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Serum levels of interleukin 1β, interleukin 8 and tumour necrosis factor α as markers of gastric cancer

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Abstract
Despite the efforts made, a serum marker reliable for the screening and follow-up of patients with gastric cancer has not yet been identified. The aim of this preliminary study was to test the role of pro-inflammatory cytokines interleukin 1β, interleukin 8 and tumour necrosis factor α in patients with gastric cancer and in control groups. The statistical analysis of cytokines serum levels in the group with gastric cancer versus control groups has shown considerable differences ($p < 0.001$) in their mean rates. The results indicate that the cytokines interleukin 1β, interleukin 8 and tumour necrosis factor α might perhaps act as diagnostic markers in patients with gastric cancer. Therefore, it is hypothesized that after more extended trials, their use in the screening and prognostic assessment of these patients could be a possibility.

Keywords: *gastric cancer, cytokine, markers*

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Introduction

Gastric cancer is the 14th leading cause of death worldwide and it is the second among neoplastic diseases (Murray & Lopez 1997). Although several markers, either with prognostic or screening aims, have been tested, so far the most sensitive one is still being searched for. Traditional markers such as carcinoembryonal antigen (CEA) and carbohydrate antigen 19.9 (CA 19-9) (Wahren & Hamemberg 1991) have not been very useful, although CA 72-4 has shown a superior reliability (Mattar et al. 2002). According to some authors (Ley et al. 2001), pepsinogen I (PGI), which is particularly accurate in the screening of patients with atrophic gastritis (Correa et al. 1976, Knight et al. 1996), has some limitations, such as its limited prognostic role, its complexity and the expensive costs of its determination, as well as the difficulty in identifying its right cut-off. Literature data (Forones et al. 2001, Chong et al. 2002) point out that cytokines, especially interleukin 1β (IL-1β), interleukin 8 (IL-8) and tumour necrosis factor-α (TNF-α), can play a significant role in gastric carcinogenesis.

The endpoint of this preliminary study was to value the serum levels of the three cytokines in patients with gastric cancer versus control groups in order to state,

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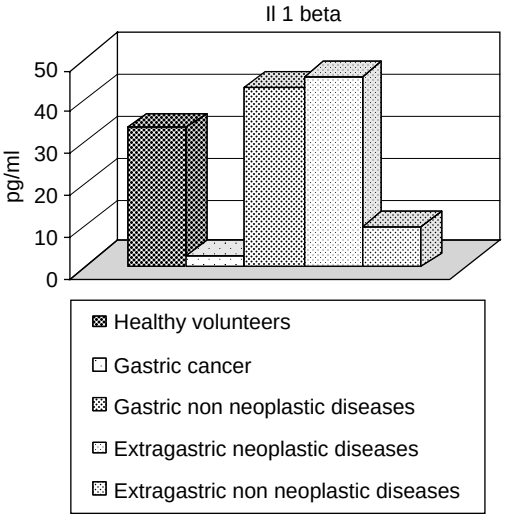

Figure 1. IL-1 beta mean levels.

according to the critical analysis of the findings, their potential role as reliable markers in the identification and follow-up of patients with such a neoplasy.

Materials and methods

From 1 January to 31 December 2003, 66 patients were enrolled: 15 (22.7%) healthy volunteers, 10 (15.2%) with gastric cancer, 13 (19.7%) with gastric non-neoplastic diseases, 14 (21.2%) with extragastric neoplastic diseases and 14 (21.2%) with extragastric non-neoplastic diseases. Tables I–V show each patient characteristic stratified into groups, which were homogeneous according to age (Table VI). The

