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POTENTIAL DIAGNOSTIC MARKERS IN NODULAR LESIONS OF THE THYROID GLAND: AN IMMUNOHISTOCHEMICAL STUDY

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Aims: The differential diagnosis of thyroid nodules in routine practice can be problemmatic for both pathologists and clinicians. Effective treatment requires a determination of the biological nature of the lesions. For this reason, ancilliary diagnostic markers along with histological examination of the nodules may be useful. The objective of this study was to evaluate the diagnostic usefulness of novel markers in the diagnosis of hyperplastic and neoplastic nodules.

Methods: Forty eight thyroid lesions forming four diagnostic groups including adenomatous goiters (AS), follicular adenomas (FA), follicular (FC) and papillary carcinomas (PC) were examined using standard immunohistochemical methods. Monoclonal antibodies against galectin-3, matrix metalloproteinases (MMPs) -2 and -7 and endothelial markers CD31 and CD105 were used.

Results: The cytoplasmatic expression of galectin-3 was positive in all cases of papillary carcinoma. Moreover, statistically significant differences between fused groups of benign (AS and FA) and malignant lesions (FC and PC) were found Fischer's exact test ($p = 0.0001$). No significant differences in cytoplasmic expression of MMPs -2 and -7 and in vascular density assessed by using of both endothelial markers between benign lesions and malignant tumors were revealed.

Conclusions: Galectin-3 appears to be a useful marker in the diagnosis of papillary carcinoma only. The matrix metalloproteinases-2 and -7 are not helpful in distinguishing hyperplastic and neoplastic thyroid nodules. Endothelial markers do not appear to be suitable for thyroid differential diagnosis. A panel of antibodies in the differential diagnosis of thyroid nodular lesions would seem most suitable and further studies with larger sets of patients are awaited.

INTRODUCTION

Thyroid nodules of various biological nature occur in approximately 10 % of persons, predominantly females. The nodules represent heterogenous group that comprises both non neoplastic and neoplastic lesions with varying biological behavior. For this reason, the differential diagnosis of thyroid nodules presents a number of pitfalls. Diagnostic difficulties are caused by morphological similarities of biologically diverse lesions, such as papillary hyperplasia vs. encapsulated papillary carcinoma or hyperplastic vs. neoplastic nodule. Moreover, unusual histologic criteria of malignancy, namely capsular and/or vascular invasion of minimally invasive follicular carcinoma and nuclear characteristics of papillary carcinoma, make differential diagnosis challenging. Fewer problems fortunately, exist in the diagnosis of widely invasive follicular carcinoma. Difficulties in the interpretation of histological criteria may lead to interobserver variability among pathologists in discriminating between minimally invasive follicular carcinoma and follicular adenoma[1, 2]. These lesions may pose a diagnostic challenge even to more experienced pathologists[3-5]. Precise pathological diagnosis is essential to optimal treatment of thyroid tumors. Therefore, using ancilliary diagnostic markers along with histological examination of thyroid nodules of uncertain biological behavior may be helpful.

Several thyroid diagnostic markers have been studied recently. Many reports have focused on the accurate differentiation of follicular thyroid tumors using cytological, immunohistochemical and molecular methods[2]. Markers such as thyreoperoxidase (TPO)[6], dipeptidylpeptidase IV (DPPIV, CD26) (ref.[7-10]), high molecular weight cytokeratin, CK 19 (ref.[4, 11, 12]), galectin-1, galectin-3 (ref.[4, 11-13]), cyclin D1 (ref.[14, 15]), proliferative marker MIB-1 (ref.[16]), vascular endothelial growth factor (VEGF)[17], cyclooxygenase-2, p63 (ref.[18]), CD15, CD57 (ref.[12]), have been evaluated as potential diagnostic markers in thyroid neoplasias[12, 19]. However, none of the mentioned markers were found entirely satisfactory in the differentiation of hyperplastic and neoplastic lesions as well as between benign and malignant thyroid nodules. The aim of this study was to evaluate expression of galectin-3, matrix metalloproteinases (-2 and -7), endothelial markers CD31 and CD105 in thyroid nodular lesions and to determine their diagnostic usefulness in the differentiation of thyroid lesions.

MATERIAL AND METHODS

Forty-eight thyroid nodule formalin-fixed paraffin-embedded tissue sections were examined immunohistochemically to detect the expression of three tumour markers and two endothelial markers. According to WHO classification the thyroid lesions were devided into 4 groups: adenomatous goiters (12 cases), follicular adenomas (12 cases), follicular carcinomas (12 cases) and papillary carcinomas (12 cases). The clinicopathological characteristics of studied groups and cases are summarized in Table 1.

Monoclonal antibodies against galectin-3 (Novocastra, clone 9C4, 1:100) MMP-2 (NeoMarkers, clone Ab-7, 1:50), MMP-7 (NeoMarkers, clone ID2, 1:200), CD31 (DakoCytomation, clone A, 1:10) and CD105 (DakoCorporation, clone SN6h, 1:1500, 1:700) were detected by avidin-biotin method as previously described[20].

