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Review

Advances in Intraluminal Exfoliative Cytology of Gastric Cancer: Oncologic Implication of the Sixth Metastatic Route (Metastasis VI)

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Abstract. *Historically, analysis of intragastric exfoliative cytology (IEC) of gastric cancer (GC) was used with a diagnostic intent only. With the successful advent of endoscopic biopsy, the rate of detection of GC has improved worldwide and, as a consequence, IEC has been progressively abandoned. Today, however, there is a renewed interest in this field of research, as witnessed by several pertinent publications. As discussed in this review, in fact, currently the importance of analyzing IEC in patients with early and advanced GC seems to reside in its clinicopathological and prognostic significance. In fact, compared to non-sloughing tumors, GC exhibiting intragastric exfoliation was recently associated with an aggressive tumor phenotype (characterized by deeper infiltration of the gastric wall, lymph nodal or distant metastases, angiolymphatic and perineural invasion) and poorer prognosis. Adoption of IEC examination in routine practice might help identify patients at higher risk of developing local recurrence and peritoneal metastasis from early and advanced GC, optimizing their treatment and improving quality of life and life expectancy.*

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The study of intragastric exfoliative cytology (IEC) of gastric carcinoma (GC) started in 1882 when Rosenbach reported for the first time the presence of tumor cells in the gastric lavage of individuals affected with this type of malignancy (1). Coinciding with the worldwide success and spread of Papanicolaou staining, the heyday of IEC was from the 1940s to the 1960s; since the 1970s, succumbing to the more accurate results in diagnosis provided by the combination of endoscopy with biopsy, it was progressively abandoned (2, 3). Over recent years, however, a renewed interest in IEC has been generated. In 2013, in fact, Xie and colleagues found isolated cancer cells infiltrating the mesogastrium of resected GC specimens; such a finding in the mesogastrium led the authors to identify a metastatic pathway different from the four traditional routes (direct invasion, hematogenous spread, lymphatic dissemination, trancoelomic seeding) and to call it Metastasis V (4, 5). Concomitantly, our and other study groups have decided to focus on and persist in IEC for GC: for the first time in history, however, what was investigated was not the diagnostic role of IEC but other unreported aspects, such as its clinicopathological, prognostic and predictive significance (5-8), as well as prophylactic or therapeutic potential (9, 10). Compared with its non-exfoliative counterpart, in fact, GC exhibiting intraluminal seeding has been associated with traditional aggressive features of malignancy (depth of gastric wall invasion, lymph nodaI and distant metastasis, lymphovascular and perineural emboli) and poorer prognosis (shorter survival and time to tumor progression) (11-12). Of interest, such results have been confirmed so far for early (13) and advanced (3) GC tumors, as well as GC submitted to neoadjuvant therapy (14). For such oncologicaI discoveries and implications, IEC of GC was recently identified as the sixth

Table I. *Main clinicopathological findings of literature dealing with intragastric exfoliative cytology of gastric cancer (GC).*

Ref.	Study type	GC, n	GC	GL1		Gastric status	GL method	GL volume; solution
				Early GC	Advanced GC			
3,13	P	96	48%	31%	51%	*In vivo*	Blind	1 l; Saline
5	P	38	34%	14%	42%	*In/ex vivo*	Blind	200 ml; Saline
9	P	89	58%	58%	70%	*In vivo*	FGS	250 ml; RL/DW
12	P	142	23%	10%	35%	*In vivo*	Blind	50 ml; Saline
19	P	48	27%	27%	No case	*Ex vivo*	No GL	No GL

DW: Distilled water; FGS: fibergastrosope; GL: gastric lavage; GL1: gastric lavage positive for cancer cells; P: prospective; Ref.: reference; RL: Ringer's lactate.

metastatic route attained by GC (Metastasis VI) (15). This review presents a discussion on some of the aforementioned original topics surrounding GC EIC.

Literature on Collection Techniques in IEC

There are two main ways of collecting gastric contents: The blind and the endoscopic (or direct-vision) washing method (16, 17). For the former, numerous variants have been described concerning both the type (normal saline; Ringer's lactate; acetate buffer, with or without mucolytic agents such as α-chymotrypsin) and quantity (from a few milliliters to 1 liter) of solution to flush (3, 16, 17). The direct-vision technique requires the use of fibergastroscope, thereby resulting in a more time-consuming and expensive procedure (3).

Literature on Analysis Techniques of IEC

The blind method permits only intragastric free-floating cancer cells to be retrieved, hence, the subsequent cytopathological analysis is called luminal fluid cytology (also known as washing, liquid, liquid-based or lavage cytology) (3, 16, 17). On the other hand, fibergastroscopy allows not only the simple technique of gastric lavage, but also mechanical abrasion of the gastric walls: this is the case of brushing (or brush) cytology (17). More recently, a further examination called stamp or imprint cytology has been developed for preoperative biopsy or surgical specimens (18, 19).

