Impact of pregnancy on prognosis of differentiated thyroid cancer: clinical and molecular features

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Abstract

Objective: Differentiated thyroid cancer (DTC) commonly occurs in women of child-bearing age and represents the second most frequent tumor diagnosed during pregnancy only behind breast cancer. It is possible that associated physiological changes could favor tumor development and growth. However, few data are available about the outcome of DTC related to pregnancy, leading to conflicting results.

Methods: Among the study population, 340 patients with DTC <45 years old were retrospectively studied. Patients were divided into three groups according to the time of tumor diagnosis in respect of pregnancy. Group 1, diagnosis of DTC at least 2 years after delivery; group 2, diagnosis during pregnancy or within the second year after delivery; and group 3, nulliparous patients at the time of diagnosis. We evaluated clinical outcome and immunohistochemical expression of estrogen receptor α (ER α), ER β , progesterone receptor, and aromatase. We also analyzed the gene expression of *NIS* (*SLC5A5*) and the prevalence of *BRAF*^{V600E} mutations.

Results: Persistence/recurrence of disease was significantly higher in group 2 patients than control groups ($P=0.023$). No significant differences were observed in other clinical parameters. Furthermore, no differences among the groups were recorded about ER pattern, *NIS* expression, and *BRAF* mutations.

Conclusions: Persistence/recurrence of DTC is significantly higher in pregnant patients, suggesting that pregnancy could really exert a negative prognostic role in patients with DTC. The underlying mechanisms are not yet clarified and further studies are required. Our results suggest that a more careful follow-up is needed when diagnosis of DTC occurs during pregnancy or shortly after.

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Introduction

Differentiated thyroid cancer (DTC) is a relatively rare neoplasia. It represents 3.6% of all malignant tumors in the USA (SEER Cancer Statistics Review, National Cancer Institute Surveillance, Epidemiology, and End Results; 1975–2005, available from <http://seer.cancer.gov>) and it is generally characterized by good prognosis. Consequently, studies evaluating the prognosis of this tumor

have to consider a wide number of cases and a long-term follow-up to highlight the differences in survival or disease recurrence rate. The majority of relapses usually occurs within 5 years from the initial treatment, and only sporadic cases have been subsequently documented (1, 2). Despite the low incidence, in the USA, DTC represents the second most frequently diagnosed tumor

during pregnancy, only after breast cancer. In women of child-bearing age, about 10% of thyroid carcinomas are diagnosed during pregnancy or early after delivery (SEER Cancer Statistics Review, National Cancer Institute Surveillance, Epidemiology, and End Results; 1975–2005, available from <http://seer.cancer.gov>). These findings have led us to hypothesize that during this period the presence of several physiologic changes, such as hormonal secretion, growth factors, and negative iodine balance, could create a favorable environment for the development and growth of tumors.

However, only a few studies about the outcome of DTC related to pregnancy have been published. A recent review (3) has reported that pregnancy is not generally described in literature as a determining condition for prognosis of DTC, neither in terms of DTC-related death (4), nor of overall survival (5, 6).

Nevertheless, these findings are in contrast with the more recent study published by Vannucchi *et al.* (7), who reported that DTC in pregnant women had a significant increase of persistent/recurrent disease than those in nonpregnant patients. Since the parameters and the methodology in each study were very different, their results were not easily comparable. In fact, the studies conducted by Yasmeen *et al.* (5) and Herzon *et al.* (6) focused mainly on the overall survival, while the study of Moosa & Mazzaferri (4) had DTC-related death and disease recurrence, evaluated by biopsy or by I-131 uptake in distant sites, as primary focus. On the contrary, Vannucchi *et al.* (7) have evaluated persistent/recurrent DTC through more sensitive tests, such as basal and stimulated thyroglobulin (Tg) levels after exogenous thyroid-stimulating hormone (TSH) injection (recombinant human TSH (rh-TSH), Thyrogen, Genzyme Corporation, Sanofi Company, Cambridge, MA, USA), which have not been used in the other studies and may – at least partially – explain the different conclusions.