Quantitative analysis of galectin-3, MMP-2 and -7 and vascular density was carried out using a light microscope Olympus BX-40 equipped with a 40x magnification objective and special eye-piece 10x10 mm grid. The percentage of galectin-3 (galectin-3 index) and MMPs positive neoplastic cells was determined by counting at least 1000 tumor cells per slide in randomly selected fields of tumors. Immunoreactivity for galectin -3, MMP-2 and MMP-7 was scored semiquantitatively in areas of tumor and expressed as percentage of positive cells with thresholds (absent = 0; 0.1–25 (%) = 1; 25.1–50 (%) = 2; 50.1–75 (%) = 3 and 70.1–100 (%) = 4). The number of microvessels was evaluated semiquantitatively applying method described by Weidner[21]. The microvessel density (MD) was assessed in at least 3 areas (so called "hot spots") with the highest density of CD31 and CD105 respectively. Positively stained isolated endothelial cells or cell clusters with or without visible lumina were counted as separate microvessels. The highest microvessel counts were used for statistical evaluation.

The statistical analysis was performed using ANOVA, Kruskal-Wallis test, Fischer exact test and Wilcoxon (Mann-Whitney) test (p-value < 5 %).

RESULTS

Galectin-3 expression in thyroid nodules

Galectin-3 expression was estimated in follicular cells and neoplastic cells of follicular cell origin, respectively. Galectin-3 staining pattern was pancytoplasmatic and finely granulated. Normal thyroid tissue revealed galectin-3 positivity only in endothelial cells and intrafollicular macrophages. The results of galectin-3 expression in studied different types of thyroid nodules are shown in

Table 1. Clinicopathological features of studied groups of lesions.

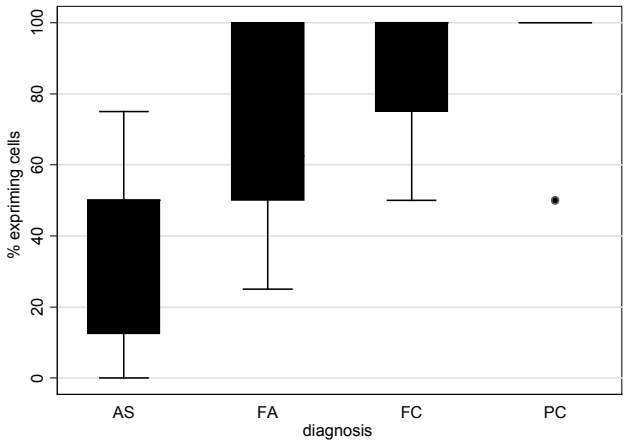
Type of lesion	No.	Sex		Age			Size of examinated lesion (mm)		
		F	M	Med	SD	Min – Max	Med	SD	Min – Max
Adenomatous goiter (AG)	12	10	2	57	13,7	23 – 70	27,5	13,7	10 –50
Follicular adenoma (FA)	12	10	2	43,5	11,4	31 – 69	18,5	19,8	10 – 80
Follicular carcinoma (FC)	12	9 – 3		55,5	19,2	24 – 77	33,5	15,2	15 – 60
Papillary carcinoma (PC)	12	10 – 2		60	21,9	20 – 84	29	10,4	18 – 50

F, female; M, male; Med, median; SD, standard deviation; Min-Max, rate

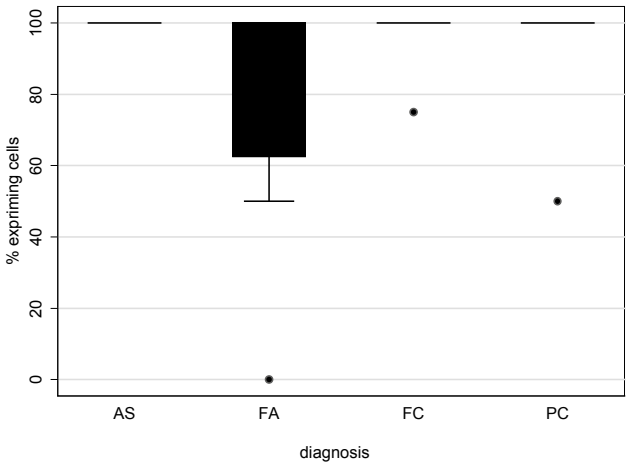
Table 2. Values of microvessel density in studied lesions.

type of lesion	CD31					
	n	mean	median	SD	Min	max
AG	12	101	100	45	46	188
FA	12	89	91	16	61	108
FC	12	119	108	35	87	202
PC	12	102	101	24	68	156

