Endocrine Pathology (2019) 30:113–117
https://doi.org/10.1007/s12022-019-9567-6

Revisiting the Significance of Prominent C Cells in the Thyroid

Talia L. Fuchs [1,2] · Stephen E. Bell [1] · A. Chou [1,2,3] · Anthony J. Gill [1,2,3]

Published online: 29 January 2019

Abstract

C cell hyperplasia is considered a precursor lesion for hereditary forms of medullary thyroid carcinoma. It has therefore been suggested as a morphological marker to distinguish hereditary from sporadic medullary thyroid carcinoma and to triage genetic testing in resource poor settings. However, numerous definitions for C cell hyperplasia have been suggested, and there is surprisingly little data regarding the number of C cells present in thyroid glands removed for conditions other than medullary carcinoma. We therefore sought to investigate the specificity of different criteria for C cell hyperplasia. We examined the number of C cells and solid cell nests (ultimobranchial body remnants) present in 118 completion thyroidectomy specimens from patients without medullary carcinoma and with no risk factors for MEN2. Morphological review was performed on all H&E-stained slides, and immunohistochemistry for calcitonin was performed on one block from each case. Solid cell nests were found in 4 (3.3%) of thyroids. Increased numbers of C cells sufficient to fulfil criteria for C cell hyperplasia were found in 5 (4.2%) to 36 (30.5%) cases depending on the criteria used. We conclude that large numbers of C cells are commonly found in thyroids not associated with medullary carcinoma. Therefore, regardless of which criteria are used, the presence of C cell hyperplasia is not a specific marker for hereditary medullary thyroid carcinoma.

Keywords Medullary thyroid carcinoma · C cell hyperplasia · MEN2

Introduction

The thyroid gland is derived from the floor of the primitive pharynx and develops as a down growth in the region of the developing tongue [1]. C cells are a minor component of the thyroid, comprising less than 0.1% of cell mass in the normal gland [2]. In contrast to the rest of the organ, C cells originate from the ultimobranchial body, which is derived from the fourth or fifth pharyngeal pouch. C cells synthesise and store calcitonin [2]. Solid cell nests (SCNs) are intrathyroidal remnants of the ultimobranchial body. Increased numbers of C cells are frequently found in the thyroid adjacent to SCN, and their presence can be used as a marker for a region rich in C cells [3]. The reported incidence of SCN varies with the number of sections of the thyroid examined [3], with an incidence of up to 61% when the entire gland is examined histologically [4].

Medullary thyroid carcinoma (MTC) is a rare but aggressive form of thyroid carcinoma derived from C cells. It accounts for less than 5% of all thyroid malignancies [5]; however, it is responsible for a disproportionately high number of deaths [6]. These tumours typically secrete calcitonin, but have been known to produce a number of other peptides [1]. Approximately 25% of MTC are hereditary and occur in the setting of the autosomal dominant hereditary cancer syndrome multiple endocrine neoplasia type 2 (MEN2) caused by activating germline *RET* mutations [6–8]. In these cases, but not in sporadic MTC, the C cell hyperplasia-to-neoplasia progression is considered to be the hallmark of carcinogenesis [7]. Because prophylactic complete thyroidectomy can either cure or prevent MTC in individuals with MEN2, it is currently recommended that all patients who present with MTC should be offered germline mutation testing for the *RET* proto-oncogene to facilitate cascade testing of family members [8].

In the era before the widespread availability of genetic testing, the presence of large numbers of C cells displaying a nodular pattern of growth in the non-neoplastic thyroid (nodular C cell hyperplasia) was used as a marker of MEN2 [9]. Although most guidelines now recommend universal germline RET mutation for all patients presenting with MTC

✉ Anthony J. Gill
affgill@med.usyd.edu.au

[1] Cancer Diagnosis and Pathology Group, Kolling Institute of Medical Research, St Leonards, NSW 2065, Australia

[2] NSW Health Pathology, Department of Anatomical Pathology, Royal North Shore Hospital, Pacific Highway, St Leonards, NSW 2065, Australia

[3] University of Sydney, Sydney, NSW 2006, Australia

[6, 8], in the resource poor setting, nodular C cell hyperplasia may still be used to triage genetic testing.

