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Calcitonin levels in autoimmune atrophic gastritis-related hypergastrinemia

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Abstract

Purpose Calcitonin (Ct) is currently the most sensitive biochemical marker of C-cell disease (medullary thyroid cancer [MTC] and C-cell hyperplasia), but its specificity is relatively low. Our aim was to examine whether autoimmune atrophic gastritis (AAG) and chronic hypergastrinemia, with or without chronic autoimmune thyroiditis (AT), are conditions associated with increased Ct levels.

Methods Three groups of patients were consecutively enrolled in this multicentric study: group A consisted of patients with histologically-proven AAG (n = 13; 2 males, 11 females); group B fulfilled the criteria for group A but also had AT (n = 92; 15 males, 77 females); and group C included patients with AT and without AAG (n = 37; 6 males, 31 females).

Results Median Ct levels did not differ between the three groups. Ct levels were undetectable in: 8/13 cases (61.5%) in group A, 70/92 (76.1%) in group B, and 27/37 (73.0%) in group C. They were detectable but ≤ 10 ng/L in 4/13 (30.8%), 20/92 (21.7%) and 7/37 (18.9%) cases, respectively; and they were > 10 ng/L in 1/13 (7.7%), 2/92 (2.2%) and 3/37 (8.1%) cases, respectively (P = 0.5). Only three patients had high Ct levels (> 10 ng/L) and high gastrin levels and had an MTC. There was no correlation between Ct and gastrin levels (P = 0.353, r = 0.0785).

Conclusions High gastrin levels in patients with AAG do not explain any hypercalcitoninemia, regardless of whether patients have AT or not. This makes it mandatory to complete the diagnostic process to rule out MTC in patients with high Ct levels and AAG.

Keywords Calcitonin · Hypergastrinemia · Autoimmune atrophic gastritis · Gastrin

Introduction

Calcitonin (Ct) is currently the most sensitive biochemical marker of C-cell disease (MTC and C-cell hyperplasia) [1], but its positive predictive value (PPV) is limited (around 8%

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at a threshold of 10 ng/L) due to the low prevalence of MTC (0.23% of thyroid nodules). When interpreting a mild rise in Ct levels, it is essential to exclude immunoassay interferences and secondary hypercalcitoninemia [2]. Several pathological and physiological conditions have been associated with a rise in Ct levels, including smoking, medications, and non-thyroid disorders [3]. The association between hypercalcitoninemia and thyroid diseases, such as autoimmune thyroiditis (AT) and differentiated thyroid carcinoma, has been quite extensively contested in several recent studies [4–6], and the relationship between hypercalcitoninemia and chronic thyroid disease is still dubious. It has been demonstrated that the human thyroid releases Ct in response to acute systemic gastrin administration both in MTC patients and, to a lesser degree, in healthy individuals [7]. Gastrin is fundamental in regulating gastric acid secretion. Gastrin is released by G-cells in the pyloric antral mucosa in the form of a 101-amino acid precursor (preprogastrin), then processed to become biologically-active amidated gastrin-17 and gastrin-34 [8]. The pentagastrin (Pg) stimulation test was commonly used in the past to distinguish MTC from secondary hypercalcitoninemia because Pg can mimic gastrin and stimulate Ct production, acting on the cholecystikinin (CCK)-B receptors (CCK-B)/gastrin receptor, expressed on the surface of MTC (even in its early stages), and on hyperplastic C-cells [11]. However, while gastrin and its synthetic analogue Pg affect Ct levels when injected at pharmacological doses, lower doses or physiological stimulation (i.e., a protein test meal) are not equally stimulating [9]. Higher than normal fasting gastrin serum concentrations are found in various clinical conditions, including autoimmune atrophic gastritis (AAG), a chronic disease involving destruction of the parietal cells in the corpus-fundus of the stomach. This leads to hypochlorhydria, followed by an increased gastrin output from G-cells in the antrum, which in turn promotes enterochromaffin-like (ECL) hyperplasia, possibly progressing to neoplastic disease, and type 1 neuroendocrine tumors (NETs) [10, 11]. AAG is characterized by the presence of two types of auto-antibody, anti-parietal cell antibodies (APCA) and anti-intrinsic factor antibodies, and by a strong association with other autoimmune disorders, one the most frequent of which is AT [12]. AAG is diagnosed on the strength of a combination of APCA positivity and histological criteria [11, 13, 14]. The chronic use of proton pump inhibitors (PPI) like omeprazole is also a known cause of secondary hypercalcitoninemia, although the mechanism is gastrin-mediated and not fully understood. Albeit with some contradictions and uncertainty, chronic hypergastrinemia is commonly assumed to cause secondary hypercalcitoninemia, not only in the context of PPI treatment [15, 16], but also in relation to AAG [17–22]. This assumption is based on old studies involving radioimmunoassay (RIA) platforms for measuring Ct levels [23–25].

