

Clinical usefulness of gastric-juice analysis in 2007: the stone that the builders rejected has become the cornerstone

Antonio Tucci, MD, Michele Bisceglia, MD, Massimo Rugge, MD, PhD, Primiano Tucci, BSWE, Attilio Marchegiani, MD, Giovanni Papadopoli, MD, Alessia Spada, BStat, Antonio Villani, MD, Gianmaria Pennelli, MD, Pietro Fusaroli, MD, Giancarlo Caravelli, MD, Tiziana Catalano, MD, Vincenzo Cennamo, MD, Massimo Cianci, MD, Corrado De Fanis, MD, Carlo Fabbri, MD, Giuseppe Feliciangeli, MD, Giuseppe Gizzi, MD, Antonio Spadaccini, MD, Giancarlo Caletti, MD

Castel S Pietro Terme, San Giovanni Rotondo, Padova, San Severo, Foggia, Larino, Napoli, Sciacca, Bologna, Pescara, Ancona, Vasto, Italy

Background: Gastric juice is usually discarded during upper-GI endoscopy.

Objective: By using a novel device, the Mt 21-42, we evaluated the potential of this important organic fluid in clinical practice, exploring its contribution to diagnosis *Helicobacter pylori* infection and atrophic gastritis of the oxyntic mucosa (AGOM).

Design and Patients: A multicenter study (17,907 patients; 10 endoscopy units) estimated the frequency of diagnosis of AGOM and *H pylori* infection in routine endoscopic practice. A prospective study (216 patients) at 1 of these units aimed to determine the real prevalence of these conditions and the possible benefits of gastric juice analysis. We considered gastric juice pH and ammonium concentration, endoscopic and histologic features, serologic parameters for atrophy and *H pylori*, gastric acid secretion, and costs.

Results: We found that *H pylori* infection and, even more markedly, AGOM were greatly underdiagnosed in routine endoscopic practice (20.1% and 0.8% vs 49.1% and 12.5% in the prospective study, respectively), because of the intrinsic limitations of the conventional tests and lack/inappropriateness of biopsy planning. Gastric-juice analysis proved to be a cheap, simple, and effective way to prevent such underdiagnosis and allowed detection of atrophic gastritis and *H pylori* in 96% and 98% of cases, and saved costs (cost-effectiveness ratio 209 vs 274-5047).

Conclusions: Gastric juice provided a valuable source of clinicopathologic information that, properly analyzed, allowed detection of the main risk factors for gastric cancer (*H pylori* and atrophic gastritis), overcoming the diagnostic limitations associated with these conditions and also producing time and cost savings. (Gastrointest Endosc 2007;66:881-90.)

Gastric juice (“the stone that the builders rejected” [Matthew 21:42]) is usually thrown away during upper-GI endoscopy. However, it could provide valuable information, especially about *Helicobacter pylori* infection and atrophic gastritis of the oxyntic mucosa (AGOM), which cannot be detected by simple endoscopic examination. The identification of these 2 risk factors for gastric

cancer^{1,2} demands that endoscopy is complemented by an urease test and histology in all patients, with both widespread and specific gastric biopsy sampling.³⁻⁶ However, this is not routine practice. In patients with normal/mild endoscopic findings, endoscopists usually take only a few antral samples or do not perform biopsies. Consequently, many cases of *H pylori* infection and, even more markedly, of AGOM can escape detection. However, to perform multiple biopsies in all patients would be expensive and require considerable time and patience, and would prove to be unnecessary in the substantial proportion of patients found to have neither *H pylori* infection nor gastritis.

A solution to the impasse could be the prediction of *H pylori*-infection and AGOM “during” endoscopy;

Abbreviations: AGOM, atrophic gastritis of the oxyntic mucosa; BAO, basal acid output; CL, confidence limits; ECL, enterochromaffin-like; H&E, hematoxylin and eosin; Ig, immunoglobulin; PAO, peak acid output; SD, standard deviation; UBT, urea breath test.

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complementary tests could then be carried out only in selected patients, and a more appropriate biopsy plan could be adopted if AGOM is suspected. *H pylori* infection and AGOM produce changes in gastric-juice ammonium concentrations and pH, so it is theoretically possible to predict those conditions by evaluating such changes during endoscopic examination. This is extremely difficult in practice; the process of measurement itself (in particular with ammonium), the peculiar characteristics of the gastric-juice matrix, and the extremely short time available for the evaluation make this assessment much more problematic than it seems and also explains why these determinations have not been used in clinical practice.⁷ Recently, a new device, the Mt 21-42 (prototype) was developed to specifically overcome this difficulty; it measures gastric-juice pH and ammonium concentration automatically in real time and can be connected to any endoscopic apparatus.^{7,8}

By exploiting the capabilities of this device, we designed a study to (a) evaluate the potential of gastric-juice analysis in clinical practice, especially its contribution to the diagnosis of *H pylori* infection and AGOM; and (b) evaluate the detection of these conditions in routine endoscopic practice to determine whether they are underdiagnosed.

PATIENTS AND METHODS

The experimental design included a multicenter retrospective study, involving several endoscopic units, to estimate the frequency of a diagnosis of AGOM and *H pylori* infection in routine endoscopic practice, and a prospective study at 1 of these units (by using an Mt 21-42 prototype), to evaluate the actual prevalence of these conditions and to explore the usefulness of gastric-juice analysis. Issues concerning the etiopathogenesis and nosology of AGOM were not addressed.

Multicenter retrospective study

At 10 Italian units (belonging to university departments or regional hospitals), the endoscopic/histologic diagnoses of patients who had undergone upper-GI endoscopy during a 1-year period were reviewed. The parameters evaluated were (a) the number of gastroscopies performed, (b) the diagnoses of *H pylori* infection and AGOM, and (c) gastric adenocarcinomas detected. The last parameter was investigated to determine whether an appropriate relationship existed between the risk factor (AGOM) and the risk event (adenocarcinoma).

The purpose of the retrospective study was not to produce data for a strict and rigorous statistical comparison with those of prospective study but simply and solely to furnish information concerning the status of a diagnosis of *H pylori* infection and AGOM in routine endoscopic practice (because this aspect, in particular, AGOM, has never been evaluated in a large multicenter study).

Capsule Summary

What is already known on this topic

- Urease testing and gastric biopsy are required for the diagnosis of *Helicobacter pylori* infection and atrophic gastritis, 2 risk factors for gastric cancer.

What this study adds to our knowledge

- In a prospective study of 216 patients undergoing endoscopy, gastric-juice analysis proved to be a cheap, simple, and effective way to prevent underdiagnosis of atrophic gastritis and *H pylori* in 96% and 98% of cases, respectively.

