REVIEW

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Pathogenesis, diagnosis and management of thyroid nodules in children

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Abstract

According to the literature thyroid nodules are quite rare in the first two decades of life. However, there are some exceptions, relating to areas with an iodine deficiency or affected by radioactive fallout, where the risk of nodules and carcinomas is increased. Therefore, it is a great challenge for the physician to distinguish between benign and malignant lesions preoperatively, and not only in these areas of greater risk. A careful work-up, comprising the patient's history, clinical examination, laboratory tests, thyroid ultrasound, scintigraphy, fine-needle aspiration biopsy (FNAB) and molecular studies, is mandatory to improve the preoperative diagnosis. The differential diagnosis should also include benign thyroid conditions such as: (i) congenital hypothyroidism due to dyshormonogenesis or ectopy, (ii) thyroid hemiagenesis, (iii) thyroglossal duct cyst, (iv) simple goiter, (v) cystic lesion, (vi) nodular hyperplasia, (vii) follicular adenoma, (viii) Graves' disease and (ix) Hashimoto thyroiditis, all of which can predispose to the development of thyroid nodules. The majority of thyroid carcinomas derive from the follicular cell (papillary, follicular, insular and undifferentiated (or anaplastic) thyroid carcinoma), whereas medullary thyroid carcinoma derives from calcitonin-producing cells. Inherited forms of thyroid cancer may occur, especially in relation to medullary thyroid carcinoma. FNAB is a critical factor in establishing the preoperative diagnosis. However, we should keep in mind the fact that a conventional cytological evaluation can miss the neoplastic nature of a lesion and the employment of immunocytochemical and molecular studies of aspirates from FNAB can give us a more precise diagnosis of neoplasia in thyroid nodules once they are detected.

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Introduction

Thyroid nodules are uncommon in children before puberty (1.5% or less) (Kirkland *et al*. 1973, Rallison *et al*. 1975, Scott & Crawford 1976, Yip *et al*. 1994, Millman & Pellitteri 1997). Any nodule discovered in such an age group should therefore be viewed with suspicion and the diagnostic approach should be more aggressive in children than in adults (Scott & Crawford 1976, Silverman *et al*. 1979, Ridgway 1991) because they are more often malignant than in adults (Belfiore *et al*. 1989). The mean incidence of thyroid carcinomas in childhood thyroid nodules which were operated on is summarized in Table 1 and shows an overall 26.4% risk of cancer.

The sex distribution in a group of all-adult patients with thyroid carcinoma is different from that in children. In adults, women outnumber men 4:1, whereas in children below 15 the ratio of girls to boys is 1.5:1 and in patients aged 15–20 the female/male ratio is 3:1 (Attie 1996). The available data show that males and children under 10 years are at higher risk of cancer, and this is in agreement with data from other authors (Yip *et al*. 1994). Age is also the major determinant of recurrence in pediatric differentiated thyroid carcinoma, particularly in those younger than 10 years (Alessandri *et al*. 2000, Jarzab *et al*. 2005).

Thyroid nodular disease (TND) comprises a wide spectrum of disorders including a solitary nodule, multinodular goiter (MNG), nodular goiter observed in autoimmune thyroid disease (AITD), i.e. chronic lymphocytic thyroiditis (Hashimoto thyroiditis (HT)) or Graves' disease (GD) and also occurring in the form of nonpalpable thyroid nodules. In

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Table 1 Incidence of thyroid carcinoma in childhood thyroid nodules

Report	Number	%	References
1	69/138	50.0	Hayles *et al.* (1960)
2	9/44	20.4	Adams (1967)
3	9/38	23.7	Psarras *et al.* (1972)
4	12/30	40.0	Kirkland *et al.* (1973)
5	6/36	16.7	Scott & Crawford (1976)
6	10/49	20.4	Valentin *et al.* (1986)
7	12/58	20.7	Desjardins *et al.* (1987)
8	11/109	9.2	Belfiore *et al.* (1989)
9	7/32	21.9	Fowler *et. al.* (1989)
10	10/57	17.5	Raab *et al.* (1995)
11	41/148	27.7	Attie (1996)
12	17/52	32.7	Lafferty & Batch (1997)
13	26/71	36.6	Millman & Pellitteri (1997)
11	5/24	20.8	Lugo-Vicente *et al.* (1998)
12	15/93	16.1	Hung (1999)
13	7/60	11.7	Wasikowa *et al.* (1999)
14	3/31	9.7	Arda *et al.* (2001)
15	4/18	22.2	Blackburn *et al.* (2001)
16	37/155	23.9	Niedziela *et al.* (2004)
Overall	299/1134	26.4	

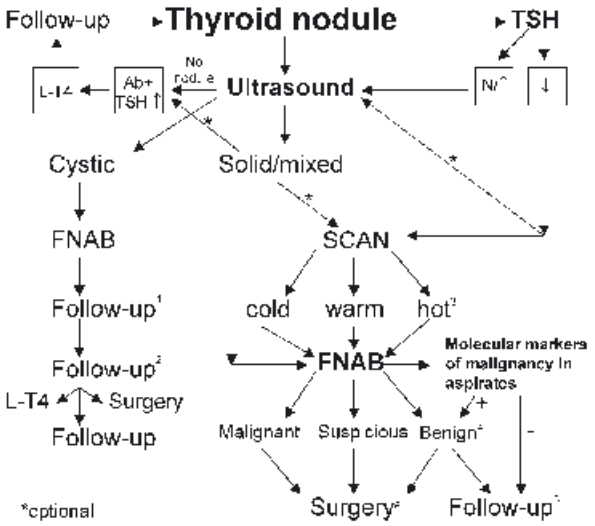

Figure 1 A possible diagnostic work-up in palpable thyroid nodules.[1]Control visit after 4 weeks (clinical examination plus ultrasound; FNAB – if indicated by, for example, palpable solid remnants of the cyst or peripherally localized solid tissue within the cystic lesion). [2]Next visit after 6–8 weeks and then a 3 month interval (supplementary L-T4 therapy if indicated; surgery if relapse). [3]Higher risk of malignancy in nonclassic subtype. [4]Consider molecular markers: if positive–surgery, if negative–surgery in cold and hot nodules vs follow-up in a warm nodule. [5]Next visit after 4–6 weeks and then a 3 month interval (L-T4 – if indicated and consider FNAB or direct surgery if tumor enlargement or suspicious appearance on US). [6]Any palpable solid/or mixed, cold/or hot nodule should be removed even with benign cytology but the time and the extent of surgery may differ, depending on all the diagnostic data.

both autoimmune disorders (HT and GD) the pediatrician should beware because a neoplastic lesion may be small and difficult to detect when palpating any gland with an altered consistency. Thyroid nodules may be detected during a routine physical examination, can be discovered by the patient himself or found incidentally during imaging techniques of the neck for thyroid or nonthyroid disorders. Firm fibrous or stony hard nodules, especially if fixed to surrounding structures and not moving on swallowing, or if paralysis of the vocal cords is present, are highly suggestive of carcinoma (Blum 1978, Ingbar & Woeber 1985). However, such forms of TND are now being observed quite rarely (Niedziela 2002). The aim of the present paper is to summarize the clinical management of thyroid nodules in children and to propose a work-up which is very likely to diagnose benign or malignant thyroid neoplasia, preoperatively. The standard diagnostic protocol of thyroid nodules consists of: (i) patient's history including the prior existence and treatment of a benign thyroid disease, (ii) clinical examination, (iii) laboratory tests, (iv) thyroid ultrasound (US), (v) scintigraphy (SC), (vi) fine-needle aspiration biopsy (FNAB) and (vii) molecular studies employed for the detection of malignancy as a part of clinical research (Salas 1995, Korman & Niedziela 2001, Koutras 2001, Wiersinga 2001, Niedziela 2002, Halac & Zimmerman 2005). I would propose a diagnostic protocol (Fig. 1) based on the increasing numbers of children with TND who were diagnosed in Poland, a country with endemic goiter due to iodine deficiency (Korman *et al.* 1999, Niedziela 2002, Niedziela *et al.* 2004). With this protocol many of the patients are qualified for surgery because of the high prediction for thyroid neoplasia. Special attention will be given to the usefulness of thyroid US in this diagnostic protocol and also for the subsequent follow-up.

All the available patient data, and not just one or a few factors, are important when choosing the best therapeutic strategy to adopt for each individual child. It is important to realize that if a malignant lesion is detected preoperatively then the need for reoperation is unlikely. However, if a nodule is diagnosed preoperatively as false-negative in terms of cancer, then reoperation is necessary and the risk of iatrogenic effects (hypoparathyroidism, injury to the laryngeal nerve) is greater.

Patient's history

Complaints, such as pain, tenderness, compression of the respiratory tract, problems with swallowing or inappropriate fixation of the neck, are not reported by the majority of young patients with thyroid nodules. No cases of permanent vocal fold paralysis were found in a group of 37 children with thyroid carcinoma in our region in the years 1996–2000 (Niedziela 2002). However, this feature has been reported in adults by some authors (Pacini & DeGroot 2001, Hegedus *et al.* 2003). There are also insufficient data to support the idea that rapid enlargement of a thyroid nodule is pathognomic of malignancy. The majority of thyroid tumors grow slowly and any rapid enlargement is probably the result of a hemorrhage into the nodule, which may be accompanied by pain and further degenerative changes within the nodule. If pain, heat and redness of the skin over the nodule are found, then suppurative thyroiditis is very likely and a full and precise inflammatory work-up should be performed (Rabska-Pietrzak *et al.* 1998, Niedziela 2002, Gawrysiak & Niedziela 2005).

Exposure to radiation – external or internal

There was a significant increase in the number of thyroid nodules in children in the 1950s, apparently as a result of previous irradiation of the head, neck and upper thorax used as a form of therapy for childhood conditions such as acne, enlarged tonsils and hemangiomas (Duffy & Fitzgerald 1950, Hempelmann 1968, De Groot & Paloyan 1973, Refetoff *et al.* 1975, Favus *et al.* 1976). After radiation ceased to be used for the treatment of these conditions the peak of thyroid carcinomas, which occurred between 1954 and 1960, declined by half (White & Smith 1986). External radiation is still used for the treatment of several childhood disorders such as in patients before bone marrow transplantation (BMT) and for patients with Hodgkin's lymphoma (HL).

