ABSTRACT

DOCTORAL THESIS

BENIGN AND MALIGNANT THYROIDAL NODULAR FORMATIONS TREATMENT IN CORRELATION WITH THE PROGNOSTIC BIOMARKERS EVALUATION

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Key words: thyroid, thyroidal nodules, levothyroxine, synthesis anti-thyroid agents, thyroidal carcinoma.

PART I
CURRENT STAGE OF KNOWLEDGE IN THE FIELD OF THE THERAPEUTIC MANAGEMENT OF THYROIDAL NODULAR DISEASES

CHAPTER I
THE THYROID – EMBRYOLOGICAL, ANATOMICAL AND PHYSIOLOGICAL DATA

There are presented the embryology, the anatomy and the physiology of the thyroid gland, also describing the synthesis, the secretion, the transport and the metabolism of the thyroidal hormones. It is also presented the action mechanism of the thyroidal hormones at the level of the nuclear receptors and the adjustment mechanisms of the thyroid gland function.

CHAPTER II
THYROIDAL NODULAR DISEASE – UPDATED INFORMATION

Thyroidal nodules are frequently met entities in the medical practice, their majority being benign (only 5-10% of the cases are malignant lesions).

The thyroidal cancer represents approximately 1% of the overall cancer cases in the developed countries and represents the most frequent type of malignancy within the endocrine system. The thyroidal cancer appears by choice in youth and middle-aged adults and it is rarely met in children (the nodules prevalence is approximately 1-2%).

Factors influencing the appearance and the evolution of the thyroidal nodules are hormonal factors involving the chronic stimulation through TSH and other hormonal disorders, iodine input, ionising radiations, the life style, goitrogens and genetically determined factors. Genetically determined factors can intervene in two courses: by favouring cellular proliferation by oncogenes (mutations of the receptor TSH, mutations of the thyrosin kinase receptor involving TRK, RET, and MET genes, mutations of the ras genes, mutations of the gene codifying Gs and Gi proteins) and through inhibition of the cellular proliferation by antioncogenes (genes p15 and p16, gene p53, PTEN).

CHAPTER III
CURRENT STAGE REGARDING DIAGNOSIS AND TREATMENT OF THE THYROIDAL NODULAR DISEASE

Setting the diagnosis for thyroidal nodular formations is a complex process which uses a large range of methods and investigations, with the purpose of establishing as early and as righteous as possible the benign or malignant character of these, respectively the histopathological form and the real extension of the disease, because a treatment which was righteous applied in an early stage provides the healing chance.

A more detailed knowledge about the genetic mechanisms involved in the appearance and evolution of thyroidal cancers has enabled the introduction of new therapies aiming at these
mechanisms: aiming mechanisms (achieved through inhibitors of protooncogenes, angiogenesis inhibitors, growth inhibitors and apoptosis stimulators), redifferentiation therapy, gene therapy.

PART II
PERSONAL CONTRIBUTIONS

CHAPTER IV
CLINICAL STUDY PROTOCOL

The purpose of this doctoral thesis is that of performing a clinical study to analyse the possibilities of righteous diagnosis framing of both benign and malignant thyroidal nodular formations and at the same time it should be evaluated the efficiency of the therapeutic protocol applied depending upon the balancing of the thyroidal functional status and correlated with the dynamics evolution of certain biomarkers (calcitonins, thyroglobulins).

Material and method
The study performed was a prospective one and took place over the period January 2007 – March 2010.

The study lot was made up of a number of 324 patients, among which 271 were set the diagnosis of benign thyroidal nodular formations and 53 of malignant thyroidal nodular formations.

The patients’ selection criterion was represented by the presence during the clinical/echographic examination of at least one nodular formation at the level of the thyroid gland.

Patients were investigated through clinical examination and paraclinic examinations, subsequently being administrated the treatment and the surveillance of the evolution over the course of time of different investigated parameters.