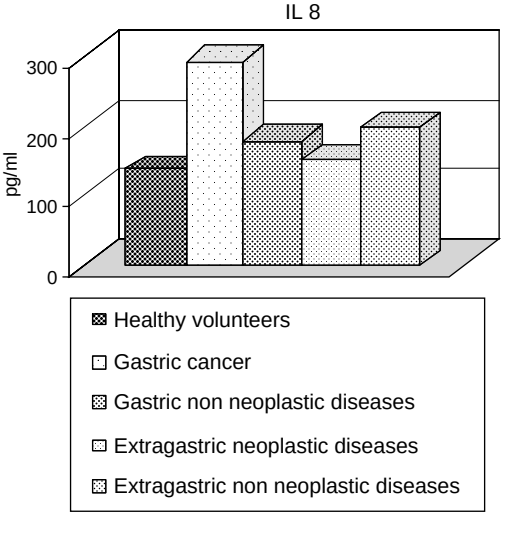

Figure 2. IL-8 mean levels.

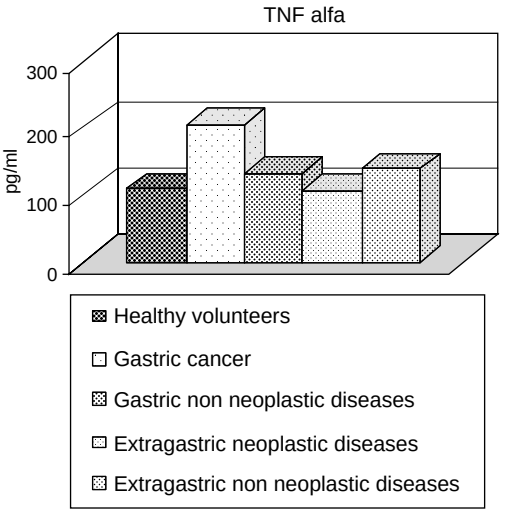

Figure 3. TNF-α mean levels.

selection of the groups of patients to insert in the study was carried out in an attempt to demonstrate the sensitivity and specificity of the markers for gastric cancer.

The presence of *Helicobacter pylori* was searched for in every case of gastric cancer by using Giemsa's stain.

The histological classification of non-neoplastic gastric lesions was made according to the Revised Sydney System (Dixon et al. 1996).

After informed consent, IL-1β, IL-8 and TNF-α were dosed in each patient from a sample of venous blood taken on fasting, which was then centrifuged and kept at a temperature of −80°C. The following techniques were used: IL-1β, IL-8 and TNF-α were measured in a solid-phase sandwich enzyme linked-immunosorbent assay (BioSource Int., Inc., Camarillo, CA, USA) on the Mago Plus DW analyser (Delta Biologicals Srl, Pomezia, Italy). Analytical imprecision for IL-1β was 4.5% CV at a level of 60.2 pg/ml and 4.7% CV at 194.9 pg/ml, for IL-8 3.9% CV at 74.92 pg/ml and

Table I. Healthy volunteers.

Patient	Sex	Age (years)	IL-1β	IL-8	TNF-α
GT	M	62	38	139	116
AI	M	70	29	128	112
RF	M	48	33	140	120
TS	M	50	41	146	110
MN	M	81	29	136	118
PO	M	44	39	126	106
RL	M	65	20	135	115
GE	F	60	19	141	99
CI	F	53	30	130	95
TM	F	60	27	140	108
RC	F	47	34	135	95
UL	F	57	40	147	107
PR	F	61	47	138	98
SD	F	76	42	147	120
IA	F	55	33	129	108

Table II. Patients with gastric cancer.

Patient	Sex	Age (years)	Tumour site	Dimension (cm)	Metastasis	Macrosopic feature (Bormann)	Histological exam	HP	IL-1β	IL-8	TNF-α
MM	F	76	body	7 × 6	liver, gallbladder	B3	intestinal moderately differentiated T3, N3	+	41	94	99
UE	F	64	body	6	liver	B2	intestinal well differentiated T3, N3	–	1	310	180
VM	F	68	antrum	5		B4	intestinal moderately differentiated T3, N2	+	2	270	210
GA	F	89	antrum	4 × 3		B2	intestinal moderately differentiated T3, N2	–	6	240	190
CG	F	53	stump	plastic linitis		B4	anaplastic T3, N2	–	4	316	240
FR	F	64	stump	plastic linitis		B4	intestinal well differentiated T2, N1	–	2	240	220
MG	F	68	antrum body	10		B1	intestinal well differentiated T2, N0	+	2	290	210
FG	M	72	body	7 × 7		B1	intestinal moderately differentiated T2, N2	–	2	294	197
MF	M	70	antrum	4 × 2		B1	intestinal well differentiated T2, N1	–	5	312	201
PF	M	42	antrum	4		B3	signet ring cell poorly differentiated T2, N2	–	4	265	198