Patients undergoing BMT preceded by radiation therapy are at increased risk of developing thyroid cancer (Rovelli *et al.* 1997, Cohen *et al.* 2001) and they should therefore be followed closely by periodic thyroid US. Sklar *et al.* (2000) reported that patients with HL who were treated with radiation had a higher risk of developing not only thyroid cancer and nodules but also hypo- and hyperthyroidism. The majority of their patients (95%) received more than 1000 cGy of radiation, thus showing the dose-dependent effects. All nodules, if larger than a few millimeters in diameter, should thereafter be evaluated with FNAB in such patients (Halac & Zimmermann 2005).

Childhood exposure to external radiation has also been associated with hyperparathyroidism, salivary gland neoplasms and neural tumors of the head and neck. Children treated with radiotherapy to the neck, or exposed to environmental radiation, are at risk of developing cancer later in life (Nikiforov & Fagin 1997, Eden *et al.* 2001). Looking long-term, individuals who develop one radiation-associated neoplasm may be at increased risk of developing a second one in later life (Inskip 2001).

An internal uptake of radioiodine 131 occurred in the Ukraine, Belarussian and neighboring regions, including Poland, following the Chernobyl disaster in 1986 (Williams 1996). Iodine-deficient subjects in Poland, particularly children who, being at a very dynamic stage of development, were extremely sensitive to this exposure. The children in Belarus responded earlier to this exposure (Baverstock *et al.* 1992, Kazakov *et al.* 1992, Demidchik *et al.* 1994, Leenhardt & Aurengo 2000) as did those in the Ukraine (Likhtarev *et al.* 1995, Tronko N *et al.* 1996, Tronko MD *et al.* 1999). Children who accumulated smaller amounts of radioiodine, as a result of living farther from the disaster, appear to have responded later (latent period) (Kirkland *et al.* 1973). We know from the literature that young children, below the age of 2, are the most sensitive for the risk of induction of radiation-induced papillary thyroid carcinoma (PTC) (Nikiforov *et al.* 1996, Pacini *et al.* 1997, Wiersinga 2001). Of a cohort of our patients, the children aged between 1 and 2 years of postnatal life at the time of Chernobyl disaster showed the highest incidence of cancer. However, of the 15 children in our study who were born after Chernobyl and subsequently operated on, five were fetuses at the time of the catastrophe, whereas the other ten were born after 1 January 1987 and thus missed direct irradiation (Niedziela 2002). Italian children, although exposed to Chernobyl fallout, were farther away and did not present any increase in the incidence of thyroid cancer (Chiesa *et al.* 2004).

The prior existence and treatment of a benign thyroid disease

Congenital hypothyroidism (CH) and the risk of cancer

There is an increased risk of thyroid nodules in children with CH due to dyshormonogenesis or to

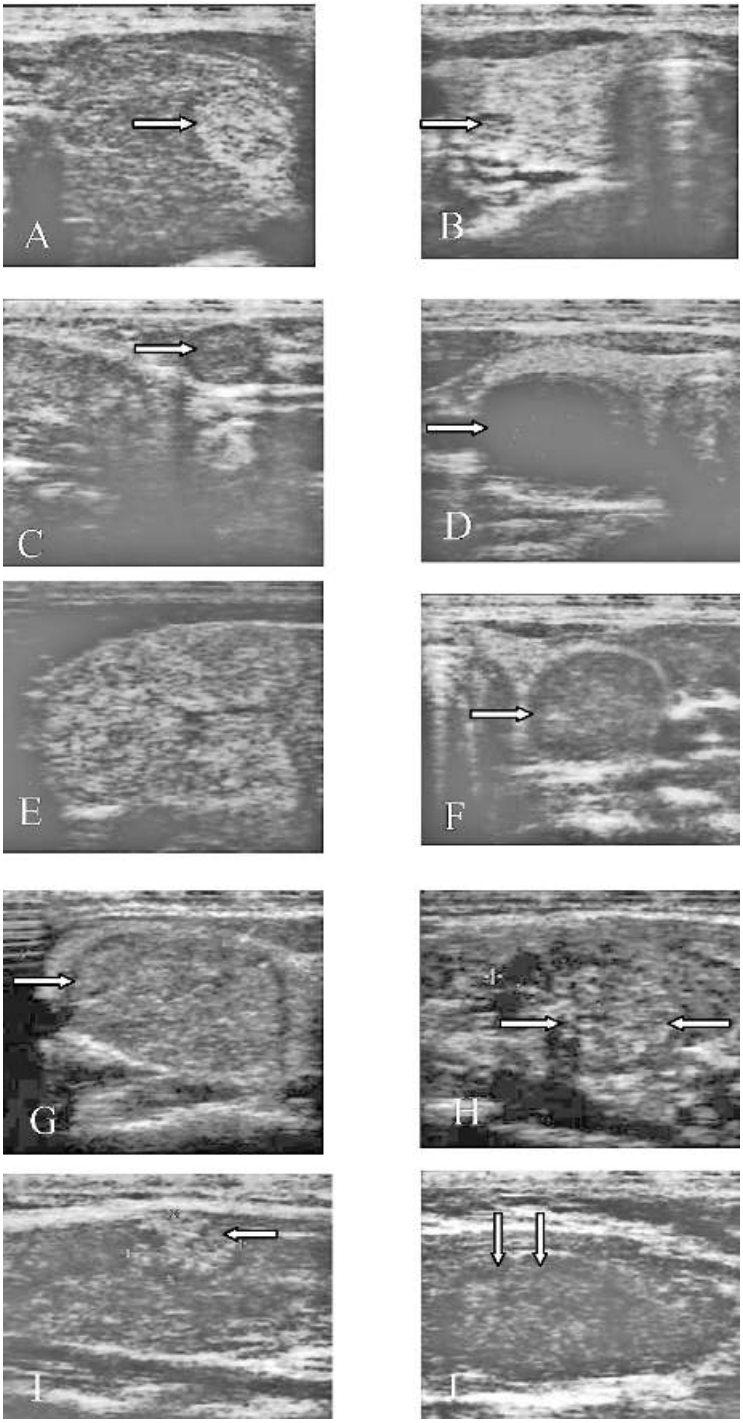

Figure 2 Ultrasound imaging of selected forms of thyroid nodular disease. (A) Nodule in congenital hypothyroidism (solid hyperechogenic vs hypoechogenic extranodular area after L-T4 withdrawal for 2 weeks). (B) Hemiagenesis of thyroid (enlarged right lobe with a small mixed lesion). (C) Hyalinizing thyroglossal duct cyst localized outside thyroid. (D) Cystic nodule (unechogenic lesion). (E) Familial multinodular goiter (solid isoechogenic lesions). (F) Oxyphilic follicular adenoma (solid hypoechogenic lesion). (G) Atypical follicular adenoma (solid hypoechogenic lesion). (H) Papillary thyroid carcinoma coexisting with Graves' disease (solid hyperechogenic lesion vs hypoechogenic thyroid). (I) Papillary thyroid carcinoma coexisting with autoimmune Hashimoto thyroiditis (solid hyperechogenic lesion vs hypoechogenic thyroid). (J) Papillary thyroid carcinoma coexisting with autoimmune Hashimoto thyroiditis (solid hypoechogenic lesion within the less hypoechogenically remaining part of thyroid).

an iodine transporter defect. Both disorders can lead to neoplastic transformation in the thyroid if the thyrotropin (TSH) level is raised for a prolonged period as a result of inappropriate L-thyroxine (L-T4) adjustment (Medeiros-Neto & Stanbury 1994). Usually incidentalomas occur but, in some cases, either palpable nodules responding to L-T4 therapy (TSH-dependent mechanism) or thyroid neoplasms (benign or malignant; TSH-independent mechanism) were observed (Fig. 2A). The majority of thyroid carcinomas in CH patients have been of the follicular type (Potter & Morris 1935, McGirr *et al*. 1959, Crooks *et al*. 1963, Medeiros-Neto & Oliveira 1970, Cooper *et al*. 1981, Watanabe 1983, Medeiros-Neto *et al*. 1998, Niedziela 2002), but one case of PTC was reported by Yashiro *et al*. (1987).

The severity of the disease varies but, in the late-onset form, the clinical course is generally mild or moderate. Goiter is only present in a minority of CH patients soon after birth and, in these children, dyshormonogenesis (mainly due to a thyroperoxidase (TPO) defect) or diminished iodine transport (due to an Natrium-Iodide symporter defect) are very likely (Gruters 1992).

The relevance of TPO in follicular thyroid carcinoma (FTC) is strongly supported by the findings that TPO gene expression is suppressed in differentiated thyroid carcinomas (Tanaka *et al*. 1996). In the affected CH patients, either follicular carcinoma (Medeiros-Neto *et al*. 1998, Niedziela 2002), follicular adenoma (FA) (Kotani *et al*. 1999, Niedziela *et al*. 2001, Niedziela 2002) or an MNG (Nascimento *et al*. 2003) were diagnosed.

Thyroid hemiagenesis (TH) and the risk of cancer

In general, this rare clinical state is found more frequently in females and in the left lobe. There are data in the literature showing that the coexistence of TH with thyroid cancer is possible in adults (Huang *et al*. 2002, Pizzini *et al*. 2005) but this has not been reported in children. TH is not directly involved in tumorigenesis. However, the reduced volume of the gland may be more pronounced during puberty when there is a greater need for thyroid hormones, thereby leading to a relatively insufficient thyroid function. This may stimulate a compensatory mechanism of thyroid hyperplasia (and goiter formation) to balance the hormone demand for normal body development. Such a unilateral goiter mimics a thyroid nodule and therefore should be screened carefully, since the coexistence of a nodule is likely (Fig. 2B). This is not a common clinical situation but it should be considered in the differential diagnosis of TND. Supplementation with L-T4 is effective in reducing thyroid volume and thus reducing the likelihood of subsequent nodule formation.

