CLINICAL STUDY

Nodular disease and parafollicular C-cell distribution: results from a prospective and retrospective clinico-pathological study on the thyroid isthmus

Giampaolo Papi^{1,4}, Giulio Rossi², Salvatore Maria Corsello¹, Stefania Corrado², Guido Fadda³, Carlo Di Donato⁴ and Alfredo Pontecorvi³

¹Chair of Endocrinology, Catholic University of Rome, Rome, Italy, ²Institute of Pathology, University of Modena and Reggio Emilia, Modena, Italy,

³Institute of Anatomic Pathology, Catholic University of Rome, Rome, Italy and ⁴Department of Internal Medicine, Azienda USL of Modena, Ospedale 'Ramazzini', Via S Giacomo 2, 41012 Carpi, Modena, Italy

(Correspondence should be addressed to G Papi at Dipartimento di Medicina Interna, Azienda USL of Modena; Email: papigiampaolo@hotmail.com)

Abstract

Objective: The isthmus represents a peculiar, as yet partially unexplored, thyroid gland area.

Aim of the study: To assess i) the prevalence and clinico-pathological features of solitary thyroid isthmus nodules (STIN); ii) the frequency of medullary thyroid carcinoma (MTC) arising from the isthmus; and iii) the C-cell distribution in the isthmus of patients with MTC and benign nodular thyroid disease (NTD).

Subjects and methods: Patients referred from 2006 to 2008 for STIN were prospectively recruited, and underwent serum calcitonin (C_t) measurement and fine needle aspiration cytology (FNAC). MTCs diagnosed from 1993 to 2005 were retrospectively searched. Immunohistochemistry was performed using anti- C_t antibodies on lateral lobes and isthmi of 50 benign NTD and 50 MTC cases.

Results: From 1993 to 2005, 150 patients underwent surgery for MTC. All patients had the neoplasm located in lateral thyroid lobes, none in the isthmus. In the 3 years following, 192 STIN patients (40 (21%) males, 152 (79%) females; mean age: 46.2 ± 7.1 years; 6.4% of NTD subjects) were recruited. All had normal C_t concentrations. FNAC was malignant or suspicious for malignancy in 14 (7.3%) patients. Histology found malignancy in 17 (9%) cases, MTC in none. C cells were disclosed in lateral thyroid lobes of 100% MTC and 77% benign NTD patients; isthmi were free of C cells in either group.

Conclusions: STINs are significantly less likely to be MTC in patients presenting with sporadic disease. Therefore, C_t screening is not warranted in these subjects. Nonetheless, STINs are more likely to be neoplastic and deserve equal attention as those of the lateral lobes.

European Journal of Endocrinology 162 137–143

Introduction

Nodular thyroid disease (NTD) is frequently observed in the clinical practice (1). Indeed, on thyroid ultrasound, NTD prevalence approaches 50% in iodine-deficient areas (2). Fortunately, malignant neoplasm is rarely seen, accounting for 5% or less of thyroid nodules (1, 2). Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor, regarded as the most aggressive of well-differentiated thyroid carcinomas, with a survival rate of 50% at 10 years (3). MTC arises from parafollicular C cells, which are responsible for both the production and the release of calcitonin (C_t), a peptide hormone (4). MTC is sporadic in about 75% of the cases, whereas its familial counterpart – isolated or in the context of MEN type 2 syndrome – accounts for the remainder of the cases (5).

Several studies (6–12) on sporadic MTC have demonstrated that the earlier the diagnosis the better the prognosis of MTC. C_t has been shown to represent a sensitive marker for MTC, leading to the early diagnosis of the neoplasm even when fine needle aspiration cytology (FNAC) discloses benign features (as occurs in nodules smaller than 10 mm).

