

Review

Measuring Intra gastric Tumor Markers in Gastric Cancer Patients: a Systematic Literature Review on Significance and Reliability

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Abstract. *As of 2017, no serum tumor marker has shown high levels of sensitivity or specificity for early detection, classification, staging, prediction and prognosis of patients affected by gastric cancer. In this regard, since 1975 several authors have investigated the gastric juice or gastric lavage of patients with gastric adenocarcinoma in order to determine the concentrations of intra gastric tumor markers and discover the perfect antigen for this cancer. To date, however, a systematic review of the literature on intra gastric tumor markers is still unreported. After a thorough search, we found important as well as unimportant findings and have come to clearly defined conclusions. We believe that describing the current state of knowledge achieved by the scientific community in this particular field of research could augment information on the complex pathobiology of gastric cancer and entail a deeper understanding of its unpredictable malignant behavior.*

As of 2017, Gastric cancer (GC) represents the second cause of cancer-related death worldwide (1). Such an

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inauspicious result reflects the scarce improvements on diagnosis and treatment made for this tumor during the recent years. The prognosis parallels the severity of the cancer stage detected at the time of diagnosis: the higher the stage, the poorer the survival. Differently from the Eastern world, in Western countries GC is still detected at advanced phases of disease resulting in problems of significant mortality and elevated medical health care costs related to hospitalization and treatment (2). Furthermore, patients diagnosed suffering from the same cancer stage often show divergent survival and response to oncologic or surgical treatment among each other (2). With this regard, differences in already known clinicopathologic and prognostic features seem to explain such a heterogeneity of clinical situations in most cases (3). Other times, however, GC behavior diverges from the theoretical prediction probably due to other unknown underlying pathogenetic (both genetic and epigenetic) mechanisms. Filling these knowledge gaps is desperately needed in order to ameliorate the prognosis of GC patients (3). With this regard, as of 2017, the attempts made by the scientific community at identifying at least one serum biological marker with high levels of sensitivity and specificity for early diagnosis as well as prediction and/or prognosis of gastric adenocarcinoma appear fruitless. As a consequence, for this study we decided to direct our attention to the literature dealing with intra gastric tumor markers in patients affected by gastric adenocarcinoma in order to discover new knowledge and possible utility deriving from this, not so well known, field of oncologic research, especially in terms of prediction, prognosis and survival of GC patients.

Specific introduction to intragastric markers. First, it is important to say that gastric juice (GJ) or gastric lavage (GL) of GC patients have been rarely investigated as a field of study in the past as well as in the present. Traditionally, GJ started being analyzed with intent of cytological diagnosis of GC at the beginning of 1900 by Marini (4, 5). Such a practice reached large popularity by the mid-1900s (6-8) but, through the years, was progressively abandoned and replaced by the combination of gastroscopy with biopsy, which soon proved to be the most reliable diagnostic test for GC concerning both sensitivity and specificity (9-11). Through the years, clinical laboratories improved the methods of determining intragastric tumor antigens changing from qualitative to quantitative detection method, from manual add sample to automatic detection, from ELISA to chemiluminescence. Regarding the determination of GJ/GL tumor markers of GC patients, only 19 dedicated works exist in the world literature so far and this field of oncologic research covers a very limited span of time (approximately 40 years) (12-30). The main features pertaining to the heritage of this scientific niche are summarized in Table I.

Materials and Methods

We investigated the current literature dealing with intragastric tumor markers in the presence of gastric cancer or other gastric diseases. We found only 19 related works. This survey is illustrated in more detail in the following section and summarized in Table I.