Moreover, Vannucchi *et al.* (7) observed a significantly higher immunohistochemical expression of the estrogen receptor α (ER α) in tumors sampled from pregnant women, compared with the control groups. With special reference to hormone receptor expression in thyroid tumors, a recent study (8) on the immunohistochemical expression of ER and androgen receptor (AR) in DTC showed that ER α was acquired or increased in tumor samples as compared with the corresponding normal tissue, whereas AR and ER β expression was decreased in tumors compared with the surrounding normal tissue. These patterns appeared also to be associated with the

clinical behavior, being the high expression of ER α and AR and the low expression of ER β associated with a more aggressive phenotype.

In such a controversial situation, we therefore designed the present study to characterize at clinical, phenotypical, and molecular levels DTC cases in pregnancy as compared with matched control groups.

Subjects and methods

Patients

We retrospectively evaluated more than 1200 medical records of patients with DTC treated and followed-up from 2001 to 2011 at the Nuclear Medicine Department of Mauriziano Hospital, which covers up to 80% of all radioiodine ablations with I-131 (RAI) performed in the Piedmont region. This allowed us to obtain an extremely homogeneous population, representative of DTC epidemiology in the fertile women population.

Among them, 340 women were selected according to the following inclusion criteria:

- age ≤ 45 years at the time of surgery
- total thyroidectomy
- I-131 radioiodine ablation
- Levo thyroxine (L-T₄) TSH-suppressive therapy (TSH ≤ 0.1 mU/l) (9)
- follow-up ≥ 1 year
- rh-TSH test during follow-up or persistent disease (Tg measured under suppressive therapy with L-T₄ (S-Tg) > 2 ng/ml)

Patients were divided into three groups according to the time of diagnosis of DTC with respect to pregnancy. Group 1 included women ($n=152$, median age 40, range 25–45) with diagnosis of DTC at least 2 years after delivery. Group 2 included women ($n=38$, median age 35, range 26–41) with diagnosis of DTC during pregnancy or within 2 years after delivery. Group 3 included nulliparous patients at the time of diagnosis ($n=150$, median age 30, range 15–45).

Tumors were classified following the World Health Organization classification (10), staged according to the 6th edition of TNM staging (American Joint Committee on Cancer (AJCC)) (11), and classified as low and high risk according to the European Consensus Statement criteria (9). ETA guidelines divide patients into three groups: very low, low, and high risk, but the first group was not represented in our series because it includes patients with no indications for RAI.

Remission or persistent/recurrent disease was defined according to the European and American guidelines for the management of DTC (9, 12):

- remission: S-Tg and Tg measured after stimulation with rh-TSH (rh-TSH-Tg) $<0.6 \mu\text{g/l}$, negative anti-Tg antibody (AbTg), and normal neck ultrasound.
- persistent/recurrent disease: at least one of the following criteria:
 - S-Tg $>2 \mu\text{g/l}$.
 - rh-TSH-Tg $>2 \mu\text{g/l}$.
 - persistence of AbTg >4 years with a trend to increase (an increasing antibody production or new antibodies appearance as a consequence of an increase in autoantigen production) (13, 14)
 - neck or distant metastasis
 - radioiodine uptake outside thyroid bed.

Serum Tg levels were measured during $L\text{-T}_4$ withdrawal, immediately before RAI and after 12 months of $L\text{-T}_4$ suppressive therapy. Then, patients received one injection of rh-TSH (0.9 mg i.m., Thyrogen, Genzyme Corporation, Sanofi Company) for 2 consecutive days; serum samples for TSH and Tg measurements were collected on day 0 (before first rh-TSH administration), day 3, and day 4. Neck ultrasonography was performed 6 and 12 months after RAI. TSH levels were evaluated using a chemiluminescent immunoassay (Access Immunoassay Systems, Beckman Coulter, Inc., Brea, CA, USA). Tg levels were determined using chemiluminescent immunoassay (Access Immunoassay Systems, Beckman Coulter, Inc.), with a functional sensitivity of $0.6 \mu\text{g/l}$; AbTg were detected with chemiluminescent immunoassay (Access Immunoassay Systems, Beckman Coulter, Inc.). On the basis of Tg assay, we considered $0.6 \mu\text{g/l}$ as the cut-off value between undetectable and measurable Tg levels, according to Mazzaferri *et al.* (1).

