 1995) signal through STAT3. Additionally, IL-4 signals through STAT3 (Rolling et al., 1996), and herein the soluble IL-4R was inversely associated with having 3 polyps as well as tending to be inversely associated with the presence of a tubular adenoma indicating that STAT3 signaling may be inhibited under conditions of high sIL-4R. Serum leptin, which also activates STAT3 (Bjorbaek et al., 1997), was previously positively associated with increased odds of having 3 polyps as well as increased odds of having a tubular adenoma (Comstock et al., 2014a).

Serum levels of IL-8, an angiogenic protein that is also known as CXCL8, were positively associated with the odds of having an adenoma compared to no polyp. Macrophages, endothelial cells and tumors produce IL-8 drawing granulocytes and activating neutrophils to the local environment. IL-8 induces VEGF expression leading to angiogenesis (Kotzya, 2012; Singh et al., 2010; Todorovic-Rakovic and Milovanovic, 2013). This chemokine also stimulates tumor cell invasiveness and cancer progression (Fernando et al., 2011; Palena et al., 2012; Todorovic-Rakovic and Milovanovic, 2013; Waugh and Wilson, 2008). In a study of colorectal tissue, IL-8 mRNA and protein was more highly expressed in samples from colorectal tumors compared to those isolated from areas of inflammatory colorectal conditions or adenomas of the colon/rectum (Rubie et al., 2007). In combination with the fact that serum IL-8 levels were associated with colorectal carcinoma and polyps, these results suggest that increased IL-8, both locally and systemically, is important in the development of colorectal polyps as well as colorectal cancer.

Inflammatory cytokines also activate proliferative responses during cancer initiation steps. In this study, levels of IL-7 were positively associated with the presence of an adenoma. This is potentially related to the ability of this cytokine to promote proliferation of a variety of cell types (Namen et al., 1988; Vollger and Uittenbogaart, 1993). It may do this through STAT signaling (Van Der Plas et al., 1996) as well as through activation of phosphoinositide-3-kinase (PI3K) (Dadi and Roifman, 1993). Furthermore, IL-7 can stimulate production of other inflammatory cytokines (Appasamy, 1993).

The immune system can inhibit as well as enhance the tumorigenicity of pre-cancerous or cancerous cells (Chow et al., 2012). Two serum proteins, MCP-3 and sIL-4R, inversely associated with colorectal polyps in the present analysis likely contribute cytotoxic skewing effects. MCP-3, also known as CCL7, recruits and activates most types of immune cells, including NK cells (Alam et al., 1994; Allavena et al., 1994; Loetscher et al., 1996; Noso et al., 1994; Sozzani et al., 1994; Taub et al., 1995; Uguccioni et al., 1995; Xu et al., 1995). In fact, MCP-3 stimulates tumor regression (Fioretti et al., 1998; Wetzel et al., 2001), and herein high serum MCP-3 levels were associated with a decreased odds of having 3 polyps compared to having no polyps demonstrating that MCP-3 can be protective at high serum concentrations. Additionally, serum sIL-4R was negatively associated with the odds of having 3 polyps compared to no polyps and tended to be negatively associated with the odds of having an adenoma compared to no polyps. These associations are likely due to the inhibitory effect of this soluble cytokine receptor on IL-4 signaling. Through inhibition of IL-4, sIL-4R creates an environment more conducive to polyp/tumor regression. Anti-IL-4 therapeutic antibodies have been demonstrated to reprogram tumor-associated macrophages, monocytes and other cells toward cytotoxic phenotypes in mammary cancer (Denardo et al., 2009). Thus, the inverse relationship between serum MCP-3 and sIL-4R and colorectal polyp number could potentially be due to their cytotoxic skewing effects.

On the other hand, cytokines, such as IL-10, which are typically regulatory (O'garra and Vieira, 2007) are elevated in cancer (Mustea et al., 2006; Sato et al., 1996). Some IL-10 polymorphisms are associated with cancer (Howell and Rose-Zerilli, 2006). In the current study population, serum IL-10 tended to be positively associated with having an adenoma compared to no polyp. MDC, also known as CCL22, is associated with tumor promotion because it attracts regulatory T cells to the tumor site (Faget et al., 2011). The presence of these regulatory T cells inhibits other immune cells, specifically cytotoxic immune cells. The positive relationship trends between IL-10 and polyp type as well as between MDC and polyp number could potentially be due to their anti-cytotoxic skewing effects. The time during polyp formation and progression determines disease trajectory. Inflammation and the immune system are important throughout tumor development, from initiation to metastasis, but many of the specifics are unknown (Coussens et al., 2013; Fan et al., 2013). The association of specific serum cytokine concentrations with the presence of colorectal polyps supports the hypothesis that serum cytokines likely play a role in colorectal cancer initiation and progression. However, our results do not address the effects of changes in serum cytokines with progression because we only analyzed one point in time for each participant and did not analyze the associations of cytokine combinations with polyp number or the presence of adenoma. Different cytokines were inversely associated with polyp number (MCP-3, sIL-4R) than were positively associated with the presence of an adenoma (IFN- α 2,