Thyroglossal cyst and the risk of cancer

Thyroglossal duct cysts are the most common developmental anomalies present during thyroid development (Weiss & Orlich 1991). They are usually localized in the midline between the base of the tongue and the hyoid bone. However, a mediastinal localization has also been reported (Reed Larsen 1998). According to LiVolsi (1974), 7% of the population have persistent abnormalities of this duct but, according to McHenry *et al*. (1993), the risk of cancer development is minimal (less than 1%). Thyroid carcinoma of such origin has only been reported in eight children to date (Patti *et al*. 2000). My own personal data relating to these cysts support the benign finding but it is difficult to predict these tumors' behavior in later years. Three of these children underwent surgery (Fig. 2C). Of the other three cases of thyroglossal duct cysts, total regression occurred spontaneously in all but one, in whom the cyst disappeared with L-T4 therapy.

Simple goiter and the risk of cancer

It is difficult to prove that a simple goiter may predispose to further neoplastic change or even the development of cancer. There are some physiological conditions (growth spurt, pregnancy, breast-feeding) in which the total production of thyroid hormones needs to increase. If a relative iodine deficiency and/or a reduction in thyroid hormone levels occurs in such states overstimulation of the gland with high-normal levels of TSH are possible, followed by goiter formation. High-normal TSH levels, if persisting for a long time, may also initiate microfocal lesions within the thyroid. Such a process can be manifested clinically in a macroscopic form several years later as either a solitary nodule or multiple nodules with benign characteristics (Studer & Derwahl 1995, Derwahl *et al*. 1999).

Thyroid cyst and the risk of cancer

Cysts are the most frequently encountered solitary palpable nodules which do not have a neoplastic background and which are included in the benign

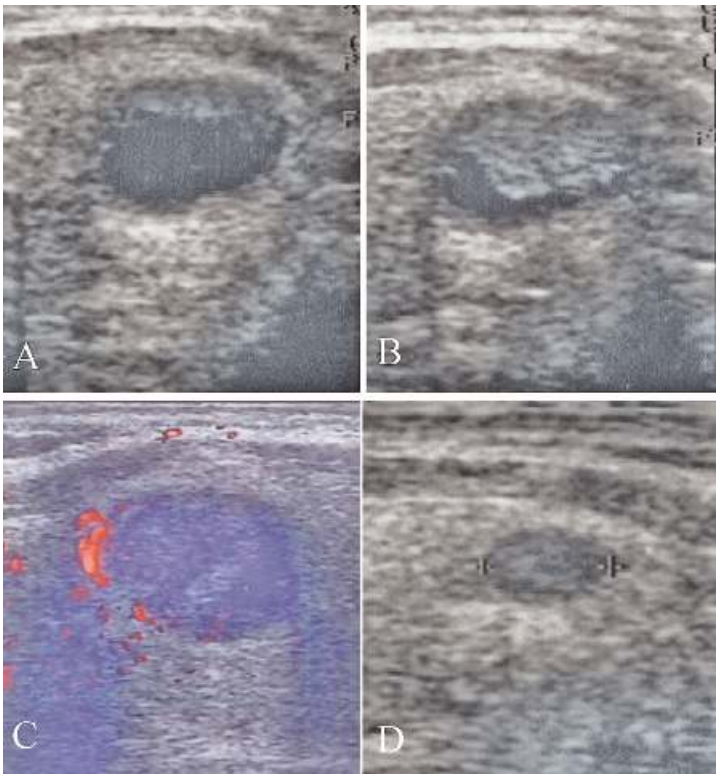

Figure 3 Ultrasonographic dynamics in the course of a benign non-neoplastic lesion (hemorrhagic cyst). From appearance (A) via hematoma post-FNAB (B), hematoma on Doppler (type I vascularization – lack of intranodular vascular network) (C), to almost complete resorption within 6 weeks (D). Longitudinal projection (from Niedziela 2002).

degenerative thyroid diseases. This is an inappropriate idea because there is a great heterogeneity of these disorders in children, ranging from benign pure cysts (Fig. 2D and Fig. 3), to malignant lesions. Yoskovitch *et al*. (1998) in a study of 24 children with cystic lesions found cancer in two and FAs in four others. It is difficult to draw any conclusions on the basis of this single paper because of the small numbers involved and because the findings included the mixed lesions (solid-cystic) which are so commonly related to neoplasia. Pure cysts should undergo a standard diagnostic work-up with US-guided FNAB and cytological evaluation. If there are solid structures connected to the cyst wall, or close to it, then such material should be obtained for further analysis to exclude cancer (Niedziela 2002, Papotti *et al*. 2002).

Nodular hyperplasia and FA and the risk of cancer

It can be difficult to distinguish a hyperplastic, non-neoplastic nodule from an FA and some pathologists use the term colloid or adenomatous nodule (Derwahl & Studer 2000, 2002). Hyperplastic nodules (Fig. 2E) are polyclonal in origin, whereas solitary nodules are monoclonal and are therefore true benign neoplasms (Apel 1995). Polyclonality of a hyperplastic nodule is a result of proliferation of groups of cells (Derwahl & Studer 2000), whereas a monoclonal neoplastic tumor is formed by proliferation and expansion of a single cell (Wainscoat & Fey 1990). An FA is classified as a benign neoplasm but some subtypes may be potentially malignant, e.g. FAs of Hürtle cell origin (Fig. 2F) or atypical FAs (Fig. 2G). The risk of local regrowth (relapse) of an FA, as in the case of a fetal or embryonal adenoma, cannot be ruled out. In the literature, the term FA with an undetermined prognosis also exists (Rosai *et al*. 1992). These tumors cause diagnostic problems for pathologists since they do not fulfill all the criteria for FTC, e.g. invasion of the tumor capsule through the whole thickness and/or vessel invasion (Fig. 2F and G). The suspicious features are as follows: (i) partial infiltration of the capsule, (ii) the presence of normal thyrocytes within the capsule or within neighboring lymph nodes, (iii) the growth of an oxyphilic tumor into the thickened capsule, but

without crossing its border, or (iv) the presence of a neoplastic lesion within the capsule (Rosai *et al*. 1992, Niedziela 2002). Recently the Chernobyl Pathologists Group suggested that tumors with 'borderline' features, should be classified either as 'a well-differentiated tumor of uncertain malignant potential (WDT-UMP)' if they exhibit questionable PTC-type nuclear changes, with or without questionable capsular penetration, or as 'a follicular tumor of uncertain malignant potential (FT-UMP)' if they show questionable capsular penetration without nuclear changes (Williams 2000, Hirokawa *et al*. 2002, Papotti *et al*. 2004). Complicated histological descriptions, such as those above, should alert the clinician to the need for careful clinical follow-up, because the behavior of thyrocytes remaining after partial thyroidectomy is unpredictable. This problem is significant since these thyroid tumors occur at such an early age and consequently the life-long prognosis is difficult to determine. In our population of children this type of thyroid tumor was quite frequent and I prefer them to be treated (after surgery) as benign lesions, i.e. with L-T4 supplementation to maintain a normal TSH level, but to be just as careful and alert to change as in malignant lesions (Niedziela 2002). Abnormal thyroid growth appears to be complex, depending on many factors, especially TSH. However, the process is initiated without TSH stimulation in the majority of neoplastic thyroid tumors.

Thyroid carcinoma in the course of GD

The various approaches to GD designed to avoid progression to neoplasia are surgery vs radioiodine vs antithyroid drugs (ATDs) (Rivkees *et al*. 1998, Kraiem & Newfield 2001). It is well known that long-term treatment of GD with ATDs can predispose to further development of a malignant lesion within the enlarged thyroid (Dobyns *et al*. 1974). Palpable nodules in our young patients with GD were diagnosed as PTCs (Fig. 2H) (Niedziela & Korman 2002, 2003). It is possible that the relatively higher iodine intake from a prophylaxis program was responsible for these two different disorders (GD and thyroid carcinoma) involving two independent pathogenetic links.

Thyroid carcinoma in the course of chronic lymphocytic autoimmune thyroiditis (HT type)

HT type thyroiditis, unlike the atrophic type, is usually manifested by a goiter with increased firmness of the whole gland on palpation, usually in a diffuse form but occasionally in a focal form.

Based on data in the literature, autoimmune thyroiditis is considered to be a condition preventing the expansion of a neoplasm (Loh *et al*. 1999). A nodule in HT may progress to carcinoma, especially PTC, but it can also be associated with a benign neoplasm. Iodine prophylaxis in a previously iodine-deficient area and its relative excess in the diet should be considered as responsible for both HT and carcinoma (Bravermann 1994, Franceschi 1998, Stanbury *et al*. 1998, Feldt-Rasmussen 2001, Niedziela & Korman 2003). In several of our patients with HT we detected thyroid neoplasms, both benign FAs (Fig. 2I) and carcinomas (only PTC) (Fig. 2J) at the time of HT confirmation (Niedziela & Korman 2003). These findings therefore do not support the so-called 'protective role' of thyroiditis (Loh *et al*. 1999).

The question arises as to whether the thyroiditis preceded the nodule or vice versa. New data coming from molecular studies of the BRAF mutations, the molecular marker of PTC, indicate that the detection of activated mutation of this gene in a patient with a prior HT may be helpful in predicting progress to PTC, even in the absence of a palpable nodule (Kim *et al*. 2005). Long-term follow-up may help us in the further identification of true-positive risk factors for neoplasia.

In all our patients with thyroid neoplasms the diagnosis of AITD was established at the time of TND evaluation and, in the case of GD, before the introduction of ATDs. To date, no direct link has been proved but it is suggested, in the literature, that a solitary nodule (or nodules) in the course of GD may lead to quite an aggressive cancer (Rieger *et al*. 1989, Pellegriti *et al*. 1998), whereas in HT the nodule may appear as an cancer, either in an occult form or as a lymphoma (Loh *et al*. 1999).