Now, in the light of more thorough analyses, the literature on Ct response to gastrin in cases of AAG is particularly conflicting [9, 25–28]

The aim of the present study was to measure Ct levels in a large, multicentric series of patients with hypergastrinemia in the context of histologically-proven AAG, with or without chronic AT. A recently-introduced two-site/two-step chemiluminescent immunometric assay (CLIA) was used for this purpose.

Patients and methods

Patients

From January 2020 to March 2022, patients consecutively seen at any of the centers involved (i.e. Endocrinology and Gastroenterology Units at Padua University Hospital [Institution 1], the Endocrinology and Diabetology clinics at Verona Hospital [Institution 2], and the Endocrinology clinic at Trieste Hospital [Institution 3]) were considered, and those eligible for one of the following groups were enrolled. Group A included patients with a history of a histologically-confirmed diagnosis of AAG. Group B consisted of patients meeting the criteria for group A who also had a history of AT. At least two of the following three criteria had to be met to confirm cases of AT: positivity to anti-thyroid peroxidase (AbTPO) or anti-thyroglobulin antibodies (AbTG); ultrasound (US) findings consistent with chronic thyroiditis; and subclinical or clinical hypothyroidism. Patients enrolled in group C had to have a history of AT but no AAG. The following exclusion criteria were also considered: (i) refusal to give informed consent to the study; (ii) age < 18 years; (iii) kidney failure, hyperparathyroidism or lung cancer; (iiii) beta-blocker and/or glucocorticoid use. The study was performed in accordance with the guidelines of the Helsinki Declaration and all participants gave their written informed consent. The study was approved by the Local Ethical Committee at Padua Hospital (the institution proposing and leading the present study)—*Comitato Etico per la Sperimentazione Clinica* (CESC) approval code: 3312/AO/14 prot. 0034435.

To help assign patients to the appropriate groups, all those with AAG underwent TSH, AbTPO and AbTG measurements, and thyroid US. On the other hand, patients with a diagnosis of AT were tested for APCA, and those found positive underwent esophagogastroduodenoscopy (EGD) with histological examination of biopsy material, and chromogranin A (CgA) testing to exclude any concomitant autoimmune gastritis.

Gastrin and serum Ct levels were assayed in all patients. Differential diagnostics were performed to rule out MTC in those whose Ct levels exceeded the reference range. In the

event of MTC being strongly suspected, patients underwent surgery in accordance with the latest ATA guidelines.

Serum analyses

Serum Ct levels were measured using the same kit in the chemiluminescence analyzer (CLIA LIAISON CT II-Gen, DiaSorin Inc., Stillwater, MN, USA) at all participating centers. The method has an analytical sensitivity of ≤ 1 ng/L, a functional sensitivity of ≤ 3 ng/L, and an upper reference limit of > 10 ng/L. Gastrin measurements were performed at Institution 1 on all serum samples collected at the three participating centers, using RIA (DIAsource ImmunoAssay S.A., Louvain-la-Neuve, Belgium) with a limit of detection (LoD) of 8.06 pmol/L, a limit of quantitation (LoQ) of 11.95 pmol/L, and reference values between 11 and 54 pmol/L. The assays used for the other measurements, distinguished for Institutions, are provided in supplemental materials.

