Dossier: Thyroid tumors

From the bench to the bedside. Galectin-3 immunodetection for improving the preoperative diagnosis of the follicular thyroid nodules

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Abstract

The authors discuss the principal aspects concerning the preoperative characterization of thyroid nodules, in particular those with follicular histology, and illustrate the potential clinical impact of a new diagnostic test-method, named “galectin-3 thyrotest”, which is based on the immunodetection of galectin-3 molecule in cytological specimens derived from thyroid nodular lesions. This diagnostic test method, which consistently improve the accuracy of conventional cytology, has been recently ylidated in a large international multicenter study and is going to impact hardly the clinical management of patients bearing thyroid nodular diseases. The rationale of this new diagnostic approach, the possibility to improve its performance by using large needle aspiration biopsy (LNAB) in selected cases, together with technical and operative details are presented and discussed.

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1. Introduction

Fine needle aspiration (FNA) biopsy is an essential procedure in the diagnosis and management of thyroid nodules. Ultrasound-guided thyroid FNA with disposable 20/21 gauge needles has many advantages including the simplicity of the technique, good accuracy, minimal patient discomfort, low cost and availability as an office procedure. Application of this method to the preoperative assessment of the thyroid nodules has contributed greatly to select appropriate patients for surgical resection of the lesions [1,2]. However, although most papillary carcinomas, colloid cystic hyperplasias and different forms of thyroiditis are generally easily diagnosed by FNA cytology, preoperative distinction between benign (hyperplastic or adenomatous nodules) and malignant follicular lesions is very difficult, if not impossible, on cytological bases alone [3,4].

This diagnostic limit is mostly due to the overlapping cytological features of benign and malignant follicular tumors and more specifically to the fact that a reliable distinction between these lesions requires the histological demonstration of capsular and/or vascular invasion. It is easily understandable that the aforementioned morphological aspects of malignancy cannot be appreciated on cytological smears. Moreover, up to date, no clinical, radiological or laboratory test is sensitive and specific enough to distinguish whether a follicular neoplasia identified on FNA-cytology is benign or malignant. As a consequence, patients with cytological report of “follicular nodule” or “follicular proliferation not otherwise specified” are frequently referred to surgery more for diagnostic purpose rather than for therapeutic necessity. The current clinical management to follicular thyroid nodules, however, has a heavy social cost for both patients and health system. In fact less than 10–12% of the surgically resected lesions will be definitively classified as carcinomas at the final histo-

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logical evaluation. This means that the large majority of thyroid nodules are overtreated. The availability of reliable markers for detecting transformed thyrocytes may allow an accurate preoperative diagnosis of thyroid cancer and consequently the appropriate clinical treatment.

Structural and functional studies on new candidate molecules, which can be potentially used as molecular targets for human malignancies, contributed greatly to improve the diagnostic accuracy of conventional cytology in several solid tumors [5,6] as well as to develop new therapeutic strategies in human oncology [7]. Useful markers of thyroid tumors can be divided into two major categories: those related to cell types (TTF-1, calcitonin, CEA, chromogranin) and those related to the type of pathology. Among the latter, the most promising markers of thyroid cancer seem to be galectin-3 and HBME-1 [8–12].

2. Galectin-3 and the preoperative characterization of thyroid nodules

Recently several laboratories demonstrated that galectin-3, a β-galactosil-binding protein involved in regulating cell cycle and apoptosis, thyroid cell transformation and tumor progression, was consistently expressed in the cytoplasm of malignant transformed thyrocytes [9–11,13]. The availability of specific monoclonal antibodies (mAbs) to detect galectin-3 in vitro and in vivo allowed the development of an easy and cheap immunocytochemical method, which combines a morphological and phenotypical evaluation of thyroid cells. This test method worded “galectin-3 thyrotest”, has been recently validated in a large multicenter study [11] and is going to improve consistently the clinical management of patients bearing thyroid nodules.

In the aforementioned international multicenter study, expression of galectin-3 has been evaluated in more than 1000 thyroid tissue specimens including 226 cytologic preparations obtained by thyroid FNA from thyroid nodules of patients candidates to surgery [11]. Although galectin-3 expression was lost in some poorly differentiated thyroid carcinomas, as well as in several anaplastic carcinomas, it was expressed in almost all of the well-differentiated carcinomas of follicular origin. This fact is very important because for the reasons reported above, benign and malignant follicular lesions cannot be distinguished on conventional cytological bases alone.

In Table 1 are shown sensitivity and specificity of galectin-3 immunodetection in discriminating benign versus malignant thyroid lesions, as reported in the current literature.

The best performance of galectin-3 thyrotest observed in the prospective analysis is due to the fact that undifferentiated galectin-3 negative thyroid neoplasms are quite rare in this collection of cases. Moreover, anaplastic and poorly differentiated thyroid cancers are generally promptly diagnosed by conventional cytomorphological methods. It appears that integration of galectin-3 immunostaining with the conventional cytological and clinical diagnostic procedures represents a sensitive inexpensive and reliable diagnostic approach for preoperatively identifying thyroid malignancies.

An optimized galectin-3 thyrotest for evaluation of galectin-3 expression on conventional cytological smears is currently in use in several specialized centers for the treatment of thyroid diseases. This test-method includes a purified mAb to galectin-3 (Mabtech, Naka, Sweden) and a biotin-free immunoperoxidase detection system.