Several different criteria for the diagnosis of C cell hyperplasia have been proposed—summarised in Table 1. Briefly, Tashjian et al. [2] defined C cell hyperplasia as four or more C cells in a cluster. Williams et al. [10] defined C cell hyperplasia as greater than six C cells per follicle. Ekblom et al. [11] defined C cell hyperplasia as a cluster of 20 or more cells seen in one or several neighbouring follicles, with clusters of 5 to 10 cells being regarded as suspicious for hyperplasia. Albores-Saavedra et al. [12] defined C cell hyperplasia as clusters of at least 50 C cells in one low-power field. In the previous (2004) WHO classification, it was noted that the definition of C cell hyperplasia was controversial, but the preferred criteria were more than 6 cells in several foci from both lobes for nodular C cell hyperplasia and more than 50 cells in one lower powered field in both lobes for diffuse hyperplasia [5]. In the most recent WHO 2017 classification, C cell hyperplasia was defined as > 6–8 C cells per cluster in several foci with > 50 C cells per low-power field [13], which represents a combination of the criteria initially proposed by Williams [10] together with those of Albores-Saavedra [12]. Several groups have noted that using some of these criteria C cell hyperplasia may also occur with significant frequency in patients with MTC without germline RET mutation [6, 14] or with no history of MTC [13, 15, 16].

Recently, we have received several consultation cases where prominent C cells fulfilling some or all of these criteria have been noted by the referring pathologist in the absence of a personal or family history of medullary thyroid carcinoma or any other features to suggest MEN2. In our opinion, the incidental identification of numerous C cells in these cases with no other features to warrant genetic testing for MEN2 but still fulfilling some or all of the published criteria for C cell hyperplasia was unlikely to be associated with germline *RET* mutation and caused unnecessary concern.

We suspect that sufficient numbers of C cells to fulfil most criteria for C cell hyperplasia may not be an uncommon incidental finding and are of little clinical significance. To test this hypothesis, we therefore sought to critically reassess the incidence of C cell hyperplasia using different criteria in thyroids from individuals without MTC and with no risk factors for MEN2.

Table 1 Incidence of C cell hyperplasia according to different criteria

Criteria	Number with C cell hyperplasia
Groups of ≥ 4 C cells [2]	36 (30.5%)
≥ 6 C cells per follicle [10]	16 (13.6%)
Clusters of ≥ 20 C cells [11]	5 (4.2%)
≥ 50 C cells per low-power field [12]	31 (26.3%)

Methods

Completion thyroidectomies from patients with no personal or familial history of MEN2 or familial MTC were obtained from Royal North Shore Hospital archives. The age, gender, and indication for completion thyroidectomy were recorded. Completion thyroidectomies were chosen for the purposes of our study because they tend to contain minimal, if any, other pathologies and are generally sampled sparingly (our laboratory protocol recommends five blocks per lobe unless specific pathologies are identified macroscopically).

All the H&E-stained slides from the completion thyroidectomies were re-screened for the presence of morphologically identifiable C cells or SCN. In addition, a single block from each thyroid was selected for immunohistochemistry for calcitonin in order to identify C cells. The selected block was chosen as the most likely to contain C cells on the basis of the findings in the slide (for example, the presence of possible C cells or SCN identified morphologically). If no C cells or SCNs were found, the block was selected on the basis of the usual distribution of C cells in the middle to upper lateral lobes of the thyroid gland [17].

Immunohistochemistry was performed using a rabbit polyclonal antibody directed against synthetic calcitonin (Dako A0576, Denmark) at a dilution of 1:800 and without antigen retrieval or pre-treatment. Staining was performed on an automated platform (Leica BONDMAX, Leica Microsystems, Melbourne, Australia) using a polymer-based detection system (Leica Define Detection DS9713, Wetzlar), and the number of calcitonin-positive C cells was recorded by two independent observers blinded to each other's results and the results of the two observers were averaged to derive the final figure. This study was approved by the local institutional ethics committee.

Results

There were 118 completion thyroidectomies that fulfilled the study criteria and were chosen for inclusion in the study cohort. The average age was 49.6 years, median age 47, and age range 20 to 85. Eighty-eight patients (75%) were female. Sixty-four (54.2%) of the examined lobes were left, and 55 (45.8%) were right. The recorded indications for the original hemi-thyroidectomy preceding completion thyroidectomy were as follows: papillary thyroid cancer in 55 (46.6%) cases, follicular thyroid cancer in 17 (14.6%), Hurthle cell carcinoma in 8 (6.7%), multinodular goitre in 7 (5.96%), and not specified in 31 (26.2%).