CHAPTER V
DESCRIPTIVE ANALYSIS OF THE LOTS OF PATIENTS INCLUDED IN THE STUDY

The purpose of this analysis was that of establishing the frequency of the thyroid nodules and the benign/malignant report as compared with the cases overall with nodular thyroidal pathology and of correlating the achieved results with the data already existing in the specialty literature.

All the patients included in the study had had determined the TSH value (thyroidal stimulation hormone), resulting three groups of patients: 259 cases of euthyroidism (79.93%), 48 cases of hypothyroidism (14.82%) and 17 cases of hyperthyroidism (5.25%).

The structure by sexes of the lots of patients has revealed an obviously higher prevalence of the feminine gender as compared with the masculine one. The study lot was made up of 31 (9.57%) male patients and 293 (90.43%) female patients. With regard to the patients’ assignment by genders and by age groups there can be noticed that the highest frequency of the thyroidal nodules (both benign and malignant) is met in the group age 45-54 years old, both in men and in women.

After having performed the clinical and imagistic examination in patients included in the study it was ascertained that a number of 233 (71.91%) patients showed a sole nodule, and 91 (28.09%) showed multinodular goiter.

As far as the nodules nature, patients in the study were classified as follows:
♦ benign nodules in 271 (83.64%) of patients;
♦ malignant nodules in 53 (16.36%) of patients;
Of the overall cases involving malignant nodules, after having analysed the hystopathological bulletins, the following types of thyroidal carcinoma have been identified: papillary carcinoma 45 cases (84.91%), medullar carcinoma 4 cases (7.55%), follicular carcinoma 2 cases (3.77%) and undifferentiated carcinoma 2 cases (3.77%). Only cases involving thyoidal primary lesions were included in the study.

CHAPTER VI
CLINICAL AND THERAPEUTIC STUDY OF SUBJECTIVE AND OBJECTIVE ONSETS IN HYPERTHYROIDISM OR HYPOTHYROIDISM IN PATIENTS WITH BENIGN THYROIDAL NODULAR FORMATIONS

In hyperthyroidism and hypothyroidism case, the efficiency of the applied treatment can be appreciated by the improvement of the entire set of signs and symptoms caused by the excess or the deficit of circulating thyroidal hormones.

I have quantified the entire set of both subjective and objective onsets showed by the 17 patients’ lot with benign thyroidal nodules and hyperthyroidism at the beginning of the treatment, every month, every 3 months and every 6 months of treatment with synthesis antithyroid agents (thiamazole, carbimazole), beta blocking agents (metoprolol, propranolol), anxiolytic (bromazepam, alprazolam). The patients were haematologically monitored to detect neutropenia, as side effect of the treatment with synthesis antithyroid agents.

For the lot of 48 patients with hypothyroidism I have quantified the characteristic symptomatology and I have monitored the improvement every 1, 3 and 6 months. The administrated treatment was as follows: levothyroxine, diuretics (hydrochlorothiazide), cerebral vasoregulation compounds containing gingko biloba, neurotropes (piracetam), coronary vasodilators (trimetazidine, nitro-glycerines, isosorbide dinitrate) and glucocorticoids in patients with the set diagnosis of self-immune thyroiditis.

It has been found that by the applied therapeutic protocol it was achieved the improvement of both subjective and objective onsets in most of the patients of the two lots.

CHAPTER VII
STUDY OF THYROIDAL MORPHOLOGICAL AND CYTOLOGICAL MODIFICATIONS IN PATIENTS WITH THYROIDAL NODULAR FORMATIONS

In patients with thyroidal nodular lesions thyoidal morphology was investigated by means of the scintigraphic examination with $^{131}$I (in a 118 patients’ lot) and of the thyroidal echography. In patients with the set diagnosis of malignant thyroidal nodular formations, the thyroidal area showed by scintigraphy has either normal sizes or it is enlarged, showing catching areas either lowered or absent.

