

# The Role of Serum Chromogranin A in Diarrhoea Predominant Irritable Bowel Syndrome

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## Abstract

**Background & Aims:** Elevated serum chromogranin A (CgA) levels have been reported co-incidentally in a small group of irritable bowel syndrome (IBS) patients (n=19). Our aim was to ascertain the prevalence of elevated CgA in diarrhoea predominant Rome II IBS (D-IBS) patients and investigate if this could be a marker for octreotide therapy. **Methods:** Patients with Rome II D-IBS were recruited prospectively and investigated as per British Society Guidelines including serial CgA levels (u/l). Patients with refractory symptoms and elevated CgA were considered for further investigation +/- octreotide therapy. **Results:** 219 patients were recruited (68% females, mean age 45 years). 81% (n=177) of IBS patients had normal CgA levels (0-20u/l). Whilst 12.3% (n=27) had values between 20-60u/l, 6.8% (n=15) had CgA levels >60u/l; 96% (26/27) with initial CgA level of 20-60u/l had repeated CgA levels which normalised. One patient (3.7%) had a gastric adenocarcinoma. In the 15 patients with elevated CgA levels >60u/l, 8 normalised on repeated testing. In the other 7, there were no cases of carcinoid, n=1 gastric leiomyoma, n=1 rectal tumour and 4 patients had persistently elevated CgA levels but with improvement of symptoms. In one patient, octreotide was commenced which resulted in normalisation of CgA and symptoms. **Conclusion:** CgA levels appear to be transiently elevated in D-IBS. Future work assessing CgA in patients with refractory D-IBS may potentially identify individuals who will benefit from octreotide therapy.

## Keywords

Diarrhoea predominant – irritable bowel syndrome  
– serum chromogranin A

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## Introduction

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal (GI) tract that affects 10-15% of the western population and has a female predominance [1, 2]. Patients with IBS present with symptoms of abdominal pain or discomfort associated with an altered bowel habit [2, 3]. There has been progress in the understanding and treatment of patients with IBS including the development of symptom based criteria such as the Manning and Rome criteria and the expansion of treatment strategies [3, 4]. Despite this, IBS symptoms may have a considerable impact on patients' quality of life and productivity [5].

Serum chromogranin A (CgA) is a 68kDa protein of 439 amino-acid residues encoded on chromosome 14 [6]. It is produced by a variety of normal and malignant neuroendocrine and sympathetic neuronal cells. Elevated serum concentrations of chromogranin A are seen in most neuroendocrine tumours (most notably pheochromocytoma and carcinoid). Chromogranin A is a well-known protein constituent in granules of neuroendocrine cells and levels increase dramatically in patients with neuroendocrine tumours [7]. Serum concentrations show a strong correlation with tumour mass within the individual patient, and thus can be used for monitoring the response to treatment [8]. Other causes of raised CgA include small cell lung cancer, prostate, breast and colon cancer. Chromogranin A may be released with increased sympathoadrenal drive and this can be seen in conjunction with increased catecholamine production. For this reason patients with essential hypertension, heart failure or liver failure have also been reported to have elevated levels of CgA [9]. Finally, poor renal clearance and proton pump inhibitor (PPI) therapy may both falsely elevate CgA levels [6].

The relationship between CgA and the GI tract is still not clearly delineated. CgA is now considered to be the most sensitive biochemical marker for carcinoid – this is irrespective of the location in the GI tract. In patients with gastrinoma (the Zollinger-Ellison syndrome) elevated CgA levels appear to reflect the associated gastrin-mediated enterochromaffin-like cell hyperplasia, rather than the actual

size of the tumour [10]. Interestingly, enterochromaffin cell hyperplasia has also been demonstrated on rectal biopsy in patients with post-infective IBS (stool culture positive for *Campylobacter*) [11]. Enterochromaffin cells are a plausible mediator of diarrhoea predominant symptoms as they are known to play a pivotal part in the control of gut motility and secretions [12]. We wondered if this is the biological plausibility for elevated serum chromogranin A levels in patients with IBS?

Ocreotide has already been shown to be beneficial in patients with refractory post-chemotherapy diarrhoea and AIDS related diarrhoea (randomised controlled studies). Case series have suggested a possible benefit in graft versus host disease, post-gastrectomy dumping related diarrhoea, short gut syndrome and functional diarrhoea. Ocreotide's effects of reducing GI motility, intestinal fluid and electrolyte transport make it a hypothetically viable option in the treatment of diarrhoea predominant IBS [13].

Our aim was to ascertain the prevalence of elevated CgA in diarrhoea predominant Rome II IBS (D-IBS) patients. As a secondary goal, we also investigated if persistently elevated CgA levels could be used as a marker for refractory IBS patients that could potentially benefit from octreotide therapy.

