Prognostic Role of Intragastric Cytopathology and Microbiota in Surgical Patients with Stomach Cancer

Edoardo Virgilio1,2, Enrico Giarnieri1, Elisabetta Carico1, Monica Montagnini2, Sandra Villani2, Michele Fiorenti1, Marco Cavallini2, Filippo Montali3,4, Renato Costi1

1Department of Medicine and Surgery, University of Parma, Parma, Italy; 2Department of General Surgery, di Vallo Hospital, Fidenza (PR), Italy; 3Department of Clinical and Molecular Medicine, Faculty of Medicine and Psychology, University “Sapienza”, St. Andrea Hospital, Rome, Italy; 4Department of Anesthesiology and Reanimation, St. Andrea Hospital, Rome, Italy; 5Department of Medical and Surgical Sciences and Translational Medicine, Faculty of Medicine and Psychology, University “Sapienza,” Rome, Italy; 6Department of Experimental Medicine, University of L’Aquila, L’Aquila, Italy

Abstract

Background: In the last decade, analysis of malignant cells and flora in gastric lavage (GL) has provided interesting data on pathogenesis of gastric cancer (GC). For this study, combining such two aspects into one cyto-microbiologic category, we tested the prognostic role of the presence/absence of cancer cells (GL1/GL0) and bacterial microbiota (MB1/MB0) in our GC population. Material and Methods: Between April 2012 and August 2019, 79 surgical patients with GC were prospectively investigated with the determination of GL MB. Results: Compared with GL1 MB0, GL1 MB1 strongly correlated with advanced GC, portended poorer overall survival (OS) (45.8 months vs 20.5 months, P = 0.049), and resulted a significant (P = 0.008) and an independent (P = 0.013) prognostic factor unfavorable for OS. Conclusion: In the light of our results, the cyto-microbiologic parameter of GL MB should be used to gain a better prognosis of GC patients. Administration of antimicrobial treatment for MB1 subjects should be entertained because it could reduce the risk of oncogenesis.

Keywords: Fluid cytology, gastric cancer, gastric microbiota, gastrointestinal cytology, non-gynecologic cytopathology

Introduction

Diverging from other adenocarcinomas affecting the enteral tube, gastric cancer (GC) carcinogenesis is poorly understood impeding the identification of efficient measures for early diagnosis, curative treatment, and reliable prognosis.1–4 Consequently, as of 2021, GC is still the third leading cause of cancer-related mortality in the world (783,000 deaths per year).5,6 Since the last decade, cytologic and molecular analysis of gastric lavage (GL) of GC patients has provided interesting results.7–17 Gastric bacterial microbiota (MB) represents another original issue for GC research drawing medical attention.18–22 The stomach lumen, in fact, is not sterile and physiologically hosts a rich MB (approximately 108–1010 colony forming units per gram content) mainly composed of the genus Lactobacillus, Clostridium, Propionibacterium, Streptococcus, and Staphylococcus.21 In the presence of Helicobacter pylori (H. pylori)-positive gastritis and pre-cancerous lesions, MB composition is deeply subverted with an important increase of Lactobacillus, Clostridium, and Pseudomonas and a major decrease of Streptococcus and Bacteroides.23,24 Subsequently, penetrating through epithelial mucosa and activating immune system activation, these taxa could co-promote tumor transformation and growth in concert with H. pylori and other factors.18,25 In this study, we combined endoluminal cytology and microbiology into one examination and investigated the clinicopathologic significance and prognostic role of this mixed innovative item: the “GL MB” parameter.

Materials and Methods

We prospectively analyzed the clinicopathologic data of 79 GC patients who were admitted between April 2012 and August 2019 to our Division of General Surgery. Our study followed the principles of the Declaration of Helsinki (as revised in

How to cite this article: Virgilio E, Giarnieri E, Carico E, Montagnini M, Villani S, Fiorenti M, et al. Prognostic role of intragastric cytopathology and microbiota in surgical patients with stomach cancer. J Cytol 2021;38:82-7.

