Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions

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Summary

Background Thyroid cancer is the most common endocrine malignant disease, but preoperative diagnosis remains a challenge. Fine-needle aspiration cytology has greatly improved the clinical management of thyroid nodules, but the preoperative characterisation of follicular lesions is very difficult. Many patients are thus referred to surgery more for diagnosis than for therapeutic necessity. We undertook an international multicentre study to assess the usefulness of immunohistocytochemical staining for two potential markers of malignant thyrocytes.

Methods Expression of galectin-3 and CD44v6 was tested on 1009 thyroid lesions (tissue specimens and cytological cell-blocks) and 226 fresh cytological samples obtained preoperatively by ultrasound-guided fine-needle aspiration of thyroid nodules (prospective analysis). The test used monoclonal antibodies specific for CD44v6 and galectin-3, the indirect avidin-biotin complex immunoperoxidase method, and 3-amino-9-ethyl-carbazole as substrate.

Findings The sensitivity, specificity, positive predictive value, and diagnostic accuracy of this test method (for coexpression of the two markers) in the prospective analysis were 88%, 98%, 91%, and 97%, respectively. The sensitivity and specificity of galectin-3 immunodetection alone in discriminating benign from malignant thyroid lesions were more than 99% and 98% respectively, and the positive predictive value and diagnostic accuracy were 92% and 99%.

Interpretation The integration of galectin-3 immunostaining with conventional cytomorphological and clinical diagnostic procedures represents a sensitive and reliable diagnostic approach for preoperative identification of thyroid carcinomas. This test method improves the diagnostic accuracy of conventional cytology and provides the molecular basis for a new nosological assignation of the not yet classified thyroid neoplasms of indeterminate malignant behaviour.

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Introduction

Fine-needle aspiration cytology is well established in the primary diagnosis of benign and malignant thyroid disorders.[1–3] However, there is general agreement that this procedure has some inherent limitations related to inadequate sampling and overlapping cytological features between benign and malignant follicular lesions.[4–8] The morphological distinction of hyperplastic adenomatous nodules, well-differentiated follicular carcinomas, and follicular variants of papillary carcinoma is difficult, even for cytologists with extensive experience of thyroid fine-needle aspiration.[7–9] Because specific criteria are required for the diagnosis of follicular carcinoma, particularly the unequivocal demonstration of capsular penetration and vascular invasion, well-differentiated follicular malignant lesions can also be difficult to distinguish from adenomas by histology.[9,10] This is the reason why cytology reports are commonly worded "probable benign follicular nodule" or "follicular nodule not otherwise specified". As a consequence, the majority of patients with these lesions are referred for surgery. The psychological and social cost of this clinical approach is high for the patients, as well as for the health-care system, especially since less than 10% of the resected lesions will be definitively classified as carcinomas.[2,9,10]

Attempts to improve the preoperative diagnosis of thyroid nodules by use of strict instructions for obtaining adequate specimens and inclusion of clinical characteristics (such as sex, dimension of the nodule, character of the gland by palpation) have been reported.[11–13] To date, however, even with optimum cytological preparation, no clinical, radiological, or laboratory test is sensitive and specific enough to distinguish reliably whether a follicular lesion identified by fine-needle aspiration is benign or malignant.

If reliable markers for detecting malignant thyrocytes are available, accurate preoperative diagnosis of thyroid cancer and appropriate clinical treatment should be possible. Several molecules have been identified by immunochemistry and reverse-transcriptase PCR as potential targets for immunocytodiagnosis of thyroid malignant disease. Among these, CD44v6 and galectin-3 seem to be promising.[14–17] CD44 is a polymorphic family of immunologically related cell-surface glycoproteins, which have a functional role in regulating several physiological and pathophysiological processes, including cell-cell and cell-matrix interactions, cell migration, and tumour growth and progression.[15,18] CD44 can be expressed on the cell surface as a standard receptor (CD44s, the putative receptor for hyaluronic acid), as well as multiple isoforms (CD44v), the expression of which is qualitatively and quantitatively altered during tumour growth and progression.[15,18–20] Interestingly, under normal conditions, only CD44s is expressed on the cell surface of non-proliferating thyrocytes.[14–17] By contrast, previous studies showed at molecular[14,21] and protein level[14,22–25] that expression of the β-galactosil-binding protein galectin-3, which is also involved in regulating cell-cell and cell-matrix interactions, is restricted to malignant transformed

thyroid cells. This study aimed to assess, in a large multicentre analysis, the clinical usefulness of a novel and promising immunocytomorphological approach to the preoperative characterisation of thyroid nodules.