## Methods

### Patients

Patients that fulfilled the Rome II criteria for D-IBS (Appendix 1) were prospectively recruited from the outpatient setting. Ethical approval was obtained from the North Sheffield Ethics Committee. Information on patient demographics and past medical history including prescribed medication were noted. Patients with IBS were investigated as per British Society of Gastroenterology (BSG) guidelines [3]. These included blood tests: full blood count, electrolytes, inflammatory markers, thyroid function tests, calcium, antibodies for coeliac disease and either a rigid or flexible sigmoidoscopy. Depending on the clinician, some patients also had additional tests such as a colonoscopy, gastroscopy and duodenal biopsies, SeCHAT scan to exclude bile salt malabsorption, lactose hydrogen breath tests to exclude lactose malabsorption and glucose hydrogen breath tests to exclude bacterial overgrowth. In addition, serial CgA levels were also measured.

### Measurement of serum chromogranin A (CgA)

Serial CgA levels were measured using a competitive radioimmunoassay with the use of purified full-length human chromogranin A [14, 15]. Plasma CgA immunoreactivity is known to be remarkably stable in vitro, readily surviving prolonged heating at 37 degrees C, as well as repeated freezing and thawing [15]. The normal adult concentration is between 2-20u/L. This is an arbitrary level suggested by the manufacturers [15]. There is no 'healthy' control comparison in the published literature. We chose the level of >60u/l as significant because in a random population sample you may expect 5% of individuals to have an elevated CgA level – this

assumption is based on the range usually being given as 2 standard deviations from the mean. This could suggest that patients with levels greater than 60 have a significant and unexplained level of serum CgA.

Patients who were found to have elevated serum CgA of >60u/L on repeated testing were investigated for the presence of other pathology including a neuroendocrine tumour. Investigations which were directed by patients' symptoms included endoscopy, radiology, fasting gut hormones, urinary 5-hydroxyindole acetic acid, meta iodo Benzyl Guanidine (mIBG) and octreotide scans.

### Intervention

All patients recruited with D-IBS were treated according to BSG guidelines on the management of IBS. Patients with refractory symptoms and elevated CgA levels were considered for octreotide therapy. The data was analysed using SPSS version 15. A non parametric test was used to compare mean CgA levels between sexes.

## Results

A total of 219 patients were recruited over a two year period (October 2004 to October 2006). There were 150 females (68.5%) with a mean age of 45 years (range 17-88 years). The median duration of symptoms in the D-IBS patients recruited was 18 months (range 8-30 months). CgA levels were followed up for a median duration of 7 months (range 5-16 months). Eighty one percent of IBS patients (n=177) had normal CgA levels (0-20u/l). Whilst 12.3% (n=27) had values between 20-60u/l, 6.8% (n=15) had CgA levels >60u/l. Figure 1 shows a scatter plot of the distribution of CgA levels in patients with levels greater than 60u/l,  $p=0.457$ .

There was no significant association between gender and CgA values,  $p=0.46$ ; median CgA levels for females was 11.5u/l (IQ 8.25) and for males was 10.5u/l (IQ 7.5) respectively. However the correlation between age and elevated CgA was poor,  $r=0.2$  ( $p=0.9$ ).

Ninety six percent (26/27) of patients with initial CgA level of 20-60u/l had repeated CgA levels which returned to normal with resolution of symptoms on standard therapy. One patient (3.7%) developed symptoms of abdominal pain and weight loss, and gastroscopy revealed a gastric adenocarcinoma.

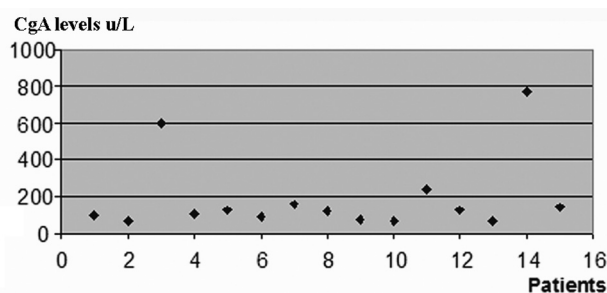
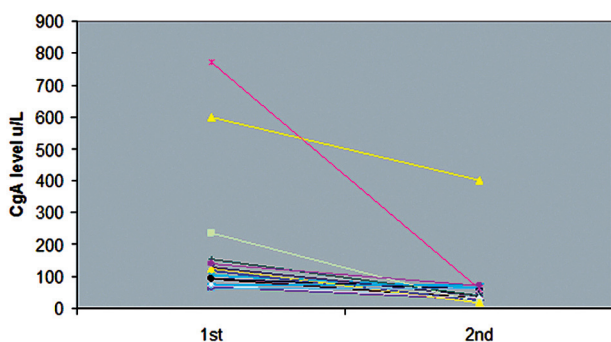


Fig 1. Scatter plot of patients with CgA levels > 60u/l (n=15)