Based on such evidence, the European Thyroid Association (ETA), developing the guidelines for the management of differentiated thyroid carcinoma (13), recommended the use of C_t screening in all patients with thyroid nodule(s). In 2006, the American Thyroid Association (ATA) published its guidelines addressing thyroid nodules (14), where C_t screening was not recommended due to its low specificity, the high prevalence of thyroid nodules in the general population, the rarity of MTC, and the costs of the test. In June 2009, the ATA

had published specific guidelines for the diagnosis and the management of MTC (15) and, again, although recent studies from the United States have confirmed the European observations and cost-effectiveness of the test (16), C_t screening was not recommended.

Immunohistochemical studies (17, 18) conducted in autopsy series from 1990s failed to demonstrate any evidence of C cells in the isthmus of normal thyroid glands. Similarly, no C cells were detected at immunohistochemical examination in the isthmus of patients who underwent total thyroidectomy for MTC or C-cell hyperplasia (CCH) (19).

To the best of our knowledge, no studies – either retrospective or prospective – have been conducted so far to assess both prevalence and clinico-pathological features of solitary thyroid isthmus nodules (STIN). Based on the hypothesis that in the absence of C cells in the thyroid isthmus MTC should not occur, the present study was designed to answer the following questions: what are prevalence and clinico-pathological features of STIN? What is the frequency of MTC in patients with STIN? What is the distribution of parafollicular C cells in the thyroid of subjects with MTC and benign NTD?

Patients and methods

Aim of the study

The aim of the present study was to assess i) the prevalence and clinico-pathological features of STIN; ii) the frequency of MTC in STIN; and iii) C-cell distribution in the isthmus of patients with MTC and benign NTD.

Study design

To assess the prevalence and clinico-pathological characteristics of STIN, a 3-year prospective study was conducted, whereas to evaluate the frequency of MTC in patients with STIN, both a retrospective and a prospective study were performed.

- i) *Retrospective study.* A database research having 'MTC' and 'histology' as keywords, and spanning the period from January 1993 to December 2005, was made from the files of the sections of Pathology of both the Catholic University of Rome and the University of Modena and Reggio Emilia, Italy. Once MTC cases were selected, the following data were retrospectively sought: age, sex, the neoplasm origin (sporadic or hereditary), and the location of the neoplasm within the thyroid gland.
- ii) *Prospective study.* During the period from January 1, 2006 to December 31, 2008, 4723 consecutive patients with suspected NTD were referred to the Division of Endocrinology, 'A. Gemelli' Hospital – Catholic University of Rome, and to the

Endocrine Outpatient Clinics of 'Ramazzini' Hospital – Azienda USL of Modena, Italy. *Inclusion criteria* were a) male and female patients; b) solitary palpable nodule of the thyroid isthmus; c) solitary nonpalpable nodule of the thyroid isthmus ≥ 10 mm in its largest diameter; d) solitary nonpalpable isthmus nodule smaller than 10 mm, showing ultrasound features suspicious for malignancy (microcalcifications, undefined margins, and intra-nodular vascularization). *Exclusion criteria* were a) the presence of multiple thyroid nodules; b) solitary thyroid nodules affecting the lateral lobes (including those located in the boundary zones between the lateral thyroid lobes and the isthmus); c) nonpalpable isthmus nodule smaller than 10 mm lacking ultrasound features suspicious for malignancy; d) patients previously evaluated for nodular goiter by FNAC and/or C_t measurement; e) patients reporting a family history of MEN and those previously thyroidectomized for sporadic MTC; f) patients with hyper- and hypothyroidism without nodules; g) patients in follow-up for thyroid diseases; and h) patients not confirmed to have thyroid diseases.

To evaluate the distribution of parafollicular C cells in the thyroid glands of subjects with either MTC or benign NTD, 50 consecutive cases of MTC (both familial and sporadic) found through the retrospective database research and 50 cases of nodular goiter consecutively diagnosed after total thyroidectomy during the 3-year prospective study were selected.

Patients

During the 12-year period of the retrospective study, 21 288 thyroidectomies were performed in our institutions.