Table III. Patients with gastric non-neoplatic diseases.

Patient	Sex	Age (years)	Anamnesis	Gastroscopy	HP	Histology	IL-1β	IL-8	TNF-α
RD	F	69	epigastralgia	hyperemic mucosa with antral erosions	–	low acute gastritis	31	130	96
VN	F	45	epigastralgia, cholelithiasis	mucosal hyperemia	+	low acute gastritis	46	92	104
GE	F	78	epigastralgia, cholelithiasis	hyperemic antral mucosa	+	middle acute gastritis	63	197	91
FF	F	81	epigastralgia, melena	sliding jatus hernia, pyloric ulcer	–	high acute gastritis	2	401	301
AG	M	62	epigastralgia gastrectomy 20 years earlier for ulcer; choledocholithiasis	mucosal hyperemia	–	low chronic gastritis	2	310	220
CS	M	35	epigastralgia	mucosal hyperemia	–	low acute gastritis	35	145	124
CG	M	75	melena; BII gastrectomy in 1974 for gastric ulcer	anastomic erosions	–	high acute gastritis	3	296	214
CS	M	59	epigastralgia; BII gastrectomy in 1977 for gastric ulcer	low mucosal hyperemia	–	low chronic gastritis	50	156	98
CF	M	81	melena	hyperemia of gastric mucosa and pyloric erosions	–	high acute gastritis	45	145	101
FG	M	45	epigastralgia, cholelithiasis	hyperemia gastric mucosa	–	low acute gastritis	38	164	112
IG	M	47	retrosternal pirosis	mucosal hyperemia	–	middle acute gastritis	60	156	110
MB	M	75	anaemia, epigastralgia	three angiodysplasiae (subcardial, body and prepyloric)	–		36	165	132
MC	M	58	epigastralgia	hyperemia gastric mucosa	–	low acute gastritis	60	165	125

Table IV. Patients with extragastric neoplastic diseases.

Patient	Sex	Age (years)	Neoplasm	IL-1β (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)
DS	F	64	colon carcinoma Dukes C	9	178	135
TL	F	50	rectal cancer Dukes B	7	189	128
IC	F	50	ovarian carcinoma Stage III	12	195	115
TM	F	79	colon carcinoma Dukes D	15	201	127
OL	F	67	bladder carcinoma T2, G3	11	197	131
RN	F	58	ovarian carcinoma Stage III	8	178	140
PA	F	58	colon carcinoma Dukes C	9	182	142
EN	M	70	bladder carcinoma T3, G3	11	180	140
UF	M	51	renal carcinoma Grade II (Fuhrman classification)	10	190	154
CL	M	50	pancreatic cancer Stage IIB	9	187	146
EN	M	84	colon carcinoma Dukes D	7	201	138
LE	M	66	colon carcinoma Dukes B	11	198	145
FF	M	55	colon carcinoma Dukes C	9	192	161
AV	M	58	pancreatic cancer Stage IV	10	180	157

Table V. Patients with extragastric non-neoplastic diseases.

