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Contrasting serum biomarker profiles in two Colombian populations with different risks for progression of premalignant gastric lesions during chronic *Helicobacter pylori* infection

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Abstract

Background: Colombians in coastal Tumaco have a lower incidence of *Helicobacter pylori*-associated gastric cancer compared to individuals from Tuquerres in the high Andes. This is despite nearly universal prevalence of *H. pylori* infection and chronic gastritis

Methods: *H. pylori* infection was confirmed by Steiner stain and serology using African and European-origin strains. Gastric histology, and serum inflammatory biomarkers in dyspeptic Tumaco or Tuquerres patients were evaluated to predict progression of gastric lesions.

Results: *H. pylori* infection was nearly universal by Steiner stain and serology. IgG response to European-origin *H. pylori* strains were greater than African-origin. High gastric cancer-risk Tuquerres patients, compared to low-risk Tumaco, had significant odds ratios for lesion progression associated with serum IL-5, trefoil factor 3 (TFF3), and low pepsinogen I/II ratio. Sensitivity and specificity for these parameters was 63.8% and 67.9%, respectively, with correctly classifying patients at 66.7%. Most odds ratios for 26 other biomarkers were significant for the

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town of residency, indicating an environmental impact on Tumaco patients associated with decreased lesion progression.

Conclusion: An IL-5 association with progression of gastric lesions is novel and could be evaluated in addition to TFF3 and pepsinogen I/II ratio as a non-invasive prognostic screen. Results suggest Tumaco patients were exposed to infectious diseases beyond *H. pylori* such as the documented high incidence of helminthiasis and toxoplasmosis.

Impact: Results support a prior recommendation to evaluate TFF3 and pepsinogen I/II together to predict aggressive gastric histology. Our data indicate IL-5 should be further evaluated as prognostic parameter.

Keywords

Colombia; *Helicobacter pylori*; gastric cancer; serum biomarkers

Introduction

The increased risk for gastric adenocarcinoma secondary to chronic gastritis caused by *Helicobacter pylori* infection has been extensively studied in Colombia [1, 2]. Colombia is representative of the 'altitude enigma' in Latin America where adults living in coastal areas appear to be partially protected against *Helicobacter pylori*-induced atrophic gastritis and intestinal metaplasia and subsequently have a 25-fold lower life-long incidence of gastric adenocarcinoma compared to cohort countrymen/women living at higher elevations [2, 3]. Colombians living in coastal, tropical Tumaco, historically have a lower risk for *H. pylori*-associated gastric cancer compared to patients of Tuquerres in the high Andes despite similar, near universal *H. pylori* infection and associated chronic gastritis. We and others have previously suggested that protection against development of gastric adenocarcinoma in *H. pylori*-infected Tumaco patients could be partially explained by differences in human and *H. pylori* genetics [4–6], *H. pylori* strain-associated virulence factors, regional diets, and the gastric microbiome [7–9]. Also, a higher exposure of Tumaco residents to intestinal helminth and protozoal parasites, including toxoplasmosis, evoked a predominant anti-inflammatory Th2-like IgG1 response to *H. pylori* [10, 11].

With the objective to establish serum markers of inflammation that would correlate with the cancer risk for each of these Colombian populations, we first determined that *H. pylori* infection was prevalent, as previously established [10]. We then analyzed serum levels of inflammation-related cytokines and chemokines and analyzed their potential association with progression of gastric pathology as detailed by Correa [12].

MATERIALS AND METHODS

Study Populations.

Human subjects with dyspepsia (n=163) underwent endoscopic gastric biopsy (Table 1). Patients from Tuquerres were predominantly mestizos of Spanish–Amerindian ancestry and were mostly from families engaged in agriculture. Tumaco patients living at sea level were predominantly of African–Spanish ancestry and came from families in which the main

occupations were related to fishing. Participation was voluntary and informed consent was obtained from all participants. Except for dyspepsia, patients did not have other significant illnesses; they had not received proton pump inhibitors, H_2-receptor antagonists, or antibiotics during the 30 days preceding endoscopy. The Ethics Committees of the participating hospitals in Nariño and the Universidad del Valle in Cali, Colombia and the Institutional Review Board of Vanderbilt University approved all study protocols, and all experiments were performed in accordance with the relevant guidelines and regulations.

Confirming prevalence of *H. pylori* infection.

H. pylori was assessed in all gastric biopsies using the Steiner silver stain, and was considered positive when *H. pylori* was observed in at least one biopsy from the same individual. Additionally, sera were assayed for serum IgG to *H. pylori* by ELISA as previously reported [10]. Control sera from patients that were PCR positive (n=3) or negative (n=3) in biopsy tissue for *H. pylori* were used to establish a cutoff for seroconversion which exceeded the mean OD (optical density) + 3 standard deviations of negative controls. Target antigens were outer membrane preparations from two African-origin strains (#5010 and #5024, mixed together based on total protein concentration) and two European-origin strains (#5056 and #5086, also mixed together) with virulence properties established by multilocus sequence typing [5, 13].

Luminex and ELISA measurements of biomarkers.

Sera from 156 of the 163 adults were assayed using the Bio-Rad 27-Plex Human Serum Cytokine kit as specified by the manufacturer. Seven samples with duplicative demographics were deleted to efficiently use the kit capacity. The following were assayed: IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, Basic FGF, Eotaxin-1, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGFββ, RANTES, TNF-α, and VEGF. R&D Systems Quantikine ELISA kits were used to measure RANTES, as the latter exceeded the range of the Luminex standard curve for the majority of samples. TFF3 and pepsinogens I and II were measured using ELISA kits (BioVendor, Candier, NC; Alpco Diagnostics, Salem NH), respectively.