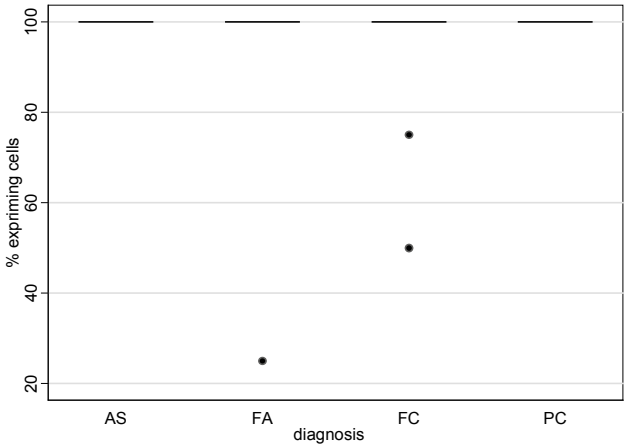
AG, adenomatous goiter; FA, follicular adenoma; FC, follicular carcinoma; PC, papillary carcinoma; SD, standard deviation



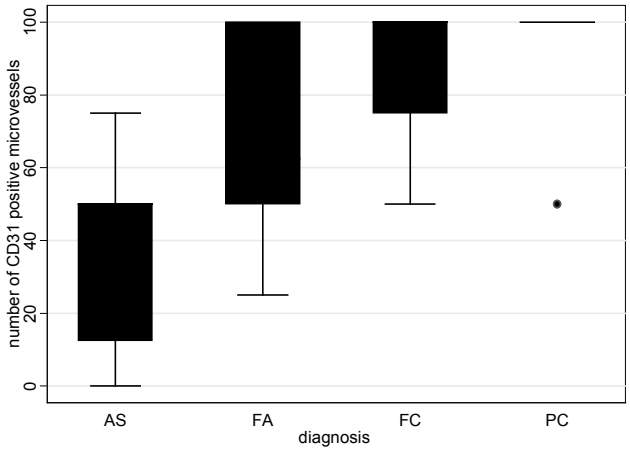
Graph 1. Expression of galectin-3 in individual diagnostic groups.



Graph 2. Expression of MMP-2 in individual diagnostic groups.



Graph 3. Expression of MMP-7 in individual diagnostic groups.



Graph 4. Distribution of microvessels density in individual lesions.

Chart 1. More than 75 % of galectin-3 positive cells was found in 10 of 12 (83.3 %) of papillary carcinomas, in 7 of 12 (58.3 %) of follicular carcinomas, and in 4 cases (33.3 %) of follicular adenoma. All cases of adenomatous goiter expressed galectin-3 in less than 75 % of cells.

Comparison of galectin-3 expression in studied groups revealed statistically significant differences between follicular adenomas and follicular and papillary carcinomas vs. adenomatous goiter as well as between papillary carcinomas vs. follicular adenomas. Suprisingly, no significant differences were found between follicular adenomas and follicular carcinomas ($p = 0.0648$) as well as between follicular carcinomas and papillary carcinomas ($p = 0.3120$). Differences in galectin-3 expression between groups of benign (AS+FA) and malignant (FC+PC) thyroid lesions were statistically significant ($p=0.0001$). Moreover, malignant lesions presented predominantly with diffuse type of galectin-3 distribution within nodule while hyperplastic and benign nodules were prone to focal galectin-3 distribution, if any.

MMPs expression in thyroid nodules

Both markers (MMP-2 and MMP-7) showed in the vicinity of tested nodules focal follicular cell expression as well as positivity in both endothelial and lymphoid cells especially in the Hashimoto thyroiditis.

The results of matrix metalloproteinase-2 immunohistochemical analysis of thyroid nodules are shown in Chart 2. All cases of adenomatous goiter as well as 11 of 12 cases of follicular carcinoma and papillary carcinoma respectively showed high expression (>75 % of cells). In the group of follicular adenoma the MMP-2 positivity (threshold >75 % od neoplastic cells) has been found in 7 of 12 cases. No statistically significant differences in the expression of MMP-2 between various thyroid nodular lesions were revealed in our study either between benign

and malignant lesions or between both types of malignant thyroid tumors.

Matrix metalloproteinase-7 expression showed similar results (Chart 3) as MMP-2. The expression of this marker in more than 75 % cells was found in the majority of lesions without respect to their biological behaviour.

Assessment of microvessel density
Pan-endothelial marker CD31

Microvessels were visualized by CD31 pan-endothelial antigen and scored in three "hot spots" of every lesion of all groups. Microvessel density values (range, mean, median, standard deviation) of studied lesions are presented in Table 2. The distribution of mean MD in the individual groups is displayed in Chart 4.

No significant difference in amount of CD-31 positive vessels was shown between the studied groups in our study.

Endothelial marker CD 105

The expression of CD105 was found in 1 case of follicular adenoma and in 3 cases of follicular carcinomas and so the results were not suitable to statistical analysis. Notably, the expression in follicular carcinomas (mean microvessel density 91.5) was more than twice as high as in follicular adenoma (mean density of vessels 43.67).

