

## REVIEW

## MOLECULAR MARKERS FOR WELL-DIFFERENTIATED THYROID CANCER

*S.M. Cherenko, M.B. Gorobeyko\*, V.G. Savchenko*

*Ukrainian Research and Practical Centre of Endocrine Surgery,  
Endocrine Organ and Tissue Transplantation, Kyiv 02175, Ukraine*

## МОЛЕКУЛЯРНЫЕ МАРКЕРЫ ВЫСОКОДИФФЕРЕНЦИРОВАННОГО РАКА ЩИТОВИДНОЙ ЖЕЛЕЗЫ

*С.М. Черенько, М.Б. Горобейко\*, В.Г. Савченко*

*Украинский научно-практический центр эндокринной хирургии,  
трансплантации эндокринных органов и тканей, Киев 02175, Украина*

The determination of serum thyroglobulin (sTg) is considered as the most effective marker for post-operative follow-up in well differentiated thyroid cancer (DTC) and must be carried out together with whole-body radioiodine scanning and detection of antibodies to Tg. The detection of serum Tg mRNA has the most valued prognostic effect. Increase of the sTg level in pre-operative period could testify the occurrence of follicular cancer or Hurtle-cell cancer. The detection of cytokeratin 20 and 19 mRNA is known to have good predictable value also. The detection of Tg and thyrotrophic hormone receptors by PCR has nearly 100% diagnostic effectiveness. The dynamic detection of calcitonin is known to serve as the most significant method for post-operative follow-up of medullary thyroid cancer (the detection of Ret mutation, CEA, chromogranin A serves as additional methods). The Ret/PTC expression in DTC tissues indicates the presence and the level of neoplastic transformation. The use of MoAb 47 and DAP in FNAB improves the results of diagnosis. The determination of E-cadherin/catenin adhesion complex, CK-19 and CK-20, galectin-3 and S-100 protein is effective for diagnosis of diffuse sclerosing variant. Low level of nm23-H1 expression and absence of p53 mutations are proving the low risk of DTC recurrence and of the development of metastases.

**Key Words:** Thyroid cancer, tumor markers, diagnosis, follow-up, FNAB.

Тироглобулин (ТГ) является наиболее эффективным маркером мониторинга послеоперационных больных с дифференцированным раком щитовидной железы (ДРЩЖ). Его определение необходимо проводить одновременно со сканированием изотопом йода и выявлением антител к ТГ. Большую прогностическую ценность имеет определение мРНК ТГ в циркулирующей крови. Повышение уровня сывороточного ТГ на дооперационном этапе может свидетельствовать о наличии фолликулярного рака или рака из клеток Хюртля. Эффективным является также определение мРНК цитокератинов 20 и 19 в периферической крови. Определение рецепторов ТГ и тиреотропного гормона в циркулирующей крови методом полимеразной цепной реакции имеет высокую диагностическую эффективность. Достоверным маркером медуллярного рака щитовидной железы является динамическое определение уровня кальцитонина. Выявление мутаций RET-онкогена, CEA, хромографина А можно рассматривать в качестве дополнительных критериев диагностики. Экспрессия RET/PTC в тканях ДРЩЖ свидетельствует о наличии злокачественного процесса и степени его агрессивности. При цитологическом изучении тонкоигольных биоптатов целесообразно использование МКАТ-47 для выявления ДАП, определение цитокератинов 19 и 20 типа. В диагностике диффузно-склерозирующего варианта ПРЩЖ важную роль играет определение Е-кадхеринового комплекса, галектина-3 и белка S-100. Сниженный уровень nm23-H1 и отсутствие мутации p53 свидетельствуют о низком риске возникновения рецидива или развития метастазов.

**Ключевые слова:** рак щитовидной железы, онкомаркеры, диагностика, мониторинг, FNAB.

The determination of tumor-specific markers in peripheral blood or in the tissue of thyroid gland (TGI) belongs to the new quickly developing field of modern endocrine oncology. The research in this field opens new ways for early diagnosis of well-differentiated thyroid cancer (DTC), elaboration of screening systems, timely

detection of metastasis and disease recurrence [1]. Tumor markers may be determined in tumor tissue during the histological study or in cytological examination of the punctate after fine-needle aspiration biopsy (FNAB) and in peripheral blood. For convenience oncomarkers may be divided on serum and tissue (i.e. genetic) markers.