Methods

Monoclonal antibodies and immunochemical assay

An extensive immunophenotypical analysis was done on 1009 thyroid lesions, comprising well-characterised formalin-fixed and paraffin-embedded tissue specimens (618 cases) and cell-blocks (165 cases) from selected benign and malignant lesions (retrospective analysis), as well as fresh cytological samples (226 cases) obtained preoperatively by ultrasound-guided fine-needle aspiration, from patients with palpable thyroid nodules, candidates for surgical resection (prospective study).

Monoclonal antibodies to CD44v6 (R&D System Inc, Minneapolis, MN, USA) and galectin-3 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) were used in immunohistocytochemistry according to the manufacturers' instructions, after antigen-retrieval microwave treatment of tissues and cell substrates, in 0·01 mol/L citrate buffer, pH 6·0. Immunohistocytochemistry used an indirect avidin-biotin complex immunoperoxidase method (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA, USA). Slides were incubated overnight with selected monoclonal antibodies at 4°C in a moist chamber. Purified monoclonal antibody directed at the CD44v6 specific epitope was used at concentrations of 10–20 mg/L and monoclonal antibody to galectin-3 was diluted 1 in 200 to 1 in 500, depending on the antibody concentration in the batch.

The enzymatic activity was visualised with 3-amino-9-ethyl-carbazole.[14] Slides were counterstained with Mayer's haematoxylin and mounted in Glycergel (Dako Corporation, Carpinteria, CA, USA) for microscopy. The positive cases were classified as + when staining was restricted to 10% or less of the lesion (histological preparations) or 10% or less of the thyroid cells (cytological smears); ++ for staining in 11–49% of the lesion or thyroid cells; and +++ for staining in more than 50% of the lesion or thyroid cells.

Six internationally qualified institutions took part in the study. Specific guidelines for the immunohistocytochemical assay and its evaluation, and for the morphological classification of thyroid lesions,[9] were distributed to the participants. The immunohistocytochemical evaluation and the definitive histological characterisation of the resected thyroid lesions were done independently by at least two experienced pathologists. This study was carried out according to the ethical guidelines of the Declaration of Helsinki. Specific approval was also obtained from each institutional scientific board.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of the proposed immunodiagnostic method were assessed both for galectin-3 and CD44v6 coexpression and for galectin-3 expression alone. The histomorphological diagnosis was taken as the gold standard. Sensitivity was defined on the basis of thyroid cancer immunodetection as: the number of carcinomas with positive results as a percentage of the total number of thyroid cancers. Specificity was defined as the number of benign thyroid lesions with negative results as a percentage of the total number of benign lesions. Frequency was the number of thyroid cancers divided by the total number of individuals in our study. The positive and negative predictive values were respectively calculated as: the number of carcinomas with positive results as a percentage of the total number of cases with positive results, and the number of benign lesions with negative results as a percentage of the total number of cases with negative results. Diagnostic accuracy was defined as (frequency×sensitivity)+(1-frequency×specificity). STAT statistical software (version 6) was used to calculate these variables.

Results

Retrospective analysis of thyroid samples

618 samples of thyroid tissue taken from patients who had undergone surgical resection of the thyroid gland for benign or malignant lesions were tested for galectin-3 and CD44v6 expression (table 1).

In 75 cases of normal thyroid tissues, neither CD44v6 nor galectin-3 molecules were expressed. Among 287 histologically defined benign thyroid tissue samples, comprising different forms of thyroiditis, nodular hyperplasias, adenomas, and the normal cases, 280 did not express galectin-3, whereas increased expression of CD44v6 was detected in proliferating lesions (hyperplasias and adenomas).

Five of seven instances in which galectin-3 was heterogeneously expressed were follicular adenomas in which no capsular penetration or vascular invasion was visible on histology. In these cases, galectin-3 immunostaining was restricted to less than 20% of the cells, which were generally clustered immediately beneath the tumour capsule. In two cases of Hashimoto's thyroiditis, galectin-3-positive thyrocytes were seen intermingled with activated lymphoid follicles. Of these seven galectin-3-positive cases, six coexpressed CD44v6.