Submitted: 21-Dec-2020; Accepted: 24-Mar-2021; Published: 11-May-2021

Address for correspondence: Dr. Edoardo Virgilio, Department of Medicine and Surgery, University of Parma, via Gramsci 14, 43125, Parma, Italy. E-mail: aresedo1992@yahoo.it, edoardo.virgilio@unipr.it

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLMedknow_reprints@wolterskluwer.com

Access this article online

Quick Response Code:
Website: www.jcytol.org
DOI: 10.4103/JOC.JOC_238_20
Brazil 2013); individual informed consent was obtained from all participants before enrolment. All the participants have been followed until April 2020 or death. All the procedures of nasogastric tube insertion with subsequent GL were conducted by the same operator: in brief, under general anesthesia and before surgical act, the GL was collected under sterile conditions through a nasogastric tube and immediately transported to laboratory and cytopathology service.\cite{15} The following cytomorphological criteria were considered pathognomonic of malignancy: nuclear changes (atypia, anisokaryosis), increased and/or abnormal mitotic figures, high nucleus-to-cytoplasm ratio, nucleolar hypertrophy or multiplicity, highly condensed nuclear chromatin, cytosolic vacuoles (signet-ring cells), pleomorphism, hypertrophy, presence of aggregates, and pseudopapillary [Figure 1].\cite{19} Gastric microbiota (cocci, bacilli, hyphae, and spores) was microscopically evaluated on the same smears prepared for cytologic examination and stained according to the Papanicolaou method [Figures 2 and 3].\cite{26} *Helicobacter pylori* (*H. pylori*) status was further examined in those GL samples showing bacilli by our bacteriology laboratory technicians; bacterial features such as Gram-negative staining, helical or spiral shape, flagellar filaments, diameter of about 0.5 μm, and positive correlation with preoperative gastric biopsies were considered consistent with the microbiologic diagnosis of *H. pylori* infection. Histopathology of surgical specimens was described following the 8th edition of AJCC TNM Staging System.\cite{27} Metastatic lymph node ratio (LNR) was classified into a 4-tier system: LNR0 (0.0), LNR1 (>0–0.3), LNR2 (>0.3–0.6), and LNR3 (>0.6).\cite{29}

**Statistics**

Statistical analysis was performed using MedCalc Statistical Software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium). Categorical, ordinal, and continuous variables were compared using the Chi-square, Kruskal-Wallis, logistic regression, Pearson correlation coefficient, and Student’s *t*-test. Overall survival (OS) was evaluated as the time from GL collection to death from any cause.\cite{10} Survival curves were interpreted and compared through the Kaplan-Meier method and the log-rank test. Univariate and multivariate analyses were performed with one-way ANOVA test and Cox proportional hazards model to identify powerful association and independency among prognostic factors. *P* values <0.05 were considered statistically significant.

**Results**

The main clinicopathologic characteristics of the studied population as well as the associations with the “GL MB” parameter are listed in Table 1. Considering all the entertained subgroups (GL1 MB1, GL1 MB0, GL0 MB1, and GL0 MB0), the median follow-up was 17.8 months (range: 62–0). Among the 39 patients with GL malignant cells (GL1) (49%), bacterial microbiota was present (MB1) and absent (MB0) in 33 and 6 patients, respectively. In the group without GL cancer cells (GL0) (51%), MB1 was found in 32 and MB0 in 8 cases. At a median follow-up of 33.9 months, 33 (85%) patients died. Considering all the subgroups (GL1 MB1, GL1 MB0, GL0 MB1, and GL0 MB0), the median overall survival was 17.8 months (range: 62–0). Considering all the patients were followed until April 2020 or death, the median overall survival was 17.8 months (range: 62–0). Considering all the patients were followed until April 2020 or death, the median overall survival was 17.8 months (range: 62–0).