Patient	Sex	Age (years)	Disease	IL-1β	IL-8	TNF-α
FG	F	18	appendicitis	28	141	98
GG	F	34	cholelithiasis	36	136	94
LA	F	22	cholelithiasis, appendicitis	46	148	120
RL	F	44	transphenteric anal fistula	92	106	102
SG	F	68	hemorrhoidal disease	52	116	86
CV	F	68	nephrolithiasis	40	196	94
BM	F	26	chronic appendicitis	40	160	110
AG	M	67	anal fistula	39	150	100
DA	M	69	incisional hernia	36	156	110
GF	M	77	inguinal hernia	42	158	96
MC	M	74	inguinal hernia	65	166	132
NA	M	69	cholelithiasis	48	158	99
PA	M	28	umbilical hernia	61	165	130
VL	M	16	inguinal hernia	45	171	114

Table VI. Age: mean, standard deviation and 95% confidence interval.

	Age (years)		
	Mean	SD	CI 95%
Healthy volunteers	59.27	10.59	53.40–65.13
Gastric cancer	64.60	11.64	57.55–75.64
Gastric non-neoplastic diseases	62.31	10.60	58.88–67.74
Extragastric neoplastic diseases	58.57	13.61	44.93–52.21
Extragastric non-neoplastic diseases	61.43	10.84	55.17–67.69

Table VII. Ilβ: mean, standard deviation and 95% confidence interval.

	IL-1β		
	Mean	SD	CI 95%
Healthy volunteers	33.40	7.98	37.82–37.82
Gastric cancer	2.90	0.72	2.41–3.13
Gastric non-neoplastic diseases	43.13	11.77	36.04–50.27
Extragastric neoplastic diseases	45.71	9.04	40.49–50.94
Extragastric non-neoplastic diseases	9.87	2.11	8.64–11.07

5.3% CV at 991.8 pg/ml and for TNF-α 5.2% CV at 58.0 pg/ml and 3.9% CV at 459.0 pg/ml.

For IL-1β, IL-8 and TNF-α, the mean, standard deviation and 95% confidence interval (CI) were calculated for each group. The assumption of a normal distribution for continuous variables was tested by a Kolmogorov–Smirnov test of goodness of fit. Non-normally distributed variables were compared by Kruskal–Wallis, NPC Test and with multiple comparisons.

P-values <0.05 were considered as being statistically significant.

Results

Tables I–V show the marker levels in each group of patients. IL-1β mean levels were 33.4 pg/ml in healthy volunteers, 2.90 pg/ml in patients with gastric cancer, 43.13 pg/ml in patients with gastric non-neoplastic disease, 45.71 pg/ml in patients with extragastric neoplastic disease and 9.87 pg/ml in those with extragastric non-neoplastic diseases. IL-8 mean levels were 137.14 pg/ml in healthy volunteers, 283.10 pg/ml in patients with gastric cancer, 178.61 pg/ml in patients with gastric non-neoplastic diseases, 151.93 pg/ml in patients with extragastric neoplastic diseases and 189.14 pg/ml in those with extragastric non-neoplastic diseases. TNF-α mean levels were 108.47 pg/ml in healthy volunteers, 204.50 pg/ml in patients with gastric cancer, 132.92 in patients with gastric non-neoplastic diseases, 106.07 in patients with extragastric neoplastic diseases and 139.93 in those ones with extragastric non-neoplastic diseases.

Each marker evaluation allowed one to determinate statistical differences ($p < 0.001$) in the mean levels obtained in the group of patients with gastric cancer versus the other groups (Tables VII–X), while the arranged use of the biomarkers have not improved their sensitivity and specificity.

Table VIII. IL-8: mean, standard deviation and 95% confidence interval.

	IL-8		
	Mean	SD	CI 95%
Healthy volunteers	137.14	6.78	133.37–140.89
Gastric cancer	283.10	28.17	262.94–303.25
Gastric non-neoplastic diseases	178.61	32.83	162.74–194.49
Extragastric neoplastic diseases	151.93	22.57	138.89–164.96
Extragastric non-neoplastic diseases	189.14	8.48	184.24–194.04

Table IX. TNF-α: mean, standard deviation and 95% confidence interval.