Diagnosis by histology	Total (n=618)	Number positive for:		
		CD44v6	Galectin-3	CD44v6 and galectin-3
Normal thyroid	75	0	0	0
Graves' disease	1	0	0	0
Hashimoto's thyroiditis	4	1	2*	1
De Quervain's thyroiditis	3	0	0	0
Other forms of thyroiditis	22	2†	0	0
Nodular hyperplasia	50	21	0	0
Oncocytic adenoma	7	2	1‡	1
Follicular adenoma and variants	125	54	4‡	4
Minimally invasive follicular carcinoma	40	33	37§	30
Widely invasive follicular carcinoma	17	16	17	16
Oncocytic carcinoma (follicular and papillary)	13	7	13	7
Papillary carcinoma and variants	201¶	178¶	195	173
Anaplastic carcinoma	20	8	18	8
Insular carcinoma and other poorly differentiated carcinomas	20	11	13	10
Medullary carcinoma and variants	7	1	3	1
Follicular neoplasms of indeterminate malignant behaviour	13	7	11	7

*Clusters of cells expressing galectin-3 were detected intermingling with lymphoid nodules. †Scattered or isolated CD44v6-positive cells. ‡Variable/heterogeneous staining of <20% of the cells, at the periphery of the follicular nodule (beneath the tumour capsule). §In the three negative lesions, a limited focus of capsular invasion was shown only in the original histological preparation. This morphological feature of malignancy was lost in the tissue sections used for immunostaining. ¶One case was not assessed for CD44v6 (it was galectin-3 negative).

Table 1: **Retrospective analysis of CD44v6 and galectin-3 expression on resected thyroid lesions**

