Abstract
**Background and Aim:** In the gastric mucosa, pepsinogen II (PgII) is produced/secreted by glands in the mucus-secreting antral and cardia compartments, but also by the chief cells and the oxyntic glands. Increasing PgII serum levels are associated with the whole spectrum of gastric inflammatory diseases, including gastritis induced by *Helicobacter pylori* (*H. pylori*). This review critically addresses the clinical value of PgII serology for assessing gastric mucosal inflammation, and as a marker of *H. pylori* status, in both naïve *H. pylori*-positive patients and after eradication therapy.

**Results:** A search in PubMed/Scopus records yielded 39 out of 1,190 published scientific studies meeting the selection criteria for this study. In the studies considered, PgII levels were significantly associated with non-atrophic gastric inflammatory lesions (p-values: 0.025-0.0001). *H. pylori*-positive patients had significantly higher PgII levels than *H. pylori*-negative individuals (p-values: 0.05-0.0001). While a significant drop in serum PgII levels is consistently reported in *H. pylori*-eradicated patients (p-values: from 0.05 to 0.0001), inconsistencies in the related negative and positive predictive values significantly lower the clinical reliability of PgII testing by comparison with other available non-invasive tests.

**Conclusions:** PgII serology may provide clinically useful information on gastric inflammatory diseases, particularly if they are non-atrophic. PgII serology is inconsistent, however, for the purposes of distinguishing patients whose *H. pylori* eradication therapy is successful from those who remain infected.
INTRODUCTION

Acid secretion is the distinctive function of the gastric mucosa. Hydrochloric acid is secreted by parietal cells (also known as oxyntic cells) that are located mainly (95%) in the oxyntic mucosa (fundus and body regions), and partly in the mucus-secreting antral mucosa (50%)2-3.

Pepsinogens are aspartic proteinases synthesized/secreted by the gastric chief cells. They have a major role in the digestive process. Pepsinogen I (PgI; pepsinogen A) is only produced/secreted by the chief cells of the oxyntic glands. As both parietal cells and chief cells are located within the oxyntic glands, PgI is considered a reliable proxy of the stomach's acid-secreting capacity, and ultimately as a consistent surrogate marker of maximal acid output. Pepsinogen II (also known as progastricsin [PGC], or pepsinogen C) is produced/secreted by glands in the mucus-secreting antral and cardia compartments, but also by the chief cells mainly included in the oxyntic glands. Both pepsinogens are autocatalytically activated when the acidity level drops below pH 5, leading to the exposure of active site, and they are rapidly inactivated by the post-pyloric alkaline pH. While both pepsinogens are excreted mainly into the stomach lumen, very small amounts (about 1%) spread into the bloodstream, enabling their serological detection. A large body of literature has examined the reliability of serological assessments of PgI and PgII as a proxy of the gastric gland function, and on the clinical value of PgI, PgII and the PgI/II ratio as markers of gastric mucosa inflammation and atrophy (the latter consistently believed to be associated with gastric cancer risk).

This comprehensive review focuses on the clinical value of PgII as a serological marker of gastric inflammatory disease, and H. pylori gastritis, in particular. The following issues are critically addressed: (a) PgII serology in the setting of gastritis (be it atrophic or non-atrophic); (b) PgII and H. pylori status; and (c) PgII before and after successful or unsuccessful H. pylori eradication treatment.

MATERIALS & METHODS

A search for the available literature electively addressing PgII serology was conducted in the PubMed and Scopus databases up to December 31st 2020. The search included the following keywords: pepsinogen II, PgII, pepsinogen C, PgC, with “and” as a Boolean operator, in relation to atrophic gastritis, non-atrophic gastritis, gastritis activity, H. pylori status, H. pylori eradication, “and” PgII sensitivity, PgII specificity, and cut-off values. All scientific articles including the above-mentioned keywords were considered, whatever the method used to measure PgII levels (radioimmunoassay, ELISA or chemiluminescence). For each study, sensitivity and specificity values (with 95% confidence intervals [95% CI]) were both checked, together with positive and negative predictive values. Only scientific articles in English were considered. Studies lacking essential information that could not be obtained directly from the authors were also excluded.

