NEW ACHIEVEMENTS IN THE DIAGNOSIS AND TREATMENT OF THYROID NODULES AND CANCER: THE CONTRIBUTION OF THE ENDOCRINOLOGY RESEARCH UNIT OF SIENA.

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Abstract. Among solid malignancies, thyroid cancer is the one showing the greatest increase in incidence in the last 10-15 years. The most likely explanation for this increase is better detection as a consequence of more diffuse screening of thyroid disorders in the general population, although an intervention from environmental carcigogens may be considered. Fortunately, most of these cancers are detected in early phase and have an excellent long term prognosis when treated appropriately. However, about 10-15% of the cases have aggressive features at presentation, tend to progress rapidly and eventually will kill the patient. Thus, the effort of the scientific community in recent yeas has been devoted to better understanding of the natural biology of the disease and to develop new therapeutic strategies for difficult cases. This research is culminated in better definition of the oncogenes responsible of thyroid cancer, in the use of molecular diagnosis to direct different therapies and in experimental trials with new drugs of the tyrosine kinase inhibitor family. All together these new achievements are offering new benefits in the management of thyroid ancer.
Keywords: Thyroid cancer, thyroglobulin, recombinant human TSH, oncogenes, tyrosine kinase inhibitors, fine needle aspiration cytology.

Thyroid nodules have a high prevalence, occurring in more than 20% of the general population worldwide, especially in iodine deficient areas and in people older than 50 years (1). According to the method of detection, the prevalence of thyroid nodules ranges between 4% by palpation to 70% by neck ultrasonography (2-4). Neck ultrasound is widely used in clinical practice in several thyroidal and non-thyroidal diseases and it is responsible for the large increase detection of thyroid nodules and cancers, which is becoming a social issue for public health. Indeed, recent epidemiological data have shown that thyroid cancer is the human cancer at the largest increase in annual rate among all human solid cancers (5, 6). Considering that only 5-10% of thyroid nodules are of malignant nature, it is apparent that the clinical issue is the cost-effective differential diagnosis between benign nodules (the large majority, usually requiring no therapy) and the malignant ones (the small minority, deserving appropriate treatment). Fine-needle aspiration cytology (FNAC) is widely accepted as the most accurate, sensitive, specific and cost-effective diagnostic procedure in the assessment of thyroid nodules and helps to select people for surgery (4). Routine use of FNAC in the evaluation of thyroid nodules has reduced the need for thyroidectomy by 20-50% while increasing the yield of thyroid cancer diagnosis by 15-45% (7). Despite its relatively good sensitivity and specificity, FNAC has some limitations related to inadequate or indeterminate sampling. Inadequate sampling may occur in 10-20% of the aspirates and it is usually due to small size of the nodules, abundant vascular component or low experience of the operator. Indeterminate lesions account for nearly 20% of all FNAC which cannot be differentiated in benign or malignant based on cytological features and refer to an heterogeneous group of "follicular lesions" including follicular lesion of indeterminate significance (FLUS), follicular/Hürthle cell neoplasm (FN/HCN), and follicular lesions suspicious for malignancy, which correlate with increasing risk of malignancy of 5-10%, 20-30%, and 50-75%, respectively (8). Due to the lack of definitive diagnosis, most patients with indeterminate cytology undergo surgery, although only 8-17% of them will be found to have thyroid cancer (9, 10). Therefore, a significant proportion of patients in this category are submitted to unnecessary thyroidectomy and are exposed to the risk of surgical complications, contributing to the increase in medical costs.

To overcome this limitation, several diagnostic approaches complementary to FNAC, have been suggested. Ultrasound features have been analyzed and scoring systems have been created to predict the likelihood of malignancy in thyroid nodules (11, 12). To date, studies of various combinations of these features have failed to provide sufficient sensitivity and specificity to advocate their routine use. An attractive alternative has been the search of molecular markers of malignancy by immunocytochemistry, such as galectin 3 and HBME-1 (13, 14). Despite preliminary enthusiasm, extensive experience with these markers failed to provide clear evidence of better diagnostic accuracy compared to traditional cytology.