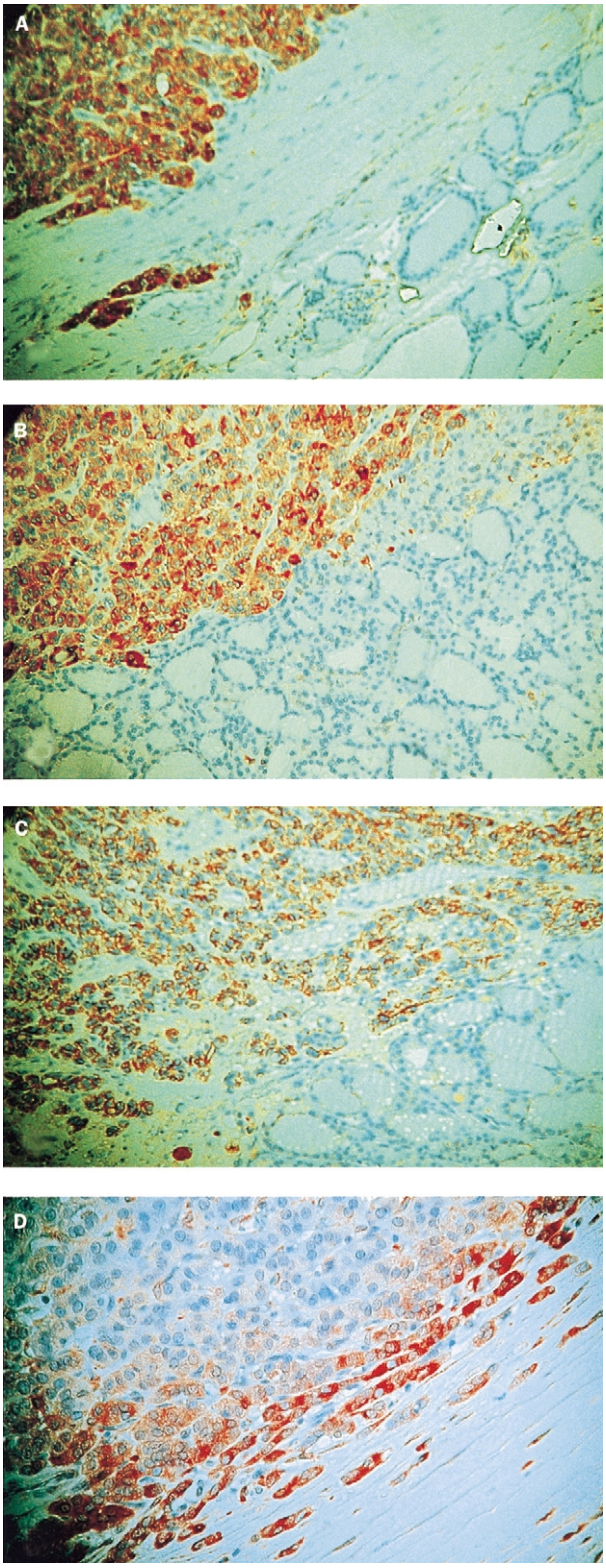

Figure 1: **Immunohistochemical detection of galectin-3 and CD44v6**

A,B=galectin-3-expressing follicular carcinoma at the border with normal thyroid tissue (A) and hyperplastic thyroid follicles (B); normal and hyperplastic follicles are visible on the right side of the pictures. C=CD44v6 expression in the lesion shown in A. D=a follicular/trabecular solid tumour, morphologically classified as "follicular neoplasm with indeterminate malignant behaviour", showing penetrating galectin-3-positive cells; these cells were mostly distributed at the periphery of the lesion, beneath the tumour capsule. Magnification A–C ×380; D ×400.

Variable	Galectin-3	Galectin-3 and CD44v6
Sensitivity (%)	94	79
Specificity (%)	98	98
Positive predictive value (%)	98	98
Negative predictive value (%)	94	81
Frequency (%)	52	52
Diagnostic accuracy(%)	96	88

This analysis excludes "follicular neoplasms of intermediate malignant behaviour" and medullary carcinomas. Five follicular adenomas (four classified under follicular adenomas and one oncocytic adenoma in table 1) coexpressing galectin-3 and CD44v6 were analysed as benign lesions, in accordance with the previous histological classification (gold standard).

Table 2: **Discrimination between benign and malignant thyroid lesions by immunodetection of CD44v6 and galectin-3 in retrospective study of thyroid histological samples**

Among 311 malignant thyroid lesions of differing histological types and degree of differentiation (medullary carcinomas excluded), 293 (94·2%) were positive for galectin-3 and 253 (81·4%) were positive for CD44v6 (figure 1). This marker was undetectable in about 50% of undifferentiated carcinomas, including anaplastic, insular, and other poorly differentiated malignant lesions. Most of the 22 CD44v6-negative papillary carcinomas were also poorly differentiated lesions. Galectin-3 was also not expressed in six of 201 papillary carcinomas and in nine of 40 undifferentiated tumours (table 1).

Among 40 minimally invasive follicular carcinomas, the result for galectin-3 was negative in three cases in which we could not demonstrate capsular invasion by histology. This morphological feature of malignancy was clearly revealed in the original haematoxylin-eosin preparation but it was lost in the adjacent tissue sections used for immunostaining.

For 13 suspicious follicular lesions, which were morphologically categorised as "follicular neoplasms of indeterminate malignant behaviour" because no morphological features of malignancy were clearly shown on histology, immunohistochemistry showed variable expression of CD44v6 and galectin-3 in seven and 11 cases, respectively (table 1, figure 1D). As expected, these markers were not useful for detecting medullary carcinomas (table 1), in accordance with the origin of these tumours from a different cell lineage (the calcitonin-secreting cells). The statistical analysis

Diagnosis by histology	Total (n=165)	Number positive for:		
		CD44v6	Galectin-3	CD44v6 and galectin-3
Thyroiditis	4	0	0	0
Nodular hyperplasia	32	2	0	0
Oncocytic adenoma	11	4	2*	2
Follicular adenoma and variants	35	15	3*	3
Minimally invasive follicular carcinoma	1	1	1	1
Widely invasive follicular carcinoma	18	17	15	14
Oncocytic carcinoma (follicular and papillary)	13	8	12	8
Papillary carcinoma and variants	46	43	45	42
Insular carcinoma and other poorly differentiated carcinomas	4	2	1	1
Anaplastic carcinoma	1	1	1	1

*In four of the five galectin-3-positive cases among the oncocytic and follicular adenoma categories, no morphological features supporting malignancy were found on histology of the corresponding resected lesions. A limited focus of capsular penetration was observed in one case.

Table 3: **Retrospective analysis of cell-blocks from fine-needle aspiration cytology**

Variable	Galectin-3	Galectin-3 and CD44v6
Sensitivity (%)	90	81
Specificity (%)	94	94
Positive predictive value (%)	94	93
Negative predictive value (%)	91	83
Frequency (%)	50	50
Diagnostic accuracy(%)	92	87

Five cases of follicular adenomas coexpressing CD44v6 and galectin-3 were analysed as benign, according to the absence of morphological features of malignancy, as reported by the original histological reports (gold standard).

Table 4: **Discrimination between benign and malignant thyroid lesions by immunodetection of CD44v6 and galectin-3 on cell-blocks from fine-needle aspiration cytology in retrospective analysis**

for this retrospective phenotypic study is shown in table 2.