RESULTS

After removing duplicates, 1,190 records were identified, among which there were 35 full-text articles that included the sensitivity, specificity and cut-off values for the relationship between PgII levels and: i) gastritis; ii) H. pylori status; and iii) H. pylori eradication. In all but three of the studies, PgII was tested together with PgI and/or in
conjunction with other serological variables (anti-\textit{Hp} antibodies, gastrin 17 [Gastropanel]) (Fig. 1).

**Fig. 1**

Flow chart of literature search and selection

- \textit{PgII} serology in patients with gastric inflammatory lesions (with or without mucosal atrophy)

Table 1 summarizes the results obtained by 9 studies in which \textit{PgII} levels were tested in different gastritis phenotypes (non-atrophic \textit{versus} atrophic), and compared with those of healthy controls. One further study only considered patients with atrophic gastritis (not those with non-atrophic gastritis or healthy controls).

### Table 1: Pepsinogen II (\textit{PgII}) in normal gastric mucosa, and in non-atrophic and atrophic gastritis phenotypes (\textdagger atrophic and non-atrophic gastritis collapsed together; \textdaggerdbl diagnosis established endoscopically; nr= not reported)

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Year</th>
<th>Patients (n.)</th>
<th>\textit{PgII} (micrograms/L)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal mucosa</td>
<td>Non-atrophic gastritis</td>
</tr>
<tr>
<td>Broutet \textsuperscript{13}</td>
<td>2003</td>
<td>222</td>
<td>8.7</td>
<td>12.5</td>
</tr>
<tr>
<td>Germanà \textsuperscript{11}</td>
<td>2005</td>
<td>287</td>
<td>9.0±8.0</td>
<td>15.7±10.4</td>
</tr>
<tr>
<td>Sun \textsuperscript{10}</td>
<td>2007</td>
<td>841</td>
<td>7.4</td>
<td>12.8</td>
</tr>
<tr>
<td>Haj-Sheykholeslami\textsuperscript{27}</td>
<td>2008</td>
<td>294</td>
<td>6.6±2.8</td>
<td>13.9±9</td>
</tr>
<tr>
<td>He\textsuperscript{8}</td>
<td>2011</td>
<td>1200</td>
<td>6.6</td>
<td>12.4</td>
</tr>
<tr>
<td>Syrianen \textsuperscript{63}</td>
<td>2017</td>
<td>107</td>
<td>9.5±4.6</td>
<td>17.3±10.1</td>
</tr>
<tr>
<td>Han\textsuperscript{84}</td>
<td>2018</td>
<td>107</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
In all cases, PgII levels were significantly higher in patients with non-atrophic gastritis (range: 12.4 to 17.3 micrograms/L) than in healthy controls (range: 5.8 to 12.7 micrograms/L). In three of the five studies comparing atrophic versus non-atrophic gastritis, higher PgII levels were associated with non-atrophic gastritis. In a large study by He and coworkers, the status of the gastric mucosa was only assessed endoscopically: patients with both non-atrophic and atrophic gastritis showed significantly higher PgII serum levels than controls. Taken together, the studies considered suggest that PgII is consistently associated with gastric inflammatory lesions (more than with atrophy). Such an association is in keeping with the results obtained by Kuipers et al., who found a significant direct association between the amount of inflammatory infiltrate (as assessed histologically) and PgII serum levels.

- **PgII serology by H. pylori status**

Table 2 shows the relationship between serum PgII levels and *H. pylori* status. Seventeen studies (4 from Japan; 2 from China; 1 from Korea; 5 from Europe; 1 from the USA; 1 from South America; 1 on populations of South and Southeast Asia; 1 from Iran; and one from India) tested the clinical usefulness of PgII as a marker of active *H. pylori* infection.