Prospective study

Patients. A total of 216 consecutive outpatients referred (T. Masselli Hospital, San Severo) for diagnostic upper-GI endoscopy were included (99 men, 117 women; mean age [\pm SD] 47 ± 17 years). Exclusion criteria were the following: medication affecting gastric-acid secretion, medication with antimicrobials within the previous 4 weeks, and gastric surgery. There were 2 groups of healthy controls for evaluation of endocrine cells (7 men, 8 women; mean age 50 ± 11 years) and for gastric-acid secretion (12 men, 10 women; mean age 49 ± 18 years). The subjects in the first group were selected from among those undergoing upper-GI endoscopy for unrelated reasons (eg, iron deficiency anemia, retrosternal discomfort, suspected abnormalities at radiology); they were all endoscopically normal and negative for *H pylori*. Most of the subjects in the second group took part in a previous study, and the description of the series is given elsewhere.⁹ All the patients gave their informed consent before entering the study.

Endoscopy and biopsies. All patients underwent endoscopy. Two sets of biopsy specimens were taken in each patient: 1 for routine histology (including 2 specimens from the gastric antrum and 2 from the fundus) and 1 for the informed pathologist (2 specimens from the antrum, 2 from the great curve, and 4 from the fundus). One antral biopsy specimen was also taken for the urease test (CP test; Yamanouchi-Pharma, Milan, Italy).

Mt 21-42. This innovative device allows detection of *H pylori* infection and hypochlorhydria/achlorhydria during endoscopy. Interposed between the endoscope and the suction system, it analyses samples of the aspirated gastric juice within 2 minutes. The operating principle of the device is based on the determination of the pH and the ammonium concentration of gastric juice, the first parameter being correlated with acid-secretion status and the second with the presence of *H pylori*. A detailed description of the device and its validation studies are reported elsewhere.^{7,8}

Histology. The first set of biopsy specimens was processed routinely and analyzed by staff pathologists

(7 attending pathologists, with 8–15 years' experience), who were all unaware of the study. The second set was processed separately and examined by a chief pathologist (M.B.) with 22 years' experience and who was informed about the study. In addition to hematoxylin and eosin (H&E), other stains were used with this second set: (a) histochemical argyrophilic Sevier-Munger stain and (b) immunohistochemical with monoclonal antibodies to chromogranin A and polyclonal to human gastrin. The parameters assessed were as follows: *H pylori* density, chronic inflammation, activity, glandular atrophy, intestinal metaplasia, enterochromaffin-like (ECL) cells, and gastrin-producing cells. The first 5 features were evaluated according to the updated Sydney System,¹⁰ the ECL cells were assessed by using the Solcia classification,¹¹ and the evaluation of G cells was performed by counting the number of cells per linear millimeter of mucosa (hyperplasia being defined as the G-cell density increased by more than twice the standard deviation (SD) of the control group). Besides evaluating the second set of biopsy specimens, the informed pathologist also reviewed the first set of samples, giving a personal grading to each of the investigated parameters. All the discordant cases were collegially reviewed at the end of the study at a staff meeting of the informed and blinded pathologists, and a definitive diagnosis was established. Furthermore, to estimate the accuracy of the assessment of atrophy, 101 slides (from 9 patients with moderate-severe AGOM, 9 with a mild form, and 9 who were negative for AGOM) were also blindly examined by a pathologist (M.R.) with expertise in atrophic gastritis. From the comparison, the result was that there was agreement about the presence/absence of atrophy in 91.7% of antral, 89.5% of corpus, and 87.5% of fundic samples. Among the concordant cases, the scores were similar in 95.5% of antral, 100% of corpus, and 90.5% of fundic samples. The kappa values for atrophy at different sites were as follows: antrum 0.81 (95% confidence limits [CL] 0.56, 1.06); corpus 0.75 (95% CL 0.43, 1.01); and fundus 0.67 (95% CL 0.49, 0.85). The overall agreement for AGOM (considered only as present/absent) was high (96.3%; $K = 0.84$, 95% CL 0.63, 1.05).

Urea breath test. The urea breath test (UBT) was done in 135 patients, by using 75 mg of ¹³C-urea in 200 mL citric acid solution. A ¹³CO₂/¹²CO₂ ratio >3.9% of baseline was considered positive.

Serologic analyses. The following determinations were performed in all the patients: basal gastrin (Gastrin-Radioimmunoassay; ICN, Aurora, Ohio), intrinsic factor antibodies (57Co radioassay-IF-bAb solid-phase; Diagnostic Products Corporation, Los Angeles, Calif), parietal cell antibodies (Autostat-TM-II anti-GPC; Hycor-Biomedical, Edinburgh, UK), vitamin-B₁₂ and folate (chemiluminescence-system AGS-180-VB12 and Folate; Bayer, Tarrytown, NY), pepsinogen (radioimmunoassay-Pepsik; Sorin Biomedica, Saluggia, Italy), immunoglobulin (Ig) G anti-*H pylori* (Helori-test; Eurospital, Trieste, Italy).⁸

Acidimetry. Acidimetry was done in 24 patients with hypochlorhydria on Mt 21-42 analysis (9 men, 15 women; mean [SD] age 54 ± 14 years) and in 22 healthy controls. Among the patients, 12 showed AGOM on histology (6 moderate-severe and 6 mild) and 12 did not. Basal acid output (BAO) and peak acid output (PAO) were calculated.¹²

Case definition. Patients were considered to have *H pylori* infection if all or all but 1 of the reference tests (histology, urease test, UBT, IgG anti-*H pylori*) were positive. If 2 tests were discordant, then the final diagnosis was established after revision of the case after review of the test (eg, histology) or repetition (eg, UBT) or further testing (eg, Giemsa stain, Western blot analysis).

AGOM was diagnosed if the oxyntic mucosa (corpus and/or fundus samples of the first and/or second set of biopsy specimens) showed glandular atrophy (grading ≥ 1) on histologic examination. In accordance with current criteria,^{10,15} glandular atrophy was defined as the loss of appropriate glands and was graded as mild (= 1), moderate (= 2), and severe (= 3). Particular attention was paid to the mild forms of glandular atrophy; specimens with "indefinite atrophy"¹⁵ and those not including full-thickness mucosa, and/or not properly fixed or sectioned, were not considered for the computation.

Because etiopathogenesis and nosology of AGOM were not issues of concern in the present study, no topographic, phenotype, or pathophysiologic classification or subdivision was made (although data regarding topography, biochemical markers, and physiology are reported and detailed in specific sections). Furthermore, to guarantee an accurate assessment of atrophy, a collegial review of the discordant cases and a comparison with a pathologist expert in atrophic gastritis were also performed.