**Figure 1:** Cluster of gastric cancer cells exfoliated into gastric lavage (Papanicolaou stain, 9100 oil immersion. Magnification: 44× field of view)

**Figure 2:** Gastric lavage gastric cancer cells with numerous cocci (Papanicolaou stain, 9100 oil immersion. Magnification: 44× field of view)

**Figure 3:** Malignant cells exfoliated into gastric lavage with cocci, bacilli, and neutrophils (Papanicolaou stain, 9100 oil immersion. Magnification: 44× field of view)
months (range: 2–77) of the 46 dead patients, 21 subjects were GL1 MB1, 2 GL1 MB0, 20 GL0 MB1, and 3 GL0 MB0; of the 33 alive subjects, 12 were GL1 MB1, 4 GL1 MB0, 12 GL0 MB1, and 5 GL0 MB0. The Kaplan-Meier model showed significant differences of OS between GL1 MB1 and GL1 MB0 groups (20.5 vs 45.8 months, respectively, \( P = 0.049 \)) [Figure 4]. Precisely, in GL1 MB1 group, following surgery, there were 12 alive and 21 dead patients. For 7 alive patients, less than 10 months passed by from intervention. As for deaths, 10 occurred after 10 months from surgery, 13 after 20 months, 19 after 30 months, and 20 after 40 months. Concerning GL1 MB0 group, 2 deaths occurred after 19 and 24 months while 4 patients are still alive after 6, 38, 42, and 62 months. GL1 MB1 strongly correlated with advanced disease (T3-T4 with \( P = 0.049 \) and Stage 3-4 with \( P = 0.035 \)) [Table 1]. At univariate analysis, the GL1 MB1 parameter resulted a significant prognostic factor for OS (\( P = 0.008 \)) [Table 2]. Furthermore, multivariate analysis revealed GL1 MB1 as an independent prognostic factor of OS (\( P = 0.013 \) with an overall model fit of \( P < 0.001 \), Table 3). In addition, GL1 MB1 significantly associated with the preoperative diagnosis of \( H. pylori \) infection (\( P = 0.011 \), Table 1).

### DISCUSSION

In the last decade, cytologic and molecular investigation of GL has provided interesting findings in terms of diagnosis, screening, prognosis, and treatment of GC patients.\[1-17,24-30\]

Concerning the cytologic aspect, as suggested by numerous

### Table 1: Clinicopathologic characteristics of the 79 gastric cancer patients related with the combined “gastric lavage cancer cells/microbiota” ("GL1/GL0 MB1/MB0") parameter

