

Variation in Chromogranin A Assays in Gastric Carcinoid TYPE 1

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ABSTRACT

AIM: Chromogranin A (CgA) is used in the diagnosis and follow-up of patients with neuroendocrine tumours. Clinical interpretation of CgA results may be affected by the heterogeneity between available assays. There is debate over the accuracy of CgA assays in gastric carcinoid type 1 (GC1). Herein, we compare two different CgA assays, the commercial CgA assay, DAKO (DAKO, Denmark A/S, Glostrup, Denmark) and the Imperial Supra-regional Assay Service radioimmunoassay (SAS Hammersmith Hospital, Imperial College, London) to determine their accuracy in the diagnosis of GC1.

METHODS: Patients diagnosed with GC1 and available plasma CgA measurements according to two different assays (SAS and DAKO) were retrospectively reviewed. CgA values were ranked in 4 groups: (1) normal values; (2) increase < 2 Upper Limit of Normal (ULN); (3) increase between 2-5 ULN; (4) increase > 5 ULN.

RESULTS: 26 patients (17 female, 9 male), mean age 55

years ± 11.75, were identified. At diagnosis, median CgA-DAKO were significantly higher than median CgA-SAS (81, normal range < 27 IU/L versus 34.5 pmol/L, normal range < 60 pmol/L, $T=35.5$, $p < 0.001$). When ranking the data, the results confirmed that median CgA-DAKO was significantly more accurate than median CgA-SAS: 3 versus 1, $T=0$, $p < 0.001$. Sensitivity was 77% and 7.7% for CgA-DAKO and CgA-SAS, respectively. Pearson correlation between gastrin levels and both CgA-DAKO and CgA-SAS were non-significant [$r(26) = 0.08$ and $r(26) = 0.2$, $p > 0.05$ for CgA-DAKO and CgA-SAS, respectively].

CONCLUSIONS: CgA-DAKO shows a better sensitivity than CgA-SAS for the diagnosis of GC1. Accurate diagnostic biomarkers may identify those patients who may benefit from a closer endoscopic follow-up in cases of raised neuroendocrine markers. Further prospective studies are needed highlighting the difference in diagnostic sensitivity between assays.

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Key words: Chromogranin A; Gastric carcinoid type 1; Accuracy; Sensitivity

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INTRODUCTION

Gastric carcinoids (GCs) are neoplasms which derive from the enterochromaffin-like cell (ECL) of the gastric oxyntic mucosa and show an annual incidence of 0.59-0.6275/100,000 inhabitants^[1,2], accounting for 8.7% of all gastrointestinal neuroendocrine tumours (NETs)^[1,3]. Their reported frequency has increased over the last 50 years, mainly due to improved diagnostic techniques and better awareness of the disease^[1].

GCs include three main types characterized by different clinico-

pathological features and prognosis^[4]: GC type 1 (GC1) associated with chronic atrophic gastritis and hypergastrinemia, GC type 2 associated with hypergastrinemia due to gastrinoma and sporadic tumours (GC type 3), which represent 15% of GC, are not related to hypergastrinemia, and are characterized by a more aggressive course, presenting with lymph node and distant metastases in more than 50% of cases^[5]. GC type 1 (GC1) are the most common (65-75%) gastric NETs and are mainly (65-75%) found in women^[6]. They can be multiple and are considered to be indolent in nature with 5-year survival rate not different from an age-matched normal population^[7]. However, a subset of GC1, less than 5%, may develop advanced disease, with lymph node and/or hepatic metastases^[8] and only four cases of tumour-related death have been reported in literature so far^[9-12].

Often GC1 do not show any specific signs and/or symptoms and are incidentally diagnosed during routine esophago-gastro-duodenoscopy^[13] with diagnosis confirmed by immuno-histochemical staining.