Cost analysis. The cost analysis focused on patients undergoing endoscopy and evaluated diagnostic cost only; long-term outcomes and quality of life issues were intentionally not considered. Cost, effectiveness, and cost-effectiveness ratio were evaluated, all expressed per 100 patients.¹⁴ For the Mt 21-42, 3 options were considered: (1) cost of the test (euro 0.4), (2) cost of the test plus histology (required when the device identified hypochlorhydria or when gastric focal lesions or features suggesting antral atrophy were detected endoscopically) (euro 125.8), (3) option 2 plus the Mt 21-42 amortization cost over 1 year (euro 133.3), calculated by dividing the estimated cost of the device (which is not a commercial product yet) by the annual volume of cases performed.

Statistical analysis

The statistical evaluations were performed by using the SPSS Statistical Package (SPSS Inc, Chicago, Ill). The tests used were as follows: the Student t (comparing means for normally distributed variables): χ^2 test or the Fisher exact test (discrete variables), the Mann-Whitney U test (histologic gradings and acid-secretion values), the Spearman

TABLE 1. Diagnostic parameters of the tests used in the diagnosis of AGOM

Parameter	Mt 21-42	Endoscopy	Routine histology	Gastrin	Pepsinogen	IFA	PCA	Vitamin B ₁₂	Folate
Sensitivity	96.3 (89.2, 100.0)*	14.8 (1.4, 28.2)	38.5 (19.8, 57.2)	40.7 (22.2, 59.3)	40.7 (22.2, 59.3)	3.7 (0, 10.8)	33.3 (15.6, 51.1)	22.2 (6.5, 37.9)	11.1 (0, 23.0)
Specificity	85.7 (80.7, 90.7)	99.5 (98.4, 100.0)	97.9 (95.8, 99.9)	98.4 (96.6, 100)	98.4 (96.6, 100)	100.0 (100.0, 100.0)	94.7 (91.5, 97.9)	99.5 (98.4, 100.0)	99.5 (98.4, 100.0)
Positive predictive value	49.1 (36.1, 62.1)	80.0 (37.6, 96.4)	71.4 (45.4, 88.3)	78.6 (52.4, 92.4)	78.6 (52.4, 92.4)	100.0 (20.7, 100.0)	47.4 (27.3, 68.3)	85.7 (48.7, 97.4)	75.0 (30.1, 95.4)
Negative predictive value	99.4 (96.6, 99.9)	89.1 (84.2, 92.6)	92.0 (87.5, 95.0)	92.1 (87.5, 95.1)	92.1 (87.5, 95.1)	87.9 (82.9, 91.6)	90.9 (86.0, 94.1)	90.0 (85.1, 93.3)	88.7 (83.7, 92.3)

Values are percent (95% CL).

IFA, Intrinsic factor antibodies; PCA, parietal cells antibodies.

* $P < .001$ between Mt 21-42 and the other tests; χ^2 test.

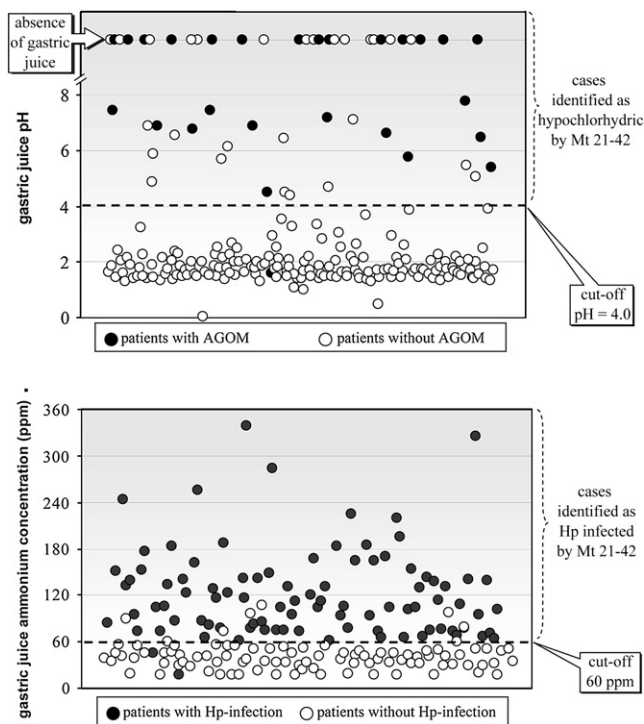


Figure 1. Relationship between gastric-juice pH values detected by Mt 21-42 and the presence or absence of AGOM (upper chart), and between gastric-juice ammonium concentrations and *H pylori* status (lower chart).

or Pearson correlation (relationship between variables), and the Cohen kappa (agreement between pathologists).

RESULTS

Multicenter retrospective study: diagnosis of AGOM and *H pylori* infection at routine endoscopy

A total of 17,907 cases were reviewed (9269 men, 8638 women; mean age 51 ± 19 years). Overall, 10,218 patients

(57%) underwent gastric biopsy for histology and/or urease testing, in line with the 56% shown (by the Italian Society for Digestive Endoscopy) in the 2001 Italian Census. The diagnosis of *H pylori* infection occurred (by histology and/or urease test) in 3597 patients (20.1%, range 11.0%–37.0%), whereas a histologically confirmed diagnosis of AGOM occurred in only 326 (1.82%, range 0.35%-3.59%), including 151 new diagnoses (0.84%, range 0.35%-1.47%) and 175 referrals for follow-up of known AGOM (0.98%, range 0.0%-3.06%). The number of gastric adenocarcinomas detected was 146 (0.82%, range 0.61%-1.16%), 64 (43.8%) were located in the antrum, 62 (42.5%) in the body, and 20 (13.7%) in the cardia. The prevalence difference between adenocarcinoma and new AGOM was small (0.02%), an anomalous pattern that suggested notable underdetection of the AGOM risk factor. When patients with previous gastric surgery or those undergoing operative/emergency endoscopy were excluded (n = 1590), the detection rates for AGOM and *H pylori* remained low (0.93% and 22.0%, respectively).

Prospective study

Prevalence of AGOM. In 27 of 216 patients (12.5%), the histologic evaluation by the informed pathologist showed AGOM. Two cases (0.93%) of gastric adenocarcinoma were also detected. The difference in prevalence between AGOM and adenocarcinoma was 11.57% ($P < .001$ vs multicenter study).