Results

Literature on intragastric CEA (analyzed from gastric juice). Carcinoembryonic antigen (CEA) represents the endogastric tumor marker which was first investigated by the medical community and the one better analyzed to date as witnessed by the large amount of dedicated works (12-24, 26-30). Historically, CEA was immunohistochemically detected both in normal and neoplastic gastric mucosa in 1972, but it was not until 1975 that Go and colleagues pioneered the determination of CEA levels in gastrointestinal secretions (12). In fact, until that year, CEA was measured in several biological materials such as saliva, pancreatic juice, colonic washing, stool and urine, but not in GJ (12). They examined 28 healthy patients and found that their GJ CEA levels were considerably lower than colonic ones thereby putting forward the hypothesis that higher than normal levels of CEA could result from cancer and can be used to detect cancer (12). In 1976, two different groups respectively headed by Vuento and Molnar, analyzed for the first time the GJ from patients affected with gastric pathologies such as gastric ulcer, atrophic gastritis and GC and compared the results with normal adults (13, 14). The former enrolled 5 GC patients, the latter 9 GC cases. Vuento and colleagues recorded higher CEA activity in GJ of patients with non-malignant gastric disease (470 ng/ml) than in normal or GC

ones (respectively 300 and 255 ng/ml): the authors related such confounding results to the laborious radioimmunologic isolation of CEA from other CEA-like substances such as Fetal sulphoglycoprotein antigen (FSA), CEA-associated protein (CEX), NCA (non-specific cross-reacting antigen), normal glycoprotein (NGP) or colonic carcinoembryonic antigen 2 (CCEA-2) (13). On the other hand, Molnar *et al.* registered more confidential data, since healthy controls and peptic ulcer patients had lower levels of GJ CEA (respectively 0-2.2 and 0.9-2.2 ng/ml) than GC patients (2.8-16.0 ng/ml) (14). In the same study, the authors demonstrated a statistically significant association between colon adenocarcinoma cases with CEA-positive colonic mucus aspirates ($p<0.01$); in light of these results, they suggested the usefulness of assaying CEA in all those fluids bathing tumors for the detection of gastrointestinal malignancies (14). In 1978, Kawaharada and colleagues, studying 22 GC patients, were the first authors to include Early GC (EGC) in the analysis of GJ CEA finding a statistically significant increase as compared with normal subjects (15). However, paralleling the conclusions drawn by Vuento *et al.*, the authors pointed out that GJ CEA measurements may be more or less heterogeneous when different radioimmunometric methods are employed, including immunodiffusion (micro-Ouchterlony's technique) and gel filtration (14,15). In the same year, Fujimoto and colleagues significantly demonstrated how CEA levels in GJ were higher in 28 GC patients than those suffering from peptic ulcer ($p<0.01$) and, precisely, more elevated for GC involving the serosa compared with EGC (2.6 versus 9.6 ng/ml respectively) (16). They also introduced the utilization of "the one step sandwich method" by Hirai as radioimmunoassay test and, paralleling what Sugarbaker demonstrated in the large bowel because of obstructing colon cancer, suggested how endogastric or endoduodenal obstruction exerted by the tumor itself could augment the intragastric concentration of tumor markers (16). In 1979, Bunn and colleagues found far higher levels of intragastric CEA in GC patients than patients suffering from benign gastric diseases (respectively, 900 ng/ml for GC, 21.4 ng/ml for gastritis, 11.9 ng/ml for gastric ulcer, 7.3 ng/ml for duodenal ulcer) but this finding failed to reach statistical significance (17). They also found no correlation between the gastric immunoreactive CEA and microscopic or macroscopic appearance of the tumor or the extent of disease (17). Nevertheless, the authors were so enthusiastic about the trend of their results that they encouraged future studies to perform gastric CEA for the screening and follow-up of patients at high risk for GC, identification of early relapse in gastrectomized patients and valuation of response to chemotherapy with or without surgery (17). Tatsuta and associates, reporting a study on 58 GC patients in 1980, were the first to assess that the determination of GJ CEA was not a good indicator of EGC because of the very low sensitivity of this test (46.6%); more importantly, in so doing, they definitely pointed at the imperfection and fallaciousness of gastric CEA

Table I. Review of the world literature dealing with intragastric tumor markers.