More recently, screening methods based on the search of molecular markers of malignancy have attracted the

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attention of several research groups, based on the elevated knowledge of the genetic alterations associated with thyroid tumorigenesis. Distinct genetic alterations in several proto-oncogenes have been associated with different histotype of thyroid cancer (papillary, follicular and medullary thyroid cancer) in the form of point mutations or gene rearrangements and altogether are present in nearly 90-95% of all thyroid cancer, but not in benign nodules. The genetic changes are usually mutually exclusive and most of the times are specific to different histotypes. Oncogenic RAS activation results from point mutations, affecting the GTP-binding domain (codons 12-13) in exon 1 or the GTPase domain (codon 61) in exon 2, (15, 16). The frequency of RAS mutations varies from 7-62% in different series (17-19).

BRAF activates the MAPK cascade thus promoting the transcription of target genes involved in cell proliferation, survival and apoptosis (20). The most common BRAF mutation is the substitution V600E which has been found in nearly 40% of papillary thyroid cancer (21).

The p53 gene encodes a nuclear transcription factor that plays a central role in the regulation of cell cycle, DNA repair and apoptosis. Alterations in the p53 tumor suppressor gene by inactivating point mutations, deletions or insertions usually involved exons 5-8 and have been described in poorly differentiated and undifferentiated thyroid carcinoma with frequencies of 22-83% in different series (22, 23).

The RET protooncogene is a 21-exon gene located on the proximal long arm of chromosome 10 that encodes a tyroisine kinase receptor. RET proto-oncogene mutations are represented by intra- and inter-chromosome rearrangements in which the tyrosine kinase domain of RET gene is under the promoter of an ubiquitary gene (24). Although many rearrangements (almost 20) have been described, RET/PTC1, RET/PTC2 and RET/PTC3 are the most frequently found in papillary thyroid cancer ranging from 3% to 60% in different series (25-27).

The PAX8 gene encodes a transcription factor essential for the genesis of thyroid follicular cell and regulation of thyroid specific gene expression. PAX8/PPAR rearrangement has been identified in a significant proportion of FTC (36–45%), follicular adenoma (FA; 4–33%), follicular variant of PTC (37.5%) or Hürthle cell carcinoma (28-31).

TRK rearrangements derive from intrachromosomal rearrangements of NTRK1 with TMP3 or TPR and have been found in approximately 10% of thyroid cancer (32).

On the basis of these observation, the search of genetic alterations in FNAC samples have attracted researcher's attention and for the first time the revised guidelines for the management of thyroid cancer published by ATA in 2009 (33) provided a level C recommendation in support of the use of molecular markers to help the management of patients with indeterminate cytology.

Many studies have demonstrated the feasibility of mutation detection in FNA samples from thyroid nodules and their contribution in improving the diagnostic accuracy of FNAC. Search of BRAF mutation in cytological material has been firstly reported by Cohen (34) with good results and subsequently confirmed by other authors (35-37). However, BRAF mutation account for no more than 30-40% of papillary thyroid carcinomas and thus cannot cover the entire spectrum of thyroid malignancies. Other authors have searched for a panel of oncogene mutations, including BRAF, RET/PTC and TRK (38-41). All these authors concluded that the identification of BRAF or RET/PTC refines the diagnosis of papillary thyroid cancer especially in FNAC with indeterminate cytology. However, the ideal tool should be to search for all oncogenes involved in thyroid tumorigenesis. This approach has been developed in our laboratory by Cantara et al. (42), who analyzed the presence of BRAF, RAS, RET/PTC, TRK, and PAX8/PPARγ mutations in 235 FNAC and the corresponding tumoral tissues taken at surgery. Mutations were found in 28.5% of the cytological samples and were always confirmed in the tissue sample. The presence of mutations at cytology was associated with cancer 91.1% of the times and with follicular adenoma 8.9% of the times, but never with hyperplastic nodules. Similarly to Nikiforov results, BRAF or RET/PTC mutations were always associated with cancer, while RAS mutations were mainly associated with cancer (74%) but also with follicular adenoma (26%). The diagnostic performance of molecular analysis was superior to that of traditional cytology, with better sensitivity and specificity, and the combination of the two techniques further contributed to improve the total accuracy (95.7%), compared to molecular analysis (92.8%) or traditional cytology (83.0%) alone. Overall the association of oncogene analysis and traditional cytology increases the diagnostic detection of thyroid cancer and may help to choose the best therapeutic approach.