**Serum markers.** The estimation of the serum thyroglobulin (sTg) level is the most significant marker in post-operative monitoring of the patients with DTC [2]; the effectiveness of the assay is higher if it is applied simultaneously with [<sup>131</sup>I]-scanning ([<sup>131</sup>I]-Sc). The prognostic values of those methods were evaluated in 4–5 year studies [3]. In 6 months after post-operative radioiodine therapy (RIT) the diagnostic specificity of [<sup>131</sup>I]-Sc and sTg was 87% and 26%, respectively. The

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\*Correspondence. Fax (+380) 44 560 8369

**Abbreviations used:** CEA — carcinoembryonic antigen; CK — cytokeratin; DTC — well differentiated thyroid cancer; EMA — epithelial membrane antigen; FTC — follicular thyroid cancer; FNAB — fine needle aspiration biopsy; [<sup>131</sup>I]-Sc — [<sup>131</sup>I]-scanning; MTC — medullary thyroid cancer; PTC — papillary thyroid cancer; RIT — radioiodine therapy; sTg — serum thyroglobulin; Tg — thyroglobulin; TGI — thyroid gland; TPO — thyroid peroxidase.

recurrence of the disease wasn't detected in 4-year period among 95% of Tg(-) and 47% of Tg(+) patients which have been shown to have residual tissue after [<sup>131</sup>I]-Sc. Thus, prognostic value of sTg determination is lower than that of [<sup>131</sup>I]-Sc method, but during first scanning the estimation of Tg status is extremely important for prognosis. According to the data [6], the results of the primary scanning, the stage and propagation of the disease should be mentioned if sTg level is high and the residual tissue is not detected by [<sup>131</sup>I]-Sc. In patients with lung or lymph node metastases the application of high dose [<sup>131</sup>I]-therapy may be effective. According to the data [7], in patients with persisting DTC or with its recurrence the sensitivity of sTg detection assay reached 91% and specificity — 99%. The best results are obtained if TSH level is high; thus, it looks reasonable to apply TSH-stimulating therapy [7]. Also the presence of anti-Tg antibodies should be mentioned [4, 5]. The multicentric study, carried in France and aimed on standartization of DTC markers application, gave similar conclusions [8]. However, it should be noted that sTg levels are most precise when patients are hypothyroid and may be unreliable in patients with anti-Tg antibodies [9–12]. As a comparative method RT-PCR for Tg mRNA detection was proposed. Tg mRNA was detected in 79% (26 from 33) patients who were cured with thyroxine and had radioiodine-determined residual tissue; at the same time the sTg was detected only in 36% (12 from 33) of patients. In the group of patients with radioiodine-sensitive residual tissue Tg mRNA was detected in 7 patients from 35 (20%) as well as in 14 patients (100%) with metastases including 2 patients with high level of anti-Tg-antibodies. Thus, the determination of Tg mRNA was found to be more valid than that for sTg [13, 14]. The detection of thyroid-stimulating hormone receptor (TSH-R) and Tg gene expression revealed by PCR technique in circulating tumor cells in DTC patients was demonstrated to be potentially effective method, too [15]. The comparison of these methods with standard FNAB has demonstrated the advantage of genetic studies, which have higher sensitivity and specificity.

A lot of publications has been devoted for the detection of Tg receptors (TgR) and thyroid peroxidase (TPO) in the tissues of thyroid adenomas or carcinomas. It was shown that TgR and TPO levels are significantly higher in benign tumors of thyroid tissue [16, 17]. Tumor-specific rearrangement of *Ret* gene was observed also in the samples of papillary thyroid cancer (PTC) and was designated as *Ret/PTC* 1, 2 and 3. Taking to account all data mentioned above one may conclude that those markers could be detected in the peripheral blood of the patients with DTC. Really, Tallini et al. [18] have shown that Tg, TPO and *Ret/PTC*1 mRNA may be detected in peripheral blood of patients with TGI pathology; the presence of named markers correlated with DTC diagnosis, but didn't have high diagnostic value (Tg and TPO were determined in 54.2% of patients with DTC). Clinical manifestation of cancer was absent in 8 from 13 patients, the signs of extraorganic invasion or regional metastases were present in

4 patients. *Ret/PTC*1 mRNA was detected only in 1 case (5%) of DTC; in the same case sTg and TPO were determined. The frequency of TgR and TPO and *Ret/PTC*1 mRNA determination was notably higher in malignant tumors than in benign tumors of thyroid gland.

