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DIAGNOSTIC VALUE OF MOLECULAR MARKERS IN EVALUATION OF THYROID NODULES

For obtaining the degree of a Doctor of Medicine

Speciality - Surgery

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LIST OF ABBREVIATIONS

ATC - Anaplastic thyroid cancer

cAMP - Cyclic adenosine monophosphate

CD56 - Neural Cell Adhesion molecule 56

CG - Colloid goiter

CI - Confidence interval

CNB - Core-needle biopsy

COX-2 - Cyclooxygenase-2

CT - Computed tomography

DIT - Diiodotyrosine

DTC - Differentiated thyroid cancer

E-CAD - Epithelial cadherin

FC - Follicular cancer

FNA - Fine-needle aspiration

FS - Frozen section

HBME-1 - Hector Battifora mesothelial antigen-1

HE - Haematoxylin–eosin

ICH - Immunocytochemistry

IHC - Immunohistochemistry

Ki-67 - Antigen Ki-67

LNB - Large needle biopsy

MEN - Multiple Endocrine Neoplasia

MIT - Monoiodotyrosine

MNG - Multi nodular goiter

MRI - Magnetic resonance imaging

MTC - Medullary thyroid carcinoma

NPV - Negative predictive value

PAP - Peroxidase anti-peroxidase

PET - Positron emission computed tomography

PPV - Positive predictive value

PTC - Papillary thyroid carcinoma

p53 - protein 53

SE - Standard error

T3 - Triiodothyronin

T4 - Thyroxine

TBG - Thyroxine-binding globulin

TG - Thyroglobulin

TPO - Thyroperoxidase

TPOAb - Anti-thyroid peroxidase antibody

TRH - Thyrotropin releasing hormone

TSH - Thyroid stimulating hormone

US - Ultrasound

WHO - World Health Organisation

INTRODUCTION

Thyroid nodules are very common and are usually discovered during routine medical care. It is estimated that 5% of the general population develops clinically palpable thyroid nodules. With the emergence of ultrasound (US) impalpable thyroid nodules can be detected in 20-67% of general population (1, 2). Most of the discovered nodules are benign, however, increase in thyroid cancer incidence has been reported. Accurate diagnosis of thyroid nodules is critical for proper clinical management. Thyroid cancer represents ~5-24% of thyroid nodules and ~1-2% of all malignancies (3). Papillary thyroid carcinoma (PTC) constitutes about 80% of all thyroid malignancies (4).

Differential diagnosis of thyroid nodules could be difficult due to overlapping morphological features. As a result, up to 85% of patients with suspicious cytology, who subsequently undergo surgery, have benign lesions (5).

Today, in scientific publications many attempts have been described to find additional criteria to distinguish follicular adenomas from carcinomas, in both surgical and fine-needle aspiration (FNA) cytological specimens. A growing number of molecular or immunohistochemical (IHC) markers have been identified and tested with considerable variability in the outcomes of these studies.

In this study we evaluate the usefulness of applying the panel of six commercially available IHC markers: *Hector Battifora mesothelial antigen-1* (HBME-1), *neural cell adhesion molecule 56* (CD56), *epithelial cadherin* (E-CAD), *cyclooxygenase-2* (COX-2), *antigen Ki-67* (Ki-67) and *protein 53* (p53) on histological samples of benign and malignant thyroid nodules. The most promising markers were further tested on a FNA cytology of the thyroid.

Aim of the study: to evaluate the diagnostic value of molecular markers in management of thyroid nodules.

To achieve this goal, the following objectives were set:

1. To determine and evaluate diagnostic accuracy of molecular markers (HBME-1, COX-2, E-CAD, CD56, Ki-67 and p53) on histological samples of benign and malignant thyroid lesions.
2. To establish the panel of markers that can be recommended as an adjunct to morphology criteria.
3. To test the panel of markers before operation in thyroid FNA immunocytochemistry.
4. To develop practical recommendations in management of benign and malignant thyroid nodules.

Working hypothesis

1. Selected molecular markers (HBME-1, COX-2, E-CAD, CD56, Ki-67 and p53) are useful as an adjunct to morphology criteria of thyroid pathology.
2. Expression of each marker differs and the combination of several markers raises the diagnostic accuracy.
3. It is possible to develop technology for immunocytochemical markers on FNA samples.
4. Preoperatively clinically and radiologically indeterminate findings of thyroid nodules could be supplemented by immunocytochemical investigation of FNA biopsy samples.

Scientific and practical diagnostic novelty

From six molecular markers previously not thoroughly analysed and with equivocal published diagnostic value, HBME-1, E-CAD and CD56, in our study showed promising results to differentiate between benign and malignant thyroid nodules. Technology for immunocytochemical (ICH) analysis of thyroid FNA biopsy material was customised and applied. In the similar way biopsy material could be analysed in the other fields. To our knowledge, this is the first time in Latvia when

technology of ICH analysis of FNA biopsy material of the thyroid was used.

Personal contribution

The author was involved in all stages of the study, including the study design, selection of the markers, thyroid operations with further patients management as well as participating in thyroid US investigation and FNA biopsies. The literature review, scientific measurements, statistical analysis and interpretation were performed by the author. The author supervised processes of immunohistochemical visualisation and is the author of the demonstrated microphotographies.

Ethical concerns

The study was approved by the Committee of Ethics Pauls Stradins Clinical University hospital, reference Nr. 151209-3L.

1. LITERATURE REVIEW

1.1. Thyroid embryology

The thyroid is the first endocrine gland to develop in the embryo and it is first identifiable during the fourth week of gestation. It begins as an endodermal invagination of the tongue at the site of foramen cecum. It lies were the sulcus terminalis divides the tongue into anterior 2/3 (oral part) and posterior 1/3 (pharyngeal part). As the embryo lengthens and the tongue primordia grows, the thyroid diverticulum descends inferiorly to the hyoid bone and the larynx. The thyroglossal duct continues to connect the thyroid to its origin in the tongue until it reacher its destination in the neck and, its distal end may form pyramidal lobe (6). Complete failure of descent of the thyroglossal duct results in a lingual thyroid, located at the base of the tongue and may be responsible for lingual goiter. During the descent in the fifth week, the superior part of the duct degenerates, but remnants may persist and this serves as the embryological basis for the formation of the thyroglossal duct cysts as well as nodules within pyramidal lobe. About 1% of thyroglossal cysts contain papillary thyroid cancer. To avoid persistent or recurrent disease it is essential to systematically search for pyramidal lobe when performing total thyroidectomy because it is present in 30-40% of patients (7). By this time, the gland has achieved its rudimentary shape with two lobes and isthmus. During the descent in seventh week it reacher the level of cricoid cartilage and by the twelfth week of development, thyroid hormone is secreted (8, 9).

The gland is composed of two types of secretory cells: follicular cells that arise from the embryonic foregut and calcitonin-producing C cells that are derived from the neural crest and migrated from the fourth and fifth brachial pouches. The C cells eventually populate the entire gland. Tubercle of Zuckerkandl can be seen as a slight nodular thickening at the junction of the superior and middle third on the posterior surface of the gland where the lateral lobes meet the main thyroid body. As the heart, great vessels and thymus descend, it is drawn toward the superior mediastinum. The thymus separates, leaving the thyrothymic ligaments as remnants of their connection. When the endoderm from the fourth brachial pouch is pulled down in the descent of the

primitive thymus, retrosternal thyroid components are formed. Care must be taken to search for these extensions of thyroid tissue to prevent persistence or recurrence of disease when thyroidectomy is performed (10).

Ectopic thyroid tissue may occur at any point along the pathway of the descent of the thyroid. In rare instances, struma ovarii may arise and be responsible for thyrotoxicosis or malignancy with peritoneal metastasis (11).

1.2. Thyroid anatomy

The normal adult thyroid gland weighs between 15-20 grams and lies caudal to the larynx and encircles the anterior and lateral aspects of the first several rings of the trachea. The right and left lobes of the thyroid are joined at the midline by a bridge of tissue called the isthmus. The anterior surface of the thyroid is related to the deep surface of the sternothyroid, sternohyoid and omohyoid muscles. In the midline these muscles are absent. These paired muscles aid in swallowing. If the sternothyroid and sternohyoid muscles are to be divided transversely, it must be done at the cricoid level to preserve their motor nerve ansa hypoglossi. Medially, the superior part of the thyroid is related to larynx and laryngopharinx but the inferior part of the thyroid is related to the trachea and the esophagus. Laterally the gland is related to the carotid sheath, which contains the common carotid artery, the internal jugular vein, and the vagus nerve (11).

Approximately 50% of people have a pyramidal lobe, which is a remnant of the distal end of the thyroglossal duct and is usually located just to the left of midline. The normal thyroid is soft, dark wine-red in colour, and surrounded by a thin, fibrous capsule which posteriorly blends with the pretracheal fascia. A thickening of this fascia attaches the gland to the cricoid cartilage and the upper tracheal rings and is called the posterior suspensory, or Berry’s ligament (9).

1.3. Thyroid physiology

The parenchyma of the thyroid consists of two major cell types, the thyrocytes

and C cells. The thyrocytes release thyroid hormones and the C cells secrete mature calcitonin. Thyroid function is regulated by a negative feedback loop involving the hypothalamus, pituitary, and thyroid gland (12). Thyrotropin releasing hormone (TRH) is released from the hypothalamus into the portal circulation of the pituitary where it stimulates to release thyroid stimulating hormone (TSH). The two main thyroid hormones are produced by the follicular epithelial cells of the thyroid glands: thyroxine (T_4) which accounts for about 80% and to a much lesser extend (~20%) triiodothyronin (T_3). Thyroid hormones are almost entirely bound to plasma proteins and only a small percentage circulates in the free, bioavailable form. The TSH induces the resorption of stored iodinated thyroglobulin (TG) from the colloid and its transportation from the apical to the basal surface of the thyroid follicular cell. During this transport, T_4 and a smaller amount of T_3 are released from the TG molecule and then into the circulation. Thyroid hormone levels are a balance between the amount released by the thyroid gland and the amount entering the tissues, especially the liver and kidney (13).

Four T_3 nuclear receptors have been discovered: α1, α2, β1, and β2 isoforms. The β2 receptors are specific to the pituitary gland and plays a major role to the phenomenon of TSH suppression by thyroid hormone negative feedback loop (13).

Thyroid hormones are synthesized from iodide, under the control of TSH, in reactions that occur on the backbone of TG. TSH binds to membrane receptors on follicular cells and stimulates production of cyclic adenosine monophosphate (cAMP), which leads to increased uptake of iodine into the follicular cells. Thyroxine is produced by attaching iodine atoms to the ring structures of tyrosine molecules. Thyroxine T_4 contains four iodine atoms. T_3 is identical to T_4, but it has one less iodine atom per molecule. A sodium - iodine (Na/I) symporter pumps iodine (I^-) actively into the cell, which previously has crossed the endothelium. The iodine enters the follicular lumen from the cytoplasm by the transporter pendrin, in a passive manner (14). In the colloid, iodine (I^-) is oxidised to (I^0) by an enzyme called thyroperoxidase (TPO). Thyroid hormones are then synthesized by reaction with the enzyme TPO, iodine is bound to tyrosine residues within matrix of the TG. In the first reaction tyrosine residues in thyroglobulin to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). In the second reaction, MIT and DIT condense to form T_3, whereas two molecules of DIT condense to form T_4 (15). In conditions of iodine-sufficient intake, the predominant iodothyronine

synthesized by the thyroid gland is T_4 (16).

Antithyroid drugs can reduce thyroid hormone production by interfering with iodine oxidation. Iodine uptake against a concentration gradient is mediated by a sodium-iodine symporter and is linked to a sodium-potassium (Na^+/K^+). Antithyroid drugs can compete with iodine at this point.

The thyroid gland contains a very large store of thyroid hormone, which can last for several weeks in absence of the formation of new hormones (17).

TG is a polypeptide containing an average of 140 tyrosine residues. The synthesis of TG occurs exclusively in the thyroid gland, where homodimers are formed in the endoplasmic reticulum before being transported into the apical lumen of thyroid follicles. Defects of TG synthesis usually cause moderate to severe hypothyroidism in association with low circulating TG levels. Iodination of tyrosine increases with increasing extracellular concentration of iodine to a maximal rate. Above the extracellular iodine concentration (about 25 μg/dl), iodination of tyrosine is inhibited. This phenomenon is called Wolff-Chaikoff effect and is the basis for acute treatment of hyperthyroidism with exogenous iodine. However, after about 2 days there is an adaptation to this effect that spontaneously decreases the transport of iodine into the follicular cell, even in the presence of continued high plasma iodine concentration thus recurrence of euthyroidism or hyperthyroidism occurs. A minimum of 75 μg of dietary iodine intake is required daily for adequate thyroid function (6).

TPO is a membrane-bound glycoprotein that is localised to the apical membrane of the follicular cell and the peroxidase reactions occur at the cell-colloid interface (16). In the follicular lumen TPO catalyses the coupling of two DIT to form T_4 or thyroxine, or coupling of MIT and DIT to form T_3. Normally, about 80 μg of T_4 and 6 μg of T_3 are made each day.

Once released into the circulation, 99.95% of T_4 and 99.5% of T_3 are bound to several serum proteins, termed thyroxine-binding globulin (TBG), transthyretin, and albumin. TBG is a glycoprotein produced in liver that contains only one binding site per molecule. TBG is responsible for the transport of more than 3/4 of the thyroid hormone in the blood, and its levels are significantly increased by elevated levels of estrogens, as occurs in pregnancy. Thyroid hormone bound to these proteins is in equilibrium with the unbound (free) thyroid hormone - the biologically active component of circulating

T_4 and T_3 (18). In adults, the half-life of T_4 is about 7 days, because T_4 has a much higher binding affinity with its carrier proteins, whereas T_3 carrier protein bond is relatively weak, resulting in a short serum half-life of about 12 hours. The concentration of TBG is influenced by drugs, hormones and disease states. These conditions may alter the plasma concentration of total T_4 or T_3, even though the concentration of the active forms, free T_4 and T_3, may be unaltered, therefore measurement of total T_4 or total T_3 is unreliable as an indicator of thyroid function (6).

Most of free T_4 is converted to T_3 in the liver and many other tissues by the action of two deiodinases with characteristic tissue distribution. Type I deiodinase is predominant in liver, kidney, and thyroid, whereas type II is present in the central nervous system, pituitary, placenta, brown adipose tissue, cardiac and skeletal muscle, and thyroid. These differences in distribution and regulation may explain some tissue-specific variation in thyroid hormone action.

Thyroid hormone affect protein, fat and carbohydrate metabolism through several mechanisms. The major effects of thyroid hormone action occur through the intranuclear action of T_3, with T_4 being largely a prohormone (19). It is controversial whether T_4 regulates non-nuclear biologic responses, for instance, the activation of certain mitochondrial or cell-membrane enzymes. Tata with co-workers in 1960s, observed that T_3 treatment resulted in the rapid synthesis of nuclear RNA, which preceded increases in protein synthesis and mitochondrial oxygen consumption (20). The anterior pituitary, liver, brain, and heart are having high binding capacity for T_3 due to specific T_3 nuclear binding sites (19, 21). The current idea of thyroid hormone action is that its nuclear receptor binds to specific regulatory regions in target genes and regulates gene transcription in response to T_3 (19-23). The clinical manifestations of thyroid hormone action are the net result of the actions of the products of the various genes whose expression is regulated by T_3 (19).

Virtually no organ or tissue escapes the effect of thyroid hormone which occur through the intranuclear action of T_3, with T_4 being largely a pro-hormone. Sufficient amounts are necessary for brain development, normal growth and metabolism. (22, 24).

Normal amounts of thyroid hormone are necessary for the production and release of growth hormone. T_3 increases intestinal absorption of glucose and increases muscle and adipose tissue uptake of glucose as well as enhances the glycogenolytic and

hyperglycemic effect of adrenaline. Hyperthyroidism leads to a worsening of blood glucose control due to increased glycogen conversion. T_3 also speeds up the degradation of insulin.

T_3 decreases lipid stores and usually plasma lipid concentrations in case if thyroid hormone excess, hence this often results in a lowering of plasma cholesterol and triglyceride levels.

Vitamin metabolism is also affected by thyroid hormone. In case of thyroid hormone excess, requirements for water-soluble vitamins increases greatly.

Calcitonin is secreted by the parafollicular C cells located in the lateral lobes of the thyroid. It inhibits osteoclastic bone resorption, but the physiologic concentration of calcitonin have never been proven to have an important influence on calcium homeostasis. Calcitonin acts through specific cell surface receptors located predominantly on the surface of osteoclasts. These receptors have also been found in renal tubular epithelium, neural tissue, and lymphocytes (25, 26). Secretion of the hormone is increased in the presence of elevated levels of serum calcium. Calcitonin secretion can be stimulated by a number of techniques, including calcium infusion and pentagastrin infusion. As parafollicular cells are cells of neuroendocrine origin, they may lead to a neuroendocrine malignancy called medullary thyroid carcinoma. These rare tumours comprise ~ 5% of thyroid cancers and are familial in 20% of patients. Medullary thyroid cancer is a component of the Multiple Endocrine Neoplasia (MEN) Type IIa and IIb syndromes. Plasma calcitonin serves as a tumour marker to diagnose and monitor the activity of these tumours (6). Calcitonin does not cause a lowering of serum calcium levels, because in patients with MEN, in which calcitonin levels may be many thousands of times the normal level, hypocalcemia is not seen. Similarly, in patients after total thyroidectomy, calcium metabolism remain normal (27).

1.4. Etiology of thyroid nodules

In the vast majority the histological nature of thyroid nodules reveals either a cystic or solid adenoma or a colloid nodule. Both represent various stages of nodule formation and degeneration within a nodular thyroid gland. 30% of nodules represent

mixture of solid and cystic components, with pure thin-walled cysts being very rare (28). The risk of a concomitant thyroid cancer within a longstanding multinodular gland has been well investigated and is similar to that in a solitary thyroid nodule (29-34).

Most of the discovered nodules are benign, however, there are approximately 44,000 estimated new cases of thyroid cancer and 1700 estimated deaths in 2010 in the United States (35). The yearly incidence has increased from 3.6 per 100,000 in 1973 to 8.7/100,000 in 2002 - a 2.4× increase and this tendency appears to be continuing as shown in Figure 1.1. (36). This is due to an increase in papillary thyroid cancer (PTC), which increased from 2.7 to 7.7/100,000 - a 2.9× increase (36).

Data shown in Figure 1.2. represents that the major part of the yearly increase is the result of increased detection of small cancers, in this case small PTC.

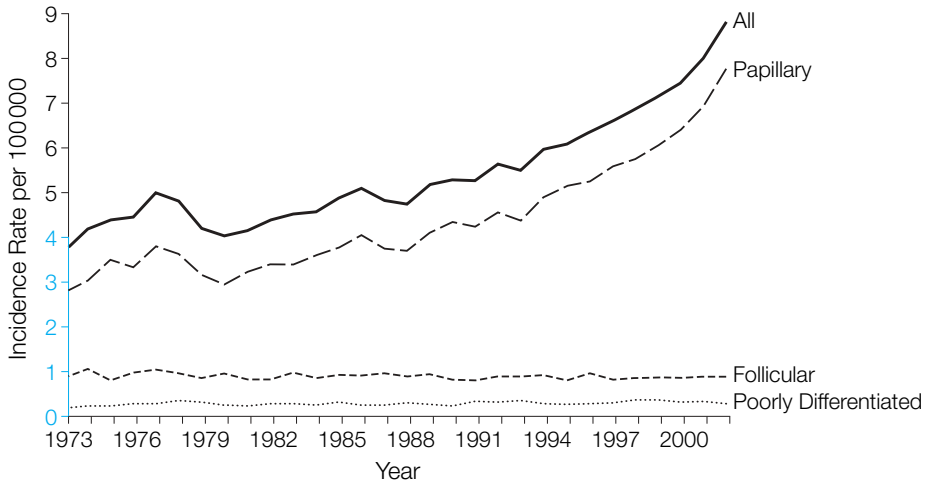

Figure 1.1. Trends in Incidence of Thyroid Cancer (1973-2002) in the United States. (Poorly differentiated indicates anaplastic and medullary cancers.) Reprinted from JAMA, May 10, 2006—Vol 295, No. 18

Benign thyroid disorders are among the most common diseases in Germany, affecting about 15 million people and leading to more than 100,000 thyroid surgeries annually (37, 38)

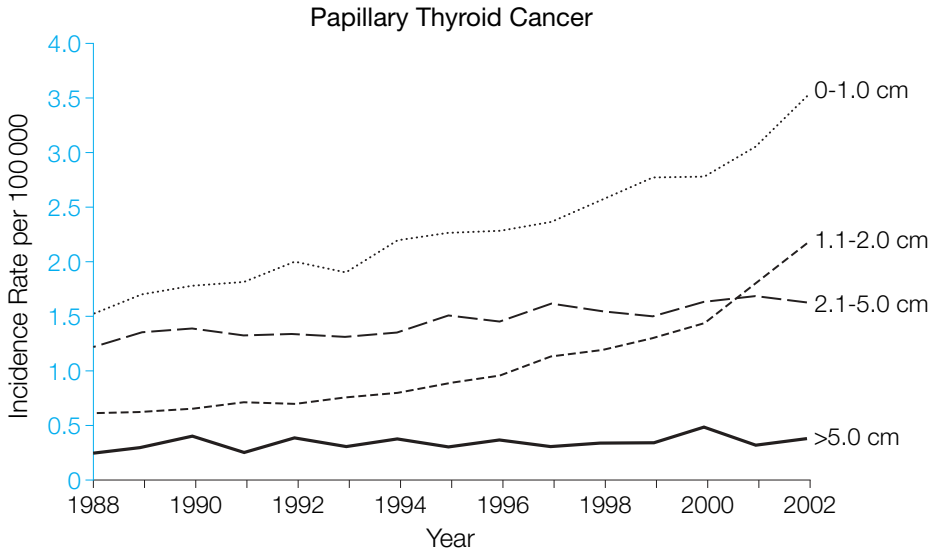

Figure 1.2. Tendency in Incidence of Papillary Thyroid Cancer by Size (1988-2002) in the United States.
Reprinted from JAMA, May 10, 2006—Vol 295, No. 18

The yearly incidence of thyroid cancer in Latvia has increased from 2.59/100,000 in 1991 to 9.39/100,000 in 2011. It is 3.62× increase and we think that this tendency will be continuing to rise (39, 40)

More than 80% of the malignancies present in palpable thyroid nodules are PTC, followed by follicular cancers (FC) and the much rarer anaplastic carcinomas. Thyroid cancer represents ~1-2% of all malignancies and 90% of all neiroendocrine tumours (3). High prevalence of PTC is accountable with to high percentage of microcarcinomas (36) which according to World Health Organisation (WHO) histological classification of tumours are papillary carcinomas 10 mm or less in its maximal diameter. Chow et al. reported data from Queen Elizabeth hospital in Hong Kong, were percentage of microcarcinomas have raised from 5.1% in period 1960-1980 to 21.7% in 1991-2000 respectively (41). Data from University of Wisconsin point out 42% prevalence of microcarcinomas and University Ferrara 40% respectively (42, 43). In conformity with it the incidence of thyroid cancer doubled over the past 30 years and 87% of the increase is due to diagnosis of small papillary cancers. Medullary thyroid carcinoma (MTC) and thyroid lymphomas are less frequent tumours (44).

Despite increasing incidence, the mortality from thyroid cancer has remained stable over the last decades. Thyroid cancer - specific mortality was approximately 0.5 death / 100,000 in 1973 and it is the same in 2002 as shown in Figure 1.3. (36).

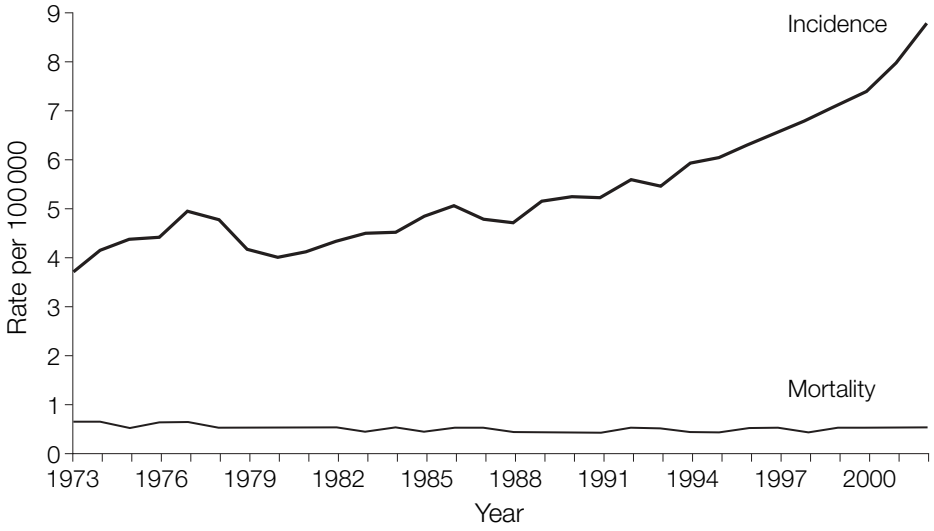

Figure 1.3. Thyroid Cancer Incidence and Mortality, 1973-2002.
Reprinted from JAMA, May 10, 2006—Vol 295, No. 18

1.5. Pathogenesis of thyroid nodules

Iodine deficiency (intake/absorption/excretion). The prevalence of thyroid nodules and multinodular goiters depends on the iodine intake, and it is lower in iodine-replete areas. It is considered that iodine deficiency is the most frequent factor contributing to development of multinodular goiters, affecting over 1.5 billion persons worldwide in 1990. However efforts by the World Health Organisation and private organisations were successful in reducing the number of persons with inadequate iodine intake (45). Even in iodine-sufficient regions the occurrence of clinically detectable nodular thyroid or sporadic goiters is observed in up to 4-7% of the population (46, 47).

External radiation to the neck during childhood is the best established environmental risk factor for the development of thyroid cancer (28, 48). Between 1940 to 1960, radiation was used as a treatment for thymic enlargement, recurrent tonsillitis,

adenoiditis, otitis media and dermatological conditions. This therapy has now clearly showed to be associated with an increased incidence of benign and malignant nodules. External radiation has been shown to increase the risk of malignancy for a thyroid nodule to approximately 40% (49), therefore, a history of neck irradiation clearly influences surgical management, lending support toward a more aggressive approach.

After the accident on April 26, 1986, at the Chernobyl nuclear power station, which is located at the north of Ukraine close to the borders of Belarus and Russia, over 10^{18} Becquerels of radioactivity were released into the environment (50). Due to changeable weather, radioactive pollutions have been detected to some extent in many European countries and even in the North America, but the heaviest contamination occurred in Belarus, Ukraine, and western parts of Russia. The spectrum of ejected isotopes included over four hundred of different radionuclides with large amounts of radiologically important ^{131}I and ^{137}Cs.

The incidence of thyroid cancer in areas around Chernobyl increased 6 - to 500× compared with previous years, depending on the distance of from the accident site. The latency period between the nuclear accident and diagnosis of thyroid cancer as relatively short ~6-7 years. Subjects less than 5 years of age or who were not born yet at the time of the nuclear accident accounted or majority of cases (51-53). A sharp increase in the incidence of thyroid cancer in exposed children in 1990–1993 peaked about a decade ago in the three most affected countries, shown in Figure 1.4. (54). The incidence rates are from 2.8/100,000 during the 1986–1989 period to 21.2/100,000 when viewing the longer time interval of 1986–1995 (55). Age at diagnosis was usually ≤ 14 years, which was younger than the sporadic thyroid cancers in children not related to the nuclear accident. The majority of cases were papillary thyroid cancers (~95%), which often showed greater aggressiveness at presentation, such as extra-thyroidal extension, venous invasion, and lymph node metastasis (53).

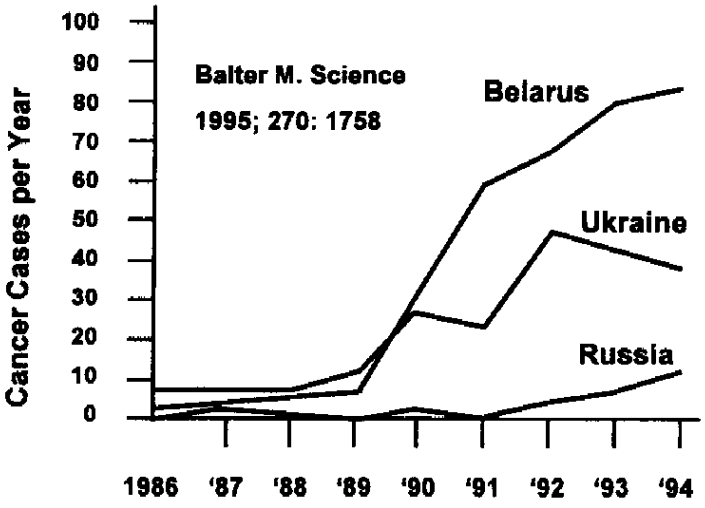

Figure 1.4. The Chernobyl disaster on 26 April 1986. Reprinted from Science. 1995; 270(5243): 1758-9

Nowadays, 20 years after the Chernobyl tragedy, incidence of thyroid cancer in children in the affected countries decreased to the levels just somewhat elevated compared to the pre-accident rate (56).

It is found that geographical factors may play a role in PTC, which has been found to have an increased incidence in iodine rich regions, while the incidence of FC is increased in iodine deficient endemic goitrous areas (57, 58).