DISCUSSION

Difficulties in differentiating follicular thyroid lesions have instigated large investigations of potential molecular markers that could reliably distinguish benign from malignant thyroid nodules[22, 23]. Several diagnostic thyroid markers have been thus evaluated but despite the advantages they possess, most also have certain limitations. None of the markers studied to date have produced unambiguous results or usefulness in every routine situation.

Our previous studies focused on thyreoperoxidase (TPO). TPO is an enzyme specific for the normal follicular cells and the structural changes of enzyme are induced in malignant transformation. TPO antibody (MoAB47) strongly binds the enzyme in normal thyroid cells in benign lesions whereas in malignant tumours the enzyme is expressed weakly if present[6, 9, 24-28]. Nevertheless in combination with other thyroid tumour markers the significance of thyreoperoxidase increases[16].

The present study was designed to evaluate the utility of diagnostic markers galectin-3, matrix metalloproteinase-2 and -7 as well as angiogenesis and their combination in the histological differentiation of the various thyroid nodules.

Galectin-3 (gal-3) belongs to the group of human lectins with 11 types of galectins defined by two properties: afinity for the beta galactosides and significant sequence homology in the carbohydrate binding site. This lectin is expressed by immune cells and by a variety of epithelial cells[29]. Despite the fact that the exact function of galectin-3 is not known, it has been shown to be implicated in many biological processes including regulation of growth and apoptosis, adhesion and premRNA processing. Galectin-3 overexpression is related to neoplastic transformation and tumor spread in several carcinomas, particularly breast, colon, tongue and liver[22, 23, 30]. Galectin-3 has been proposed as a marker that distinguishes between benign and malignant thyroid nodules; in some reports gal-3 immunostaining was absent in benign thyroid lesions and normal thyroid tissue[22]. There is general agreement that among thyroid carcinomas PC shows the most prominent and consistent galectin-3 expression[29].

In our study, we focused on assessement of the expression of galectin-3 in groups of both benign (collection of "benign" lesions comprised hyperplastic nodules as well) and malignant thyroid nodular lesions. Our observation confirmed strong expression of galectin-3 in 10 of 12 cases of papillary carcinomas and in 7 of 12 cases of follicular carcinomas. Statistically significant difference in expression of this marker was shown between groups of benign (AG + FA) and malignant lesions (FC + PC); $p = 0.0001$ by Fischer's exact test. In agreement with other reports[31, 32, 23], we found galectin-3 immunoreactivity in follicular adenomas as well; furthermore the percentage of galectin-3 positive cells and intensity of expression was higher in atypical adenomas. This fact may mirror the potential of malignant transformation in follicular lesions without morphological signs of malignancy, e.g. transcapsular and/or vascular invasion[11, 30, 32].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent structurally and functionaly related endopeptidases that are capable of degrading most of the components of the extracellular matrix[2, 33, 34]. There is evidence, that MMPs are important for the creation and maintenance of a microenvironment that facilitates growth and angiogenesis of tumours both at primary and metastatic sites[35, 36]. MMPs are expressed both in tumour cells, and in neoplastic stroma cells including fibroblasts and inflammatory cells. MMPs have been intensively studied to elucidate their possible use in diagnosis and prognosis in some tumours. Positive correlation between most tumour aggressiveness and the expression of high levels of multiple MMP family members was shown in numerous studies. Matrix metalloproteinases and their inhibitors have been studied in many tumors and malignancies in various locations such as breast[34, 37, 38], lung[39], salivary glands[39], colon[40-44]. Thus MMPs can serve as diagnostic and /or prognostic tumour markers[33-35, 45-48].

Campo et al was the first to study MMP-2 in thyroid tumors[49]. Maeta et al published a study on the expression of MMP-2 and -9 in papillary thyroid carcinomas[50] and Cho Mar et al. examined the expression of MMP-2 and -7 in benign and malignant follicular thyroid lesions[2]. All the above studies found positive staining of matrix metalloproteinases in carcinomas, benign lesions beeing negative or minimally positive. In contrast to these results, our study failed to confirm statistically significant differences in expression of MMP-2 and MMP-7 between papillary and follicular carcinomas and benign lesions of thyroid gland. Percentage of cells expressing both markers in in-

dividual groups did not differ significantly in our study. The positivity of both markers was recorded in most of the lesions without any respect to biological behavior. We found no usefulness of MMP-2 and MMP-7 in the differential diagnosis of nodular thyroid lesions.