Histological diagnosis of autoimmune gastritis

AAG was diagnosed histologically on gastric biopsy samples obtained during the EGD. According to internationally accepted criteria, for each biopsy sampling site, gastritis was distinguished as non-atrophic or atrophic (loss of glands appropriate for the site being sampled). Based on the topographical location (antrum or body) of histological changes, the extent of atrophic gastritis was staged according to the Operative Link on Gastritis Assessment (OLGA) system. Each biopsy sample from the gastric antrum and body was scored from 0 to 3, depending on the degree of atrophy of the gland (no atrophy = 0, mild atrophy = 1, moderate atrophy = 2, severe atrophy = 3). The OLGA gastritis stage was then obtained by combining the scores for antrum and body, as explained in detail elsewhere [13, 14].

Statistical analysis

The Kolmogorov–Smirnov test was used to test the normal distribution of the variables. A p value of < 0.05 was considered statistically significant. Serum Ct, gastrin and CgA levels were considered in both quantitative and categorized terms. Serum Ct levels were categorized as: undetectable (< 1 ng/L); 1–10 ng/L; or > 10 ng/L. Gastrin levels were dichotomized as: ≤ 54 pmol/L or > 54 pmol/L. CgA levels were dichotomized as ≤ 100 or > 100 ng/mL. Gastrin-17 levels were dichotomized as ≤ 7.6 or > 7.6 pmol/L. Continuous Variables that were not normally distributed were analyzed with the Mann–Whitney or Kruskal tests, as appropriate. Categorical variables were analyzed with the χ^2 test. Correlations were investigated using Spearman's rank test. All statistical analyses were run with MedCalc

version 19.2.1 (MedCalc Software Ltd, Ostend, Belgium; <https://www.medcalc.org>; 2020).

Results

Patients

Patients' characteristics are summarized in Table 1. Among AAG patients, the median duration of disease (from the diagnosis to the latest follow-up) was 6 years (interquartile range, IQR: 4–10 years). OLGA staging was available for 80 patients, and was: stage 0 in one case (1.2%); stage I in 8 (10.0%); stage II in 55 (68.8%); stage III in 14 (17.5%); and stage IV in two (2.5%). APCA status was available for 136/142 (95.8%) study participants. APCA positivity emerged for 8/10 (80%) patients tested in group A, 85/89 (95.5%) tested in group B, and 9/37 (24.3%) tested in group C. Patients found APCA-positive in group C underwent gastrin and CgA measurements: as both were within the normal ranges, and these patients had no other symptoms of concern, no EGD was performed, but only APCA and gastrin follow-up. Twelve of the 105 patients (11.4%) with AAG had been diagnosed with gastric NETs (active or in follow-up): 3/12 (25.0%) in group A, and 9/12 (75.0%) in group B (Table 2). Six (5.7%) of the 105 patients had active NET at the time of their gastrin, CgA and Ct measurements. Gastrin levels were higher than normal in all 12 patients with gastric NETs. CgA levels were available for 11/12 (91.6%) patients, and found elevated in 10/11 (90.9%). Among AT patients, the median duration of disease (from the diagnosis to the latest follow-up) was 7.5 years (IQR: 3–13.25 years). Among the 92/142 study participants with available data, 41 (44.6%) tested positive for TgAb: 24 in group B and 17 in group C. TPOAb status was available for 103/142 study participants and 83 (80.6%) of them tested positive: 54 in group B and 29 in group C. US revealed a pattern consistent with autoimmune thyroid disease in all cases of AT.

Gastrin and CgA levels

Median gastrin levels were 378.50 pmol/L (IQR: 43.24–657.89 pmol/L) in the study population as a whole. They were lower in group C than in group A or B ($P < 0.01$): 584.20 pmol/L (457.25–715.54 pmol/L) in group A; 540.00 pmol/L (IQR: 243.27–736.35 pmol/L) in group B; and 22.00 pmol/L (IQR: 16.75–30.55 pmol/L) in group C (Fig. 1A). No differences in gastrin level were observed among PPI user and not users. Median gastrin levels stratified for OLGA stages are provided in Fig. 2A. There was a trend towards higher gastrin levels in patients with OLGA stages II and III, compared with stage I, but the difference was not statistically significant in either case ($P = 0.11$ and