Histology.

Histologic diagnoses and pathology scores were reported with a range of 1–6 based on a scoring system previously described [7]. The global diagnosis for each subject was determined independently by 2 pathologists (P.C. and M.B.P.) based on all gastric biopsies from antrum, incisura angularis, and corpus, according to the updated Sydney system for gastritis, including the degree of inflammation, atrophy, and intestinal metaplasia using the recommended visual analogue scales and the Padova International Classification for dysplasia [14, 15]. Global diagnoses were scored on an ordinal scale from 1 to 6, as follows: 1, mild to moderate non-atrophic gastritis (NAG); 2, severe NAG; 3, multifocal atrophic gastritis without intestinal metaplasia (MAG); 4, intestinal metaplasia (IM); 5, dysplasia; and 6, carcinoma. Degrees of inflammation (acute and chronic) were used to determine scores in cases with NAG.

In addition, a detailed histopathology scoring system was used to quantify differences in morphological variables within each global diagnosis category, which we have shown to be a sensitive and reliable system in a long-term follow-up of patients treated for *H. pylori* infection. This system assigns numerical values to the severity of the MAG, the extent and type of IM (representing the proportion of complete and incomplete types) and the severity of the dysplastic changes. We used this system because increased severity of MAG is associated with greater cancer risk, the incomplete type of IM carries a higher risk than the complete type, and the grade of dysplasia correlates with gastric cancer risk [15–18]. The multifocal atrophic gastritis (MAG) score (3) was modified, adding the following values: indefinite for atrophy (0.25), mild (0.50), moderate (0.75), and severe (1.0). The IM score (4) was modified according to type and extension. Four different IM types were defined: complete type (0.1), mixed predominant complete type (0.2), mixed predominant incomplete type (0.3), and incomplete (colonic) type (0.4). Periodic Acid Schiff/Alcian blue and high iron diamine/Alcian blue stains were used for the assessment of the type of IM [19]. For semi-quantification of the IM, each biopsy was also scored according to the area of the histological section with IM, in a 0–3 scale as follows: negative for IM, < 30%, >30 to 60%, and >60%, respectively. The mean extension of the IM (calculated using the total number of biopsies per subject) was grouped by tertiles. Each tertile was given a value: 0.2, 0.4, or 0.6, respectively. In order to obtain a total score for IM, values for type and extension were added to the original score for IM (4). The dysplasia score (5) was modified adding the following values: indefinite (0.25), low grade (0.50), and high grade (0.75). Both the augmented histopathology score and global diagnosis are reported but the global diagnosis was used for regression analyses.

Statistical Analysis.

Differences in serum levels of *H. pylori*-specific IgG, cytokines and chemokines were assessed by STATA 15 software (StataCorp, College Station, Texas). ELISA serology results were analyzed using unpaired t tests and histograms. Logistic regression used factors including histologic diagnosis, town of residence (Tumaco vs. Tuquerres), age and sex. Histologic diagnoses and global diagnosis scores between patient groups (town, sex) were assessed by ANOVA and the nonparametric tests of Mann–Whitney and Kruskal-Wallis. Contingency tests were performed by Chi square analysis. Cytokine and chemokine values were square root transformed as needed. p values < 0.05 were considered significant.

When the dependent variable was considered to be the town, coastal Tumaco or mountainous Tuquerres were considered for analysis as a binary variable using multivariate logistic regression. When the dependent variable was considered to be the overall histological diagnosis, data were divided into three groups: 1) Non atrophic gastritis (NAG), 2) Multifocal atrophic gastritis without intestinal metaplasia (MAG) and 3) Intestinal metaplasia / Dysplasia (IM/DYS). Odds Ratios and 95% confidence intervals (CIs) were estimated by ordinal logistic regression models to assess biomarkers in relation to progression of gastric lesions. Each biomarker was assessed categorically according to the percentage of values above the lower limit of detection: biomarkers detected in 75% or more of subjects were categorized into quartiles; biomarkers detected in 50 – <75% of subjects were categorized into tertiles; and biomarkers detected in <50% of subjects were

dichotomized at the median. Cut-points were determined using the entire study population. Odds ratios (OR) for the town of residence were reported using Tuquerres as the default town. ORs were inverted (1/default OR) to aid in interpretation of the OR for Tumaco patients. The sensitivity and specificity of evaluating the pepsinogen I/II ratio, TFF3 and IL-5 were assessed independently as well in combination. Data analysis was based on 153 patients that had values for all 3 parameters. Sensitivity, specificity and area under the curve (AUC) for serum levels of pepsinogen I and II to produce the pepsinogen I/II ratio, TFF3 and IL-5 were calculated for estimating progression of gastric lesions. Histologic scores of 2 or less were considered baseline (no lesions to nonatrophic gastritis); scores of 3 (multifocal atrophic gastritis) or higher (score 4 for intestinal metaplasia, 5 for dysplasia and 6 for carcinoma) were grouped as signifying progression of gastric lesions.

Results

H. pylori infection was highly prevalent in both Tumaco and Tuquerres.

H. pylori was recorded as present or absent in gastric biopsies using the Steiner silver stain (Table 2). *H. pylori* prevalence as determined by the Steiner stain was 82% in Tumaco and 85% in Tuquerres. Serum IgG to *H. pylori* as determined by ELISA indicated that all but one patient had seroconverted (Table 2, Figure 1) consistent with a previous report [10]. Serum IgG levels were greater when the European strains were used as antigens compared to the African strains in both towns; IgG serum levels were significantly higher in Tuquerres for the European and African strains.