Both types of AITD should be viewed with suspicion and more established forms of treatment should be applied much earlier (e.g. in the form of radioiodine if the GD is still active even with treatment (i.e. if showing a hypoechogenic pattern of the gland and an elevated titer of thyroid-stimulating hormone receptor antibodies (TRAb)) or surgery if a coexisting nodule or nodules are present).

I believe that both AITDs predispose to nodules but the risk of cancer developing is difficult to forecast. Since patients in the AITD group are at risk of developing thyroid neoplasia in the future, long-term follow-up is essential, especially in areas with a relatively higher intake of iodine in the diet.

Nonpalpable thyroid nodules

With the use of US for the evaluation of thyroid and nonthyroid neck disease, the incidental discovery of previously unsuspected thyroid nodules has dramatically increased (Castro & Gharib 2005). Nonpalpable thyroid nodules, as a form of TND, appear to be of less importance in children and adolescents (Niedziela 2002) than in adults (Leenhardt *et al.* 1999). Consequently the majority of published data relate to patients older than 15 years. Based on the data of Leenhardt *et al.* (1999) and our own findings in children (Niedziela & Korman 2001) I would agree with Papini *et al.* (2002), who recommends performing FNAB on all 8–15 mm hypoechoic lesions with irregular margins, intranodular vascular spots (examined with color-Doppler) or with microcalcifications, and that they should be followed carefully, both clinically and by US, even if the cytology is benign. The time to the first control visit should not exceed 3 months. A different protocol should be applied for those individuals with accompanying risk factors, especially those radiation-related or with a familial predisposition.

Coexisting other malignancy

The coexistence of any other malignancy, such as HL, non-Hodgkin's lymphoma or any other cancer, is of great importance in terms of predicting whether a thyroid lesion is malignant or not. Interestingly, in Li Fraumeni syndrome with multiple malignant tumors, in which there is a germ-line mutation of p53, there is no higher risk of anaplastic carcinoma, even if this abnormality is found in the tumor itself (Ito *et al.* 1992).

Family history

The hereditary forms of thyroid carcinoma are less frequent than the sporadic type and are mainly related to medullary thyroid carcinoma (MTC) of C-cell origin (25% of MTC cases are hereditary vs 75% sporadic) (Raue *et al.* 1993, Eng 2000, Gimm *et al.* 2001). We should consider such a risk in a child if one of the parents is affected by MTC and carries the germ-line mutation within a ret proto-oncogene. The family members should be screened for this mutation since hereditary MTC is transmitted in an autosomal dominant mode of inheritance. If the patient has one of the following: pheochromocytoma, hyperparathyroidism, marfanoid appearance or multiple ganglioneuromas, the endocrinologist should also screen the patient for MTC as a part of either a multiple endocrine neoplasia (MEN) 2A or MEN 2B syndrome (Holmes *et al.* 1995, Eng *et al.* 1996, Gagel 1998, Eng 2000).

Familial forms of thyroid carcinoma of follicular origin are very rare and there is no single candidate gene for this predisposition (Malchoff & Malchoff 2002). The PTEN gene, if mutated, is responsible for Cowden's disease and in some cases FTC is more commonly present (Liaw *et al.* 1997) but PTC is very rarely present in these patients. It is also known that APC gene mutation for familial adenomatous polyposis and Gardner's syndrome is responsible for a higher incidence of PTC (Giardiello *et al.* 1993). The presence of PTC with oxyphilia (Canzian *et al.* 1998) or without (Bevan *et al.* 2001) may have a familial pattern in some patients. We also analyzed a family with a history of a Hurthle's cell neoplasm, a carcinoma with metastases in the mother and an adenoma in her prepubertal son, thereby supporting the familial pattern as well as the belief that untreated adenoma may progress toward carcinoma (author's unpublished observation). A higher incidence of FA was noted in patients with the MEN 1 syndrome caused by a defect in the menin gene (Thakker 1995, Trump *et al.* 1996, Pannett & Thakker 1999).

The Carney complex, an autosomal dominant syndrome which is a result of inactivated mutation of the PPKAR1A gene encoding the type Iα regulatory subunit of protein kinase A, is responsible for multiple nodules in different organs, including the thyroid, but these are generally of a benign character (Stratakis *et al.* 1997). Thyroid nodules were detected in 67% of their patients but thyroid carcinoma in only 3.8% of them.

Clinical examination

The clinical presentation of TND (solitary nodule vs MNG) may vary in different cohorts depending on the criteria, palpation or/and US, used.

Arici *et al.* (2002), in their study of 15 children with thyroid cancer, found a solitary nodule in five (33%), and an MNG in eight (53%). In our larger study of 37 thyroid carcinomas we found a much lower frequency of MNG (29.7%) in association with thyroid cancer with a predominance of solitary nodules (70.3% of all thyroid carcinomas) (Niedziela 2002).

The clinical state (euthyroidism vs hypo- or hyperthyroidism) does not appear to be a predictive factor for neoplasia. Of all the patients operated on

in our hospital in the years 1996–2000, 83.2% were clinically euthyroid, a finding similar (86.5%) to that in a group of cancer patients (Niedziela 2002, Niedziela *et al*. 2004).

The localization of thyroid cancer (right vs left lobe) is unpredictable. However, in the majority of our children who were operated on, the tumor was localized within the right lobe (68.4%), (Niedziela 2002, Niedziela *et al*. 2004). Furthermore, 66.1% of the benign lesions and 75.5% of thyroid carcinomas in the group were also localized in the right lobe. The isthmus is rarely involved in thyroid cancer.

Size of tumor does not appear to be a critical parameter in the prediction of malignancy. Usually, size of the palpable thyroid mass ranges from 1 to 5 cm (Halac & Zimmermann 2005). However, the majority of cancers have a diameter of 1.5 cm or more (Cotterill *et al*. 2001, Niedziela 2002). On the other hand, nodules >4 cm in diameter and partially cystic should be viewed with a moderate degree of suspicion (Hegedus *et al*. 2003). The dynamics of a tumor (its progressive growth in months rather than years) appears to be a more significant factor.

Both lymph node involvement and the presence of distal metastases in a patient with a thyroid nodule are highly predictive of the future outcome in patients with thyroid carcinoma. PTC mainly metastasizes to regional lymph nodes and lungs, MTC to cervical lymph nodes by the lymphatic route and FTC by a hematological route mainly to the lungs and liver (Halac & Zimmermann 2005). Yip *et al*. (1994), Samaan *et al*. (1992), Cotterill *et al*. (2001) and Arici *et al*. (2002) reported local metastases in 80, 74, 48 and 27% respectively of their patients with thyroid cancer. Moreover, Ardito *et al*. (2001) recommended a more radical treatment approach in children and adolescents due to the higher prevalence of local lymph node involvement in these cases. This is in contrast to the findings of Stankowiak-Kulpa *et al*. (2002), who found that, out of a group of 22 children with thyroid carcinoma who were analyzed between 1999 and 2001, lymph node involvement was present in only one, but additionally in another, an adolescent girl with multifocal PTC, distant metastases to the lungs were observed on the X-ray of the chest, making a total of approximately 9%. The time of nodule detection is a critical parameter in arriving at an early diagnosis. Poland was alerted by the Chernobyl catastrophe and it is very likely that, due to the aggressive screening of children with thyroid dysfunction which was promptly introduced, earlier detection was possible at a $T_{1-4}N_0M_0$ clinical grade of malignancy.

Laboratory tests

Free thyroid hormones and TSH are routinely measured in the patient's sera to evaluate the hormonal state, whether the child is euthyroid, hypo- or hyperthyroid. None of these parameters (TSH, free T4, free triiodothyronine) distinguish benign from malignant lesions but, if their levels are abnormal, they should be normalized prior to surgery, i.e. with L-T4 (if hypothyroid occurs) or with ATDs (if hyperthyroidism is detected).

Patients with thyroid cancer are usually euthyroid and rarely present with hyperthyroidism (Halac & Zimmermann 2005). Normal thyroid hormone levels predominated (86.5%) in all our patients (Niedziela 2002, Niedziela *et al*. 2004).

The question arises as to whether the calcitonin level should be assayed in all children with a thyroid nodule, since the risk of missing MTC on FNAB may occur. In this era of screening for MEN syndromes it is advisable to measure the calcitonin level in palpable, solid thyroid nodules (Deftos 2004, Elisei *et al*. 2004, Hodak & Burman 2004) and is obligatory in familial forms of thyroid nodules. The measurement of serum carcinoembryonic antigen (CEA) is also advisable in those patients with a suspicion of MTC. Unfortunately, a negative value may be found in advanced stages of the disease (Bockhorn *et al*. 2004). The level of urinary metabolites of catecholamines in a 24 h collection should also be measured, because pheochromocytoma should be excluded if the nodule is recognized as MTC. An existing pheochromocytoma or paraganglioma should be excised before thyroidectomy to avoid a hypertension crisis during surgery on the thyroid.

Serum anti-TPO antibodies (TPO-Ab) are recommended in the evaluation of thyroid nodules. Their detection is of great importance in the interpretation of cytological results. Hegedus *et al*. (2003) found no place for the routine assessment of serum thyroglobulin (Tg) and only rarely for anti-Tg antibodies (Tg-Ab) in thyroid nodules. The TR Ab titer should also be evaluated in patients with signs and symptoms of hyperthyroidism.

Pacini *et al*. (1988) found thyroid antibodies in up to 25% of the patients with thyroid cancer. Positive titers of antibodies (TPO-Ab, Tg-Ab) were detected more frequently in a group with cancer (20% predominantly Tg-Ab) than in those with FA (5%)

(Niedziela 2002). It is important to note that the thyroid, except for the nodule, was otherwise normal without US features of lymphocytic inflammation. Tg-Ab titers were also elevated in two of four children with PTC in Belgium (Blackburn *et al.* 2001) whereas TPO-Abs were absent. By contrast, in our recent analysis of patients seen in the years 2001–2004 (Niedziela & Korman 2003), i.e. a few years after the reintroduction of iodine in the diet in January 1997, we found a remarkably higher incidence of thyroid carcinoma in children with HT (and lymphocytic inflammation) and with positive titers of TPO-Abs. These data may support the hypothesis that the molecular background of cancer differed in the periods before and after iodine prophylaxis.