IL-7, IL-8) suggesting that initiation may rely on a cytotoxic or damaging environment while progression may rely on factors that promote angiogenesis and proliferation.

Strengths of the present study include the design. The study was cross-sectional with consecutive enrollment resulting in reduced enrollment bias. Additionally, only asymptomatic individuals undergoing a complete screening were enrolled, and individuals immediately participated in the study removing potential bias from loss to follow-up. We selected for healthy individuals lacking conditions known to be related to underlying inflammation to avoid detection of un-related inflammatory signals. Trained staff measured body weight and height so there was no reliance on self-report. The scope of information collected from participants about relevant confounding variables enabled factors such as smoking history to be controlled for in the analysis. Therefore, the results of this study are likely typical for men of an ethnicity and age similar to that of this well-defined study population.

However, caution must be used when interpreting the results of this study because the study had some limitations. For instance, the results may not apply to individuals of other ethnicities or females since this study was conducted in a sample of primarily Caucasian, adult males. Some data was imputed. However, these few imputations likely have minimal effects on the overall results. The associations described in this study were based on cross-sectional data thus cause cannot be assigned to any factors identified as associated with polyp number or type. Only a single time point is analyzed, but effects of cytokines on polyp formation may be related to the duration and localization of exposure. Local production is difficult to measure, and serum measurements are more useful in a clinical setting. Furthermore, although we did not detect associations between a number of cytokines with polyp number or the presence of an adenoma, this could be due to the sample size or specific characteristics of the population rather than the result of a lack of association in all circumstances. In summary, the results of this study should be verified using a larger, more representative sample size.

Further examination of the serum factors identified in this study as they relate to polyp formation and progression could elucidate pathways by which the immune system successfully prevents cancer or, alternatively, contributes to cancer. Additionally, although the identified factors may also be elevated in the serum of individuals with other tumor types, the association of specific serum cytokine concentrations with the presence of colorectal polyps suggests that these serum factors could potentially predict which individuals are likely to have polyps and therefore benefit from colonoscopy screening.

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Figure 1. The OR of having 3 polyps relative to no polyps is negatively associated with serum MCP-3 (A) and sIL-4R (B)

A, Compared to males with a serum MCP-3 concentration ≤ 7.16 pg/ml, males with a serum MCP-3 concentration > 15.98 pg/ml are 0.21 times less likely to have 3 polyps (p=0.0196) relative to no polyps. In addition for each increase in serum MCP-3 tertile, a man is 0.44 times less likely to have 3 polyps than no polyps (p=0.0126). Reference group = males with MCP-3 concentrations in the lowest tertile. B, Compared to males with a serum sIL-4R concentration ≤ 1360.0 pg/ml, males with a serum sIL-4R concentration > 1575.0 pg/ml are 0.22 times less likely to have 3 polyps (p=0.0246) relative to no polyps. In addition for

each increase in serum sIL-4R tertile, a man is 0.45 times less likely to have 3 polyps than no polyps ($p=0.0180$). Reference group = males with sIL-4R concentrations in the lowest tertile. The models were adjusted for age and smoking status (ever/never).

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Figure 2. Serum IFN-α2, IL-8 and IL-7 concentrations are associated with the presence of adenomas

A, Compared to males with a serum IFN-α2 concentration ≤ 14.98 pg/ml, males with a serum IFN-α2 concentration > 33.12 pg/ml are 2.8 times more likely to have an adenoma (p=0.0468) relative to no polyps. Further, for each increase in serum IFN-α2 tertile, an individual is 1.75 times more likely to have an adenoma than no polyp (p=0.0391).