With regards biomarkers, Chromogranin A (CgA) is a 49 kDa acidic glycoprotein, which represents one of the most abundant components of secretory granules in neuroendocrine cells, and it is secreted from multiple tumours sharing neuroendocrine differentiation. CgA is considered as the best and most sensitive general marker for the diagnosis and follow-up of (NETs), with a sensitivity of 96% and 75% in functioning and non-functioning NETs, respectively, and a specificity of 68-100%^[14]. Circulating CgA levels are a sensitive marker also for GC^[15-22] and recent studies indicate that circulating CgA levels correlate positively with ECL cell mass in patients with autoimmune chronic atrophic gastritis^[18,22]. CgA level is increased in 80-90% of patients with GC1^[11,23], but increased CgA levels may result from various conditions (i.e. proton pump inhibitor therapy, chronic atrophic gastritis type A, ECL-cell hyperplasia, renal insufficiency, untreated arterial hypertension, liver disease or inflammatory bowel disease) as well as different NETs, which may decrease its specificity (up to 68%) for GC. CgA might be more useful during surveillance and to monitor therapy response as, according to available data, CgA levels tend to decrease significantly after both medical and surgical therapy of GC^[24,25].

Clinical interpretation of CgA results may be affected by the heterogeneity between commonly available CgA assays. The diagnostic accuracy of an assay depends upon antibody specificity and the cleaved peptide forms it recognizes. As NETs might release different molecular forms of CgA, an assay is considered to have better diagnostic accuracy if it can recognize more forms^[26,27].

There are a number of different CgA assays. The aim of our study is to compare two different CgA assays, the commercial CgA assay, DAKO (DAKO, Denmark A/S, Glostrup, Denmark) and the Imperial Supra-regional Assay Service radioimmunoassay (SAS Hammersmith Hospital, Imperial College, London) to determine their accuracy in the diagnosis of GC1. The two assays use different antisera. The SAS assay is a competitive radioimmunoassay utilizing polyclonal anti-sera raised against the whole pancreastatin molecule, which is a 52-amino acid (CgA 250-301) fragment produced by dibasic cleavage of the 439 amino acid CgA peptide^[28]. DAKO is an ELISA using two polyclonal antibodies directed against a 23 kD C terminal fragment of CgA^[29].

METHODS

Patients with a confirmed diagnosis of GC1, based on immuno-histochemistry, and available plasma (CgA) measurements according

to two different assays (Imperial SAS and DAKO) were identified from a database at the Neuroendocrine Tumour Unit, Royal Free Hospital, London, United Kingdom and retrospectively reviewed.

Clinical data

Clinical data including age, gender, the tumour grade according to WHO 2010^[30], presence of metastases, somatostatin analogue therapy (SST), presence of symptoms (i.e. dyspepsia and/or features of carcinoid syndrome), presence of concomitant vitamin B12 deficiency, positivity of anti-parietal cell antibodies (APCA) were collected for all the patients. Confounding factors which could determine a raise of CgA levels independently on the presence of GC1 [i.e. ongoing therapy with proton pump inhibitors (PPI), diagnosis of arterial hypertension, renal failure, severe liver impairment and/or *Helicobacter Pylori* infection] have been recorded for each participant.

Biochemical data

All the patients had available plasma CgA measurements according to two different assays [Imperial Supra-regional Assay Service radioimmunoassay (SAS Hammersmith Hospital, Imperial College, London) and the commercial CgA assay, DAKO (DAKO, Denmark A/S, Glostrup, Denmark)] at the time of diagnosis of GC1: blood samples of the 26 patients included were taken on the same day of the gastroscopy. Samples were processed and analysed following manufacturer's recommendations for each kit^[26,28,29]. The diagnostic cut-off values for CgA recommended by the manufacturers were <60 pmol/L and <27 UI/L for CgA-SAS and CgA-DAKO respectively. CgA values were ranked in 4 groups: (1) normal values; (2) increase <2 Upper Limit of Normal (ULN); (3) increase between 2-5 ULN; and (4) increase >5 ULN.

Gastrin levels were also recorded for all the GC1 patients and ranked in 4 groups as above.

Statistical analyses

SPSS version 21 software for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Both CgA DAKO and Hammersmith were not normally distributed, therefore non-parametric tests were used to evaluate the significance of these variables. In particular, Wilcoxon Rank-Sum test was used.