Table 1 Patients' characteristics

Factor	Cat	Tot	Group			P value
			A (AAG)	B (AAG + AT)	C (AT)	
n		142	13	92	37	
Gender (column %)	F	119	11 (84.6)	77 (83.7)	31 (83.8)	0.996
Age (years), median (IQR)		57 (49–65)	65 (55–71)	59 (50–66)	52 (45–61)	0.008*
Smoker (column %)**	No	76	7 (100.0)	58 (98.3)	11 (84.6)	0.056
	Yes	3	0 (0.0)	1 (1.7)	2 (15.4)	
PPI user (column %)**	No	73	6 (85.7)	54 (91.5)	13 (100.0)	0.342
	Yes	5	1 (14.3)	4 (8.5%)	0 (0.0)	
Gastrin (pmol/L), median levels (IQR)		378.50 (43.24–657.89)	584.20 (457.25–715.54)	540.00 (243.27–736.35)	22.00 (16.75–30.55)	<0.01*
Gastrin > 54 pmol/L (%)	Yes		13/13 (100)	86/92 (93.5)	0/37 (0)	<0.01
Gastrin-17 (pmol/L), median levels (IQR)		40.00 (40–82.35)	66.90 (40.00–199.58)	40.00 (40.00–79.23)	4.00 (4.00–4.00)	0.09
Gas-trin-17 > 7.6 pmol/L (%)	Yes		11/11 (100%)	51/53 (96.2%)	–	0.3
CaA (ng/ml), median values (IQR)		144.7 (87.6–247.0)	142.2 (83.4–241.2)	157.5 (96.2–262.0)	46.6 (27.1–54.6)	0.005*
CaA > 100 ng/ml (%)	Yes		9/13 (69.2%)	46/63 (73.0%)	0/5 (0)	0.03*
Ct (ng/L), median values (IQR)		1.0 (1.0–1.26)	1.0 (1.0–1.67)	1.0 (1.0–1.01)	1.0 (1.0–4.00)	0.6
Ct, categorized						
	Ct < 1 ng/L		8/13 (61.5%)	70/92 (76.1%)	27/37 (73.0%)	0.5
	Ct ≥ 1 and ≤ 10		4/13 (30.8%)	20/92 (21.7%)	7/37 (18.9%)	
	Ct > 10		1/13 (7.7%)	2/92 (2.2%)	3/37 (8.1%)	

AAG autoimmune atrophic gastritis, AT autoimmune thyroiditis, Cat categorization, CgA chromogranin A, Ct calcitonin, F females, IQR interquartile range, M males, PPI proton pump inhibitors

*The difference was significant in the comparison between groups C and A, and between groups C and B, but not between groups B and A

**Data were not available for all cases. Reference ranges: calcitonin: upper reference limit > 10 ng/L; chromogranin A: upper reference limit > 100 ng/mL, gastrin: 11–54 pmol/L

0.29, respectively). Data on Gastrin-17 and CgA are provided in Table 1.

Calcitonin values

Median Ct levels were superimposable among different groups (Table 1 and Fig. 1B). Median Ct levels were identical and within the normal range (median value: 1.0 ng/L; IQR: 1.0–1.0 ng/L) in patients testing positive or negative for AbTPO or AbTG. Thyroid nodules were found in 33/77 (42.9%) patients who underwent US. Median Ct levels were identical and within the normal range (1.0 ng/L; IQR: 1.0–1.0 ng/L) in patients with and without thyroid nodules. Among patients with high gastrin levels (> 54 pmol/L), Ct levels were undetectable in 72/99 (72.7%) cases, detectable but within the normal range (≤ 10 ng/L) in 24/99 (24.2%),

and high (> 10 ng/L) in 3/99 (3.0%). Spearman's rank test revealed no correlation between Ct and gastrin levels ($P=0.353$, $r=0.0785$). Median Ct levels were the same (1 ng/L), and within the normal range for patients with high or normal gastrin-17 levels ($P=0.8$). Among the 65 patients with high gastrin-17 levels, 47/65 (72.3%) had undetectable Ct levels, and the other 18 (27.7%) had Ct levels between 1 and 10 ng/L (none had Ct levels > 10 ng/L). No correlation was found between Ct and gastrin-17 levels using Spearman's rank test ($r=0.126$, $P=0.317$). No differences in Ct levels emerged by OLGA staging of AAG (Fig. 2B). Among patients with NETs, Ct levels were undetectable in 8/12 (66.6%) cases, and detectable but ≤ 10 ng/L in the other 4/12 (33.4%); no patients with NETs had Ct levels > 10 ng/L. No correlation was found between Ct and CgA levels ($r=0.065$, $P=0.564$).