Immunohistochemical analysis of hormone receptors and aromatase in tumor tissues

Immunohistochemical evaluation of ER α , ER β , progesterone receptor (PGR), and aromatase was performed in 37 histological specimens selected from the three different groups (12 of group 1, ten of group 2, and 15 of group 3). The cases for immunohistochemical analysis were blinded, selected to obtain three groups homogenous in terms of age and stage, regardless of the outcome.

Immunohistochemical analyses were carried out on paraffin-embedded tissue sections of $5 \mu\text{m}$, after dewaxing, dehydration in alcohol, and rehydration in PBS, pH 7.5.

Endogenous peroxidase block was performed through immersion of the slides in 0.3% solution of methanol and hydrogen peroxide for 15 min. Then, the sections were incubated with the following monoclonal primary antibodies: ER α (clone 1D5, dilution 1:300, Dako, Glostrup, DK), ER β (clone PPG5/10, dilution 1:50, Dako), PGR (clone 636, dilution 1:300, Dako), and aromatase (clone mca2077s, dilution 1:50, Serotec, Kidlington, UK). A biotin-free, dextran chain-based detection system (EnVision, Dako) and diaminobenzidine as the chromogen were used according to standard protocols. All markers were assessed in tumoral and peritumoral tissues using H-score evaluation, which takes into account both quantitative and qualitative expression with a 0–300 range scale.

Molecular analysis

Nucleic acids isolation ► Genomic DNA was isolated from formalin-fixed, paraffin-embedded tissues using QIAamp DNA Mini Kit (Qiagen). RNA was isolated from paraffin-embedded material using the high pure RNA paraffin kit (Roche), following the manufacturer's instructions. The quantity of isolated DNA and RNA was assessed using a Biophotometer (Eppendorf, Hamburg, Germany).

BRAF point mutation analysis ► The presence of *BRAF* point mutation (V600E) was analyzed using pyrosequencing and PCR primers following previously published protocols (15). PCR amplification for the pyrosequencing assay was carried out according to standard protocols. The amplicons were mixed with sequencing primers and sequencing was performed using a PyroGold Reagent Kit (Biotage AB, Uppsala, Sweden) according to the manufacturer's protocol. Results were analyzed using the PSQ-96 MA 2.0.2 Software (Biotage AB).

Quantitative real-time PCR for the sodium/iodide symporter ► Relative cDNA quantitation of the sodium/iodide symporter (NIS; *SLC5A5*) and an internal reference gene (β -actin) were done using a fluorescence-based real-time detection method (ABI PRISM 7900 Sequence Detection System, TaqMan; Applied Biosystems/Life Technologies). β -actin primers and probe were previously published (16), whereas for *NIS* the TaqMan gene expression assay $20\times$ (*SLC5A5* Hs00166567_m1, Applied Biosystems) was used according to the manufacturer's instructions. The PCR mixture consisted of 1200 nmol/l of each primer, 200 nmol/l probe, 200 nmol/l each of dATP, dCTP, dGTP, dTTP, 3.5 mmol/l MgCl_2 , and $1\times$ TaqMan Universal PCR Master Mix to

a final volume of 20 μ l (all reagents were from PE Applied Biosystems). Cycling conditions were 50 °C for 2 min, 95 °C for 10 min, followed by 46 cycles at 95 °C for 15 s and 60 °C for 1 min. To analyze target gene expression in individual tumors, the relative gene expression levels were expressed as ratios (differences between the C_t values) between two absolute measurements (genes of interest/internal reference gene). Then, the $\Delta\Delta C_t$ values were calculated subtracting ΔC_t values of each case to the value of the normal sample expression, and converting the ratio by the $2^{-\Delta\Delta C_t}$ formula; cases were considered of low or high expression according to the median expression level obtained.

Statistical analysis

The clinical (age, outcome, number of treatments, ablation-Tg levels, and high/low risk classification) and pathological/molecular features (histology, pTNM stage, hormone receptor expression, *NIS* gene expression, and *BRAF* mutation status) were compared among the three groups of patients by using the χ^2 -test for dichotomic variables and the Mann–Whitney *U* and Kruskal–Wallis tests for continuous variables, as appropriate. The

reciprocal correlation among immunohistochemical markers was evaluated using the Spearman's test. Statistical significance was defined as $P < 0.05$.