Literature on IEC in Patients with GC

As demonstrated by previous studies, cancer cells do indeed exist in the gastric juice of patients affected with GC (including those with early GC) (9, 11, 15, 19). There are two possible ways for GC cells to be exfoliated intraluminally: one spontaneous, and one iatrogenic (after direct or indirect contact by gastroscopic biopsy, surgical manipulation or other maneuvers) (5, 19). For the former, the rate of unforced intragastric exfoliation of GC varies through the literature, ranging from 23.2% to 58% (3, 5, 9, 12, 19). For the latter, intraoperative acts or complications occurring during surgery of non-serosal GCs can spill the gastric contents (including the cancer cells exfoliated into it) into the peritoneal cavity, thereby recreating the same conditions when faced with serosal cancer: peritoneal seeding and the development of peritoneal metastasis (PM) (12, 20). The oncological significance of this possible post-surgery complication is very serious when related to the current dimension of the problem: as of 2019, in fact, half of all patients with GC who undergo radical gastrectomy die as result of PM (11, 12). In addition to duodenogastric reflux and other mechanisms, moreover, IEC may be responsible for some gastric stump cancers (including anastomotic site and remnant stomach) (12, 21). The main characteristics of the studies dealing with GC IEC are listed in Table I.

Literature on IEC of Early GC Developing Metastasis After Treatment

Early GC treated with surgery or minimally invasive techniques is associated with the development of three types of metastatic or secondary deposit: PM, port-site metastasis and metachronous gastric stump cancer. PM is a possible accident in early GC during its natural history course, as well as in the post-treatment follow-up (5, 12). Differently from advanced GC, however, early GC shows minimal infiltration of the gastric wall and no cancer cells floating in peritoneal cavity: hence, the four traditional metastatic routes cannot explain the development of PM in such an initial phase of disease and other pathways (such as Metastasis V and VI) might be involved (4-6, 13, 15, 20). In this regard, IEC (Metastasis VI) of early GC has been confirmed by several works (13, 19) (Table I) and its seeding through gastric wall perforation to the peritoneal cavity, as occurs accidentally or intentionally during endoscopic submucosal dissection or laparoscopic endoscopic cooperative surgery respectively, assessed as the main cause of early GC-related PM (22-25). As of 2019, M has been recorded in four patients with early GC who experienced gastric wall perforation during endoscopic submucosal dissection; three of them later died (22-24) (Table II). IEC of early GC can be also associated with the development of port-site metastasis after

Table II. *Literature data of patients with early gastric cancer (GC) developing metastasis potentially related to intraluminal exfoliation cytology.*

Ref.	Early GC, n	T1a/T1b, n	N-Stage	Treatment	Treatment complication	Treatment complication correction	Related metastasis type
3,13	2	1/1	N0/N1	NAT + TG DG + AT	None	None	AR and PMPM and KT
22	1	0/1	N0	ESD	GWP	ES	R and PM
23	2	1/1	N0/N0	ESD	GWP + peritonitis GWP	ES/G	PM and LM PS (P0/Cy1)
24	1	0/1	N0	ESD	GWP	Endoclip + G	PS and PM
26	1	1/0	N0	LADG	None	None	PS
27	1	0/1	N0	NAT+LADG	None	None	PS and PM

AR: Anastomotic recurrence; AT: adjuvant treatment; DG: distal gastrectomy; ES: emergency surgery; ESD: endoscopic submucosal dissection; G: gastrectomy; GWP: gastric wall perforation; KT: Krukenberg tumor; LADG: laparoscopy-assisted distal gastrectomy; LM: liver metastasis; N.A. not assessed; N0/N1: without/with lymph node metastasis; NAT: neoadjuvant treatment; PM: peritoneal metastasis; PS (PO/Cy1): peritoneal seeding (without macroscopic PM but with positive peritoneal wash cytology); R: recurrence; T1a: mucosal GC; T1b: submucosal GC; TG: total gastrectomy.

laparoscopic gastrectomy: to date, only two cases have been reported and both of them later died of PM (26, 27) (Table II). After bleeding and gastric wall perforation (with peritoneal seeding), gastric stump cancer represents the third potential complication of endoscopic submucosal dissection. To date, although no work has considered Metastasis VI among the risk factors of this type of secondary GC (28, 29), we strongly suggest it should be kept in mind and such a topic be further explored in future research. More recently, in fact, some studies have demonstrated a reduction in GC IEC performing gastric lavage with distilled water. In the future, especially in the case of GWP occurring during endoscopic submucosal dissection or laparoscopic endoscopic cooperative surgery for early GC, the intraoperative use of gastric lavage with distilled water, reducing IEC and minimizing the associated risk of intraperitoneal spillage of intragastric malignant cells, could become a cogent antitumor procedure (9).

Conclusion

In the light of the results coming from our and other study groups, IEC of GC, better known as the sixth metastatic route attained by GC (Metastasis VI), is shedding renewed light on the branch of gastrointestinal cytopathology and attracting the attention of those interested and motivated in this research area.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

All the Authors agreed with the content of the article. Dr. Virgilio conceived the presented research. Dr. Virgilio and Dr. Mercantini performed the clinical part of the research. Dr. Giarnieri, Mrs. Montagnini, Mrs. Villani and Professor Giovagnoli performed the cytopathological analysis. Dr. Virgilio and Dr. Giarnieri contributed to the interpretation of the results. Dr. Virgilio wrote the article and performed the statistical analyses. Dr. Balducci and Dr. Cavallini helped supervise the entire project.

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