Table 2 Details of patients with gastric neuroendocrine tumors (NETs)

Patient ID No	Group	Gastric NET	Gastrin (pmol/L)	Chromogranin A (ng/ml)	Calcitonin (ng/L)
8	A	Previous	609.38	180.8	1.4
10	A	Active	666.39	658.0	1.0
13	A	Previous	891.59	297.7	1.7
14	B	Active	568.53	147.8	4.6
17	B	Previous	664.00	135.6	1.0
21	B	Previous	1611.00	381.0	1.0
23	B	Active	1900.00	223.4	3.3
24	B	Previous	1065.00	127.3	1.0
47	B	Active	388.00	84.5	1.0
51	B	Active	1413.00	420.0	1.0
53	B	Active	1282.00	n.a	1.0
68	B	Previous	159.38	69.8	1.0

n.a. not available, *NET* neuroendocrine tumor. Reference ranges: calcitonin: upper reference limit > 10 ng/L; chromogranin A: upper reference limit > 100 ng/mL, gastrin: 11–54 pmol/L

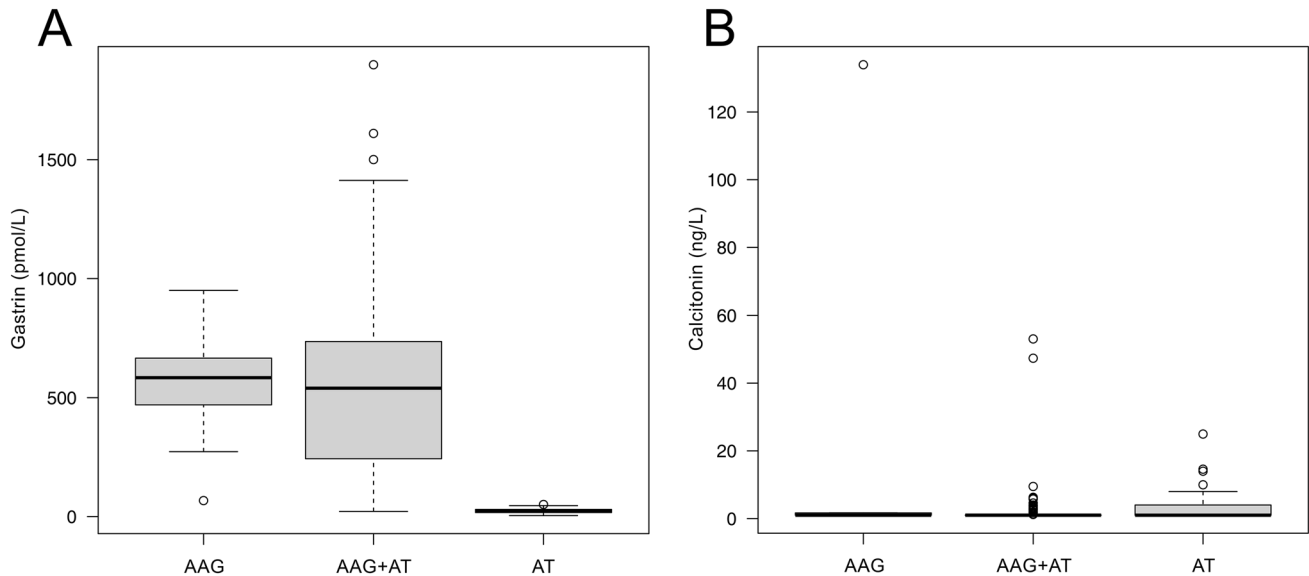


Fig. 1 Median gastrin (1A) and calcitonin (Ct) (2A) levels in the three groups of patients. Median gastrin levels were: 584.20 pmol/L (interquartile range, IQR: 457.25–715.54 pmol/L) for autoimmune atrophic gastritis (AAG) group, 540.00 pmol/L (243.27–736.35 pmol/L) for AAG+autoimmune thyroiditis (AT) group and 22.00 pmol/L (16.75–30.55 pmol/L) for AT group ($P < 0.01$). The