Gastric pathology

Higher gastric lesion scores were observed in Tuquerres males and females combined (Figure 2A) ($p < 0.03$) with a trend for the highest scores in Tuquerres males (Figure 2B) ($p<0.08$). Non-atrophic gastritis was more common in Tumaco and the premalignant change of IM was more common in Tuquerres ($p<0.02$) (Table 3). There were two cases that progressed to dysplasia in Tumaco women that were aged 47 and 56 yrs. Only one case of gastric adenocarcinoma was observed in a Tuquerres male aged 43 yrs (Table 3). Only one patient, a female aged 47 yrs., from Tumaco had normal gastric tissue and was negative for *H. pylori* by Steiner stain.

Regression analyses.

Logistic regression used factors of global diagnosis, town of residence, age and sex in adult patients. Patient age and sex were not significant factors in regression analyses and were subsequently excluded. Progression of gastric pathology, and in particular the town of patient residence, were significant factors.

IL-5, TFF3, pepsinogen II and a low pepsinogen I/II ratio were associated with progression of gastric lesions.

When the dependent variable was the overall histologic diagnosis, ordinal logistic regression provided an odds ratio which estimated either progression (greater than 1) or a decreased rate of lesion progression (less than 1) (Figure 3). The Forest plot based on histologic diagnosis demonstrated that serum levels of IL-5, TFF3, and pepsinogen II and a low

pepsinogen I/II ratio were associated with progression of gastric lesions (Table 4). Measurements of IL-5 were grouped in 4 quartiles and only the second quartile was significantly higher than the first quartile (OR 2.76, 95% CI 1.18 – 6.44, p=0.019). Most of the patients with a diagnosis of NAG had low IL-5 levels in the first quartile whereas most patients with IL-5 levels in the second quartile had IM and dysplasia. There was not a linear relationship between IL-5 levels and further progression of gastric lesions in the third or fourth quartiles. TFF3 serum levels were highest in Tuquerres patients and levels in the fourth quartile were associated with lesion progression with an OR of 2.67, 95% CI 1.10–6.50, p=0.03. Pepsinogen II was also found to be associated with progression of gastric lesions when comparing the fourth and first quartiles (OR 2.71, 95% CI 1.15 – 6.41, p=0.023). These serum values contributed to a low pepsinogen I/II ratio being associated with progression when comparing the ORs of the first to the second (OR 0.54 (0.23–1.25), p=0.001) and third (OR 0.19, (0.08–0.47), p=0.005) quartiles in Tuquerres. Inverting these ORs to predict risk of lesion progression in Tumaco (Table 4) demonstrated that these patients had high ORs of 5.16 and 3.47 for the third and fourth quartiles of the pepsinogen I/II ratio, respectively, indicating decreased rates of lesion progression. The sensitivity and specificity of evaluating the pepsinogen I/II ratio, TFF3 and IL-5 were assessed independently as well in combination. The pepsinogen I/II ratio evaluated alone yielded a sensitivity of 44.7% and specificity of 83% with 71.2% of patients correctly classified. Adding TFF3 to this analysis did not improve these results but adding serum levels of IL-5 yielded an improved sensitivity of 63.8% with a specificity of 67.9% and correctly classifying patient status at 66.7%. The area under the curve was 0.66 with a standard error of 0.04 and confidence interval of 0.58 – 0.74.

The town of residence explained most of the variance in biomarker profiles.

The Forest plot for examining the data using the town of residence as the dependent variable in logistic regression demonstrated significant differences between patients living in Tumaco versus Tuquerres (Figure 4). Given that Tumaco patients are known to be at lower risk for gastric cancer compared to residing in Tuquerres, the serum levels of the majority of biomarkers in Tumaco samples had significantly higher serum levels which yielded ORs lower than 1, representing a decreased rate of gastric lesion progression (Table 5). The greatest inverted ORs were noted for RANTES (T3 vs. T1, OR 191.67), IL-8 (Q4 vs. Q1, OR 147.25) and PDGFββ (Q3 vs. Q1, OR 105). Linear relationships between all quartiles (Q4>Q3>Q2>Q1) or tertiles (T3>T2>T1) were evident for IL-12, IL-1β, IL-1RA, IL-4, IL-6, IL-7, IL-8, MIP-1β, TFF3, RANTES, eotaxin-1, PDGFββ and TNFα.

Discussion

Gastric cancer of the intestinal type is usually preceded by a decades-long precancerous process driven by *H. pylori* infection with well-defined progressive lesions including glandular atrophy and intestinal metaplasia [12]. In the current study, serology, Steiner stain and histopathologic diagnoses supported endemic *H. pylori* infection in both areas with a higher risk for precursor lesions and gastric adenocarcinoma in Tuquerres residents. Elevations of inflammatory biomarkers in serum enabled us to evaluate association with progression of gastric pathology. Based on histologic diagnoses as the dependent variable,

regression identified serum IL-5 as a biomarker of gastric lesion progression, particularly in combination with increased serum TFF3 and pepsinogen II, consistent with a prior report [20]. In contrast, regression based on the town of residency revealed Tumaco patients had significant serum levels for the majority of biomarkers which suggested concurrent helminthiasis and potentially other infectious diseases such as exposure to toxoplasmosis, all which play important roles in Colombian public health [10].

We documented the high prevalence of *H. pylori* infection using the Steiner stain and serology. The immune IgG responses to the European strains were higher in both Tumaco and Tuquerres patients and is likely attributable to the greater inflammatory response associated with the more virulent European strains [5]. Both African and European *H. pylori* strains have been isolated from patients in Tumaco and Tuquerres and thus the serology data supports the epidemiology of mixed infections with *H. pylori*. Both Steiner stain and serology have limitations [21]. Steiner silver stain can promote false-negative results based on the quality of the biopsy and relative density of *H. pylori*. Serology is sensitive and serves as an index of *H. pylori* prior exposure but does not confirm current infection.