Table 2 Ultrasonographic features of malignancy

1. Solitary solid lesion (Fig. 4A and B)
2. Hypoechogenic (Fig. 4A and B)
3. Subcapsular localization (Fig. 4A and B)
4. Irregular margins of the lesion (Fig. 4B)
5. Invasive growth (no compression of adjacent tissues) (Fig. 4B)
6. Heterogenous nature of the lesion (solid hypo/iso) (Fig. 4C)
7. Multifocal lesions within an otherwise clinically solitary nodule (Fig. 4D)
8. Microcalcifications (<2 mm; found mainly in PTC and MTC) (Fig. 4E)
9. High intranodular flow by Doppler (with normal TSH) (Fig. 4F)
10. Suspicious regional lymph nodes accompanying thyroid nodule (Fig. 4G)

US

In the past the test chosen for first-line screening of thyroid nodules appears to have been continent-dependent. US was more common in Europe (Bennedbaeck *et al.* 1999, Bennedbaeck & Hegedus 2000) and SC was used more commonly in America (Bonnema *et al.* 2000, 2002). However, changing trends have occurred in the evaluation and management of nodular thyroid disease and two important developments are employed in thyroid nodule evaluation and management, namely US and FNAB. Thyroid US is the imaging method of choice for the evaluation of thyroid gland structure, and FNAB, as the most accurate test for nodule diagnosis, has reduced the need for scanning and for thyroidectomy, thereby reducing the health-care costs significantly (Castro & Gharib 2003, 2005, Gharib 2004). Some authors prefer to perform FNAB in euthyroid patients only and to carry out a prior SC in thyrotoxic patients (Mazzaferri 1993). US results alone cannot be accepted as true positives in terms of malignancy. However, the procedure's usefulness is considerable, if combined with the clinical data and laboratory test results. As thyroid nodules are rare disorders the use of US examination is not contraindicated on economic grounds. Moreover, it helps to complete the diagnostic protocol of a thyroid nodule and subsequently to choose the best mode of treatment (Fig. 1).

US is a safe and widely available technique, and I therefore recommend it strongly as the first-line screening diagnostic test in all pediatric patients with thyroid nodules. This should be followed by further imaging-directed tests (SC (invariably in patients with suppressed TSH) and FNAB or direct FNAB in cystic (unechogenic) lesions) (Fig. 1).

Preoperative US examination of thyroid nodules not only provides information on their size, echogeneity, echostructure and location but also contributes significantly to the differential diagnosis of benign vs malignant tumors. It is a simple, inexpensive and radiation-free method of examination of great sensitivity and specificity and is complementary to FNAB (Varverakis & Neonakis 2002). The criteria for suspected malignancy are summarized in Table 2 (Niedziela 2002, Varverakis & Neonakis 2002, Lyshchik *et al.* 2005). If multiple, solid isoechogenic or unechogenic lesions are visible, or if a peripheral halo is present, then the tumor is very likely to be benign (Niedziela 2002). Some authors believe that if the halo is thick and irregular then malignancy should be suspected (Solbiati *et al.* 1992). Color-Doppler sonography may be helpful in hyperfunctioning nodules (hot on SC and usually benign on histology), indicating an intensive vascular flow within a highly vascularized lesion (Fig. 4H), and no visible flow through the remaining, suppressed thyroid gland (Hegedus *et al.* 2003). Color-Doppler sonography is also valuable in distinguishing a cystic lesion (with no vascular flow) (Fig. 3C) from a solid neoplasm (with intranodular flow) (Fig. 4F). Cystic degeneration occurring in previously solid lesions does not determine the diagnosis, whether benign or malignant. If there is no vascular network within the nodule, the nodule is painful on palpation and the patient has a fever, then a suppurative thyroiditis is suspected. US-guided FNAB, with a subsequent cytological examination and culture of the aspirated material, helps to identify a bacterial cause of the nodule. Drainage of the abscess, plus i.v. antibiotic therapy, usually leads to a complete resorption of the lesion.

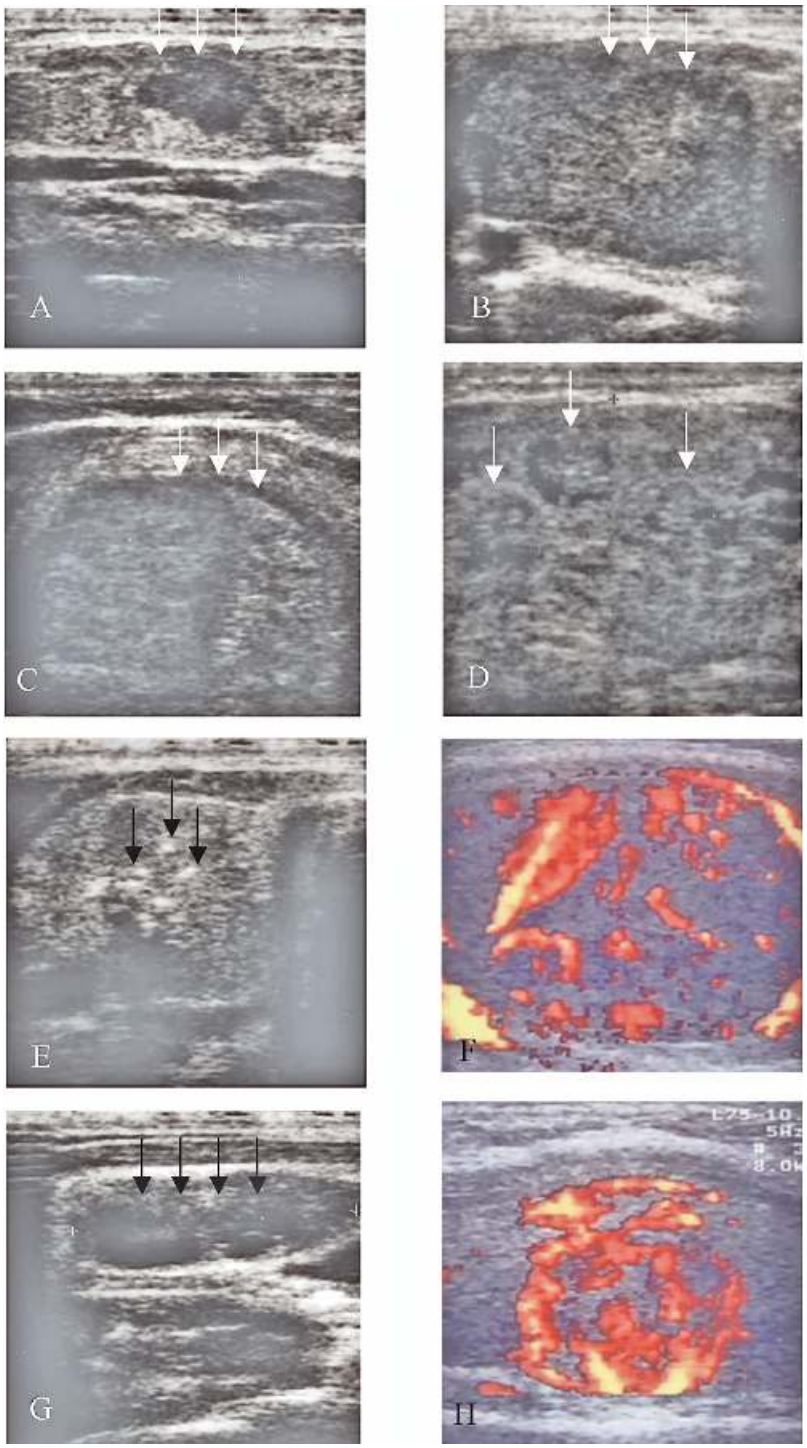

Figure 4 Ultrasound imaging of thyroid carcinoma. (A) Papillary thyroid carcinoma (solitary solid hypoechogenic lesion with irregular borders; tumor not detected on palpation). (B) Oxyphilic follicular thyroid carcinoma (solid hypoechogenic lesion with irregular borders). (C) Follicular variant of papillary thyroid carcinoma (hypo/isoechogenic pattern of the lesion). (D) Papillary thyroid carcinoma (multifocal form). (E) Papillary thyroid carcinoma with microcalcifications. (F) Follicular thyroid carcinoma (cold on scintigraphy) with a high intranodular flow with Doppler (type III vascularization – increased perinodular and intranodular). (G) Local lymph node metastases. (H) Follicular adenoma (classic hot nodule with a high vascular flow with Doppler and no visible flow in the remaining part of the thyroid.

However, laryngoscopy should be performed subsequently to exclude a fistula of the piriform fossa (Rabska-Pietrzak *et al*. 1998, Gawrysiak & Niedziela 2005).

Hypoechogenic lesions were observed in 70.3% of a group of patients with carcinoma (Niedziela 2002). In multiple nodules it is important to select the most suspicious lesion, based on US examination, for further US-guided FNAB. However, there are data in the literature showing that the dominant nodule in MNG carries the same risk of cancer as a solitary nodule (Gandolfi *et al*. 2004). US plays an important role in the diagnostic work-up of thyroid nodules. However, there are still doubts as to whether it is a sufficiently accurate method in the differentiation between benign and malignant lesions and therefore some deny its usefulness (Hegedus 2001). On the other hand, authors from regions affected by radioactive fallout are convinced that systematic US screening is a significant tool for the early detection of thyroid carcinoma due to the many indicators of the malignant process which may be detected (Drozd *et al*. 2002, Niedziela 2002, Lyschik *et al*. 2005) (Table 2).