difference was significant in the comparison between groups AT and AAG, and between groups AT and AAG+AT, but not between groups AAG+AT and AAG. Median Ct levels were 1.0 ng/L (IQR: 1.0–1.67 ng/L) for AAG group, 1.0 ng/L (1.0–1.01 ng/L) for AAG+AT group and 1.0 ng/L (1.0–4.00 ng/L) for AT group ($P = 0.6$)

Only three patients had high Ct levels (> 10 ng/L) and high gastrin levels: they had nodular thyroid disease and calcium stimulation test or Ct measurement from fine needle aspiration (FNA) washout fluid consistent with MTC, a diagnosis confirmed on histological examination (Table 3). Another three patients, all females, had high Ct levels with normal gastrin levels (Ct levels of 14 ng/L, 25 ng/L and 14.6 ng/L): two had a calcium stimulation test (one peaking at 77 ng/L and the other at 106 ng/L). All had a

non-suspicious thyroid nodular disease and are under active biochemical and US surveillance.

Discussion

The aim of our study was to examine Ct behavior in the presence of chronic hypergastrinemia due to AAG, with or without AT. We only enrolled patients with longstanding,

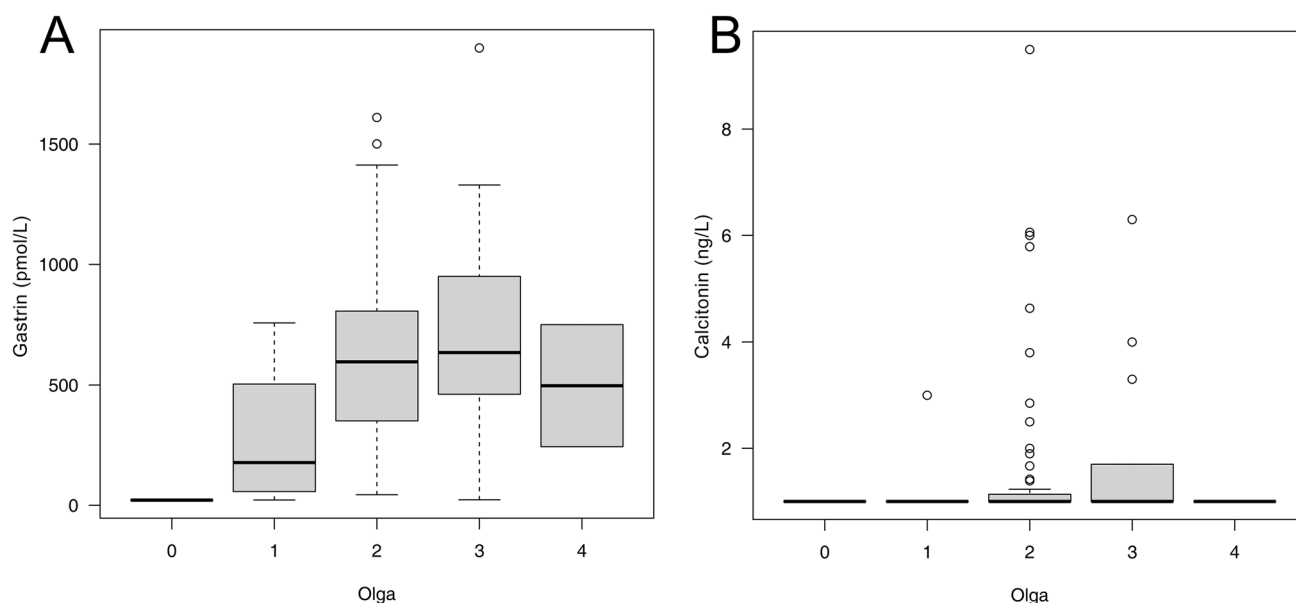


Fig. 2 Median gastrin (2A) and calcitonin (Ct) (2A) levels by Operative Link on Gastritis Assessment (OLGA) score. Median gastrin levels were: 177.34 pmol/L for stage I (IQR: 438.03–500.50 pmol/L); 596.25 pmol/L (IQR: 351.00–805.67 pmol/L) for stage II; 634.78 pmol/L (IQR: 463.89–928.25 pmol/L) for stage III; and