The availability of a purified mAb that is used at concentration ranging from 5 to 7 mg/ml is very important in order to guarantee the standardization of the method for diagnostic purpose and the reproducibility of the results. Although several not purified mAbs to galectin-3 are commercially available, other laboratories and we experienced conflicting results during their use in indirect immunoperoxidase. The variability of the antibody concentration in each batch and the consequent variability of their work dilution may cause, in some instances, tissue background and false positive results. These problems are also amplified when avidin/biotin complex immunoperoxidase staining kits are used for this purpose [14].

3. Large needle aspiration biopsy (LNAB) as useful substrate for immunodetection of galectin-3 in thyroid cells

Although cytological samples obtained from FNA should represent the most simple and convenient substrates for application of galectin-3 thyrotest, the first generation of mAbs to galectin-3 work better on formalin fixed and paraffin

| Table 1 |
| Discrimination between benign and malignant thyroid lesions by using galectin-3 thyrotest |

| Archival histological samples (retrospective analysis on 783 cases) |
|------------------|------------------|
| Sensitivity     | 94%              |
| Specificity     | 98%              |
| PPV             | 98%              |
| NPV             | 94%              |
| Prevalence      | 52%              |
| DA              | 96%              |

| Cytological samples from preoperative FNA (prospective analysis on 226 cases) |
|------------------|------------------|
| Sensitivity     | >99%             |
| Specificity     | 98%              |
| PPV             | 92%              |
| NPV             | >99%             |
| Prevalence      | 15%              |
| DA              | 99%              |

PPV = positive predictive value; NPP = negative predictive value; DA = diagnostic accuracy (from Bartolazzi et al. [11] modified).
embedded tissue specimens, with respect to the conventional cytological smears.

For this reason and for minimizing the occurrence of false negative results, galectin-3 thyrotest is currently applied on cytological material adequately processed for obtaining paraffin embedded cellblocks [15]. This procedure requires specific facilities for conventional histology.

A second generation of mAbs specific to galectin-3 is going to be created with the aim to apply this test-method to conventional cytological smears. This achievement will contribute greatly to a larger diffusion of the galectin-3 thyrotest also in those diagnostic laboratories that have not access to routine histological facilities.

On the other hand, LNAB represents an optimal substrate for galectin-3 thyrotest.

If it is true that LNAB is relatively more invasive than conventional FNA, the tissue biopsy derivation from this procedure can be easily processed as a conventional tissue specimen. LNAB provides histological detail of the nodular thyroid lesion that cannot be observed by using FNA cytology. LNAB of thyroid nodules may represent an alternative diagnostic procedure also in those instances in which FNA is inadequate for lack of cells [16].

LNAB is a percutaneous needle aspiration technique, which provides specimens adequate for both histological evaluation [17] and immunophenotypical analysis [18], although as for FNA cytology, there is no possibility with this method to get information on capsular and/or vascular invasion of follicular tumors [1].

In 1980, Carpi et al. [19] started a comparative evaluation of palpable thyroid nodules (with size >1.5 cm) by using FNA cytology and LNAB. In their experience LNAB procedure can be used safely in the routine workup of patients bearing palpable thyroid nodules [19]. Of course the association of ultrasonographic thyroid scan can improve consistently LNAB of deep thyroid lesions.

Recently, after performing more than 2000 LNAB, the authors evaluated the role of this procedure in improving the diagnostic performance of thyroid nodules, in particular in those lesions that remained undiagnosed or suspicious of malignancy after FNA [20,21]. Suspected FNA cytological findings are mainly represented by the diagnosis of microfollicular nodule, or follicular nodules with atypia. The prevalence of these cytological diagnoses varies from <10% to 30%, depending on the selection criteria [19,22,23].

In the case of nodules diagnosed as “microfollicular lesions” by FNA, a preliminary observation was that the histological evaluation of the preoperative microbiopsies from LNAB let a distinction of such lesions in two groups: the homogeneous microfollicular nodules and the mixed micro–macrofollicular nodules [19].

Likely the latter represents benign hyperplastic lesions that may be followed by clinical observation (although follicular carcinoma with mixed follicular features have been reported), whereas pure trabecular or microfollicular nodules need a more careful evaluation in order to establish their biological features [20,21].

Galectin-3 thyrotest when applied in this setting can consistently improve the clinical management of these lesions, providing the possibility to discriminate between benign and transformed thyroid cells. Moreover, the availability of extra tissue sections may let the evaluation of additional thyroid markers (i.e. HBME-1, calcitonin, chromogranin, etc.) to complete the phenotypic analysis of the lesion.

4. Conclusion

All these data suggest that LNAB represent per se a useful diagnostic approach for the preoperative evaluation of thyroid follicular nodules. In some instances this procedure may integrate FNA cytology improving the morphological detail and consequently the preoperative selection (diagnosis) of the solid-trabecular or microfollicular suspicious lesions. Galectin-3 thyrotest when applied to biopsies derived from LNAB of thyroid nodules seems to work efficiently (personal communication) and offers some advantages compared to the same analysis performed on FNA derived cytological specimens. In fact, it is a common experience that immunodetection of galectin-3, by using the currently available mAbs, is more reliable when performed on histological setting with respect to the immunocytochemistry. The galectin-3 analysis can be easily repeated on seriated tissue section of the paraffin blocks and in some instances a comparative evaluation of different tumor associated antigens may be performed.

We are confident that either preoperative galectin-3 evaluation on cellblocks obtained from FNA or on tissue microbiopsies derived from LNAB of thyroid nodules will improve, in the next future, the diagnostic accuracy of the conventional morphological methods, contributing to a better selection of patients candidated for surgery.

References