	TNF-α		
	Mean	SD	CI 95%
Healthy volunteers	108.47	8.63	103.69–113.24
Gastric cancer	204.50	16.71	192.55–216.45
Gastric non-neoplastic diseases	132.92	26.63	114.74–151.10
Extragastric neoplastic diseases	106.07	13.86	98.07–114.07
Extragastric non-neoplastic diseases	139.93	12.52	132.69–147.16

Discussion

Identification of the ideal marker for gastric cancer is still the leading aim of several trials (Ohkura 2002, Miki 2003). Physiopathological considerations (Forones et al. 2001, Beales 2002, Chong et al. 2002, Konturek et al. 2002) led us to test the levels of the three cytokines (IL-1β, IL-8 and TNF-α).

Genetic variations in pro- and anti-inflammatory cytokine genes influence an individual response to carcinogenic exposure. A pro-inflammatory cytokine genetic profile increases the risk of non-cardia gastric adenocarcinoma, but not of other upper gastrointestinal cancers by inducing a hypochlorhydric and atrophic response to *H. pylori* infection (El-Omar et al. 2003).

IL-1β, which is a strong suppressant of the gastric secretion, is responsible for a delayed elimination of bacterial toxins and of inflammatory products (Stockbruegger et al. 1984), an increase in the concentration of mutagen substances, and a reduction of vitamin C levels in gastric juice (Ruiz et al. 1994, Furuta et al. 2002). The above factors enhance the development of a severe gastritis pattern (Furuta et al. 1998), therefore a probable evolution towards cancer, pathogenetically also determined by the duodenogastric reflux, increased by the gastric pH rise (Kido et al. 2001).

Table X. Matrix of significant differences (*p*-values).

	Healthy volunteers	Gastric cancer	Gastric non-neoplastic diseases	Extragastric neoplastic diseases
IL-1β:				
Gastric cancer	0.001			
Gastric non-neoplastic diseases	0.05	0.001		
Extragastric neoplastic diseases	0.03	0.001	n.s.	
Extragastric non-neoplastic diseases	0.001	0.001	0.001	0.001
IL-8:				
Gastric cancer	0.001			
Gastric non-neoplastic diseases	0.01	0.001		
Extragastric neoplastic diseases	n.s.	0.001	0.01	
Extragastric non-neoplastic diseases	0.01	0.001	n.s.	0.02
TNF-α:				
Gastric cancer	0.001			
Gastric non-neoplastic diseases	0.03	0.001		
Extragastric neoplastic diseases	n.s.	0.001	0.04	
Extragastric non-neoplastic diseases	0.02	0.001	n.s.	0.01

n.s., Not significant.

IL-8 stimulates angiogenesis and increases the invasive activity of tumoral gastric cells (Beales & Calam 1998).

TNF-α has different biological functions such as the induction of haemorrhagic necrosis and cytotoxicity (Forones et al. 2001).

In the present study, the serum levels of the three cytokines were shown to be different with statistically significant levels in patients with gastric cancer versus the other groups ($p < 0.001$), also including the groups of patients with extragastric neoplastic diseases. Therefore, they resulted specific for this kind of neoplasy. Compared with controls, IL-1β levels have specifically recorded much lower rates, while TNF-α and IL-8 have registered much higher ones (Tables I and III).

The findings, when compared with the data in the literature, are concordant relative to IL-8 (Konturek et al. 2002), while are characterized by lower levels of IL-1β and higher of TNF-α (Kabir & Daar 1995) than the controls. A more careful analysis allows one to assume that the higher levels of IL-1β reported in the literature can depended on the concomitant presence of *H. pylori* infection, a phenomenon that, as reported in Table II, is absent in 70% of the present authors' patients with gastric cancer. The high levels of TNF-α of the patients, according to Forones et al. (2001), can be justified by the advanced stage of the cancer (Table II).

The present results lead us to state that IL-1β, IL-8 and TNF-α can probably be considered as reliable markers in patients with gastric carcinoma and therefore it is hypothesized that they have a potential role in screening and prognostic assessment.

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