497.14 pmol/L (IQR: 370.84–623.94 pmol/L) for stage IV (median gastrin levels were obviously not measurable for the one patient with OLGA stage 0 disease). Median Ct values: 1 ng/ml in all OLGA groups. The range of Ct levels was as follows: 1–3 ng/L for stage I, 1–9.5 ng/L for stage II, 1–6.3 ng/L for stage III, 1–1 for stage IV

histologically-proven AAG. This is one of the strengths of our study as it rules out the possibility of higher Ct levels being the result of an acute rise in gastrin levels.

Our results show that chronically high gastrin levels do not cause a rise in Ct levels in patients with AAG, with or without chronic thyroiditis. Using a CLIA to measure Ct, we found no correlation between Ct and gastrin levels, confirming the results obtained by other authors using other methods [9, 24, 25]. Nor did we find higher Ct levels associated with the chronically high gastrin levels of patients with long-term AAG disease, but this is in apparent contrast with prior reports. For instance, Erdogan et al. found Ct levels higher (though still within the normal range of their laboratory method) in patients with AAG or hypergastrinemia compared with healthy controls; but they found no correlation between Ct and gastrin levels. These apparently conflicting results could stem partly from different assays being used to measure Ct (an RIA platform in the previous study, as opposed to CLIA in ours). Given the acknowledged importance of the assay kit used to measure serum Ct [2], the findings of these two studies are probably not comparable. Another possible reason for the discrepancies between different reports could derive from clinical differences in hypergastrinemic patients: the previous study also included users of PPI and histamine-2 receptor blockers (H2RB), whereas we only considered patients with longstanding AAG. Acute *versus* chronic elevations in gastrin levels have different effects on Ct levels, so the inclusion of patients

with a relatively recent PPI and H2RB users could explain the difference between their previous study findings and ours. Becker et al. and Franchimont and Heyen also found Ct levels higher in many of their AAG patients (55% and 25%, respectively) [23, 25], albeit without any correlation between Ct and gastrin levels in either series. Here again, both studies used RIA to measure Ct, which is less specific than the modern non-competitive, two-site and two-step CLIA and immunoradiometric assays (IRMAs), as they also recognize Ct-like proteins and its precursors as well as Ct [2]. That said, other studies employing RIA in the past produced results in line with ours. Fahrenkrug et al. measured Ct concentrations in 13 AAG patients, all with hypergastrinemia, and in age- and sex-matched healthy controls, finding no difference between the two groups, and Ct levels were within the normal range in both [27]. Brandborg et al. likewise found no differences in serum Ct between 38 AAG patients and 32 healthy controls [26]. Another possible explanation for the diverse results in the literature concerning Ct levels in cases of high gastrin levels could lie in differences in the patterns of gastrin components. Gastrin circulates not as a single peptide, but as a combination of peptides of various lengths [29]. Only gastrin-17 and gastrin-34 are biologically active on acid secretion, however, and presumably on Ct secretion too [30]. In the event of an abnormally increased synthesis (which occurs chronically in AAG patients), G-cells release large amounts of incompletely-processed progastrin products because the processing enzymes cannot keep up with

Table 3 Details of patients with increased serum levels of calcitonin and total gastrin