| Clinicopathologic feature | Result | Association with GL1 MB1 |
|---------------------------|--------|------------------------|
| Sex                       | M: 35 (44.3%); F: 44 (55.7%) | \( P = 0.587 \) |
| Age (mean years)          | 70.7 years (range: 42-88); GL1 MB0: 61; GL1 MB1: 72 | \( P = 0.013 \) |
| Tumor Site                | proximal*: 32 (33%); distal*: 47 (67%) | \( P = 0.586 \) |
| Siewert Type              | Type 1 and 2: 12 (12.3%); Type 3 and non-Siewert cancers: 87 | \( P = 0.596 \) |
| NAT                       | 18 (18.5%); 30 (31%) | \( P = 0.689 \) |
| AT                        | T category; T1: 17; T2: 18; T3: 12; T4: 32 | \( P = 0.049 \) |
| N                         | N category; N1: 13; N2: 14; N3: 26; N1-3: 53 | \( P = 0.05 \) |
| M                         | M0: 81 (85.55%); M1: 16 (16.5%) | \( P = 0.150 \) |
| Stage                     | Category: 1; 2; 3; 4; 18 | \( P = 0.035 \) |
| G                         | G category; G1: 10; G2: 13; G3: 56 | \( P = 0.05 \) |
| Lauren Classification     | intestinal: 62 (64%); diffuse: 35 (36%) | \( P = 0.236 \) |
| WHO classification        | WHO category; tubular: 28 (29%) | \( P = 0.05 \) |
| Signet Ring Cells         | 18 (18.5%); absence: 79 (81.5%) | \( P = 0.221 \) |
| LVI                       | LV0: 52 (53%); LV1: 45 (47%) | \( P = 0.203 \) |
| PnI                       | Pn0: 73 (75%); Pn1: 24 (25%) | \( P = 0.781 \) |
| LNR                       | Category: 1; 2; 7; 3; 17; 1-3: 53 | \( P = 0.05 \) |
| N° lymph nodes            | GL1 MB0: 26.5; GL1 MB1: 24.8 | \( P = 0.745 \) |
| Gastrectomy type          | Distal: 43 (44.3%); Total: 24 (24.7%) | \( P = 0.489 \) |
| Operative time (min)      | GL1 MB0: 201; GL1 MB1: 219 | \( P = 0.557 \) |
| PLS (days)                | GL1 MB0: 8.8; GL1 MB1: 14.4 | \( P = 0.272 \) |
| Tumor size (mm)           | GL1 MB0: 28.3; GL1 MB1: 48.5 | \( P = 0.127 \) |
| Preoperative Anemia       | 47 (48.5%); absence: 50 (51.5%) | \( P = 0.131 \) |
| Postoperative Complications | 14 (14%) | \( P = 0.949 \) |
| BMI                       | GL1 MB0: 26.2; GL1 MB1: 23.7 | \( P = 0.257 \) |
| Microbiota species        | Cocei: 77; Bacilli: 9; Mixed: 11 | \( P = 0.472 \) |
| GL histiocytes            | Presence: 6 (6%); Absence: 91 (94%) | \( P = 0.534 \) |
| GL lyphae/spores          | Presence: 12; Absence: 85 (88%) | \( P = 0.482 \) |
| Presurgery Hp biopsy      | \( H_\text{p} \) presence: 5; Absence: 92 (95%) | \( P = 0.011 \) |

**GL1/GL0: Presence/absence of free malignant cells exfoliated into gastric lavage samples; MB1/MB0: Presence/absence of bacterial microbiota in gastric lavage samples; *Proximal site: Cardio‑fundic and gastric body carcinomas; distal site: antro‑pyloric cancers; NAT: Neoadjuvant therapy; AT: Adjuvant therapy; LVI: Lymphovascular invasion; PnI: Perineural invasion; LNR: metastatic lymph node ratio; PLS: Postoperative length of stay; BMI: Body mass index; GL: Gastric lavage; _Hp_: Helicobacter pylori; P and association written in bold are statistically significant (<0.05)**
authors, the oncologic value of GL derives from its privilege of collecting GC products released directly by the tumor avoiding hepatic clearance, a condition known under the name of Metastasis VI.\(^\text{[8,9,11]}\) In the presence of a patent gastrointestinal tube, the exfoliation of malignant cells into the gastric lumen (Metastasis VI) strongly suggests the possibility that other cell elements have already migrated or infiltrated the surrounding tissue following the classical routes of metastasis (invasion through vascular or lymphatic channels, lymph nodes, direct contact, intraperitoneal or mesogastric seeding).\(^\text{[9]}\) On the other hand, when obstruction by GC has occurred especially at cardia or pylorus, a number of cancerous cells, surviving for a long time due to a phenomenon called anoikis resistance, could colonize the gastric lumen, deposit on gastric or esophageal mucosa, and promote a metastasis.\(^\text{[11]}\) Moreover, in most recent years, analysis of GL and stomach acid has been enriched with a further new perspective on GC research: the gastric bacterial MB.\(^\text{[18‑20]}\) Concerning the gastric microbial community, \textit{H. pylori} infection indeed plays a pivotal role in GC carcinogenesis.\(^\text{[16‑21,23,24]}\) However, latest studies suggested that colonization of other non-\textit{H. pylori} bacteria in the stomach (such as Propionibacterium acnes, Prevotella copri, and Eubacterium cylindroides) can also stimulate GC risk by producing proinflammatory cytokines such as IL 15 and lymphocytic gastritis.\(^\text{[14‑26]}\) Taking a cue from such new literature data, for this study, we