Conventional assessment of AGOM. Routine histology detected AGOM in 10 of 27 patients (37.0%). Nine of the false-negative cases were found to have AGOM at the collegial review (avoidable false negatives). No evidence of atrophy was found in the remaining cases (unavoidable false negatives): diagnosis was possible only from the samples taken for the informed pathologist. Endoscopy identified features suggestive of AGOM (submucosal vascular network, flattening of folds, mucosal paleness) in 5

Gastric endocrine cell hyperplasia

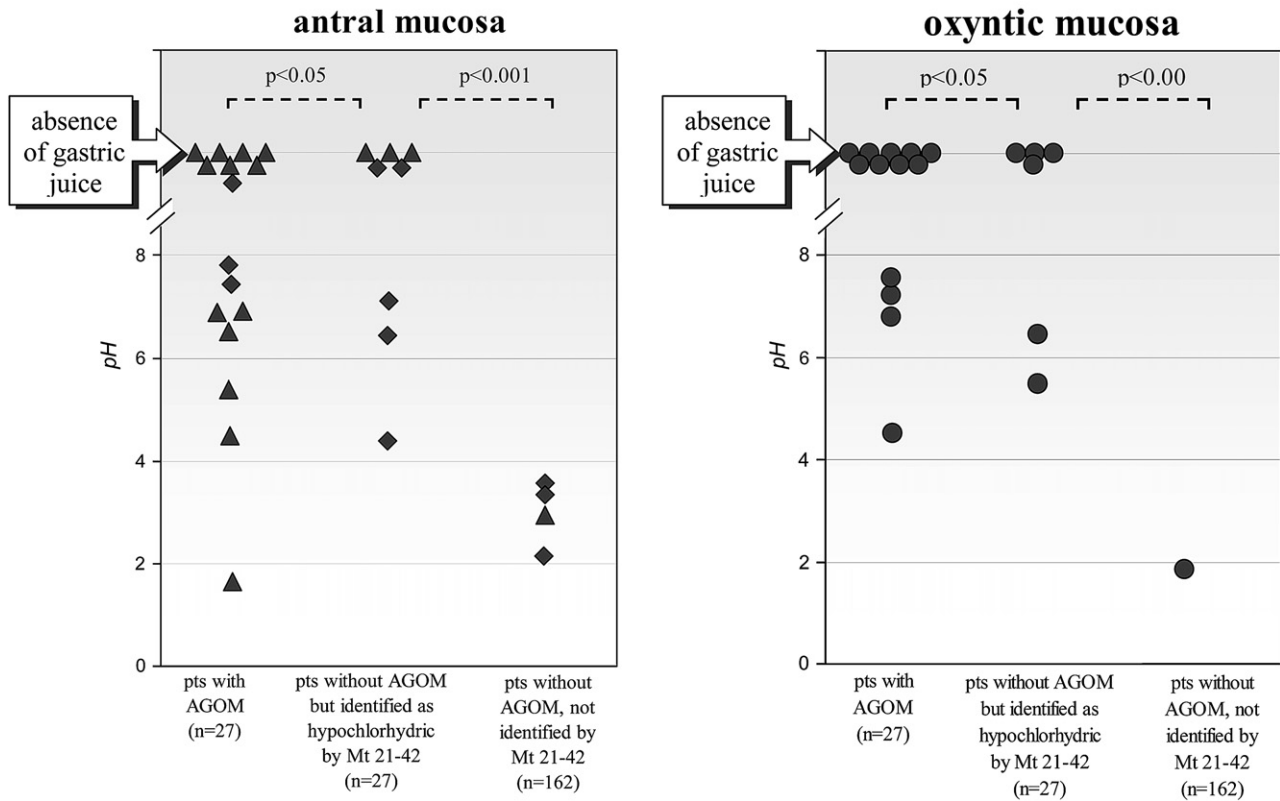


Figure 2. Relationship between endocrine-cell hyperplasia and gastric-juice pH in patients with histologically confirmed AGOM, in those without AGOM histologically but identified by the Mt 21-42 as hypochlorhydric, and in those without AGOM and not identified by the device; χ^2 test. ▲, G cells; ◆, other antral endocrine cells (chromogranin-A and/or argyrophilic-positive but gastrin-negative cells); ●, ECL cells.

TABLE 2. Diagnostic parameters of the tests used in the diagnosis of *H pylori* infection

Parameter	Mt 21-42	Urease test	Routine histology	Urea breath test	IgG anti- <i>H pylori</i>
Sensitivity	97.9* (95.0, 100.0) [97.3]†	79.2 (71.5, 87.0) [76.5]	93.4 (88.7, 98.1) [96.3]	89.7 (82.5, 96.9) [96.2]	84.0 (77.0, 91.0) [86.4]
Specificity	93.6‡ (88.7, 98.6) [92.8]	98.2 (95.7, 100.0) [97.3]	80.9 (73.6, 88.3) [81.6]	94.0 (88.4, 99.7) [97.4]	81.8 (74.6, 89.0) [88.2]
Positive predictive value	93.9 (87.3, 97.2) [93.6]	97.7 (91.9, 99.4) [96.9]	82.5 (74.7, 88.3) [84.8]	93.8 (85.2, 97.6) [98.0]	81.7 (73.4, 87.8) [88.6]
Negative predictive value	97.8 (92.3, 99.4) [97.0]	82.9 (75.5, 88.5) [79.3]	92.7 (85.7, 96.4) [95.4]	90.0 (80.8, 95.1) [94.9]	84.1 (76.0, 89.8) [85.9]

Values are percent (95% CL). Square brackets include the values calculated after excluding patients with AGOM and those who had received a previous anti-*H pylori* treatment.

* $P < .002$ vs urease test and IgG anti-*H pylori*.

† $P < .05$ vs urease test and IgG anti-*H pylori*; χ^2 test.

‡ $P < .02$ vs histology and IgG anti-*H pylori*.

patients, 4 being histologically confirmed and 1 not. Serologic analyses, overall (ie, 1 or more tests suggesting AGOM), detected AGOM in 14 of 27 patients with atrophy (51.9%). The individual sensitivity range was 3.7% to 40.7%. With mild forms of AGOM excluded from calcula-

tions, serologic tests showed increased sensitivity (gastrin 72.7%, parietal cells antibodies 63.6%, vitamin B₁₂ 54.5%, intrinsic factor antibodies 9.1%, folate 27.3%, pepsinogen 81.8%). The calculated diagnostic parameters of the tests are presented in Table 1.

TABLE 3. Histologic findings

	Patients with AGOM (n = 27)					
	Hp+ (n = 16)			Hp- (n = 11)		
	No. cases (%); grading (mean [SD])			No. cases (%); grading (mean [SD])		
	Antrum	Corpus	Fundus	Antrum	Corpus	Fundus
Chronic infiltrate	15 (94); 2.0 ± 0.7	15 (94); 1.9 ± 0.4	16 (100); 1.9 ± 0.7	6 (55)*; 1.2 ± 0.4†	10 (91); 1.7 ± 0.8	11 (100); 1.7 ± 0.5
Activity	12 (75); 1.1 ± 0.3	11 (69); 1.1 ± 0.3	11 (69); 1.2 ± 0.4	1 (9)§; 1.0 ± 0.0	2 (18)§; 1.5 ± 0.7	3 (27)*; 1.3 ± 0.6
Glandular atrophy	4 (25) 1.0 ± 0.0	12 (75); 1.3 ± 0.6	12 (75); 1.1 ± 0.3	4 (36); 1.0 ± 0.0	9 (82); 2.3 ± 0.5	11 (100); 2.4 ± 0.9
Intestinal metaplasia	5 (31); 1.2 ± 0.4	1 (6); 1.0 ± 0.0	1 (6); 1.0 ± 0.0	1 (9); 1.0 ± 0.0	2 (18); 1.0 ± 0.0	6 (55)*; 1.3 ± 0.0

Hp+, *H pylori* positive; Hp-, *H pylori* negative.