The patients analyzed had dyspepsia as the primary complaint. While there are multiple dyspepsia etiologies, gastritis attributable to chronic *H. pylori* infection appears responsible. Notably there was very low incidence of normal stomachs (1 of 163 biopsies) and only one gastric carcinoma. Our demographic selection had limitations including more female than male patients along with middle age predominating. When possible, a wider scope would include older and preferably equal numbers of male and female patients. Given the well-established predisposition of older men to gastric cancer [12], our demographics nonetheless yielded actionable information as there was elevated risk of multifocal atrophic gastritis accompanied by intestinal metaplasia in Tuquerres.

Previous gastric biopsy data on these two populations have noted elevated eosinophil density in males from Tumaco compared to Tuquerres, suggesting a protective effect against the *H. pylori* inflammatory response [22]. In both populations, eosinophil density increased with the histopathology score in the progression of lesions from normal morphology to multifocal atrophic gastritis [22], suggesting that chronic inflammatory responses to *H. pylori* infection in the gastric mucosa is modulated by concurrent helminthiasis [22]. Abundant eosinophils that bias the immune response toward a gastric mucosa Th2 phenotype could limit tissue injury. In the current study, eotaxin, noted to be higher in the low risk population, is a potent eosinophil chemoattractant that acts in synergy with IL-5 to stimulate the release of eosinophils from bone marrow and to recruit these cells into inflammatory sites, including the gastrointestinal tract [23]. Also of interest, IL-4, IL-5 and IL-13 were elevated in the Tumaco patients and are produced during tissue damage caused by helminth tissue invasion, which induces type 2 cell mediated immunity [24]. Wound-healing properties of elevated serum PDGFββ demonstrated in Tumaco patients may have contributed to a decreased rate of lesion progression.

Histologic diagnoses were significantly different by town of residence. Elevated serum IL-5, TFF3, and pepsinogen II were identified as significant risk factors for lesion progression. This ordinal logistic regression provided an OR which measured either progression (>1) or

decreased rate of progression (<1) of lesions along the cascade of NAG - MAG – IM/DYS [12]. IL-5 was the only interleukin that was significant by regression considering histologic diagnosis as the dependent variable and is a novel finding. IL-5 has been associated with allergy, *H. pylori* infection and risk for gastric carcinoma and has an important role in eosinophil and mast cell biology [25]. A second biomarker, elevated serum TFF3, stands out as a biomarker for IM, which is well supported in the literature [20] and our data. TTF3 serum levels were higher in Tuquerres compared to Tumaco patients, particularly Tuquerres men compared to Tumaco men. Two individuals (one patient from each town) with very high levels of TFF3 both had a histologic diagnosis of IM. A third biomarker we established as associated with lesion progression was elevated serum pepsinogen II. Pepsinogen I has been evaluated as a biomarker for decreased chief cell mass resulting in lower pepsinogen I and thus a lower pepsinogen I/II ratio in the peripheral blood [26]. We observed elevated pepsinogen II in Q4 vs. Q1 and Q3 vs. Q1 which appears to have similar promotional effects on lesion progression. This yielded a lower pepsinogen I/II ratio which has been traditionally used for monitoring the risk of gastric cancer [26]. Overall, the pepsinogen I/II ratio was lower and serum levels of TFF3 factor remained significantly higher in patients with more severe gastric pathology, consistent with greater progression of lesions in Tuquerres patients. This agrees with a previous report that suggested risk analysis was more predictive if the pepsinogen ratio and TFF3 were evaluated together [20]. Adding serum levels of IL-5 yielded improved the sensitivity but lowered the specificity. These results are comparable to but not as high as reported for using pepsinogen I/II ratio and TFF3 in combination [20]. A potential reason is that our study only had 1 case of carcinoma wheras the cited study had a large number of patients with gastric cancer.

The second approach was to perform logistic regression using town of residency as the dependent variable. The data obtained from the Luminex platform and ELISAs indicated there was a strong correlation between the majority of serum cytokine and chemokine levels and living in the low risk coastal region. Tumaco patients, compared to Tuquerres patients, had significantly higher serum levels of pro-inflammatory biomarkers associated with innate immunity such as IL-1β, IL-6, IL-7, IL-8, IL-12p70, MIP-1α, MIP-1β, RANTES, and TNFα. Anti-inflammatory IL-4 and IL-13 were also elevated in Tumaco patients. Seemingly paradoxical, serum levels of IL-8, often implicated in infection with virulent *H. pylori* strains, was significantly higher in coastal Tumaco. We attribute the IL-8 and other proinflammatory cytokine responses to greater exposure to a variety of enteric infections and possible tissue damage related to migrating helminths in coastal residents compared to the Andes region of Tuquerres. Consistent with tissue damage in Tumaco patients, PDGFββ, FGF basic and VEGF, all associated with wound healing, were higher in the Tumaco population. RANTES, commonly elevated in acute infections and is chemotactic for T cells, eosinophils, and basophils into inflammatory sites, was much higher in Tumaco patients, supporting the inflammatory status of these residents. The balance between proinflammatory and anti-inflammatory cytokines and chemokines may be evolutionarily important in inhibiting progression of *H. pylori*-related gastric lesions to overt cancer. Our human studies have been supported by our work in animal models to more fully understand how intestinal parasitism promotes a Th2 anti-inflammatory immune response to delay the progression of

H. pylori infection to gastric atrophy and premalignant lesions which culminate in gastric cancer [10, 11, 27, 28].

This study found a distinct difference in the inflammation status of the Tumaco and Tuquerres patients. A novel suggestion is to consider using serum IL-5, TFF3 and the pepsinogen I/II ratio to screen Colombian patients for their risk of gastritis progressing to gastric cancer. The data support the 'altitude enigma' in Latin and South America where adults living in coastal areas appear to be partially protected against *H. pylori*-induced atrophic gastritis and the cascade to intestinal metaplasia, dysplasia and cancer compared to cohort populations living at higher elevations [1–4].