The level of sTg was determined in the blood samples of pre-operative group of patients (516 persons) [19]. The medium values of Tg were the highest in the group of patients with follicular thyroid cancer (FTC) or with Hurtle-cell cancer. The diagnostic sensitivity and diagnostic specificity were respectively 71.8% and 80.4% for follicular cancer, 55.6% and 83.6% for Hurtle-cell cancer. Positive and negative prognostic value was 75.6% and 77.1% for FTC, 75% and 68.4% for Hurtle-cell cancer. So, on the pre-operative stage the determination of Tg is an important additional assay for selection of adequate treatment schedule for the patients with thyroid tumors. In another research the reliability of sTg determination on the pre-operative stage in PTC patients and standard use of Tg monitoring on post-operative stage in DTC patients have been evaluated [20]. One should note that during determination of Tg level on pre-operative stage it's necessary to consider that the degree of iodine deficiency and hormonal status of each patient may strongly influence the level of sTg. Such dependence was demonstrated in the study of sTg level in 4649 individuals from two regions of Denmark different in iodine deficiency [21].

By RT-PCR the circulating immune complexes containing cytokeratins of 20 type (CK-20) were detected in the blood of 3 from 8 patients with medullary thyroid cancer (MTC); 2 from 8 patients with FTC, 2 from 11 patients with PTC and in 25% of patients with anaplastic cancer. Thus, the determination of CK-20 by RT-PCR may improve the diagnosis of DTC and be useful for determination of circulating tumor cells [22]. The use of ferritin as tumor marker was found to be ineffective in diagnosis or monitoring of DTC [23].

Calcitonin is considered as a known marker of MTC. The detection of calcitonin and carcinoembryonic antigen (CEA) may be used for monitoring of patients with MTC and allows to detect the recurrence of the disease [8, 20]. Despite the fact that serum concentration of chromogranin A is significantly higher in patients with neuroendocrine tumors than in the cases of MTC — 80% against 46% — the diagnostic value of this marker was found to be relatively low in diagnosis of the primary or residual MTC [24]. Diagnostic sensitivity for different markers in MTC amount 46% for chromogranin A, 100% — for calcitonin, 52% — for CEA. Thus, only calcitonin may be considered as reliable marker for recurrence or metastasis. American researchers have evaluated the diagnostic value of determination of RET oncogene mutations in monitoring of MTC patients and concluded that in clinical practice this assay isn't sufficient [25]. The determination of glucosaminoglycans in the urine is a satisfactory method for monitoring patients with ophtalmopathy of endocrine genesis but not for patients with thyroid cancer [26].

**Tissue markers.** Tumor-specific rearrangement of the genes including RET protooncogene has been ob-

served in the samples obtained from PTC patients and was designated as RET/PTC 1, 2 and 3 [27, 28]. RET/PTC 1 is frequently found in the tissue of PTC (approximately in 40% of cases) [29]. According to the data [30], RET-*oncogene* transcripts may be detected in 85% of patients with PTC including follicular variant and in single cells in some tissues but not in nonmalignant TGI tissue. Also it has been shown that PTC tissues are positively stained by anti-RET/PTC-antibodies in 78% of cases, and tissues in follicular variant of PTC — in 63% of cases. The research of RET-*oncogene* expression in tissues of PTC and benign tumors of thyroid gland revealed focal or diffuse expression of RET-*oncogene* in all cases of PTC with signs of infiltrative growth [32]. The level of immunoreactivity was found to be proportional to the risk of metastases occurrence. It's necessary to note that focal or diffuse expression of RET-*oncogene* occurs also in the benign tumors of TGI. So this assay is reasonable to apply only on infiltrative growth detection in PTC with established diagnosis. The joint Italian-Byelorussian research of RET/PTC modifications was carried out on 65 patients with benign tumors of thyroid gland and on 89 patients with PTC [33]. The last group include 25 belorussian pediatric patients, which were irradiated after Chernobyl Nuclear Power Plant accident. This research demonstrated that RET/PTC mutation in the tumors of TGI isn't strictly linked to malignant phenotype, doesn't increase in radiation-induced tumors, doesn't change after radioiodine therapy or distant x-ray therapy and doesn't depend on the age of patients. From other hand, ethnic and hereditary factors may influence damage of DNA which results in activation of RET-*protooncogene*. The determination of c-RET mRNA expression and alternative splicing of c-RET mRNA in the tissues of PTC is undergoing the stage of experimental research yet [34].

TPO alterations are known as early markers of thyroid follicular tumors [35, 36]. Earlier it was shown that in FNAB samples immunohistochemical determination of TPO with the use of MoAb-47 may be applied on the evaluation of malignization degree [37–39]. In prospective research of 1620 surgically resected thyroid neoplasms the sensitivity of this assay was 97.4%, and specificity gave raise to 82% [40, 41].