Multifactorial heredity. A family history of endocrine disease and medical history which includes symptoms of pheochromocytoma or hyperparathyroidism, long standing constipation and/or diarrhea, hypertension and/or episodes of nervousness, should alert to the possibility of medullary thyroid carcinoma (MTC) in association with familial MEN Type II syndrome. MTC may be familial in 20% of the time, occurring in the MEN Type II syndromes, or may be sporadic but rarely is a non-MTC when thyroid cancers occur in two first-degree relatives. PTC is also occasionally familial and has been described with familial adenomatous polyposis (Gardner‘s syndrome) and ataxia-telaniectasia (59, 60).

Over the past decades the pathogenesis of nodular goiter formation has been intensively studied and debated. Usually it is thought that the presence of elevated TSH

level, for example in situations of iodine deficiency, is the prime stimulus leading to thyrocytes proliferation and the formation of a diffusely enlarged gland already during childhood and adolescence (61, 62). Presumably, elevated TSH level plays the key role in pathogenesis of nodular goiter, however angiogenic factors as well as signal transduction systems are investigated (63).

Lately modified idea about development of thyroid nodules has emerged, whereby the thyroid has specific impulse to form nodules with age, and that this impulse is amplified by additional factors, such as iodine deficiency and elevated TSH level, further contributing thyrocytes proliferation and nodule formation (64).

1.6. Approach to thyroid nodules

Thyroid nodules are very frequent finding and their prevalence steadily increases with age. Nodular thyroid disease refers to the presence of a solid nodule, a multinodular gland, or one or more cystic lesions. The primary goal in the evaluation of the thyroid nodule is to distinguish those nodules that require surgical intervention from those that can be safely observed. Grave's disease and chronic lymphocytic Hashimoto's thyroiditis can conduce the nodules to develop, as may subacute de Quervain's thyroiditis or an infection (65). While typically minimally aggressive, thyroid cancer can be lethal, therefore the question rises of how incidentally discovered lesions should be investigated in cost-effective and safe manner to identify the rare patient with a clinically significant malignancy.

It is estimated that 5-7% of adults have clinically detectable nodule in the thyroid and with the emergence of modern US techniques detecting thyroid nodules of a few millimetres, the frequency of nodularity was estimated at 16-67% in unselected subjects (66, 67). In autopsy series, the incidence of thyroid nodules in apparently normal thyroid glands is till 40% of cases, with nearly 40% of these nodules being larger than 2 cm (68, 69). From such studies it becomes apparent that thyroid nodules are extremely frequent in the normal population, and their prevalence increases with advancing age.

Starting at the age of 20, the prevalence of nodules detected by palpation increases by 1% for each decade of age or by 10% per decade if detected by US (70). About half of such patients present with a solitary nodule, while the other half harbours multiple nodules. When the palpation is used as the method of detection, nodules are found in 5-20% of normal population, most of which exceed the size of 1 cm, which is usually the threshold for detection by physical examination. As for the nodules detected by US, nearly 50% of patients with a clinically solitary nodule have a multinodular gland (71).

1.6.1. Clinical evaluation of thyroid nodules

Most thyroid nodules are asymptomatic and are usually discovered by the patient themselves or by the physician during routine medical care, however the absence of symptoms does not rule out malignancy. A great deal of thyroid nodules are detected during a radiological examination of the neck.

Usually medical concerns according to the thyroid nodules revolve around three questions: 1) the presence of thyroid disfunction; 2) the presence of malignancy; 3) the likelihood of a progressive increase in size of the nodule eventually leading to symptoms (28).

Evaluation of a patient with a thyroid nodule should begin with a comprehensive history and physical examination focused on the thyroid gland and cervical lymph nodes. Patients commonly report a lump in the neck found during palpation. According to single and multiple nodules, current evidence suggests that when a dominant nodule appears in a multinodular gland, the risks of malignancy are probably the same as those in a true solitary nodule (72, 73). According to Bouhabel et al. the likelihood of thyroid cancer is independent of the number of thyroid nodules. Moreover, the malignancy rate is not influenced by the distribution of the nodules or their size (74).

The factors like young and old age, male gender, history of head and neck irradiation, family history of thyroid carcinoma, rapid growth and hoarseness can predict malignancy, however malignant nodule can also be extremely slow growing, present for many years before diagnosis is made. Any nodule developing before puberty

should be viewed with suspicious. It has been estimated that more than half of all thyroid nodules in children prove to be malignant (75).

The incidence if malignancy is also higher in nodules that develop after the age of 65. Benign nodules are more common in both males and females; however, thyroid nodules are five times more frequent in females. The proportion of malignant nodules in males is twice that of females but as nodules are much more frequent in women, more cancers are still found among women (29, 65).

Very rapid development, such as over hours or days, would suggest a thyroid cyst or haemorrhage. Specific symptoms that would be worrisome of malignancy are dysphagia, voice change, coughing, choking, dyspnea, sudden increase in size and cervical lymphadenopathy (76).

Nowadays most experts are agreed that palpable solitary nodules above 1 cm should be investigated in euthyroid patients (66, 77). The kind of limit is warrant by the very low recurrence rate and the virtually absent mortality for differentiated thyroid cancers below 1 cm (28, 78).

Because of the possible presence of an autonomous nodule, Graves' disease, or Hashimoto's thyroiditis it is important to rule out hyperthyroidism or hypothyroidism by measuring a serum TSH level before going on with the evaluation of a nodule.

In a multinodular gland of a euthyroid patient a reasonable approach is to evaluate the dominant nodule. The term dominant refers to either the largest nodule or the one that has recently increased significantly in size. In elderly patients with multinodular goiters without a radiation history before adolescence and without recent changes in the size of the existing nodules or the appearance of new lesions, only nodules above 1.5 cm are recommended to evaluate (28).

It would be wrong to assume that the most precise quality of care is obtained by evaluating all thyroid nodules with FNA, apart from their size and their clinical presence (multinodular thyroid, single nodule, age of the patient, radiation history, etc.), since the probability for the presence of a cytologically "suspicious" lesions is 20%. Considering this, most of the patients will eventually undergo a thyroidectomy to exclude the presence of a cancer which is present in 10-20% of all microfollicular lesions. Hence, once the decision is made to do FNA biopsy of the nodule, the patient has an a priori probability of 10-20% for a thyroidectomy, which is unnecessary in

80-90% of the cases (28).

Thyroidectomy carriers small but significant risks of operative complications, including permanent hypoparathyroidism and damage to the recurrent laryngeal nerve, which can result in chronic aspiration and compromised voice quality. Additionally, thyroidectomy commits the patient to a lifetime of thyroid replacement therapy and a recommendation of longterm surveillance for recurrent disease. From these considerations and the clinical irrelevance of most occult papillary microcarcinomas, consequences can be drawn that nodules below 1 cm (or <1.5 cm within a multinodular goiter) should not require further evaluation in most patients. These lesions should be followed clinically unless the patient presents with specific risk factors for malignancy.

Above mentioned strategy applies to thyroid incidentalomas, nodules that are discovered on a radiological examination of the neck for non-thyroid disease or as part of the evaluation of a clinically solitary apparent thyroid nodule (28, 69).

While the discussion is appropriate for patients with nodules below 4 cm, lesions above this size are recommended to operate without necessarily performing a prior biopsy. This approach is accessible by the high potential of such nodules to become locally symptomatic. In more than 40% of lesions of this size, there are difficulties to exclude malignancy by FNA because of higher rate of false-negative cytology due to sampling issues (79).

When choices must be made between clinical follow-up, biopsy, and surgery, it is important to remember that death from cancer is a rare event however microscopic cancers seldom lead to significant diseases (80).

1.6.2. Laboratory evaluation

With the discovery of a thyroid nodule, a biochemical assessment of thyroid function is required, because clinical appraisal is not a reliable indicator of thyroid status. An obvious biochemical test that is necessary is the TSH level. The high sensitivity of the TSH assay for detecting even small thyroid dysfunction makes it the most useful test in the initial evaluation if thyroid nodules (81). According to Boelaert et al. elevated serum TSH could be associated with increased risk of malignancy in a

thyroid nodule as shown in Table 1.1. (82). A study done by Zafon et al. found that TSH levels were higher in patients with final diagnosis of DTC and there is a correlation between tumour size and TSH level (83)

Table 1.1.

TSH and the calculated risk of thyroid cancer. Forty year old woman with a solitary thyroid nodule

Serum TSH mU/L	Risk of Cancer
0.3	8.1%
0.5	8.4%
1.0	9.4%
3.0	14.6%
5.0	21.9%
6.0	26.4%

If the serum TSH is subnormal, a thyroid scintigraphy using ^{123}I should be obtained to document whether the nodule is hyperfunctioning, isofunctioning or nonfunctioning. Hyperfunctioning nodule may be part of Plummer's syndrome (toxic multinodular goiter) or multiple nodules present in a patient with Graves' disease. Hyperfunctioning nodules rarely represent malignancy.

Measuring serum levels of free thyroid hormones and anti-thyroid peroxidase antibody (TPOAb) or anti-TSH-receptor antibody should be the next diagnostic step for confirmation of thyroid dysfunction if the TSH concentration is outside the reference range (84). In case of increased serum TSH free thyroxine and TPOAb should be tested to evaluate hypothyroidism. When the serum TSH is decreased free thyroxine and triiodothyronine should be assigned to evaluate hyperthyroidism.

TPOAb should be measured in patients with high levels of TSH. Elevated serum TPOAb values and a firm, diffusely enlarged, or small thyroid suggest autoimmune or Hashimoto thyroiditis (85, 86).

Routine assessment of serum thyroglobulin for initial evaluation of thyroid

nodules is not recommended because it can be elevated in most thyroid diseases and is neither sensitive nor specific for thyroid cancer (87).

Calcitonin is a hormone that is produced in humans primarily by the parafollicular cells (C-cells) of the thyroid. The utilities of serum calcitonin assessment have been evaluated in a series of prospective, nonrandomized studies (88, 89). The use of routine serum calcitonin in patients with nodular thyroid disease may detect C-cell hyperplasia and medullary thyroid cancer (MTC) at an earlier stage; however, routine testing in all patients with unselected thyroid nodules is still debated. Calcitonin levels can be increased in patients with pulmonary or pancreatic endocrine tumours, kidney failure or autoimmune thyroid disease; other factors are alcohol consumption and smoking. If calcitonin level is increased, the test should be repeated and, if confirmed without the above-mentioned modifiers, a pentagastrin stimulation test should be added to increase specificity (88). Measurement of serum calcitonin is mandatory in patients with family history or clinical suspicious of MTC or MEN Type II syndrome.

To exclude concomitant hyperparathyroidism, serum calcium concentration should be determined preoperatively. An increased calcium concentration requires further preoperative evaluation (37)

1.6.3. Diagnostic imaging studies

Ultrasonography. Due to superficial anatomic location of thyroid gland, it may be easily assessed by US which provides information in the size and the structure of the organ and detects signs of malignancy (90). Usually transducers with a width of 7.5-9 cm and frequencies of around 10 MHz are used. In the 1980s, US came into widespread use (91). US is much more sensitive than physical examination alone and with US it is possible to detect thyroid lesions, identify their structure and measure their dimensions. It should be performed in all patients with suspected thyroid nodule or nodular goiter and combined with a US of the surrounding soft tissues and the cervical vessels. While thyroid US cannot diagnose a thyroid nodule as malignant, it can detect nodules as small as 0.2 mm in diameter as well as the presence of cervical lymphadenopathy. The position, shape, size, margins, content, echogenic pattern and vascular features of the

nodules can be described (92-94). A sonographic examination performed by the surgeon may be helpful for planning the resection.

The normal thyroid has a smooth contour and shows a fine granular homogeneous, slightly hyperechoic sonographic type. Its total volume ranges up to 18 ml in women and up to 25 ml in men. Isthmical part should not exceed 1 cm in its anteroposterior diameter and both lobes should perform a symmetric elevation during swallowing (95).

Some US characteristics associated with a higher risk of malignancy have been evaluated in a series of studies (94, 96-98). The reported specificities for predicting malignancy are 41.4 - 92.2% for marked hypoechogenicity compared to normal thyroid parenchyma, 44.2 - 95% for microcalcifications, 48.3 - 91.8% for irregular or microlobulated margins, and ~ 80% for increased intranodular vascularity (97, 98).

A shape taller than the width measured in the transverse dimension is an additional US pattern suggestive of malignant potential. Suspicious cervical lymphadenopathy is a specific but insensitive finding. No single sonographic feature or combinations of features are adequate to identify all malignant nodules; however, the coexistence of 2 or more suspicious US criteria greatly increases the risk of thyroid cancer (96, 97). US features of benign and malignant thyroid nodules are summarised in Table 1.2.

US is best used as imaging guidance for FNA which greatly increases FNA accuracy, sensitivity and specificity (7). In case of cancer diagnosis on FNA, neck US by an experienced ultrasonographer is mandatory to evaluate cervical lymphadenopathy that may not be detected by physical examination (99).

US guided FNA (Figure 1.5.) represents a hypoechogenic nodule with microcalcifications, irregular margin and mixed vascularity.

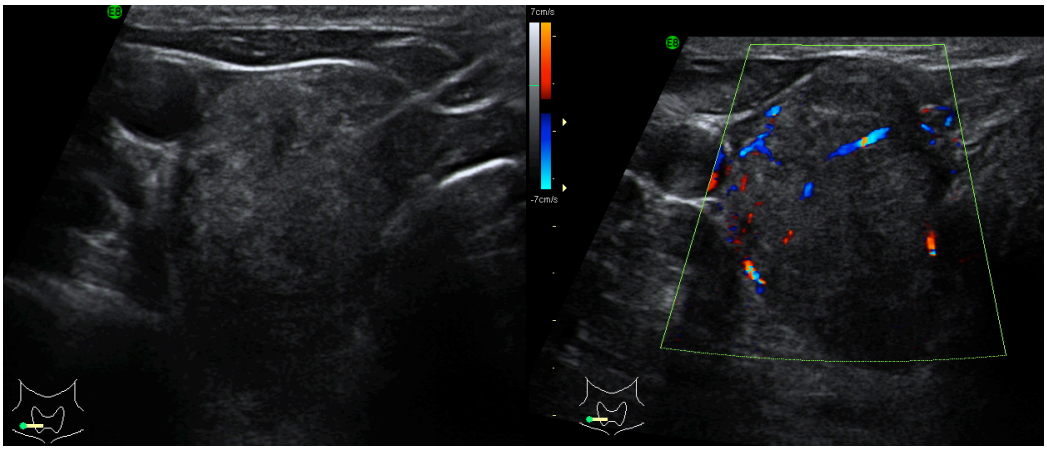

Figure 1.5. Ultrasound guided FNA of the nodule in thyroid right lobe. Note hypoechogenic nodule with microcalcifications, irregular margin with mixed vascularity. Procedure made by Kaspars Stepanovs, year 2012. Used with permission

The most common sonographic appearances of PTC are a solid or predominantly solid and hypoechoic mass, usually with infiltrative irregular margins and increased nodular vascularity. Microcalcifications are highly specific for PTC. Whereas FC is more often iso - to hyperechoic with a thick irregular halo and lack of microcalcifications. There are also certain sonographic criteria that may be predictive of a benign nodule. A pure cystic nodule or a nodule with spongiform appearance, defined as an aggregation of multiple microcystic components in more than 50% of the nodule volume, is specific for identification of benign thyroid nodule (98).

Table 1.2.

Summary of ultrasonographic features of benign and malignant nodules

Features	**Benign**	**Malignant**
Echogenicity	Normal or hypoechogenic	Hypoechogenic
Halo	Thin and well-defined	Thick, irregular or absent
Calcification	Coarse	Micro
Margin	Regular	Irregular
Invasive growth	Absent	Present
Lymphadenopathy	Absent	Present

Features	Benign	Malignant
Intranodular blood flow	Low	High

US elastography is a newly developed and promising technique that has recently been applied in the diagnostic approach to nodular thyroid disease and has shown a high sensitivity and specificity in selected patients (100-102). It evaluates the degree of distortion of a tissue under application of an external force, based upon the principle that the softer parts of tissues deform more readily than the harder parts under compression, thereby allowing objective determination of tissue consistency (103, 104). However, US elastography has some limitations because the nodule to be examined must be clearly distinguishable from other nodules. In addition, malignant lesions tend to be much harder than benign ones.

Larger prospective studies are needed to establish the diagnostic accuracy of this technique (105). At the moment available elastography studies are summarised in Table 1.3.

Table 1.3.

Notable elastography studies

Study	Patients	Nodules	Sensitivity	Specificity
Shuzhen et al., 2011	244	291	94.1	81.1
Luo et al., 2012	106	123	95	73.8
Rago et al., 2007	92	92	97	100
Hong et al., 2009	90	145	88	90
Friedrich-Rust et al., 2009	56	59	88	83
Tranquart et al., 2008	96	108	100	93.1
Sabag et al., 2010	93	148	83.2	93.9

Computed tomography (CT) and **magnetic resonance imaging (MRI)** has a limited but important role in evaluation of the thyroid gland. CT and MRI should not be used routinely in nodular thyroid disease but are best used as an adjunct in imaging of advanced thyroid pathology when retrosternal, intrathoracic, or retrotracheal extension of the glans is present. CT and MRI scanning may also be very helpful in the preoperative assessment of cervical lymphadenopathy, as well as in determining invasion or compression of the aerodigestive tracts (76).

Positron emission computed tomography (PET) scanning is being recently reported as useful method for finding thyroid incidentalomas as well as for its ability to determine malignancy of thyroid nodules. PET scanning is increasingly being used for staging and for supervision of other malignancies as a result, it may occasionally identify a thyroid lesion (106). Normally the thyroid gland is not visualised on whole body PET scan but incidental diffuse or focal increased uptakes have been reported in the large series.

Diffuse uptake indicate a benign process while focal uptake is associated with a significantly increased thyroid cancer risk (30-50% of those selected for FNA) (28, 107).

1.6.4. Thyroid scintigraphy

Until recently, radionuclide scanning had routinely been the first test used in the evaluation of the thyroid nodule but with the appearance of FNA biopsies, the importance of thyroid scans was greatly reduced. Thyroid scintigraphy allows assessment of thyroid regional function. Usually ^{123}I is the preferred isotope for this examination with the theory that malignant thyroid tissue neither traps nor incorporates iodine and appear nonfunctioning, or cold, on uptake scan. After radionuclide uptake, nodules may be classified as hyper-functioning (“hot”), hypo-functioning (“cold”), or normally functioning (“warm”). Thyroid scintigraphy has a role in patients with a low TSH level, indicating developing thyroid autonomy and hence the possible presence of a toxic adenoma. Nonfunctioning “cold” nodules are believed to have a probability of

being malignant, but according to literature the reported malignancy risk is approximately 15% (108) compared with 9% for warm nodules and about 5% in hot nodules (109). If thyroid scintigraphy is concordant with US for the identification of a clearly “hot” nodule, then that nodule does not require further evaluation with FNA as malignancy risk is extremely low. Thyroid scintigraphy can be performed for multi nodular goiter (MNG) even if the TSH level is normal, to identify cold or indeterminate areas for FNA and hot areas that do not need cytologic evaluation. Thyroid scintigraphy should be performed if a retrosternal goiter or ectopic thyroid tissue is suspected and before surgery for recurrent disease.

1.6.5. Laryngoscopy

Laryngoscopy is increasingly being advocated as a routine procedure before thyroid surgery for benign as well malignant disease. In the presence of a suspected thyroid cancer or with preexisting voice changes as well as after previous surgery in the neck area, laryngoscopy is necessary and can be supplemented by further examinations (76). On the one hand it serves as quality control and, on the other hand, to confirm any preexisting impairments, which must be considered into the decision-making regarding surgical intervention (37, 110)

1.6.6. Thyroid biopsy

Fine needle aspiration (FNA)

Thyroid nodules are common and their incidence increases with age. An estimated 40% of the United States population contain thyroid nodules, approximately half of which are solitary on physical examination (71, 111). However among them there are clinically silent thyroid cancers, usually less than centimetre in size, which are found in up to 35% of thyroid glands evaluated at autopsy or at surgery (112-114). Wherewith it would be irresponsible to remove all thyroid nodules surgically.

FNA has become the ultimate test, in association with clinical findings to select

those patients who will benefit from surgery the most. FNA is now considered to be the most accurate, cost-effective, and simplest screening method for fast diagnosis of thyroid nodules, with accuracy around 95% (31, 115, 116). It is a simple outpatient procedure which provides quick and specific information about the cytology of a nodule. PTC is the most frequently detected malignant thyroid neoplasm. Aspiration cytology diagnosis is more reliable and the non-diagnostic rate is lower when FNA biopsy is performed with US guidance, because the most common cause of a false - negative cytological diagnosis is sampling error (117, 118).

Main indications for performing FNA of the thyroid are:

- Initial diagnostic test for solitary cold thyroid nodule;
- Dominant new or enlarging cold nodule in a gland showing multinodularity;
- Nodules with suspicious US features;
- Complex or recurrent cystic nodule;
- Suspicious neck lymph nodes;
- Metastatic disease to the thyroid.

There are no specific contraindication to the procedure. The needles are enough thin (23 gauge or smaller) hereby complications such as local infections, hematoma or localised pain are exceedingly rare (119). Prophylactic antibiotic therapy is not indicated.

Among other preoperative tests it has been shown to be a better predictor of malignancy and has decreased the number of thyroid operations by about half and has increased the yield of cancer from 15-50% (70). Influence of FNA to number of thyroid operations is undeniable. It is convincingly showed also in the study by Gharib and Goeliner (120) were due to the introduction of FNA, number of operations decreases, but cancer incidence stays practically the same through more than 10 years (Figure 1.6.).

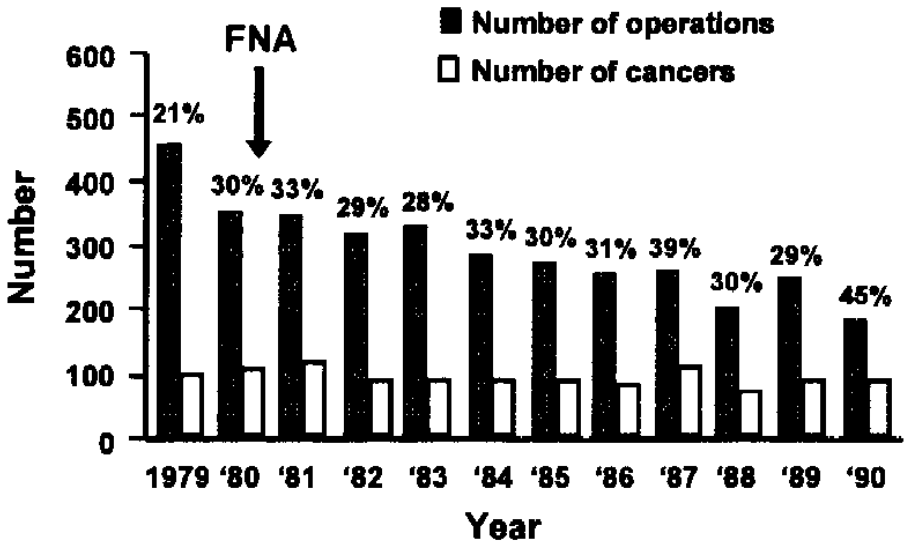

Figure 1.6. Influence of FNA to Thyroid Operations.
Reprinted from Ann Intern Med. 1993; 118(4): 282-9

FNA of the thyroid has an overall diagnostic accuracy of over 95%, with a sensitivity of over 95% and a specificity above 95% even in multinodular glands (121). While the probability of obtaining a malignant diagnosis in repeat FNA in the follow-up of an eventual benign thyroid nodule may be low, rebiopsy reportedly reduces the rate of false-negative diagnoses from a mean of 5.2% to less than 1.3% (122, 123). However the routine performance of repeat FNA in the follow-up of patients with or without any clinical changes is of limited value (124). Clinical factors may be superior in surgical decision of patients with benign nodular thyroid disease management (125, 126). Still discussions exists over whether the best samples are obtained with passage of the needle through the lesion with or without continuous suction (76). A Comparative study on FNA cytology versus fine needle capillary cytology in thyroid nodules done by Tauro with coworkers conclude that hat there was no significant difference between the two techniques. In highly cellular lesions, in which abundant material was obtained, fine needle capillary technique was more likely to be diagnostically superior, but FNA can diagnose most of the lesions. In less cellular lesions, FNA is more likely to be diagnostically superior (127).

The FNA biopsy sample must be adequate for an interpretation that yields a low false negative rate and should be reviewed by a cytopathologist with an interest in

thyroid disease. The rate of inadequate samples is mostly dependent on the experience of the physician performing the aspiration and even in the most experienced hands the rate of non-diagnostic biopsies is around 5% (67, 118). Inadequate specimen is usually caused by dilution of aspirated thyrocytes (by blood in vascularized nodules or by fluid in cystic nodules) or lesions technically difficult to biopsy (128). It is important to underline that samples with insufficient material don not provide reassurance and the procedure needs to be repeated. Indeed, in the study by McHenry et al. 10% of operated nodules with previous non-diagnostic biopsies turned out to be cancers (129). The rate of unsatisfactory samples can be decreased if more than one aspiration is performed during the first FNA (130).

A false-positive diagnosis reflects that no malignancy was detected in a surgically removed thyroid that had a positive result for malignancy in FNA. The incidence of false-positive results ranges from less than 1%-7.7% (118). Most errors are interpretative, resulting from overlapping features, inadequate specimen, degenerative changes, or inexperience of cytopathologist (131). PTC is the most common false-positive diagnosis (131).

There are no universally accepted criteria defining adequacy of a specimen. Criteria vary from a minimum of five groups of cells to as many as ten groups in each of two slides (128). The amount and type of colloid as well as the size of follicles should be described. The diagnosis - PTC by FNA is based on identification of classic nuclear, cytoplasmic, and architectural features. These features are nuclei with irregular membranes, grooving, pale chromatin, and intranuclear cytoplasmic invaginations (132). However, in cases that lack all these features, the accurate diagnosis of PTC can be diagnostically challenging.

Usually five diagnostic categories are used as indicated by the British Thyroid Association guidelines: benign, nondiagnostic, follicular lesion, suspicious, and malignant (133). FNA results are classified as benign in 60-80%; nondiagnostic in 10-15%; follicular lesions in 10-20%; suspicious in 2.5-10% and 3.5-10% are malignant (118, 134). The results of FNA biopsy are critical in deciding whether to manage the nodule medically or surgically.

Benign lesion is the most common finding and is present around 70% of all aspirates. Benign cytological findings are adenomatous or colloid nodule, hyperplastic nodule and different forms of thyroiditis as well as benign cysts (105). Once the presence of a malignant lesion in a dominant nodule has been ruled out by FNA, patients should be followed clinically. Due to the false - negative rate for a malignancy being less than 5%, a repeated FNA is not mandatory unless the nodule changes significantly in size (28).

Nondiagnostic aspirate will contain too few cells for the cytopathologist to make a diagnosis, which can be due to cyst fluid, bloody smears, or poor technique in preparing slides (67). Criteria for adequate material vary from a minimum of five groups of cells to as many as ten groups on each of two slides (128). According to definition of Hamburger, the specimen is labelled “diagnostic” if it contains a minimum of 6 groupings of well - preserved thyroid epithelial cells, consisting of at least 10 cells per group (134, 135). The rate of nondiagnostic samples is largely dependent on the experience of the physician performing the aspiration, but even in the most experienced hands the rate of nondiagnostic samples is around 5% (128).

The samples with insufficient material do not provide reassurance and the biopsy need to be repeated, because around 10% of operated nodules with previous nondiagnostic biopsies turned out to be cancers (129). Repeated FNA is worthwhile because it will provide adequate sampling in 50-70% of patients (31). Performing more than one aspiration during the first FNA decreases the rate of unsatisfactory samples (130).

Follicular lesions also called follicular neoplasms is reported in 10-20% of aspirated thyroid nodules. Follicular lesions include adenomatoid hyperplasia, follicular adenoma and carcinoma, Hürthle cell neoplasms, and the follicular variant of PTC. Definitive cytologic diagnosis between a follicular adenoma and a follicular cancer, the latter being present in less than 10-20% of follicular lesions, cannot be established with cytomorphology (32, 136, 137). It is important to perform the cytological evaluation of a thyroid nodule only after the euthyroid state of the patient has been ascertained, because the cytological features of aspirates from autonomous benign nodules may

mimic those present in follicular cancers.

Suspicious results in cytologic diagnosis are characterised by adequate cellularity and cytologic features suggesting but not fulfilling the criteria for a definitive diagnosis of malignancy. In suspicious results there are also included samples with poor cellularity or poor fixation and preservation but clear signs indicating malignancy.

Malignant results on thyroid FNA occurs in about 5% of the aspirated nodules and are characterised by malignant cytologic features that are reliably identified by cytopathologist. In the vast majority a PTC is present and thanks to its specific cytological features this diagnosis can be made with over 90-95% sensitivity and specificity. In rare cases, the cytology may suggest the presence of anaplastic cancer, MTC, a metastasis, or a lymphoma (28).

Large needle biopsy (LNB)

LNB performed without US guidance with a large-bore needle, is not recommended for thyroid nodules because of local pain and risk of cervical bleeding. It also does not add any further diagnostic information to FNA biopsy in nodules with follicular cytologic characteristics (105, 138).

Core-needle biopsy (CNB)

CNB is performed under US guidance with a 20-21 gauge cutting needle by experienced operator, may offer additional information to FNA biopsy in selected cases of thyroid or neck masses with repeated inadequate FNA cytology (139). CNB offers no additional diagnostic value in distinguishing a cellular hyperplastic nodule from a follicular adenoma or carcinoma, therefore US guided CNB should not been seen as an alternative to FNA, but as a complementary investigational tool (140, 141).