A logistic multivariable analysis was performed. Dependent dichotomous variable was tumor persistence/recurrence (1) or remission (0). Age, T, N, and multifocality of primary tumor and pregnancy (DTC diagnosis during pregnancy or within 2 years after delivery: 1; other groups: 0) were the independent variables. All these analyses were performed using STATISTICA for Windows, ver. 8.0.

Results

Clinical, biochemical, histopathological, and molecular parameters in the three groups are reported in Table 1. No significant differences were noticed in the number of treatments for achieving clinical remission, in the tumor size or extrathyroidal invasion, in the lymphnodal metastatic involvement at diagnosis, in histology, and in high risk/low risk classification of patients according to the ETA guidelines (9).

Clinical remission was obtained in 150/152 patients (98.7%) of group 1, 34/38 patients (89.5%) of group 2,

Table 1 Clinical, histological, and molecular characteristics of patients with a DTC diagnosis at least 2 years after delivery (group 1), during pregnancy or within 2 years after delivery (group 2), or before pregnancy/nulliparous (group 3).

	Group 1	Group 2	Group 3	P value
Age at diagnosis (years): median (range)	40 (25–45)	35 (26–41)	30 (15–45)	<0.001
Duration of follow-up (years): median (range)	5 (1–27)	6 (1–10)	6 (1–20)	0.31
Remission	150/152 (98.7%)	34/38 (89.5%)	143/150 (95.3%)	0.023 ^a
Persistence/recurrence	2/152 (1.3%)	4/38 (10.5%)	7/15 (4.7%)	
Number of treatments (average)	1.19	1.21	1.28	0.22
Ablation-HTG				
< 10 ng/ml ^b	127/152 (83.5%)	27/38 (71%)	110/150 (73.3%)	0.060
> 10 ng/ml ^b	25/152 (16.5%)	11/38 (29%)	40/150 (26.7%)	
High risk	68/152 (44.7%)	19/38 (50%)	79/150 (52.7%)	0.38
Low risk	84/152 (55.3%)	19/38 (50%)	71/150 (47.3%)	
TNM				
T < 3	105/152 (69%)	26/38 (68.4%)	95/150 (63.3%)	0.85
T > 3	47/152 (31%)	12/38 (31.6%)	55/150 (36.7%)	
N –	114/152 (75%)	29/38 (76.3%)	102/150 (68%)	0.54
N +	38/152 (25%)	9/38 (23.6%)	48/150 (32%)	
Histology				
High risk	54/152 (35.5%)	16/38 (42.1%)	62/150 (41.3%)	0.85
Low risk	98/152 (64.5%)	22/38 (57.9%)	88/150 (58.7%)	
ER α tumor expression	3/12 (25%)	3/10 (30%)	4/14 (28.6%)	0.96
ER β tumor expression	5/12 (41.7%)	5/10 (50%)	7/15 (46.7%)	0.92
PGR tumor expression	4/12 (33.3%)	3/10 (30%)	8/15 (53.3%)	0.419
<i>BRAF</i> ^{V600E} mutation	3/12 (25%)	4/9 (44.4%)	9/15 (60%)	0.19
<i>NIS</i> fold change < 1	8/12 (66.6%)	5/8 (62.5%)	9/13 (69.2%)	0.9

^aGroup 2 was significantly different as compared with both groups 1 and 3.

^bThe ablation-HTG cut-off was defined according to Webb *et al.* (17).

and in 143/150 patients (95.3%) of group 3. Persistent/recurrent disease was observed in 2/152 patients (1.3%) of group 1, 4/38 patients (10.5%) of group 2, and in 7/150 patients (4.7%) of group 3. Our results showed a significant difference (χ^2 , 7.532; $P=0.023$) in the outcome among the three groups, with a greater percentage of persistent disease in group 2 than in groups 1 and 3. Groups 1 and 3 did not show any significant difference. Only 4/38 patients in group 2 had cytological diagnosis while pregnant. They underwent thyroidectomy in the early *postpartum* period, achieving clinical remission, showing that the surgical delay of a few months was not a factor that could influence the worst outcome of group 2.

