Impact of Assay Epitope Specificity in Gastrinoma Diagnosis, Jens Peter Goetze and Jens F. Rehfeld (Department of Clinical Biochemistry, KB 3014, Rigshospitalet, University of Copenhagen, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark; * author for correspondence: fax 45-3545-4640, e-mail JPG@daph.net.dk)

The antral hormone gastrin regulates gastric acid secretion and growth of the gastric mucosa (1). As a result of the elaborate cellular maturation process of progastrin, the antral G cells release a mixture of different acid-stimulatory gastrins and other precursor fragments to the circulation (2). This mixture comprises gastrins-71, -52, -34, -17, -14, and -6, all of which are carboxymidated and circulate in an O-sulfated and nonsulfated form. The dominant gastrin forms in serum from healthy humans are amidated gastrin-34 and -17 [for a review, see Ref. (3)]. In cases of increased gastrin synthesis, however, as seen in patients with hypo- and achlorhydric gastritis and gastrin-producing tumors, i.e., gastrinomas, the serum or plasma pattern molecular pattern changes to larger molecular forms and more incompletely processed precursors (4-6). Hence, only little gastrin-17 may be present in serum from hypergastrinemic patients, although gastrin-17 is the predominant tissue form in healthy antral mucosa.

In clinical chemistry, the only well-validated routine use of gastrin assays is in the diagnosis of gastrinomas, i.e., the Zollinger-Ellison syndrome (7, 8). Gastrin assays for diagnostic purposes (usually RIA or ELISA) should preferably measure all forms of gastrin. Although assays specific for only one molecular gastrin form may be of interest in biochemical research, such assays pose a significant diagnostic problem. In the following, we illustrate this problem with a newly launched commercial gastrin-17-specific ELISA.

Serum was obtained from six healthy individuals (three females and three males; median age, 26 years; range, 22-28 years) before and during food intake. Individuals were served a protein-rich meal, and blood samples were collected from the cubital vein before and at set time intervals after the meal. In addition, serum was obtained from seven patients with gastrinomas who had been admitted to the local department of gastrointestinal surgery (five females and two males; median age, 52 years; range, 37-57 years). The diagnosis was ascertained by increased serum concentrations of amidated gastrin, increased basal as well as pentagastrin-stimulated gastric acid secretion, gastroduodenoscopy, selective blood sampling from the portal vein (for gastrin measurements), abdominal computed tomography scanning, and somatostatin analog (ocreotide) scintigraphy. All patients later underwent surgery, with the tumor located and histologically verified in six patients. The remaining patient had the tumor identified at autopsy. All healthy individuals and patients gave informed consent for participation in the study, and the local ethics committee approved the study.

For measurement of amidated gastrin concentrations in serum, we used a RIA utilizing an antisera raised against the C-terminal tetrapeptide sequence common to all bioactive gastrins (9). This antisera detects nonsulfated and sulfated gastrin forms with an equimolar potency. N-Terminal gastrin-17 measurements were performed using an antisera raised against the cyclized NH2 terminus of gastrin-17 (10). This assay detects the N-terminal epitope on gastrin-17, where the N-terminal residue is pyroglutamic acid. This assay detects both gastrin-17 and its C-terminal extended precursors. Measurements with a gastrin-17-specific ELISA were performed as suggested by the manufacturer (Biohit Plc, Helsinki, Finland). Briefly, diluted serum was incubated in microplates with a gastrin-17-specific antibody absorbed to the wells. A second gastrin-17-specific antibody was then added, followed by biotinylated IgG, which bound to the second antibody. Avidin-labeled horseradish peroxidase was used for signal production, and after the enzymatic reaction was terminated, the intensity of color was determined by measuring the absorbance at 450 nm. The between-assay variation (at a gastrin-17 concentration of 30.2 pmol/L) was 7.9%, and the within-assay imprecision (gastrin-17 concentration of 19.6 pmol/L) was 4.7%.

The median amidated gastrin concentration in normal serum was 10.5 pmol/L (range, 9.0-18.0 pmol/L), whereas the gastrin-17-specific ELISA measured significantly lower concentrations [median, 4.0 pmol/L; range, 0.0-8.0 pmol/L; n = 6; P < 0.005]. After food intake, the amidated gastrin concentration increased to maximum concentrations 20 min after food intake with a threefold increase in gastrin concentrations (median, 30.0 pmol/L; range, 17.0-79.0 pmol/L). The gastrin-17-specific ELISA detected almost identical concentrations after stimulation (median, 30.0 pmol/L; range, 7.0-96.0 pmol/L). We then examined sera from gastrinoma patients. On average, the gastrin-17-specific ELISA detected only 10% of the amidated gastrin concentrations (Table 1). Consequently, in five of the seven gastrinoma patients, the gastrin-17-specific ELISA measured concentrations below the median postprandial concentration. The concentrations obtained with our N-terminal gastrin-17 immunoassay and the gastrin-17-specific ELISA were highly associated, whereas we found no association between amidated gas-

<table>
<thead>
<tr>
<th>Gastrinoma patient</th>
<th>Amidated gastrin, pmol/L</th>
<th>Gastrin-17 ELISA, pmol/L</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>475</td>
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</tr>
<tr>
<td>2</td>
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</tr>
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<td>6</td>
<td>60</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>308</td>
<td>6</td>
</tr>
<tr>
<td>Median (range)</td>
<td>235 (57-475)</td>
<td>24 (6-90)</td>
</tr>
</tbody>
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trin concentrations and the results obtained with the gastrin-17-specific ELISA (Fig. 1).

The present examination illustrates that a gastrin-17-specific ELISA measures very low concentrations of gastrin in serum from gastrinoma patients. In five of seven gastrinoma patients, the concentrations were not increased beyond physiologic concentrations after meal intake, rendering biochemical diagnosis of gastrinoma difficult. The ELISA-measured gastrin concentrations were low even in normal serum before food intake. However, this mostly reflects the well-established fact that circulating gastrins in healthy individuals comprise both gastrin-34 and -17. The gastrin response to food corroborates this finding because gastrin-17 constitutes a large fraction of the serum gastrins immediately after a meal (11).

Circulating gastrins in gastrinoma patients vary extensively, and the molecular heterogeneity is accordingly unpredictable (4, 12). Ideally, a diagnostic gastrin analysis should therefore quantify all secreted forms irrespective of the degree of posttranslational processing. In fact, such a method has been developed (10). Measurement of the biologically active, amidated gastrin forms, however, has been highly sensitive in the diagnosis of gastrinoma, possibly because the initial clinical suspicion of a gastrinoma is frequently raised by the symptoms caused by bioactive, amidated gastrin (i.e., hypersecretion of gastric acid and gastroduodenal ulcers, i.e., the Zollinger–Ellison syndrome). In sera from patients with gastrinomas, however, the molecular heterogeneity of gastrins shifts toward larger, less processed forms (4, 13). Accordingly, sensitive measurement of gastrin-17 concentrations in serum or plasma becomes an inferior marker of tumor synthesis of gastrin because of the frequent false-negative results. This analytical pitfall should be kept in mind when using a heterogeneous peptide hormone in serum as a tumor marker. Assay epitope specificity is crucial in gastrinoma diagnosis because the molecular heterogeneity of gastrins in serum alters in disease.

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References