1.6.7. Frozen section

Frozen section (FS) has been in use since 1818, but it became routine after the introduction of cryostat in 1960 (142). The frozen section procedure is a pathological laboratory procedure to perform rapid microscopic analysis of a specimen. The quality of the slides produced by frozen section is of lower quality than formalin fixed, wax embedded tissue processing. While diagnosis can be rendered in many cases, fixed tissue processing is preferred in many conditions for more accurate diagnosis. Method of FS is well recognised, but only few studies have been performed to examine the role of FS in surgical planning and postoperative treatment of patients with indeterminate cytology (143-146). With the widespread application of FNA, in recent years the role of FS in the management of thyroid nodules decreases.

1.6.8. Immunohistochemistry

Immunohistochemistry (IHC) has emerged as a powerful tool for investigation which can provide additional information to the routine morphological assessment of tissues. The use of IHC to study molecular markers that define specific phenotypes has provided significant diagnostic, prognostic, and predictive information relative to status and biology of the disease. The application of antibodies to the molecular study of tissue pathology has required adaptation and refinement of IHC techniques, particularly for use in fixed tissues because of the superior morphology provided by formalin-fixed paraffin-embedded tissues, this has become the method of choice for most clinical and research studies (147).

IHC is the process of detecting antigens or proteins in cell of a tissue section with labeled antibodies as specific reagents through antigen-antibody interaction. Visualising an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction. The peroxidase-labeled antibody method, introduced in 1968, was the first practical application of antibodies to paraffin-embedded tissues (148). Alternatively, the antibody can also be tagged to a

fluorescein or rhodamine (fluorescence antibody methods) but these methods have some of the limitations.

The successful application of IHC methods to formalin-fixed paraffin-embedded tissues specimens stimulated rapid progress in this newly emerging field, and in short time came the introduction of the immunoperoxidase bridge method and the peroxidase anti-peroxidase (PAP) complex method (149, 150). Figure 1.7. represents peroxidase anti-peroxidase complex method. Several other methods for IHC staining exists, including avidin-biotin complex method, polymer-based IHC, fluorescyl-tyraminde amplification and rolling circle amplification methods.

Nowadays as immunohistochemical techniques continues to evolve, their application to surgical and research pathology is becoming increasingly valuable. Different amplification methods have made significant improvements such that many antigens, previously believed to have been lost to the process of fixation and embedding, can now be routinely demonstrated. As technology marches forward, new molecular markers are emerging that are providing the tools to generate important new discoveries. Due to this our knowledge of the underlying biology and pathogenesis of disease increases.

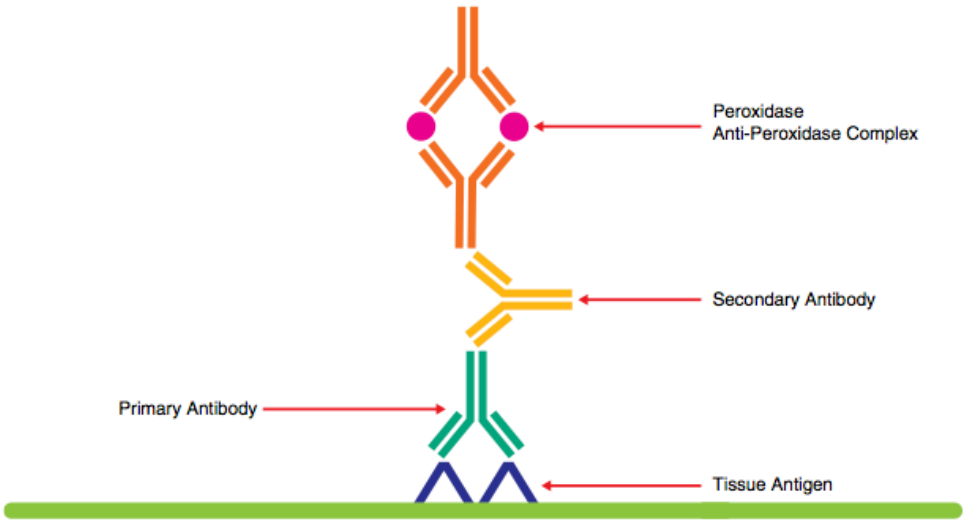

Figure 1.7. Peroxidase Anti-Peroxidase (PAP) Complex Method.
Reprinted from Education Guide Immunohistochemical (IHC) Staining Methods. Updated and Expanded Fifth Edition, G.L. Kulmar and L. Rudbeck, Editors. 2009; Carpinteria: 57-60

Hector Battifora mesothelial antigen-1 (HBME-1), first described in 1992 by Battifora et al. (151), is a mouse monoclonal antibody directed against an antigen of the microvillous surface of mesothelioma cells (152). Although this antibody was originally developed as a mesothelioma marker, it was subsequently applied to the diagnosis of malignant thyroid conditions (151, 153). Miettinen et al. performed HBME-1 immunohistochemical staining on thyroid tumours and reported HBME-1 positivity in thyroid carcinomas (154, 155) It has been found to be reactive mostly in papillary thyroid carcinoma and some follicular carcinomas, but usually negative in follicular adenomas (152). Although it shows positive for the normal tracheal epithelium and adenocarcinoma of the lung, pancreas and mammary gland (156, 157).

There are only few studies analysing HBME-1 expression in thyroid FNA material. Among them Schmitt et al. (158) analysed 32 cases of papillary thyroid cancer and 20 cases of benign thyroid nodules which all had follow-up histologic confirmation. HBME-1 detection was noted in 25 of 32 (78%) papillary thyroid cancer cases and in 1 of 20 (5%) benign thyroid nodule case.

Study by de Micco et al. (159) analysed HBME-1 on FNA smears from 200 thyroid tumours. Immunostaining of HBME-1 was negative or low grade (≤10% positive cells) in 65 out of the 109 benign nodules, and high grade (≥80% positive cells) in 44 of 59 papillary thyroid cancer and 26 of 32 cases of follicular cancer.

CD56 antigen, recognised by Leu-19 is a glycoprotein, expressed on about 15% of normal peripheral blood lymphocytes (160, 161). Its presence in natural killer cells was established in 1983 by Griffin et al. (162) and in 1985 by Hercend et al. (163). CD56 may also be expressed in activated T cells, large granular lymphocytes, specific endocrine and brain tissue, thus it is believed that CD56 antigen may be involved in cell adhesion (164). CD56 is a neural cell adhesion molecule that is present on follicular epithelial cells of the normal thyroid (164). The protein itself is neural cell adhesion molecule and is believed to affect the migratory capability of tumour cells, homophilic binding between neurons, stimulation of neurite outgrowth, and fasciculation (161, 165).

E-Cadherin (E-CAD) is transmembrane glycoprotein and belongs to family of adhesion receptors (166). E-CAD is present in most epithelial cells and appears to play an important role in epithelial integrity, in cell adhesion and differentiation, as well as in the maintenance of cell polarity and tissue architecture. E-CAD consists of 728 amino acid transmembrane polypeptide, and is Ca^{2+} dependant homophilic adhesion receptors mediating cell-cell adhesion (167).

Cell lines normally expressing E-CAD show an epitheloid morphotype and are not invasive, whereas those lacking E-CAD are fibroblastoid and have a highly invasive growth (168). These and other experimental findings indicate that E-CAD has suppressor role in tumour spreading. The role of E-CAD has also been examined in several epithelial tumors of human such as: gastric adenocarcinomas, ductal carcinomas of mammary gland, squamous cell carcinomas, hepatocellular carcinomas, meningeomas, female genital carcinomas and prostate carcinomas (167, 169).

In the thyroid very little is known about intracellular adhesiveness. The expression of E-CAD in normal thyroid was first described in 1989 by Eidelman et al. (170). Since that many new findings are published in this field. Clearly gene for the cell-cell adhesion molecule, E-CAD, is expressed in normal human thyroid tissue and the translated protein is specific for follicular cells and not detected in stromal cells. E-CAD is normally expressed in all thyrocytes to about equal amounts, because thyrocytes are typical epithelial cells for which E-CAD is considered to be specific (167, 171).

In general, it appears that E-CAD expression is retained in follicular neoplasms; it is reduced in PTC and lost in anaplastic carcinomas (167, 172-175).

COX-2 gene is an early response gene that is induced rapidly by growth factors, tumour promoters, oncogenes, and carcinogens. Multiple lines of evidence suggest that COX-2 is important in carcinogenesis (176-179). Increased COX-2 protein expression levels have been described in malignant thyroid tumours, but not in benign thyroid nodules in 2002 by Specht et al. (180). Several articles have been reported on higher COX-2 expression in PTC and FC compared to normal thyroid tissue and poorly differentiated carcinoma.

COX-2 is up-regulated in transformed cells and in many epithelial carcinomas including: stomach (176), colon (177), pancreas (178), and prostate (179), whereas

levels of COX-1 are relatively constant. The differences in immunohistochemical findings can appear due to technological details, namely, different origin of primary antibodies (181, 182).

Ki-67 antigen is preferentially expressed in the active phases of cell cycle and is recognised with monoclonal antibody MIB1. The antigen is rapidly degraded as the cell enters non proliferative state. As a marker of cellular proliferation activity, Ki-67 antigen detection has become a mainstay in the morphological assessment of tumours.

First study of Ki-67 expression in thyroid lesions is published in 1991 by Rigaud et al. (183). Low proliferative activity has been reported in thyroid tissue (184) as well as in thyroid tumours (185, 186). In addition, proliferation indicators have been found to have no prognostic significance in PTC (187).

p53 is an important tumour suppressor gene as it integrates multiple stress signals and regulates cell response to DNA damage and is capable of inhibiting cell proliferation and transformation (188, 189). Several studies have reported finding that the detection of p53 protein was a significant prognostic indicator in thyroid carcinoma (190-192). A study by Morita et al. revealed significant correlation between p53 protein over-expression and large tumour size as well as the presence of lymph node metastasis (192).

Mutations in the p53 tumour suppressor gene are present in approximately 50% of all human cancers, and they represent the most common genetic changes in malignant cells (193). Molecular analyses of thyroid tumours have usually documented mutations in the p53 tumour suppressor gene in anaplastic carcinomas. IHC detection of p53 protein is thought to be associated with the occurrence of p53 gene mutations, but p53 protein expression has been detected by IHC in papillary thyroid cancer and follicular cancer irrespective of whether any p53 gene mutations had occurred (192, 194).

In thyroid malignancies p53 is reported to serve as an independent prognostic factor for overall survival of the patient (191). The frequency of p53 protein overexpression in papillary thyroid cancer has been reported as 11– 59% (193). Mutation of p53 gene are associated with the most aggressive histologic type of thyroid

tumours, such as undifferentiated carcinoma, and that the alteration of this gene represents a late genetic event in human thyroid carcinogenesis (193).

1.7. Management and therapy

Clinical management of thyroid nodules should be guided by the results of US evaluation and FNA biopsy (1, 195).

1.7.1. Benign FNA results

Strategies for management of patients with benign FNA results vary among practitioners and institutions. There are three options for the clinician. These include surgery, observation and hormone suppression. Most thyroid nodules with benign FNA cytology and no clinical and US risk factors require follow-up because the false-negative rate is as high as 5% (124, 196-198).

It is recommended that all benign thyroid nodules be followed with clinical and US examination and TSH measurement 6-18 month after the initial FNA. Thyroid nodule growth is defined as 20% increase in nodule diameter with a minimal increase in two or more dimensions of at least 2 mm (199). The total duration of follow-up period should be at least 3-5 years. Patients with multiple thyroid nodules have the same risk of malignancy as those with a single nodule, and the same follow-up plan is used in both groups. If there is evidence of nodule growth or suspicious clinical or US changes, the repeated FNA should be performed and surgical resection considered.

If the nodule is causing symptoms, or is aesthetically displeasing to the patient, surgery may be considered. Surgery should also be considered in those patients with neck pressure, dysphagia, shortness of breath, a choking sensation, dyspnea, hoarseness or pain in the neck or who are at increased risk for thyroid cancer despite a benign FNA. Symptoms must be associated with the nodule or goiter and not with the pulmonary, cardiac or esophageal disorders or other disease (1).

If the patient does not require surgery, the nodule may either be observed or

suppressed with Levothyroxine. The goal of thyroid hormone administration is to eliminate TSH stimulation by total exogenous replacement of the body's need for thyroid hormone. This should either reduce the size of the nodule or prevent its further growth (65). Nowadays Levothyroxine suppression therapy is no longer recommended, because randomised trials have suggested that thyroid hormone suppression may result in a decrease in nodule size in some patient populations with borderline low iodine intake but not in most patients ingesting sufficient iodine (77, 200). Most nodules do not respond to suppression therapy, and because of potential side effects of long term TSH suppression, this practice has been abandoned in most countries (105).

1.7.2. Nondiagnostic FNA results

There is no universally accepted approach to follow-up of nondiagnostic thyroid FNA. Nondiagnostic biopsies occur more often with cystic nodules because of the small amount of cellular material or bloody smears. Nondiagnostic aspirates obtained from cystic and solid nodules are treated differently in follow-up strategies.

Follow-up of aspirates of cysts that contain blood and histiocytes but no epithelial component require correlation with US findings (201). Cysts with these FNA findings are at very low risk for harbouring a malignancy, and many authors have recommended that they are best managed by nonsurgical follow-up (202). The optimal timing for repeated needle aspiration is 3-6 month, unless the clinical suspicion for malignancy is high (131, 140). Reaspiration yields satisfactory results in 50-60% of cases (131). When repeat FNA still yields nondiagnostic material, close clinical and US follow-up is appropriate (201).

Solid nodules with nondiagnostic FNA results should be reaspirated and if the repeat specimens are still nondiagnostic, surgery should be considered because malignancy is eventually diagnosed in about 9% of such cases (131).

Nodules 1 cm or less in size, on the basis of auspicious clinical and US findings, may be followed up with close clinical and US control (1, 195, 201).

1.7.3. Follicular lesions by FNA biopsy

Follicular lesions category is used if definitive cytologic diagnosis of malignancy cannot be made and cytologic features indicate lesion of follicular nature. Follicular lesions appear as hypercellular specimens with microfollicular arrangement and decreased or absent colloid. Currently, no definitive morphologic criteria are available to distinguish benign from malignant follicular lesions. If nodule is classified as follicular neoplasm, repeated FNA is not recommended, while it is recommended in cases diagnosed as "atypical cells" to exclude a follicular neoplasm. About 20% of such specimens are determined to be malignant in final histology.

US features and elastography as well as molecular markers may provide adjunctive information and may improve the accuracy of cytologic diagnosis but they do not have consistent predictive value for malignancy (103, 105, 195, 203). Complete thyroidectomy is usually performed after a diagnosis of papillary or follicular carcinoma, but lobectomy alone may be suffice for small, minimally invasive tumours, and treatment depends on the clinical status of the patient. Frozen section can be useful in case of non total thyroidectomy however its usefulness in evaluation for capsular or vascular invasion is controversial (202).

An unequivocal diagnosis of follicular carcinoma is justified when subsequent histologic examination discloses capsular and/or vascular invasion. In addition, about half of the nodules in this cytologic category found to be malignant are the follicular variant of papillary thyroid cancer (204). If clinical, cytological and US features are auspicious, clinical follow up by multidisciplinary team can be considered without immediate diagnostic surgery (205).

1.7.4. Suspicious nodules by FNA biopsy

Suspicious results in cytologic diagnosis are characterised by cytologic features suggesting but not fulfilling the criteria for a definitive diagnosis of malignancy. Approximately 50-75% of cytologically suspicious lesions are subsequently diagnosed as papillary carcinomas (140). In suspicious results there are also included samples with

poor cellularity and preservation but clear signs indicating malignancy. Patients with an FNA diagnosis of suspicious nodules for malignancy should be referred for surgical consultation. Frozen section can be recommended to help guide surgical decision making (205, 206).

1.7.5. Malignant nodules by FNA biopsy

This FNA category refers to PTC, MTC, anaplastic thyroid cancer (ATC), lymphoma, and metastatic malignancy. Malignant results include samples characterised by malignant cytologic features that are reliably identified by cytopathologist. Whenever possible, the type of carcinoma should be stated in the cytologic report precisely (140, 205). Cytologic diagnosis of malignancy in a thyroid nodule should result in surgery unless clinically contraindicated or cancer is due to metastatic disease. Well-differentiated thyroid cancers, such as PTC, FC and mixed papillary-follicular carcinomas are the most common malignancies of the thyroid. Thyroid US and cytologic results should be reviewed with the patient, and treatment options should be discussed (133). Consultation with a surgeon experienced in endocrine surgical procedures should be obtained soon and surgical excision should be recommended and its potential complications discussed (105, 133). The extend of thyroid resection is controversial. Supporters of total thyroidectomy argue that lobectomy leads to increased local recurrence, although survival rates are approximately equal (65). Complete thyroidectomy is usually performed after a diagnosis of PTC or FC, but lobectomy alone may suffice for small, minimally invasive tumours, and treatment depends on the clinical status of the patient (202). For anaplastic carcinoma, lymphoma and metastatic cancers, further diagnostic workup is recommended before surgery. In selected cases CT and MRI may be performed.

2. MATERIALS AND METHODS

2.1. The study design

In the first stage of the study one hundred and sixty-three thyroidectomy specimens were selected consecutively from the files of the Institute of Pathology, Pauls Stradins Clinical University hospital, Riga, Latvia between 2006 and 2010. The diagnoses were verified by repeated histological examination.

Immunohistochemical investigation was performed using six ICH markers: HBME-1, COX-2, E-cadherin (E-CAD), CD56, Ki-67 and p53.

Inclusion criteria were:

- Differentiated thyroid cancer originating from follicular epithelial cell including all types of papillary thyroid cancer and follicular cancer;
- Unequivocal morphological findings;
- Diameter of largest nodule not less that 1 cm;
- Enough archival paraffin-embedded tissue material to analyse;
- Period from operation till immunohistochemical analysis not exceeding eight years.

Exclusion criteria were:

- Hürthle cell variant of follicular cancer;
- Undifferentiated and poorly differentiated cancer including anaplastic carcinoma and insular carcinoma;
- Diameter of largest nodule less that 1 cm;
- Medullary thyroid cancer;
- Malignant lymphoma or metastatic cancer
- Deficient archival paraffin-embedded tissue material to analyse;
- Period from operation till immunohistochemical analysis more than eight years.

In the second stage of the study sixty-eight thyroid FNA cases confirmed by subsequent surgical resection specimens, during the period of 2010-2011, were selected from the Institute of Pathology, Pauls Stradins Clinical University Hospital, Riga, Latvia.

Immunocytochemical investigation was performed using most promising markers according to the first stage results (E-CAD, CD56, HBME-1).

The study was approved by the Committee of Ethics Pauls Stradins Clinical University hospital, reference Nr. 151209-3L. Written consent was obtained from each patient before FNA procedure.

Inclusion criteria were:

- Available FNA smear as well as operation material for the same case according to the above mentioned inclusion criteria;
- Period less than one year from FNA biopsy of the thyroid till operation and final morphology.

Exclusion criteria were:

- Unavailable operation material;
- Inadequate FNA specimen;
- Period more than one year from FNA biopsy of the thyroid till operation and final morphology.

2.2. Materials

In the first stage of the study after the histological verification of diagnosis the study group consisted of 50 malignant and 113 benign thyroid lesions including 36 papillary thyroid cancers (PTC) and 14 follicular cancers (FC) as well as 36 follicular adenomas (FA) and 77 cases of colloid goiter (CG).

In the second stage of the study the study group consisted of 26 malignant and 42 benign thyroid lesions including 25 PTC and 1 FC as well as 22 FA and 20 cases of CG.

2.3. Methods

2.3.1. Tissue processing and general histological report

The tissues of operation materials were fixed in 10% neutral buffered formalin, sampled widely during the grossing of operation material, processed and embedded in paraffin blocks as described by Bancroft et al. (207).

Four-micron-thick sections of the formalin-fixed, paraffin-embedded tissues were cut with automatic microtome Microm, HM 360 (Microm Int., Walldorf, Germany) on Histobond glass slides (Menzel Glasser, Braunschweig, Germany) and stained with haematoxylin–eosin (HE) for screening (207). During screening, the archival diagnoses were verified by the examination of these slides by an independent pathologist. The diagnostics, typing and grading of thyroid pathology were performed according to the WHO classification of tumours (208) by an independent reviewer with an experience in thyroid pathology. Only cases with unequivocal histological diagnosis were included in the study group.

Papillary thyroid cancer

Definition. PTC is defined as a malignant epithelial tumour showing evidence of follicular cell differentiation, and characterised by distinctive nuclear features (209).

Diagnostic criteria. PTC were diagnosed by following histologic appearance. The nuclei of PTC are typically large, crowded, ovoid, ground-glass and grooved, with small nucleoli (209). The feature present in almost all cases of PTC is the nuclear groove formed by deep folding in the nuclear membrane (Figure 2.1.). Architecture of PTC is usually infiltrative. Many variants of PTC have been described, but most are merely morphologic variants with no prognostic significance. The tall cell, diffuse sclerosing, diffuse follicular, solid, trabecular, and dedifferentiated variants are biologically more aggressive, while the encapsulated variant is associated with a highly favourable prognosis. Follicular variant of PTC is the most frequent and is composed almost exclusively of follicles. The clinical behaviour is no different from conventional papillary carcinoma (210). In the present study, all variants of PTC were included.

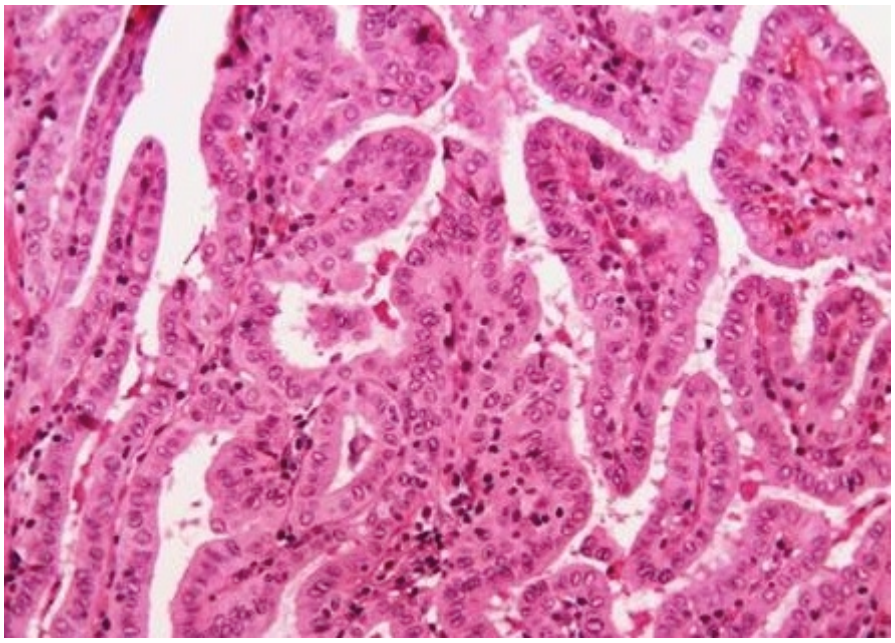

Figure 2.1. Tissue structure of papillary thyroid cancer. Note the characteristic architecture and nuclear structure. Haematoxylin-eosin, magnification ×100. Microphotography by A. Ozolins

Follicular adenoma and carcinoma

Definition. FA and FC are, respectively, benign and malignant epithelial tumours of the thyroid showing follicular cell differentiation but lacking the diagnostic features of PTC.

Diagnostic criteria. The tumour cells are cuboidal or low columnar with dark or pale-staining round nuclei. Within the tumour, delicate capillaries are present between the follicles and cell islands (211). Microphotography (Figure 2.2.) represent the structure of FA.

Criteria for distinction between FA and FC. FA and FC are encapsulated and usually indistinguishable macroscopically, except that the capsule tends to be thicker in the case of FC. It is fleshy usually light brown, sometimes having secondary changes like haemorrhage and cystic degeneration (212). In a follicular neoplasms lacking the cytoarchitectural features of PTC, the only feature that distinguishes a carcinoma from adenoma is the presence of vascular and/or capsular invasion in the former. Vascular invasion is qualified if the involved blood vessels are located within or outside the

fibrous capsule and the intravascular tumour is covered by endothelium (211).

To qualify for capsular invasion, there must be transgression of the fibrous capsule. It can be complete or incomplete (211).

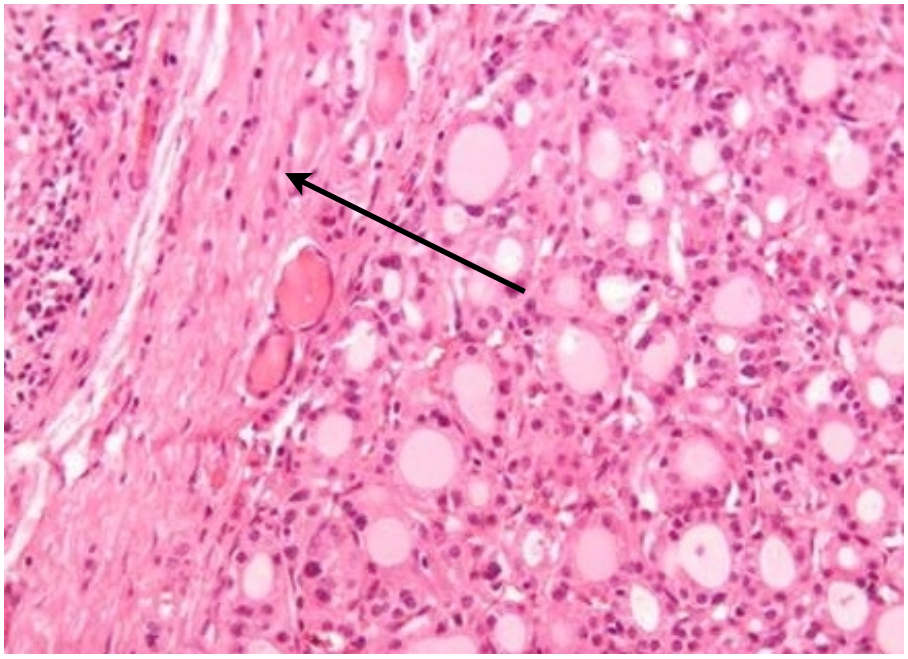

Figure 2.2. Tissue structure of follicular adenoma. Note the presence of capsule highlighted by black arrow. Haematoxylin-eosin, magnification ×100. Microphotography by A. Ozolins

Colloid goiter

CG is diagnosed when loss of normal thyroid architecture is present and abundant colloid is found. The features of other, more significant lesions must be excluded. Typical specimen is the goiter that has developed a nodular consistency. Structure usually gelatinous, colloid-rich or degenerative cystic structure. Often extensive fibrosis and calcium deposits are present. Microphotography (Figure 2.3.) represent the structure of CG.

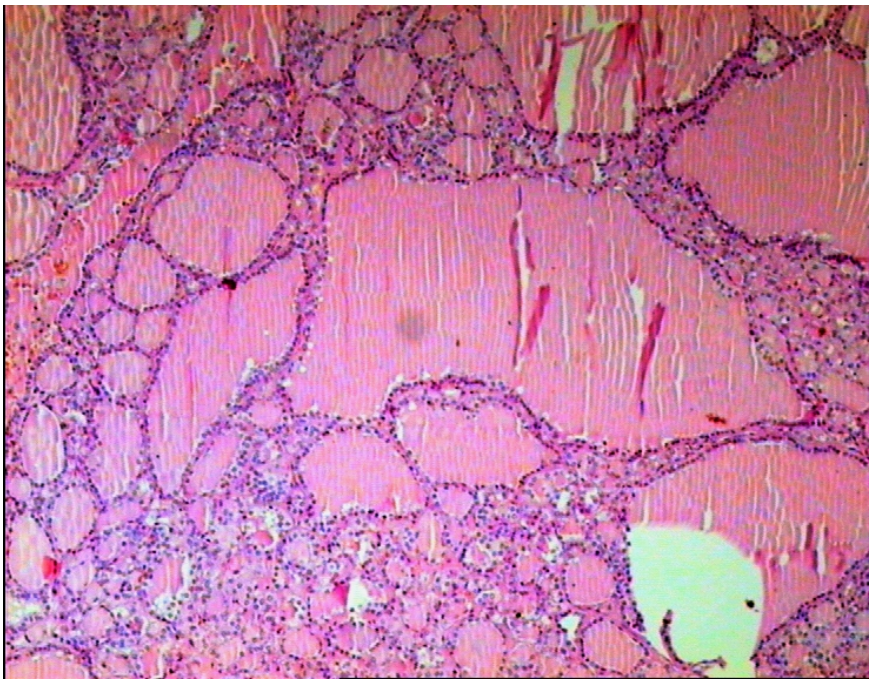

Figure 2.3. Tissue structure of colloid goiter. Haematoxylin-eosin, magnification ×100. Microphotography by A. Ozolins

If primary malignant tumour is found in the thyroid, TNM staging is applied as follows (Table 2.1., 2.2.).

Table 2.1.

Staging of malignant thyroid tumours (213)

Stage	Definition	Stage	Definition
Tx	Primary tumour cannot be assessed	Nx	Regional lymph nodes cannot be assessed
T0	No evidence of primary tumour	N0	No regional lymph node metastases
T1	Tumour ≤ 2 cm, limited to the thyroid	N1	Regional lymph node metastasis
T2	Tumour > 2 cm but ≤ 4 cm, limited to the thyroid	N1a	Metastasis to level VI nodes (pretracheal, paratracheal and prelaryngeal) nodes
T3	Tumour > 4 cm, limited to the thyroid; or tumour any size with minimal extrathyroidal extension	N1b	Metastasis to unilateral, bilateral, or contralateral cervical or upper/superior mediastinal nodes

Stage	Definition	Stage	Definition
T4a	Tumour of any size extending beyond the thyroid capsule to invade subcutaneous tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve	Mx	Distant metastasis cannot be assessed
		M0	No distant metastasis
T4b	Tumour invades prevertebral fascia or mediastinal vessels, or encases carotid artery	M1	Distant metastasis

Regional lymph nodes are the cervical and upper/superior mediastinal lymph nodes.
Undifferentiated (anaplastic) carcinomas are all considered T4.