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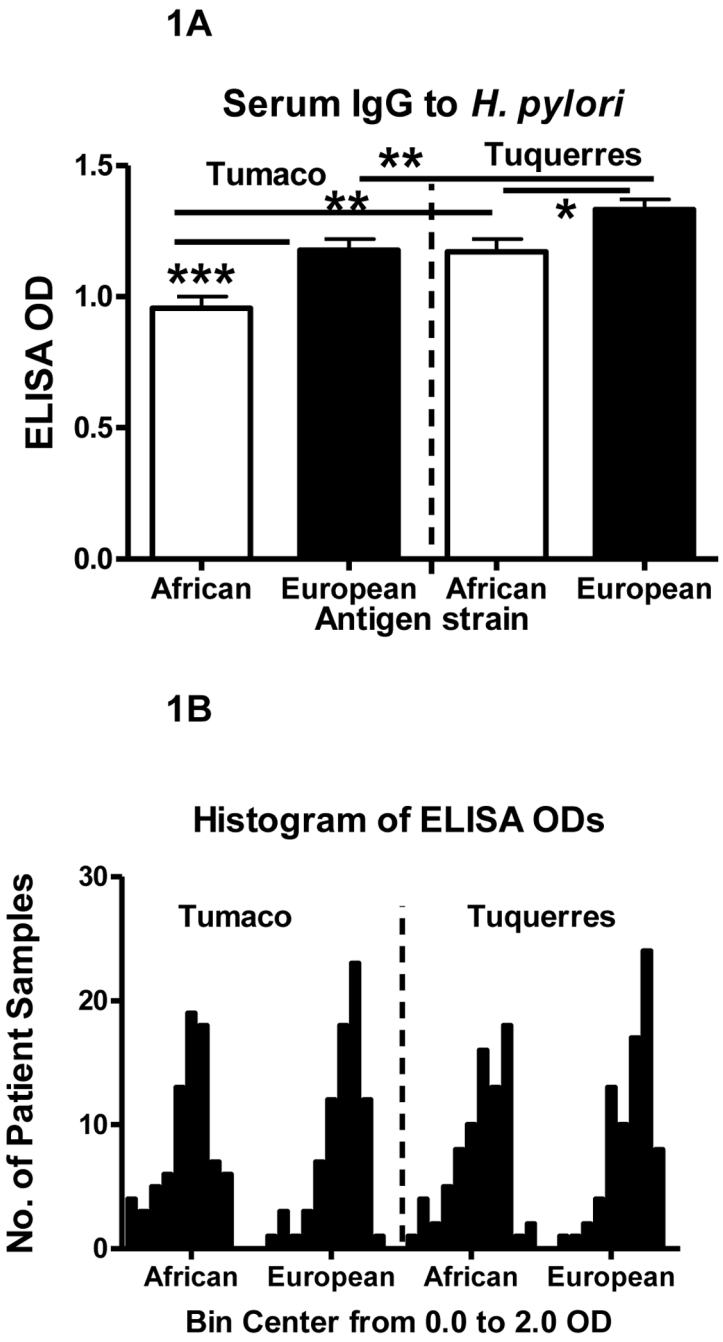

Figure 1.

A. Mean ± Std Error of patient sera from Tumaco (n=82) and Tuquerres (n=81) that were screened by ELISA for serum IgG to composite antigens processed from African or European-origin *H. pylori* strains. IgG levels were highest for European-origin strains for both locations and highest overall for Tuquerres. *p<0.05, **p<0.01, ***p<0.001. B. Data presented in Figure 1A were analyzed for subject frequency via histograms for responses to African or European-origin *H. pylori* strains.