There is evidence that dipeptidyl aminopeptidase IV (DAP IV) may play an important role in oncogenesis; cytochemical determination of DAP IV may serve as a definite marker of thyroid cell malignization [42, 43]. The sensitivity of DAP IV determination in thyroid cancer was shown to be somewhat lower than that of immunocytochemical method with the use of MoAb-47, but its specificity was nearly 90% [44].

The immunohistochemical determination of CK-19 and CK-20 is of exceptional importance for practical use. The positive reaction on CK-20 was found in 100% of MTC cases, in 9 from 12 of FTC cases, in 7 from 12 of PTC cases and in 1 from 6 cases of anaplastic cancer; CK-20 wasn't detected by RT-PCR in none from 30 cases of benign tumors [22]. CK-19 has been detected in 80% of PTC cases and in 57% of FTC cases [31]. The detection of CK-19 mRNA in the serum is

used as a marker of metastases in patients with DTC. Immunocytochemical detection of CK-19 may be performed also in the smears obtained by FNAB.

For histological research the determination of HBME-1 and CD15 antigens in tumor tissues is shown to be important, too [45]. The HBME-1-reaction was demonstrated to be positive in all cases of PTC (145/145) and FTC (27/27); on other hand, cases of nodular goiter and papillary hyperplasia either showed no reactivity or were focally positive (in 1/3 of cases). According to the data [31], HBME-1-positive reaction was observed in 70% of PTC cases, and in 45% of FTC cases. Thus, the detection of HBME-1-antigen may be used as additional marker in the diagnosis of DTC. The reaction with anti-CD15 MoAb was shown to be less specific in papillary cancer and was detected only in 50% of FTC cases [45].

The detection of galectin-3 (G-3) expression could be widely used in diagnosis of thyroid neoplasms [46]. With the use of anti-G-3 MoAb 118 samples of thyroid tumors were studied. The research revealed that in that assay normal tissue of TGI, the tissues of diffuse and nodular goiter are predominantly negative as well as adenomas with typical cytological structure (in the last case only single cells or separate groups of cells were positive); but in 100% of PTC cases the reaction was positive.

The determination of E-cadherin expression was carried out on 82 samples of follicular adenoma, 53 samples of PTC, 4 samples of FTC, 2 samples of MTC, 2 samples of anaplastic cancer [47]. It was demonstrated that E-cadherin expression is significantly lower in PTC than in follicular adenomas or in normal thyroid tissue, especially if regional lymph node metastasis are present. The detection of anomalic adhesion E-cadherin-catenin complex in PTC tissue indicates the presence of diffuse sclerosing variant of papillary cancer with signs of invasive growth [48].

The role of Akt activation in pathogenesis and progression of DTC was studied [49]. This research revealed that Akt level was significantly increased in the cases of FTC or in follicular variant of papillary cancer — in the types of DTC which are hardly diagnosed on pre- and post-operative stages.

The significant increase in argyrophilic proteins in nucleolar organizer region of cells in the 16.8% of FTC cases and 22.8% of PTC cases in comparison with follicular adenomas (3.2%) and atypic adenomas (12%) may play a role in differential diagnosis during histological study and potentially — in cytological diagnosis of atypic adenomas and FTC [50].

For improvement of DTC diagnosis the expression of epithelial membrane antigen (EMA) and S-100 protein was studied [51]. 14 cases of PTC and 13 cases of benign neoplasms were included in this research. The diffuse staining was registered in 9 cases of DTC and focal (by nuclear and cytoplasmic immunostaining of S-100) — in 3 cases. Immunostaining of EMA was detected in 11 cases of PTC and in 7 cases of benign thyroid neoplasms. Thus, the determination of S-100 protein expression may be used for diagnosis in more difficult cases.

Some methods (the detection of c-met and hTERT mRNA in the thyroid tumors) have only theoretical value for PTC diagnosis yet [52, 53]. In particular, it is known that catalytic subunit of human telomerase (hTERT — human telomerase reverse transcriptase) is activated in the majority of the malignant tumors but is found in inactive state in the cells of benign neoplasms. As it was reported [54], the detection of kinesin — kinesin receptor — is potentially effective method for cancer diagnosis (i.e. DTC). The scientists from South Korea studied Fra-1 expression in the tissues of benign and malignant thyroid tumors and demonstrated that Fra-1 expression has more pronounced character in malignant tissues of thyroid gland; but differentiation of DTC cases can't be done properly with the use of this marker [55]. The TGF $\beta$ 1 and activin A expression in thyroid tumors was studied, too [56], but practical application of those markers wasn't proposed.