Table 2.2.

Staging of thyroid tumours: stage grouping (213)

Stage	< 45 years	> 45 years
Papillary or follicular carcinoma		
I	Any T, any N, M0	T1, N0, M0
II	Any T, any N, M1	T2, N0, M0
III	-	T3, N0, M0 T1/T2/T3, N1a, M0
IV A	-	T4a, any N, M0 T1/T2/T3, N1b, M0
IV B	-	T4b, any N, M0
IV C	-	Any T, any N, M1

2.3.2. Immunohistochemistry

For immunophenotypic studies, formalin-fixed, paraffin-embedded tissues were cut in 3-µm-thick sections on electrostatically charged Histobond glass slides and incubated in 60°C for 1 h to ensure tissue adhesion to slides. Deparaffining and rehydration were carried out by routine treatment in xylene for 4 × 5 min and graded ethanol for 2 × 3 min, 99.9%; 4 × 3 min, 96% and 5 min, 70%. Endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide in methanol for 10 min. All chemicals were produced by Sigma-Aldrich (Steinheim, Germany).

After rinsing in TBS buffer (pH 7.6, Tris-buffered saline, THAM-HCl 50 mM/L, NaCl 150 mM/L) for 5 min, the slides were subjected to heat-induced antigen retrieval (HIER) treatment in domestic microwave oven for 3 × 5 min at maximum power in basic (TEG, pH 9.0, Tris base 10 mM/L, EGTA 0.5 mM/L) buffer. After HIER, the slides were allowed to cool at room temperature for 20 min in the HIER buffer.

The slides were encircled with Dako pen (Dako, Glostrup, Denmark) and transferred to magnetic immunostaining trays (CellPath plc, Newtown, UK). After the rinse with TBS buffer for 5 min, the incubation with primary antibodies (Table 2.3.) was carried out at room temperature for 60 min.

Table 2.3.

Characteristics of the antibodies

Target antigen	Monoclonal antibody	Antibody dilution
E-CAD	NCH-38	1:50
CD56	123C3	1:100
HBME-1	HBME-1	1:50
COX-2	CX-294	1:200
p53	DO-7	1:400
Ki-67	MIB-1	1:100

All antibodies were produced by Dako, Glostrup, Denmark.

Unbound primary antibodies later were removed by repeated rinses with TBS buffer 2 × 5 min. A commercially available polymeric EnVision+ System, bound with horseradish peroxidase (Dako), was used for visualisation. The slides were incubated in a humid chamber for 30 min with EnVision+ with following rinses in TBS 2 × 5 min. The colour development was obtained with 3,3-diaminobenzidine (Dako) for 10 min. The slides then were rinsed in water and counterstained in haematoxylin for 3 min. After colour development in tap water for 5 min, the slides were coverslipped using aqueous mounting medium Faramount (Dako). Positive and negative control slides were included in each run.

The evaluation and scoring was performed by the author under supervision of experienced pathologist. The expression of p53 protein and Ki-67 was evaluated in the nuclei of cancer cells. The quantitative data were obtained by computed morphometry (Kappa Metreo software) counting positive nuclei among 200 neoplastic cells and expressing the result as the percentage of positive cells. The membranous expression of E-CAD, CD56 and HBME-1 as well as cytoplasmic expression of COX-2 were scored semiquantitatively by staining the intensity and percentage of positive cells. The staining intensity was estimated as negative, 0; weakly positive, 1; moderately intensively staining, 2 or intensively positive, 3. To evaluate the expression of the considered markers in the whole analysable tissue, the expression intensity was computed as the multiplication of the percentage of positive cells by staining intensity. The lesion was considered positive for a marker when the expression intensity was at least 1.5.

2.3.3. Fine needle aspiration and immunocytochemistry (ICH)

US followed by FNA was performed by one experienced radiologist using GE Voluson E8 ultrasound machine and 11L-D linear transducer at Pauls Stradins Clinical University Hospital, Institute of Diagnostic Radiology, Riga, Latvia.

In case of a single thyroid nodule, only those nodules above 1 cm were further evaluated by FNA. In a multinodular gland the dominant nodule was evaluated or the nodule with most suspicious US findings. US characteristics associated with a higher risk of malignancy are as described previously: marked hypoechogenicity compared to normal thyroid parenchyma, microcalcifications, irregular or microlobulated margins, increased intranodular vascularity and a taller than wide shape (92-99).

The dominant nodule means either the largest nodule or the one that has recently increased significantly in size. In 2004 the Society of Radiologists in Ultrasound convened a panel of specialists from a variety of medical disciplines to come to a consensus about management of thyroid nodules presented at thyroid US (214). The specialists agreed that US-guided FNA should be considered for nodules demonstrating substantial growth on serial US examinations, even if a prior FNA result was benign.

Although it was found that rapid growth of the a nodule indicates and increased risk for malignancy (215, 216). However still specialists don't have one consensus on how to define substantial growth, nor how to monitor growth (214).

During FNA procedure patients were placed in supine position, the puncture site was prepared sterile and draped. US probe was covered sterile and disinfected with Cutasept F solution. Local anesthetic 1.0 ml Lidocaine 20 mg/ml was used. A 21-gauge needle was attached to 20 mL syringe. Under real-time visualisation needle tip was introduced in suspicious nodules. Passes were done using 5-10 ml suction. A minimum of 2 passes were employed. Needle placement was documented by taking pictures. Aspirated material was placed, smeared on Histobond adhesive glass slides and air dried.

For immunophenotypic studies the cell smears were air dried and fixed in 96% ethanol for 10 min. Endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide in methanol for 10 min. All chemicals were produced by Sigma-Aldrich (Steinheim, Germany).

After rinsing in TBS buffer (pH 7.6, Tris-buffered saline, THAM-HCl 50 mM/l, NaCl 150 mM/l) for 5 min, the slides were encircled with Dako pen (Dako, Glostrup, Denmark) and transferred to magnetic immunostaining trays (CellPath plc, Newtown, UK). After the rinse with TBS buffer for 5 min, the incubation with primary antibodies was carried out at room temperature for 60 min as described [30]. Unbound primary antibodies later were removed by repeated rinses with TBS buffer 2×5 min. A commercially available polymeric EnVision+ System, bound with horseradish peroxidase (Dako), was used for visualisation. The slides were incubated in a humid chamber for 30 min with EnVision+ with following rinses in TBS 2 × 5 min. The colour development was obtained with 3,3-diaminobenzidine (Dako) for 10 min. The slides then were rinsed in water and counterstained in haematoxylin for 3 min. Positive and negative control slides were included in each run.

2.3.4. Methods of statistical analysis

The statistical evaluation of the data was carried out using the Statistical Package for Social Sciences (SPSS® version 18.0) and Microsoft Excel 2011 (Mac) programs. In the present study descriptive statistics was used as well as 95% confidence interval for single proportion and for mean as described by Altman et al. (217). The confidence interval calculations were made by Confidence Interval Analysis (CIA) software. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

Confidence interval for the mean

The confidence interval (CI) is calculated using the mean $\bar{x}$ and its standard error SE($\bar{x}$) from a sample of size *n*. SE($\bar{x}$) usually calculated by the sample estimate of the population standard deviation divided by the square root of the sample size:

$$SE_{\bar{x}} = \frac{s}{\sqrt{n}}$$

where

s - the sample standard deviation, and

n - the size (number of observations) of the sample.

Thereby, the confidence interval is given by following formula:

$$\bar{x} - [t_{1-\alpha/2} \times \mathrm{SE}(\bar{x})] \quad \text{to} \quad \bar{x} + [t_{1-\alpha/2} \times \mathrm{SE}(\bar{x})]$$

where

$t_{1-\alpha/2}$ - the accordant value from the *t* distribution with *n-1* degrees of freedom associated with a "confidence" of 100(1-α)%;

$\bar{x}$ - mean, and

$SE(\bar{x})$ - standard error.

For a 95% confidence interval the value of *t* will be close to 2 for samples of 20 upwards. Values of *t* can be found in statistical textbooks (217).

Confidence interval for single proportion

For calculation of a single proportion, the Wilson's method is recommended. According to it, if r is the observed number of subjects with some feature in a sample of size n then the estimated proportion who have the feature is $p = r/n$. The proportion who do not have the feature is $q = 1-p$ (217). First, three quantities should be calculated:

$$A = 2r + z^2;$$

$$B = z\sqrt{z^2 + 4rq};$$

$$C = 2(n + z^2);$$

where

z - as before the appropriate value, that is $z_{1-\alpha/2}$, from the standard normal distribution.

Than the confidence interval for the population proportion is shown as:

$$(A - B)/C \text{ to } (A + B)/C.$$

This method can be used for any data. When observed events are missing, r and p are both zero, and the recommended confidence interval is 0 to $z^2/(n + z^2)$. When $r = n$ so that $p = 1$, the interval expresses as $n/(n + z^2)$ to 1. No negative values were accepted for confidence interval (217).

Sensitivity and Specificity

Sensitivity and *specificity* are statistical measures of the performance of a binary classification test. They are independent of the population of interest subjected to the test.

Sensitivity measures the proportion of actual positives which are correctly identified as such (e.g. the percentage of sick people who are correctly identified as having the condition). *Specificity* measures the proportion of negatives which are correctly identified (e.g. the percentage of healthy people who are correctly identified as not having the condition). These two measures are closely related. A theoretical, optimal prediction aims to achieve 100% sensitivity (i.e. predict all people from the sick group

as sick) and 100% specificity (i.e. not predict anyone from the healthy group as sick), however theoretically any predictor will possess a minimum error bound known as the Bayes error rate (218).

The following terms are fundamental to understanding the utility of clinical tests:

1. *True positive:* the patient has the disease and the test is positive.
2. *False positive:* the patient does not have the disease but the test is positive.
3. *True negative:* the patient does not have the disease and the test is negative
4. *False negative:* the patient has the disease but the test is negative.

Sensitivity relates to the test's ability to identify those patients with the disease.

$$Sensitivity = True\ positives\ /\ True\ positives + False\ negatives$$

If a test has high sensitivity then a negative result would suggest the absence of disease. For example, a sensitivity of 100% means that the test recognises all actual positives – i.e. all sick people are recognised as being ill. Thus, in contrast to a high specificity test, negative results in a high sensitivity test are used to rule out the disease (218).

Specificity of a clinical test refers to the ability of the test to correctly identify those patients without the disease.

$$Specificity = True\ negatives\ /\ True\ negatives + False\ positives$$

If a test has high specificity, a positive result from the test means a high probability of the presence of disease. Therefore, a test with 100% specificity correctly identifies all patients without the disease. A test with 80% specificity correctly reports 80% of patients without the disease as test negative (true negatives) but 20% patients without the disease are incorrectly identified as test positive (false positives) (218).

Positive predictive value

In statistics and diagnostic testing, the positive predictive value is the proportion of subjects with positive test results who are correctly diagnosed. It is useful to clinicians since it answers the question: ‘How likely is it that this patient has the disease given that the test result is positive?’ Its value does however depend on the prevalence of the outcome of interest, which may be unknown for a particular target population. The PPV can be derived using Bayes' theorem (219).

$$\textit{Positive predictive value} = \textit{True positives} / \textit{True positives} + \textit{False positives}$$

or

$$\textit{Positive predictive value} = \textit{True positives} / \textit{Test outcome positive}$$

where

true positive - the event that the test makes a positive prediction, and the subject has a positive result;

false positive - the event that the test makes a positive prediction, and the subject has a negative result.

Negative predictive value

In statistics and diagnostic testing, the negative predictive value (NPV) is a summary statistic used to describe the performance of a diagnostic testing procedure. The NPV of a test answers the question: ‘How likely is it that this patient does not have the disease given that the test result is negative?’ A high NPV means that when the test yields a negative result, it is most likely correct in its assessment. In the familiar context of medical testing, a high NPV means that the test only rarely misclassifies a sick person as being healthy. Note that this says nothing about the tendency of the test to mistakenly classify a healthy person as being sick (219).

Negative predictive value = True negatives / True negatives + False negatives

or

Negative predictive value = True negatives / Test outcome negative

where

true negative - the event that the test makes a negative prediction, and the subject has a negative result;

false negative - the event that the test makes a negative prediction, and the subject has a positive result.

Unlike sensitivity and specificity, the PPV and NPV are dependent on the population being tested and are influenced by the prevalence of the disease.

3. RESULTS

3.1. The results of immunohistochemical staining

In the first stage of the study 50 malignant and 113 benign thyroidectomy specimens were analysed including 36 papillary thyroid cancers and 14 follicular cancers as well as 36 follicular adenomas and 77 cases of colloid goiters. The mean age of the patients was 57 ± 14.52 (24 - 85 years). There were 20 male patients and 143 females.

Of 36 papillary thyroid cancers 26 were stage I tumours and 10 were stage II. In follicular cancers group 10 were stage I tumours and 4 were stage II.

3.1.1. Expression of E-cadherin

The mean expression of E-CAD in FA was 2.2 (95% CI = 1.92-2.48) which is significantly higher than in the tissues surrounding FA - 0.63 (95% CI = 0.39-0.87). Microphotography (Figure 3.1.) represents E-CAD expression in FA. FC also was characterised by high expression of E-CAD - 2.1 (95% CI = 1.64-2.56). Unfortunately, there was no possibility to analyse expression of E-CAD in thyroid tissue surrounding FC due to its widespread invasion.

In contrast to FA and FC, PTC showed lowest expression of E-CAD - 0.55 (95% CI = 0.34-0.75). Microphotography (Figure 3.2.) represents weak expression of E-CAD in PTC.

In CG expression of E-CAD was 1.39 (95% CI = 1.23-1.54) which is lower that FA or FC, but higher than in PTC.

Results of the expression of E-CAD in different thyroid lesions are summarised in Table 3.1.

Table 3.1.

Descriptive statistics of E-CAD expression in different thyroid lesions

Target structure	E-CAD expression intensity				
	N	Mean ± SD	95% CI	Min	Max
Follicular adenoma	36	2.20 ± 0.82	1.92 - 2.48	0.15	3
Thyroid tissue surrounding follicular adenoma	32	0.63 ± 0.71	0.39 - 0.87	0	1.5
Papillary thyroid cancer	36	0.55 ± 0.61	0.34 - 0.75	0	2.4
Thyroid tissue surrounding papillary thyroid cancer	34	1.60 ± 0.85	1.30 - 1.90	0.3	3
Colloid goiter	77	1.39 ± 0.69	1.23 - 1.54	0	3
Follicular cancer	14	2.10 ± 0.80	1.64 - 2.56	0	3

Abbreviation in the Table: N, number of measurements; SD, standard deviation; CI, confidence interval for a mean; Min, minimal observed value; Max, maximal observed value.

Taking into account the presented results, the sensitivity, specificity, PPV and NPV of E-CAD were calculated. E-CAD shows high sensitivity and specificity for separating FA from the tissue surrounding FA with values 0.888 and 0.875, respectively. PPV and NPV were 0.888 and 0.875.

The sensitivity and specificity were 0.914 and 0.891 for FA compared to PTC. For separating PTC from tissue surrounding PTC E-CAD shows 0.702 sensitivity and 0.869 specificity.

Comparison of sensitivity, specificity, PPV and NPV of E-CAD expression is summarised in Table 3.2. Microphotography's (see Figure 3.1. and Figure 3.2.) represent E-CAD expression in FA and PTC.

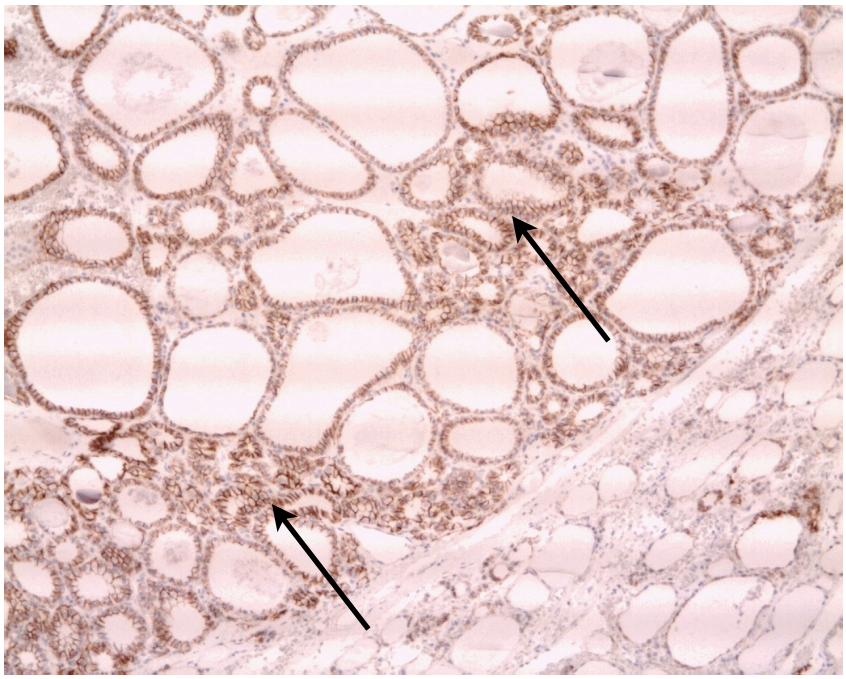

Figure 3.1. Intense membranous expression of E-CAD in follicular adenoma, highlighted by black arrows. Immunoperoxidase, magnification × 50. Microphotography by A. Ozolins

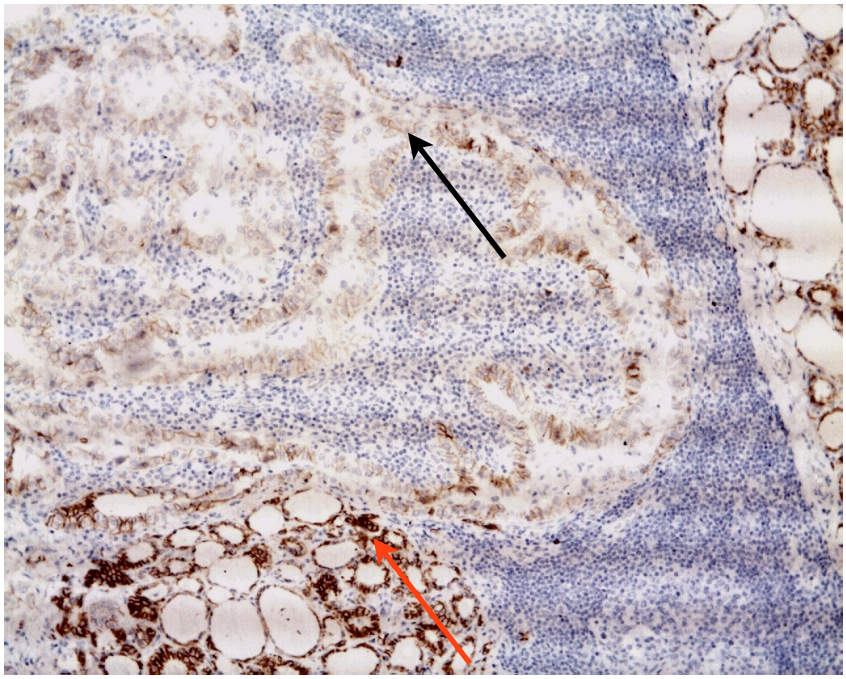

Figure 3.2. Weak membranous expression of E-CAD in papillary thyroid cancer, highlighted by black arrow. Note intense expression in the adjacent benign thyroid tissue, highlighted by red arrow. Immunoperoxidase, magnification × 50. Microphotography by A. Ozolins

Table 3.2.

Comparison of sensitivity, specificity, positive predictive value and negative predictive value of E-CAD expression in different thyroid lesions

Marker	Target structure	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
E-CAD	FA	32	4	36	0.888	0.875	0.888	0.875
	TtS FA	4	28	32				
	PTC	3	33	36	0.702	0.869	0.916	0.588
	TtS PTC	20	14	34				
	FA	32	4	36	0.914	0.891	0.888	0.916
	PTC	3	33	36				

Abbreviation in the Table: FA, follicular adenoma; TtS FA, thyroid tissue surrounding FA; PTC, papillary thyroid cancer; TtS PTC, thyroid tissue surrounding PTC; PPV, positive predictive value; NPV, negative predictive value.

The expression frequency of E-CAD was found in 32/36 (88.8%; 95% CI = 74.7-96.0%) cases of FA. In contrast, thyroid tissue surrounding FA showed very low E-CAD expression frequency - 4/32 (12.5%; 95% CI = 5.0-28.0%). Notable is the fact that expression of E-CAD in case of PTC was found only in 3/36 cases (8.3%; 95% CI = 2.8-21.8%). More frequently E-CAD expression was present in thyroid tissue surrounding PTC, namely, 20/34 (58.8%; 95% CI = 42.2-73.6%). The expression frequency of E-CAD in FC was found in 13/14 (92.8%; 95% CI = 68.5-98.7%) of cases.

Results of the expression frequency of E-CAD in different thyroid lesions are summarised in Table 3.3.

Table 3.3.

Frequency of E-CAD expression in different thyroid lesions

Target structure	E-CAD	
	Expression n (%)	95% CI
Follicular adenoma (n=36)	32 (88.8%)	74.7 - 96.0

Target structure	E-CAD	
Thyroid tissue surrounding follicular adenoma (n=32)	4 (12.5%)	5.0 - 28.0
Papillary thyroid cancer (n=36)	3 (8.3%)	2.8 - 21.8
Thyroid tissue surrounding papillary thyroid cancer (n=34)	20 (58.8%)	42.2 - 73.6
Colloid goiter (n=77)	25 (32.4%)	23.0 - 43.5
Follicular cancer (n=14)	13 (92.8%)	68.5 - 98.7

Abbreviation in the Table: n, absolute number; CI, confidence interval for a proportion.

3.1.2. Expression of CD56

The mean expression of CD56 in FA was 2.2 (95% CI = 1.88-2.51) which is significantly higher than in the tissue surrounding FA - mean value 0.95 (95% CI = 0.67-1.22) Microphotography (Figure 3.3.) represents CD56 expression in FA. FC was characterised by highest expression of CD56 - mean value 2.3 (95% CI = 1.72-2.87).

In PTC expression of CD56 was significantly lover than in the tissue surrounding PTC, namely, 0.2 (95% CI = 0.13-0.26) and 1.02 (95% CI = 0.85-1.18), respectively. Microphotography (Figure 3.4.) represents lack of CD56 expression in PTC. In CG expression of CD56 was 0.85 (95% CI = 0.70-0.99) which is lower that FA or FC, but higher than in PTC.

Results of the expression of CD56 in different thyroid lesions are summarised in Table 3.4.

Table 3.4.

Descriptive statistics of CD56 expression in different thyroid lesions

Target structure	CD56 expression intensity				
	N	Mean ± SD	95% CI	Min	Max
Follicular adenoma	36	2.20 ± 0.92	1.88 - 2.51	0.6	3
Thyroid tissue surrounding follicular adenoma	32	0.95 ± 0.77	0.67 - 1.22	0	3

Target structure	CD56 expression intensity				
Papillary thyroid cancer	36	0.20 ± 0.19	0.13 - 0.26	0	0.6
Thyroid tissue surrounding papillary thyroid cancer	34	1.02 ± 0.47	0.85 - 1.18	0.6	2.4
Colloid goiter	77	0.85 ± 0.63	0.70 - 0.99	0	3
Follicular cancer	14	2.30 ± 1.00	1.72 - 2.87	0.4	3

Abbreviation in the Table: N, number of measurements; SD, standard deviation; CI, confidence interval for a mean; Min, minimal observed value; Max, maximal observed value.

Taking into account the presented results, the sensitivity, specificity, PPV and NPV of CD56 were calculated. CD56 sensitivity, specificity for separating FA from the tissue surrounding FA were 0.882 and 0.823 as well as PPV and NPV were 0.833 and 0.875, respectively. Comparing FA and PTC the sensitivity and specificity were 1.0 and 0.857, respectively. For separating PTC from tissue surrounding PTC CD56 shows 0.530 sensitivity and 1.000 specificity.

Comparison of sensitivity, specificity, PPV and NPV of CD56 expression is summarised in Table 3.5. Microphotography's (see Figure 3.3. and Figure 3.4.) represent CD56 expression in FA and PTC.

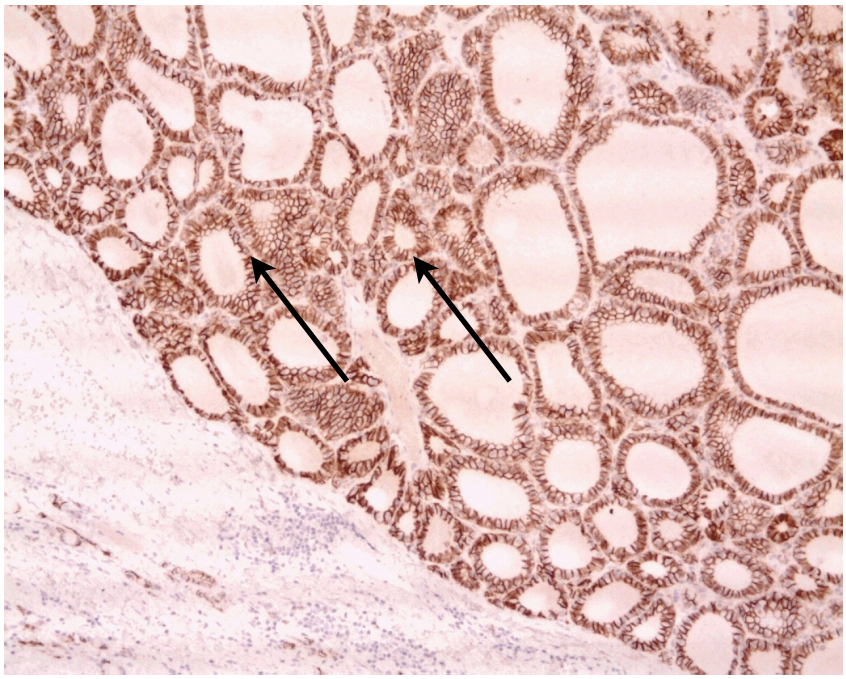

Figure 3.3. Intense membranous expression of CD56 in follicular adenoma, highlighted by black arrows. Immunoperoxidase, magnification × 50. Microphotography by A. Ozolins

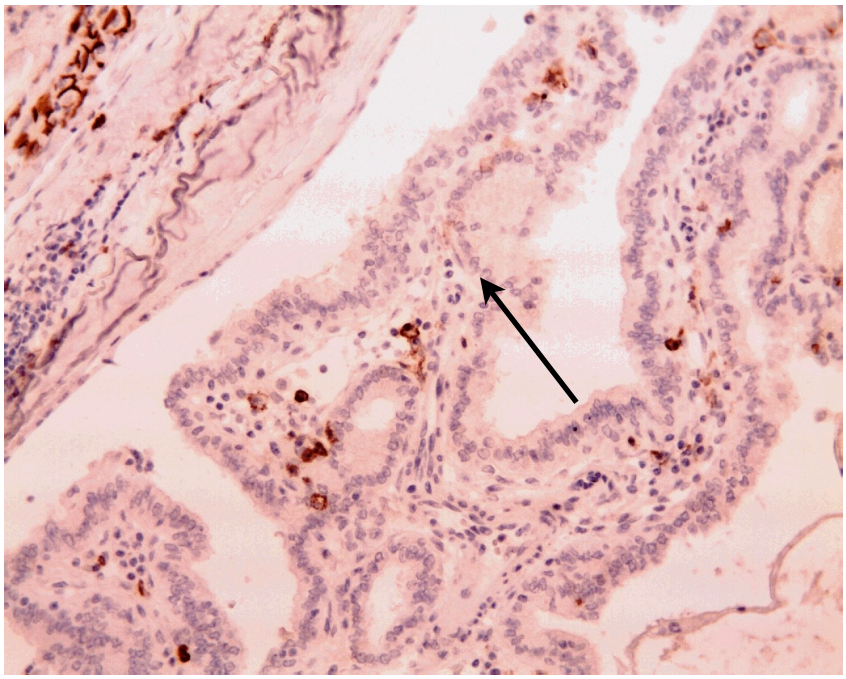

Figure 3.4. Absence of CD56 expression in papillary thyroid cancer, highlighted by black arrows. Immunoperoxidase, magnification × 100. Microphotography by A. Ozolins

Table 3.5.

Comparison of sensitivity, specificity, positive predictive value and negative predictive value of CD56 expression in different thyroid lesions

Marker	Target structure	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
CD56	FA	30	6	36	0.882	0.823	0.833	0.875
	TtS FA	4	28	32				
	PTC	0	36	36	0.530	1.000	1.000	0.058
	TtS PTC	2	32	34				
	FA	30	6	36	1.000	0.857	0.833	1.000
	PTC	0	36	36				

Abbreviation in the Table: FA, follicular adenoma; TtS FA, thyroid tissue surrounding FA; PTC, papillary thyroid cancer; TtS PTC, thyroid tissue surrounding PTC; PPV, positive predictive value; NPV, negative predictive value.

The expression frequency of CD56 was found in 28/36 (78.0%; 95% CI = 62.0-88.2%) cases of FA. In contrast, thyroid tissue surrounding FA showed comparatively low CD56 expression frequency - 4/32 (12.5%; 95% CI = 5.0-28.0%). In case of PTC there where no CD56 expression observed and only one case in thyroid tissue surrounding papillary thyroid cancer. In case of FC expression was observed 13/14 cases.