* $P < .05$ vs patients Hp+, with AGOM.

† $P < .05$ vs Hp-positive patients Hp+, with AGOM.

‡ $P < .001$ vs patients Hp+, without AGOM.

§ $P < .005$ vs Hp-positive patients with AGOM; χ^2 test.

|| $P < .005$ vs patients Hp+, with AGOM; Mann-Whitney *U* test.

Mt 21-42 assessment of AGOM. The device identified 53 cases of hypochlorhydria: 25 with gastric juice pH > 4.0 and 28 with partial/complete absence of gastric juice (Fig. 1). All but 1 patient with AGOM (96.3%) were identified with the Mt 21-42. Among those identified by Mt 21-42 analysis but without AGOM histologically were 5 patients with antral atrophy, 1 with gastric lymphoma, and 1 affected by Werlhof's disease associated with complete achlorhydria and an intense mononuclear infiltrate in the oxyntic mucosa. Furthermore, in this patient subgroup, immunohistochemistry and acid-secretion testing revealed a high rate (44.4%) of endocrine-cell hyperplasia (Fig. 2) and low values of basal-acid secretion.

***H pylori* status.** Of the patients, 106 of 216 (49.1%) were *H pylori* positive. There was an overall concordance of tests in 158 (73%); in 51 (24%), 1 test was discordant; and in 7 patients, a review was needed because 2 tests were discordant.

Conventional assessment of *H pylori* status. Overall, conventional tests showed good diagnostic performance (individual rates above 79%). The calculated diagnostic parameters are presented in Table 2. Because AGOM or previous treatment for *H pylori* may lead to false-negative and false-positive results, a further computation was done, excluding patients in these categories, but this made little change to the pattern of results (Table 2).

Mt 21-42 assessment of *H pylori* status. By using the cutoff point established in the validation studies,^{7,8} the Mt 21-42 correctly predicted *H pylori* status in all but 8 cases; 6 of the latter were false positives and 2 were false negatives (Fig. 1). In 28 cases, the diagnosis was not possible for the absence of gastric juice. The diagnostic parameters and comparison with other tests are presented in Table 2. Sensitivity and specificity were high (97.9% and 93.6%, respectively), and when the 28 patients not tested (in an intention to diagnose analysis)

were included, sensitivity and specificity remained high (86.8% and 93.6%). Indeed, in all the cases with an absence of gastric juice, the device invites (through vocal synthesized message provided during the examination) the endoscopist to perform gastric biopsies in both the antrum and the fundus. So, the diagnosis of infection cannot in any case escape.

Other data. Patient data. Of the 27 patients with AGOM, 44% (12/27) had previously undergone 1 to 10 endoscopies before atrophy was diagnosed, and 56% (15/27) had received acid-inhibiting drugs during the previous 5 years because of clinical suspicion of acid-related disorders.

Histology. Data are reported in Table 3. Among patients without AGOM, chronic infiltration and activity in the antrum, corpus, and fundus were more frequent in patients who were *H pylori* positive than in patients who were *H pylori* negative ($P < .001$). Among patients with AGOM, glandular atrophy of the oxyntic mucosa was more severe in uninfected patients than in patients who were infected ($P < .005$); intestinal metaplasia of the fundic mucosa was more frequent in patients who were *H pylori* negative than in patients who were *H pylori* positive ($P < .05$); conversely, activity in the antrum and the oxyntic mucosa was more frequent in patients who were *H pylori* positive than in patients who were *H pylori* negative ($P < .005$).

Immunohistochemistry. Endocrine-cell hyperplasia (in the antrum and/or the oxyntic mucosa) was detected in 23 of 27 patients (85.2%) with histologically confirmed AGOM, in 12 of 27 patients (44.4%) without AGOM histologically but identified by the Mt 21-42 as hypochlorhydric, and in only 5 of 162 patients (3.1%) without AGOM and not identified by the device (Fig. 2). No adenomatoid hyperplasia, dysplasia, or neoplasia was found. A correlation was detected between endocrine-cell hyperplasia and gastric-juice pH ($r = 0.609$; $P < .001$). Regardless of the

TABLE 3 (continued)

Patients without AGOM (n = 189)					
Hp+ (n = 90)			Hp- (n = 99)		
No. cases (%); grading (mean [SD])			No. cases (%); grading (mean [SD])		
Antrum	Corpus	Fundus	Antrum	Corpus	Fundus
90 (100); 1.6 ± 0.7	52 (58); 1.2 ± 0.4	47 (52); 1.2 ± 0.4	5 (5)‡; 1.0 ± 0.0	3 (3)‡; 1.0 ± 0.0	0 (0)‡; 0.0 ± 0.0
74 (82); 1.1 ± 0.4	24 (27); 1.1 ± 0.4	23 (26); 1.0 ± 0.2	1 (1)‡; 1.0 ± 0.0	1 (1)‡; 1.0 ± 0.0	0 (0)‡; 0.0 ± 0.0
8 (9); 1.4 ± 0.5	0 (0); 0.0 ± 0.0	0 (0); 0.0 ± 0.0	3 (3); 1.3 ± 0.6	0 (0); 0.0 ± 0.0	0 (0); 0.0 ± 0.0
7 (8); 1.4 ± 0.5	0 (0); 0.0 ± 0.0	0 (0); 0.0 ± 0.0	3 (3); 1.7 ± 0.6	0 (0); 0.0 ± 0.0	0 (0); 0.0 ± 0.0

presence or absence of AGOM, 34 of 41 patients with hyperplasia (83%) were identified by Mt 21-42 analysis as hypochlorhydric.

Acidimetry. Patients with AGOM showed lower gastric acid secretion (mean BAO [\pm SD] = 0.14 ± 0.27 mEq/h; PAO = 7.60 ± 10.17 mEq/h) than healthy controls (BAO = 2.80 ± 1.31 mEq/h; PAO = 23.25 ± 8.38 mEq/h) ($P < .001$). Among patients with AGOM, those with moderate-severe forms evidenced a more deteriorated acid secretory function (BAO = 0.0 ± 0.0 mEq/h; PAO = 0.04 ± 0.09 mEq/h) than patients with mild forms (BAO = 0.29 ± 0.33 mEq/h, PAO = 15.17 ± 9.49 mEq/h; $P = .059$, $P < .005$). The patient subgroup without evidence of AGOM but identified by Mt 21-42 as hypochlorhydric showed lower basal-acid output (BAO = 0.82 ± 0.99 mEq/h) than healthy controls ($P < .001$) but higher than patients with AGOM ($P < .005$) (Fig. 3). Gastric-juice pH was inversely correlated with BAO ($r = -0.72$; $P < .001$) and PAO ($r = -0.29$; $P < .05$).