### Table 2: Univariate analysis of significant prognostic factors for overall survival

| Variable | \(P\) | Variable | \(P\) |
|----------|------|----------|------|
| GL1 MB1 | 0.008 | LV1 | 0.013 |
| Stage | 0.006 | PnI | <0.001 |
| Stage 3C | 0.049 | N | 0.014 |
| Stage 3-4 | 0.048 | N2 | 0.032 |
| Stage 4 | 0.001 | N3 | 0.004 |
| Lauren type | 0.041 | PnI | <0.001 |
| LNR3 | 0.004 | LNR | 0.040 |
| M | 0.003 | Size | 0.044 |
| NAT | 0.010 | Curative surgery | 0.010 |
| Preoperative anemia | 0.021 | T3-T4 | 0.040 |
| R | <0.001 | T4 | 0.026 |
| Others | >0.05 | Others | >0.05 |

GL1 MB1: Intragastric copresence of cancer cells and bacterial microbiota; LNR: Metastatic lymph node ratio; LV1: Lymphovascular invasion; PnI: Perineural invasion; NAT: Neoadjuvant therapy; variables and \(P\) written in bold are statistically significant (<0.05)  

### Table 3: Multivariate analysis of independent prognostic factors for overall survival

| Independent variables | \(b\) | SE | Wald | \(P\) | Exp(b) | 95% CI of Exp(b) |
|-----------------------|------|----|------|------|--------|-----------------|
| GL1 MB1               | 4.9254 | 1.9995 | 6.0676 | <0.013 | 137.7445 | 2.7354 to 6936.2278 |
| T3-T4                 | -20.8581 | 10.3869 | 4.0326 | 0.0446 | 8.7384E-010 | 1.2588E-018 to 0.60 |
| T4                    | 11.3276 | 4.8025 | 5.5633 | 0.0183 | 83086.3574 | 6.7845 to 1.02E+09 |
| N2                    | 0.4916 | 2.0694 | 0.05643 | 0.8122 | 1.6349 | 0.0283 to 94.4130 |
| N3                    | -13.6155 | 9.0095 | 2.2838 | 0.1307 | 0.0000 | 2.6169E-014 to 57.0 |
| Stage 3-4             | 9.6674 | 7.2151 | 1.7953 | 0.1803 | 15793.8721 | 0.0114 to 21.9E+09 |
| Stage 4               | -18.6568 | 10.3357 | 3.2583 | 0.0711 | 7.8967E-009 | 1.2575E-017 to 4.95 |
| LV1                   | -1.8558 | 1.1352 | 2.6726 | 0.1021 | 0.1563 | 0.0169 to 1.4465 |
| PnI                   | 10.9953 | 4.7423 | 5.3758 | 0.0204 | 59593.7547 | 5.4764 to 648492966 |
| Lauren type           | 4.8801 | 2.7423 | 3.2076 | 0.0733 | 131.6499 | 0.6309 to 27471.123 |
| LNR3                  | 10.5443 | 6.4439 | 2.6767 | 0.1018 | 37961.2555 | 0.1242 to 11.6E+09 |
| M                     | 17.9615 | 8.8487 | 4.1203 | 0.0424 | 63178227.38 | 1.8553 to 2.1514E+07 |
| NAT                   | 10.5263 | 6.0707 | 3.0066 | 0.0829 | 37282.7020 | 0.2535 to 5.48E+09 |
| Curative surgery      | 5.3211 | 5.9040 | 0.8123 | 0.3675 | 204.5997 | 0.0019 to 21703212 |
| PO NS complications   | 4.1918 | 1.7152 | 5.9727 | 0.0145 | 66.1412 | 2.2933 to 1907.5679 |
| Preoperative anemia   | 4.2286 | 1.5673 | 7.2792 | 0.0070 | 68.6244 | 3.1794 to 1481.1931 |
| R                     | 15.0872 | 7.5381 | 4.3973 | 0.0360 | 73279997.730 | 2.8081 to 19.1E+012 |