Four (3.3%) of the thyroids screened were found to contain SCNs. C cells were thought to be present in six (4.8%) of the thyroids based on H&E morphology. However,

Table 2 Summary of patient characteristics according to C cell hyperplasia analysis

Indication for initial thyroidectomy and demographic details	Groups of ≥ 4 C cells [2]	≥ 6 C cells per follicle [10]	Clusters of ≥ 20 C cells [11]	≥ 50 C cells per low-power field [12]
Papillary carcinoma	22 (61.1%)	10 (62.5%)	4 (80%)	20 (64.5%)
Follicular carcinoma	7 (19.4%)	3 (18.8%)	0	6 (19.4%)
Multinodular goitre	1 (2.8%)	0	0	0
Hurthle cell carcinoma	2 (5.6%)	2 (12.5%)	1 (20%)	3 (9.7%)
Not specified	4 (11.1%)	1 (6.3%)	0	2 (6.5%)
Average age	45.5	43.3	50.2	49.6
Median age	44	43	44	47
Male	15 (41.7%)	7 (43.8%)	3 (60%)	11 (35.5%)
Female	21 (58.3%)	9 (56.3%)	2 (40%)	20 (64.5%)

immunohistochemistry confirmed the presence of C cells in only four of these blocks, all of which were also associated with SCNs. With immunohistochemistry for calcitonin, C cells were observed in 65 (54.6%) of the selected blocks. When present, the maximum number of C cells observed ranged from 1 to 85 per high-power field, with an average of 19.6 C cells per high-power field (Fig. 1).

Depending on the criteria used, the number of thyroids that demonstrated C cell hyperplasia ranged from 5 (4.2%) to 36 (30.5%)—summarised in Tables 1 and 2. Of note, C cell hyperplasia was noted in the thyroid adjacent to a SCN in two of four thyroids using Tashjian's criteria [2] and in three of four using Albores-Saavedra's criteria [15].

Discussion

In this study, we investigated the number of C cells identified in 118 completion thyroidectomy specimens from patients with no history of MTC and no risk factors for MEN2. Although these thyroids were only sparingly sampled (our laboratory protocol recommends five blocks per lobe unless specific pathologies are identified macroscopically), 65 (54.6%) thyroids were found to contain C cells on immunohistochemistry. Only four of these cases were also identified with H&E staining, confirming the poor sensitivity of routine microscopy in detecting C cells. Using immunohistochemistry, C cell hyperplasia was present in 4.2 to 30.5% of our