**Genetic markers.** The study of genetic mechanisms of PTC initiation and progression is important for prognosis of the disease and the right choice of treatment. Chromosomal aberrations (the gain in 1q-region and the loss of 9q21.3–q32-region) are reliably observed only in tumors with aggressive behavior and/or with appearance of distant metastasis which occurrence is associated with the gain in 1q-region [57]. Timely detection of those genetic alterations contributes to more radical treatment of patients and to the control in the appearance of regional and distant metastases. According to [58], in PTC the p53 expression is notably higher, than in benign nodules; the presence of BAX and p21 protein evidences the absence of p53 gene mutation. From practical point of view the absence of p53 mutation in thyroid tumors points to good prognosis (the low risk of recurrence and metastases). The decrease in *nm23-H1* gene expression correlates with high risk of appearance of metastases in a number of human carcinomas. For example, the tissue samples from 94 patients with DTC (64 — PTC, 30 — FTC) were obtained. 5 years-long follow-up was performed. The level of *nm23-H1* gene expression was shown to have reverse relation to the recurrence and metastases. Moreover, the level of *nm23-H1* gene expression was associated with the survival period. It has been concluded that *nm23-H1* immunoreactivity is more specific, but less sensitive, than AMES system for prediction of metastases; this method may be recommended as additional method for long-term follow-up of patients with FTC [59]. In the study [60], nicotinamide adenine dinucleotide phosphate oxidase (ThoX, LNOX, Duox) expression wasn't related to the type of thyroid tumor (malignant or benign). The determination of the *MYC*, *ERBB2* та *CCND1* genes expression in the early and late stages of thyroid cancerogenesis has only theoretical significance yet [61].

Thus, one may conclude the next: 1. The blood serum, somatic and parenchymal cells of thyroid gland possess numerous specific biological markers for DTC which could play an important role in the DTC diagnosis. 2. The practical application of oncomarkers in diagnosis depends on specificity and sensitivity of the as-

say, the complexity of the technique and its cost-effectiveness. 3. The oncomarker diagnosis in DTC should be considered as a perspective way to improve the treatment of thyroid tumors.

## REFERENCES

1. Ghossein RA, Scher HI, Gerald WL, Croce C. Detection of circulating tumor cells in patients with localized and metastatic prostatic carcinoma. Clinical implication. *J Clin Oncol* 1995; **13**: 1195–200.
2. Dugelloff AJ, Hershman JM. Medical therapy for differentiated thyroid carcinoma. *Endocr Rev* 1994; **15**: 500–15.
3. Roelants V, De Nayer P, Bouckaert A, Beckers C. The predictive value of serum thyroglobulin in the follow-up of differentiated thyroid cancer. *Eur J Nucl Med* 1997; **24**: 722–7.
4. Duren M, Siperstein AE, Shen W, Duh QY, Morita E, Clark OH. Value of stimulated serum thyroglobulin levels for detecting persistent or recurrent differentiated thyroid cancer in high- and low-risk patients. *Surgery* 1999; **126**: 13–9.
5. Ruter A, Smeds S, Lennquist S. Value of serum thyroglobulin measurement in patients operating on for well differentiated thyroid carcinoma. *Eur J Surg* 1998; **164**: 665–7.
6. Pacini F, Agate L, Elisei R, Capezzone M, Caccarelli C, Lippi F, Molinaro E, Pinchera A. Outcome of differentiated thyroid cancer with detectable serum Tg and negative diagnostic (131)I whole body scan: comparison of patients treated with high (131)I activities versus untreated patients. *J Clin Endocrinol Metab* 2001; **86**: 4092–7.
7. Shaha AR, Ferlito A, Rinaldo A. Distant metastases from thyroid and parathyroid cancer. *J Otorhinolaryngol* 2001; **63**: 243–9.
8. Pichon MF, Basuyau JP, Gory-Delabaere G, Eche N, Daver A, Blanc-Vincent MP, Riedinger JM, Deneux L, Bidart JM. Standards, options and recommendations for blood tumor markers in thyroid cancers. *Bull Cancer* 2001; **88**: 775–92.
9. Robbins J, Merino MJ, Boice JJD, Schwarz W, Thomas P. Thyroid cancer: a lethal endocrine neoplasm. *Ann Intern Med* 1991; **115**: 133–47.
10. Spencer CA, Wang CC. Thyroglobulin measurement: techniques, clinical benefits and pitfalls. *Endocrinol Metab Clin North Am* 1996; **24**: 841–64.
11. Hjiyannakis P, Mundy J, Harmer C. Thyroglobulin antibodies in differentiated thyroid cancer. *Clin Oncol* 1999; **11**: 240–4.
12. Maussier ML, Danese D, D'Errico G, Garganese MC, Pontecorvi A, Lemmo G. Clinical and laboratory follow-up in differentiated thyroid carcinoma. *Rays* 2000; **25**: 239–44.
13. Ringel MD, Ladenson PW, Levine MA. Molecular diagnosis of recurrent thyroid cancer by amplification of thyroglobulin messenger ribonucleic acid in peripheral blood. *J Clin Endocrinol Metab* 1998; **83**: 4435–42.
14. Haber RS. The diagnosis of recurrent thyroid cancer — a new approach [editorial comment]. *J Clin Endocrinol Metab* 1998; **83**: 4435–42.
15. Arturi F, Russo D, Giuffrida D, Ippolito A, Perrotti N. Early diagnosis by genetic analysis of differentiated thyroid cancer metastases in small lymph nodes. *J Clin Endocrinol Metab* 1997; **82**: 1638–41.
16. Bertaux F, Noel M, Malthiery Y, Marvin MR, Pritsker T. Demonstration of a heterogeneous transcrip-