Reference group = males with IFN-α2 concentrations in the lowest tertile. B, Compared to males with a serum IL-7 concentration ≤ 3.54 pg/ml, males with a serum IL-7 concentration > 12.06 pg/ml are 3.3 times more likely to have an adenoma (p=0.0277) relative to no

polyps. Further, for each increase in serum IL-7 tertile, an individual is 1.82 times more likely to have an adenoma than no polyp ($p=0.0288$). Reference group = males with IL-7 concentrations in the lowest tertile. C, Compared to males with a serum IL-8 concentration 6.76 pg/ml, males with a serum IL-8 concentration > 10.74 pg/ml are 4.0 times more likely to have an adenoma ($p=0.0158$) relative to no polyps. Further, for each increase in serum IL-8 tertile, an individual is 1.85 times more likely to have an adenoma than no polyp ($p=0.0213$). Reference group = males with IL-8 concentrations in the lowest tertile. All models were adjusted for age and smoking status (ever/never).

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Table 1Participant[†] characteristics[‡]

	Age (years)	BMI (kg/m ²)	Waist Circumference (inches)
No Polyps n=69	57 (48-65)	28.4 (21.7-39.1)	40.1 (30.0-55.0)
Any Polyps n=57	57 (50-65)	31.3 (19.2-45.6)	42.9 (29.8-57.5)
3 Polyps n=23	57 (50-65)	32.5 (19.2-45.6)	43.7 (31.8-57.5)
Adenoma n=37	57 (50-65)	32.3 (19.2-45.6)	43.6 (29.8-57.5)

[†] All participants (n=126) were male, >96% Caucasian[‡] Reported as mean (range)

Table 2Cytokine concentrations[‡]

	No Polyps n=69	Any Polyps n=57	3 Polyps n=23	Adenoma n=37
IFN-α2 (pg/ml)	18.1 (1.9-445.0)	28.1 (0.0-211.0)	28.1 (5.2-94.2)	33.8 (0.0-205.0)
IL-7 (pg/ml)	6.0 (0.0-136.0)	7.4 (0.0-84.3)	5.7 (0.0-35.8)	9.8 (0.0-57.9)
IL-8 (pg/ml)	7.1 (1.5-68.8)	9.1 (0.8-78.0)	8.0 (0.8-19.4)	9.8 (0.8-44.1)
IL-10 (pg/ml)	5.5 (0.0-166.0)	6.4 (0.0-175.0)	3.7 (0.0-81.3)	10.2 (0.0-106.0)
sIL-4R (pg/ml)	1475.0 (825.0-2965.0)	1415.0 (840.0-2380.0)	1345.0 (1090.0-2105.0)	1415.0 (840.0-2380.0)
MCP-3 (pg/ml)	11.6 (0.0-372.0)	8.3 (0.0-249.0)	6.8 (0.0-58.6)	8.3 (0.0-221.0)
MDC (pg/ml)	963.0 (449.0-2108.0)	1171.0 (128.0-2663.0)	1287.0 (546.0-2663.0)	1082.0 (615.0-2663.0)

[‡]Reported as median (range)

Table 3

Correlations among variables

	Age	BMI	Waist Circumference	IFN- α 2	IL-7	IL-8	IL-10	MCP-3	MDC	sIL-4R
Age		0.05992 0.5051	0.12274 0.1709	-0.02928 0.7458	-0.09279 0.3034	-0.10938 0.2246	-0.00865 0.9237	-0.13564 0.1315	0.06766 0.4534	-0.1787 0.0453
BMI	0.05992 0.5051		0.89497 <.0001	0.2963 0.0008	0.21651 0.0153	0.24287 0.0064	0.22796 0.0106	-0.01473 0.8705	0.18045 0.044	-0.05608 0.5328
Waist Circumference	0.12274 0.1709	0.89497 <.0001		0.26075 0.0033	0.17625 0.0493	0.19151 0.0324	0.13998 0.1195	-0.00054 0.9952	0.18967 0.0341	-0.05974 0.5064
IFN- α 2	-0.02928 0.7458	0.2963 0.0008	0.26075 0.0033		0.52252 <.0001	0.48437 <.0001	0.36054 <.0001	0.46496 <.0001	0.27646 0.0018	0.0412 0.6483
IL-7	-0.09279 0.3034	0.21651 0.0153	0.17625 0.0493	0.52252 <.0001		0.57216 <.0001	0.71784 <.0001	0.60345 <.0001	0.15337 0.0877	0.34088 0.0001
IL-8	-0.10938 0.2246	0.24287 0.0064	0.19151 0.0324	0.48437 <.0001	0.57216 <.0001		0.50852 <.0001	0.44129 <.0001	0.20139 0.0243	0.14322 0.1111
IL-10	-0.00865 0.9237	0.22796 0.0106	0.13998 0.1195	0.36054 <.0001	0.71784 <.0001	0.50852 <.0001		0.4536 <.0001	0.09633 0.2852	0.23793 0.0075
MCP-3	-0.13564 0.1315	-0.01473 0.8705	-0.00054 0.9952	0.46496 <.0001	0.60345 <.0001	0.44129 <.0001	0.4536 <.0001		0.10277 0.2541	0.19531 0.029
MDC	0.06766 0.4534	0.18045 0.044	0.18967 0.0341	0.27646 0.0018	0.15337 0.0877	0.20139 0.0243	0.09633 0.2852	0.10277 0.2541		-0.17364 0.0528
sIL-4R	-0.1787 0.0453	-0.05608 0.5328	-0.05974 0.5064	0.0412 0.6483	0.34088 0.0001	0.14322 0.1111	0.23793 0.0075	0.19531 0.0290	-0.17364 0.0528	