Cost analysis. The results are presented in Table 4. Compared with the other diagnostic strategies, the new technology showed the highest effectiveness (60.2 vs 1.9-51.9) and the best cost-effectiveness ratio (209 vs 274-5047). The difference was particularly marked for AGOM (1048 vs 2008-7226). Inclusion of 1 year's amortization costs for the device did not substantially change the results (Table 4).

Usefulness of gastric-juice analysis. Analysis of the data revealed that an endoscopic strategy based on gastric-juice analysis, with gastric biopsy only in selected patients, was more effective and less expensive than both routine endoscopy and endoscopy with traditional complementary tests performed in all patients.

In our series, excluding 78 patients who needed a gastric biopsy (43 identified by Mt 21-42 analysis, 25 with gastric focal lesions or antral atrophy on endoscopy, and 10 with both indications), in the remaining 138 cases (80 with normal/mild endoscopic findings, 44 with hiatal her-

nia or incompetent cardias, and 14 with duodenal ulcer/erosions), histology and urease test did not provide substantial information in addition to that already obtained through gastric-juice analysis. Histology detected 3 cases of antral atrophy (1 moderate and 2 mild) but no important modifications, or only a chronic/active inflammatory infiltrate, in the remaining 135. Conversely, without the support of gastric-juice analysis, complementary tests would have missed 63% of the AGOM cases and 1 of *H pylori* infection. In the first scenario (endoscopy plus gastric-juice analysis), the overall cost for 216 patients would have been euro 27,170, whereas, in the second (endoscopy plus complementary tests), it would have been euro 39,701 (greater by 46%).

DISCUSSION

Our study demonstrated that *H pylori* infection and, even more, AGOM can easily go undetected in routine endoscopic practice. This may be of great consequence, because both diseases are important risk factors for gastric cancer.^{1,2} The current practice of performing gastric biopsies in patients with focal lesions and only in an unselected proportion of patients without macroscopic findings may account for significant underdiagnosis of pathologic conditions. This is confirmed by the discrepancy between the prevalences of *H pylori* infection and AGOM in our prospective study (49.1% and 12.5%) and those seen in routine endoscopic practice (20.1% and 0.8%), as well as by the anomalously small difference in prevalence (0.02%) between AGOM and gastric adenocarcinoma at routine endoscopy.

Paradoxically, the problem persists (although less markedly) when biopsies and complementary tests are performed in all patients. In our study, such an approach improved the detection of *H pylori* but still failed to

Gastric acid secretion

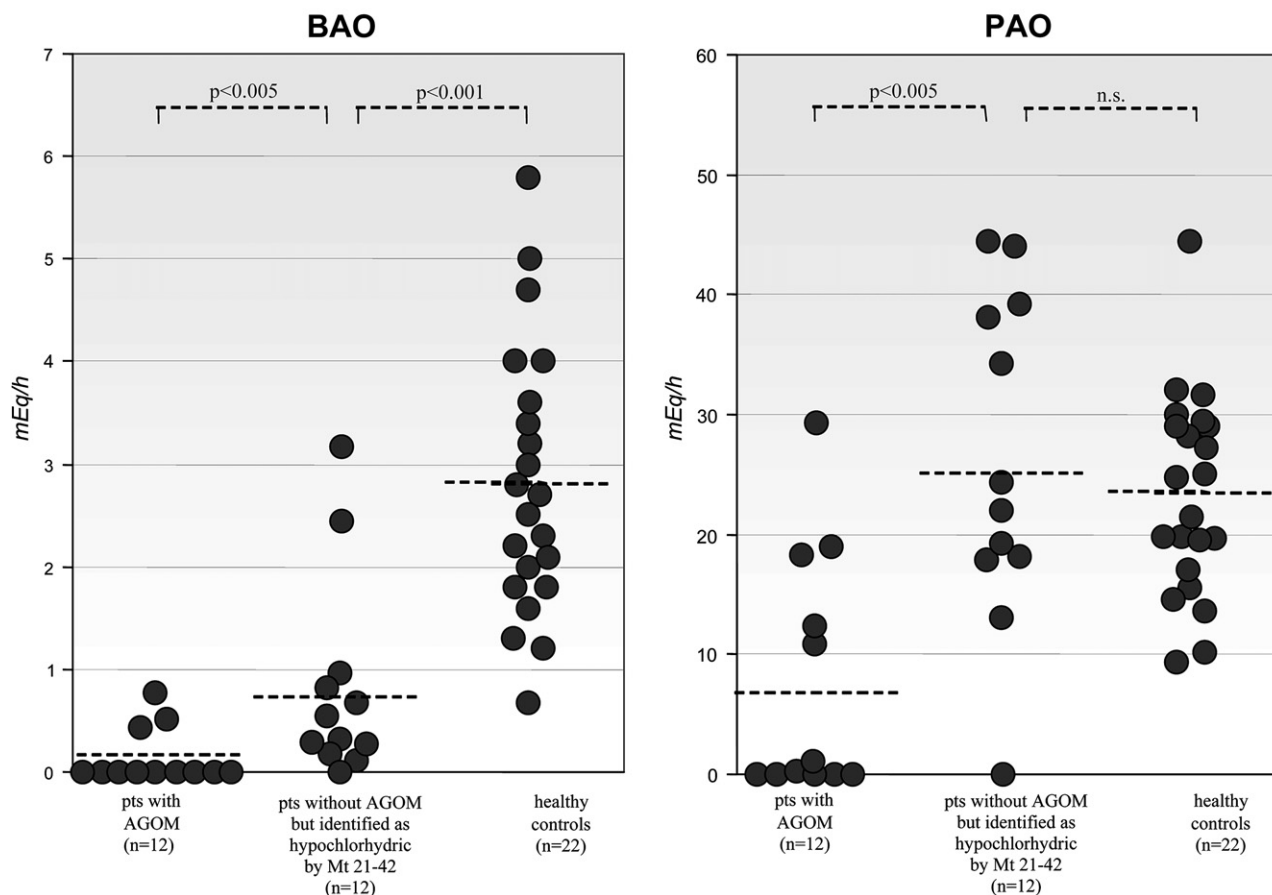


Figure 3. BAO and PAO values in patients with AGOM, in those without histologic evidence of AGOM but identified as hypochlorhydric by Mt 21-42, and in healthy controls. Mann-Whitney *U* test.

detect AGOM in 63% of cases. The patchy distribution of lesions and the low reliability of general pathology departments for detection of AGOM may account for these outcomes.^{9,15,16} This is confirmed by the discrepancy we found between routine and “accurate” histology, consistent with previous reports.³⁻⁶ The combination of endoscopy with histology is a necessary but not sufficient condition for the correct assessment of atrophy. To match expected outcomes, other requirements include, eg, larger numbers of biopsy samples and sites, pathologist awareness, additional stains.