Results of the expression frequency of CD56 in different thyroid lesions are summarised in Table 3.6.

Table 3.6.

Frequency of CD56 expression in different thyroid lesions

Target structure	CD56	
	Expression n (%)	95% CI
Follicular adenoma (n=36)	28 (78.0%)	62.0 - 88.2
Thyroid tissue surrounding follicular adenoma (n=32)	4 (12.5%)	5.0 - 28.0
Papillary thyroid cancer (n=36)	0 (0%)	0 - 9.6
Thyroid tissue surrounding papillary thyroid cancer (n=34)	1 (3.0%)	0.5 - 15.0
Colloid goiter (n=77)	10 (13.0%)	7.2 - 22.2
Follicular cancer (n=14)	13 (92.8%)	68.5 - 99.0

Abbreviation in the Table: n, absolute number; CI, confidence interval for a proportion.

3.1.3. Expression of HBME-1

The expression of HBME-1 was absent in benign thyroid lesions including FA, CG and pericancerous tissue but was notably high in PTC with the average intensity 2.80 (95% CI = 2.68-2.91) and 4/14 cases of FC with the average intensity 0.90 (95% CI = 0.10-1.70). Microphotographs represents lack of HBME-1 expression in FA (Figure 3.5.) and intense HBME-1 expression in PTC and FC (Figure 3.6., 3.7.). Results of the expression of HBME-1in different thyroid lesions are summarised in Table 3.7.

Table 3.7.

Descriptive statistics of HBME-1 expression in different thyroid lesions

Target structure	HBME-1 expression intensity				
	N	Mean ± SD	95% CI	Min	Max
Follicular adenoma	36	0.09 ± 0.37	0 - 0.22	0	1.8
Thyroid tissue surrounding follicular adenoma	32	0.001 ± 0.003	0 - 0.002	0	0.03
Papillary thyroid cancer	36	2.80 ± 0.33	2.68 - 2.91	2.1	3
Thyroid tissue surrounding papillary thyroid cancer	34	0.006 ± 0.03	0 - 0.016	0	0.15
Colloid goiter	77	0	0	0	0.15
Follicular cancer	14	0.90 ± 1.40	0.10 - 1.70	0	2.85

Abbreviation in the Table: N, number of measurements; SD, standard deviation; CI, confidence interval for a mean; Min, minimal observed value; Max, maximal observed value.

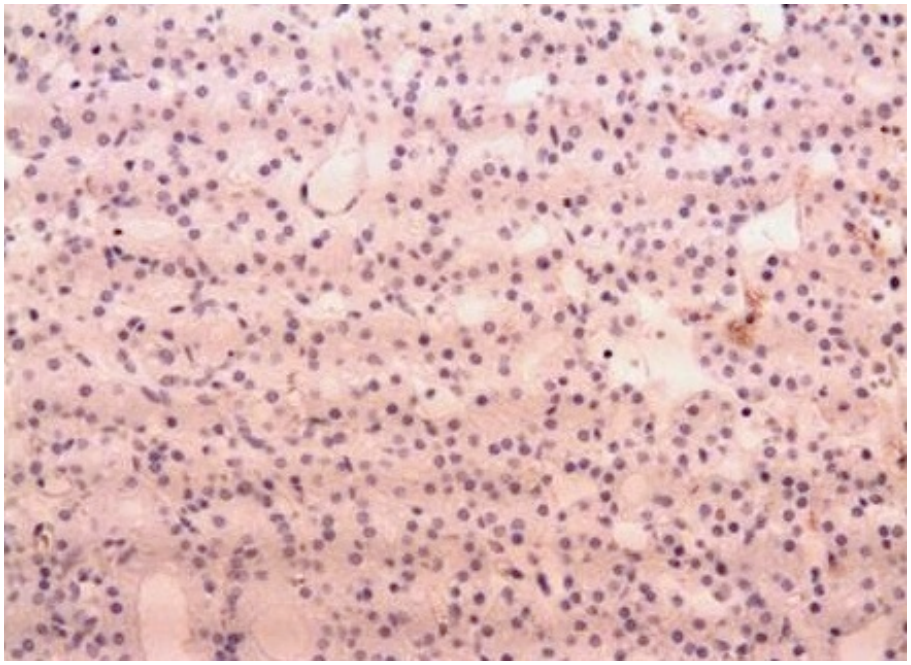

Figure 3.5. Lack of HBME-1 in follicular adenoma. Immunoperoxidase, anti-HBME-1, magnification ×100. Microphotography by A. Ozolins

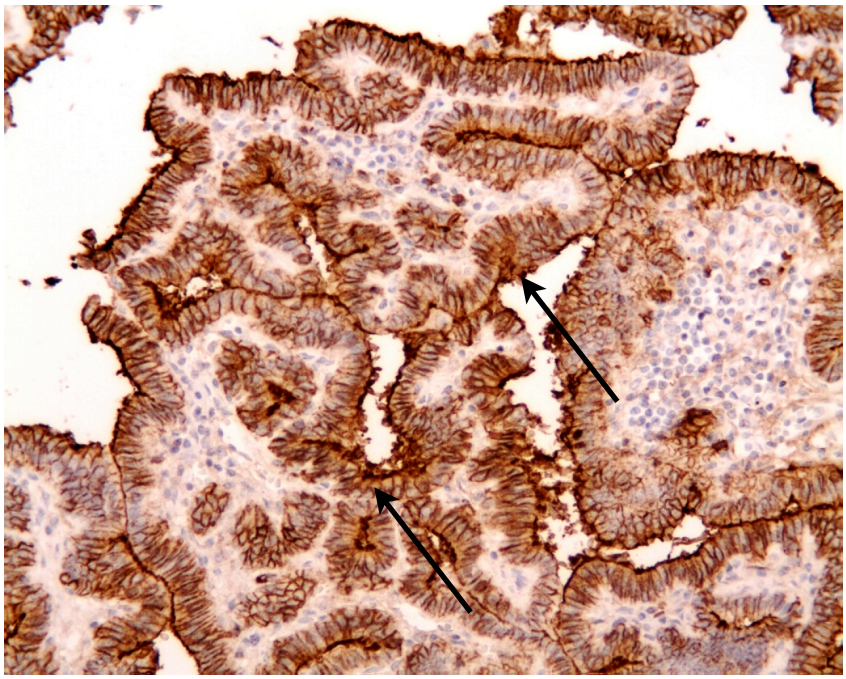

Figure 3.6. Intense membranous expression of HBME-1 in papillary thyroid cancer, highlighted by black arrows. Immunoperoxidase, anti-HBME-1, magnification × 100. Microphotography by A. Ozolins

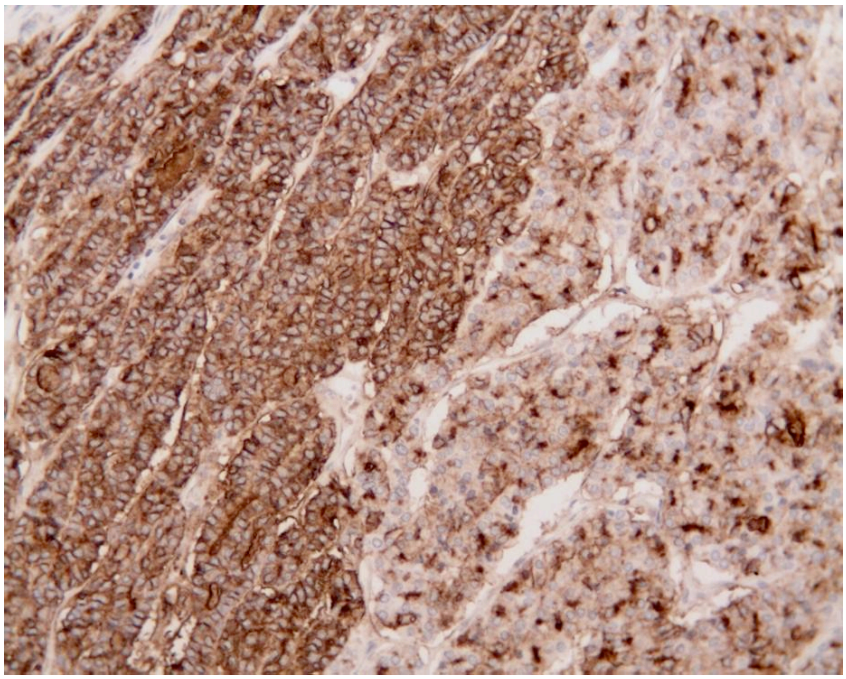

Figure 3.7. Intense heterogeneous expression of HBME-1 in follicular cancer. Immunoperoxidase, anti-HBME-1, magnification × 100. Microphotography by A. Ozolins

Taking into account the presented results, the sensitivity, specificity, PPV and NPV of HBME-1 were calculated. Sensitivity, specificity, PPV and NPV for separating PTC from the tissue surrounding PTC using HBME-1 is 1.000. To differentiate FA from PTC sensitivity and specificity is 1.000 and 0.973. Besides PPV and NPV is 0.970 and 1.000, respectively. HBME-1 has an extremely high value in the differential diagnostics of PTC showing high ability to discriminate between PTC and FA or benign tissues.

Results of the expression of HBME-1 and comparison of sensitivity, specificity, PPV and NPV are summarised in Table 3.8. Microphotography images (see Figure 3.5., 3.6. and 3.7.) represent HBME-1 expression in FA, PTC and FC.

Table 3.8.

Comparison of sensitivity, specificity, positive predictive value and negative predictive value of HBME-1 expression in different thyroid lesions

Marker	Target structure	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
HBME-1	FA	1	35	36	0.522	0.000	0.972	0,000
	TtS FA	0	32	32				
	PTC	36	0	36	1.000	1.000	1.000	1.000
	TtS PTC	0	34	34				
	FA	1	35	36	1.000	0.973	0.970	1.000
	PTC	36	0	36				

Abbreviation in the Table: FA, follicular adenoma; TtS FA, thyroid tissue surrounding FA; PTC, papillary thyroid cancer; TtS PTC, thyroid tissue surrounding PTC; PPV, positive predictive value; NPV, negative predictive value.

The expression frequency of HBME-1 was found only in 1/36 (3%; 95% CI = 0.5-14.1%) cases of FA and in none of thyroid tissue surrounding FA as well as CG. While in PTC expression was present in 36/36 (100%; 95% CI = 90.3-100%) but there where no expression in thyroid tissue surrounding PTC. FC showed expression of HBME-1 in 4/14 cases (28.5%; 95% CI = 11.7-54.6%).

Results of the expression frequency of HBME-1 in different thyroid lesions are summarised in Table 3.9.

Table 3.9.

Frequency of HBME-1 expression in different thyroid lesions

Target structure	HBME-1	
	Expression n (%)	95% CI
Follicular adenoma (n=36)	1 (3.0%)	0.5 - 14.1
Thyroid tissue surrounding follicular adenoma (n=32)	0 (0%)	0 - 10.7
Papillary thyroid cancer (n=36)	36 (100%)	90.3 - 100.0
Thyroid tissue surrounding papillary thyroid cancer (n=34)	0 (0%)	0 - 10.1
Colloid goiter (n=77)	0 (0%)	0 - 4.7
Follicular cancer (n=14)	4 (28.5%)	11.7 - 54.6

Abbreviation in the Table: n, absolute number; CI, confidence interval for a proportion.

3.1.4. Expression of COX-2

The expression of COX-2 was low in all lesions and did no show any statistical significant differences between groups. Results of the expression of COX-2 are summarised in Table 3.10.

Table 3.10.

Descriptive statistics of COX-2 expression in different thyroid lesions

Target structure	COX-2 percentage of positive cells				
	N	Mean ± SD	95% CI	Min	Max
Follicular adenoma	36	0.25 ± 0.41	0.11 - 0.39	0	0.53
Thyroid tissue surrounding follicular adenoma	32	0.12 ± 0.24	0.03 - 0.20	0	0.31

Target structure	COX-2 percentage of positive cells				
Papillary thyroid cancer	36	0.21 ± 0.38	0.08 - 0.34	0	0.51
Thyroid tissue surrounding papillary thyroid cancer	34	0.20 ± 0.32	0.09 - 0.31	0	0.47
Colloid goiter	77	0.34 ± 0.42	0.24 - 0.43	0	0.72
Follicular cancer	14	0.34 ± 0.67	0 - 0.73	0	0.85

Abbreviation in the Table: N, number of measurements; SD, standard deviation; CI, confidence interval for a mean; Min, minimal observed value; Max, maximal observed value.

3.1.5. Expression of Ki-67

The expression of Ki-67 was generally low, not reaching 5%. However, there were statistically significant differences between PTC with expression 2.36 (95% CI = 2.07-2.64) and the surrounding tissues with expression 0.99 (95% CI = 0.83-1.14). In FC expression was 3.62 (95% CI = 3.00-4.25) which is significantly higher than in FA 1.07 (95% CI = 0.85-1.29) or CG 0.69 (95% CI = 0.56-0.82).

Overall proliferative activity was significantly higher in cancers. Results of the expression of Ki-67 are summarised in Table 3.11.

Table 3.11.

Descriptive statistics of Ki-67 expression in different thyroid lesions

Target structure	Ki-67 percentage of positive cells				
	N	Mean ± SD	95% CI	Min	Max
Follicular adenoma	36	1.07 ± 0.65	0.85 - 1.29	0.1	1.7
Thyroid tissue surrounding follicular adenoma	32	0.75 ± 0.52	0.56 - 0.93	0	1.2
Papillary thyroid cancer	36	2.36 ± 0.85	2.07 - 2.64	0.2	4.7
Thyroid tissue surrounding papillary thyroid cancer	34	0.99 ± 0.45	0.83 - 1.14	0	1.3
Colloid goiter	77	0.69 ± 0.57	0.56 - 0.82	0	1.0
Follicular cancer	14	3.62 ± 1.10	3.00 - 4.25	0.4	4.9

Abbreviation in the Table: N, number of measurements; SD, standard deviation; CI, confidence interval for a mean; Min, minimal observed value; Max, maximal observed value.

3.1.6. Expression of p53

No expression of p53 was found in any of the group.

3.2. The results of immunocytochemical staining

In the second stage of the study we picked three of six markers (E-CAD, CD56 and HBME-1) analysed in the first stage who showed most promising and acceptable results. Twenty-six malignant and 42 benign thyroid FNA cases confirmed by subsequent surgical resection were included. Study group consisted of 25 papillary thyroid cancers, 1 follicular cancer as well as 22 follicular adenomas and 20 cases of colloid goiters.

The mean age of the patients was 54 ± 13.96 (22 - 77 years). There were 6 male patients and 62 females. Of all cancers 24 were stage I tumours and 2 were stage II.

3.2.1. Expression of E-cadherin in FNA material

The expression by immunocytochemistry of E-CAD was found in 16/22 (72.7%; 95% CI = 52.0-87.0%) cases of FA. In contrast, PTC showed very low E-CAD expression 2/25 (8%; 95% CI = 2.2-25.0%). In case of CG expression of E-CAD was 2/20 (10%; 95% CI = 2.8-30.1%). No expression was found in FC.

Results of the immunocytochemical staining of E-CAD are summarised in Table 3.12. Microphotography (Figure 3.8.) represents cytoplasmic expression of E-CAD in a group of thyroid epithelial cells.

Table 3.12.

Frequency of E-CAD expression in FNA material of different thyroid lesions

Target structure	E-CAD	
	Expression n (%)	95% CI
Follicular adenoma (n=22)	16 (72.7%)	50.0 - 88.0
Colloid goiter (n=20)	2 (10%)	1.75 - 33.1
Papillary thyroid cancer (n=25)	2 (8%)	1.40 - 27.5
Follicular cancer (n=1)	0 (0%)	0 - 95.0

Abbreviation in the Table: n, absolute number; CI, confidence interval for a proportion.

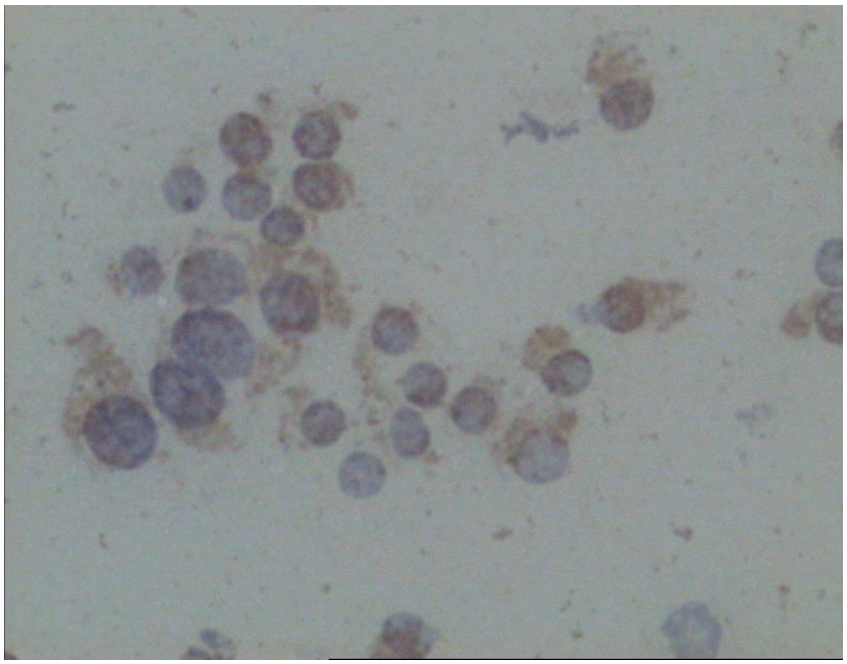

Figure 3.8. Cytoplasmic expression of E-CAD in a group of thyroid epithelial cells. Immunoperoxidase, anti-E-CAD, magnification × 400. Microphotography by A. Ozolins

3.2.2. Expression of CD56 in FNA material

The expression by immunocytochemistry of CD56 was found in 12/22 (54.5%; 95% CI = 34.6-73.0%) cases of FA. In contrast, PTC showed very low CD56 expression

1/25 (4%; 95% CI = 0.7-20.0%). In case of CG expression of CD56 was 1/20 (5%; 95% CI = 0.9-23.6%) No expression was found in FC.

Results of the immunocytochemical staining of CD56 are summarised in Table 3.13. Microphotography (Figure 3.9.) represents intense membranous expression of CD56 in a single epithelial cell.

Table 3.13.

Frequency of CD56 expression in FNA material of different thyroid lesions

Target structure	CD56	
	Expression n (%)	95% CI
Follicular adenoma (n=22)	12 (54.5%)	34.6 - 73.0
Colloid goiter (n=20)	1 (5%)	0.9 - 23.6
Papillary thyroid cancer (n=25)	1 (4%)	0.7 - 20.0
Follicular cancer (n=1)	0 (0%)	0 - 79.3

Abbreviation in the Table: n, absolute number; CI, confidence interval for a proportion.

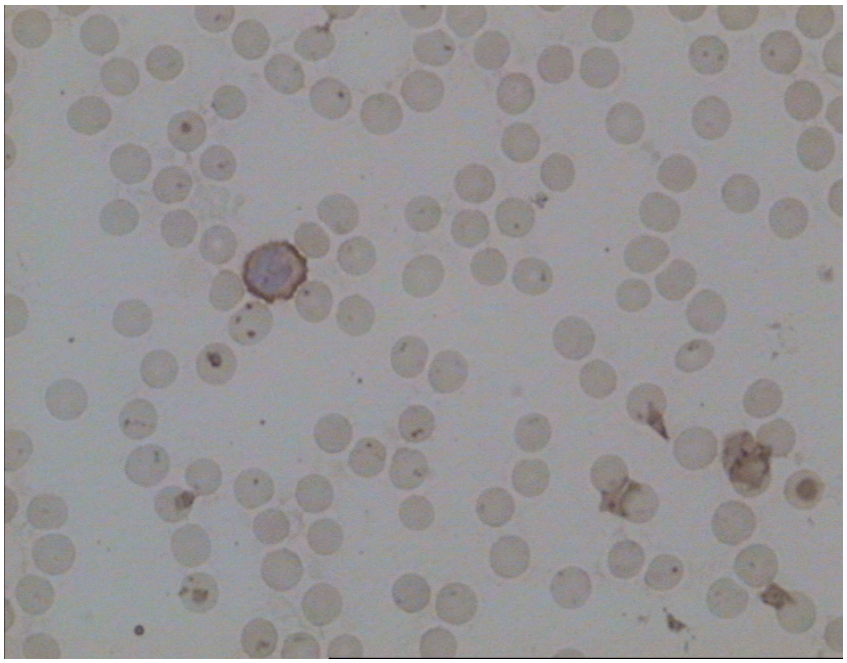

Figure 3.9. Intense membranous expression of CD56 in a single epithelial cell despite the rich presence of red blood cells in the clearly suboptimal smear. Immunoperoxidase, anti-CD56, magnification × 400. Microphotography by A. Ozolins

3.2.3. Expression of HBME-1 in FNA material

The expression by immunocytochemistry of HBME-1 in PTC was significantly higher than in another thyroid lesions included in the study 24/25 (96%; 95% CI = 80.4-99.3%). HBME-1 expression was absent in FA and CG as well as in the only case of FC. Results of the immunocytochemical staining of HBME-1 are summarised in Table 3.14.

Table 3.14.

Frequency of HBME-1 expression in FNA material of different thyroid lesions

Target structure	HBME-1	
	Expression n (%)	95% CI
Follicular adenoma (n=22)	0 (0%)	0 - 15.0
Colloid goiter (n=20)	0 (0%)	0 - 16.1
Papillary thyroid cancer (n=25)	24 (96%)	80.4 - 99.3
Follicular cancer (n=1)	0 (0%)	0 - 80.0

Abbreviation in the Table: n, absolute number; CI, confidence interval for a proportion.

4. DISCUSSION

Thyroid nodules are fairly common findings in clinical practice affecting approximately 40% of the population between 30 and 60 years old in the United States, besides thyroid cancer is the most common endocrine malignancy. Luckily most of these nodules are benign tumors or hyperplastic lesions and only a minority are malignant or suspicious tumours that require surgery. Therefore it is important to identify these benign lesions for proper management and to realise best possible benefit for the patients. For post-operative management of patients with thyroid nodules, accurate diagnosis is very critical as well. Errors in any of the patients management stage can lead to significant psycho-social problems and unnecessary increase in healthcare cost (152).

The local importance of the problem is emphasised by the growing cases of thyroid cancer in Latvia (220). Trend in increasing occurrence of thyroid cancer and division between sex is represented in Figure 4.1. According to data provided by Central Statistical Bureau of Latvia there is a 3.3× increase in thyroid cancer cases comparing year 1990 (n=59) with 2011 (n=195). According to data of year 2011 incidence of thyroid cancer in women is 5× more than in man (220).

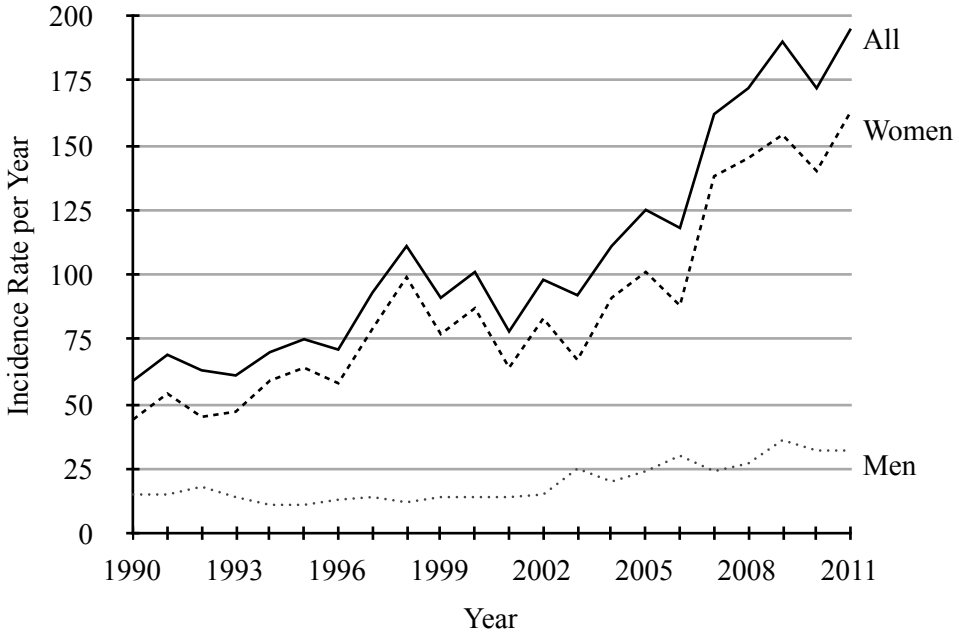

Figure 4.1. Cases of Thyroid Cancer (1990-2011) in Latvia

The decision whether to observe the nodules or to refer patient to surgical operation, is based on clinical information, thyroid US, scintigraphy, and FNA diagnosis. Although thyroid cancer constitutes one of the curable cancers, the differential diagnosis can often be ambiguous.

The current standard in the diagnosis of thyroid lesions is by histologic examination of routine HE stained sections. However, it is widely known that the interpretation of follicular lesions can be quite difficult (152).

As early as 1982 it was stated that FNA is the most sensitive and specific test for diagnosis of thyroid nodules. FNA of the thyroid is widely used since it has been proven to be a safe, inexpensive, and reliable diagnostic procedure.

First FNA of the thyroid in Latvia was performed in 1991 by Peteris Prieditis. In early years there were only some cases of thyroid FNA no exceeding 20-30 per year (221). Nowadays FNA of the thyroid are performed in several places, namely, in Pauls Stradins Clinical University hospital, Clinic Teika, Riga Eastern Hospital Latvian Oncology Centre, and much less in Latvian cites Valmiera and Liepaja. Most of all thyroid FNA are performed in Clinic Teika - 1200 per year (data of year 2011), then follows Riga Eastern Hospital Latvian Oncology Centre - 700 per year and 400 per year in Pauls Stradins Clinical University hospital (222).

In fact, the present consensus is that thyroid FNA biopsy is the procedure of choice for evaluation of nodules therefore cytologic interpretation can play a very important role in further clinical management of the patient. There are well-known limitations in the role of thyroid FNA, most importantly its inability to differentiate benign from malignant follicular neoplasms, since the final diagnosis rests on the histologic identification of capsular and / or vascular invasion, not available for cytology. Histologic evaluation of surgically resected follicular lesions can be challenging as well because identification of invasion can be tricky due to incomplete capsular penetration, equivocal vascular invasion or technical difficulties due to processing or sectioning artefacts (223-225). Another difficult situation can develop when some but not all the diagnostic nuclear features of papillary thyroid cancer are present.

A successful FNA biopsy rests on several factors such as trained, dedicated specialists to perform the FNA and read the smears, as well as maintain constant

interaction with radiologists, endocrinologists, surgeons, and oncologists. The use of ancillary techniques is also of importance. Since FNA cytology itself is not a reliable method to differentiate between benign and malignant follicular tumors or lesions, these patients usually undergo surgical resection, while only about 10% of them will after all have malignant tumors (5).

According to aforementioned reasons, during the last several years researchers have focused on finding molecular or IHC markers that could help in the distinction between benign and malignant lesions of the thyroid.

Recent advances on molecular diagnostics, such as immunocytochemistry, enzyme activity assays and real-time polymerase chain reaction, allowed a further analysis of FNA material in attempt to differentiate benign from malignant thyroid nodules. ICH was introduced to the practice of pathology in the early 1970s, however, in thyroid pathology, originally its use has been restricted. Gradually ICH found new applications in aspiration cytology. First, it has been introduce to obtain thin layer slides in cervical cytology and due to excellent results in this field, have encouraged a wider application to almost all cytological branches, including thyroid FNA biopsy (226).

New techniques have been introduced to thyroid FNA procedure to enhance its diagnostic yield and improve the accuracy. In spite of these improvements, many writes that even in “the right hands” the rate of inadequate smears is rarely lower that 10% and there remain difficulties in cytodiagnosis of follicular-derived lesions. For this reason, the characterisation of follicular thyroid nodules is widely considered as the “gray zone of FNA cytology” (31, 131, 202, 227). A precise diagnosis of papillary thyroid cancer in FNA material is practicable with the use of contemporary cytology techniques and immunocytochemistry, which is the valuable tool for correct and accurate diagnosis.

Much attention has shifted to identifying molecular or IHC markers that can help to distinguish adenomatous colloid nodules or follicular adenoma from follicular carcinoma on one hand, and papillary thyroid cancer from follicular neoplasm on the other hand (228).

During the last several years, a growing number of IHC markers have been tested in histologic and, to a lesser amount, on FNA samples with variable success rates (154, 229-232). Mostly the evaluation of IHC markers have been conducted on surgically resected thyroid specimens. However, similar studies were also done on FNA

cytologic specimens using cell block preparations (231, 233, 234). Generally, the studies have shown similar results of markers expression between surgical specimens and FNA cell block sections.

The diagnosis of papillary thyroid cancer in FNA specimens is usually unsophisticated when classic cytologic features are present (235, 236). However, in clinical practice there are often situation when it is difficult to make an unequivocal cytologic diagnosis of papillary thyroid cancer. The diagnostic difficulties are related to observation that some of typical cytologic features of papillary thyroid cancer (nuclear grooves, giant cells, psammoma bodies, papillary fragments) can also be observed in nonneoplatic lesions and follicular neoplasms of the thyroid (237). Adenomatous nodules, follicular adenomas and hot autonomous nodules also can show papillary fragments, nuclear enlargement, and nuclear grooves. Thus, benign lesions can occasionally be misdiagnosed as PTC. A marker that would readily differentiate papillary thyroid cancer from nonneoplatic thyroid lesions and follicular neoplasms would be useful in resolving thees common diagnostic dilemmas.