Tumour angiogenesis, the formation of tumour new blood vessels, is necessary for the growth and metastasis of solid tumours[51]. In recent years, progress has been made in the identification of regulators of angiogenesis and in detection of markers of microvessels density in tumours. Von Willebrand factor (FVIII), CD34 and CD31 are panendothelial markers commonly used in the evaluation of tumor vessel density. In the last decade, studies of tumour angiogenesis have also focused on another endothelial marker – CD105 known as endoglin. As mentioned previously we chose two of listed markers – CD31 and CD105 to analyse neovascularisation. *CD31* plays a role in angiogenesis through its involvement in endothelial cell-cell and cell-matrix interactions and signal transduction[52-54]. CD31 is involved in tumor stroma angiogenesis and its expression on endothelial cells correlates with metastatic potential in some tumors[55]. *Endoglin (CD105)* is a cell-surface glycoprotein most recently identified as an optimal indicator of proliferation of human endothelial cells. Consistently elevated levels of CD105 expression were detected on human microvascular endothelium and on vascular endothelial cells in tissues undergoing active angiogenesis, such as regenerating and inflamed tissues or tumors[56]. In contrast to panendothelial markers, CD105 is expressed only in the newly formed blood vessels and it has been documented that its expression is limited to the newly formed blood vessels in brain, lung, breast, stomach and colon cancers[53].

Markers of angiogenesis were viewed as a promising area of investigation as well. Early reports on breast carcinoma presented angiogenesis as one of the strongest prognostic factors[21], but these results were not subsequently confirmed[57]. Despite a number of studies published on angiogenesis in different tumor types, endocrine neoplasms have not been subjected to extensive investigation. There are few reports on tumor angiogenesis in the hypophysis[58], in pheochromocytomas[59], in thyroid papillary carcinoma[60, 61] and medullary carcinoma[62]. Ryska et al examined microvessel density in the thyroid gland by using endothelial marker CD31. In this study, they found no significant differences in angiogenesis between follicular adenomas and carcinomas and between papillary carcinomas on one hand and follicular tumors on the other hand[63]. One fact that can explain the variability of the results is that there is no endothelial antigen that is absolutely specific and sensitive and also ubiquitous for vessels in all organs[63, 64]. CD31 has been shown to be reliable marker with relatively high sensitivity, but it does not distinguish neoformed microvessels from preexisting capillary bed[63]. Endoglin (CD105) was believed to be a promising specific marker of newly formed vessels and it has been later shown to be an independent predictor of survival in patients with various tumor types[53] e.g. colorectal cancer[51], endometrial cancer[65], prostate cancer[66]. Endoglin is currently confirmed to be more useful marker than CD31 to identify proliferating endothelium involved in tumor angiogenesis[51]. There have been no references about using CD105 in thyroid lesions yet.

This study focused on evaluation of two different angiogenetic markers CD31 and CD105 in neovascularisation of thyroid nodules. The simple technique of manual capillary counting described by Weidner et al is regarded as the "golden standard" by many investigators[67]. By this method, we found no significant differences between CD31 vascular density in benign and malignant thyroid nodules. We found large variability in vessel count in the group of adenomatous goiters (minimal count 46, maximal 188). Various degree of degeneration, character of follicular component (macrofollicular versus microfollicular nodules) and the variable proportion of stromal and follicular component can explain this interface variation. Although CD31 expression in group of follicular carcinomas was higher than in follicular adenomas the difference was not statistically significant to allow use of this marker to differentiate these tumors. Expression of CD 105 exclusively on newly formed vessels has been described in brain, lung, breast, stomach, endometrium and colorectal cancer[51, 53, 56, 65, 68, 69]. In relation to the earlier presented expression of CD105 in the above–mentioned tumors, the results of our thyroid study were surprising. The expression of CD105 was detected only in one case of FA (mean value of 3 "hot spots" was 43.67) and in two FC (mean value = 91.5). The significance of the endoglin in thyroid tumors should be object of further investigation.

CONCLUSIONS

None of the investigated markers (gal-3, MMP-2, MMP-7, CD31 and CD105) used in our study has been validated as specific and sensitive enough to use in discriminating of thyroid nodules. However, expression of galectin-3 has been found in malignant thyroid neoplasms, mainly papillary carcinomas and it seems that detection of galectin-3 in thyroid tumors might helpful in the differential diagnosis of thyroid nodules. From these results, markers MMP-2 and MMP-7 are not helpful in distinguishing of thyroid nodules with variable biologic behavior and angiogenesis is not suitable as either a diagnostic or prognostic indicator in the evaluation of thyroid nodules either.

An ideal tumor marker has not been discovered yet, but there are many markers of note for differential diagnosis of thyroid lesions. Thus, we recommend using a panel of markers in routine practice.