RESULTS

A total of 26 patients, 17 female and 9 male, mean age 55 years old±11.75, with a confirmed diagnosis of GC1 and available CgA measurements at the time of diagnosis according to both assays (DAKO and Hammersmith) were identified. Patients' epidemiological and clinical characteristics are shown in table 1.

Review of histopathology samples showed that all patients had well differentiated gastric neuroendocrine neoplasms with linear and micronodular ECL-cell hyperplasia on a background of gastric atrophy with immunohistochemical expression of chromogranin except for two patients in whom histopathology review was unavailable.

None of the patients on this study did have surgery, as the GC1 are usually treated as benign conditions and the polyps are not removed but on active surveillance. Only three patients were started on SST because of GC recurrence in two patients and presence of lymph node metastases in the other one.

At diagnosis, median CgA-DAKO values were significantly higher than median CgA-SAS (81, normal range<27 IU/L versus 34.5 pmol/

Table 1 Patients' epidemiological and clinical characteristics.	
Variable	26 GC1 patients
Gender	
Male	9 (34.6%)
Female	17 (65.4%)
Histological grade	
Low	23 (88.5%)
Intermediate	3 (11.5%)
High	0
Symptoms	
Asymptomatic	13 (50%)
Dyspepsia	13 (50%)
Carcinoid syndrome	0
Vitamin B12 deficiency	14 (53.8%)
APCA	
Positive	16 (61.5%)
Negative	5 (19.2%)
Missing data	5 (19.2%)
Presence of metastases	1 (3.8%)
SST therapy	3 (11.5%)
Ongoing PPI therapy	6 (23.1%)
Presence of arterial hypertension	9 (34.6%)
Presence of renal insufficiency	0
Concomitant H. Pylori infection	0
Severe liver failure	0

L, normal range <60 pmol/L) T=35.5, $p < 0.001$, Gender, histological grade, ongoing PPI therapy or concomitant diagnosis of arterial hypertension were not found to be confounding factor.

When ranking the data, the results confirmed median CgA-DAKO significantly more accurate than median CgA-SAS: 3 versus 1, T=0, $p < 0.001$ (Figure 1). 6 patients (23%) showed CgA-DAKO values within normal limits, whereas 20 patients (77%) had increased values [5 (19%) <2 ULN, 8 (31%) between 2-5 ULN, 7 (27%) >5 ULN]. With regards ranked CgA-SAS, 24 patients (92.3%) showed values within normal limits, whereas only 2 patients (7.7%) had increased CgA values (1 <2 ULN and 1 between 2-5 ULN) (Figure 2). This resulted in a suboptimal sensitivity for CgA-SAS of 7.7%, with 2/26 GC1 patients who resulted as true positive and 24 out of 26 patients who came back as false negative. When analysing CgA-DAKO, we found 20 out of 26 true positive and 6 out of 26 false negative with an overall sensitivity of 77%.

Mean gastrin levels were 350.8 ± 236.6 pmol/L (normal range <40 pmol/L). When combining CgA-DAKO and gastrin measurements, overall sensitivity increased up to 88%.

Pearson correlation between gastrin levels and both CgA-DAKO and CgA-SAS were non-significant. Correlation DAKO $r(26) = 0.08$, $p > 0.05$ and Cg Hammersmith $r(26) = 0.2$, $p > 0.05$.

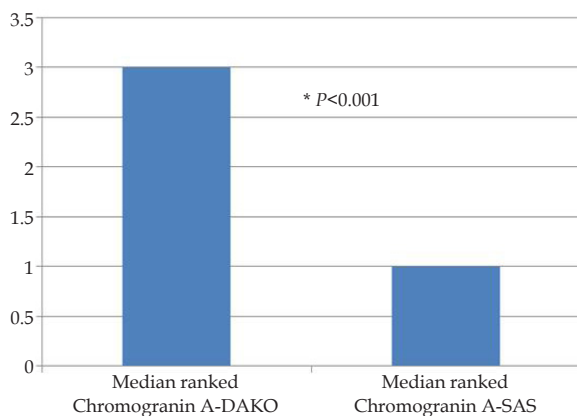


Figure 1 Median ranked Chromogranin A-DAKO versus median ranked Chromogranin A-Imperial Supra-regional Assay Service radioimmunoassay (SAS Hammersmith Hospital, Imperial College, London).