Considering the data in literature, of a large number of investigated IHC markers, only a few have emerged as potentially useful and clearly no single “magic” marker that distinguishes follicular adenomas from carcinomas has yet been found. In fact, several reports on this topic have provided conflicting results. Variances are mainly due to the apparently false-positive staining of some of the markers in normal thyroid or adenomas (230, 238). To solve the aforementioned issues, we designed this study on a relatively large number of histologically proven thyroid follicular lesions, and tested a panel of commercially available IHC markers (HBME-1, CD56, E-CAD, COX-2, Ki-67 and p53).

Most studies using **HBME-1** for diagnosis of thyroid tumours have been performed in tissue samples and only a few studies using FNA material have been carried out (153, 155, 158, 159, 238, 239).

According to our data on tissue samples, HBME-1 had a high expression level in papillary thyroid cancer and 4/14 in follicular cancer. High HBME-1 expression was present also on FNA samples. There was no HBME-1 expression in benign lesions of surgical tissue samples as well as in FNA material. This agrees with the study by Nasr et

al. (153) on thyroidectomy specimens showing positive expression of HBME-1 in 49/51 (96%) of papillary thyroid cancer, whereas normal thyroid tissue was consistently negative. Overall, HBME-1 in his study showed 96% sensitivity and 93% specificity for PTC.

Although Miettinen's study analysing operation material showed HBME-1 expression in 145/145 papillary thyroid cancer and 27/27 follicular cancer. In contrast, 33% cases of nodular goitres and papillary hyperplasia either showed no reactivity or were focally positive (155). Notable is the fact that all cases of FC were with high expression. In our study only 4/14 cases of FC had high expression level. This difference could be explainable with different antibody clone. However we believe that the invariably high expression suggests the possibility of technological failure.

A study by Saleh et al. (240) performed immunocytochemical staining of HBME-1 on cell block sections of thyroid FNA. They concluded that HBME-1 also had a high immunoexpression level in malignant tumours 24/27 (88.8%) compared to benign lesions 12/44 (27.3%). This agree with our study as well with the previous studies showing a high rate of immunoexpression of HBME-1 in malignant thyroid tumours (154, 229, 241).

Scognamiglio et al. (242) analysed HBME-1 expression in 78 cases of papillary thyroid cancer, including 49 classic papillary thyroid cancer and 29 cases of follicular variant of papillary thyroid cancer. Results showed expression of HBME-1 in 43 (88%) cases of classic papillary thyroid cancer and 29 (86%) of follicular variant of papillary thyroid cancer. The main conclusion agrees with our finding that diffuse and intense membranous staining with HBME-1 strongly supports the diagnosis of papillary thyroid cancer. Similar study was performed by Cheung et al. (243) who reported HBME-1 positivity in 38/54 (70%) classic papillary thyroid cancer and 38/84 (45%) follicular variant of papillary thyroid cancer with no expression in 40 nodular hyperplasia cases and 35 follicular adenomas. In follicular cancer HBME-1 expression was found in 2/4 (50%). Comparing to our study HBME-1 expression in papillary thyroid cancer is notably lower. Previous studies reinforces and prove our approach not dividing papillary thyroid cancer for analysis into different its variants for example follicular variant of papillary thyroid cancer.

A large study by Saggiorato et al. (230) retrospectively analysed expression of HBME-1, galectin-3, thyroperoxidase and cytokeratin-19 in 125 consecutive FNA samples of indeterminate diagnoses of "follicular thyroid neoplasm", and compared with their corresponding surgical specimens, including 33 follicular cancer, 42 papillary thyroid cancer and 50 follicular adenomas. Statistical analysis confirmed that galectin-3 and HBME-1 were the most sensitive (92% and 80% respectively) and specific (94% and 96% respectively) molecules.

Study by Liang et al. demonstrated overexpression of HBME-1 in thyroid carcinomas, with 92% of cases showing positive expression compared with 29% for thyroid adenomas. Main conclusion of the author agrees with finding in our study indicating that HBME-1 could be considered a good marker for distinguishing between benign and malignant differentiated thyroid tumours.

Prasad et al. (239) very similar to our study demonstrated HBME-1 expression in 57/67 (85%) papillary thyroid cancer, 3/6 (50%) follicular cancer and only 1/102 (1%) non-neoplastic thyroid lesions stained for HBME-1. Under the term non-neoplastic thyroid lesions author included 29 nodular goiters, 14 diffuse thyrotoxic hyperplasia and 59 normal thyroid tissues.

A total number of 83 FNA cases were included in the study by Pazaitou-Panayiotou et al. (244). HBME-1 similarly as in our study showed a predominantly strong membranous pattern and was positive in 65 out of 83 cases (86.7%) of papillary thyroid cancer.

Some other investigators have reported confident results of HBME-1 expression in papillary thyroid cancer and some follicular cancer, but low or negative expression in benign thyroid tissue (175, 245-247).

Several studies have investigated the expression of **E-CAD** in thyroid malignancies. It is recognised that the reduction of E-CAD expression is associated with thyroid neoplasms (167, 172-175).

Analysing our data papillary thyroid cancer demonstrated a significant reduction in E-CAD expression when compared to FA or thyroid tissue surrounding papillary thyroid cancer. In contrast, follicular cancer showed no significant differences in E-CAD expression compared to follicular adenoma and the expression was generally very

high. As mentioned before, there was only one case of follicular carcinoma to analyse in second stage of the study in FNA material. The low and heterogenous expression of E-CAD in papillary thyroid cancer and intensive in follicular adenoma or follicular cancer confirms the results of Brabant et al. (167) where in 9 of 16 patients with papillary thyroid cancer the level of E-CAD was clearly reduced, which was particularly evident when compared to normal tissue located near the tumour. Still expression of E-CAD in papillary thyroid cancer was highly variable. In follicular cancer (n=6) E-CAD expression also varied considerably. Expression was rather in high levels well above average which is similar finding in tissue of various benign thyroid diseases, including normo- and hyperfunctioning follicular adenomas. Considering these findings author concludes that E-CAD is a marker of thyroid differentiation, because in anaplastic carcinomas (n=6), which are most malignant group of thyroid tumours, the expression of E-CAD was very weak or undetectable. Here follows that loss of E-CAD expression and/or defective posttranscriptional control appear to be restricted to undifferentiated and metastatic thyroid tumours. In our study anaplastic carcinomas or metastatic thyroid tumours were not included, otherwise results of Brabant et al. are very similar.

Our study agree with previously reported data by Soares et al. (172) who analysed E-CAD expression in surgically removed material of follicular adenomas (n=52), follicular cancer (n=8), papillary thyroid cancer (n=18) and poorly differentiated carcinoma (n=3). The results showed moderate to strong membranous expression in every neoplastic cell of follicular cancer, whereas in papillary thyroid cancer the immunoreactivity was comparatively less intense and negative areas were frequently observed. The main conclusion of the study by Soares et al. states that reduced E-CAD expression means reduced differentiation and increased aggressiveness of the tumour.

Confident results are showed by Dahlman et al. (248) from Karolinska Hospital, Sweden where the role of integrins and E-CAD were evaluated on the normal thyroid gland and in different type of tumours including six follicular adenomas, seven follicular cancers, ten papillary thyroid cancer and four anaplastic carcinomas. Results of the study agree with other published as well as with our study were E-CAD displayed a strong staining of the lateral cell borders in all tissues examined, except in anaplastic

carcinomas, in which the staining was weak and in some specimens absent. Anaplastic carcinomas were not included in our study due to rarity of the disease.

Unlike results previously reported moreover different results from our study are showed by Smyth et al. (249) who analysed E-CAD expression in operation material of 31 papillary thyroid cancers, 12 follicular cancers and 16 follicular adenomas. Contrary to results of our study follicular cancer demonstrated a significant reduction in E-CAD expression when compared to normal thyroid or follicular adenoma. In contrast, papillary thyroid cancer showed no significant reduction in expression. This difference in expression of follicular cancer and papillary thyroid cancer has not been reported previously, and although the data differs from previously published works as well as from results of our study. One of the explanation of such a differences in expression is possibly explained by different technological approach, namely, Smyth et al. for obtaining results used reverse transcription-polymerase chain reaction.

Choi et al. (175) analysed expression of E-CAD in tissue material of the thyroid including 67 papillary thyroid cancers, 30 follicular cancers and 9 colloid goiters. Choi et al. clearly shows diffusely positive expression of E-CAD in papillary thyroid cancer and similarly in follicular cancer. Expression in colloid goiter is found in maximum intensity in 100% of cases. Comparatively in our study the expression of papillary thyroid cancer at the same time was not so high as in case of follicular cancer. Higher E-CAD reactivity for the follicular cancer could be explained with use of special biotin blocking system. In our study, due to different cut-off level and more sensitive technology, we were able to demonstrate differencies in E-CAD expression in papillary thyroid cancer and benign lesions.

Considerable study is published by Batistatou et al. (250) who analysed E-CAD expression in thyroid tissue blocks of eighty papillary thyroid cancers. Immunoreactivity for E-CAD was detected in the membranes of non-neoplastic follicular cells. In papillary thyroid cancer low expression was detected in 37.5% (15/40) of the cases, intermediate expression in 40% (16/40), and high expression in 20% (8/40). These findings are rather similar to our study, only the possible mechanisms of E-CAD inactivation are still under investigation. Batistatou et al. mentions that irreversible molecular alterations of the E-CAD are infrequent in thyroid

tumours, while methylation is frequent in papillary thyroid cancer and translational pathways may also be involved in the reduction of E-CAD expression in the thyroid.

Contradictory results are published by Liang et al. (187) who analysed E-CAD expression in tissue specimens from 45 papillary thyroid cancers, 26 follicular cancers and 48 follicular adenomas. According to results of the study negative or weakly positive expression of E-CAD was met in follicular adenomas as well as in both types of carcinomas. These findings are contrary to most of the published data as well to results of our study.

One of the few studies published so far of E-CAD expression in FNA material of the thyroid are made by Pazaitou-Panayiotou et al. (244). E-CAD expression was analysed in 83 FNA cases diagnosed as papillary thyroid cancer and results showed positive E-CAD in 5 out of 83 (6%) cases, and 79 (93.97%) cases showed loss of expression. All controls retained their normal expression. These findings are like results of our study. The author concludes that loss of expression of E-CAD may provide an objective diagnostic tool, and its use may be extremely useful in the diagnosis of papillary thyroid cancer, especially in doubtful cases.

Original study was done by Erdem et al. (251) who analysed tissue material of 79 patients with papillary thyroid cancer. Interestingly marker expression intensity was analysed in following groups: age groups (<35 age; 35-55 age; >55 age), stage of the tumour (stage I, II, III and IV), diameter of the tumour (<2 cm; 2-4 cm; >4cm) as well as patients showing presence of thyroid gland capsule invasion and lymph node metastasis.

The trend of E-CAD expression in papillary thyroid cancer was as reported previously and similarly to our study, but when comparing to expression differences in different study groups mentioned above, there were no statistically significant differences.

In our study, statistically significant differences was observed regarding E-CAD expression in benign thyroid tissue surrounding follicular adenoma and papillary thyroid cancer with upregulation in tissue adjacent to papillary thyroid cancer. The differences can be explained by the interaction between invasive tumour and surrounding tissue in contrast to non-invasive neoplasia. Hypothetically, it can influence

the diagnostics significantly, with possible beneficial meaning in diagnostic pathology, by negative - in FNA.

So far there are very few studies published concerning **CD56** expression in thyroid neoplasms. Notable is the fact that there are no study published about CD56 expression in such a number of FC cases and only one study exists were CD56 expression is analysed in thyroid FNA material.

Similar results to our finding was published by Migita et al. (252) where CD56 expression intensity was analysed in thyroidectomy specimens. Comparatively confident results of the study showed less than 10% cell expression of CD56 in papillary thyroid cancer whereas colloid goitre and follicular adenoma showed approximately 70% of the cells positive results. These results are similar to our study except for papillary thyroid cancer where it was remarkably decreased.

Study by Zeromski et al. (164) analysed CD56 expression in benign samples of human thyroid collected during surgery. According to study results CD56 expression showed positive results on all benign thyroid tissue examined (simple goiter, Grave's disease, Hashimoto's thyroiditis). Author is speculating that CD56 is involved in the morphogenesis, the growth and the function of thyroid gland in both, normal and pathological conditions, by promoting homotypic cell adhesion (164).

Recent study from the group of researchers form Canada leaded by El Demellawy et al. (161) collected 185 cases of different thyroid gland lesions and concluded that diffuse CD56 expression was consistently present in normal, lesional, and neoplastic follicular epithelium, except for PTC, including the follicular variant. All cases of papillary thyroid cancer showed absent of CD56 expression, whereas in all non-papillary thyroid cancer groups, the CD56 expression were membranous and diffuse, involving 100% of the thyrocytes in the lesion. Interestingly that CD56 expression was retained in follicular cancer which is exactly the same finding as in our study but only here two cases of follicular cancer are present. El Demellawy et al. concludes that the use of CD56 is extremely helpful in selecting cases of papillary thyroid cancer (including follicular variant) from other follicular cell-derived thyroid lesions/tumours, with 100% sensitivity and 100% specificity. Adding this marker to IHC

panel will assist in the making of papillary thyroid cancer diagnosis, particularly follicular variant.

The only study published so far about CD56 expression analysis in thyroid FNA is made by Pazaitou-Panayiotou et al. (244). CD56 expression was analysed in 83 FNA cases diagnosed as papillary thyroid cancer and results showed positive CD56 in 3 out of 83 (3.6%) cases, and 80 (96.4%) cases showed loss of expression. All controls retained their normal expression. These findings are like results of our study. The author concludes that CD56 can assist in decision making about the benign or malignant nature of the aspirated material. Loss of the expression seems to agree with the presence of PTC.

Most recent study done by Kim et al. (253) who analysed CD56 staining on 72 papillary thyroid cancers. Results revealed similar findings to previously published data moreover similar to our study that non-neoplastic areas of all 72 cases showed positive CD56 expression along the plasma membrane; however staining was reduced or absent in 65 papillary thyroid cancers (90.3%). In this study only papillary thyroid cancers were included, besides the CD56 staining did not reveal any significant relationship with clinicopathologic features ($p>0.05$).

Contrary to our work results of **COX-2** expression in thyroid lesions are published by Specht et al. (180). Author analysed IHC expression of COX-2 in 28 different thyroid specimens. Immunohistochemical analysis of representative cases of thyroid cancer (12 papillary thyroid cancers, 1 follicular cancer) revealed that COX-2 expression was multifocal and moderate to strong in intensity in the majority of cases. Staining for COX-2 according to results was negligible in normal tissue. In tumour tissue, expression of COX-2 was localised to tumour cells, but not to surrounding stromal cells or infiltrating inflammatory cells. These results are in great contradiction to our results where the difference in COX-2 expression between colloid goiter, follicular adenoma, papillary thyroid cancer and follicular cancer was not remarkable and not statistically significant. Furthermore it is well known that technological variations exceed the biological differences regarding COX-2 analysis by IHC (254).

Very similar finding and conclusions with our study was published by Kim et al. (181) where COX-2 expression was studied immunohistochemically in 19 papillary

thyroid cancers, 8 follicular cancers, 14 follicular adenomas, and 8 colloid goiters. COX-2 staining was not present in any of the colloid goiters. In contrast, COX-2 staining was observed in all of papillary thyroid cancers. Moreover, 7 of 8 follicular cancers and 11 of 14 follicular adenomas showed COX-2 staining. Kim et al. concludes that COX-2 is not useful as a marker of malignancy since its expression was evident in follicular adenomas and in papillary thyroid cancer and follicular cancer as well. Wherewith author speculates that the enzyme could be involved in the early process of thyroid tumorogenesis. In our study we didn't find such a differences in expression intensities between groups, but overall usefulness of the marker is also found to be very dubious.

Study published by Casey et al. (255) also reveals confident results about COX-2 usefulness in decision making of different thyroid lesions. In agreement with our study Casey et al. reports that COX-2 are expressed in benign as well as in malignant thyroid tissues. Yet papillary thyroid cancer and follicular cancer expressed higher levels of COX-2 compared to follicular adenoma and colloid goiter still COX-2 probably is not useful in the IHC diagnosis of thyroid malignancies. Similar results to Casey et al. and our study are reported by Fuhrer et al. (256) were COX-2 expression is found similarly in benign as well as in malignant thyroid tissues.

Haynik et al. (182) published yet the only study were COX-2 expression is analysed exclusively in follicular cancer and follicular adenoma. Results revealed positive COX-2 staining in 9 of 34 follicular cancers and 2 of 7 follicular adenomas Normal thyroid tissue surrounding tumour did not stain for COX-2. In summary author concludes the data suggest that such expression of COX-2 may correlate with increased tumour recurrence and death and future studies need to be conducted to determine if selective COX-2 inhibitors can also play some role in the management of a subset of follicular neoplasms of the thyroid.

Garcia-Gonzales et al. (257) studied IHC expression of COX-2 in a total of 174 samples of human thyroid. Among them 33 follicular adenomas, 39 papillary thyroid cancers, 15 follicular cancers and 15 colloid goiters. In this study COX-2 expression was frequently up-regulated in thyroid carcinomas, whereas COX-2 expression was limited in benign thyroid lesions. Twenty-seven (68.8%) of papillary thyroid cancers showed COX-2 immunoexpression. Garcia-Gonzales et al. speculates that this fact,

together with negative expression of COX-2 in 30.8% of papillary thyroid cancers tested, indicates that COX-2 is not always useful as a marker of malignancy. Although expression in follicular cancer was higher there were no statistically significant differences comparing to follicular adenoma. Hypothesis was highlighted from the study group about the possibility that some follicular adenoma up-regulating COX-2 could in fact be "malignant" tumours that have not yet revealed morphologic criteria of malignancy.

The only study published so far about COX-2 expression analysis in thyroid FNA is made by Krawczyk-Rusiecka et al. (258). 45 thyroid FNA specimens were analysed including 23 cases of papillary thyroid cancers and 22 cases of benign thyroid lesions. Contrary to our data Krawczyk-Rusiecka et al. reports a significantly higher expression of COX-2 in papillary thyroid cancers when compared to benign thyroid lesions ($p=0.021$). No relationship was found between COX-2 expression and patients age and sex. Author concludes that usefulness of COX-2 as a marker of thyroid malignancy is provocative and its potential role in carcinogenesis still arises significant interest.

Very recent study concerning COX-2 expression in papillary thyroid cancer is published by Erdem et al. (251). Marker expression intensity was analysed by age groups (<35 age; 35-55 age; >55 age), stage of the tumour (stage I, II, III and IV), diameter of the tumour (<2 cm; 2-4 cm; >4cm) as well as presence of capsule invasion and lymph node metastasis. When COX-2 expression was evaluated based on age groups, 42.9 ± 36% of 1+, 2+ and 3+ stained cells belonged to patients under 35 years old, 45.3 ± 26.2% were from the 35-55 year old age group and 62.8 ± 24.8% fell into the above 55 years of age group. As the age increased, the rate of staining also increased ($p=0.05$). The trend of COX-2 expression in papillary thyroid cancer was as reported previously and similarly to our study, but when comparing to expression differences in different study groups mentioned above, there were no statistically significant differences. No correlation was observed when COX-2 expression was analysed based on capsule invasion and lymph node metastasis ($p>0.05$).

The differences in immunohistochemical findings can appear due to technological details, namely, different origin of primary antibodies (181, 182).

Analysing **Ki-67** very confident results, which are in common with findings of our study, are published by Mehrotra et al. (184). Ki-67 protein expression were assessed by IHC in formalin-fixed, paraffin-embedded thyroid tissues from 128 patients with histologic diagnoses of papillary thyroid cancer (n = 38), follicular cancer (n = 22), follicular adenoma (n = 33), and colloid goiter (n = 35). As a result Ki-67 labeling was higher in follicular cancer and papillary thyroid cancer than in follicular adenoma or colloid goiter. The Ki-67 discriminated between follicular cancer and follicular adenoma ($P < 0.0001$). However, Ki-67 overlapped widely between the four histologic groups, and the expression of these proteins was also noted to be heterogenous within these lesions. Mehrotra et al. concluded that Ki-67 cannot currently be reliably applied as preoperative markers to distinguish benign from malignant thyroid lesions.

Spectacular study was published in 2010 by Ito et al. (185) where relationship between Ki-67 labelling index and the biological behaviour of papillary thyroid cancer (n=371) was analysed. Ito et al. IHC investigated Ki-67 labelling index in their primary lesions and compared this finding with various clinicopathological features, including patient prognosis. As a result Ki-67 labelling index was ≤1% in 213 patients (57%) and among the remaining 158, only 35 showed Ki-67 labelling index >3%. Ito et al. concludes that Ki-67 was associated with patient age, massive extrathyroid extension, and distant metastasis at surgery. Of 363 patients without distant metastasis at surgery, 54 (15%) showed carcinoma recurrence during follow-up (average 124 months) and the disease-free survival of patients with Ki-67>1% was significantly worse than that of those with Ki-67<1% ($p < 0.0001$). Ki-67 labelling index was recognised as an independent prognostic factor for the disease-free survival of patients. This study is notable because of large series of papillary thyroid cancer cases analysed and long patients follow-up. In any case our study results are alike, because Ki-67 labelling index as well was low not reaching 5%.

Similar results to our study is published by Liang et al. (187) who analysed more that 10 IHC markers on thyroid tissue samples from 119 patients including 71 papillary thyroid cancers, 26 follicular cancers and 48 follicular adenomas. According to the results Ki-67 expression was not significantly different between the groups and immunopositivity for Ki-67 among the carcinomas was highly variable, ranging from + to +++. Author speculates that these results may be related to antibody selection,

inability of the antibody to recognise a protein with altered configuration, or antigen retrieval methodology. As conclusion it makes it unlikely that Ki-67 alone will be of value and practical use in differentiating benign from malignant differentiated thyroid tumours.

Results of Pujani et al. (186) agrees with our study were author evaluated the role of the proliferative marker Ki-67 in nonneoplastic and neoplastic lesions of the thyroid, with a special emphasis on the distinction between follicular adenoma and follicular cancer. One hundred cases of thyroid lesions, including 50 nonneoplastic and 50 neoplastic lesions, were retrieved and Ki-67 immunostaining was performed. Ki-67 labeling index showed a progressive rise from multinodular goiter to benign to malignant neoplasms. A statistically significant difference was observed in Ki-67 counts between multinodular goiter vs papillary thyroid cancer ($P < 0.05$) and follicular adenoma vs follicular cancer ($P < 0.05$). The correlation between mitotic counts and Ki-67 labeling index was found to be significant. Author concludes that Ki-67 was found to be useful in differentiating between follicular adenoma and follicular cancer, but since the sample size of the study was small, larger studies are needed to confirm this observation as well as to assign a cutoff value.

One of the first work published according to **p53** IHC expression in differentiated thyroid tumours is done by Dobashi et al. (191). In his study a total of 110 cases of thyroid carcinomas were examined IHC to evaluate the overexpression of mutant forms of p53 protein considering their relationship with their histological subtypes. Overall, IHC detected nuclear p53 expression in 22.7% of the thyroid carcinomas. A significant difference in the positivity of p53 among histological subtypes was noted; the positivity was 11.1% of the cases in papillary thyroid cancer, 14.3% in follicular cancer, 40.9% in poorly differentiated carcinoma, and 63.6% in undifferentiated carcinoma. No IHC positivity was found in adjacent non-neoplastic tissues or in benign lesions, including follicular adenoma and colloid goiter. These results suggest that overexpression of p53 is not a responsible factor for the oncogenesis itself, but rather that it plays a crucial role in aggressive subtypes of thyroid carcinomas. Additionally, the distinct entity of poorly differentiated carcinoma, previously categorised in the well-differentiated carcinoma under the name of papillary or

follicular carcinoma, was statistically confirmed. It is hard to compare these results to our study because no expression of p53 was found in any of the groups. Possible explanation could be the absence of undifferentiated tumours in study group as well as differences in commercially available antibodies.

Confident results are published by Morita et al. (192) who analysed p53 expression in 68 patients in whom thyroidectomy with lymph node dissection had been performed due to papillary thyroid cancer. Results of IHC staining showed overexpression of p53 protein in papillary thyroid cancer, and revealed a statistically significant correlation between overexpression of p53 and large tumour size, presence of lymph node metastasis, and the mean number of lymph node metastases. So far this is the only study analysing such a number of lymph nodes (n=196).

One of the recent studies on p53 immunoexpression in thyroid pathologies is published in 2011 by Tan et al. (190) were differences of the expression among the papillary thyroid cancer, follicular cancer, and follicular adenoma were analysed. Thirty-nine thyroid tissue specimens with the diagnoses of the papillary thyroid cancer, follicular cancer, and follicular adenoma were included in the study. The expression of p53 was increased statistically significant in papillary thyroid cancer. The author concludes that p53 could be usable in favour of the diagnosis of the papillary thyroid cancer.

p53 according to our study was not expressed in any of the groups. This could be associated with lack of undifferentiated cancer morphologies included as well as lack of lymph node metastasis included. Important factors include possible differences in primary antibody due to different manufacturers.

Differential diagnosis of thyroid nodules and treatment options still remains actual from medical and socio-economic point of view. Possibly this topic will be actual for a very long time. Nowadays recent trends in molecular diagnostics and radiological examinations plays a crucial role in the choice of management of nodular thyroid.

We believe that no single immunohistochemical marker by itself is completely sensitive and specific to differentiate between benign and malignant thyroid tumours, therefore the most appropriate panel of markers is wanted and many studies are made in this field. Due to possible technical problems or processing issues usually panel of two

or more markers is used. The relatively low cost IHC test of commercially available markers comparing to costs of real-time polymerase chain reaction can help to optimise the management of patients with thyroid nodules and to avoid unnecessary surgery. Nevertheless, there are still question to answer and additional studies are needed toward the quest of identifying useful markers in case of thyroid pathology.

5. CONCLUSIONS

1. As significant immunophenotypic differences are found in nodular thyroid diseases, immunohistochemistry can have valuable diagnostic implications
2. The panel consisting of three immunohistochemical markers, HBME-1, E-CAD and CD56, can reliably distinguish papillary thyroid carcinoma from follicular tumours (follicular adenoma and follicular cancer).
3. Our results indicate that expression of HBME-1 is found in malignant lesions only. It is also the most sensitive and specific single marker in papillary thyroid cancer.
4. CD56 and E-CAD can assist in decision making about benign and malignant nature of the aspirated material. Loss of expression seems to agree with the presence of papillary thyroid cancer and distinguishes it from follicular tumours. Both of them are characterised by high expression of CD56 and E-CAD.
5. Although proliferation activity significantly differs between benign and malignant thyroid lesions, the practical use of Ki-67 marker is difficult due to generally low values.
6. The lack of p53 expression in our study may be due to lower level of malignancy, as our study group did not include undifferentiated tumours.
7. According to our study, the difference in COX-2 expression between groups was not remarkable and not significant.
8. Developed FNA technologies reveals different frequencies of E-CAD, HBME-1 and CD56 expression in follicular adenoma and papillary thyroid cancer.

6. PRACTICAL RECOMMENDATIONS

1. The established technologies for immunohistochemical analysis of histological material should be introduced for practical diagnostic use.
2. For evaluation of FNA smears we recommend the use of created immunocytochemical visualisation technique.
3. We recommend the established panel of molecular markers (HBME-1, E-CAD and CD56) as an additional criteria to diagnose thyroid cancer in preoperative FNA when US characteristics associated with higher risk of malignancy are not conclusive.
4. Taking into account the results of marker expression in FNA, it is possible to evaluate the indications for thyroid operation and its extend more appropriate.

7. REFERENCES

1. Gharib H, Papini E. Thyroid nodules: clinical importance, assessment, and treatment. Endocrinol Metab Clin North Am. 2007;36(3):707-35, vi. Epub 2007/08/04.
2. Topliss D. Thyroid incidentaloma: the ignorant in pursuit of the impalpable. Clin Endocrinol (Oxf). 2004;60(1):18-20. Epub 2003/12/18.
3. Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995 [see commetns]. Cancer. 1998;83(12):2638-48. Epub 1999/01/05 21:59.
4. Finley DJ, Arora N, Zhu B, Gallagher L, Fahey TJ, 3rd. Molecular profiling distinguishes papillary carcinoma from benign thyroid nodules. J Clin Endocrinol Metab. 2004;89(7):3214-23. Epub 2004/07/09.
5. Haugen BR, Woodmansee WW, McDermott MT. Towards improving the utility of fine-needle aspiration biopsy for the diagnosis of thyroid tumours. Clin Endocrinol (Oxf). 2002;56(3):281-90. Epub 2002/04/10.
6. Prinz RA, Staren ED. Embryology, Anatomy and Physiology of Thyroid. In: J.L.Harrison, E.D.Staren, R.A.Prinz, editors. Endocrine Surgery. Texas U.S.A.: Landes Bioscience; 2000. p. 1-9.
7. Delbridge L. Total thyroidectomy: the evolution of surgical technique. ANZ J Surg. 2003;73(9):761-8. Epub 2003/09/06.
8. Larsen WJ, Sherman LS. Human embriology. New York: Churcill Livingstone; 2001.
9. Oertli D, Udelsman R. Embryology and Surgical Anatomy of the Thyroid and Parathyroid Glands. In: William B, Rizzolo J, editors. Surgery of the Thyroid and Parathyroid Glands. NewYork: Springer; 2007. p. 13-20.
10. Sackett WR, Reeve TS, Barraclough B, Delbridge L. Thyrothymic thyroid rests: incidence and relationship to the thyroid gland. J Am Coll Surg. 2002;195(5):635-40. Epub 2002/11/20.
11. Clark OH, Duh QY, Kebebew E. Surgical Anatomy and Embryology of the Thyroid and Parathyroid Glands and Recurrent and External Laryngeal Nerves. In: Henry J-F, editor. Textbook Of Endocrine Surgery. Philadelphia: Elsevier Saunders; 2005. p. 9-15.
12. Wartofsky L. The thyroid gland. In: Becker KL, editor. Principles and practice of endocrinology and metabolism. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 308-471.
13. Davies TF. A Guide to the Physiology and Testing of Thyroid Function. In: E.Schwartz A, Pertsemlidis D, Gagner M, editors. Endocrine Surgery. New York: Mercel Dekker Inc.; 2004. p. 75-85.