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REFERENCES

1. Rosai J, Carcangiu ML, DeLellis RA. Tumors of thyroid gland. In Rosai J, Sobin H: Atlas of tumor pathology, 3rd Series, Fascicle 5. Washington, DC: A.F.I.P., 1992.
2. Cho Mar K, Eimoto T, Tateyama H, Arai Y, Fujiyoshi Y, Hamaguchi M. Expression of matrix metalloproteinases in benigh and malignant follicular thyroid lesions. Histopathology 2006; 48:286-94.
3. Williams ED. Two proposals regarding the terminology of thyroid tumors. Int J Surg Pathol 2000; 8:181-3.
4. De Matos PS, Ferreira AP, de Oliveira Facuri F, Assumpcao LVM, Metze K, Ward LS. Usefulness of HBME-1, cytokeratin 19 and galectin-3 immunostaining in the diagnosis of thyroid malignancy. Histopathology 2005; 47:391-401.
5. Stephenson TJ. Papillary carcinoma of the thyroid: a tumour still with no benign neoplastic counterpart. Histopathology 2001; 39:536-38.
6. Kholova I, Ryska A, Ludvikova M, Cap J. Thyroid peroxidase in the differential diagnosis of thyroid gland lesions. A marker of biological behavior or differentiation? Cesk Pathol 2004; 40(1):18-21 (in Czech).
7. Kholova I, Ludvikova M, Ryska A, Hanzelkova Z, Cap J, Pecen L et. al. Immunohistochemical detection of dipeptidylpeptidase IV (CD26) in thyroid neoplasia using biotinylated tyramine amplification. Neoplasma 2003; 50(3):159-164.
8. Kholova I, Ryska A, Ludvikova M, Cap J, Pecen L. Dipeptidyl peptidase IV expression in thyroid cytology: retrospective histologically confirmed study. Cytopathology 2003; 14(1):27-31.
9. Kholova I, Ludvikova M, Ryska A, Topolcan O, Pikner R, Pecen L et. al. Diagnostic role of markers dipeptidyl peptidase IV and thyroid peroxidase in thyroid tumors. Anticancer Res 2003; 23(2A):871-75.
10. Kholova I, Ryska A, Ludvikova M, Pecen L, Cap J. Dipeptidyl peptidase IV (DPP IV, CD 26): a tumor marker in cytologic and histopathologic diagnosis of lesions of the thyroid gland. Čas Lék Česk 2003; 142(3):167-71 (in Czech).
11. Beesley MF, McLaren KM. Cytokeratin 19 and galectin-3 immunohistochemistry in the differential diagnosis of solitary thyroid nodules. Histopathology 2002; 41:236-43.
12. Laco J, Ryska A. The use of immunohistochemistry in the differential diagnosis of thyroid gland tumors with follicular growth pattern. Cesk Patol. 2006;42(3):120-4 (in Czech).
13. Ito Y, Yoshida H, Tomoda C, Miya A, Kobayashi K, Matsuzuka F, et al. Galectin-3 expression in follicular tumours: an immunohistochemical study of its use as a marker of follicular carcinoma. Patology 2005; 37:296-8.
14. Temmim L, Ebraheem AK, Baker H, Sinowatz F: Cyclin D1 protein expression in human thyroid gland and thyroid cancer. Anat Histol Embryol 2005; ISSN 0340-2096.
15. Lantsov D, Meirmanov S, Nakashima M, Kondo H, Saenko V, Naruke Y, et al. Cyclin D1 overexpression in thyroid papillary microcarcinoma: its association with tumour size and aberrant beta-catenin expression. Histopathology 2005; 47:247-56.
16. Ludvikova M, Ryska A, Hovorkova E, Pikner R. Role of proliferative marker MIB-1 in diagnosis and prognosis of thyroid gland tumors. Česk Patol 2002; 38(1):4-10 (in Czech).
17. Pikner R, Ludviková M, Ryška A, Kholová I, Holubec L Jr, Topolčan O, et al. TPS, thymidine kinase, VEGF and endostatin in cytosol of thyroid tissue samples. Anticancer Res 2005; 25:1517-22.
18. Unger P., Ewart M, Wang BY, Gan L, Kohtz SD, Burstein DE: Expression of p63 in papillary thyroid carcinoma and in Hashimoto´s thyroiditis: A pathobiologic link? Hum Pathol 2003; 34:764-9.
19. Ludvikova M, Holubec LJr, Topolčan O. Proliferative markers in diagnosis and prognosis of thyroid tumors: a comparative study of MIB-1 and topoisomerase II-α immunostaining. Anticancer Res 2005; 25:1835-40.