Such an approach, however, is too expensive, complex, and time consuming for routine use, and would prove unnecessary in the substantial proportion of patients found to have neither *H pylori* infection nor atrophy. However, it is unacceptable that patients with preneoplastic conditions should pass undiagnosed, even after an invasive procedure. That 44% of patients with AGOM had undergone previous endoscopies without detection of atrophy is of serious concern, and that 56% of them had been treated with acid-inhibiting drugs is embarrassing.

Real-time gastric-juice analysis seems to represent an appropriate solution. It allows the detection of both conditions in nearly all cases, with further advantages: (1) avoiding complementary gastric tests in many patients; (2) adopting an appropriate biopsy plan in patients with suspicious conditions (addressing the problem of patchy distribution); (3) reducing costs and time; (4) assessing *H pylori* and AGOM, even when biopsy is contraindicated (eg, because of coagulopathy); and (5) detecting other conditions associated with hypochlorhydria (eg, gastric lymphoma, antral atrophy, gastric endocrine cell hyperplasia, basal gastric acid hyposecretion). Concerning the latter issue, the patient subgroup identified by Mt 21-42 analysis but with no AGOM histologically, is particularly interesting. The high rate of gastric endocrine cell hyperplasia and the low BAO values in these patients prompts many possible interpretations (eg, initial forms of AGOM, functional disorders), and recalls the debate regarding the role of hypochlorhydria (rather than atrophy) in gastric neoplastic transformation.^{17,18} Interestingly, most of these patients had normal-mild endoscopic

TABLE 4. Cost-effectiveness of different diagnostic pathways (the parameters evaluated were expressed as number per 100 patients)

Diagnostic pathways	Cost Euro (per 100 patients)	Effectiveness			Cost/effectiveness		
		No. patients correctly diagnosed (per 100 patients)*			Euro/diagnosis		
		<i>H pylori</i>	AGOM	Total	<i>H pylori</i>	AGOM	Total
EGD	9590	0	1.9	1.9	n.e.	5047	5047
EGD + urease test	13,730	39.1	1.9	41.0	351	7226	335
EGD + histology	17,760	45.8	4.7	50.5	388	3779	352
EGD + histology + urease test	18,380	47.2	4.7	51.9	389	3911	354
EGD + IgG anti- <i>H pylori</i> + pepsinogen + gastrin	13,050	41.2	6.5	47.7	317	2008	274
EGD + Mt 21-42							
Test alone	9630	43.1	12.0	55.1	223	803	175
+ Histology only in selected patients (36%)†	12,579	48.2	12.0	60.2	261	1048	209
+ Histology only in selected patients (36%) + amortization cost of the device (in 1 y)	13,329	48.2	12.0	60.2	277	1111	221

n.e., Not evaluable.

The costs of individual diagnostic procedures were taken from the National Sanitary Tariff²⁸ and from an estimation carried out by a working group set up by the Italian Society for Digestive Endoscopy (SIED).²⁹

*The number of cases per 100 patients with *H pylori* infection and AGOM was 49.1 and 12.5, respectively.

†"Selected patients" included those identified by Mt 21-42 as hypochlorhydric and those with endoscopic evidence of gastric focal lesions (eg, ulcer, polyp, erosions) or features suggestive of antral atrophy.

features and, probably, without gastric juice analysis, many of them would have remained unidentified.

One of the best values of gastric-juice testing is the possibility of providing pathophysiologic information in real time (ie, during endoscopy). This greatly enhanced practice and decision making during the procedure. In the case of Mt 21-42, such property is provided by a dedicated system that allows measurement of the parameters of interest (ammonium and pH) in less than 2 minutes. The validity of these parameters for detecting *H pylori* infection and hypochlorhydric conditions is clearly confirmed in the present study and in many other studies,¹⁹⁻²² and has been rigorously analyzed and extensively discussed in our previous reports.^{7,8,23}

In this context, an intriguing, but not surprising, finding of our study was the poor diagnostic performance of serologic methods in the diagnosis of AGOM, which contrasts with what has been reported in other studies. In fact, the contradiction is only apparent and it can be explained by an intrinsic limitation of serologic techniques related to their low sensitivity for the mild forms of AGOM. This was reported repeatedly.²⁴ It can also be deduced from the fact that most of the commercially available assays are based on the determination of more than 1 parameter to compensate for this limitation, and often it is clearly indicated that their use is limited to the assessment of

"moderate-severe" or "advanced" forms of atrophy.²⁵⁻²⁷ This weakness, however, does not substantially affect the value of serology, which remains a useful tool, particularly in patients who are not undergoing endoscopy and in large-scale screening programs. On the contrary, gastric-juice analysis is targeted only to patients undergoing upper-GI endoscopy in whom serology, as well as other conventional nonbiopsy-based tests (eg, UBT), are not routinely performed.

Finally, we consider costs. Usually, technologic improvements entail a proportionate cost increase; real-time gastric juice analysis achieves an advance with a considerable reduction of costs. Our data demonstrated that the endoscopic strategy based on gastric-juice analysis was more cost effective than routine endoscopy and even more so than endoscopy with traditional complementary tests performed in all patients. The potential cost savings (46%) and the large number of upper-GI endoscopies performed every day throughout the world, indicate the potential economic impact of such a strategy.

In conclusion, the results of the present study demonstrated that gastric juice is a valuable source of clinicopathologic information that, properly analyzed, allowed detection of the main risk factors for gastric cancer (*H pylori* and atrophic gastritis), overcome the diagnostic limitations associated with these conditions and also

produced time and cost savings. In this view, the new testing (real-time gastric-juice analysis) could represent a useful integrative tool for endoscopy.

DISCLOSURE

None of the authors have any commercial associations or conflicts of interest. Regarding the Mt 21-42, Antonio Tucci is the inventor and patent owner of the principle device. The Mt 21-42 device is a prototype, not yet a commercial product.