Immunocytochemical analysis of cell-blocks

Cell-blocks were selected according to the availability of the definitive histological diagnosis on the corresponding resected lesions, which was taken as the gold standard. Among the benign thyroid lesions, galectin-3 was not detected in any cases of thyroiditis or nodular hyperplasia (table 3). This molecule was expressed in five of 46 follicular adenomas (including two oncocytic adenomas), in which the histomorphological features were not compatible with malignancy (no capsular penetration or vascular invasion were observed on histology of the resected lesions). Among 83 cell-blocks from thyroid cancers of differing histological type and degree of differentiation, galectin-3 was detected in 75 (90%) cases. Among the 18 widely invasive follicular carcinomas, three were galectin-3 negative; these results were confirmed by immunohistochemistry on tissue sections obtained from the respective resected lesions.

All of the three galectin-3-negative follicular carcinomas showed trabecular areas with barely differentiated follicular structures. Among the oncocytic carcinomas, one lesion negative for both CD44v6 and galectin-3 has been classified as a false-negative result. The immunohistochemical analysis of the corresponding surgical specimen clearly showed expression of both galectin-3 and CD44v6. In this case, fine-needle aspiration probably failed to provide a representative and adequate cytological sample of the lesion. Furthermore, three poorly differentiated carcinomas of insular type did not show any galectin-3 expression. On the other hand, increased expression of CD44v6 was detectable in adenomas as well as in thyroid malignant disorders. Altogether, CD44v6 was expressed in 72 (87%) of the 83 carcinomas, and coexpressed with galectin-3 in 67 (81%). Galectin-3 and CD44v6 were not coexpressed in benign lesions, except for five of 46 follicular adenomas. For such cases, the corresponding surgically resected lesions were reassessed by histology. Extensive and accurate morphological analysis, on several tissue sections derived from these lesions, showed a limited focus of capsular penetration in one of the cases (data not shown). Sensitivity, specificity, negative and positive predictive values, and diagnostic accuracy of this retrospective immunocytochemical assay are shown in table 4. In this statistical analysis the follicular adenomas coexpressing CD44v6 and galectin-3 were considered benign in accordance with the original histological report.

Diagnosis by histology	Total (n=226)	Number positive for:		
		CD44v6	Galectin-3	CD44v6 and galectin-3
Thyroiditis	2	0	0	0
Nodular hyperplasia	151	45	1	1
Oncocytic adenoma	4	2	0	0
Follicular adenoma and variants	35	21	2	2*
Minimally invasive follicular carcinoma	5	4	5	4
Widely invasive follicular carcinoma	6	6	6	6
Oncocytic carcinoma (follicular and papillary)	5	3	5	3
Papillary carcinoma and variants	12	11	12	11
Metastatic carcinomas (to lymph nodes)	6	6	6	6

*In these two cases no capsular penetration or vascular invasion was detected by histology in the respective resected lesions.

Table 5: **Prospective study of CD44v6 and galectin-3 expression on fresh cytological specimens**