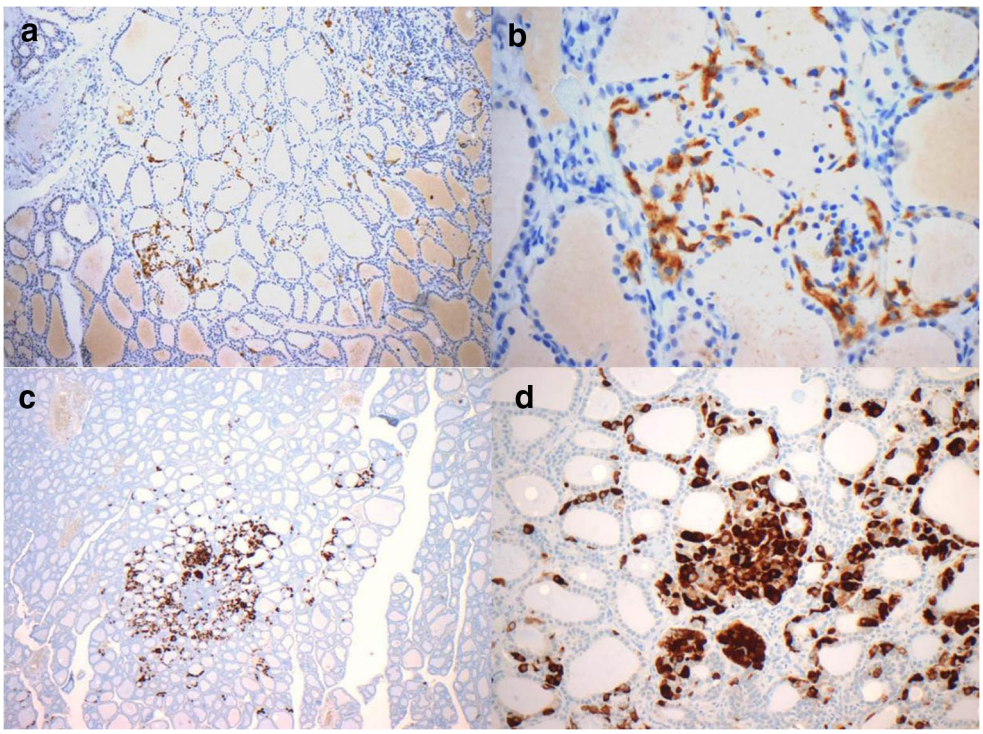

Fig. 1 **a**, **b** Calcitonin immunohistochemistry in a thyroid with low numbers of C cells. **c**, **d** Calcitonin immunohistochemistry in thyroid containing large numbers of C cells, fulfilling all criteria for C cell hyperplasia [calcitonin immunohistochemistry original magnifications: **a** × 100, **b** × 400, **c** × 100, **d** × 400]

cases, depending on the criteria used for diagnosis. Of particular note, the criteria of groups of ≥ 4 C cells proposed by Tashjian at al [2] and ≥ 50 C cells per low-power field proposed by Albores-Saavedra et al. [12] were fulfilled in 30.5% and 26.3% of thyroids in this study respectively clearly lack specificity and therefore have little or no significance when identified in routine clinical practice. Given that our cohort is significantly larger than those used in other studies on C cell hyperplasia and medullary thyroid carcinoma [6, 7, 9, 12, 14], and comprised individuals without germline *RET* mutations and with no risk factors for MEN2, and that the thyroids were sampled less intensely than other cases in which medullary thyroid carcinoma was identified or MEN2 suspected, these findings provide further evidence that C cell hyperplasia occurs with significant frequency in the general population and is therefore not a specific predictor of the presence of germline *RET* mutations. As it applies to our previous consultation cases, we therefore conclude that the incidental identification of C cell hyperplasia in patients with no risk factors for MEN2 is not an indication for *RET* mutation testing.

As outlined above, the C cell hyperplasia-to-neoplasia progression is the hallmark of inherited forms of MTC in the setting of familial cancer syndromes associated with *RET* proto-oncogene germline mutations [7]. This is supported by the frequent finding of primary C cell hyperplasia in association with tumours from patients with heritable forms of MTC [13]. However, as we and others have demonstrated, C cell hyperplasia is often identified in thyroids removed for reasons other than MTC [6, 12, 14]. Moreover, the absence of C cell hyperplasia in patients with MTC in no way excludes the possibility of MEN2 [6]. For these reasons, we support the current American Thyroid Association (ATA) guidelines which recommend germline RET mutation testing be offered to all MTC patients regardless of other clinical or pathologic features including C cell hyperplasia [8].

Although the natural history of C cell hyperplasia not associated with familial MTC is unknown, the incidental observation of benign and malignant follicular-cell derived tumours (mainly papillary carcinoma) adjacent to C cell hyperplasia has suggested alternative hypotheses [13]. These include the possibility that thyroid follicular cell-derived tumours might induce reactive C cell hyperplasia and possibly its evolution into MTC, or the possible existence of a common genetic background responsible for both follicular cell-derived and sporadic parafollicular neoplastic lesions [6, 7]. To investigate this possibility, we deliberately confined the study to cases of completion thyroidectomies which by their nature contained minimal pathology in that lobe. We therefore suspect that an alternative explanation for the identification of numerous C cells adjacent to other tumours is a recognition and sampling bias. That is, pathologists take more blocks and look more carefully at the thyroid adjacent to lesions than non-neoplastic thyroids.

Outside the setting of familial disease, there is no evidence that C cell hyperplasia can progress to MTC. A study by Saggiorato et al. [7] analysed the presence of *RET* gene point mutations in a cohort of 24 cases of C cell hyperplasia, including cases with a purely reactive aetiology, as well as those associated with non-familial MTC. In Saggiorato et al.'s study, there were no RET mutations in 24 cases of C cell hyperplasia not associated with MEN2, but a somatic RET mutation was identified in three concomitant medullary thyroid carcinomas.

One limitation of our study is the lack of comparison with cases of sporadic and hereditary MTC. This would significantly enhance the strength of our findings and is an area of potential future research.