SC

Thyroid scans provide information on iodine-trapping function. In this era of high-resolution US machines, SC is of less importance. However, in the author's opinion it can still serve as an additional test to help make the final preoperative decision in terms of the extent of surgery. It seems logical that cystic lesions (Fig. 3A–D) (unechogenic on US) or mixed, (solid-cystic) but predominantly cystic are not an indication for immediate SC, because with very few exceptions the result is quite obvious for predictive purposes (a cold area). It is also of limited value if the nodule is small (~1 cm in diameter or less) especially if present in an enlarged thyroid gland. Such a small nodule may be visualized if cold and if located peripherally. I would recommend SC in a mixed/or solid lesion if TSH is reduced because it may be a hot nodule with degenerative changes (mixed). I would also recommend this method in such a clinical state (decreased TSH) even if the palpable nodules were small (~7–10 mm), since it may confirm the autonomous nature of the nodule and thus suggest a benign histopathology in iodine sufficient areas (Fig. 1).

In western Poland we observed a high percentage (29.0%) of thyroid carcinoma within hot nodules in the late 1990s (Niedziela *et al*. 2002*a*). It is very likely

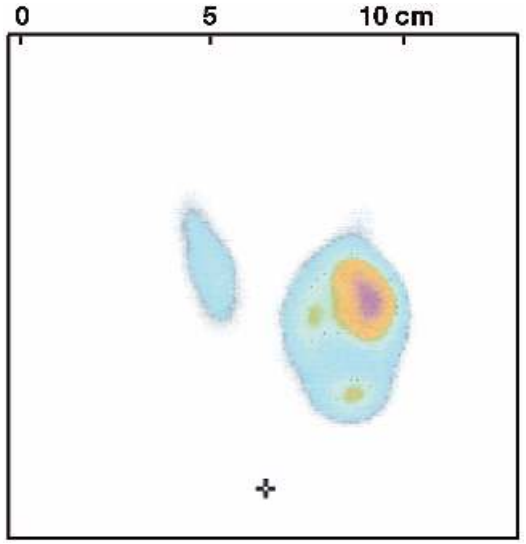

Figure 5 Thyroid scan (^{99m}Tc) – non-classic hot nodule in the left lobe.

that this was a result of long-term iodine deficiency, from 1980 to December 1996, followed by a relative increase in iodine intake due to the introduction of an obligatory program of iodine prophylaxis in January 1997 (Niedziela *et al*. 2002*a*, Niedziela *et al*. 2004). Iodine excess plays a role in a higher incidence of hot nodules (Jonckheer *et al*. 1992, Delange & Lecomte 2000, Niedziela *et al*. 2002*a*) as well as of PTCs (Harach *et al*. 1985, Harach & Williams 1995). In classic hot nodules (with radionuclide uptake only in the area corresponding to the nodule) thyroid carcinoma was detected in 5.9%. By contrast, the incidence of carcinoma in the group of non-classic hot nodules (with minimal radionuclide uptake in the extranodular area) (Fig. 5) was 57.1%. In other words, 88.9% of all the cancers in this group of nodules were found in non-classic forms (Niedziela *et al*. 2002*a*). The significance of so-called non-classic hot nodules in our patients should be emphasized, since they carry a higher risk of thyroid carcinoma (Niedziela *et al*. 2002*a*). This is in agreement with Harach *et al*. (2002), who wrote that untreated hot nodules can progress to carcinoma. The molecular background of non-classic nodules may differ somewhat from classic hot nodules in that the former lose high expression of the symporter responsible for iodine or technetium influx during the test. Surgical treatment is advisable for all children and adolescents with autonomously functioning thyroid nodules because of the risks of hyperthyroidism and thyroid carcinoma (Croom *et al*. 1987, Niedziela

et al. 2002*a*). Poland is on the way to achieving optimal levels of iodine intake as a result of the National Program of Iodine Prophylaxis and, since its introduction, the incidence of hot nodules has significantly declined to single cases per year. This is in agreement with observations made in those other communities which introduced similar prophylactic programs several years ago (Jonckheer *et al*. 1992, Delange & Lecomte 2000), thereby confirming their value.

According to Desjardins (1987) up to 30% of cold nodules are malignant (28.8% in our series; Niedziela 2002). The need for SC still exists if nodules are solid on US, especially if they coexist with GD (Meller & Becker 2002), or if ectopy or an autonomous nodule is suspected in a CH patient (Fig. 1).

At present, it is a great challenge for physicians to detect malignant lesions in the thyroid and therefore cytological examination is essential.

FNAB

There are two major indications for biopsy in children with thyroid nodules: (i) as a diagnostic procedure and (ii) for therapeutic purposes. To date, only a few papers published in English have documented series involving the use of FNAB for diagnosing thyroid nodules in children (Raab *et al*. 1995, Degnan *et al*. 1996, Lugo-Vicente *et al*. 1998, Khurana *et al*. 1999, Al-Shaikh *et al*. 2001, Amrikachi *et al*. 2005). FNAB is carried out to obtain adequate cell material but, in some cases, the rapid influx of blood from highly vascularized tumors (hot nodules, neoplasms with an abundant vascular network) demands more investigations on the same day, which can be particularly difficult in children. The application of an analgesic cream minimizes one disadvantage of this method, that of pain during the puncture, especially in very young children. Preferably those less than 10 years of age should undergo excisional biopsy under general anesthesia (Van Vliet *et al*. 1987, Koch & Sarlis 2001, Bettendorf 2002). In adults, FNAB can also be employed for ethanol injection into hot nodules (Paracchi *et al*. 1992) especially in nodules whose initial volume was less than 15 ml (Lippi *et al*. 1996).

Follicular lesions (Fig. 6A), one of the most common diagnoses, may suggest a hyperplastic nodule, FA, follicular carcinoma or a follicular variant of PTC. However, other histological diagnoses are also possible. In the author's experience a suspicious lesion such as that in Fig. 6B is almost always neoplastic, either benign or malignant, on histopathology (Niedziela 2002). If malignant cells,

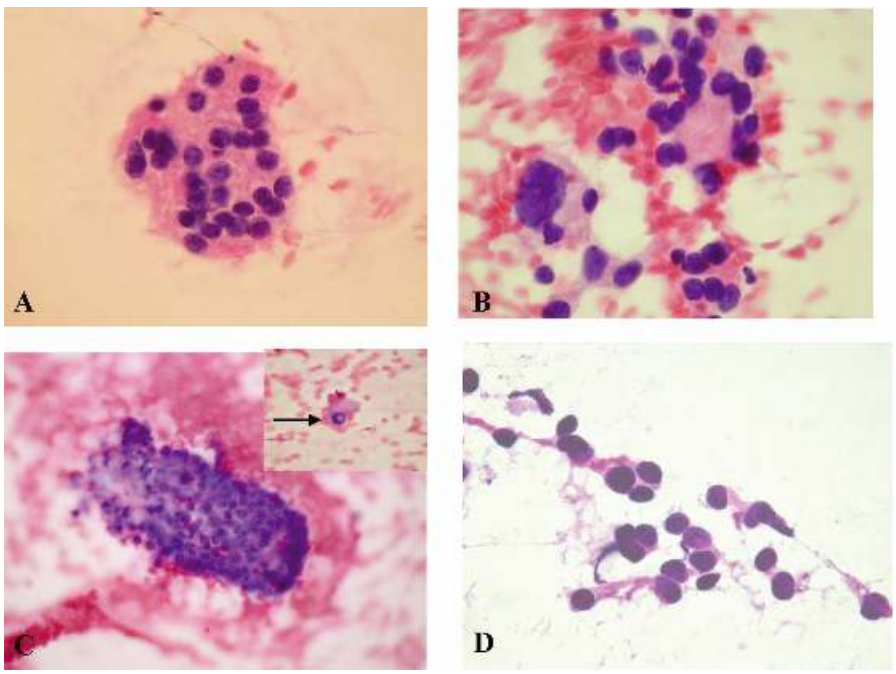

Figure 6 Cytological picture of selected forms of thyroid nodular disease in children suspected of neoplasia, either benign or malignant (H&E staining). (A) Follicular lesion. (B) Suspicious result. (C) Papillary thyroid carcinoma (one papilla and a single cell with intranuclear vacuole). (D) Medullary thyroid carcinoma.

indicating thyroid cancer, are present (e.g. PTC (Fig. 6C) or MTC (Fig. 6D)) then total thyroidectomy with elective central lymph node removal is obligatory (Polish Guidelines 2001). If the material obtained from the thyroid nodule is insufficient for diagnosis then a second biopsy is recommended.

Overall, FNAB is the most reliable and cost-effective method of distinguishing benign from suspicious or malignant thyroid nodules (Castro & Gharib 2003).

Interpretation of FNAB

PTC, the most common type of thyroid cancer, as well as medullary and anaplastic thyroid carcinoma, may be diagnosed preoperatively from cytological examination of biopsy material. It is necessary here to mention some aspects of false-positive results for malignancy in conventional cytology in some clinical conditions. One of these is HT in the hypothyroid phase, whether clinical or subclinical. Normalization of TSH is mandatory, prior to FNAB, because if it is elevated, it promotes goiter development and could be responsible for morphological changes in epithelial follicular cells. If nodules are not present then L-T4 therapy is recommended, before FNAB, to normalize the TSH level, which in turn normalizes the stimulation of thyroid epithelial cells. Otherwise the resulting overstimulated follicular cells may lead to a false-positive result at cytology, with nuclear grooves and other features suggesting PTC (Kini 1996, Gould *et al*. 1989, Chhieng *et al*. 1997). A careful clinical follow-up 4–6 weeks later and a subsequent visit, which should include an US check, after a 3 month interval, is advisable to avoid unnecessary thyroidectomy. If, with this correction based on clinical and US examinations, there is still a need for a FNAB then it should be directed to the most suspect area within the thyroid, i.e. to a detected nodular region. FNAB should be performed directly if a solitary palpable lesion is present (Fig. 2I) or if a suspected hypoechogenic area is detected with US (Fig. 2J). The effects of ATDs given before FNAB should also be considered in the interpretation of biopsy specimens, otherwise the cytological conclusion may be inaccurate.