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Year</th>
<th>Patients (n.)</th>
<th>PgII in Hp-ve patients (micrograms/L)</th>
<th>PgII in Hp+ve patients (micrograms/L)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broutet 13</td>
<td>2003</td>
<td>266</td>
<td>8.45 ± 3.7</td>
<td>15.5 ± 5.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Di Mario 7</td>
<td>2004</td>
<td>313</td>
<td>8.6 ± 3.7</td>
<td>16.8 ± 7.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Germanà 11</td>
<td>2005</td>
<td>287</td>
<td>8.9 ± 7.9</td>
<td>15.9 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nurgalieva 24</td>
<td>2006</td>
<td>20</td>
<td>9.1 ± 8.5 ‡</td>
<td>42 ± 40 ‡</td>
<td>0.001</td>
</tr>
<tr>
<td>Kim 22</td>
<td>2007</td>
<td>1,485</td>
<td>8.0 ± 5.8</td>
<td>17.5 ± 10.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haji-Sheykholeslami 27</td>
<td>2008</td>
<td>481</td>
<td>9.8 ± 9</td>
<td>15.0 ± 9.9</td>
<td>nr</td>
</tr>
<tr>
<td>He 8</td>
<td>2011</td>
<td>2,022</td>
<td>6.6</td>
<td>14.0</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Huang 21</td>
<td>2016</td>
<td>2,814</td>
<td>11.50 ± 7.45</td>
<td>18.09 ± 8.68</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Kumar 28</td>
<td>2016</td>
<td>168</td>
<td>13.97 ± 8.14 †</td>
<td>18.78 ± 12.63 †</td>
<td>0.034</td>
</tr>
<tr>
<td>Osumi 17</td>
<td>2017</td>
<td>650</td>
<td>24.7 ± 12.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kawamura 18</td>
<td>2019</td>
<td>45</td>
<td>24.7 ± 13.1</td>
<td>16.3 ± 8.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Kikuchi 19</td>
<td>2019</td>
<td>6.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syrianen 23</td>
<td>2019</td>
<td>80</td>
<td>9.5 ± 4.6</td>
<td>17.3 ± 10.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fernandez Botran 25</td>
<td>2020</td>
<td>203</td>
<td>8.2</td>
<td>17.8</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
In all but one 29 of the studies, *H. pylori*-positive patients had significantly higher PgII levels (range: 6.6 to 13.9 micrograms/L in Hp-ve individuals versus 14.0 to 24.7 micrograms/L in Hp+ve cases). These features were basically consistent in various epidemiological contexts.

Focusing on the relationship between PgII and CagA status, in keeping with results previously obtained by Broutet et al13, Okuda et al recently confirmed that serum PgII levels were significantly higher in CagA-seropositive infection (PgII levels: 9.0 micrograms/L in CagA-negative individuals versus 15.3 micrograms/L in CagA-positive ones; p= 0.001). This difference retained a borderline significance even when cancer patients were considered25.

- **PgII serology in H. pylori eradication**

Table 3 summarizes the results of the studies addressing the changes (if any) in patients' serum PgII levels after they had been given successful versus unsuccessful *H. pylori* eradication therapy.

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Year</th>
<th>Patients (n.)</th>
<th>Serum PgII values (micrograms/L)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before eradication therapy</td>
<td>After eradication therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>successful</td>
<td>unsuccessful</td>
</tr>
<tr>
<td>Hunter 29</td>
<td>1993</td>
<td>nr</td>
<td>13.3±0.8</td>
<td>7.9±0.7</td>
</tr>
<tr>
<td>Tanaka 30</td>
<td>2004</td>
<td>9</td>
<td>21.9±3</td>
<td>10.3±2</td>
</tr>
<tr>
<td>Plebani 31</td>
<td>1992</td>
<td>49</td>
<td>Increased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Pilotto 32</td>
<td>1996</td>
<td>88</td>
<td>21.58±1.97</td>
<td>14.34 ± 1.75</td>
</tr>
<tr>
<td>Plebani 33</td>
<td>1996</td>
<td>192† 97‡</td>
<td>18±1</td>
<td>8±1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13±1</td>
<td></td>
</tr>
<tr>
<td>Di Mario 7</td>
<td>2004</td>
<td>70</td>
<td>17.5±7.5</td>
<td>8.2±2.0</td>
</tr>
<tr>
<td>Gatta 34</td>
<td>2011</td>
<td>228</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Osumi 17</td>
<td>2017</td>
<td>650</td>
<td>24.7±12.1</td>
<td>7.5±3.5</td>
</tr>
</tbody>
</table>

Serum PgII levels (tested between 1 and 2 months after completing the eradication therapy) dropped significantly only in patients whose treatment was successful, whereas the other patients’ PgII levels remained much the same as before. Notably, these results were consistent whatever the method used to measure PgII (radioimmunoassay or ELISA).