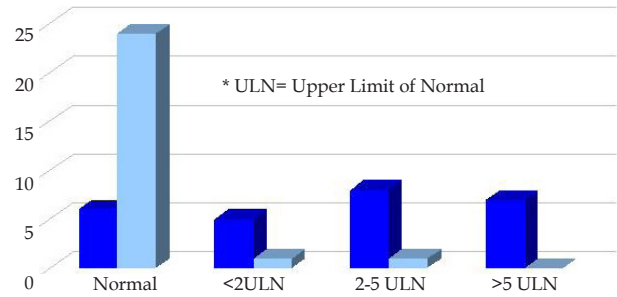


Figure 2 Frequencies of patients at different ranked Chromogranin A DAKO and Imperial Supra-regional Assay Service (SAS) assays.

DISCUSSION

This study shows that median CgA-DAKO levels are significantly higher than CgA-SAS at diagnosis of GC1 (with a median value raised 3 fold the ULN) and show a better sensitivity. CgA-SAS has a poor sensitivity for the diagnosis of GC1 leading to false negative results in more than 92% of patients with GC1. To our knowledge, this is the first study which compares two different CgA assays in the specific setting of GC1.

The possible explanation of these results may depend on the different nature of the two assays and the fact that they usually recognize different epitopes. It has been suggested that the assays which use antibodies raised against pancreastatin including CgA-SAS have a higher specificity for NETs with liver metastases^[31], although these results have not been confirmed in the recent study by Ramachandran *et al*^[27]. However, our results might reflect the finding of Ardill^[31] as none of our GC1 patients had liver metastases.

Gastrin is a hormone secreted by G cells, that are located in the gastric antrum and stimulate the parietal cells to produce hydrochloric acid. Hypergastrinemia can occur in patients who have a gastrinoma or in response to achlorhydria, typically in patients with atrophic gastritis or in the case of long-term PPI therapy^[32]. Hypergastrinemia is the cause of ECL hyperplasia and the spectrum of ECL hyperplasia include three different degrees of hyperplasia (simple, linear and micronodular), dysplasia, and GC1^[33,34]. When combining CgA-DAKO with gastrin we found increased sensitivity for the diagnosis of GC1, which is in keeping with the current knowledge that gastrin levels are always elevated in GC1^[13]. Moreover, current European Neuroendocrine Tumor Society guidelines suggest to test both CgA and gastrin at GCI diagnosis^[35]. Our study suggests that the use of gastrin and CgA-DAKO as diagnostic markers in GC1 is superior to CgA-SAS.

As GC1 although indolent can show a slowly progressive process in regard of polyp growth as well as a recurring disease after polypectomy, and also an increased risk of gastric cancer, endoscopic follow-up should be performed every 12 to 24 months^[13]. The availability of accurate diagnostic biomarkers may allow the identification of those patients who may benefit from a closer endoscopic follow-up with accurate biopsy samples in cases of raised NET markers.

One of the strength of our study is the fact that concomitant confounding factors (mainly PPI and arterial hypertension), which might increase CgA levels independently on the presence of GC1, have not affected the results. Moreover, given the lack of data on sensitivity of different CgA assays in the setting of GC1, our results might suggest that CgA-DAKO assay. May have utility in the follow up of patients with chronic atrophic gastritis as it is more sensitive for the detection of GC1.

Limitations of our study include the small sample size and the lack

of a control group which does not allow us to calculate the specificity of the two different assays analyzed. In summary, our study shows that CgA-DAKO has a significantly higher sensitivity for the diagnosis of GC1, whereas the use of CgA-SAS in the follow up of patients with chronic atrophic gastritis might underestimate GC1 in more than 92% of cases. However, further prospective studies with larger sample size and follow up data as well as the inclusion of a control group, are needed to draw more solid conclusions.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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