\(b\): Regression coefficient beta; SE: Standard error; Wald: \(b/SE2\); \(Exp(b)\): Exponentiation of the beta coefficient; CI: Confidence interval; LNR: Metastatic lymph node ratio; NAT: Neoadjuvant therapy; NS: Non-surgical; variables and \(P\) written in bold are statistically significant (<0.05)
wanted to enrich our previously reported line of research (the cytopathologic analysis of GL from GC patients) by combining it with examination of intragastric MB: as a consequence, we assessed the cyto-microbiologic parameter of “GL MB.” In our patient population, analysis of this character provided original and interesting results. In fact, subjects showing GL1 and MB1 had poorer survival compared with GL1 MB0 group (20.5 vs 45.8 months, respectively, \( P = 0.049 \)) [Figure 4]; such a result could confirm a pro-tumorigenic role of some gastric microbiota as suggested by previous studies.[34-36] This is also corroborated by the fact that, in our series, MB1 in conjunction with GL1 strongly correlated with tumor aggressiveness in advanced phase of disease (T3-T4 with \( P = 0.049 \) and Stage 3-4 with \( P = 0.035 \)) [Table 1]. Furthermore, the GL1 MB1 parameter resulted a significant prognostic factor for OS in univariate analysis (\( P = 0.008 \), Table 2) and an independent prognostic factor of OS at multivariate analysis (\( P = 0.013 \) with an overall model fit of \( P < 0.001 \), Table 3).

In other words, the absence of bacterial microbiota (MB0) in GL1 GC patients seemed to be a protective factor. In addition, GL1 MB1 was significantly associated with the preoperative diagnosis of \( H. \) pylori infection (\( P = 0.011 \), Table 1).