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Year</th>
<th>Patients (n.)</th>
<th>Serum PgII values (micrograms/L)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before eradication therapy</td>
<td>After eradication therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>successful</td>
<td>unsuccessful</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Cut-off values, sensitivity, and specificity for PgII in assessing the success of *H. pylori* eradication therapy († population over>65 years old; nr= not reported in the publication; ‡ according to Youden’s Index)
As for the PgII cut-off values for judging the success of *H. pylori* eradication therapy, most studies suggested a 23-25% decrease from a patient’s PgII levels before the treatment. In an adult population, He\(^8\) established a cut-off at 10.25 µg /L, significantly higher than the one proposed by Pilotto et al. in a geriatric population based on Youden’s index (5.1 µg /L)\(^32\). Depending on the cut-off adopted, the test’s specificity ranged between 62% and 96.6%. Two studies reporting the positive (PPV) and negative (NPV) predictive values for PgII levels as an indicator of *H. pylori* eradication generated inconsistent results (PPV = 98-67.6%; NPV = 71-74%) \(^7, 8\).

**DISCUSSION**

Based on what was known about functional gastric physiopathology, IM Samloff suggested in the early 1980s that serum pepsinogens (I and II) could be useful serological markers of various kinds of gastric mucosal damage \(^34\). At around the same time, Ichinose and coworkers proposed a radioimmunoassay method for measuring serum pepsinogens driven by the same biological rationale\(^35\). The promise of such non-invasive diagnostic procedures prompted a (possibly excessive) enthusiasm for the idea of a “serological biopsy”. \(^34\)

Over the years before *H. pylori* was discovered, PgII serology had been suggested as a non-invasive test potentially capable of providing clinically useful information on a variety of gastric diseases, including non-atrophic and atrophic gastritis, gastric polyps, and gastric cancer (GC). \(^10, 16, 28, 36, 37, 38, 39, 40, 41, 42\)

After the discovery of *H. pylori*, further studies proposed testing pepsinogens (PgI and PgII) to identify patients harboring (advanced) atrophic gastritis, which had been widely recognized as the *H. pylori*-triggered field of cancerization in the so-called “epidemic” gastric cancer. A functional test for exploring the “efficiency” of the gastric mucosa thus came to be inappropriately perceived as revealing a “cancer marker”.

Far more studies on the clinical impact of testing serum pepsinogens have focused on PgI (with 5,953 records, as opposed to 1,117 records for PgII), while PgII is largely tested “in support” of PgI values (as in the PgI/PgII ratio), as a marker of oxyntic atrophy \(^43-44, 45\). This review aims to restore serum PgII testing to its original (appropriate) clinical-biological role, examining both its clinical usefulness and its operative limitations.

In 2004, DY Graham \(^46\) unequivocally documented seroconversion (from anti-Hp-negative to anti-Hp-positive, with a prevalence of 94% in 4 weeks), and the onset of gastric mucosal inflammation in a model of acute *H. pylori* infection in a group of volunteers. A significant increase in serum levels of PgII (but not PgI) was also documented within the first two weeks of these volunteers becoming infected (PgII 9.1±8.5 µg /L at the baseline,
18±16 after 7 days [p=0.008], and 42±40 after 14 days [p=0.001]). These results are consistent with those obtained by Lorente and coworkers in an in vitro study showing that H. pylori (irrespective of the strain involved) promotes PgII secretion from human peptic cells via an intracellular pathway mediated by calcium and nitric oxide. 47

