Diagnostic test pepsinogen I and combination with tumor marker CEA in gastric cancer

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Abstract. Gastric cancer (GC) is the fifth leading cause of cancer and the third leading cause of cancer-related mortality globally. Human pepsinogens (HP) are considered promising serological biomarkers for the screening of atrophic gastritis (AG) and GC. HP are biochemically and immunochemically classified into two groups: pepsinogen I (PG I) and PG II. Carcinoembryonic antigen (CEA) is a glycoprotein, which is present in normal mucosal cells but increased amounts are associated with adenocarcinoma, especially colorectal cancer. CEA in combination with other tumour markers can be used in pre-operative staging and thereby assist in the planning of the type of surgery required and future management options. The purpose of this study was to diagnose test PG I and combination with tumor marker CEA in 32 patients suspected with GC. There was a significant difference in levels of CEA between GC group with non-GC with a value p <0.001. PGI sensitivity was 70.58% and specificity 93.3%. The sensitivity of PGI and CEA combination of 94.1% and specificity 80%. The area of AUC obtained was 92.7% at 95% confidence interval (82.7-100%). This AUC value indicated that the value of diagnostic accuracy of the PGI and CEA combinations of 92.7%.

1. Introduction
Gastric cancer (GC) is the fifth leading cause of cancer and the third leading cause of cancer-related mortality globally.[1] Globally, ischaemic heart disease, stroke, chronic obstructive lung disease, lower respiratory infections, trachea, bronchus, lung and GC have remained the top killers during the past decade. Chronic diseases cause increasing numbers of deaths worldwide.[2]
The clinical symptoms in the early stages of GC are not specific; therefore, a large number of patients with early GC do not seek appropriate medical care until the disease has progressed.[3] GC is one of the most frequent malignant tumors with high mortality due to the lack of convenient methods for early screening and diagnosis in clinical practice. Gastroscopy with biopsy is currently an efficient method for the diagnosis of GC, but it is not appropriate for screening GC because of its discomfort and high cost. The detection of serum tumor markers is simple in cancer screening and diagnosis, but it is difficult to be a conventional method for gastric cancer screening for its poor sensitivity and specificity, only valuable in the prognostic evaluation of patients with GC.[4] The development of tools for the early diagnosis of GC and precancerous lesions of GC is important for reducing mortality, increasing survival rates, and improving quality of life.[5] Endoscopy and biopsy are the reference standards for diagnosis and screening of GC and precancerous lesions of GC, but their use is limited for population-wide screening due to their invasiveness. Subsequently, it is necessary to identify novel, simple, cost-effective and manipulable screening methods for GC and precancerous lesions of GC.[6] Serum pepsinogen (SPG) test provides a valuable method for detecting GC.[7] Human pepsinogens (HP) are considered promising serological biomarkers for the screening of atrophic gastritis (AG) and GC. HP are proenzymes for pepsin, a digestive enzyme produced by gastric chief cells. HP are biochemically and immunochemically classified into two groups: pepsinogen I (PGI) and pepsinogen II (PGII).[8] PG originating from gastric mucosa can be classified into two immunochemically distinct groups: pepsinogen I (PG I) and PG II, which are mostly secreted into the gastric lumen and nearly 1% of them are leaked into the blood circulation. SPG levels reflect the morphological and functional status of gastric mucosa. SPG tests served as a useful marker for the prevalence of GC in a cross sectional setting.[9] SPG has been commonly accepted as a useful biomarker for GC screening and AG diagnosis.[10] Carcinoembryonic antigen (CEA) is a glycoprotein, which is present in normal mucosal cells but increased amounts are associated with adenocarcinoma, especially colorectal cancer. CEA therefore has a role as a tumour marker. CEA levels are useful in assessing prognosis (with other factors), detecting recurrence (especially for disease that cannot be evaluated by other means) and monitoring treatment in people with colorectal cancer. CEA is particularly recommended for postoperative follow-up of patients with stage II and III colorectal cancer if further surgery or chemotherapy is an option. CEA may be elevated in colorectal cancer, and it is most clinically useful.[11] CEA in combination with other tumour markers can be used in pre-operative staging and thereby assist in the planning of the type of surgery required and

Figure 1. Mortality and global health estimates [2].
future management options.[12] The purpose of this study was to diagnose test PG I and combination with tumor marker CEA in patients with GC.

2. Materials and Methods
This study was a cross-sectional study design with an observational analytic type on eighty consecutive suggestive gastric cancer patients that were admitted to General Hospital Haji Adam Malik Medan. Suspected cases of GC based on the criteria of NCNN 2013. Inclusion criteria were patients with suggestive GC that comes to the surgical and internal medicine departments, no surgery has been performed, have no received chemotherapy, willing to do biopsy procedure and willing to take part in the research. Exclusion criteria were patients with malignancy in other organs. Biopsy results, serum CEA levels and serum PGI levels were recorded. This study was approved by local ethics committee.

2.1. Criteria NSNN 2013
NSNN criteria for gastric cancer are weight loss for no reason, nausea and vomitus, epigastric pain, dysphagia, burning sensation when eating, melena and hematemesis, lost of appetite, pale and mass in the abdominal.

2.2. Definition of GC
Diagnosis of GC on based on the results of histopathology examination of the current sample from endoscopy which is adjusted by Laurent criteria or WHO criteria.