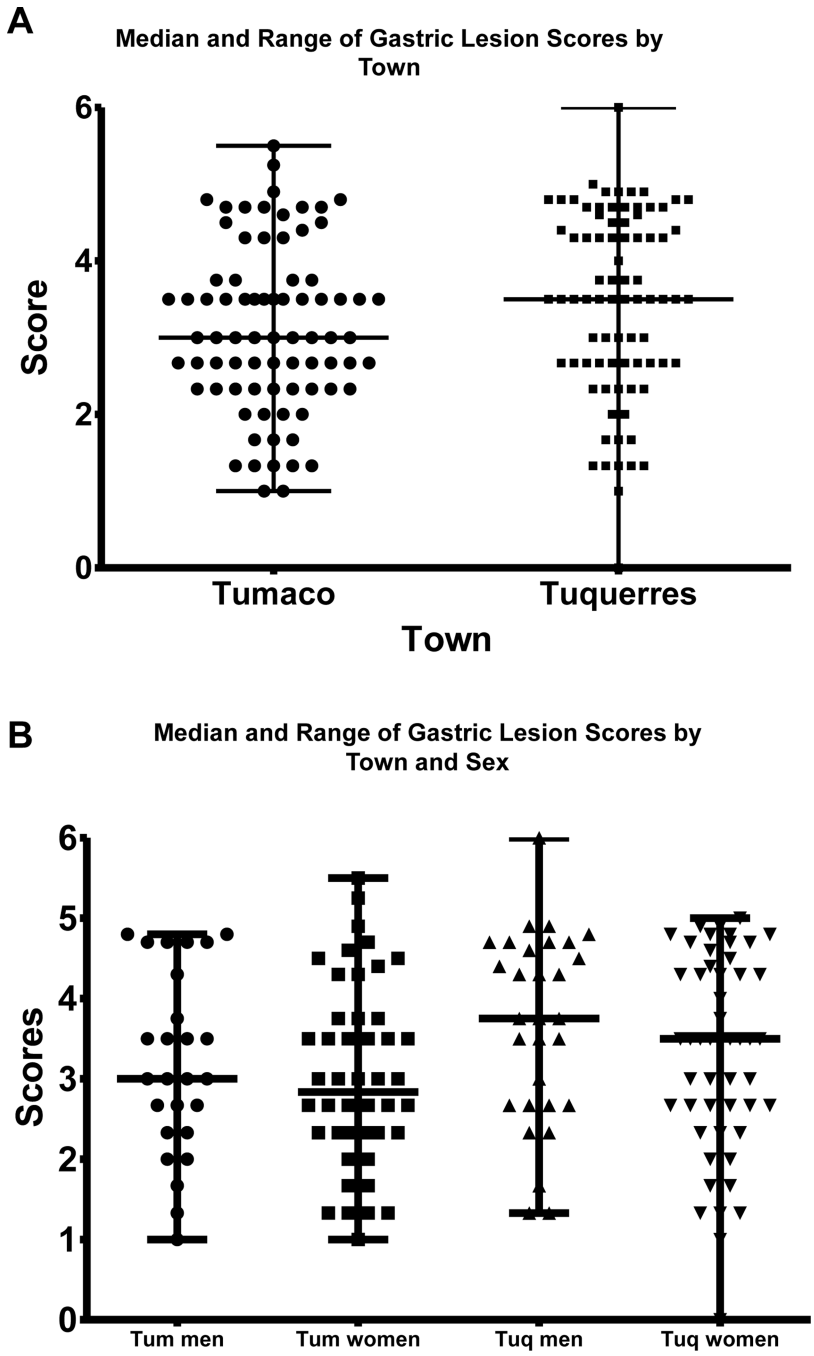

Figure 2.

A. Median and range of gastric lesion histological scores from patients residing in Tumaco (n=82) and Tuquerres (n=81). Lesion scores were higher in Tuquerres $p<0.03$. B. Median and range of gastric lesion histological scores analyzed by town of residence and patient sex. Tumaco (Tum) men n=26, Tumaco women n=56, Tuquerres (Tuq) men n=30, Tuquerres women n= 51. Lesion scores trended higher in men from Tuquerres compared to other groups ($p<0.08$).