During the 3-year period of the prospective study, 3978 patients with thyroid nodules were evaluated at our institutions. Of these, 256 (6.4%) presented with a solitary nodule of the thyroid isthmus. In total, 222 patients fulfilled the criteria of the study design but 30 dropped out of the study either because they refused FNAC and/or surgery ($n=8$) or because they were lost to follow-up after the first visit ($n=22$). Of the remaining 192 patients, 111 (58%) were recruited by the Institute of Endocrinology of the Catholic University of Rome, and 81 (42%) were recruited by the Endocrine Outpatient Unit of 'Ramazzini' Hospital of AUSL Modena. When matched for gender, age, C_t , and TSH concentrations, no significant differences were found among the patients recruited by the two different institutions.

All patients satisfying the inclusion criteria underwent the following procedures: a) blood sample for the measurement of serum TSH and C_t concentrations and b) ultrasound-guided FNAC.

When the TSH value was abnormal, serum-free thyroxine (FT₄) and free triiodothyronine concentrations were also measured. Furthermore, patients with suppressed serum TSH concentrations underwent ^{99m}Tc-thyroid scan, to detect autonomously functioning nodules.

Surgery was always recommended if one of the following criteria was met: a) FNAC diagnostic or suspicious for malignancy (indeterminate included); b) repeated (>2) nondiagnostic FNACs; and c) complaints of compressive symptoms (dysphagia and dyspnea). All patients gave informed consent to the diagnostic and therapeutic procedures.

Methods

CT assay Serum C_t concentrations were measured in the laboratories of the institutions involved in the study, using the same two-site chemiluminescence immunoassay (immunoluminometric assay, Nichols Institute Diagnostics, San Juan Capistrano, CA 92675, USA).

Ultrasound and fine needle aspiration biopsy

Thyroid ultrasound was performed utilizing a Philips En Visor C machine (Philips Medical Systems SpA, Monza, MI, Italy). Ultrasound-guided fine needle aspiration biopsy was performed on nodular areas with 23–27 gauge needles, using a 7.5–13 MHz probe. Multiple direct smears were prepared, and May-Grunwald-Giemsa and Papanicolaou stainings were employed.

Surgery Patients recruited in the 3-year prospective study design with a benign FNAC and compressive symptoms underwent isthmusectomy. In case of a suspicious or repeatedly nondiagnostic FNAC, frozen sections of the nodule(s) were obtained for intraoperative histological examination to decide the definitive surgical extension. In all patients undergoing surgery following a cytological diagnosis of malignancy, total thyroidectomy was the procedure of choice completed by a lymphadenectomy in case of lymph node involvement.

Histological and immunohistochemical examination of surgical specimens Given that isthmus has no standard size (20), in this study isthmus region was considered as the thyroid tissue interposed between the two lateral lobes.

MTC cases found through the retrospective 12-year database research were all reviewed.

In the 100 cases of total thyroidectomy selected to identify the presence of C cells throughout the isthmus and the lateral thyroid lobes, microscopic slides were obtained by three formalin-fixed and paraffin-embedded 5 mm large specimens of thyroid tissue devoid of

nodules: one derived from the middle of the isthmus, one from the middle of the right thyroid lobe, and one from the middle of the left thyroid lobe. Immunohistochemical studies were performed on such tissues by means of an automated immunostainer (Benchmark, Ventana, Tucson, AZ, USA) using antibodies directed against C_t (polyclonal: Ventana; microwave antigen retrieval). CCH was defined as the presence of more than 50 C cells in a single low-power field (×10 magnification) in both thyroid lobes (21), of solid nests of C cells with >7 cells per nest, or of follicles completely surrounded by C cells.

Statistical analysis

Results are reported as mean ± s.d. All statistics were conducted using the commercially available software program SPSS for Windows, release 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Retrospective study

Of 21 288 cases of thyroidectomy performed in our institutions in the period from January 1993 to December 2005, 150 (0.7%) MTCs were discovered: 126 of the sporadic form and 24 of the hereditary form (nine with familial MTC (FMTC) and 15 with MEN 2A). The diagnosis of MTC was confirmed by our pathologists in all the 150 cases. Sixty-five (43.3%) patients were males, 85 (56.7%) were females, with a female to male ratio of 1.3 to 1, ranging in age from 9 to 83 years (mean: 55.2 ± 7.2 years; median: 59 years). The results of cytological examination before surgery are reported in Table 1. The neoplasm was located within the right lateral lobe of the thyroid gland in 84 (56%) cases and in the left lateral lobe in 66 (44%) cases; it was detected in the thyroid isthmus in none of the cases.