With regards to the expression of hormone receptors (Fig. 1), the percentage of intratumoral and peritumoral expression of ER α in the 37 histological samples was globally low, with no detection of significant differences between the

groups ($P=0.96$). ER β showed a high expression in the peritumoral tissue in a large number of cases, while in tumoral tissue its expression was quite variable, similarly in the three groups ($P=0.82$). PGR expression was mostly negative in peritumoral tissue, while it was quite variable in tumoral tissue, in a similar way in the three groups ($P=0.41$). A significant correlation was observed in tumor tissues between ER α and PGR (Spearman's R value, $R=0.49$; $P=0.002$). Aromatase expression was found to be negative both on peritumoral and tumoral tissue in all the samples analyzed. *BRAF*^{V600E} mutation, known as a negative prognostic factor (18), was detected in 25% in group 1, 44.4% in group 2, and 60% in group 3 (average of whole samples=43%). The difference was not statistically significant ($P=0.191$), showing that the worst outcome observed in the patients of group 2 is independent from *BRAF* mutation. However, *BRAF* was mutated in 100% of patients

Figure 1

Immunohistochemical analysis of hormone receptors. A case of multifocal papillary carcinoma (group 3) ((a) H&E, original magnification 40 $\times$), with the predominant nodule of the

follicular variant ((b) H&E, original magnification 200 $\times$), and high expression of ER α (c), ER β (d), and PGR (e) ((c, d and e) immunoperoxidase, original magnification 200 $\times$).

Table 2 Logistic regression analysis for persistence/recurrence of DTC.

Variable	RR (95% CI)	P value
Pregnancy	1.26 (0.97–1.55)	0.09
Erβ-positive staining	0.69 (0.38–1.00)	0.06
Presence of <i>BRAF</i> mutation	1.46 (1.16–1.77)	0.005
High <i>NIS</i> expression	0.66 (0.36–0.96)	0.03

with persistence of disease and in 37.5% of patients in remission, irrespective of the group.

NIS gene expression levels were also not different in the three groups ($P=0.82$) nor associated with *BRAF* mutation status ($P=0.55$).

Logistic multivariable analysis performed on the whole population (thus excluding molecular analyses) showed pregnancy (group 2) as the unique independent variable for persistent/recurrent DTC prediction. The relative risk (RR) was 1.12 (95% CI, 1.02–1.22; $P=0.02$). Age, T, N, and multifocality of the primary tumor did not enter the model.

In the 37 patients with molecular and immunohistochemical data available, *BRAF* mutation and low *NIS* expression were strong independent predictors of persistence/recurrence of DTC (Table 2), whereas ERα and PGR did not enter the model. Pregnancy and ERβ positivity were of borderline statistical significance..

Power analysis was carried out grouping the entire population into patients with DTC during or within 2 years after delivery (38 subjects) vs all other patients (302 subjects), with values of 79 and 87%, by two-sided and one-sided test respectively.

Discussion

Thyroid cancer discovered during pregnancy represents a challenge for the clinicians because, at present, there are still no reliable data available supporting a specific management of pregnancy-associated DTCs. Currently, pregnant patients with a cytologically suspicious thyroid nodule for DTC do not require surgery during pregnancy except in cases of rapid nodular growth and/or the appearance of lymph node metastases (19).

Most studies showed that pregnancy did not worsen the prognosis of DTC. In four studies, the prognosis of women with DTC diagnosed either during pregnancy or within the first *postpartum* period was compared with that of women diagnosed at another time as controls. In three of these works (4, 5, 6), no difference was found in DTC prognosis between pregnant women and control groups.

However, in the fourth study (7), Vannucchi *et al.* reported a significantly worse outcome in pregnant patients. As a matter of fact, they observed 60% of recurrent/persistent disease in pregnant women (group 2) vs 4.2% in women with DTC diagnosed more than 1 year after delivery (group 1) and 13.1% in nulliparous patients (group 3). Moreover, a higher expression of ERα in tumor samples of pregnant women was reported.