tion pattern of thyroglobulin mRNA in human thyroid tissues. *Biochem Biophys Res Commun* 1995; **198**: 586–92.

17. **Otha K, Endo T, Onayata T.** The mRNA levels of thyrotropin receptor, thyroglobulin and thyroid peroxidase in neoplastic human thyroid tissue. *Biochem Biophys Res Commun* 1991; **174**: 1148–53.

18. **Tallini G, Ghossein RA, Emanuel J, Gill J, Kinder B, Dimich AB, Costa J, Robbins R, Burrow GN, Rosai J.** Detection of thyroglobulin, thyroid peroxidase, and RET/PTC1 mRNA transcripts in the peripheral blood of patients with thyroid disease. *J Clin Oncol* 1998; **16**: 1158–66.

19. **Hocevar M, Auersperg M.** Role of serum thyroglobulin in the pre-operative evaluation of follicular thyroid tumors. *Eur J Surg Oncol* 1998; **24**: 553–7.

20. **Okamoto T.** Tumor markers for endocrine neoplasms. *Gan To Kagaku Ryoho* 2001; **28**: 561–5.

21. **Knudsen N, Bulow I, Jorgensen T, Perrild H, Ovesen L, Laurberg P.** Serum Tg – a sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. *J Clin Endocrinol Metab* 2001; **86**: 3599–603.

22. **Weber T, Lacroix J, Weitz J, Amnan K, Magener A, Holtting T, Klar E, Herfarth C, von Knebel Doeberitz M.** Expression of cytokeratin 20 in thyroid carcinomas and peripheral blood detected by reverse transcription polymerase chain reaction. *Br J Cancer* 2000; **82**: 156–7.

23. **Slavnov VN, Markov VV, Kovalenko AE, Kovpan NA, Kvachenuk AN.** Tumor markers in diagnostics of thyroid cancer. *Lyikarska Sprava* 1997; **4**: 31–4 (in Russian).

24. **Franke WG, Pinkert J, Runge R, Bredow, Wunderlich G, Koch R.** Chromogranin A: an additional tumor marker for postoperative recurrence and metastases of medullary thyroid carcinomas. *Anticancer Res* 2000; **20**: 5257–60.

25. **Puxeddu E, Fagin JA.** Genetic markers in thyroid neoplasia. *Endocrinol Metab Clin North Am* 2001; **30**: 493–513.

26. **Reinhardt MJ, Moser E.** An update on diagnostic methods in the investigation of diseases of the thyroid. *Eur J Nucl Med* 1996; **23**: 587–94.

27. **Grieco M, Santoro M, Berlingieri MT, Cosci B, Miccoli P, Romei T.** PTC is a novel rearranged form of the RET proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell* 1990; **60**: 557–63.

28. **Santoro M, Dathan NA, Melillo RM, Bernet V, Burman KD, Kohn LD.** Molecular defects in thyroid carcinomas: role of the RET oncogene in thyroid neoplastic transformation. *Eur J Endocr* 1995; **133**: 513–22.

29. **Vigiletto G, Chiappetta G, Fukunada FG.** RET/PTC oncogene activation is an early event in thyroid carcinogenesis. *Oncogene* 1995; **11**: 1207–10.