2.3. Measurement of PGI
Serum PGI examination was performed by using immunoluminometric assay.

2.4. Measurement of CEA
Serum CEA examination was performed by using immunoluminometric assay CMIA Metode with Architect Plus C4100.

2.5. Statistical Methods
Data analysis was performed through bivariate and multivariate analyses using the SPSS 22nd version (SPSS Inc., Chicago) with a 95% confidence interval. Bivariate analysis was performed using Mann-Whitney U test with significance p<0.05. The cut off value of PGI and CEA is determined by the Receiver Operating Characteristics (ROC) curve. The value of diagnostic accuracy described by value of AUC. In the preliminary data there were 39 patients, which then were found 7 patients did not meet the Criteria NSNN 2013. Our study were conducted with 32 patients from December 2016 until May 2017 (Table 1).

| Age (year) | Ca (positive) | Non Ca (negative) | Total |
|------------|---------------|-------------------|-------|
| ≤ 50 years | 6 (18.8%)     | 2 (6.3%)          | 8 (25%) |
| > 50 years | 11 (34.4%)    | 13 (40.6%)        | 24 (75%) |
| Total      | 17 (53.1%)    | 15 (46.9%)        | 32 (100%) |

All patients with GC, most commonly encountered with low PGI levels (≤ 41.48 ng/mL) (Table 2) were 12 out of 17 them (70.58%), whereas in non-GC they have lower levels of PGI levels were found only 1 of 15 subjects (6.66%). Associations between clinicopathologic factors and peritoneal disease were examined with chi square test.
Table 2. Distribution of patients based on PGI dan CEA.

| Level CEA | Level PGI ≤ 41.48 ng/mL (Positive) | >41.48 ng/mL (Negative) | Total |
|-----------|-------------------------------------|-------------------------|-------|
| ≥ 3.28 ng/mL (Positive) | 12 (37.5%) | 7 (21.87%) | 19 (59.37%) |
| <3.28 ng/mL (Negative) | 1 (3.13%) | 12 (37.5%) | 13 (40.63%) |
| Total | 13 (40.63%) | 19 (59.37%) | 32 (100%) |

3. Results and Discussion

Based on the ROC curve (Figure 2) obtained the sensitivity of PGI and CEA combination of 94.1% and specificity 80%. The area of AUC obtained is 92.7% at 95% confidence interval (82.7-100%). This AUC value indicated the value of diagnostic accuracy of the PGI and CEA combinations of 92.7%.

![ROC Curve](image)

Our findings is supported by F Feng L Sun Z Liu S Liu G Zhen G Xu M Guo X Lian D Fan H Zhang [13], they reported that CEA levels are associated with tumor depth, TNM staging and metastasis to the liver in patients with GC. When tested chi square test, the results showed that there was a significant difference from PGI levels between GC group with non-GC with p value <0.001. Patients with the highest GC were elevated CEA (≥ 3.28 ng/mL) of 16 out of 17 subjects (94.11%), whereas in non-GC were elevated CEA levels were found in only 3 of 15 subjects (20%). Further tested chi square test, the also results showed that there was a significant difference in levels of CEA between GC group with non-GC with a value p <0.001. PGI sensitivity was 70.58% and specificity 93.3%. The area of AUC indicated by the AUC obtained 81.6% in confidence interval of 95% (66.4-96.7%). This AUC value indicated the value of diagnostic accuracy of PGI that was equal to 81.6%. CEA sensitivity of 94.11% and specificity 80%. The total area proved by the AUC obtained was 89.8% at the 95% confidence interval (78.4-1.012%). This AUC value showed that the diagnostic accuracy value of CEA of 89.8%. The sensitivity of PGI and CEA combination is 94.1% and specificity 80%. The area of AUC obtained was 92.7% at 95% confidence interval (82.7-100%). This
AUC value indicated that the value of diagnostic accuracy of the PGI and CEA combinations of 92.7%. This findings suggested that the use of a combination of PGI and CEA examinations increases diagnostic accuracy by 2.9% which indicated that PGI and CEA examinations have been singularly shown to be good enough test results with nearly the same level of diagnostic ability. Y Liang W Wang C Fang SS Raj W-M Hu Q-W L Z-W Zhou [14] obtained a combination of several tumor markers (CEA, CA19-9, and CA72-4) in diagnosing gastric carcinoma increased the sensitivity of the examination, while the diagnostic accuracy did not improve at all. However, J Yu S Zhang and B Zhao [15] received positive rates for the combination of tumor markers (CEA, CA19-9, and CA72-4) increased significantly from individual examinations (44.91% vs. 22.69, 18.98, and 22.69% with p <0.005). PGI alone is often combined with antibodies to H. pylori to determine the risk of GC. There has been reported that in individual marker tests higher risk was observed in subjects with positive PGI levels (HR 3.2, CI: 95%, 2.7-4.7) and positive H. pylori antibodies (HR 3.2, CI: 95%; 2.0 - 5.2), but when combined, the highest risk in patients with positive PGA levels with negative H. pylori antibodies (HR 2.43, CI: 95%; 1.86 - 3.12).[16] These results confirmed why early detection of GC is still a matter that needs to be further investigated more intensively in order to prevent delayed diagnosis in patients.

4. Conclusions
Increased diagnostic accuracy values only 2.9% which indicated that PGI and CEA examinations alone have been good enough to diagnose GC.

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