14. Mian C, Lacroix L, Alzieu L, Nocera M, Talbot M, Bidart JM, Schlumberger M, Caillou B. Sodium iodide symporter and pendrin expression in human thyroid tissues. Thyroid. 2001;11(9):825-30. Epub 2001/09/29.
15. McLachlan SM, Rapoport B. The molecular biology of thyroid peroxidase: cloning, expression and role as autoantigen in autoimmune thyroid disease. Endocr Rev. 1992;13(2):192-206. Epub 1992/05/01.
16. Bjorkman U, Ekholm R, Denef JF. Cytochemical localization of hydrogen peroxide in isolated thyroid follicles. J Ultrastruct Res. 1981;74(1):105-15. Epub 1981/01/01.
17. Larsen PR, Ingbar SH. The thyroid gland. In: Wilson D, Foster D, editors. Williams Textbook of Endocrinology, 8th ed. Philadelphia: Saunders; 1992. p. 357.
18. Engler D, Merkelbach U, Steiger G, Burger AG. The monodeiodination of triiodothyronine and reverse triiodothyronine in man: a quantitative evaluation of the pathway by the use of turnover rate techniques. J Clin Endocrinol Metab. 1984;58(1): 49-61. Epub 1984/01/01.
19. Clark OH, Duh QY, Kebebew E. Thyroid physiology. In: Roderick Clifton-Bligh, Delbridge L, editors. Textbook Of Endocrine Surgery. Philadelphia: Elsevier Saunders; 2005. p. 3-8.
20. Tata JR, Ernster L, Lindberg O, Arrhenius E, Pedersen S, Hedman R. The action of thyroid hormones at the cell level. Biochem J. 1963;86:408-28. Epub 1963/03/01.
21. Lazar MA. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr Rev. 1993;14(2):184-93. Epub 1993/04/01.
22. Brent GA. The molecular basis of thyroid hormone action. N Engl J Med. 1994;331(13):847-53. Epub 1994/09/29.
23. Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev. 2001;81(3):1097-142. Epub 2001/06/28.
24. Chin WW. Molecular mechanisms of thyroid hormone action. Thyroid. 1994;4(3): 389-93. Epub 1994/01/01.
25. Body JJ, Glibert F, Nejai S, Fernandez G, Van Langendonck A, Borkowski A. Calcitonin receptors on circulating normal human lymphocytes. J Clin Endocrinol Metab. 1990;71(3):675-81. Epub 1990/09/01.
26. Takahashi N, Akatsu T, Sasaki T, Nicholson GC, Moseley JM, Martin TJ, Suda T. Induction of calcitonin receptors by 1 alpha, 25-dihydroxyvitamin D3 in osteoclast-like multinucleated cells formed from mouse bone marrow cells. Endocrinology. 1988;123(3):1504-10. Epub 1988/09/01.
27. R.Clifton-Bligh, L.Delbridge. Thyroid Physiology. In: Clark OH, Duh QY, Kebebew E, editors. Textbook Of Endocrine Surgery. Philadelphia: Elsevier Saunders; 2005. p. 3-8.
28. Procopiou M, A.Meier C. Evaluation of Thyroid Nodules. In: D.Oertli, R.Udelsman, editors. Surgery of the Thyroid and Parathyroid Glands. New York: Springer; 2007. p. 45-57.

29. Belfiore A, La Rosa GL, La Porta GA, Giuffrida D, Milazzo G, Lupo L, Regalbuto C, Vigneri R. Cancer risk in patients with cold thyroid nodules: relevance of iodine intake, sex, age, and multinodularity. Am J Med. 1992;93(4):363-9. Epub 1992/10/01.
30. Cole WH. Incidence of carcinoma of the thyroid in nodular goiter. Semin Surg Oncol. 1991;7(2):61-3. Epub 1991/03/01.
31. Gharib H. Fine-needle aspiration biopsy of thyroid nodules: advantages, limitations, and effect. Mayo Clin Proc. 1994;69(1):44-9. Epub 1994/01/01.
32. Gharib H. Changing concepts in the diagnosis and management of thyroid nodules. Endocrinol Metab Clin North Am. 1997;26(4):777-800. Epub 1998/01/16.
33. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, Panunzi C, Rinaldi R, Toscano V, Pacella CM. Risk of malignancy in nonpalpable thyroid nodules: predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab. 2002;87(5):1941-6. Epub 2002/05/08.
34. Tollin SR, Mery GM, Jelveh N, Fallon EF, Mikhail M, Blumenfeld W, Perlmutter S. The use of fine-needle aspiration biopsy under ultrasound guidance to assess the risk of malignancy in patients with a multinodular goiter. Thyroid. 2000;10(3):235-41. Epub 2000/04/25.
35. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60(5): 277-300. Epub 2010/07/09.
36. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA. 2006;295(18):2164-7. Epub 2006/05/11.
37. Musholt TJ, Clerici T, Dralle H, Frilling A, Goretzki PE, Hermann MM, Kussmann J, Lorenz K, Nies C, Schabram J, Schabram P, Scheuba C, Simon D, Steinmuller T, Trupka AW, Wahl RA, Zielke A, Bockisch A, Karges W, Luster M, Schmid KW. German Association of Endocrine Surgeons practice guidelines for the surgical treatment of benign thyroid disease. Langenbecks Arch Surg. 2011;396(5):639-49. Epub 2011/03/23.
38. Dralle H. [Thyroid incidentaloma. Overdiagnosis and overtreatment of healthy persons with thyroid illness?]. Chirurg. 2007;78(8):677-86. Epub 2007/07/14. Inzidentalome der Schilddruse. Uberdiagnostik und -therapie gesunder Schilddrusenkranker?
39. POPULATION AND MAIN DATA OF VITAL STATISTICS [http://data.csb.gov.lv]. Central Statistical Bureau of Latvia. 2012.
40. CASES OF MALIGNANT NEOPLASMS BY SELECTED SITES [http:// data.csb.gov.lv]. Central Statistical Bureau of Latvia. 2011.
41. Chow SM, Law SC, Chan JK, Au SK, Yau S, Lau WH. Papillary microcarcinoma of the thyroid-Prognostic significance of lymph node metastasis and multifocality. Cancer. 2003;98(1):31-40. Epub 2003/07/02.

42. Cheema Y, Olson S, Elson D, Chen H. What is the biology and optimal treatment for papillary microcarcinoma of the thyroid? J Surg Res. 2006;134(2):160-2. Epub 2006/06/20.
43. Roti E, Rossi R, Trasforini G, Bertelli F, Ambrosio MR, Busutti L, Pearce EN, Braverman LE, Degli Uberti EC. Clinical and histological characteristics of papillary thyroid microcarcinoma: results of a retrospective study in 243 patients. J Clin Endocrinol Metab. 2006;91(6):2171-8. Epub 2006/02/16.
44. Sherman SI. Thyroid carcinoma. Lancet. 2003;361(9356):501-11. Epub 2003/02/14.
45. Gaitan E. Intervention policy in endemic goitre areas. Thyroidology. 1990;2(3):113-9. Epub 1990/12/01.
46. Pinchera A, Aghini-Lombardi F, Antonangeli L, Vitti P. [Multinodular goiter. Epidemiology and prevention]. Ann Ital Chir. 1996;67(3):317-25. Epub 1996/05/01. Gozzo multinodulare. Epidemiologia e prevenzione.
47. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol (Oxf). 1977;7(6):481-93. Epub 1977/12/01.
48. Schneider AB. Radiation-induced thyroid tumors. Endocrinol Metab Clin North Am. 1990;19(3):495-508. Epub 1990/09/01.
49. Delbridge L. Solitary thyroid nodule: current management. ANZ J Surg. 2006;76(5): 381-6. Epub 2006/06/14.
50. Demidchik YE, Saenko VA, Yamashita S. Childhood thyroid cancer in Belarus, Russia, and Ukraine after Chernobyl and at present. Arq Bras Endocrinol Metabol. 2007;51(5): 748-62. Epub 2007/09/25.
51. Pacini F, Vorontsova T, Demidchik EP, Molinaro E, Agate L, Romei C, Shavrova E, Cherstvoy ED, Ivashkevitch Y, Kuchinskaya E, Schlumberger M, Ronga G, Filesi M, Pinchera A. Post-Chernobyl thyroid carcinoma in Belarus children and adolescents: comparison with naturally occurring thyroid carcinoma in Italy and France. J Clin Endocrinol Metab. 1997;82(11):3563-9. Epub 1997/11/14.
52. Tronko MD, Bogdanova TI, Komissarenko IV, Epstein OV, Oliynyk V, Kovalenko A, Likhtarev IA, Kairo I, Peters SB, LiVolsi VA. Thyroid carcinoma in children and adolescents in Ukraine after the Chernobyl nuclear accident: statistical data and clinicomorphologic characteristics. Cancer. 1999;86(1):149-56. Epub 1999/07/03.
53. Nikiforov Y, Gnepp DR, Fagin JA. Thyroid lesions in children and adolescents after the Chernobyl disaster: implications for the study of radiation tumorigenesis. J Clin Endocrinol Metab. 1996;81(1):9-14. Epub 1996/01/01.
54. Balter M. Chernobyl's thyroid cancer toll. Science. 1995;270(5243):1758-9. Epub 1995/12/15.

55. Heidenreich WF, Bogdanova TI, Biryukov AG, Tronko ND. Time trends of thyroid cancer incidence in Ukraine after the Chernobyl accident. J Radiol Prot. 2004;24(3): 283-93. Epub 2004/10/30.
56. Wium P, Lund E, Reitan JB. [Medical consequences following the Chernobyl nuclear accident]. Tidsskr Nor Laegeforen. 2007;127(19):2564-6. Epub 2007/10/11. Medisinske konsekvenser av Tsjernobyl-ulykken.
57. Williams ED, Doniach I, Bjarnason O, Michie W. Thyroid cancer in an iodide rich area: a histopathological study. Cancer. 1977;39(1):215-22. Epub 1977/01/01.
58. Cuello C, Correa P, Eisenberg H. Geographic pathology of thyroid carcinoma. Cancer. 1969;23(1):230-9. Epub 1969/01/01.
59. Plail RO, Bussey HJ, Glazer G, Thomson JP. Adenomatous polyposis: an association with carcinoma of the thyroid. Br J Surg. 1987;74(5):377-80. Epub 1987/05/01.
60. Ohta S, Katsura T, Shimada M, Shima A, Chishiro H, Matsubara H. Ataxia-telangiectasia with papillary carcinoma of the thyroid. Am J Pediatr Hematol Oncol. 1986;8(3):255-7. Epub 1986/01/01.
61. Dumont JE, Ermans AM, Maenhaut C, Coppee F, Stanbury JB. Large goitre as a maladaptation to iodine deficiency. Clin Endocrinol (Oxf). 1995;43(1):1-10. Epub 1995/07/01.
62. Foley TP, Jr. Goiter in adolescents. Endocrinol Metab Clin North Am. 1993;22(3): 593-606. Epub 1993/09/01.
63. Viacava P, Bocci G, Tonacchera M, Fanelli G, DeServi M, Agretti P, Berti E, Goletti O, Aretini P, Resta ML, Bevilacqua G, Naccarato AG. Markers of cell proliferation, apoptosis, and angiogenesis in thyroid adenomas: a comparative immunohistochemical and genetic investigation of functioning and nonfunctioning nodules. Thyroid. 2007;17(3):191-7. Epub 2007/03/27.
64. Derwahl M, Studer H. Multinodular goitre: 'much more to it than simply iodine deficiency'. Baillieres Best Pract Res Clin Endocrinol Metab. 2000;14(4):577-600. Epub 2001/04/06.
65. Sabel MS, Staren ED. Solitary Thyroid Nodule. In: J.L.Harrison, E.D.Staren, R.A.Prinz, editors. Endocrine Surgery. Texas U.S.A.: Landes Bioscience; 2000. p. 10-7.
66. Tan GH, Gharib H. Thyroid incidentalomas: management approaches to nonpalpable nodules discovered incidentally on thyroid imaging. Ann Intern Med. 1997;126(3): 226-31. Epub 1997/02/01.
67. Gharib H, Goellner JR. Fine-needle aspiration biopsy of thyroid nodules. Endocr Pract. 1995;1(6):410-7. Epub 1995/11/01.
68. Pacini F, Burroni L, Ciuoli C, Di Cairano G, Guarino E. Management of thyroid nodules: a clinicopathological, evidence-based approach. Eur J Nucl Med Mol Imaging. 2004;31(10):1443-9. Epub 2004/09/15.

69. Burguera B, Gharib H. Thyroid incidentalomas. Prevalence, diagnosis, significance, and management. Endocrinol Metab Clin North Am. 2000;29(1):187-203. Epub 2000/03/25.
70. Mazzaferri EL. Management of a solitary thyroid nodule. N Engl J Med. 1993;328(8): 553-9. Epub 1993/02/25.
71. Brander A, Viikinkoski P, Nickels J, Kivisaari L. Thyroid gland: US screening in a random adult population. Radiology. 1991;181(3):683-7. Epub 1991/12/01.
72. Hagag P, Strauss S, Weiss M. Role of ultrasound-guided fine-needle aspiration biopsy in evaluation of nonpalpable thyroid nodules. Thyroid. 1998;8(11):989-95. Epub 1998/12/16.
73. Cusick EL, Krukowski ZH, MacIntosh CA, Matheson NA. Risk of neoplasia and malignancy in "dominant" thyroid swellings. BMJ. 1991;303(6793):20-2. Epub 1991/07/06.
74. Bouhabel S, Payne RJ, Mlynarek A, Hier M, Caglar D, Tamilia M. Are solitary thyroid nodules more likely to be malignant? Journal of otolaryngology - head & neck surgery = Le Journal d'oto-rhino-laryngologie et de chirurgie cervico-faciale. 2012;41(2): 119-23. Epub 2012/05/10.
75. Zohar Y, Strauss M, Laurian N. Adolescent versus adult thyroid carcinoma. Laryngoscope. 1986;96(5):555-9. Epub 1986/05/01.
76. Suliburk J, Delbridge L. Thyroid Nodule. In: Morita SY, Dackiw APB, Zeiger MA, editors. Endocrine Surgery. US: The McGraw-Hill Companies; 2010. p. 2-16.
77. Gharib H, Mazzaferri EL. Thyroxine suppressive therapy in patients with nodular thyroid disease. Ann Intern Med. 1998;128(5):386-94. Epub 1998/03/07.
78. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med. 1994;97(5):418-28. Epub 1994/11/01.
79. Tuttle RM, Lemar H, Burch HB. Clinical features associated with an increased risk of thyroid malignancy in patients with follicular neoplasia by fine-needle aspiration. Thyroid. 1998;8(5):377-83. Epub 1998/06/12.
80. Wang C, Crapo LM. The epidemiology of thyroid disease and implications for screening. Endocrinol Metab Clin North Am. 1997;26(1):189-218. Epub 1997/03/01.
81. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyrotropin (TSH) assays. Clin Chem. 1996;42(1):140-5. Epub 1996/01/01.
82. Boelaert K, Horacek J, Holder RL, Watkinson JC, Sheppard MC, Franklyn JA. Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. J Clin Endocrinol Metab. 2006;91(11):4295-301. Epub 2006/07/27.
83. Zafon C, Obiols G, Baena JA, Castellvi J, Dalama B, Mesa J. Preoperative thyrotropin serum concentrations gradually increase from benign thyroid nodules to papillary

thyroid microcarcinomas then to papillary thyroid cancers of larger size. Journal of thyroid research. 2012;2012:530721. Epub 2011/08/24.

84. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP, Spencer CA, Stockigt JR. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13(1):3-126. Epub 2003/03/11.

85. Hegedus L, Bonnema SJ, Bennedbaek FN. Management of simple nodular goiter: current status and future perspectives. Endocr Rev. 2003;24(1):102-32. Epub 2003/02/18.

86. Carle A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Jorgensen T, Laurberg P. Thyroid volume in hypothyroidism due to autoimmune disease follows a unimodal distribution: evidence against primary thyroid atrophy and autoimmune thyroiditis being distinct diseases. J Clin Endocrinol Metab. 2009;94(3):833-9. Epub 2008/12/18.

87. Date J, Feldt-Rasmussen U, Blichert-Toft M, Hegedus L, Graversen HP. Long-term observation of serum thyroglobulin after resection of nontoxic goiter and relation to ultrasonographically demonstrated relapse. World J Surg. 1996;20(3):351-6; discussion 7. Epub 1996/03/01.

88. Costante G, Meringolo D, Durante C, Bianchi D, Nocera M, Tumino S, Crocetti U, Attard M, Maranghi M, Torlontano M, Filetti S. Predictive value of serum calcitonin levels for preoperative diagnosis of medullary thyroid carcinoma in a cohort of 5817 consecutive patients with thyroid nodules. J Clin Endocrinol Metab. 2007;92(2):450-5. Epub 2006/11/23.

89. Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A, Pacini F. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab. 2004;89(1):163-8. Epub 2004/01/13.

90. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, Cronan JJ, Doubilet PM, Evans DB, Goellner JR, Hay ID, Hertzberg BS, Intenzo CM, Jeffrey RB, Langer JE, Larsen PR, Mandel SJ, Middleton WD, Reading CC, Sherman SI, Tessler FN. Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. Ultrasound quarterly. 2006;22(4):231-8; discussion 9-40. Epub 2006/12/06.

91. Rojeski MT, Gharib H. Nodular thyroid disease. Evaluation and management. N Engl J Med. 1985;313(7):428-36. Epub 1985/08/15.

92. Petrone L, Mannucci E, De Feo ML, Parenti G, Biagini C, Panconesi R, Vezzosi V, Bianchi S, Boddi V, Di Medio L, Pupilli C, Forti G. A simple us score for the identification of candidates to fine needle aspiration of thyroid nodules. J Endocrinol Invest. 2011. Epub 2011/10/07.

93. Lee YH, Kim DW, In HS, Park JS, Kim SH, Eom JW, Kim B, Lee EJ, Rho MH. Differentiation between benign and malignant solid thyroid nodules using an US classification system. Korean J Radiol. 2011;12(5):559-67. Epub 2011/09/20.
94. Kwak JY, Koo H, Youk JH, Kim MJ, Moon HJ, Son EJ, Kim EK. Value of US correlation of a thyroid nodule with initially benign cytologic results. Radiology. 2010;254(1):292-300. Epub 2009/12/19.
95. Walter W, Herman E. Diagnostic Imaging of the Thyroid and Radioiodine Therapy. In: D.Oertli, R.Udelsman, editors. Surgery of the Thyroid and Parathyroid Glands. New York: Springer; 2007. p. 32-44.
96. Kwak JY, Han KH, Yoon JH, Moon HJ, Son EJ, Park SH, Jung HK, Choi JS, Kim BM, Kim EK. Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. Radiology. 2011;260(3):892-9. Epub 2011/07/21.
97. Cappelli C, Castellano M, Pirola I, Cumetti D, Agosti B, Gandossi E, Agabiti Rosei E. The predictive value of ultrasound findings in the management of thyroid nodules. QJM. 2007;100(1):29-35. Epub 2006/12/21.
98. Moon WJ, Jung SL, Lee JH, Na DG, Baek JH, Lee YH, Kim J, Kim HS, Byun JS, Lee DH. Benign and malignant thyroid nodules: US differentiation--multicenter retrospective study. Radiology. 2008;247(3):762-70. Epub 2008/04/12.
99. Kouvaraki MA, Shapiro SE, Fornage BD, Edeiken-Monro BS, Sherman SI, Vassilopoulou-Sellin R, Lee JE, Evans DB. Role of preoperative ultrasonography in the surgical management of patients with thyroid cancer. Surgery. 2003;134(6):946-54; discussion 54-5. Epub 2003/12/12.
100. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, Zoppo A. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid. 2008;18(5):523-31. Epub 2008/05/10.
101. Rubaltelli L, Corradin S, Dorigo A, Stabilito M, Tregnaghi A, Borsato S, Stramare R. Differential diagnosis of benign and malignant thyroid nodules at elastosonography. Ultraschall Med. 2009;30(2):175-9. Epub 2008/05/23.
102. Friedrich-Rust M, Sperber A, Holzer K, Diener J, Grunwald F, Badenhoop K, Weber S, Kriener S, Herrmann E, Bechstein WO, Zeuzem S, Bojunga J. Real-time elastography and contrast-enhanced ultrasound for the assessment of thyroid nodules. Exp Clin Endocrinol Diabetes. 2010;118(9):602-9. Epub 2009/10/27.
103. Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: new developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab. 2007;92(8):2917-22. Epub 2007/05/31.
104. Tranquart F, Bleuzen A, Pierre-Renoult P, Chabrolle C, Sam Giao M, Lecomte P. [Elastosonography of thyroid lesions]. J Radiol. 2008;89(1 Pt 1):35-9. Epub 2008/02/22. Elastographie ultrasonore des lesions thyroidiennes.

105. Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedus L, Vitti P. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association medical guidelines for clinical practice for the diagnosis and management of thyroid nodules. J Endocrinol Invest. 2010;33(5 Suppl):1-50. Epub 2010/11/26.
106. Chu QD, Connor MS, Lilien DL, Johnson LW, Turnage RH, Li BD. Positron emission tomography (PET) positive thyroid incidentaloma: the risk of malignancy observed in a tertiary referral center. Am Surg. 2006;72(3):272-5. Epub 2006/03/24.
107. Schoder H, Yeung HW. Positron emission imaging of head and neck cancer, including thyroid carcinoma. Semin Nucl Med. 2004;34(3):180-97. Epub 2004/06/18.
108. La Rosa GL, Belfiore A, Giuffrida D, Sicurella C, Ippolito O, Russo G, Vigneri R. Evaluation of the fine needle aspiration biopsy in the preoperative selection of cold thyroid nodules. Cancer. 1991;67(8):2137-41. Epub 1991/04/15.
109. Hegedus L. Clinical practice. The thyroid nodule. N Engl J Med. 2004;351(17): 1764-71. Epub 2004/10/22.
110. Randolph GW, Kamani D. The importance of preoperative laryngoscopy in patients undergoing thyroidectomy: voice, vocal cord function, and the preoperative detection of invasive thyroid malignancy. Surgery. 2006;139(3):357-62. Epub 2006/03/21.
111. Christensen SB, Ericsson UB, Janzon L, Tibblin S, Trell E. The prevalence of thyroid disorders in a middle-aged female population, with special reference to the solitary thyroid nodule. Acta Chir Scand. 1984;150(1):13-9. Epub 1984/01/01.
112. Harach HR, Franssila KO, Wasenius VM. Occult papillary carcinoma of the thyroid. A "normal" finding in Finland. A systematic autopsy study. Cancer. 1985;56(3):531-8. Epub 1985/08/01.
113. Mazzaferri EL, de los Santos ET, Rofagha-Keyhani S. Solitary thyroid nodule: diagnosis and management. Med Clin North Am. 1988;72(5):1177-211. Epub 1988/09/01.
114. Pelizzo MR, Piotto A, Rubello D, Casara D, Fassina A, Busnardo B. High prevalence of occult papillary thyroid carcinoma in a surgical series for benign thyroid disease. Tumori. 1990;76(3):255-7. Epub 1990/06/30.
115. Reeve TS, Delbridge L, Sloan D, Crummer P. The impact of fine-needle aspiration biopsy on surgery for single thyroid nodules. Med J Aust. 1986;145(7):308-11. Epub 1986/10/06.
116. Campbell JP, Pillsbury HC, 3rd. Management of the thyroid nodule. Head Neck. 1989;11(5):414-25. Epub 1989/09/01.
117. Wu M, Burstein DE, Yuan S, Nurse LA, Szporn AH, Zhang D, Genden E. A comparative study of 200 fine needle aspiration biopsies performed by clinicians and cytopathologists. Laryngoscope. 2006;116(7):1212-5. Epub 2006/07/11.

118. Wu HH, Jones JN, Osman J. Fine-needle aspiration cytology of the thyroid: ten years experience in a community teaching hospital. Diagn Cytopathol. 2006;34(2):93-6. Epub 2006/03/04.
119. Baloch ZW, LiVolsi VA. Fine-needle aspiration of thyroid nodules: past, present, and future. Endocr Pract. 2004;10(3):234-41. Epub 2004/08/18.
120. Gharib H, Goellner JR. Fine-needle aspiration biopsy of the thyroid: an appraisal. Ann Intern Med. 1993;118(4):282-9. Epub 1993/02/15.
121. Levy EG, Greenlee C, Mandel S, Kaplan M. Should you always trust FNA interpretations? Thyroid. 2000;10(3):279-80. Epub 2000/04/25.
122. Chehade JM, Silverberg AB, Kim J, Case C, Mooradian AD. Role of repeated fine-needle aspiration of thyroid nodules with benign cytologic features. Endocr Pract. 2001;7(4):237-43. Epub 2001/08/11.
123. Dwarakanathan AA, Staren ED, D'Amore MJ, Kluskens LF, Martirano M, Economou SG. Importance of repeat fine-needle biopsy in the management of thyroid nodules. Am J Surg. 1993;166(4):350-2. Epub 1993/10/01.
124. Erdogan MF, Kamel N, Aras D, Akdogan A, Baskal N, Erdogan G. Value of re-aspirations in benign nodular thyroid disease. Thyroid. 1998;8(12):1087-90. Epub 1999/01/27.
125. Liel Y, Ariad S, Barchana M. Long-term follow-up of patients with initially benign thyroid fine-needle aspirations. Thyroid. 2001;11(8):775-8. Epub 2001/08/30.
126. Merchant SH, Izquierdo R, Khurana KK. Is repeated fine-needle aspiration cytology useful in the management of patients with benign nodular thyroid disease? Thyroid. 2000;10(6):489-92. Epub 2000/07/25.
127. Tauro LF, Lobo GJ, Fernandes H, George C, Aithala PS, Shenoy D, Shetty P. A Comparative Study on Fine Needle Aspiration Cytology versus Fine Needle Capillary Cytology in Thyroid Nodules. Oman medical journal. 2012;27(2):151-6. Epub 2012/04/13.
128. Burch HB. Evaluation and management of the solid thyroid nodule. Endocrinol Metab Clin North Am. 1995;24(4):663-710. Epub 1995/12/01.
129. McHenry CR, Walfish PG, Rosen IB. Non-diagnostic fine needle aspiration biopsy: a dilemma in management of nodular thyroid disease. Am Surg. 1993;59(7):415-9. Epub 1993/07/01.
130. Belfiore A, La Rosa GL. Fine-needle aspiration biopsy of the thyroid. Endocrinol Metab Clin North Am. 2001;30(2):361-400. Epub 2001/07/11.
131. Kini SR. Specimen adequacy and assessment, reporting system. Thyroid Cytopathology: An Atlas and Text. Philadelphia: Lippincott Williams & Wilkins; 2008. p. 17-26.

132. Chandan VS, Faquin WC, Wilbur DC, Khurana KK. The role of immunolocalization of CD57 and GLUT-1 in cell blocks in fine-needle aspiration diagnosis of papillary thyroid carcinoma. Cancer. 2006;108(5):331-6. Epub 2006/09/01.
133. Watkinson JC, British Thyroid A. The British Thyroid Association guidelines for the management of thyroid cancer in adults. Nucl Med Commun. 2004;25(9):897-900. Epub 2004/08/21.
134. Redman R, Zalaznick H, Mazzaferri EL, Massoll NA. The impact of assessing specimen adequacy and number of needle passes for fine-needle aspiration biopsy of thyroid nodules. Thyroid. 2006;16(1):55-60. Epub 2006/02/21.
135. Hamburger JI. Diagnosis of thyroid nodules by fine needle biopsy: use and abuse. J Clin Endocrinol Metab. 1994;79(2):335-9. Epub 1994/08/01.
136. Gharib H, Goellner JR, Zinsmeister AR, Grant CS, Van Heerden JA. Fine-needle aspiration biopsy of the thyroid. The problem of suspicious cytologic findings. Ann Intern Med. 1984;101(1):25-8. Epub 1984/07/01.
137. Schlinkert RT, van Heerden JA, Goellner JR, Gharib H, Smith SL, Rosales RF, Weaver AL. Factors that predict malignant thyroid lesions when fine-needle aspiration is "suspicious for follicular neoplasm". Mayo Clin Proc. 1997;72(10):913-6. Epub 1997/11/14.
138. Pisani T, Bononi M, Nagar C, Angelini M, Bezzi M, Vecchione A. Fine needle aspiration and core needle biopsy techniques in the diagnosis of nodular thyroid pathologies. Anticancer Res. 2000;20(5C):3843-7. Epub 2001/03/28.
139. Renshaw AA, Pinnar N. Comparison of thyroid fine-needle aspiration and core needle biopsy. Am J Clin Pathol. 2007;128(3):370-4. Epub 2007/08/22.
140. Baloch ZW, LiVolsi VA, Asa SL, Rosai J, Merino MJ, Randolph G, Vielh P, DeMay RM, Sidawy MK, Frable WJ. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagn Cytopathol. 2008;36(6):425-37. Epub 2008/05/15.
141. Zhang S, Ivanovic M, Nemcek AA, Jr., Defrias DV, Lucas E, Nayar R. Thin core needle biopsy crush preparations in conjunction with fine-needle aspiration for the evaluation of thyroid nodules: a complementary approach. Cancer. 2008;114(6):512-8. Epub 2008/11/07.
142. Ibanez ML, Russell WO, Chang JP, Speece AJ. Cold chamber frozen sections for operating room diagnosis and routine surgical pathology. Lab Invest. 1960;9:98-109. Epub 1960/01/01.
143. Leteurtre E, Leroy X, Pattou F, Wacrenier A, Carnaille B, Proye C, Lecomte-Houcke M. Why do frozen sections have limited value in encapsulated or minimally invasive follicular carcinoma of the thyroid? Am J Clin Pathol. 2001;115(3):370-4. Epub 2001/03/13.