Prospective study

Of 192 subjects with solitary isthmus nodule recruited in the study, 40 (21%) were males and 152 (79%) were females, with a female-to-male ratio of 3.8 to 1.

Table 1 Results of fine needle aspiration cytology in 150 patients with medullary thyroid carcinoma.

Cytological examination	
Repeatedly nondiagnostic	0
Benign	21
Indeterminate	11
Suspicious for malignancy	40
Malignant	74
Not performed	4
Total	150

Table 2 Results of fine needle aspiration cytology (FNAC) in 192 patients with solitary isthmic nodules and consequent treatment.

	Repeatedly nondiagnostic	Benign	Indeterminate	Suspicious for malignancy	Malignant
Total	2 (1.1%)	171 (89.1%)	5 (2.6%)	7 (3.6%)	7 (3.6%)
Submitted to surgery	2	27	5	7	7
Treated by TSH-suppressive therapy	0	31	0	0	0
Followed without therapy	0	113	0	0	0

FNAC, fine needle aspiration cytology; TSH, thyrotropin.

The mean age was 46.2 ± 7.1 years (range: 21–84 years; median: 49 years). Mean serum TSH concentrations were 3.7 ± 2.3 mIU/l. One hundred and eighty-six patients (97%) were euthyroid, four patients (2%) were hypothyroid (three with subclinical and one with overt hypothyroidism) due to Hashimoto's thyroiditis, and two patients (1%) were overtly hyperthyroid due to Graves' disease. Mean serum C_t concentrations were 2.2 ± 0.4 pg/ml and were normal in all 192 patients. Thyroid ultrasound detected hypoechoic nodules in 156 patients (81%), isoechoic nodules in 27 (14%), and hyperechoic nodules in 9 (5%). The mean diameter of isthmic nodules was 29.5 ± 8 mm (range: 8–52 mm). The results of cytological examination are reported in Table 2. Fourteen (7.3%) nodules harbored cytological features suspicious for or consistent with papillary thyroid carcinoma (PTC). In no case was a cytological diagnosis of MTC given. In total, 113 subjects were followed up with observation only, 31 (with cytological diagnosis of 'benign colloid nodule') were treated by $L-T_4$ TSH-suppressive therapy, 48 underwent thyroidectomy (total thyroidectomy in 26 cases and isthmusectomy in 22 cases) (Table 2). Histological examination demonstrated a benign nodule in 31 cases and a malignant neoplasm in 17 (9%) cases. Of the 17 cases

diagnosed with malignancy, 16 were PTCs (of which, eight (50%) were smaller than 10 mm in their largest diameter), and one was a follicular carcinoma. In no case was there evidence of a MTC.

Immunohistochemical study

In Fig. 1, the results of immunohistochemical study are reported.

A positive reaction to C_t immunostaining was observed in the lateral thyroid lobes of all 50 (100%) patients with both sporadic and hereditary MTC. CCH (Fig. 2A) was demonstrated in 14 out of 14 (100%) patients affected by hereditary MTC (FMTC or MEN2A), and in 26 out of 36 (72%) subjects with sporadic MTC. On the contrary, no C_t -positive cells were seen in the isthmi of either group (Fig. 2B). Similarly, in 38 out of 50 (76%) patients with benign NTD, C_t reaction was positive within the lateral thyroid lobes; however, contrary to the picture found in MTC patients, in these benign NTD cases, C cells appeared as sparse or even isolated (Fig. 2C) and CCH criteria were never met. Again, no C_t -positive cells were found in the isthmi of this patient group (Fig. 2D).

Figure 1 Summary of immunohistochemical examination results in 50 patients with medullary thyroid carcinoma (MTC) and 50 patients with benign nodular thyroid disease (NTD).