30. **Lahr G, Stich M, Schutze K, Blumel P, et al.** Diagnosis of papillary thyroid carcinoma is facilitated by using an RT-PCR approach on laser-microdissected archival material to detect RET oncogene activation. *Pathology* 2000; **68**: 218–26.

31. **Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL.** Immunohistochemical diagnosis of papillary thyroid carcinoma. *Mod Pathol* 2001; **14**: 338–42.

32. **Mai KT, Landry DC, Thomas J, Yazdi HM, Perkins DG, Odell PF.** Ret oncogene protein expression in papillary thyroid carcinoma and related lesions. *Tumori* 2001; **87**: 166–72.

33. **Elisei R, Romei C, Vorontsova T, Cosci B, Veremeychik V, Kuchinskaya E, Basolo F, Demidchik EP, Miccoli P, Pinchera A, Pacini F.** RET/PTC rearrangement in thyroid nodules: studies in irradiated and not ir-

radiated, malignant and benign thyroid lesions in children and adults. *J Clin Endocrinol Metab* 2001; **86**: 3211–6.

34. **Fluge O, Haugen DR, Akslen LA, Marstad A, Santoro M, Fusco A, Varhaug JE, Lillehaug JR.** Expression and alternative splicing of c-ret RNA in papillary thyroid carcinomas. *Oncogene* 2001; **15**: 885–92.

35. **De Micco C, Ruf J, Chrestian MA, Gros N, Henry JF, Carayon P.** Immunohistochemical study of thyroid peroxidase in normal, hyperplastic and neoplastic human thyroid tissues. *Cancer* 1991; **67**: 3036–41.

36. **Garcia S, Vassko V, Henry JF, De Micco C.** Comparison of thyroid peroxidase expression with cellular proliferation in thyroid follicular tumors. *Thyroid* 1998; **8**: 745–9.

37. **Christensen L, Blichert-Toft M, Brandt M, Lange M.** Thyroperoxidase (TPO) immunostaining of the solitary cold thyroid nodules. *Clin Endocrinol* 2000; **52**: 797–806.

38. **De Micco M, Kopp F, Vassko V, Grino M.** *In situ* hybridization and immunohistochemistry study of thyroid peroxidase expression in thyroid tumors. *Thyroid* 2000; **10**: 109–15.

39. **Faroux MJ, Theobald S, Pluot M, Patey M, Menzies D.** Evaluation of the monoclonal antibody anti-thyroperoxidase MoAb 47 in the diagnostic decision of cold thyroid nodules by fine-needle aspiration biopsy. *Pathol Res Pract* 1997; **193**: 705–12.

40. **De Micco M, Carayon P, Conte Devolx B, Denizot A, Henry JF.** Thyroid peroxidase (tpo) immunocytochemistry as a screening test for malignancy in thyroid cytology: a prospective evaluation in 1620 resected thyroid nodules. *J Endocrinol Invest* 1998; **21**: 5.

41. **Lange M, Blichert-Toft M, Christensen LH, Brandt M, Sneppen SB, Ravnsbaek J, Molleup CL, Strange L, Jensen F, Kirkegaard J, Hansen HS, Sorensen SS, Feldt-Rasmussen UF.** TPO immunostaining of the solitary, cold thyroid nodules. *Ugeskr Laeger* 2001; **163**: 4198–201.

42. **Umeki K, Tanaka T, Yamamoto L, Aratake Y, Kotani T, Sakamoto F, Noguchi S, Ohtaki S.** Differential expression of dipeptidyl aminopeptidase IV and thyroid peroxidase in neoplastic thyroid tissues. *Endocrinol J* 1996; **43**: 53–60.

43. **Aratake Y, Kotani T, Tamura K, Araki Y, Kuriyayashi T, Konoe K, Ohtaki S.** Dipeptidyl aminopeptidase IV staining of cytologic preparation to distinguish benign from malignant thyroid diseases. *Am J Clin Pathol* 1991; **96**: 306–10.

44. **Zoro P, Vassko V, Garcia S, Pazart L, Aho S, De Micco C.** Malignancy markers in the cytological diagnosis of thyroid nodules: dipeptidyl aminopeptidase IV (DAP IV). *Ann Pathol* 1996; **16**: 261–5.

45. **Miettinen M, Karkkainen P.** Differential reactivity of HBME-1 and CD15 antibodies in benign and malignant thyroid tumors. Preferential reactivity with malignant tumors. *Virchows Arch* 1996; **459**: 213–9.