Ref	Country	Antigen	GJ/GL	Comparison with serum	N° of GC patients	Presence of control group	P of outcomes
12	USA	CEA	GJ	Yes	None	Yes	n/a
13	Finland	CEA	GJ	No	5	Yes	n/a
14	USA	CEA	GJ	Yes	17	Yes	n/a
15	Japan	CEA	GJ	No	22	Yes	SS
16	Japan	CEA	GJ	Yes	28	Yes	SS
17	USA	CEA	GJ	Yes	25	Yes	NS
18	Japan	CEA	GJ	Yes	58	Yes	NS/NS/NS/SS
19	Japan	CEA	GJ	Yes	39	Yes	SS/SS
20	Italy	CEA	GJ	Yes	8	Yes	SS/NS
21	Sweden	CEA	GJ	Yes	6	Yes	NS
22	Italy	CEA	GJ	No	25	Yes	SS
23	Japan	CEA	GJ	No	56	Yes	SS/SS/SS/NS
24	China	CEA/Ig	GJ	No	Total amount of 93 patients (including GC and control group)		SS/SS/NS/SS/NS
25	Italy	Ca 19.9	GJ	No	23	Yes	SS but low Se and Sp
26	Italy	CEA/Ca 19.9/Ca 72.4	GJ	Yes	59	Yes	NS
27	Turkey	CEA/Ca 19.9	GJ	Yes	139	Yes	NS/NS
28	Italy	CEA/Ca 19.9	GJ	No	23	Yes	NS/SS
29/30	Italy	CEA/Ca 19.9/Ca 72.4/Ca 50	GL	Yes	38	Yes	NS/SS/NS/SS but low Se

GJ: Gastric juice; GL: gastric lavage; GC: gastric cancer; P: probability of statistical significance of the designated outcome/outcomes (SS) in relation to GC; SS if $p < 0.05$; NS: not significant ($p > 0.05$); n/a: not applicable; Ig: immunoglobulin; Se: sensitivity of the intragastric test; Sp: specificity of the intragastric test.

just like serum CEA titers (18). The same year, on the contrary, Satake and colleagues suggested the analysis of GJ CEA as a useful adjunct to the diagnosis of early malignant changes in the gastric mucosa: in fact, there was a significant difference between benign gastric disease and EGC ($p < 0.01$) as well as between benign and late GC ($p < 0.001$) (19). Of interest, all the following pertinent literature (namely Nitti *et al.* in 1983, Borch *et al.* in 1987, Amadori *et al.* in 1987 and, again, Tatsuta *et al.* in 1988) was in accordance with Satake's perspective: in fact, in all these works, elevated GJ CEA levels significantly correlated with those types of gastric intestinal metaplasia showing the potential for neoplastic transformation and, later on, this appeared to be the actual significance of the gastric CEA test (20-23). In 1986 Wang and associates proposed the combined determination of CEA and immunoglobulin to improve the diagnostic accuracy of GC and the identification of precancerous lesions; although this was an interesting suggestion, no continuation succeeded in line with their model of analysis (24). All the succeeding work dealing with intragastric CEA concomitantly investigated other tumor antigens and are reported hereafter and listed in Table I (26-29).

Literature on intragastric Ca 19.9 (analyzed from gastric juice). In 1988, Farinati and associates pioneered the measurement of another tumor marker in the GJ of 23 Italian patients affected with GC: the carbohydrate antigen 19.9

(Ca 19.9) (25). In their study, even though statistical significance was attained for all groups considered (23 GC patients, 57 patients affected by chronic atrophic gastritis and 55 healthy controls), the rise in GJ levels of this antigen was not sharp enough to allow a clear cut distinction between groups and the test showed low sensitivity and specificity (65% and 71 % respectively): for these reasons the authors discouraged the routine use of this marker in clinical practice (25). Four further studies examined endogastric Ca 19.9 in association with other antigens, as discussed hereafter and summarized in Table I (26-29).

Literature on intragastric Ca 72.4 (analyzed from gastric juice). Similar disappointing results were registered in 1998 by Tocchi *et al.* for another GJ tumor marker in 59 Italian patients with GC: Ca 72.4 (26). In fact, the differences in GJ levels of this tumor marker (as well as CEA and Ca 19.9) between GC and peptic ulcer patients were not significant and then the presence of Ca 72.4 in the GJ was deemed not to play any prognostic role (26).