An adequate (true-positive) diagnosis can be made in more than 90% of undifferentiated, medullary and papillary carcinomas using FNAB (Kini 1996). An FTC cannot be distinguished preoperatively by FNAB from a hyperplastic nodule, an FA or a follicular variant of PTC (Kini 1996, Hamburger 1994). Ardito *et al*. (2001) concluded that the preoperative work-up of children and adolescents with thyroid nodules requires FNAB as the initial diagnostic test, since malignancy was detected in 73.3% of their lesions, prior to surgery. Zimmermann (1997) noted a false-negative result from biopsy in only 2% of his aspirates. Corrias *et al*. (2001) found a high degree of sensitivity (95%), specificity (86.3%) and accuracy (90.4%) of FNAB in relation to histological diagnosis and they therefore also advocate the use of this method as a diagnostic test in euthyroid patients with thyroid nodule(s). Raab *et al*. (1995) stated that FNAB is useful in the management of pediatric thyroid nodules because of its high diagnostic accuracy and minimal invasiveness. Arda *et al*. (2001) assert that surgery should only be performed in patients with malignant or suspicious cells and that it has no place in patients whose previous FNAB revealed benign cells. All the patients with suspicious or malignant FNAB results in our series were found to have adenomas or carcinomas post-operatively. However, cancer was also detected in tumors with a benign preoperative cytology (Niedziela 2002).

As there is therefore a risk of false-negative cytological results with earlier methods of investigation, including FNAB cytological examination, a more accurate preoperative diagnostic test is still required in TND (Haugen *et al*. 2002, Bojunga & Zeuzem 2004).

Molecular studies employed for the detection of malignancy

Each FNAB aspirate, in parallel with conventional cytological evaluation, may be subjected to RT-PCR in the search for the expression of different neoplastic markers within the aspirated cells (Gasbarri *et al*. 1999, Russo *et al*. 1999, Takano *et al*. 1999, Takano & Amino 2002).

These markers include telomerase (Haugen *et al*. 1997), Hector Baltifora Mesothelial cell (HBME-1) (Sack *et al*. 1997), galectin-3 (Gasbarri *et al*. 1999, Bartolazzi *et al*. 2001, Saggiorato *et al*. 2001, Kovacs *et al*. 2003), CD44v6 (Gasbarri *et al*. 1999) and cytokeratin-19 (Khurana *et al*. 2003). However, these do not provide a precise identification of the various thyroid cancer subtypes. Some markers have also been detected in PTC (ret/PTC translocations (Cheung *et al*. 2001), platelet-derived growth factor (Yano *et al*. 2004)) and FTC (PAX8-PPARγ1 (Kroll *et al*. 2000)). Additionally,

a number of markers such as trefoil factor 3 (TFF3) (Takano *et al*. 2004), Tg (Giordano *et al*. 2004) and TPO (Tanaka *et al*. 1996) have diminished expression in cancer. Galectin-3 was originally a very promising marker in aspirates from thyroid nodules but its practical value was reduced by its falsely positive expression in MNG (Cvejic *et al*. 1998), FA (Bernet *et al*. 2002) and HT (Niedziela *et al*. 2002*b*). The false positives in HT occurred not only in cases with a poor clinical manifestation of the condition but with an obvious thyroid nodule on palpation, but also occurred in patients with the classic form of HT (Niedziela *et al*. 2002*b*). Galectin-3 and HBME-1 expression (Sagioratto *et al*. 2005) in tandem has been shown to have a high sensitivity for cancer detection on cytological smears broadly described as 'follicular neoplasm'. The presence of other markers in tumor tissue such as calcitonin and ret protein supports a diagnosis of MTC (Takano *et al*. 1999). Expression profile analysis of several genes with microarrays helps to screen many candidate genes as biomarkers of malignancy (Finley *et al*. 2004). However, such analyses are quite expensive, not easily available and may only serve a small number of patients. Since we have seen thyroid cancer in children born after 1987, the effects of the Chernobyl catastrophe, such as the rearrangement of ret/PTC3 in PTC (Grieco *et al*. 1990, Nikiforov *et al*. 1997, Fagin 2004*a*), appear to be limited and support the need to look for a universal marker of cancer, independent of radiation. According to Penko *et al*. (2005) ret/PTC rearrangements are the most frequent molecular abnormalities in childhood PTC.

The recently reported BRAF gene mutations are present in a high percentage of melanoma and colon carcinoma cells (Davies *et al*. 2002) and in 70% of PTC cases and anaplastic thyroid carcinoma of PTC origin (Cohen *et al*. 2003). BRAF gene mutation (T1799A) in exon 15 creates 80% of all its mutations in cells of different cancers (Davies *et al*. 2002). This mutation is a somatic mutation in sporadic PTC and anaplastic thyroid carcinoma of PTC origin (Kimura *et al*. 2003, Xu *et al*. 2003). In particular, this mutation occurs most frequently in the PTC columnar (77%), in classic PTC (60%) and rarely (12%), in the follicular variant of PTC (Xing 2005*a*). The first two subtypes have a greater tendency to metastasize to the lymph nodes and are more aggressive in their development, and it follows that analysis of the BRAF gene is therefore of great diagnostic and prognostic value. NB No BRAF mutations have been detected in the other thyroid carcinoma subtypes indicating that this is probably a diagnostic test of great importance (Xing 2005*a*). BRAF gene mutation leads to the origin of oncogene BRAF (the active form of this protein), which activates MEK kinase, followed by the activation of MAPK kinase (Duesbery *et al*. 1999). Permanent, uncontrolled activation of this signal cascade has the promitogenic effect responsible for inappropriate cell proliferation and differentiation into neoplasia (Avruch *et al*. 2001, Fagin 2004*b*).

The T1799A BRAF mutation is not a germline mutation in familial nonmedullary thyroid cancer (Xing 2005*b*). Several papers have shown that the T1799A mutation in exon 15 of the BRAF gene does not occur as frequently in children and adolescents with PTC as in adults (Lima *et al*. 2004, 2005 Miao *et al*. 2004, Nikiforova *et al*. 2004). Summarized data on the occurrence of BRAF mutations in childhood PTC are shown in Table 3.

The newly detected fusion oncogene AKAP9/BRAF, a result of intrachromosomal recombination, also leads to the activation of the pathway and thus may serve as another diagnostic tool in

Table 3 Prevalence of BRAF mutation in childhood papillary thyroid carcinoma

	Exposed to radiation		Non-exposed		
Report	Number	%	Number	%	**References**
1	4/34	11.8	1/17	5.9	Lima *et al.* (2004)
2	0/15	0	1/31	3.2	Kumagai *et al.* (2004)
3	2/55	3.6	–	–	Nikiforova *et al.* (2004)
4	–	–	0/14	0	Penko *et al.* (2005)
5	1/27	3.7	0/8	0	Powell *et al.* (2005)
6	–	–	4/20	20.0	Rosenbaum *et al.* (2005)
7	1/5	20.0	–	–	Xing (2005*b*)
Overall	8/136	5.9	6/90	6.7	

preoperative studies, particularly in BRAF-negative young patients with a previous history of radiation exposure (Ciampi *et al*. 2005).

A revealing feature of PTC is that the mutations in the associated genes are mutually exclusive. Several workers have examined PTCs for concordance of ret/PTC, NTRK, BRAF and RAS mutations. Altogether, 177 PTC cases have been studied and one of these alterations was present in about 70% of the tumors (Kimura *et al*. 2003) However, no single PTC had a mutation in more than one of these genes. This lack of overlap provides compelling genetic evidence that a mutation of MAPK signaling components is required for transformation to PTC (Soares *et al*. 2003). Based on histological evidence from microscopic lesions in the thyroid these MAPK-directed pathological events probably occur early in the course of tumor development (Nikiforova *et al*. 2003*a*). Moreover, PTCs with *BRAF* mutations have more aggressive properties, present more often with extra thyroidal invasion and at a more advanced clinical stage, and can give rise to undifferentiated or anaplastic carcinomas (Namba *et al*. 2003, Nikiforova *et al*. 2003*a*). These data indicate that *BRAF* mutations may be an alternative tumor-initiating event in PTC and that tumors with this genotype carry a less favorable prognosis (Fagin 2004*b*). We cannot exclude the possibility that overactivation of the ret/PTC–RAS–BRAF pathway could be located beyond BRAF, in distal elements of this transductory system (i.e. MEK–MAPK). Their genes may also undergo mutation leading to neoplastic transformation. The lack of autoinhibitory domains within the BRAF gene, CR1 and CR2, might be an additional cause of the permanent stimulation of the MAPK transductory pathway (Fusco *et al*. 2005). Selective kinase inhibitors acting on distal effectors of the MAPK pathway could be particularly well suited for PTCs that do not respond to conventional treatment (Fagin 2005).

A genetic factor in FTCs is that between 20 and 50% of them harbor an interchromosomal translocation that fuses the PAX8 gene with the PPARγ gene. PAX8/PPARγ is believed to act as an oncoprotein, in part through dominant-negative inhibition of the function of the wild-type copy of PPARγ (Kroll *et al*. 2000, Gregory Powell *et al*. 2004). FTCs that do not have the PAX8/PPARγ recombination are often associated with RAS mutations, although there is no obvious explanation for why these two distinct oncogenic steps are mutually exclusive (Nikiforova *et al*. 2003*b*). It is also not yet clear if these mutations occur early in tumorigenesis, although this appears likely because both PAX8/PPARγ rearrangements and RAS mutations are also found in a small number of FAs.

The recently published data on serum DNA methylation markers in patients with thyroid carcinoma suggest that they may serve as a novel tool for the differential diagnosis of solid thyroid nodules and for monitoring thyroid cancer recurrence. However, false-positive results may occur, just as they may in patients with benign cystic thyroid nodules (Hu *et al*. 2006).

Clearly, clinical difficulties in distinguishing thyroid carcinoma from benign lesions are still present, particularly in the case of FTC vs FA or the follicular variant of PTC (Cerutti *et al*. 2004). If the diagnosis of malignant tumor based on the results from the diagnostic work-up is unclear, then some other tests, such as CT/MRI of appropriate regions/organs and radiographs of the chest, are required to search for the presence of distant metastases. Overall therefore we still need and are desperately looking for a more precise marker of thyroid carcinoma, especially in children.