144. Chen H, Nicol TL, Udelsman R. Follicular lesions of the thyroid. Does frozen section evaluation alter operative management? Ann Surg. 1995;222(1):101-6. Epub 1995/07/01.
145. Paphavasit A, Thompson GB, Hay ID, Grant CS, van Heerden JA, Ilstrup DM, Schleck C, Goellner JR. Follicular and Hurthle cell thyroid neoplasms. Is frozen-section evaluation worthwhile? Arch Surg. 1997;132(6):674-8; discussion 8-80. Epub 1997/06/01.
146. Kingston GW, Bugis SP, Davis N. Role of frozen section and clinical parameters in distinguishing benign from malignant follicular neoplasms of the thyroid. Am J Surg. 1992;164(6):603-5. Epub 1992/12/01.
147. Key M. Immunohistochemistry Staining Methods. In: Kulmar GL, Rudbeck L, editors. Education Guide | Immunohistochemical (IHC) Staining Methods Updated and Expanded Fifth Edition. Carpinteria, California: Dako North America; 2009. p. 57-60.
148. Nakane PK. Simultaneous localization of multiple tissue antigens using the peroxidase-labeled antibody method: a study on pituitary glands of the rat. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 1968;16(9):557-60. Epub 1968/09/01.
149. Mason TE, Phifer RF, Spicer SS, Swallow RA, Dreskin RB. An immunoglobulin-enzyme bridge method for localizing tissue antigens. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 1969;17(9):563-9. Epub 1969/09/01.
150. Sternberger LA, Hardy PH, Jr., Cuculis JJ, Meyer HG. The unlabeled antibody enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 1970;18(5):315-33. Epub 1970/05/01.
151. Sheibani K, Esteban JM, Bailey A, Battifora H, Weiss LM. Immunopathologic and molecular studies as an aid to the diagnosis of malignant mesothelioma. Hum Pathol. 1992;23(2):107-16. Epub 1992/02/01.
152. Saleh HA, Jin B, Barnwell J, Alzohaili O. Utility of immunohistochemical markers in differentiating benign from malignant follicular-derived thyroid nodules. Diagn Pathol. 2010;5:9. Epub 2010/02/26.
153. Nasr MR, Mukhopadhyay S, Zhang S, Katzenstein AL. Immunohistochemical markers in diagnosis of papillary thyroid carcinoma: Utility of HBME1 combined with CK19 immunostaining. Mod Pathol. 2006;19(12):1631-7. Epub 2006/09/26.
154. Sack MJ, Astengo-Osuna C, Lin BT, Battifora H, LiVolsi VA. HBME-1 immunostaining in thyroid fine-needle aspirations: a useful marker in the diagnosis of carcinoma. Mod Pathol. 1997;10(7):668-74. Epub 1997/07/01.

155. Miettinen M, Karkkainen P. Differential reactivity of HBME-1 and CD15 antibodies in benign and malignant thyroid tumours. Preferential reactivity with malignant tumours. Virchows Arch. 1996;429(4-5):213-9. Epub 1996/11/01.
156. Wick MR. Immunophenotyping of malignant mesothelioma. Am J Surg Pathol. 1997;21(12):1395-8. Epub 1997/12/31.
157. Riera JR, Astengo-Osuna C, Longmate JA, Battifora H. The immunohistochemical diagnostic panel for epithelial mesothelioma: a reevaluation after heat-induced epitope retrieval. Am J Surg Pathol. 1997;21(12):1409-19. Epub 1997/12/31.
158. Schmitt AC, Cohen C, Siddiqui MT. Paired box gene 8, HBME-1, and cytokeratin 19 expression in preoperative fine-needle aspiration of papillary thyroid carcinoma: diagnostic utility. Cancer Cytopathol. 2010;118(4):196-202. Epub 2010/08/24.
159. de Micco C, Savchenko V, Giorgi R, Sebag F, Henry JF. Utility of malignancy markers in fine-needle aspiration cytology of thyroid nodules: comparison of Hector Battifora mesothelial antigen-1, thyroid peroxidase and dipeptidyl aminopeptidase IV. Br J Cancer. 2008;98(4):818-23. Epub 2008/01/24.
160. Lanier LL, Testi R, Bindl J, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. J Exp Med. 1989;169(6): 2233-8. Epub 1989/06/01.
161. El Demellawy D, Nasr AL, Babay S, Alowami S. Diagnostic utility of CD56 immunohistochemistry in papillary carcinoma of the thyroid. Pathol Res Pract. 2009;205(5):303-9. Epub 2009/01/21.
162. Griffin JD, Hercend T, Beveridge R, Schlossman SF. Characterization of an antigen expressed by human natural killer cells. J Immunol. 1983;130(6):2947-51. Epub 1983/06/01.
163. Hercend T, Griffin JD, Bensussan A, Schmidt RE, Edson MA, Brennan A, Murray C, Daley JF, Schlossman SF, Ritz J. Generation of monoclonal antibodies to a human natural killer clone. Characterization of two natural killer-associated antigens, NKH1A and NKH2, expressed on subsets of large granular lymphocytes. J Clin Invest. 1985;75(3):932-43. Epub 1985/03/01.
164. Zeromski J, Bagnasco M, Paolieri F, Dworacki G. Expression of CD56 (NKH-1) differentiation antigen in human thyroid epithelium. Clin Exp Immunol. 1992;89(3): 474-8. Epub 1992/09/01.
165. El Demellawy D, Nasr A, Alowami S. Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of the thyroid. Diagn Pathol. 2008;3:5. Epub 2008/02/08.
166. Hirohashi S, Kanai Y. Cell adhesion system and human cancer morphogenesis. Cancer Sci. 2003;94(7):575-81. Epub 2003/07/05.

167. Brabant G, Hoang-Vu C, Cetin Y, Dralle H, Scheumann G, Molne J, Hansson G, Jansson S, Ericson LE, Nilsson M. E-cadherin: a differentiation marker in thyroid malignancies. Cancer Res. 1993;53(20):4987-93. Epub 1993/10/15.
168. Vleminckx K, Vakaet L, Jr., Mareel M, Fiers W, van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell. 1991;66(1):107-19. Epub 1991/07/12.
169. Mitselou A, Ioachim E, Peschos D, Charalabopoulos K, Michael M, Agnantis NJ, Vougiouklakis T. E-cadherin adhesion molecule and syndecan-1 expression in various thyroid pathologies. Exp Oncol. 2007;29(1):54-60. Epub 2007/04/14.
170. Eidelman S, Damsky CH, Wheelock MJ, Damjanov I. Expression of the cell-cell adhesion glycoprotein cell-CAM 120/80 in normal human tissues and tumors. Am J Pathol. 1989;135(1):101-10. Epub 1989/07/01.
171. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science. 1991;251(5000):1451-5. Epub 1991/03/22.
172. Soares P, Berx G, van Roy F, Sobrinho-Simoes M. E-cadherin gene alterations are rare events in thyroid tumors. Int J Cancer. 1997;70(1):32-8. Epub 1997/01/06.
173. von Wasielewski R, Rhein A, Werner M, Scheumann GF, Dralle H, Potter E, Brabant G, Georgii A. Immunohistochemical detection of E-cadherin in differentiated thyroid carcinomas correlates with clinical outcome. Cancer Res. 1997;57(12):2501-7. Epub 1997/06/15.
174. Graff JR, Greenberg VE, Herman JG, Westra WH, Boghaert ER, Ain KB, Saji M, Zeiger MA, Zimmer SG, Baylin SB. Distinct patterns of E-cadherin CpG island methylation in papillary, follicular, Hurthle's cell, and poorly differentiated human thyroid carcinoma. Cancer Res. 1998;58(10):2063-6. Epub 1998/05/30.
175. Choi YL, Kim MK, Suh JW, Han J, Kim JH, Yang JH, Nam SJ. Immunoexpression of HBME-1, high molecular weight cytokeratin, cytokeratin 19, thyroid transcription factor-1, and E-cadherin in thyroid carcinomas. J Korean Med Sci. 2005;20(5):853-9. Epub 2005/10/15.
176. Ristimaki A, Honkanen N, Jankala H, Sipponen P, Harkonen M. Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res. 1997;57(7):1276-80. Epub 1997/04/01.
177. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107(4):1183-8. Epub 1994/10/01.
178. Tucker ON, Dannenberg AJ, Yang EK, Zhang F, Teng L, Daly JM, Soslow RA, Masferrer JL, Woerner BM, Koki AT, Fahey TJ, 3rd. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. Cancer Res. 1999;59(5):987-90. Epub 1999/03/10.

179. Yoshimura R, Sano H, Masuda C, Kawamura M, Tsubouchi Y, Chargui J, Yoshimura N, Hla T, Wada S. Expression of cyclooxygenase-2 in prostate carcinoma. Cancer. 2000;89(3):589-96. Epub 2000/08/10.
180. Specht MC, Tucker ON, Hocever M, Gonzalez D, Teng L, Fahey TJ, 3rd. Cyclooxygenase-2 expression in thyroid nodules. The Journal of clinical endocrinology and metabolism. 2002;87(1):358-63. Epub 2002/01/15.
181. Kim SJ, Lee JH, Yoon JS, Mok JO, Kim YJ, Park HK, Kim CH, Byun DW, Suh KI, Yoo MH. Immunohistochemical expression of COX-2 in thyroid nodules. The Korean journal of internal medicine. 2003;18(4):225-9. Epub 2004/01/14.
182. Haynik DM, Prayson RA. Immunohistochemical expression of cyclooxygenase 2 in follicular carcinomas of the thyroid. Arch Pathol Lab Med. 2005;129(6):736-41. Epub 2005/05/26.
183. Rigaud C, Bogomoletz WV. Apparent lack of usefulness of monoclonal antibody Ki-67 in thyroid tumour pathology. Relation to histological typing and classification. Pathol Res Pract. 1991;187(2-3):198-200. Epub 1991/03/01.
184. Mehrotra P, Gonzalez MA, Johnson SJ, Coleman N, Wilson JA, Davies BR, Lennard TW. Mcm-2 and Ki-67 have limited potential in preoperative diagnosis of thyroid malignancy. Laryngoscope. 2006;116(8):1434-8. Epub 2006/08/04.
185. Ito Y, Miyauchi A, Kakudo K, Hirokawa M, Kobayashi K, Miya A. Prognostic significance of ki-67 labeling index in papillary thyroid carcinoma. World J Surg. 2010;34(12):3015-21. Epub 2010/08/13.
186. Pujani M, Arora B, Pujani M, Singh SK, Tejwani N. Role of Ki-67 as a proliferative marker in lesions of thyroid. Indian journal of cancer. 2010;47(3):304-7. Epub 2010/07/01.
187. Liang HS, Zhong YH, Luo ZJ, Huang Y, Lin HD, Luo M, Su HX, Zhou SB, Xie KQ. Comparative analysis of protein expression in differentiated thyroid tumours: a multicentre study. The Journal of international medical research. 2009;37(3):927-38. Epub 2009/07/11.
188. Martinez J, Georgoff I, Martinez J, Levine AJ. Cellular localization and cell cycle regulation by a temperature-sensitive p53 protein. Genes & development. 1991;5(2): 151-9. Epub 1991/02/01.
189. Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. Cell. 1989;57(7):1083-93. Epub 1989/06/30.
190. Tan A, Etit D, Bayol U, Altinel D, Tan S. Comparison of proliferating cell nuclear antigen, thyroid transcription factor-1, Ki-67, p63, p53 and high-molecular weight cytokeratin expressions in papillary thyroid carcinoma, follicular carcinoma, and follicular adenoma. Annals of diagnostic pathology. 2011;15(2):108-16. Epub 2011/02/15.

191. Dobashi Y, Sakamoto A, Sugimura H, Mernyei M, Mori M, Oyama T, Machinami R. Overexpression of p53 as a possible prognostic factor in human thyroid carcinoma. Am J Surg Pathol. 1993;17(4):375-81. Epub 1993/04/01.
192. Morita N, Ikeda Y, Takami H. Clinical significance of p53 protein expression in papillary thyroid carcinoma. World journal of surgery. 2008;32(12):2617-22. Epub 2008/10/07.
193. Donghi R, Longoni A, Pilotti S, Michieli P, Della Porta G, Pierotti MA. Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. J Clin Invest. 1993;91(4):1753-60. Epub 1993/04/01.
194. Nikiforov YE. Genetic alterations involved in the transition from well-differentiated to poorly differentiated and anaplastic thyroid carcinomas. Endocr Pathol. 2004;15(4): 319-27. Epub 2005/02/01.
195. Kwak JY, Kim EK, Kim HJ, Kim MJ, Son EJ, Moon HJ. How to combine ultrasound and cytological information in decision making about thyroid nodules. Eur Radiol. 2009;19(8):1923-31. Epub 2009/03/12.
196. Orlandi A, Puscar A, Capriata E, Fideleff H. Repeated fine-needle aspiration of the thyroid in benign nodular thyroid disease: critical evaluation of long-term follow-up. Thyroid. 2005;15(3):274-8. Epub 2005/03/24.
197. Ylagan LR, Farkas T, Dehner LP. Fine needle aspiration of the thyroid: a cytohistologic correlation and study of discrepant cases. Thyroid. 2004;14(1):35-41. Epub 2004/03/11.
198. Carmeci C, Jeffrey RB, McDougall IR, Nowels KW, Weigel RJ. Ultrasound-guided fine-needle aspiration biopsy of thyroid masses. Thyroid. 1998;8(4):283-9. Epub 1998/05/20.
199. Brauer VF, Eder P, Miehle K, Wiesner TD, Hasenclever H, Paschke R. Interobserver variation for ultrasound determination of thyroid nodule volumes. Thyroid. 2005;15(10):1169-75. Epub 2005/11/11.
200. Zelmanovitz F, Genro S, Gross JL. Suppressive therapy with levothyroxine for solitary thyroid nodules: a double-blind controlled clinical study and cumulative meta-analyses. J Clin Endocrinol Metab. 1998;83(11):3881-5. Epub 1998/11/14.
201. Marqusee E, Benson CB, Frates MC, Doubilet PM, Larsen PR, Cibas ES, Mandel SJ. Usefulness of ultrasonography in the management of nodular thyroid disease. Ann Intern Med. 2000;133(9):696-700. Epub 2000/11/14.
202. Layfield LJ, Cibas ES, Gharib H, Mandel SJ. Thyroid aspiration cytology: current status. CA Cancer J Clin. 2009;59(2):99-110. Epub 2009/03/13.
203. Rago T, Di Coscio G, Basolo F, Scutari M, Elisei R, Berti P, Miccoli P, Romani R, Faviana P, Pinchera A, Vitti P. Combined clinical, thyroid ultrasound and cytological features help to predict thyroid malignancy in follicular and Hupsilonrthle cell thyroid lesions: results from a series of 505 consecutive patients. Clin Endocrinol (Oxf). 2007;66(1):13-20. Epub 2007/01/05.

204. Baloch ZW, Sack MJ, Yu GH, Livolsi VA, Gupta PK. Fine-needle aspiration of thyroid: an institutional experience. Thyroid. 1998;8(7):565-9. Epub 1998/08/26.
205. Fadda G, Basolo F, Bondi A, Bussolati G, Crescenzi A, Nappi O, Nardi F, Papotti M, Taddei G, Palombini L, Group S-IICW. Cytological classification of thyroid nodules. Proposal of the SIAPEC-IAP Italian Consensus Working Group. Pathologica. 2010;102(5):405-8. Epub 2011/03/03.
206. Haymart MR, Greenblatt DY, Elson DF, Chen H. The role of intraoperative frozen section if suspicious for papillary thyroid cancer. Thyroid. 2008;18(4):419-23. Epub 2008/03/21.
207. Bancroft JD, Gamble M. Theory and practice of histological techniques, international edition.: Churchill Livingstone, Edinburgh; 2003.
208. DeLellis RA. Pathology and genetics of thyroid carcinoma. J Surg Oncol. 2006;94(8): 662-9. Epub 2006/11/30.
209. Chan JK. Papillary carcinoma of thyroid: classical and variants. Histol Histopathol. 1990;5(2):241-57. Epub 1990/04/01.
210. Zidan J, Karen D, Stein M, Rosenblatt E, Basher W, Kuten A. Pure versus follicular variant of papillary thyroid carcinoma: clinical features, prognostic factors, treatment, and survival. Cancer. 2003;97(5):1181-5. Epub 2003/02/25.
211. LiVolsi VA, Asa SL. The demise of follicular carcinoma of the thyroid gland. Thyroid. 1994;4(2):233-6. Epub 1994/01/01.
212. DeMay RM. Follicular lesions of the thyroid. W(h)ither follicular carcinoma? Am J Clin Pathol. 2000;114(5):681-3. Epub 2000/11/09.
213. Wittekind C. [2010 TNM system: on the 7th edition of TNM classification of malignant tumors]. Der Pathologe. 2010;31(5):331-2. Epub 2010/08/13. TNM-System 2010 : Zur 7. Auflage der TNM-Klassifikation maligner Tumoren.
214. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, Cronan JJ, Doubilet PM, Evans DB, Goellner JR, Hay ID, Hertzberg BS, Intenzo CM, Jeffrey RB, Langer JE, Larsen PR, Mandel SJ, Middleton WD, Reading CC, Sherman SI, Tessler FN. Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. Radiology. 2005;237(3):794-800. Epub 2005/11/24.
215. Alexander EK, Hurwitz S, Heering JP, Benson CB, Frates MC, Doubilet PM, Cibas ES, Larsen PR, Marqusee E. Natural history of benign solid and cystic thyroid nodules. Ann Intern Med. 2003;138(4):315-8. Epub 2003/02/15.
216. Grant CS, Hay ID, Gough IR, McCarthy PM, Goellner JR. Long-term follow-up of patients with benign thyroid fine-needle aspiration cytologic diagnoses. Surgery. 1989;106(6):980-5; discussion 5-6. Epub 1989/12/01.
217. Altman D, David M, Trevor B, Martin G. Statistics with Confidence - Confidence Intervals and Statistical Guidelines. Bristol: British Medical Journal; 2000.

218. Altman DG, Bland JM. Diagnostic tests. 1: Sensitivity and specificity. BMJ. 1994;308(6943):1552. Epub 1994/06/11.
219. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ. 1994;309(6947): 102. Epub 1994/07/09.
220. CASES OF MALIGNANT NEOPLASMS BY SELECTED SITES [database on the Internet]. 2011.
221. Prieditis P. Personal communication. First FNA of the Thyroid in Latvia. 20.03.2012.
222. Prieditis P, Saulite J. Personal communication. Number of Thyroid FNA Performed per Year in Latvia. 20.03.2012.
223. Kesmodel SB, Terhune KP, Canter RJ, Mandel SJ, LiVolsi VA, Baloch ZW, Fraker DL. The diagnostic dilemma of follicular variant of papillary thyroid carcinoma. Surgery. 2003;134(6):1005-12; discussion 12. Epub 2003/12/12.
224. Shih SR, Shun CT, Su DH, Hsiao YL, Chang TC. Follicular variant of papillary thyroid carcinoma: diagnostic limitations of fine needle aspiration cytology. Acta Cytol. 2005;49(4):383-6. Epub 2005/08/30.
225. Wu HH, Jones JN, Grzybicki DM, Elsheikh TM. Sensitive cytologic criteria for the identification of follicular variant of papillary thyroid carcinoma in fine-needle aspiration biopsy. Diagn Cytopathol. 2003;29(5):262-6. Epub 2003/11/05.
226. Zhang Y, Fraser JL, Wang HH. Morphologic predictors of papillary carcinoma on fine-needle aspiration of thyroid with ThinPrep preparations. Diagn Cytopathol. 2001;24(6): 378-83. Epub 2001/06/08.
227. Chow LS, Gharib H, Goellner JR, van Heerden JA. Nondiagnostic thyroid fine-needle aspiration cytology: management dilemmas. Thyroid. 2001;11(12):1147-51. Epub 2002/08/21.
228. Filie AC, Asa SL, Geisinger KR, Logani S, Merino M, Nikiforov YE, Clark DP. Utilization of ancillary studies in thyroid fine needle aspirates: a synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. Diagn Cytopathol. 2008;36(6):438-41. Epub 2008/05/15.
229. Mase T, Funahashi H, Koshikawa T, Imai T, Nara Y, Tanaka Y, Nakao A. HBME-1 immunostaining in thyroid tumors especially in follicular neoplasm. Endocr J. 2003;50(2):173-7. Epub 2003/06/14.
230. Saggiorato E, De Pompa R, Volante M, Cappia S, Arecco F, Dei Tos AP, Orlandi F, Papotti M. Characterization of thyroid 'follicular neoplasms' in fine-needle aspiration cytological specimens using a panel of immunohistochemical markers: a proposal for clinical application. Endocr Relat Cancer. 2005;12(2):305-17. Epub 2005/06/11.
231. Fadda G, Rossi ED, Mule A, Miraglia A, Vecchio FM, Capelli A. Diagnostic efficacy of immunocytochemistry on fine needle aspiration biopsies processed by thin-layer cytology. Acta Cytol. 2006;50(2):129-35. Epub 2006/04/14.

232. Asa SL. The role of immunohistochemical markers in the diagnosis of follicular-patterned lesions of the thyroid. Endocr Pathol. 2005;16(4):295-309. Epub 2006/04/22.
233. Leung SW, Bedard YC. Immunocytochemical staining on ThinPrep processed smears. Mod Pathol. 1996;9(3):304-6. Epub 1996/03/01.
234. Rossi ED, Raffaelli M, Minimo C, Mule A, Lombardi CP, Vecchio FM, Fadda G. Immunocytochemical evaluation of thyroid neoplasms on thin-layer smears from fine-needle aspiration biopsies. Cancer. 2005;105(2):87-95. Epub 2005/03/03.
235. Kini SR, Miller JM, Hamburger JI, Smith MJ. Cytopathology of papillary carcinoma of the thyroid by fine needle aspiration. Acta Cytol. 1980;24(6):511-21. Epub 1980/11/01.
236. Kaur A, Jayaram G. Thyroid tumors: cytomorphology of papillary carcinoma. Diagn Cytopathol. 1991;7(5):462-8. Epub 1991/01/01.
237. Baloch ZW, Gupta PK, Yu GH, Sack MJ, LiVolsi VA. Follicular variant of papillary carcinoma. Cytologic and histologic correlation. Am J Clin Pathol. 1999;111(2):216-22. Epub 1999/02/04.
238. Papotti M, Rodriguez J, De Pompa R, Bartolazzi A, Rosai J. Galectin-3 and HBME-1 expression in well-differentiated thyroid tumors with follicular architecture of uncertain malignant potential. Mod Pathol. 2005;18(4):541-6. Epub 2004/11/06.
239. Prasad ML, Pellegata NS, Huang Y, Nagaraja HN, de la Chapelle A, Kloos RT. Galectin-3, fibronectin-1, CITED-1, HBME1 and cytokeratin-19 immunohistochemistry is useful for the differential diagnosis of thyroid tumors. Mod Pathol. 2005;18(1):48-57. Epub 2004/07/24.
240. Saleh HA, Feng J, Tabassum F, Al-Zohaili O, Husain M, Giorgadze T. Differential expression of galectin-3, CK19, HBME1, and Ret oncoprotein in the diagnosis of thyroid neoplasms by fine needle aspiration biopsy. Cytojournal. 2009;6:18. Epub 2009/10/15.
241. Nga ME, Lim GS, Soh CH, Kumarasinghe MP. HBME-1 and CK19 are highly discriminatory in the cytological diagnosis of papillary thyroid carcinoma. Diagn Cytopathol. 2008;36(8):550-6. Epub 2008/07/12.
242. Scognamiglio T, Hyjek E, Kao J, Chen YT. Diagnostic usefulness of HBME1, galectin-3, CK19, and CITED1 and evaluation of their expression in encapsulated lesions with questionable features of papillary thyroid carcinoma. Am J Clin Pathol. 2006;126(5):700-8. Epub 2006/10/20.
243. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL. Immunohistochemical diagnosis of papillary thyroid carcinoma. Mod Pathol. 2001;14(4):338-42. Epub 2001/04/13.
244. Pazaitou-Panayiotou K, Mygdakos N, Boglou K, Kiziridou A, Chrisoulidou A, Destouni C. The Immunocytochemistry Is a Valuable Tool in the Diagnosis of Papillary Thyroid Cancer in FNA's Using Liquid-Based Cytology. Journal of oncology. 2010;2010:963926. Epub 2010/11/06.

245. Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N, Smit JW. Combined immunostaining with galectin-3, fibronectin-1, CITED-1, Hector Battifora mesothelial-1, cytokeratin-19, peroxisome proliferator-activated receptor-{gamma}, and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. European journal of endocrinology / European Federation of Endocrine Societies. 2008;158(3):375-84. Epub 2008/02/27.

246. Wiseman SM, Melck A, Masoudi H, Ghaidi F, Goldstein L, Gown A, Jones SJ, Griffith OL. Molecular phenotyping of thyroid tumors identifies a marker panel for differentiated thyroid cancer diagnosis. Ann Surg Oncol. 2008;15(10):2811-26. Epub 2008/07/10.

247. Torregrossa L, Faviana P, Camacci T, Materazzi G, Berti P, Minuto M, Elisei R, Vitti P, Miccoli P, Basolo F. Galectin-3 is highly expressed in nonencapsulated papillary thyroid carcinoma but weakly expressed in encapsulated type; comparison with Hector Battifora mesothelial cell 1 immunoreactivity. Hum Pathol. 2007;38(10):1482-8. Epub 2007/06/29.

248. Dahlman T, Grimelius L, Wallin G, Rubin K, Westermark K. Integrins in thyroid tissue: upregulation of alpha2beta1 in anaplastic thyroid carcinoma. European journal of endocrinology / European Federation of Endocrine Societies. 1998;138(1):104-12. Epub 1998/02/14.

249. Smyth P, Sheils O, Finn S, Martin C, O'Leary J, Sweeney EC. Real-time quantitative analysis of E-cadherin expression in ret/PTC-1-activated thyroid neoplasms. Int J Surg Pathol. 2001;9(4):265-72. Epub 2003/02/08.

250. Batistatou A, Charalabopoulos K, Nakanishi Y, Vagianos C, Hirohashi S, Agnantis NJ, Scopa CD. Differential expression of dysadherin in papillary thyroid carcinoma and microcarcinoma: correlation with E-cadherin. Endocr Pathol. 2008;19(3):197-202. Epub 2008/08/05.

251. Erdem H, Gundogdu C, Sipal S. Correlation of E-cadherin, VEGF, COX-2 expression to prognostic parameters in papillary thyroid carcinoma. Experimental and molecular pathology. 2011;90(3):312-7. Epub 2011/02/22.

252. Migita K, Eguchi K, Kawakami A, Ida H, Fukuda T, Kurata A, Ishikawa N, Ito K, Nagataki S. Detection of Leu-19 (CD56) antigen on human thyroid epithelial cells by an immunohistochemical method. Immunology. 1991;72(2):246-9. Epub 1991/02/01.

253. Kim JH, Kim YH, Han JH, Lee KB, Sheen SS, Lee J, Soh EY, Park TJ. Silencing of homeobox B9 is associated with down-regulation of CD56 and extrathyroidal extension of tumor in papillary thyroid carcinoma. Hum Pathol. 2012. Epub 2012/01/10.

254. Strumfa I. Ciklooksigenāzes-2 Proteīna Ekspresija Barības Vada un Kuņģa Audzējos: Riga Stradins University; 2005.

255. Casey MB, Zhang S, Jin L, Kajita S, Lloyd RV. Expression of cyclooxygenase-2 and thromboxane synthase in non-neoplastic and neoplastic thyroid lesions. Endocr Pathol. 2004;15(2):107-16. Epub 2004/08/10.

256. Fuhrer D, Eszlinger M, Karger S, Krause K, Engelhardt C, Hasenclever D, Dralle H, Paschke R. Evaluation of insulin-like growth factor II, cyclooxygenase-2, ets-1 and thyroid-specific thyroglobulin mRNA expression in benign and malignant thyroid tumours. European journal of endocrinology / European Federation of Endocrine Societies. 2005;152(5):785-90. Epub 2005/05/10.

257. Garcia-Gonzalez M, Abdulkader I, Boquete AV, Neo XM, Forteza J, Cameselle-Teijeiro J. Cyclooxygenase-2 in normal, hyperplastic and neoplastic follicular cells of the human thyroid gland. Virchows Arch. 2005;447(1):12-7. Epub 2005/06/11.

258. Krawczyk-Rusiecka K, Wojciechowska-Durczynska K, Cyniak-Magierska A, Adamczewski Z, Galecka E, Lewinski A. COX-2 expression in papillary thyroid carcinoma (PTC) in cytological material obtained by fine needle aspiration biopsy (FNAB). Thyroid Res. 2011;4(1):3. Epub 2011/01/11.