46. **Neidobitek C, Neidobitek F, Lindenberg G, Bachler B, Neudeck H, Zuschneid W, Hopfenmuller W.** Expression of galectin-3 in thyroid gland and follicular cell tumors of the thyroid. A critical study of its possible role in preoperative differential diagnosis. *Pathology* 2001; **22**: 205–13.

47. **Naito A, Iwase H, Kuzushima T, Nakamura T, Kobayashi S.** Clinical significance of E-cadherin in thyroid neoplasm. *J Surg Oncol* 2001; **76**: 176–80.

48. **Rocha AS, Soares P, Seruca R, Maximo V, Matias-Guiu X, Cameselle-Teijeiro J, Sobrinho-Simoes M.** Abnormalities of the E-cadherin/catenin adhesion complex in classical papillary thyroid carcinoma and in its diffuse sclerosing variant. *J Pathol* 2001; **194**: 358–66.

49. Ringel MD, Hayre N, Saito J, Saunier B, Schupert F, Burch H, Bernet V, Burman KD, Kohn LD, Saji M. Overexpression and overactivation of Akt in thyroid carcinoma. *Cancer Res* 2001; **61**: 6105–11.
50. Bukaeva IA, Smirnova EA, Pavlovskaja AI, Makanin MA, Ol'khovskaia IG, Raikhlin NT. Significance of argyrophilic proteins of the nucleolar organizer region in differentiation of benign and malignant growth of thyroid epithelial tumors. *Arkh Patol* 2001; **63**: 15–8 (in Russian).
51. Kilicarslan B, Pesterelli EH, Oren N, Sargin FC, Karpuzoglu G. Epithelial membrane antigen and S-100 protein expression in benign and malignant papillary thyroid neoplasms. *Adv Clin Path* 2000; **4**: 155–8.
52. Fluge O, Haugen DR, Lillehaug JR, Varhaug JE. Difference in patterns of Met expression in papillary thyroid carcinomas and nonneoplastic thyroid tissue. *World J Surg* 2001; **25**: 623–31.
53. Chou SJ, Chen CM, Harn HJ, Chen CJ, Liu YC. *In situ* detection of hTERT mRNA relates to Ki-67 labeling index in papillary thyroid carcinoma. *J Surg Res* 2001; **99**: 75–83.
54. Rodnin NV, Tichoncova IO, Nemazaniy IO, Gorlova LM, Komissarenko IV. Serological identification of antigens that determine autoimmune reaction in human thyroid cancer. *Exp Oncol* 2000; **22**: 135–8 (in Ukrainian).
55. Kim YH, Oh JH, Kim NH, Choi KM, Kim SH, Baik SH, Choi DS, Lee ES. Fra-1 expression in malignant and benign thyroid tumor. *Korean J Intern Med* 2001; **16**: 93–7.
56. Schulte KM, Jonas C, Krebs R, Roher HD. Activin A and activin receptors in thyroid cancer. *Thyroid* 2001; **11**: 3–14.
57. Kjellman P, Lagercrantz S, Hoog A, Wallin G, Larsson C, Zedenius J. Gain of 1q and loss of 9q21.3-q32 are associated with a less favorable prognosis in papillary thyroid carcinoma. *Genes Chromosomes Cancer* 2001; **32**: 43–9.
58. Hermann S, Sturm I, Mrozek A, Klosterhalfen B, Hauptmann S, Dorken B, Daniel PT. Bax expression in benign and malignant thyroid tumors: dysregulation of wild-type P53 is associated with a high Bax and P21 expression in thyroid carcinoma. *Int J Cancer* 2001; **15**: 805–11.
59. Zafon C, Obiols G, Castellvi J, Tallada N, Galofre P, Gemar E, Mesa J, Simo R. nm23-H1 immunoreactivity as a prognostic factor in differentiated thyroid carcinoma. *J Clin Endocrinol Metab* 2001; **86**: 3975–80.
60. Caillou B, Dupuy C, Lacroix L, Nocera M, Talbot M, Ohayon R, Deme D, Bidart JM, Schlumberger M, Virionn A. Expression of reduced nicotinamide adenine dinucleotide phosphate oxidase (ThoX, LNOX, Duox) genes and proteins in human thyroid tissues. *J Clin Endocrinol Metab* 2001; **86**: 3351–8.
61. Beiche I, Franc B, Vidaud D, Vidaud M, Lide-reau R. Analyses of *MYC*, *ERBB2*, and *CCND1* genes in benign and malignant thyroid follicular cell tumors by real-time polymerase chain reaction. *Thyroid* 2001; **11**: 147–52.