In conclusion, we demonstrate that depending on the criteria used, C cell hyperplasia occurs in between 4.2 and 30.5% of thyroids resected for reasons other than MTC in patients with no risk factors for MEN2. Therefore, it is a relatively common incidental finding, and we do not believe that its identification in the absence of MTC or other suggestive clinical features warrants genetic counselling and testing for MEN2. Furthermore, in the modern era where genetic testing is readily available in most centres, we do not believe that morphologic criteria for C cell hyperplasia have any role in triaging genetic testing for germline *RET* mutations. That is, we support the current ATA recommendations that advise all patients with MTC be offered genetic counselling and testing irrespective of the presence or absence of C cell hyperplasia [8].

Compliance with Ethical Standards

This study was approved by the local institutional ethics committee.

Conflict of Interest The authors declare that they have no conflict of interest.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Chan JKC (2007) Tumours of the thyroid and parathyroid glands. In: Fletcher CDM (ed) Diagnostic histopathology of tumors, 3rd edn. Churchill Livingstone Elsevier, New York, pp.1223–1232.
2. Tashjian AH, Wolfe HJ, Voelkl EF (1974) Human calcitonin: immunologic assay, cytologic localization and studies on medullary thyroid carcinoma. Am J Med 56:840–849.
3. Harach HR (1988) Solid cell nests of the thyroid. Journal of Pathology 155:191–200.
4. Harach HR (1985) Histological markers of solid cell nests of the thyroid. With emphasis on their expression in thyroid ultimobranchial-related tumors. Acta Anatomica 124:111–116.
5. Gimm O, Morrison CD, Suster S et al (2004) Multiple endocrine neoplasia type 2. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds)

Pathology and genetics of tumours of endocrine organs. IARC Press, Lyon, pp.211–217.
6. Reagh J, Bullock M, Andrici J, Turchini J, Sioson L, Clarkson A, Watson N, Sheen A, Lim G, Delbridge L, Sidhu S, Sywak M, Aniss A, Shepherd P, Ng D, Oei P, Field M, Learoyd D, Robinson BG, Clifton-Bligh RJ, Gill AJ (2017) NRASQ61R mutation-specific immunohistochemistry also identifies the HRASQ61R mutation in medullary thyroid cancer and may have a role in triaging genetic testing for MEN2. Am J Surg Pathol, 41:75–81.
7. Saggiorato E, Rapa I, Garino F, Bussolati G, Orlandi F, Papotti M, Volante M (2007) Absence of RET gene point mutations in sporadic thyroid C-cell hyperplasia. J Mol Diag 9(2):214–219.
8. Wells SA Jr, Asa SL, Dralle H et al (2015) Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid 25:567–610.
9. Wolfe HJ, Melvin KEW, Cervi-Skinner SJ, al Saadi AA, Juliar JF, Jackson CE, Tashjian Jr AH (1973) C-cell hyperplasia preceding medullary thyroid carcinoma. NEJM 289:437–441.
10. Williams ED, Ponder BJ, Craig RK (1987) Immunohistochemical study of calcitonin gene-related peptide in human medullary carcinoma and C-cell hyperplasia. Clin Endocrinol 27:107–114.
11. Ekblom M et al (1987) Familial and sporadic medullary thyroid carcinoma: clinical and immunohistochemical findings. Quarterly Journal of Medicine 247:899–910.
12. Albores-Saavedra J, Monforte H, Nadji M, Morales AR (1988) C-cell hyperplasia in thyroid tissue adjacent to follicular cell tumors. Hum Pathol 19(7):795–799.
13. LiVolsi V, DeLellis R, Komminoth P et al (2017) Multiple endocrine neoplasia type 2. In: Lloyd RV, Osamura RY, Kloppel G, Rosai J (eds) WHO classification of tumours of endocrine organs, 4th edn. IARC Press, Lyon, pp.248–250.
14. Guyetant S, Rousselet M-C, Durigon M et al (1997) Sex-related C-cell hyperplasia in the normal human thyroid: a quantitative autopsy study. J Clin Endocrinol Metab 82:42–47.
15. Albores-Saavedra JA, Krueger JE (2001) C-cell hyperplasia and medullary thyroid microcarcinoma. Endocrine Pathology 12:365–377.
16. Guyetent S, Blechet C, Saint-Andre JP (2006) C-cell hyperplasia. Annals of Endocrinology (Paris) 67:190–197.
17. Scognamiglio T (2017) C-cell and follicular epithelial cell precursor lesions of the thyroid. Arch Pathol Lab Med 141:1646–1652.