Figure 2 Results of immunohistochemical examination using antibodies against calcitonin (C_t -Ab). (A) C-cell hyperplasia in the lateral thyroid lobe of a patient with medullary thyroid carcinoma (MTC) (C_t -Ab, 40 $\times$). (B) No reactivity in the thyroid isthmus of a patient with MTC (C_t -Ab, 40 $\times$). (C) Sparse and isolated C cells in the lateral thyroid lobe of a patient with benign nodular thyroid disease (C_t -Ab, 40 $\times$). (D) No reactivity in the thyroid isthmus of a patient with benign nodular thyroid disease (C_t -Ab, 40 $\times$).

Discussion

General considerations

To the best of our knowledge, this study represents the first work investigating the clinico-pathological characteristics of solitary nodules arising from the thyroid isthmus, and in particular their association with MTC.

The thyroid isthmus and the parafollicular C cells

In our series, we have not found either parafollicular C cells or C cell-derived tumors in the thyroid isthmi of patients with both benign NTD and MTC. To perform immunohistochemical investigations, we used antibodies against C_t because several studies have shown that this is the most effective method to detect parafollicular C cells (16, 17, 22). During embryonic development, C cells migrate from the ventral parts of the fourth branchial pouch or the ultimobranchial bodies into the thyroid gland that descends caudally from the median anlage (23). Within the thyroid, C cells have a peculiar distribution. Indeed, autopsy studies conducted on normal thyroid glands in the 1970s and 1980s, when specific anti- C_t antibodies were not available yet (24), found that parafollicular C cells were distributed symmetrically in both lateral lobes, concentrated in a zone in the upper two-thirds of the lateral lobes bilaterally. When the isthmus was investigated, few scattered cells or even no cells were seen. Subsequently, studies using more accurate techniques for C_t immunostaining confirmed previous

observations, failing to detect C cells in the thyroid isthmus of patients with CCH (15, 16). CCH is defined as the presence of more than 50 C cells in a single low-power field ($\times 10$ magnification) in both thyroid lobes (21), of solid nests of C cells with > 7 cells per nest, or of follicles completely surrounded by C cells. Although the occurrence of CCH has been reported in patients with benign diseases (25), some authors consider CCH as a preneoplastic event, especially in patients with hereditary MTC. In the surgical series of Wahl *et al.* (19), the isthmi of 58 patients with both normal and elevated pre-operative serum C_t concentrations (due to either CCH or MTC) were investigated by immunohistochemical studies; C_t -positive cells were seen in none of them. The authors concluded that isthmus-preserving total bilobectomy removes all C cells and that total thyroidectomy is not necessary in patients with CCH. Because of the few papers published in the literature on this topic and the small number of patients with FMTC included in our series, the possibility that patients with RET proto-oncogene germline mutations present with multifocal disease involving the isthmus cannot be definitively ruled out and should be investigated by further studies.

The routine measurement of serum C_t concentrations in patients with solitary isthmus nodule

MTC is a malignant tumor of the thyroid gland that appears as nodule(s) and represents $\sim 5\%$ of all thyroid neoplasms (3, 26). MTC arises from the neural crest-derived parafollicular C cells (4), presents in the familial form with a genetic mutation in 25% of the cases (1, 5), and is the most aggressive of differentiated thyroid carcinomas, with a survival rate at 10 years approaching 40–50% and depending on the tumor stage at diagnosis (3, 27). In the literature, a convincing body of evidence exists showing that when the neoplasm is confined within the thyroid and thyroidectomy with dissection of the central lymph node compartment is performed, the chance of definitive cure is high (3, 10, 28). Therefore, early detection of MTC is crucial to perform the best surgical approach before extra-thyroidal dissemination and to save the patient's life.

Actually, two diagnostic tools are available for the evaluation of patients with NTD, including those with MTC: FNAC and measurement of human C_t concentrations. Previous European studies recruiting large series of patients with NTD (6–12) demonstrated that it is possible to diagnose MTC earlier with C_t screening than FNAC, particularly in patients with nodules smaller than 10 mm (microMTCs). Hence, the ETA has recommended C_t screening in all subjects with NTD (13). Although authors from the United States have also confirmed the European observations and even the cost-effectiveness of C_t screening (14), as yet the ATA does not recommend it, mainly due to its high costs (15).