REFERENCES

- Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:829-32.
- Kamada T, Hata J, Sugiu K, et al. Clinical features of gastric cancer discovered after successful eradication of *Helicobacter pylori*: results from a 9-year prospective follow-up study in Japan. *Aliment Pharmacol Ther* 2005;21:1121-6.
- Rugge M, Cassaro M, Pennelli G, et al. Atrophic gastritis: pathology and endoscopy in the reversibility assessment. *Gut* 2003;52:1387-8.
- Dursun M, Yilmaz S, Yukselen V, et al. Evaluation of optimal gastric mucosal biopsy site and number for identification of *Helicobacter pylori*, gastric atrophy and intestinal metaplasia. *Hepatogastroenterology* 2004;51:1732-5.
- Testoni PA, Bonassi U, Bagnolo F, et al. In diffuse atrophic gastritis, routine histology underestimates *Helicobacter pylori* infection. *J Clin Gastroenterol* 2002;35:234-9.
- Satoh K, Kimura K, Taniguchi Y, et al. Biopsy sites suitable for the diagnosis of *Helicobacter pylori* infection and the assessment of the extent of atrophic gastritis. *Am J Gastroenterol* 1998;93:569-73.
- Tucci A, Tucci P, Marchegiani A, et al. Mt 21-42: development and validation of an automatic device proposed for the endoscopic diagnosis of *Helicobacter pylori* infection and atrophic gastritis. *Digestion* 2005;72:33-42.
- Tucci A, Tucci P, Biscaglia M, et al. Real time detection of *Helicobacter pylori* infection and atrophic gastritis. Comparison between conventional methods and an innovative device (Mt 21-42) performing gastric juice analysis during endoscopy. *Endoscopy* 2005;37:966-76.
- Tucci A, Poli L, Biasco G, et al. *Helicobacter pylori* infection and gastric function in patients with fundic atrophic gastritis. *Dig Dis Sci* 2001;46:1573-83.
- Dixon MF, Genta RM, Yardley JH. Classification and grading of gastritis. The updated Sydney System. International workshop on the histopathology of gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161-81.
- Solcia E, Bordi C, Creutzfeldt W, et al. Histopathological classification of nonantral gastric endocrine growths in man. *Digestion* 1988;41:185-200.
- Tucci A, Corinaldesi R, Stanghellini V, et al. *Helicobacter pylori* infection and gastric function in patients with chronic idiopathic dyspepsia. *Gastroenterology* 1992;103:768-74.
- Rugge M, Correa P, Dixon MF, et al. Gastric mucosal atrophy: interobserver consistency using new criteria for classification and grading. *Aliment Pharmacol Ther* 2002;16:1249-59.
- Makris N, Crott R, Fallone CA, et al. Cost-effectiveness of routine endoscopic biopsies for *Helicobacter pylori* detection in patients with non-ulcer dyspepsia. *Gastrointest Endosc* 2003;58:14-22.
- Christensen AH, Gjørup T, Hilden J, et al. Observer homogeneity in the histologic diagnosis of *Helicobacter pylori*. Latent class analysis, kappa coefficient, and repeat frequency. *Scand J Gastroenterol* 1992;27:933-9.
- Graham DY. Is real-time testing for *Helicobacter pylori* and corpus atrophy clinically useful in 2005? *Endoscopy* 2005;37:1006-7.
- Haruma K, Yoshihara M, Sumii K, et al. Gastric acid secretion, serum pepsinogen I, and serum gastrin in Japanese with hyperplastic polyps or polypoid-type early gastric carcinoma. *Scand J Gastroenterol* 1993;28:633-7.
- Yoshihara M, Haruma K, Sumii K, et al. The relationship between gastric secretion and type of early gastric carcinoma. *Hiroshima J Med Sci* 1995;44:79-82.
- Furuta T, El Omar EM, Xiao F, et al. Interleukin 1beta polymorphisms increase risk of hypochlorhydria and atrophic gastritis and reduce risk of duodenal ulcer recurrence in Japan. *Gastroenterology* 2002;123:92-105.
- Annibale B, Capurso G, Lahner E, et al. Concomitant alterations in intragastric pH and ascorbic acid concentration in patients with *Helicobacter pylori* gastritis and associated iron deficiency anaemia. *Gut* 2003;52:496-501.
- Farinati F, Cardin F, Di Mario F, et al. Perendoscopic gastric pH determination. Simple method for increasing accuracy in diagnosing chronic atrophic gastritis. *Gastrointest Endosc* 1987;33:293-7.
- Andersen J, Strom M. A technique for screening of achlorhydria and hypochlorhydria during upper gastrointestinal endoscopy. *Scand J Gastroenterol* 1990;25:1084-8.
- Tucci A. Real-time testing for *Helicobacter pylori* and atrophy of the gastric body: reply to the editorial of D. Graham. *Endoscopy* 2006;38:285-6.
- Ricci C, Vakil N, Rugge M, et al. Serological markers for atrophic gastritis in asymptomatic patients infected with *Helicobacter pylori*. *Am J Gastroenterol* 2004;99:1-6.
- Vaananen H, Vauhkonen M, Helske T, et al. Non-endoscopic diagnosis of atrophic gastritis with a blood test. Correlation between gastric histology and serum levels of gastrin-17 and pepsinogen I: a multicentre study. *Eur J Gastroenterol Hepatol* 2003;15:885-91.
- Lindgren A, Lindgren A, Lindstedt G. Advantages of serum pepsinogen A combined with gastrin or pepsinogen C as first-line analytes in the evaluation of suspected cobalamin deficiency: a study in patients previously not subjected to gastrointestinal surgery. *J Intern Med* 1998;244:341-9.
- Sanduleanu S, Bruine AD, Biemond I, et al. Ratio between serum IL-8 and pepsinogen A/C: a marker for atrophic body gastritis. *Eur J Clin Invest* 2003;33:147-54.
- Decreto n.81 of the President of the Republic on the National Sanitary Tariff. Official Gazette of the Italian Republic, Ordinary Supplement n.128, 17 February 1992.
- Rossi A, Battaglia G, Fregola G. Revisione del tariffario delle prestazioni endoscopiche. *Giornale Italiano di Endoscopia Digestiva* 2006;1(Suppl 1):5-17.

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Current affiliations: Gastrointestinal Unit of Castel S. Pietro Terme Hospital (A.T., P.T., P.F., G. Caletti), University of Bologna, AUSL of Imola, Department of Pathology (M.B.), Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo (FG), Department of Pathology (M.R., G. Pennelli), University of Padova, Padova, Endoscopic Unit (A.M., G. Papadopoli), T. Masselli Hospital, San Severo (FG), Department of Statistics (A. Spada), University of Foggia, Foggia, Biochemical Laboratory (A.V.), Vietri Hospital, Larino (CB), Gastroenterology Unit (G. Caravelli), II University of Naples, Naples, Digestive and Thoracic Endoscopic Unit (T.C.), Giovanni Paolo II Hospital, Sciacca (AG), Gastroenterology Unit (V.C.), Maggiore Hospital, Bologna, Department of Internal Medicine (M.C., G.G.), Sant'Orsola Hospital, Bologna, Gastroenterology Unit (C.D.F.), S. Spirito Hospital, Pescara, Gastroenterology Unit (C.F.), Bellaria Hospital, Bologna, Department of Gastroenterology (G.F.), Umberto I Hospital, Ancona, Gastroenterology Unit (A. Spadaccini), S. Pio da Pietrelcina Hospital, Vasto (CH), Italy.

Reprint requests: Antonio Tucci, MD, S.P. Lesina-Ripalta, km 0.5, 71010 Lesina, FG (Italy).