The present review likewise documented significantly higher serum PgII levels in gastritis patients who were infected with H. pylori (from 14.1 to 24.7 µg/L) than in those who were not (below 10 µg/L in all but three studies) 8,10,17,27,35,49. As expected (given the association between H. pylori infection and mucosal inflammation), serum PgII levels were always significantly higher in the patients carrying the infection, regardless of the laboratory method used (colorimetric, radioimmunoassay, ELISA, chemiluminescence). 10,36,37,49,50,51,52 The increase in PgII levels also correlated with the amounts of all the types of inflammatory cells involved in the histological phenotype of gastritis. 16

PgII has also been proposed as a marker of successful H. pylori eradication therapy ever since 1992 (years before more sensitive/specific tests become available) 31. Judging from the findings of the present review (Table 4), PgII serum levels drop significantly after H. pylori eradication therapy, but the reliability of serological testing as an indicator of successful eradication suffers from inconsistencies in its sensitivity and specificity. As a cut-off that can be safely assumed to indicate that eradication therapy has been successful, most studies suggest a 25% drop in PgII levels after the treatment 7,17,29. That said, the sensitivity and specificity values can vary considerably in adult populations (from 82% to 100%, and from 62% to 96.6%, respectively), reflecting the numerous situations that might potentially influence the results (Table 4). Much the same can be said of the results that emerged when PgII was tested in a selected population of geriatric patients. 32,53 The variability seen in how PgII levels respond to the eradication effort may plausibly have to do with the fact that the efficacy of the treatment in down-modulating or clearing the inflammatory lesions (and lowering PgII levels as a consequence) may not necessarily reflect a successful eradication of the infection 54,55,56. On the other hand, the successful eradication of H. pylori may not always be associated with patients’ inflammatory lesions disappearing (and their serum PgII levels dropping) 57,58,59,60,61.

Overall, the findings of the present review do not alter the skeptical conclusion reached by M. Tarek Al-Assi in 1999, who wrote: “Despite a significant fall in serum markers of H. pylori infection in the groups of successfully treated individuals, no marker (PgII among others) tested in this study approached the reported accuracy of the urea breath test” 62.

In conclusion, this review supports the reliability of PgII as a marker of gastric mucosal inflammation, particularly in non-atrophic gastritis caused by H. pylori infection. A drop in PgII levels of at least 25% (within 2 months after completing eradication therapy) supports a down-modulation of a patient’s inflammatory lesions. On the other hand, PgII is a considerably less reliable indicator of the success of H. pylori eradication therapy. Its clinical usefulness is further lessened by the availability of more sensitive and specific (and even less invasive) test methods. When coupled with Pgi levels (and the Pgi/PgII ratio, as in the ABC classification 44 and MAPS 45), then PgII levels are useful as a marker of both gastric inflammation and H. pylori infection.
Forse meglio togliere la frase o rimodularla
The value of PgII in association with PgI testing ... ma toglierei anche questo
REFERENCES


3) Engevik AC, Kaji I, Goldenring JR. The Physiology of the Gastric Parietal Cell Physiol Rev. 2020 Apr 1;100(2):573-602.


8) He CY, Sun PL, Gong YH, Xu Q, Dong NN, Yuan Y. Serum pepsinogen II: a neglected but useful biomarker to differentiate between diseased and normal stomachs. J Gastroenterol Hepatol 2011; 26: 1039–1046


15) Han YM, Chung SJ, Choi JM, Lee C, Kim JS. Long-term outcome of group D patients with negative serum anti-Helicobacter pylori antibody and positive serum pepsinogen test


28) Kumar S, Kumari N, Mittal RD, Ghoshal UC. Pepsinogen-II 100 bp ins/del gene polymorphism and its elevated circulating levels are associated with gastric cancer,


44) Pimentel-Nunes P, Libânio D, Marcos-Pinto R. et al. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019 Endoscopy 2019; 51(04): 365-388


52) Kadkhodaei S, Siavoshi F, Noghabi KA. Mucoid and coccoid Helicobacter pylori with fast growth and antibiotic resistance Helicobacter. 2020; 25(2):e12678