At present, the recession of the world economy invariably affects the decisions of the Western governments on health policy. Thus, we do believe that, on the one hand and owing to actual evidence, C_t screening should be performed in NTD patients and, on the other hand, the problem of cost deserves special consideration. In a recent study recruiting a large series of patients with NTD, we have identified a subgroup, i.e. subjects younger than 40 years, who should not be candidates to undergo the routine measurement of C_t because in these individuals (without a family history of MEN) we did not observe any cases of MTC. Now, based on the results of the present study, we propose that another patient subgroup, i.e. NTD subjects with solitary nodules of the thyroid isthmus, should not undergo C_t testing routinely. In Italy, the total cost of a single basal CT measurement averages 45€. In our 3-year prospective study, of 3978 patients referred with NTD to our institutions, no sporadic MTCs were disclosed in the 875 patients younger than 40 years (data not shown). The cost avoidance of not performing the C_t test in our patients aged <40 years and in those presenting with solitary isthmic nodules (1062 subjects altogether) would have saved 47 790€ (about \$66 900).

The thyroid isthmus and nonparafollicular C cell-derived tumors

This is the first study investigating the clinicopathological characteristics of patients with solitary isthmic nodule. In our series, the prevalence of nodules located within the thyroid isthmus only was low with respect to the nodules located in the thyroid lateral lobes. Indeed, patients with a solitary isthmic nodule accounted for 6.4% of the whole NTD population recruited in the study. The female to male ratio of ~4 to 1 is similar to that found in previous NTD prevalence studies (29). Nonetheless, the mean and median age of our patient group – 46.2 years and 49 years respectively – averages that reported in other NTD series (29).

However, our results have also demonstrated some differences compared to nonselected NTD populations recruited previously. For instance, in our series, a primary malignant neoplasm was found in 9% of the patients with solitary isthmic nodules. Previous studies (30, 31) have reported a frequency of cancer lower than 5% in subjects with NTD. Further prospective studies are needed to confirm whether subjects with solitary isthmic nodule truly present a higher risk of thyroid cancer than subjects with nodules located in the lateral lobes. Interestingly, in our series, almost all tumors were PTCs. Such data demonstrate that thyroid isthmic nodules behave as their lateral thyroid lobe counterparts. Indeed, since goiter prevention policy by iodine supplementation has become a priority in the western countries (in Italy, a specific law has been passed recently to make iodine salt supplementation

mandatory), a decrease in the incidence of follicular thyroid carcinomas has been observed (32). On the other hand, an increase in the frequency of PTCs has been shown worldwide (33–35). This event is likely due to the thyroid nodule management effects after the nuclear Chernobyl accident, as reflected by the fact that half of PTCs in our series were microcarcinomas (36).

Conclusions

Lesions of the isthmus are significantly less likely to be MTC in patients who present with sporadic disease. Therefore, C_t screening is not warranted in these subjects. Nonetheless, STIN are more likely to be neoplastic and deserve equal attention as those of the lateral lobes.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgements

The authors are deeply indebted to Prof. Sylvia L Asa, MD, PhD, Pathologist-in-Chief and Medical Director, Laboratory Medicine Program, University Health Network, University of Toronto, Canada, for her skilful comments and suggestions.