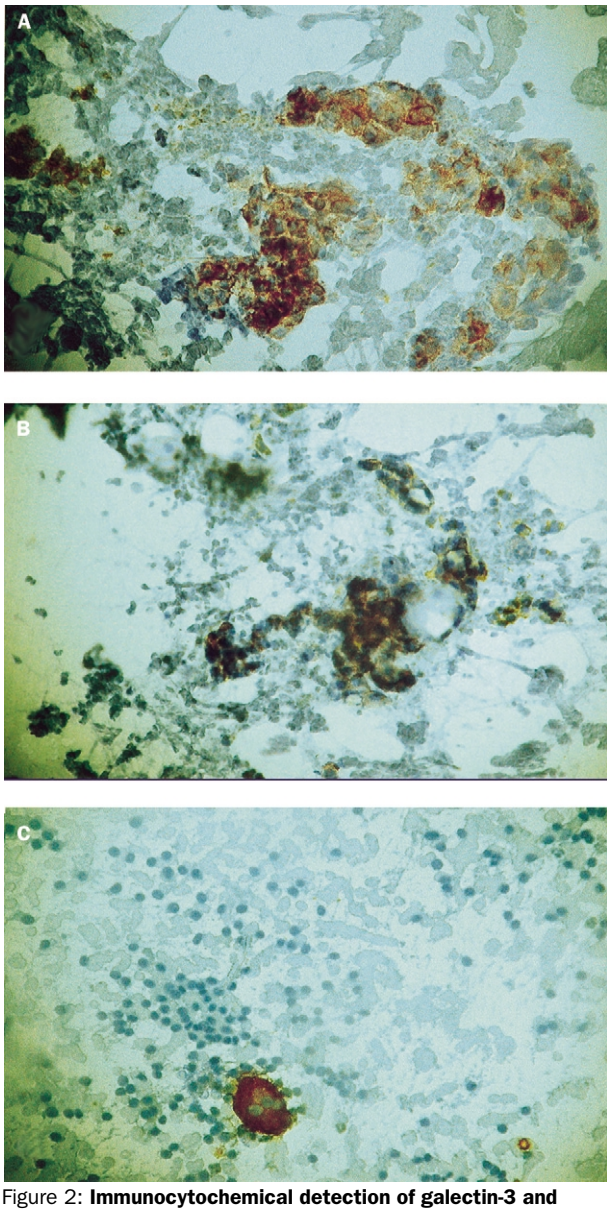

Figure 2: **Immunocytochemical detection of galectin-3 and CD44v6 on fresh cytological samples from preoperative fine-needle aspiration**

CD44v6 (A) and galectin-3 (B) expression in malignant cells from follicular carcinoma. Immunocytology from a nodular lesion histologically categorised as follicular hyperplasia, showing several galectin-3-negative thyrocytes (C). In this picture an internal positive control is represented by the galectin-3-positive histiocyte. Magnification A and C ×400; B ×380.

Variable	Galectin-3	Galectin-3 and CD44v6
Sensitivity (%)	100	88
Specificity (%)	98	98
Positive predictive value (%)	92	91
Negative predictive value (%)	100	98
Frequency (%)	15	15
Diagnostic accuracy(%)	99	97

Two adenomas and one hyperplastic nodule showing galectin-3 expression were analysed as benign according to the definitive conventional histological classification.

Table 6: **Discrimination between benign and malignant thyroid lesions by CD44v6 and galectin-3 immunodetection on fresh cytological material from preoperative fine-needle aspiration**

Prospective analysis

226 patients with palpable thyroid nodules, who were referred for surgery for clinical reasons, underwent ultrasound-guided, fine-needle aspiration. Cytological specimens were prepared for the conventional morphological evaluation as well as for the immunocytochemical assay. The respective resected lesions were examined histologically. As expected, the majority (126/151 [83·4%]) of the nodular hyperplasias in the context of colloid goitres, and ten of 12 papillary carcinomas, were promptly diagnosed by conventional cytology alone (data not shown). In all of the other lesions showing follicular morphological features, conventional cytodiagnosis was not conclusive. The generic definition of "follicular nodule" was reported in 25 of 151 (16·5%) of the nodular hyperplasias, in all of the follicular adenomas and carcinomas, and in two follicular variants of papillary carcinoma (data not shown). This finding strongly confirms the limits of conventional cytology in discriminating between benign and malignant follicular lesions.

Galectin-3 immunostaining was able to identify all of the true follicular thyroid malignancies, including five minimally invasive follicular carcinomas (table 5). CD44v6 was coexpressed with galectin-3 in the majority of these cases. Among 192 benign thyroid samples, galectin-3 was immunodetected in a few scattered thyrocytes (<8% of the cells) in one of 151 hyperplastic nodules, as well as in two of 35 follicular adenomas. Increased expression of CD44v6 was observed in proliferating benign and malignant thyroid lesions (figure 2). Statistical analyses of galectin-3 and CD44v6 immunodetection in discriminating benign and malignant thyroid lesions are shown in table 6.

Intercentre variability

To assess intercentre variability we analysed galectin-3 immunodetection on two homogeneous groups of lesions: the thyroid carcinomas (all histological types) and benign thyroid lesions (including normal thyroid, thyroiditis, hyperplastic lesions, and adenomas).

The sensitivity of galectin-3 in detecting thyroid cancer ranged from 87% to 100% (centre A 87%; B 93%; C 92%; D 100%; E 97%; F 100%). Centre A contributed the highest number of undifferentiated and insular thyroid carcinomas, some of which were galectin-3 negative (table 1). These rare tumours were sparsely represented in the collections of the other groups. The low intercentre variability was confirmed for the specificity of the test (A 93%; B 82%; C 99%; D 99%; E 97%; F 100%). Centre B contributed only 17 adenomas to the total of benign thyroid lesions, and three of these adenomas expressed galectin-3.