In order to verify these conflicting results, we selected a homogeneous population, dividing patients into three groups according to the criteria adopted by Vannucchi *et al.* We extended group 2 to women with DTC diagnosis within 2 years after delivery instead of 1 year, arbitrarily assuming that in tumors with low biological aggressiveness, such as DTC, pregnancy-induced hyperestrogenism may exert its tissue activity in a longer period. To our knowledge, no published data are available on this issue. Moreover, in our population the rate of persistent/recurrent disease in patients diagnosed within 1 year or between 1 and 2 years after delivery was very similar (9.5%, 2/21 cases and 11.7%, 2/17 cases respectively). However, all the patients (14/14) diagnosed between 2 and 3 years after delivery displayed clinical remission.

Consistent with the data reported by Vannucchi *et al.*, we confirmed a significant correlation between pregnancy and a worse outcome of DTC ($P=0.023$), representing the unique independent variable for persistent/recurrent disease prediction.

Indeed, thyroid cancer diagnosed during pregnancy (group 2) was found to be significantly associated with persistence or relapse of DTC compared with those diagnosed more than 2 years after delivery (group 1) or before pregnancy (group 3).

Taken together, recent evidence has supported the hypothesis that pregnancy may negatively affect the prognosis of DTC. The discrepancy with previous studies could be attributed to the different criteria used for the outcome evaluation, as suggested elsewhere (3). Previous papers used the overall survival, DTC-related death, and disease recurrence (evaluated by biopsy or whole-body scan) as outcome criteria, which were probably not appropriate for a long survival disease with frequent indolent course. In the present study, according to Vannucchi *et al.*, the persistence/recurrence of disease was investigated using more sensitive and precocious markers, such as basal and rh-TSH-stimulated thyroglobulin, and neck ultrasonography, as suggested by European and American guidelines (9, 12).

Nevertheless, the worst outcome in patients of group 2 cannot be referred to a higher prevalence of a worse

staging at the time of diagnosis or to a more aggressive histological phenotype because, in our study, no significant differences in the examined clinical and morphological parameters were observed.

The mechanisms by which pregnancy could affect the DTC outcome are not easily explainable. In order to verify whether molecular and/or phenotypical features influence the results above, we tested the protein expression of sex hormone receptors, as well as the gene expression of *NIS* and the prevalence of *BRAF* mutations in the three groups. Indeed, we cannot support the negative prognostic role of estrogens, as previously suggested (7), considering that our results did not show any significant expression of ER α and no differences among the three groups were observed. The discrepancy between these results has to be clarified, but a difference in the methodological approach could be considered. For example, different antibody dilutions were used in the two works (1:300 vs 1:100 dilution). However, it has to be noted that the good correlation between the low expression of ER α and PGR justifies the reliability of our findings. The immunohistochemical analysis was performed also for the detection of ER β , showing a variable expression without any significant difference among the three groups of patients. Furthermore, aromatase expression was generally very low, leading us to rule out its potential pathophysiological role.

In the multivariable logistic regression analysis, *BRAF*^{V600E} mutations were associated with a worse prognosis, but their similar distribution among the groups excludes a pathophysiological role on the poorer outcome of group 2 patients.

We hypothesized that the worse outcome of group 2 could be explained by a lower response to radioiodine therapy. As shown by the multivariable logistic regression analysis, lower expression of *NIS* is associated with a higher persistence/recurrence of DTC, but its distribution was not different among the three groups, excluding a role in affecting the outcome of group 2.