References

- 1 Hegedus L. Clinical practice: the thyroid nodule. *New England Journal of Medicine* 2004 **351** 1764–1771.
- 2 Ezzat S, Sarti DA, Cain DR & Braunstein GD. Thyroid incidentalomas. Prevalence by palpation and ultrasonography. *Archives of Internal Medicine* 1994 **154** 1838–1840.
- 3 Kebebew E, Ituarte PHG, Siperstein AE, Duh QY & Clark OH. Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. *Cancer* 2000 **88** 1139–1148.
- 4 Ashworth M. The pathology of preclinical medullary thyroid carcinoma. *Endocrine Pathology* 2004 **15** 227–231.
- 5 Leboulleux S, Baudin E, Travagli JP & Schlumberger M. Medullary thyroid carcinoma. *Clinical Endocrinology* 2004 **61** 299–310.
- 6 Pacini F, Fontanelli M, Fugazzola L, Elisei R, Romei C, Di Coscio G, Miccoli P & Pinchera A. Routine measurement of serum calcitonin in nodular thyroid diseases allows the preoperative diagnosis of unsuspected sporadic medullary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 826–829.
- 7 Rieu M, Lame MC, Richard A, Lissak B, Sambort B, Vuong-Ngoc P, Berrod JL & Fombour JP. Prevalence of sporadic medullary thyroid carcinoma: the importance of routine measurement of serum calcitonin in the diagnostic evaluation of thyroid nodules. *Clinical Endocrinology* 1995 **42** 453–460.
- 8 Niccoli P, Wion-Barbot N, Caron P, Henry JF, de Micco C, Saint Andre JP, Bigorgne JC, Modigliani E & Conte-Devolx B. Interest of