Patient ID No	Basal calcitonin (ng/L)	Stimulated calcitonin peak on calcium test (ng/L)	Gastrin (pmol/L)	US pattern, FNAC	<i>RET</i> gene mutation	Type of surgery	Histological examination
7	134.0	175.0 (5')	419.0	Right nodule, 10 mm, hypo-echoic with hyperechoic spots, uneven, peri- and intranodular vascularization, increased consistency on elastosonography FNAC = TIR4* (suspected malignancy), calcitonin from eluate > 2000 ng/L	No germline mutations	TT, prophylactic CC lymphadenectomy	(1) MTC, with amyloid stroma, 12 mm, right lobe 5/20 lymph nodes with metastasis T1bN1a (stage III for M0) (2) C-PTC, 1 mm, right lobe (3) FV-PTC, 8 mm, left lobe
27	47.4	235.0 (2')	500.0	Right nodule, 19 mm, isoechoic, homogeneous, defined margins	No germline mutations	TT, prophylactic CC lymphadenectomy	MTC, with amyloid stroma, 10 mm, right lobe T1aN0 (stage I for M0) 0/24 lymph nodes with metastasis
30	53.1	538.0 (2')	362.0	Left nodule, 12 mm, hypo-echoic with hyperechoic spots, uneven, peri- and intra-nodular vascularization, increased consistency on elastosonography 1st FNAC 2017 = TIR3A* (undetermined significant low risk), 2nd and 3rd FNAC = TIR2* (benign)	No germline mutations	TT, prophylactic CC lymphadenectomy	(1) MTC, with CCH, 8 mm, left lobe T1aN0 (stage I for M0) (2) PTC, 1 mm, isthmus 0/25 lymph nodes with metastasis

* = According to the 2014 Italian classification of thyroid cytology. Reference ranges: calcitonin: upper reference limit > 10 ng/L; gastrin: 11–54 pmol/L

CC central compartment, CCH C-cell hyperplasia, C-PTC classic papillary thyroid carcinoma, FNAC fine needle aspiration cytology, FV-PTC follicular variant papillary thyroid carcinoma, MTC medullary thyroid carcinoma, TT total thyroidectomy, US ultrasound

progastrin maturation. RIA kits generally measure total gastrin, however (as was the case in our study), and a different prevalence of the two biologically active isoforms in the total gastrin count – depending partly on the cause and duration of the hypergastrinemia – might affect the Ct response. On

the other hand, no correlation emerged between gastrin-17 and Ct in our sample. We also checked whether OLGA stage (an indirect parameter of G-cell activity and atrophy) was associated with Ct levels. We found a trend towards a wider range of Ct levels for patients with OLGA stages II and III,

but they were always within the normal range, and the differences with respect to OLGA stages I and IV were not statistically significant. We concluded that the trend identified probably related to the greater numerosity of patients with OLGA stages II and III in our sample.

We considered the presence or absence of AT in our patients because previously-published data suggested that the condition could cause a spurious rise in Ct levels related to C-cell hyperplasia, which is common in AT patients [31–33]. The C-cell hyperplasia would lead to hypercalcitoninemia in some cases [33], with a reportedly greater frequency of higher than normal Ct levels among patients with chronic thyroiditis [31]. Given the frequent association between AAG and AT, it was important to exclude the latter as possible confounding factor in our analysis. A previous study found that AAG patients with AT had higher Ct levels than those with AAG but no AT [24]. Conversely, our AAG patients with AT did not have higher Ct levels than those without AT. Our results are in line with recent reports of no difference in Ct levels in the presence or absence of AT [4] and/or thyroid autoantibodies [6]. On the other hand, Ct deficiency has sometimes been described in AT patients [34, 35]. It is reportedly more pronounced in advanced stages [35, 36], presumably following the C-cell destruction related to lymphocytic infiltration and fibrosis of the gland itself, since it is not encountered in cases of secondary hypothyroidism [37].

Our study has various limitations: the duration of AAG and AT was calculated from the date of diagnosis; gastrin-17 was not available for all patients; and, most importantly, the study had a cross-sectional design. It would be very interesting to conduct a longitudinal cohort study, monitoring Ct and gastrin levels over time in patients with AAG and/or AT, to gather more definitive evidence of their behavior.

In conclusion, based on our data, chronically high gastrin levels do not explain any concurrent rise in Ct levels, at least in the context of AAG, with or without concomitant AT. The only three patients in our study with higher than normal levels of both Ct and gastrin had MTC. In our opinion, the main message to be drawn from our study is that diagnostics to exclude MTC are mandatory in patients revealing high Ct levels and hypergastrinemia, at least in the context of AAG and/or AT.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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Research involving human participants and/or animals The study was performed in accordance with the guidelines of the Helsinki Declaration.

Informed consent All participants gave their written informed consent.

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