At the present, the use of molecular biology for the evaluation of genetic alteration in FNAC samples seems a real promise. The few limitations represented by issues related to the technique (RNA recovery and subjective interpretation of the results) and to the operator (time consuming procedures and need of specialized laboratories), will be solved in future studies. After a diagnosis of thyroid cancer has been made, the next step is to ensure the more appropriate treatment for a disease that is becoming more and more frequent. Indeed, thyroid cancer, although representing less than 1 % of all human cancers, is the human neoplasm showing the highest rate of increase in the last 20 years, with an incidence of approximately 15 new cases per 100,000 people per year. Since most of these cancers are detected when they are very small (<1 cm), most experts believe that the main reason for the increase is a better diagnostic procedures and extensive scrutiny of the thyroid gland in the general population, although the influence of environmental factors cannot be excluded. Nowadays, the most frequent type of thyroid cancer is papillary, the less aggressive and the most curable of all thyroid cancers. It is essential that

these cancers be cured with the less aggressive procedures, avoiding over treatment. Initial treatment for thyroid cancer is total thyroidectomy and dissection of involved lymph node compartments. After total thyroidectomy, patients with differentiated thyroid cancer are treated with 131I activities aimed at ablating any remnant thyroid tissue and potential microscopic residual tumor. This procedure decreases the risk of locoregional recurrence. Radioiodine ablation is recommended in high risk patients and in low risk patients, while there is no indication in very low risk patients (those with unifocal T1 tumors, less than 1 cm in size, with favorable histology, no extrathyroidal extension and lymph node metastases). Effective thyroid ablation requires stimulation by TSH. In the past, this was achieved by withdrawing l-thyroxine rendering the patient severely hypothyroid. In the last 10 years, this procedure has been substituted with a more effective method that avoid the need of hypothyroidism. This method is based on the administration of recombinant human TSH (rhTSH) while the patient is on levo-thyroxine (LT4) therapy. Recent multicenter and prospective studies, including ours, have demonstrated that this preparation is highly effective and safe and that the rate of successful ablation is similar to that obtained with LT4 withdrawal when using 3700 MBq (100 mCi) or 1850 MBq (50 mCi) of 131I . Based on these results the use of rhTSH has been approved in Europe by the European Medicine Agency (EMEA) and in USA by the FDA.

After thyroid ablation, aim of follow-up is the early discovery and treatment of persistent or recurrent disease. To this purpose, we can rely on a specific and sensitive serum tumor marker, thyroglobulin (Tg) which is able to predict whether a patient is free of disease or has persistent or recurrent disease. Our laboratory has contributed in the definition of the use of Tg in the follow-up of thyroid cancer patients. At 6 to 12 months after surgery and thyroid ablation, the follow-up is based on physical examination, neck ultrasound, rhTSH stimulated serum Tg measurement. At this time most (nearly 80%) of the patients will belong to the low risk categories and will disclose normal neck ultrasound and undetectable (< 1.0 ng/ml) stimulated serum Tg in the absence of serum Tg antibodies. These patients may be considered in complete remission and their rate of subsequent recurrence is very low (<1.0% at 10 years). Patients in remission may be shifted from suppressive to replacement LT4 therapy, with the goal of maintaining a serum TSH level within the normal range. The subsequent follow-up of these patients should be based on yearly physical examination, serum Tg measurement on replacement LT4 and neck ultrasound.

The few patients with evidence of persistent disease, or with detectable levels of serum Tg increasing with time, require imaging techniques for the localization of disease and appropriate treatment, including therapeutic doses of 131I. Included in this category are the 5-10% of DTC patients that presented with local or distant metastases at diagnosis and an additional 5-10% that develop recurrent disease during follow-up. When appropriately treated, 2/3 of those patients with local disease and 1/3 of those with distant disease may achieve complete remission. Treatment of loco-regional disease is based on the combination of surgery and radioiodine therapy. External beam radiotherapy may be indicated when complete surgical excision is not possible or when there is no significant radioiodine uptake in the tumor. Distant metastases are more successfully cured if they take up radioiodine, are of small size located in the lungs (not visible at X-rays). Lung macronodules may benefit from radioiodine therapy but the definitive cure rate is very low. Bone metastases have the worst prognosis even when aggressively treated by combination of radioiodine therapy and external beam radiotherapy. Whenever radioiodine therapy is not effective and the disease progress, enrollment of the patients in experimental trials with tyrosine kinase inhibitor should be the treatment of choice. We are currently involved in several such clinical trials, enrolling patients with metastatic, refractory thyroid cancer, who are treated with tyrosine kinase inhibitor, obtaining promising results. Thanks to these trials, two of these drugs have been approved by the European agencies and are currently on the market.

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