- routine measurement of serum calcitonin: study in a large series of thyroidectomized patients. The French Medullary Study Group. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 338–341.
- 9 Vierhapper H, Raber W, Bieglmayer C, Kaserer K, Weinhäusl A & Niederle B. Routine measurement of plasma calcitonin in nodular thyroid diseases. *Journal of Clinical Endocrinology and Metabolism* 1997 **85** 1589–1593.
 - 10 Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A & Pacini F. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10 864 patients with nodular thyroid disorders. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 163–168.
 - 11 Vierhapper H, Niederle B, Bieglmayer C, Kaserer K & Baumgartner-Parzer I. Early diagnosis and curative therapy of medullary thyroid carcinoma by routine measurement of serum calcitonin in patients with thyroid disorders. *Thyroid* 2005 **15** 1267–1272.
 - 12 Papi G, Corsello SM, Cioni K, Pizzini AM, Corrado S, Carapezzi C, Fadda G, Baldini A, Carani C, Pontecorvi A & Roti E. Value of routine measurement of serum calcitonin concentrations in patients with nodular thyroid disease: a multicenter study. *Journal of Endocrinological Investigation* 2006 **29** 427–437.
 - 13 Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JWA & Wiersinga W. European Thyroid Cancer Taskforce. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. *European Journal of Endocrinology* 2006 **154** 787–803.
 - 14 Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri E, McIver B, Sherman SI & Tuttle RM. American Thyroid Association Guideline Taskforce. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2006 **16** 109–142.
 - 15 American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M & Wells SA Jr. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* 2009 **19** 565–612.
 - 16 Cheung K, Roman SA, Wang TS, Walker HD & Sosa JA. Calcitonin measurement in the evaluation of thyroid nodules in the United States: a cost-effectiveness and decision analysis. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2173–2180.
 - 17 Biddinger PW, Brennan MF & Rosen PP. Symptomatic C-cell hyperplasia associated with chronic lymphocytic thyroiditis. *American Journal of Surgical Pathology* 1991 **15** 599–604.
 - 18 Guyétant S, Rousselet MC, Durigon M, Chappard D, Franc B, Guerin O & Saint-André JP. Sex-related C cell hyperplasia in the normal human thyroid: a quantitative autopsy study. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 42–47.
 - 19 Wahl RA, Vorländer C, Kriener S, Pedall J, Spitz M & Hansmann ML. Isthmus-preserving total bilobectomy: an adequate operation for C-cell hyperplasia. *World Journal of Surgery* 2006 **30** 860–871.
 - 20 Carcangiu ML. Section XI, Thyroid. In *Histology for Pathologists*, 3rd edn, ch 44, pp 1129–1148. Ed SE Mills. Philadelphia, USA: Lippincott Williams & Wilkins, 1990.
 - 21 Rosai J, Carcangiu ML & DeLellis RA. *Tumors of the Thyroid Gland*, 3rd series, Fascicle 5, pp 223–229. AFIP, Armed Forces Institute of Pathology: Washington, DC 1990.
 - 22 Schmid KW, Kirchmair R, Ladurner D, Fischer-Colbrie R & Böcker W. Immunohistochemical comparison of chromogranins A and B and secretogranin II with calcitonin and calcitonin gene-related peptide expression in normal, hyperplastic and neoplastic C-cells of the human thyroid. *Histopathology* 1992 **21** 225–232.
 - 23 Sadler TW & Langman J. Medizinische embryologie die normale menschliche entwicklung und ihre fehlbildungen. *Thieme* 2003 **10** 330–335.
 - 24 Wolfe HJ, Voelkel EF & Tashjian AH. Distribution of calcitonin-containing cells in the normal adult human thyroid gland: a correlation of morphology with peptide content. *Journal of Clinical Endocrinology and Metabolism* 1974 **38** 688–694.
 - 25 Guyétant S, Wion-Barbot N, Rousselet MC, Franc B, Bigorgne JC & Saint-André JP. C-cell hyperplasia associated with chronic lymphocytic thyroiditis: a retrospective quantitative study of 112 cases. *Human Pathology* 1994 **25** 514–521.
 - 26 Heshmati HM, Gharib H, van Heerden JA & Sizemore GW. Advances and controversies in the diagnosis and management of medullary thyroid carcinoma. *American Journal of Medicine* 1997 **103** 60–69.
 - 27 Santoro M, Melillo RM, Carlomagno F, Vecchio G & Fusco A. RET: normal and abnormal functions. *Endocrinology* 2004 **145** 5448–5451.
 - 28 Gharib H, McConahey WM, Tieg RD, Bergstralh EJ, Goellner JR, Grant CS, van Heerden JA, Sizemore GW & Hay ID. Medullary thyroid carcinoma: clinicopathologic features and long-term follow-up of 65 patients treated during 1946 through 1970. *Mayo Clinic Proceedings* 1992 **67** 934–940.
 - 29 Fleming JB, Lee JE, Bouvet M, Schultz PN, Sherman SI, Sellin RV, Friend KE, Burgess MA, Cote GJ, Gagel RF & Evans DB. Surgical strategy for the treatment of medullary thyroid carcinoma. *Annals of Surgery* 1999 **230** 697–707.
 - 30 Tan GH & Gharib H. Thyroid incidentalomas; management approaches to nonpalpable nodules discovered incidentally on thyroid imaging. *Annals of Internal Medicine* 1997 **126** 226–231.
 - 31 Filetti S, Durante C & Torlontano M. Nonsurgical approaches to the management of thyroid nodules. *Nature Clinical Practice. Endocrinology & Metabolism* 2006 **2** 384–394.
 - 32 Kondo T, Ezzat S & Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nature Reviews. Cancer* 2006 **6** 292–306.
 - 33 Burgess JR & Tucker P. Incidence trends for papillary thyroid carcinoma and their correlation with thyroid surgery and thyroid fine-needle aspirate cytology. *Thyroid* 2006 **16** 47–53.
 - 34 Colonna M, Guizard AV, Schvartz C, Velten M, Raverdy N, Molinie F, Delafosse P, Franc B & Grosclaude P. A time trend analysis of papillary and follicular cancers as a function of tumour size: a study of data from six cancer registries in France (1983–2000). *European Journal of Cancer* 2007 **43** 891–900.
 - 35 Enewold L, Zhu K, Ron E, Marrogi AJ, Stojadinovic A, Peoples GE & Devesa SS. Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980–2005. *Cancer Epidemiology, Biomarkers and Prevention* 2009 **18** 784–791.
 - 36 Leenhardt L, Grosclaude P & Cherie-Challine L. Thyroid Cancer Committee. Increased incidence of thyroid carcinoma in France: a true epidemic or thyroid nodule management effects? Report from the French Thyroid Cancer Committee *Thyroid* 2004 **14** 1056–1060.

Received 28 August 2009

Accepted 29 September 2009