**Fig 2.** First and second CgA levels in patients with values > 60 u/L

Figure 2 shows the trend of CgA levels in patients with initial values >60u/L. In 8 of these 15 patients the CgA levels normalised over time. In the other 7 patients, CgA remained elevated >60u/l on repeated testing. These 7 patients accounted for 3.2% of the whole group (7/219). These patients underwent further investigation including endoscopy, radiology, fasting gut hormones, urinary 5-hydroxyindole acetic acid, mIBG and octreotide scans to exclude other pathology. There were no cases of carcinoid, however, a gastric leiomyoma (n=1) and a rectal adenocarcinoma (n=1) were found in two patients in this group (patients aged 87 and 77, respectively). In 4 patients CgA levels remained elevated between 60-80u/l but with improvement/resolution of symptoms on standard treatment (for example, antispasmodics/ anti-diarrhoeals). In one patient (of this group of 7), octreotide was commenced for persistence of IBS symptoms. The initiation of octreotide has resulted in normalisation of CgA and resolution of bowel habit/symptoms in this patient. The response has been sustained over the 20 months of follow up. Table I tabulates the categories of patients with CgA levels and the outcome.

**Table I.** Categories of patients with associated serum chromogranin A values

CgA categories	CgA <20u/L	CgA 20-60u/L	CgA >60u/L
Number of patients	n=177	n=27	n=15
Mean age	41 years	54 years	61 years
Outcome	-	96% of patients (n=26/27) CgA values normalised	CgA values normalised in 8, remained >60u/l in 4 patients but with improvement of symptoms on standard therapy. 1 patient commenced on octreotide with resolution of symptoms.
Other pathology found	-	Gastric adenocarcinoma (n=1)	Gastric leiomyoma (n=1) Rectal adenocarcinoma (n=1)

**Discussion**

This is the first study to demonstrate in a large cohort that

elevated CgA levels occur in patients who fulfil the Rome II criteria for D-IBS. These results support the findings of Militello et al [16] who assessed the positive and negative predictive value of using CgA to recognise neuroendocrine tumours and made a comparison against neuron-specific enolase (n=55) [16]. The authors used 19 patients affected by irritable bowel (not categorised according to accepted IBS criteria) as controls. Militello et al reported a coincidental observation that CgA may be raised in this sub-group of patients with IBS, which is in keeping with our findings [16]. However, there was no delineation of the type of IBS based on the Rome criteria in their study. In addition, their IBS group only consisted of 19 patients [16]. In our study of 219 patients with D-IBS, significantly elevated CgA levels were found in 6.8% (n=15).

Dunlop et al found increased enterochromaffin cells in patients with post infectious IBS compared to asymptomatic subjects. The observation that elevated enterochromaffin cell counts persist several months after gastrointestinal infection may have a role in the pathogenesis of causing new bowel symptoms [11]. It could be postulated that patients with D-IBS have enterochromaffin hyperplasia which results in their elevated CgA levels. However, the reason behind the elevated CgA in only a proportion of patients with D-IBS remains unclear. One plausible explanation could be the differential replication of the enterochromaffin cells in these patients. The process of cell differentiation through the Notch signalling pathways may explain the range of CgA levels that we observed [17]. Enterochromaffin cell counts have also been known to decrease in time [11], that may be an explanation of the decline in subsequent measurement of CgA in our patients.

A novel and unexpected observation from our study was that persistently elevated CgA levels could also signify the presence of alternative pathology, particularly in older patients. A gastric leiomyoma and rectal adenocarcinoma were diagnosed in patients with persistently elevated CgA levels. Previous investigators have described the presence of CgA immunoreactivity in colorectal cancer cells. The suggestion in such patients is that the presence of an elevated level of serum CgA may indicate a degree of neuroendocrine differentiation within the tumour. This may predict a lack of response or poor prognosis [18].

Finally, an elevated CgA in D-IBS patients in the absence of other pathology could be a marker for response to octreotide therapy. However in our study, as only one such patient qualified for octreotide, no firm recommendations can be made based on our results. We believe that further studies are required to validate our findings and perhaps specifically concentrating on serial CgA measurements in patients with persistent/refractory IBS symptoms.

**Conclusion**

Chromogranin A levels appear to be transiently elevated in diarrhoea predominant IBS. Future work assessing CgA in patients with refractory diarrhoea predominant IBS

may potentially identify individuals who will benefit from octreotide therapy.

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Funding obtained from Novartis Educational Research Fund for IBS. RS wrote the initial draft, all five authors were involved in the subsequent revisions and final draft. David S Sanders, Consultant Gastroenterologist & Honorary Reader University of Sheffield is the guarantor for this manuscript.

### Conflicts of interest

No conflict of interest.

### Appendix 1

Rome II diagnostic criteria\* for irritable bowel syndrome

Recurrent abdominal pain or discomfort\*\* at least 3 days a month in the past 3 months

associated with two or more of the following:

Improvement with defecation

Onset associated with a change in frequency of stool

Onset associated with a change in form (appearance) of stool

\* Criteria fulfilled for the past 3 months with symptom onset at least 6 months before diagnosis.

\*\*Discomfort means an uncomfortable sensation not described as pain.

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