20. Ludvíkova M, Topolčan O, Kholova I, Rydlova M, Holubec L: Can tumor markers be helpful in thyroid nodules diagnosis? Plzeň. Lék. Sborn. 2005; Suppl.80:71-4.
21. Weidner N, Semple JP, Welch WR, Folkman J. Tumour angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 1991; 324:1-8.
22. Gaffney RL, Carney JA, Sebo TH, Erickson LA, Volante M, Papotti M, et al. Galectin-3 expression in hyalinizing trabecular tumors of the thyroid gland. Am J Surg Pathol 2003; 27:494-8.
23. Mehrotra P, Okpokam A, Bouhaidar R, Johnson SJ, Wilson JA, Davies BR, et al. Galectin-3 does not reliably distinguish benign from malignant thyroid neoplasms. Histopathology 2004; 45:493-500.
24. 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.
25. De Micco C. Thyroid cytology: evaluation and perspectives. Annals of Endocrinology Paris. 1993; 54:258-63.
26. De Micco C, Zoro P, Henry JF. Malignancy markers in the cytodiagnosis of thyroid nodules. Thyroid peroxidase. Annals of Pathology 1994; 14:378-83.
27. De Micco C, Vasko V, Garcia S, Zoro P, Denizot A, Henry JF. Fine-needle aspiration of thyroid follicular neoplasm: Diagnostic use of thyroid peroxidase immunocytochemistry with monoclonal antibody 47. Surgery 1994; 116:1031-35.
28. Umeki K, Tanaka T, Yamamoto I, Aratake Y, Kotani T, Sakamoto F, et al. Differential expression of dipeptidylpeptidase IV (CD26) and thyroid peroxidase in neoplastic thyroid tissues. Endocr J 1996; 43:53-60.
29. Cvejic D, Savin S, Petrovic I, Tatic S, Krkovic K, Havelka M. Galectin-3 expression in papillary microcarcinoma of the thyroid. Histopathology 2005; 47:209-14.
30. Buttery R, Monaghan H, Salter CM, Sethi T. Galectin-3: differential expression between small-cell and non-small-cell lung cancer. Histopathology 2004; 44:339-44.
31. Orlandi F, Saggiorato E, Pivano G, Puligheddu B, Termine B, Cappia S, et al. Galectin-3 is a presurgical marker of human thyroid carcinoma. Cancer Research 1998; 58:3015-20.
32. Cvejic D, Savin S, Golubovic S, Paunovic I, Tatic S, Havelka M. Galectin-3 and carcinoembryonic antigen expression in medullary thyroid carcinoma: possible relation to tumour progression. Histopathology 2000; 37:530-35.
33. Curran S and Murray GI. Matrix metalloproteinases in tumour invasion and metastasis. J Pathol 1999; 189:300-8.
34. Manello F. Natural bio-drugs as matrix metalloproteinase inhibitors: New perspectives on the horizon? Recent Patents on Anti-Cancer Drug Discovery 2006; 1:91-103.
35. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: Biologic activity an clinical implications. J Clin Oncol 2000; 18:1135-49.
36. Takahashi E, Tateyama H, Akatsu H, Fukai I, Yamakawa Y. Expression of matrix metalloproteinases 2 and 7 in tumor cells correlates with the World Health Organisation classification subtype and clinical stage of thymic epithelial tumors. Hum Pathol 2003; 34:1253-8.
37. Aribas A. Matrix metalloproteases and tumor invasion. NEJM 2005; 352:2020-1.
38. Nguyen N, Kuliopulos A, Graham RA, Covic L. Tumor-derived Cyr61 (CCN1) promotes stromal matrix metalloproteinase-1 production and protease-activated receptor 1-dependent migration of breast cancer cells. Cancer Res 2006; 66:2658-65.
39. Nagel H, Laskawi R, Wahlers A, Hemmerlein B. Expression of matrix metalloproteinases MMP-2, MMP-9 and their tissue inhibitors TIMP-1, -2, and -3 in benign and malignant tumours of the salivary gland. Histopathology 2004; 44:222-31.
40. Tien YW, Lee PH, Hu RH, Hsu SM, Chang KJ. The role of gelatinase in hepatic metastasis of colorectal cancer. Clin Cancer Res 2003; 9:4891-96.
41. Ishida H, Murata N, Tada M, Okada N, Hashimoto D, Kubota S, et al. Determining the levels of matrix metalloproteinases-9 in portal and peripheral blood is useful for predicting liver metastases of colorectal cancer. Jpn J Clin Oncol 2003; 33:186-91.