The new millennium started with Duraker and colleagues who reported in 2002 the largest patient population ever tested for GJ tumor markers (27). In fact, they analyzed for CEA and Ca 19.9 the GJ obtained from 139 GC patients compared to 54 patients with benign gastroduodenal disease and a healthy control group of 46 subjects. Considering the

very low rate of positivity of GJ CEA and Ca 19.9 in GC patients (16.5% and 27.3% respectively) as well as the absence of statistical significance between mean GJ CEA and Ca 19.9 levels of the examined groups, the authors assigned no diagnostic or prognostic value to these intragastric tumor markers (27). Only one further article focused on measurement and significance of intragastric Ca 72.4 as stated hereafter and in Table I (29).

The capsular method. Since the first study on gastric tumor marker, the procedure of getting samples of GJ from GC patients has always been through nasogastric or orogastric catheter (12-27). With this regard, in 2003, Muretto and colleagues first described the successful employment of an endogastric capsule for measuring CEA and Ca 19.9 in GJ of 23 Italian GC patients (28). They also compared the results with 21 patients affected by benign gastric disease and 6 more patients with gastric epithelial dysplasia (28). The authors found that GJ Ca 19.9 values were significantly higher in patients with cancer than in patients with precancerous lesions ($p < 0.001$), nevertheless, considering the existing data, they also had cognizance of the doubtful value of this test in differentiating between precancerous lesions and GC (28). Although interesting and innovative, the capsular technique was not followed by other similar attempts and, to date, it remains the relevant study dealing with this topic (28).

Literature on intragastric CEA, Ca 19.9, Ca 72.4 and Ca 50 (analyzed from gastric lavage). In 2016, our study group contributed to the topic of intragastric biomolecular markers with two further innovations (29, 30). Firstly, the biological material of study was not GJ but gastric lavage (GL), obtained by washing the gastric lumen of 38 GC patients with at least 500 ml of saline solution (or water) before surgery or other kinds of interventions; the results were compared with a control group of 41 patients (29). Secondly, we were the second research group to determine intragastric Ca 72.4 in GC patients and, most importantly, the first one to measure Ca 50 (29). Based on ROC curve analysis, differently from GL CEA and Ca 72.4, GL Ca 50 and GL Ca 19.9 of GC patients attained a statistically significant cut-off value compared to GL levels recorded for non-GC patients ($p < 0.0096$ and $p < 0.002$ respectively); however, the low statistical sensitivity of the tests make their adoption no feasible in clinical practice (48.4% and 51.4% respectively) (29).

Discussion

As of 2016, differently from other malignancies such as colorectal cancer or hepatocellular carcinoma, GC seems to remain orphan of a highly related tumor marker. Given the former disappointments deriving from scientific research on

serum tumor markers, since 1975 many authors have been investigating GJ or GL of GC patients in order to find out the ideal antigen of this disease. This sort of research has certainly added novel and notable information to the knowledge of the pathobiology of GC, such as the possibility of better identifying and monitoring patients with precancerous lesions furnished by analysis of GJ CEA (19-23). However, none of the aforementioned works managed to materialize the ideal tumor antigen including CEA, Ca 19.9, Ca 72.4 or Ca 50 (12-30). The intragastric determination on tumoral antigens, in fact, appears to be undermined by the low sensitivity of the test itself (18, 25, 26, 29, 30). This is likely to be determined by numerous reasons, such as the inconstant polarized secretion of tumor peptides into the gastric lumen, the fluctuations of intragastric concentrations in the presence or absence of neoplastic obstruction, differences in methods of sampling, evolution of radiomunological instruments and analyses, the limited sizes of the reported patient populations (12-30). Indeed, traditionally, GJ CEA has been the biologic marker more extensively and longer evaluated (12-24, 26-30).

Conclusion

Further investigations accomplished with more sophisticated laboratory tools determining the other intragastric antigens, herein cited or not, could reveal novel important prognostic dowels hitherto unknown for this dismal disease.

Conflicts of Interest

The Authors declare no conflicts of interest.

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