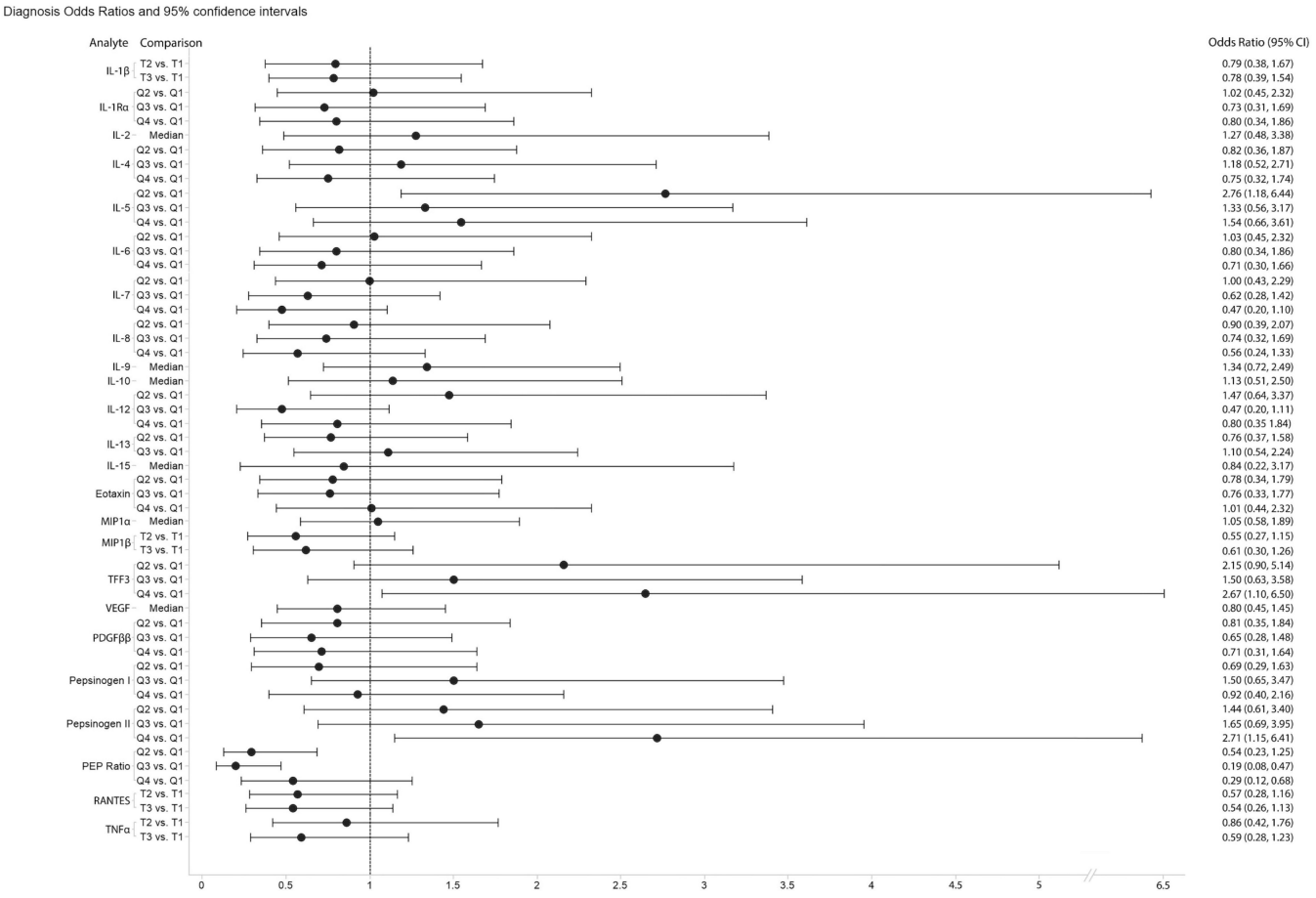

Figure 3.

Summary plot of associations between circulating serum biomarkers and histologic diagnosis. Comparisons were between the lowest and the next highest tertile or quartile. Each circle represents an odds ratio (OR) estimate, and the length of the horizontal line between the whiskers represents the 95% confidence interval (CI). Circulating markers are ordered by class and the numeric results are shown on the rightmost column. n=156 samples.

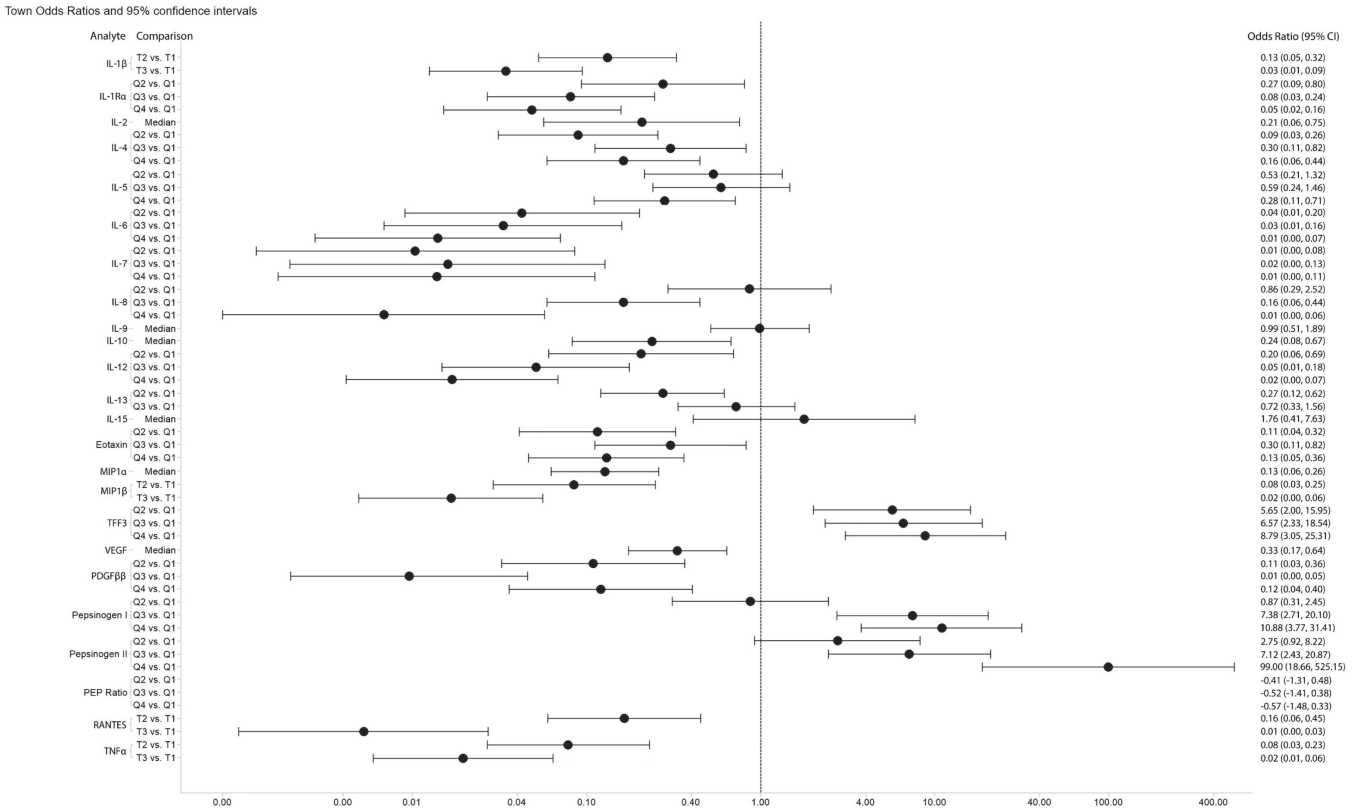

Figure 4.

Summary plot of associations between circulating serum biomarkers and town of residence. Comparisons were between the lowest and the next highest tertile or quartile. Each circle represents an odds ratio (OR) estimate, and the length of the horizontal line between the whiskers represents the 95% confidence interval (CI). Circulating markers are ordered by class and the numeric results are shown on the rightmost column. n=156 samples.

Table 1.

Demographics of patients from Tumaco and Tuquerres.

Adults	n	Age range (yrs)	Median age (yrs)
Tumaco Females	56	40–59	48.5
Tumaco Males	26	42–62	52
Total Tumaco	82		
Tuquerres Females	51	40–60	46
Tuquerres Males	30	40–57	49
Total Tuquerres	81		

Table 2.

Prevalence of *H. pylori* (Hp) infection as determined by Steiner stain and serology.