Concluding remarks

A careful work-up is essential in all patients younger than 15 with suspected thyroid disorders. We now live in an era when the number of children and adolescents with AITD is increasing. In Poland, the transition period from iodine deficiency to adequacy resulted in the detection of an increase in solitary nodules and MNG. On the other hand, iodine sufficiency resulted in a higher incidence of AITD in an adaptive (transient) period. These new observations lead to a more suspicious protocol of TND, because the clinical course of affected thyroid glands is difficult to predict. The iodine given as a prophylaxis may be responsible for the increase in AITD and may have provoked the development of PTC, the only type of thyroid cancer detected to date in these patients. On the other hand, based on a review of the literature, children with CH are at risk of developing FTC, the absolutely predominant type of thyroid cancer found in children with dyshormonogenesis. FTC observed in two patients with HL may suggest a mechanism of cancer formation independent of radiation (Niedziela 2002). Twenty-five percent of MTCs in adults are an hereditary form and the condition requires evaluation of all family members, because of the risk of familial MTC or MEN 2A and MEN 2B syndromes (Brandi *et al*. 2001). Screening for serum calcitonin of all children with thyroid

nodules is also necessary, since false-negative FNAB results may occur (Bugalho *et al*. 2005). With the increasing availability of molecular techniques we are now able to screen not only the affected patients but also the other members of their families for ret proto-oncogenes, thus providing a new therapeutic model for the early intervention, or even prevention, of the clinical manifestation of disease (Marsh *et al*. 1997, Santoro *et al*. 2004). Surgical treatment should be advocated as soon as possible in carriers of this rare disease because the aggressiveness is difficult to predict. The exclusion of pheochromocytoma in these patients is also obligatory to avoid a life-threatening emergency during thyroidectomy.

In addition there is a need to screen for asymptomatic thyroid cancers those children who have been exposed to X-ray treatment of the head and neck or who have received high-dose total-body irradiation earlier in their lives (Shafford *et al*. 1999, Eden *et al*. 2001). It is important to remember that the latent period between exposure and the appearance of thyroid cancer may be up to 30–40 years (Nikiforov & Fagin 1997, Inskip 2001).

I strongly believe that the primary treatment of thyroid nodules should be surgical. Palpable thyroid nodules should not be treated with L-T4 suppressive therapy because of (i) the absence of proven successful clinical data, (ii) the risk of hyperthyroidism, (iii) the risk of bone loss, (iv) adverse cardiac effects and (v) >95% of palpable thyroid nodules are histologically neoplastic and therefore should be primarily removed for a good long-term prognosis (Singer *et al*. 1996, Gharib 1997, Gharib & Mazzaferri 1998, Lugo-Vicente & Ortiz 1998, Koutras 2001, Niedziela 2002).

The majority of patients with thyroid nodules are euthyroid. However, those who are hyper- or hypothyroid require therapy with ATDs or L-T4 respectively, to reach hormonal euthyroidism (normal levels of free thyroid hormones) prior to surgery. In rare cases, the coexistence of AITD and PTC may be detected at the time of diagnosis, i.e. before the introduction of any treatment. Hot nodules, being predominantly toxic adenomas, should be treated in children primarily with surgery since they are a step toward progression to FTC if left untreated (Suarez 1998, Kroll *et al*. 2000, Vecchio & Santoro 2000, Gimm 2001). The most important risk factors predisposing to childhood thyroid cancer are summarized in Table 4 and the US features of thyroid cancer are shown in Table 2.

Table 4 Factors strongly suggesting the malignant thyroid tumor

1. Age <10 years
2. Male gender
3. Firm solitary nodule, fixed to adjacent tissues
4. Rapid growth of the nodule (even on L-T4 treatment)
5. Paralysis of vocal folds
6. Regional lymph node enlargement
7. Distant metastases (lungs, bones)
8. Preexisting serious thyroid benign thyroid disease (CH, HT, GD, FA)
9. External irradiation of head and neck or total body irradiation (HL, BMT, others)
10. Prior exposure to internal radiation (e.g. to radioactive ^{131}I from Chernobyl disaster)
11. Others:
    ↑ calcitonin and ↑ CEA (for MTC)
    hyperparathyroidism with coexisting thyroid tumor (for MEN 2A)
    pheochromocytoma with coexisting thyroid tumor (for MEN 2A and 2B)
    multiple ganglioneuromas with coexisting thyroid tumor (for MEN 2B)
12. Family history in terms of MTC, MENs or familial nonmedullary thyroid carcinoma
13. US features of malignancy
14. Cold nodule on scintigraphy (otherwise solid on US)
15. Malignant cytology
16. Positive biomarkers of malignancy in aspirates (BRAF mutation, AKAP9-BRAF, ret/PTC, RAS mutation, PAX8/PPARγ, HBME-1, galectin-3, cytokeratin (19)

Future perspectives

US and radionuclide scanning have been used routinely to screen thyroid nodules but many reports question their reliability (Garcia *et al*. 1992, Kneafsey *et al*. 1994, Sabel *et al*. 1997). FNAB results can prevent unnecessary thyroid surgery in children but, based on the author's experience, all nodules cold on SC or solid on US should be excised, even if the cytology is benign. A benign tumor if left untreated (e.g. an FA) is a potential candidate for tumor progression toward thyroid carcinoma of the follicular type. More than 90% of the solitary nodules removed surgically in our series were found histologically to be neoplasms and therefore it is not logical to leave such tumors in the necks of children. Postoperative treatment with L-T4 is safe, well-tolerated and easy to monitor. FNAB, although not perfect, is currently the best method of establishing the final preoperative diagnosis. As we advance from the conventional strategy with cytological evaluation, which can miss the neoplastic nature of a lesion, the employment of immunocytochemical and molecular studies in aspirates from FNAB nodules (Domingues *et al*. 2005)

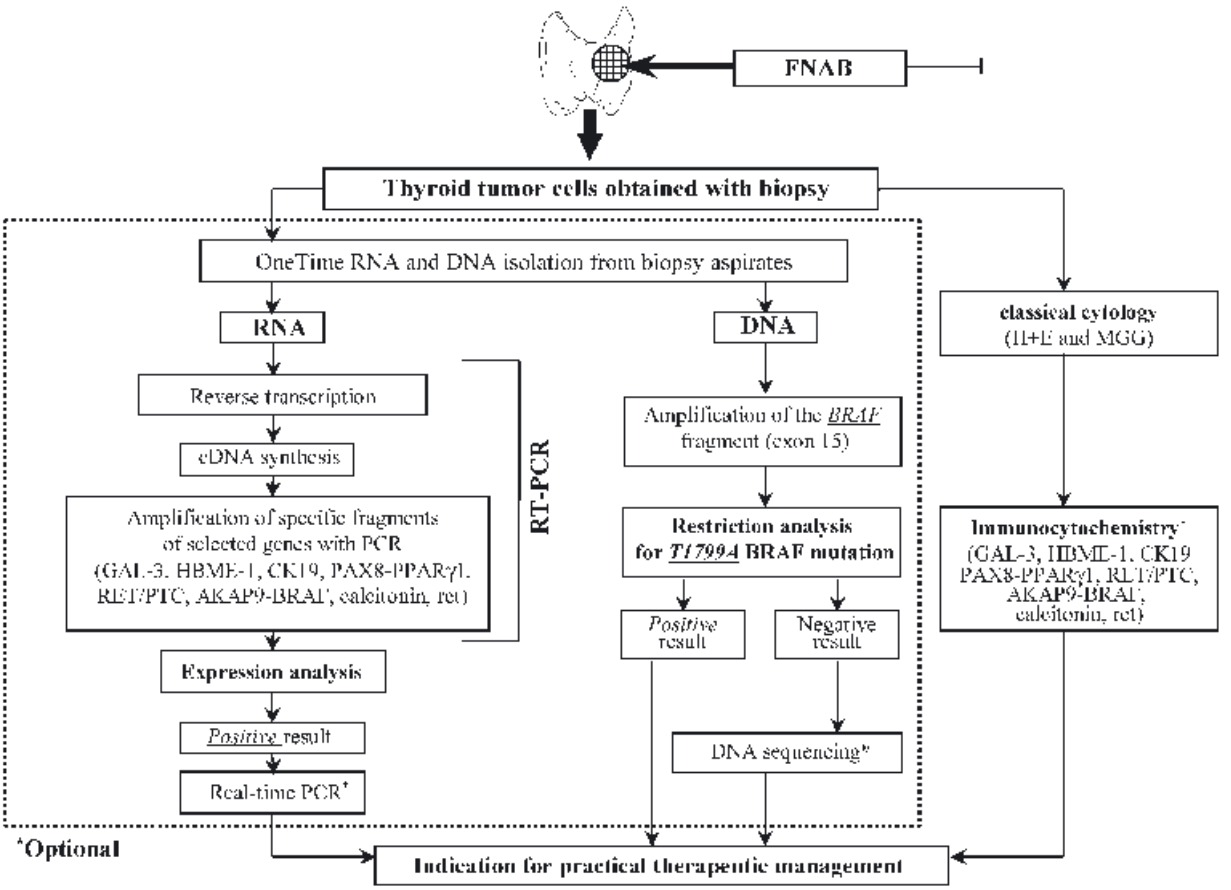

Figure 7 Preoperative study design (diagnostic work-up) of thyroid nodules based on cells obtained from biopsy.

should offer much greater precision in establishing the degree of risk of thyroid neoplasia and may help in choosing the best clinical management (Fig. 7). Expression profile studies, in terms of an accurate TND diagnosis (Finley *et al*. 2004, Giordano *et al*. 2005, Weber *et al*. 2004), are of great value, but are too expensive to be used as a standard preoperative test at present. Finally, detecting the presence of a combination of a limited number of genetic markers and an investigation into alterations of the BRAF gene may be reliable methods of preoperatively determining the malignant potential of thyroid nodules (Ciampi & Nikiforov 2005, Rosen *et al*. 2005).

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