CD44v6 expression analysis was comparatively assessed on thyroid carcinomas (all histological types) and proliferative benign lesions (nodular hyperplasias and adenomas), because CD44v6 is a marker of deregulated thyrocyte proliferation (table 1). The sensitivity of CD44V6 immunodetecion for carcinomas ranged from 72% to 100% (A 72%; B 91%; C 94%; D 100%; E 84%; F 72%), but for benign proliferative lesions the range was 38% to 100%. This finding strongly discourages the use of CD44v6 immunodetection for discriminating among benign proliferative thyroid lesions. CD44v6 may be expressed transiently during benign cell proliferation. Moreover, we cannot exclude potential interference between specific pharmacological treatments administered for benign thyroid diseases and CD44v6 expression in untransformed thyrocytes.

Discussion

An estimated 4% of people in the USA between the ages of 30 and 60 years have one or more palpable thyroid nodules. Most of these lesions are benign, so the indication for their surgical removal should be as narrow as possible.[8,9] Although the majority of papillary carcinomas and most forms of thyroiditis are easily detected by fine-needle aspiration cytology, preoperative discrimination between benign and malignant follicular lesions is still very difficult.[8–13] In this study, six independent institutions worked together to validate the findings reported in a previous pilot study in which an immunocytodiagnostic approach to preoperative diagnosis of thyroid nodules was proposed.[14] The method, which integrates conventional fine-needle aspiration cytology with a simple and inexpensive immunophenotypical assay, is based on the detection of two markers, which are preferentially expressed during deregulated cell growth (CD44v6) and malignant transformation (galectin-3) of thyrocytes.[14–25] In this study, we found that immunodetection of galectin-3 on thyroid cells is a highly specific and sensitive strategy for identifying malignant lesions of the thyroid, on conventional histological and cytological preparations. Until now, there have been no clinical or laboratory tests that can reliably detect malignant thyroid lesions preoperatively. Although CD44v6 may strengthen the significance of galectin-3 expression on well-differentiated follicular lesions, this marker was not expressed in some papillary carcinomas, nor in about 50% of the undifferentiated malignant lesions. These rare lesions were not considered in the previous pilot study.[14] Although these tumours can easily be identified by conventional fine-needle aspiration cytology, the usefulness of CD44v6 immunodetection should be reconsidered in the light of our results.

However, galectin-3-positive cells were detected in five of 132 well-characterised follicular adenomas (table 1), and 11 of 13 (85%) of the so-called "follicular neoplasms of indeterminate malignant behaviour" were found to express galectin-3 also. These suspicious lesions, in which no morphological features of capsular penetration or vascular invasion are visible, cannot be classified with confidence as benign.[9] Galectin-3-positive cells observed in these neoplasms were confined beneath the tumour capsule, sometimes in invasive fashion (figure 1D). For these reasons, we are tempted to consider galectin-3-positive and morphologically suspicious follicular adenomas as potential early cancers, in which capsular penetration and vascular invasion are not yet apparent. The intriguing possibility of detecting early thyroid carcinomas with the help of galectin-3 immunostaining is supported by another important observation. In the group of the minimally invasive

follicular carcinomas, galectin-3-positive cells were mostly confined to the areas showing focal capsular penetration. These findings, together with the high sensitivity, specificity, and predictive value of this test,[14,22–25] suggest that galectin-3-expressing suspicious adenomas are potential early thyroid cancers with molecular evidence of transformation. For this reason, we suggest that such lesions should be classified as PETC-MET. Although these lesions will be treated according to good clinical practice, the expression of galectin-3 provides an objective criterion for improving the nosological assignation of the not yet classified thyroid neoplasms.

The finding of galectin-3-positive cells in a small number of cases of Hashimoto's thyroiditis deserves a separate discussion. Association between Hashimoto's thyroiditis and different types of thyroid cancers, as well as the discovery of RET/PTC1 and RET/PTC3 (the papillary-carcinoma-specific gene rearrangements), in several of these instances, has been reported.[26–29] However, no well-substantiated link between these findings and development of thyroid cancer has been made. In the few galectin-3-positive cases reported here, clusters of galectin-3-positive cells were intermingled with activated lymphoid follicles. Do these cells bring about an active immune response against early transformed thyrocytes? This hypothesis needs to be tested in a larger morphological, molecular, and epidemiological study, also in the light of the recently discovered PAX-PPARγ1 translocation, reported to be specific for follicular carcinoma.[30]

Our multicentre analysis shows that galectin-3 immunostaining represents a valuable adjunct to fine-needle aspiration cytology, which will help clinicians to take a decision. Nevertheless, this test method cannot be considered as a substitute for the conventional morphological and clinical assessment of each specific case.