Steiner Stain	Hp neg	Hp pos	n	% Positive
Tumaco	15	67	82	82%
Tuquerres	12	69	81	85%
Serology				
Tumaco	1	80	81	99%
Tuquerres	0	80	80	100%

Table 3.

Histologic Diagnosis.

	Normal	NAG	MAG	IM	Dysplasia	Carcinoma
Tumaco	0	47*	18	15	2	0
Tuquerres	1	31	17	31*	0	1

NAG = Nonatrophic gastritis; MAG = multifocal atrophic gastritis without intestinal metaplasia; IM = intestinal metaplasia.

*p<0.02

Table 4.

Biomarkers with significant p values from regression based on histologic diagnosis.

Analyte	Comparison	OR*	p value	Low CI	High CI	Inverted OR**
IL-5	Q2 vs. Q1	2.76	0.019	1.18	6.44	0.36
TFF3	Q4 vs. Q1	2.67	0.03	1.10	6.50	0.37
Pepsinogen II	Q4 vs. Q1	2.71	0.023	1.15	6.41	0.37
PEP I/II Ratio	Q2 vs. Q1	0.19	0.001	0.08	0.47	5.16
PEP I/II Ratio	Q3 vs. Q1	0.29	0.005	0.12	0.68	3.47

*OR is the odds ratios for Tuquerres as the default town.

**The Inverted OR is an alternative approach to evaluate ORs in Tumaco.

Table 5.

Biomarkers with significant p values from regression based on town of residence.

Analyte	Comparison	OR*	p value	Confidence Interval Range		Inverted OR**
TFF3	Q2 vs. Q1	5.65	0.001	2.00	15.95	0.18
TFF3	Q3 vs. Q1	6.57	0.001	2.33	18.54	0.15
TFF3	Q4 vs. Q1	8.79	0.001	3.05	25.31	0.11
Eotaxin	Q2 vs. Q1	0.11	0.001	0.04	0.32	8.72
Eotaxin	Q3 vs. Q1	0.30	0.019	0.11	0.82	3.32
Eotaxin	Q4 vs. Q1	0.13	0.001	0.05	0.36	7.75
IL-10	Median	0.24	0.007	0.08	0.67	4.25
IL-12	Q2 vs. Q1	0.20	0.011	0.06	0.69	4.90
IL-12	Q3 vs. Q1	0.05	0.001	0.01	0.18	19.69
IL-12	Q4 vs. Q1	0.02	0.001	0.00	0.07	59.50
IL-13	Q2 vs. Q1	0.27	0.002	0.12	0.62	3.67
IL-1β	T2 vs. T1	0.13	0.001	0.05	0.32	7.67
IL-1β	T3 vs. T1	0.03	0.001	0.01	0.09	29.33
IL-1RA	Q2 vs. Q1	0.27	0.018	0.09	0.80	3.67
IL-1RA	Q3 vs. Q1	0.08	0.001	0.03	0.24	12.37
IL-1RA	Q4 vs. Q1	0.05	0.001	0.02	0.16	20.63
IL-2	Median	0.21	0.017	0.06	0.75	4.86
IL-4	Q2 vs. Q1	0.09	0.001	0.03	0.26	11.24
IL-4	Q3 vs. Q1	0.30	0.019	0.11	0.82	3.32
IL-4	Q4 vs. Q1	0.16	0.001	0.06	0.44	6.20
IL-5	Q4 vs. Q1	0.28	0.008	0.11	0.71	3.57
IL-6	Q2 vs. Q1	0.04	0.001	0.01	0.20	23.64
IL-6	Q3 vs. Q1	0.03	0.001	0.01	0.16	30.39
IL-6	Q4 vs. Q1	0.01	0.001	0.00	0.07	71.69
IL-7	Q2 vs. Q1	0.01	0.001	0.00	0.08	96.73
IL-7	Q3 vs. Q1	0.02	0.001	0.00	0.13	63.33
IL-7	Q4 vs. Q1	0.01	0.001	0.00	0.11	73.08
IL-8	Q3 vs. Q1	0.16	0.001	0.06	0.44	6.20
IL-8	Q4 vs. Q1	0.01	0.001	0.00	0.06	147.25
MIP1α	Median	0.13	0.001	0.06	0.26	7.87
MIP1β	T2 vs. T1	0.08	0.001	0.03	0.25	11.85
MIP1β	T3 vs. T1	0.02	0.001	0.00	0.06	60.43
PDGFββ	Q2 vs. Q1	0.11	0.001	0.03	0.36	9.21
PDGFββ	Q3 vs. Q1	0.01	0.001	0.00	0.05	105.00
PDGFββ	Q4 vs. Q1	0.12	0.001	0.04	0.40	8.31
Pepsinogen I	Q3 vs. Q1	7.38	0.001	2.71	20.10	0.14

Analyte	Comparison	OR*	p value	Confidence Interval Range		Inverted OR**
Pepsinogen I	Q4 vs. Q1	10.88	0.001	3.77	31.41	0.09
Pepsinogen II	Q3 vs. Q1	7.12	0.001	2.43	20.87	0.14
Pepsinogen II	Q4 vs. Q1	99.00	0.001	18.66	525.15	0.01
RANTES	T2 vs. T1	0.16	0.001	0.06	0.45	6.08
RANTES	T3 vs. T1	0.01	0.001	0.00	0.03	191.67
TNFα	T2 vs. T1	0.08	0.001	0.03	0.23	12.82
TNFα	T3 vs. T1	0.02	0.001	0.01	0.06	51.70
VEGF	Median	0.33	0.001	0.17	0.64	3.02

*OR is the odds ratios for Tuquerres as the default town.

**The Inverted OR is an alternative approach to evaluate ORs in Tumaco.