In the light of our results, the mixed cyto-microbiological test on GL seems quite interesting to perform in GC patients, especially from a prognostic and therapeutic point of view. Our findings, in fact, strengthening the carcinogenic role executed by Metastasis VI and \( H. \) pylori but also suggesting the cooperation between such features and the other non-\( H. \) pylori pro-oncogenic germs within the endogastric microenvironment, showed that GL1 MB1 GC patients had a poorer OS in comparison with GL1 MB0 GC subjects.[18-25,32-36] In this regard, the treatment of non-\( H. \) pylori bacteria could exert a conspicuous benefit for individuals with related precancerous gastric lesions (such as lymphocytic gastritis), just as already proven by antibiotic therapy for \( H. \) pylori infection.[36,37] Further studies dealing with GC patients showing malignant endogastric exfoliation in combination with intragastric microbiota (GL1 MB1 GC subjects) are needed to corroborate our data.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Ai S, Sun F, Liu Z, Yang Z, Wang J, Zhu Z, et al. Change in serum albumin level predicts short-term complications in patients with normal preoperative serum albumin after gastrectomy of gastric cancer. ANZ J Surg 2019;89:E298-301.
2. Kanaji S, Kakeji Y. Is laparoscopic distal gastrectomy a feasible procedure for elderly patients with gastric cancer? J Invest Surg 2019;31:546-7.
3. Pezzì E, Ferri M, Rapazzotti Onelli M, Mercantini P, Corigliano N, Duranti E, et al. Prognostic significance of 18q LOH in sporadic colorectal carcinoma. Am Surg 2011;77:38-43.
4. Ferri M, Lorenzon L, Rapazzotti Onelli M, La Torre M, Mercantini P, Virgilio E, et al. Lymph node ratio is a stronger prognostic factor than microsatellite instability in colorectal cancer patients: Results from a 7 year follow-up study. Int J Surg 2013;11:1016-21.
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Fears EJ. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
6. Virgilio R, Jorgensen MS, Attwood K, Almery T, Elii EF, Collibeaum DT, et al. Racial disparities in outcomes among Asians with gastric cancer in the USA. Anticancer Res 2020;40:881-9.
7. Virgilio E, Giarnieri E, Giovagnoli MR, Montagnini M, Proietti A, D’Urso R, et al. Gastric lavage malignant cells (yGL) and hypohemoglobinemia (yAnemia) as new systems of tumor regression grading and prognostic prediction for gastric cancer after neoadjuvant treatment. Anticancer Res 2019;39:1019-27.
8. Virgilio E, Giarnieri E, Montagnini M, Villani S, Giovagnoli MR, Mercantini P, et al. Advances in intraluminal exfoliative cytology of gastric cancer: Oncologic implication of the sixth metastatic route (Metastasis VI). Anticancer Res 2019;39:4019-22.
9. Virgilio E, Giarnieri E, Giovagnoli MR, Montagnini M, Proietti A, D’Urso R, et al. Presence of cancer cells in gastric lavage of gastric cancer patients as an indicator of advanced disease, predictor of tumour aggressive phenotype and independent prognostic factor for poor survival: The endoluminal metastatic pathway of gastric cancer and GL0/GL1 classification. Cytopathology 2018;29:41-8.
10. Virgilio E, Balducci G, Mercantini P, Giarnieri E, Giovagnoli MR, Mercantini P, et al. Preoperative gastric lavage in gastric cancer patients undergoing surgical, endoscopic or minimally invasive treatment: An oncological measure preventing peritoneal spillage of intragastric cancer cells and development of related metastases. Med Hypotheses 2018;114:30-4.
11. Virgilio E, Proietti A, D’Urso R, Cardelli P, Giarnieri E, Montagnini M, et al. Measuring intragastric tumor markers in gastric cancer patients: A systematic literature review on significance and reliability. Anticancer Res 2017;37:2817-21.
12. Virgilio E, Giarnieri E, Montagnini M, D’Urso R, Proietti A, Mesiti A, et al. Detection of cancer cells and tumor markers in gastric lavage of patients with gastric cancer: Do these findings have a clinicopathological significance and oncological implication? Med Hypotheses 2016;94:1-3.
13. Virgilio E, Giarnieri E, Montagnini M, D’Urso R, Proietti A, Mesiti A, et al. Analyzing gastric lavage of gastric cancer patients: A prospective observational study on cytopathology and determination of intragastric CEA, Ca 9.9, Ca 72.4 and Ca 50. Acta Cytol 2016;60:161-6.
14. Virgilio E, Balducci G, Mercantini P, Giarnieri E, Giovagnoli MR, Montagnini M, et al. Utility of nasogastric tube for medical and surgical oncology of gastric cancer: A prospective institutional study on a new and precious application of an old and economic device. Anticancer Res 2018;38:433-9.
15. Virgilio E, Giarnieri E, Giovagnoli MR, Montagnini M, Proietti A, D’Urso R, et al. Gastric juice microRNAs as potential biomarkers for screening gastric cancer: A systematic review. Anticancer Res 2018;38:613-6.
Virgilio, et al.: Intragastric malignant cells with microbiota

17. Virgilio E, Giarnieri E, Giovagnoli MR, Montagnini M, Proietti A, D’Urso R, et al. Long non-coding RNAs in the gastric juice of gastric cancer patients. Pathol Res Pract 2018;214:1239-46.

18. Pichon M, Burucoa C. Impact of the gastro-intestinal bacterial microbiome on Helicobacter-associated diseases. Healthcare (Basel) 2019;7:34.

19. Engstrand L, Graham DY. Microbiome and gastric cancer. Dig Dis Sci 2020;65:865-73.

20. Schüttke M, Malfertheiner P, Schulz C. What is the relevance of gastric microbiota beyond H. pylori? Curr Treat Options Gastro 2019;17:619-27.

21. Schulz C, Schüttke K, Mayerle J, Malfertheiner P. The role of the gastric bacterial microbiome in gastric cancer: Helicobacter pylori and beyond. Ther Adv Gastroenterol 2019;12:1756284819894062.

22. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, Costa JL, Carneiro F, Machado JC, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut 2018;67:226-36.

23. Zilberstein B, Quitanilha AG, Santos MA, Pajecki D, Moura EG, Arruda Alves PR, et al. Digestive tract microbiota in healthy volunteers. Clinics (Sao Paulo) 2007;62:47-54.

24. Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R, Mantilla A, Torres J. Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. Sci Rep 2014;4:4202.

25. Thorrell K, Bengtsson-Palme J, Liu OH, Palacios Gonzales RV, Noookaev I, Rabeneck L, et al. In vivo analysis of the viable microbiota and Helicobacter pylori transcriptome in gastric infection and early stages of carcinogenesis. Infect Immun 2017;85:e00331-17.

26. Hashemi MR, Rahnavard M, Bikhdeli B, Dehghani Zahedani M, Iranmanesh F. Touch cytology in diagnosing Helicobacter pylori: Comparison of four staining methods. Cytopathology 2008;19:179-84.

27. Jiang Y, Tu R, Lu J, Zhang Y, Zhu J, Tang W, et al. Proposed modification of the 8th edition of the AJCC staging system for gastric cancer. J Invest Surg 2020;33:932-8.

28. Lorussio D, Linsalata M, Pezzolla F, Berloco P, Osella AR, Guerra V, et al. Duodenogastric reflux and gastric mucosal polyp-like in the non-operated stomach and in the gastric remnant after Billroth II gastric resection. A role in gastric carcinogenesis? Anticancer Res 2000;20:2197-201.

29. Virgilio E, Proietti A, D’Urso R, Cardelli P, Giarnieri E, Giovagnoli MR, et al. Elevated gastric juice carbohydrate antigen (Ca 72.4) is an independent prognostic factor of poor survival for gastric cancer patients. Anticancer Res 2020;40:1691-5.

30. Virgilio E, Giarnieri E, Giovagnoli MR, Montagnini M, Villani S, Proietti A, et al. Combined analysis of intragastric malignant exfoliation and Ca 72.4 concentration in stomach adenocarcinoma: The “GL1 Ca 72.4” parameter. Acta Cytol 2020;64:563-71.

31. Virgilio E, D’Antonio C, Baldacci G. Mesogastrum recurrence as expression of the fifth metastatic route of gastric cancer. Med Hypotheses 2013;80:498-500.

32. Chung AY, Chow PK, Yu WK, Ho JM, Chan HS, Wong WK, et al. Prevalence of Helicobacter pylori in gastric cancer in a South-East Asian population by 14C-urea breath test. ANZ J Surg 2001;71:574-6.

33. Benberin V, Bektayeva R, Karabayeva R, Lebedev A, Akemeyeva K, Palohimeo L, et al. Prevalence of H. pylori infection and atrophic gastritis among symptomatic and dyspeptic adults in Kazakhstan. A hospital-based screening study using a panel of serum biomarkers. Anticancer Res 2013;33:4595-602.

34. Gunathilake MN, Lee J, Choi II, Kim YI, Ahn Y, Park C, et al. Association between the relative abundance of gastric microbiota and the risk of gastric cancer: A case-control study. Sci Rep 2019;9:13589.

35. Dias-Jacome E, Libanio D, Borges-Canha M, Galagarah A, Pimentel-Nunes P. Gastric microbiota and carcinogenesis: The role of non-helicobacter pylori bacteria-a systematic review. Rev Esp Enferm Dig 2016;108:530-40.

36. Petra CV, Rus A, Dumitrașcu DL. Gastric microbiota: Tracing the culprit. Cluj Med 2017;90:369-76.

37. Monstein HJ, Tjyejung A, Kraft CH. Profiling of bacterial flora in gastric biopsies from patients with Helicobacter pylori-associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16S rDNA sequence analysis. J Med Microbiol 2000;49:817-22.