Patients with cellular follicular smears that are morphologically suspicious can be referred to surgery with more confidence when galectin-3-positive follicular cells are detected. On the other hand, galectin-3-negative lesions will be referred to surgery only if clinical and pathological opinion agree. In cases for which such agreement is lacking, repeated clinical examinations (close follow-up), together with conventional fine-needle aspiration cytology and assessment of galectin-3 expression, will direct the therapeutic decision.

The wider application of this test method should help to prevent unnecessary surgical procedures and the use of radiolabelled iodine compounds for diagnosis of thyroid cancers. In the near future, the potential diagnostic value of serum concentrations of galectin-3 as a specific indicator of thyroid cancer should also be investigated.

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Contributors

Armando Bartolazzi initiated the project, designed and wrote the study protocol, monitored data collection and analysis, and edited the paper. Alessandra Gasbarri, Marco Paolo Martegani, Fabrizio del Prete, Raffaele Tecce, Teresa Lucante, and Pier Giorgio Natali proposed this diagnostic method in a previous pilot study, showing the rationale at molecular and protein level. They contributed by coordinating the prospective and retrospective studies at the Regina Elena Cancer Institute, Rome. Olle Larsson, Gianni Bussolati, Mauro Papotti, Ashraf Khan, Aldo Vecchione, Tiziana Pisani, Francesco Nardi, Antonella Marzullo, Enrico Saggiorato, Ferdinando Marandino, Anna Tofani, Hidenori Inohara, Yuikiro Honjo, and Fabio Orlandi coordinated activities and data collection at their centres and provided morphological characterisation of the lesions, evaluation of immunohistocytochemical staining, and final histological reports. Rino Bellocco contributed to statistical analysis and revision of the results.

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Clinical picture: The man allergic to money

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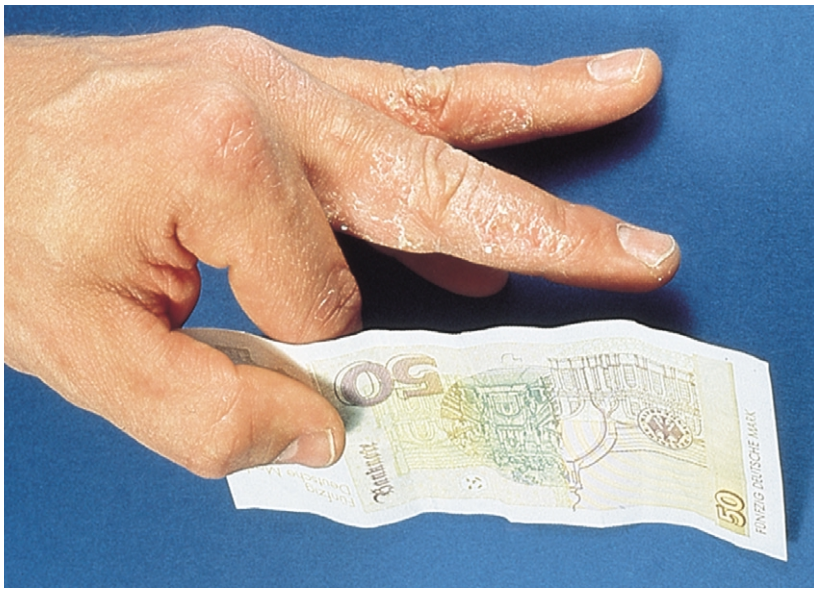

A 35-year-old cashier presented with bilateral hand eczema, suggestive of an allergic reaction to bank notes. Patch testing revealed sensitisation towards the dyes 4-phenylenediamine, dispersion orange 3, 4-aminoazobenzene and bismarck brown r. These dyes are used as printing colours and for staining synthetic materials, wax, leather, and textiles. The central printing office in Berlin declined to comment on the chemical composition of German bank notes, so we patch tested all denominations. The patient had an eczematous reactions to the brownish 50 deutsche mark (DM) note, but not to the blue 10 DM notes, or other bills. Thus, the patient had a contact allergy only to 50 DM notes.Certainly, this man will be one of the few people in Germany looking forward to the abolition of the DM due to the introduction of the EURO.

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