42. Curran S, Dundas SR, Buxton J, Leeman MF, Ramsay R, Murray GI. Matrix metalloproteinase/tissue inhibitors of matrix metalloproteinase phenotype identifies poor prognosis colorectal cancers. Clin Cancer Res 2004; 10:8229-34.
43. Pesta M, Holubec L Jr, Topolcan O, Cerna M, Rupert K, Holubec LS, et al. Quantitative estimation of matrix metalloproteinases 2 and 7 (MMP-2, MMP-7) and tissue inhibitors of matrix metalloproteinases 1 and 2 (TIMP-1, TIMP-2) in colorectal carcinoma tissue samples. Anticancer Res 2005; 25:3387-91.
44. Waas ET, Wobbes T, Ruers T, Lomme RM, Hendriks T. Circulating gelatinases and tissue inhibitor of metalloproteinase-1 in colorectal cancer metastatis liver disease. Eur J Surg Oncol 2006; 32:756-63.
45. Massi D, Franchi A, Ketabchi S, Paglierani M, Pimpinelli N, Santucci M. Expression and prognostic significance of matrix metalloproteinases and their tissue inhibitors in primary neuroendocrine carcinoma of the skin. Hum Pathol 2003; 34:80-8.
46. Holubec L, sen et al. Colorectal carcinoma. Praha: Grada Publishing 2004; ISBN 80-247-0636-9:25-27.
47. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: Moving target for therapeutic intervention. J Clin Invest 1999; 103:1237-41.
48. Nabeshima K, Inoue, T, Shimao Y, Sameshima T. Matrix metalloproteinases in tumor invasion: Role for cell migration. Pathology International 2002; 52:255-64.
49. Campo E, Merino MJ, Liotta L. Distribution of the 72-kD type IV colagenase in nonneoplastic and neoplastic thyroid tissue. Hum Pathol 1992; 23:1395-1401.
50. Maeta H, Ohgi S, Terada T. Protein expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinase 1 and 2 in papillary thyroid carcinomas. Virchows Arch 2001; 438:121-128.
51. Saad RS, Liu YL, Nathan G, Celebrezze J, Medich D, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in colorectal cancer. Mod Pathol 2004; 17:197-203.
52. De Lisser HM, Christofidou-Solomidou M, Strieter RM, Burdick MD, Robinson CS, Wexler RS, et al. Involvement of endothelial PECAM-1/CD31 in angiogenesis. Am J Pathol 1997; 151:671-77.
53. Minhajat R, Mori D, Yamasaki F, Sugita Y, Satoh T,Tokunaga. Organ-specific endoglin (CD105) expression in the angiogenesis of human cancers. Pathol Int 2006; 56:717-23Erdem O, Taskiran C, Onan MA, Erdem M, Guner H, Ataoglu O. CD105 expression is an independent predictor of survival in patients with endometrial cancer. Gynecol Oncol 2006; 103:1007-11.
54. Kondo S, Scheef EA, Sheibani N, Sorenson CM. PECAM-1 isoform-specific regulation of kidney endothelial cell migration and capillary morphogenesis. Am J Physiol Cell Physiol 2007; 292: C2070-83.
55. Polverini PJ. Cellular adhesion molecules. Am J Pathol 1996; 148:1023-1029.
56. Fonsatti E, Maio M. Highlights on endoglin (CD105): from basic findings towards clinical applications in human cancer. J Transl Med 2004; 2:18.
57. Mayers MM, Seshadri R, Raymond W, McCaul K, Horsfall DJ: Tumor microvascularity has no independent prognostic significance for breast cancer. Pathology 1998; 30:105-10.
58. Jugenburg M, Kovacs K, Stefaneanu L, Scheithauer BW. Vasculature in nontumorous hypophyses, pituitary adenomas, and carcinomas: A quantitative morphologic study. Endocr Pathol 1995; 6:115-124.
59. Liu Q, Djuricin G, Staren ED, Gattuso P, Gould VE, Shen J, et al. Tumor angiogenesis in pheochromocytomas and paragangliomas. Surgery 1996; 150:938-43.
60. Ishiwata T, Iino Y, Takei H, Oyama T. Morishita Y. Tumor angiogenesis as an independent prognostic indicator in human papillary thyroid carcinoma. Oncol Rep 1998; 5:1343-8.
61. Lennard CM, Patel A, Wilson J, Reinhardt B, Tuman C, Fenton C, et al. Intensity of vascular endothelial growth factor expression is associated with increased risk of recurrence and decreased disease-free survival in papillary thyroid cancer. Surgery 2001; 129:552-8.
62. Fontanini G, Vignati S, Pacini F, Pollina L, Basolo F: Microvessel count: an indicator of poor outcome in medullary thyroid carcinoma but not in other types of thyroid carcinoma. Mod Pathol 1996; 9:636-41.
63. Ryska A, Ludvikova M, Klaboch J, Laco J, Kholova I, Topočan O. Angiogenesis in thyroid gland tumors. Biomarkers and Environment 2002; 5:24-31.
64. Kuzu I, Bicknell R, Harris AL, Jones M, Gatter KC, Mason DY. Heterogenity of vascular endothelial cells with relevance to diagnosis of vascular tumors. J Clin Pathol 1992; 45:143-8.
65. Erdem O, Taskiran C, Onan MA, Erdem M, Guner H, Ataoglu O. CD105 expression is an independent predictor of survival in patients with endometrial cancer. Gynecol Oncol 2006; 103:1007-11.
66. Wikstrom P, Lissbrant IF, Stattin P, Egevald L, Bergh A. Endoglin (CD105) is expressed on immature blood vessels and is a marker for survival in prostate cancer. Prostate 2002; 51:268-75.
67. Ranefall P, Wester K, Busch Ch, Malmström PU, Bengtsson E: Automatic quantification of microvessels using unsupervised image analysis. Anal Cell Pathol 1998; 17:83-92.
68. Nikiteas NI, Tzanakis N, Theodoropoulos G, Atsaves V, Christoni Z, Karakitsos P, Lazaris AC, Papachristodoulou A, Klonaris C, Gazouli M. Vascular endothelial growth factor and endoglin (CD-105) in gastric cancer. Gastric Cancer 2007; 10:12-7.
69. Erdem O, Erdem M, Erdem A, Memis L, Akyol G. Expression of vascular endothelial growth factor and assessment of microvascular density with CD 34 and endoglin in proliferative endometrium, endometrial hyperplasia, and endometrial carcinoma. Int J Gynecol Cancer 2007; 17:1327-32.