In conclusion, our results, obtained in a large homogeneous population, confirm that pregnancy could really exert a negative prognostic role, at least in terms of risk of persistent disease or recurrence, in patients with DTC. Further studies are needed to clarify the pathophysiological mechanisms. At the present state of our knowledge, a more careful follow-up is needed when diagnosis of DTC occurs during pregnancy or shortly after. However, the impact on DTC prognosis is not so heavy to justify the reconsideration of the American guidelines for the management of thyroid cancer during pregnancy (19).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Mazzaferri EL, Robbins RJ, Spencer A, Braverman LE, Pacini F, Wartofsky L, Haugen BR, Sherman SI, Cooper DS, Braunstein GD *et al*. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 1433–1441. (doi:10.1210/jc.2002-021702)
- Durante C, Montesano T, Torlontano M, Attard M, Monzani F, Tumino S, Costante G, Meringolo D, Bruno R, Trulli F *et al*. Papillary thyroid cancer: time course of recurrences during postsurgery surveillance. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 636–642. (doi:10.1210/jc.2012-3401)
- Alves GV, Santin AP & Furlanetto TW. Prognosis of thyroid cancer related to pregnancy: a systematic review. *Journal of Thyroid Research* 2011 **2011** 691719. (doi:10.4061/2011/691719)
- Moosa M & Mazzaferri EL. Outcome of differentiated thyroid cancer diagnosed in pregnant women. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2862–2866. (doi:10.1210/jcem.82.9.4247)
- Yasmeen S, Cress R, Romano PS, Xing G, Berger-Chen S, Danielsen B & Smith LH. Thyroid cancer in pregnancy. *International Journal of Gynaecology and Obstetrics* 2005 **91** 15–20. (doi:10.1016/j.ijgo.2005.06.022)
- Herzon FS, Morris DM, Segal MN, Rauch G & Parnell T. Coexistent thyroid cancer and pregnancy. *Archives of Otolaryngology – Head and Neck Surgery* 1994 **120** 1191–1193. (doi:10.1001/archotol.1994.01880350009002)
- Vannucchi G, Perrino M, Rossi S, Colombo C, Vicentini L, Dazzi D, Beck-Peccoz P & Fugazzola L. Clinical and molecular features of differentiated thyroid cancer diagnosed during pregnancy. *European Journal of Endocrinology* 2010 **162** 145–151. (doi:10.1530/EJE-09-0761)
- Magri F, Capelli V, Rotondi M, Leporati P, La Manna L, Ruggiero R, Malovini A, Bellazzi R, Villani L & Chiovato L. Expression of estrogen and androgen receptors in differentiated thyroid cancer: an additional criterion to assess the patient's risk. *Endocrine-Related Cancer* 2012 **19** 463–471. (doi:10.1530/ERC-11-0389)
- Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, 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. (doi:10.1530/eje.1.02158)
- Hedinger C. In *Histological Typing of Thyroid Tumors: WHO International Histological Classification of Tumors*, 2nd edn. Berlin–Heidelberg–New York: Springer–Verlag, 1988.
- UICC. In *TNM Classification of Malignant Tumours*, 6th edn. New York: Wiley Liss, 2002.
- Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI & Tuttle RM. Management

- guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2006 **16** 109–142. (doi:10.1089/thy.2006.16.109)
- 13 Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, Grasso L & Pinchera A. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Annals of Internal Medicine* 2003 **139** 346–351. (doi:10.7326/0003-4819-139-5_Part_1-200309020-00010)
 - 14 Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS & Nicoloff JT. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1121–1127. (doi:10.1210/jcem.83.4.4683)
 - 15 Volante M, Rapa I, Gandhi M, Bussolati G, Giachino D, Papotti M & Nikiforov YE. RAS mutations are the predominant molecular alteration in poorly differentiated thyroid carcinomas and bear prognostic impact. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 4735–4741. (doi:10.1210/jc.2009-1233)
 - 16 Ceppi P, Volante M, Novello S, Rapa I, Danenberg KD, Danenberg PV, Cambieri A, Selvaggi G, Saviozzi S, Calogero R *et al.* ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. *Annals of Oncology* 2006 **17** 1818–1825. (doi:10.1093/annonc/mdl300)
 - 17 Webb RC, Howard RS, Stojadinovic A, Gaitonde DY, Wallace MK, Ahmed J & Burch HB. The utility of serum thyroglobulin measurement at the time of remnant ablation for predicting disease-free status in patients with differentiated thyroid cancer: a meta-analysis involving 3947 patients. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 2754–2763. (doi:10.1210/jc.2012-1533)
 - 18 Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, Kim KW, Hahn SK, Youn YK, Kim KH *et al.* The association of the BRAF(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. *Cancer* 2012 **118** 1764–1773. (doi:10.1002/cncr.26500)
 - 19 Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S *et al.* Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and *postpartum*. *Thyroid* 2011 **21** 1081–1125. (doi:10.1089/thy.2011.0087)

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