Note: Top number is the Spearman correlation coefficient. P values are shown under the correlation coefficient.

Table 4

Association of cytokines with having 3 polyps relative to no polyps

	No Polyp n	3 Polyps n	OR (95% CI)	Trend OR (P trend)
MCP-3 (pg/ml)				
7.2	19	13	1	
> 7.2 to 16.0	24	6	0.43 (0.13-1.38)	0.44
> 16.0	26	4	0.21 (0.06-0.78)	(0.0126)
sIL-4R (pg/ml)				
1360.0	20	12	1	
> 1360.0 to 1575.0	20	7	0.46 (0.14-1.48)	0.45
> 1575.0	29	4	0.23 (0.06-0.83)	(0.018)
MDC (pg/ml)				
889.5	27	6	1	
> 889.5 to 1224.5	26	4	0.68 (0.17-2.72)	1.86
> 1224.5	16	13	3.14 (0.95-10.37)	(0.0555)
IFN- α 2 (pg/ml)				
15.0	25	6	1	
> 15.0 to 33.1	26	7	1.06 (0.31-3.67)	1.37
> 33.1	18	10	1.95 (0.58-6.59)	(0.3104)
IL-7 (pg/ml)				
3.5	24	9	1	
> 3.5 to 12.1	26	8	0.81 (0.26-2.49)	0.95
> 12.1	19	6	0.95 (0.28-3.24)	(0.8735)
IL-8 (pg/ml)				
6.8	30	6	1	
> 6.8 to 10.7	19	10	2.66 (0.81-8.75)	1.35
> 10.7	20	7	1.85 (0.53-6.48)	(0.3272)
IL-10 (pg/ml)				
3.0	26	8	1	
> 3.0 to 11.5	22	9	1.41 (0.45-4.38)	1.03
> 11.5	21	6	1.09 (0.31-3.78)	(0.9002)

Note: Model is adjusted for age and ever/never smoked.

Table 5

Association of cytokines with the presence of a tubular adenoma relative to no polyps

	No Polyp n	Adenoma n	OR (95% CI)	Trend OR (P trend)
IFN- α 2 (pg/ml)				
15.0	25	9	1	
> 15.0 to 33.1	26	8	0.82 (0.27-2.50)	1.75
> 33.1	18	20	2.80 (1.01-7.73)	(0.0391)
IL-7 (pg/ml)				
3.5	24	8	1	
> 3.5 to 12.1	26	11	1.29 (0.43-3.84)	1.82
> 12.1	19	18	3.32 (1.14-9.66)	(0.0288)
IL-8 (pg/ml)				
6.8	30	6	1	
> 6.8 to 10.7	19	16	4.29 (1.41-13.13)	1.85
> 10.7	20	15	3.98 (1.30-12.24)	(0.0213)
IL-10 (pg/ml)				
3.0	26	9	1	
> 3.0 to 11.5	22	12	1.65 (0.58-4.74)	1.6
> 11.5	21	16	2.63 (0.927-7.47)	(0.083)
sIL-4R (pg/ml)				
1360.0	20	14	1	
> 1360.0 to 1575.0	20	16	0.98 (0.37-2.57)	0.61
> 1575.0	29	7	0.35 (0.12-1.04)	(0.0653)
MCP-3 (pg/ml)				
7.2	19	15	1	
> 7.2 to 16.0	24	12	0.72 (0.27-1.94)	0.68
> 16.0	26	10	0.48 (0.17-1.32)	(0.1362)
MDC (pg/ml)				
889.5	27	12	1	
> 889.5 to 1224.5	26	9	0.76 (0.27-2.14)	1.4
> 1224.5	16	16	2.00 (0.73-5.50)	(0.2119)

Note: Model is adjusted for age and ever/never smoked.