In patients having hyperthyroidism associated, RIC indicated increased cut-offs both every 2 hours, and every 24 hours, the catching being quick and excessive. In cases with diagnosis set of toxic adenoma, the thyroidal scintigram emphasized a limited catching only at the level of the thyroid nodule. In patients with diagnosis set of toxic multinodular goiter the scintigraphic appearance is of catching areas together with hypo or uncatching areas (scintigraphic appearance of “chess table”).

The thyroidal echography was performed in all patients included in the study, this being the method by which there was objectivised the presence of thyroid nodules, their number, dimension and localisation. The result of Chi-square test is smaller than 12.59, the significance threshold 95% of this
test, therefore there is not a significant difference with regard to the nodules and goiter spread depending on sizes and malignancy.

**CHAPTER VIII**

**CARDIOVASCULAR MODIFICATIONS EVALUATION IN PATIENTS WITH THYROIDAL NODULAR FORMATIONS BEFORE AND AFTER THE TREATMENT**

An important stage of the study is represented by the clinical and paraclinical evaluation of the cardiovascular apparatus in patients in the study lot with thyroidal tumour formations which have associated the thyroidal hypofunction and hyperfunction and it consisted in auscultation of cardiac noises, blood pressure cut-offs establishment, electrocardiograms (ECG) interpretation. After the initial evaluation, on the moment of the inclusion in the study of patients, I had achieved the re-evaluation of the cardiovascular parameters after 6 months and I recorded variations occurred following treatment administration.

Patients with thyroidal nodular formations and hyperthyroidism were re-evaluated after 6 months of treatment by clinical examination and EKG performance and it was noticed the persistence of the following modifications: throbbing in 3 patients (17.64%), high blood pressure in 2 patients (11.76%), atrial fibrillation in a patient (5.88%).

There was performed a re-evaluation of patients with thyroidal nodular formations and hypothyroidism after 6 months of treatment and it was found that for a number of 5 patients (10.41%) the subjective symptomatology of precordialgia type persisted, TA maintained high for a number of 8 patients (16.66%), and electric modifications on the ECG route persisted in 11 patients (22.91%).

**CHAPTER IX**

**EVALUATION OF THE METABOLISMS: GLUCIDIC, LIPIDIC, CALCIUM AND MAGNESIUM IN PATIENTS WITH BENIGN THYROIDAL NODULAR FORMATIONS BEFORE AND AFTER THE TREATMENT**

In patients with benign nodular formations with hyper and hypothyroidism, I have noticed metabolic unbalances in the beginning and after 3 months of endocrine specific treatment. Patients in whom metabolic unbalances did not improve after 3 months received medication aiming at the respective metabolic disorder.

**CHAPTER X**

**RESEARCHES ON THE EVOLUTION OF THE THYROIDAL NODULAR FORMATIONS THROUGH MEDICAMENTARY AND/OR SURGICAL TREATMENT**

I monitored the evolution under medicamentary and/or surgical treatment for the thyroidal nodular formations and I estimated their efficiency.

For a number of 17 patients the thyroidal nodular lesions were associated with hyperthyroidism. For the treatment of these patients I used synthesis antithyroid agents (ATS), beta-blocking agents, sedatives and I monitored the evolution of TSH and FT4 cut-offs under this treatment. The thyroidal function normalisation, which was achieved every 3 months in a patient (5.89%), every 6 months in 5 patients (29.42%), and for a number of 11 patients (64.71%) the normalisation occurred after 12 months.

The treatment of patients with hypothyroidism consisted in administration of levothyroxine in dosage adapted to each patient depending on the clinical form and the metabolic and visceral complications.
Of the lot of 206 patients with thyroidal nodular formations and euthyroidism I selected a group of 142 patients (68.93%) in which I applied a suppression treatment with levothyroxine for 12 months, for the purpose of detecting modifications of the nodular formations dimensions. Of the 27 patients in whom the thyroidal nodules decreased as sizes under the levothyroxine treatment, for a number of 16 patients (59.26%) the effect was maintained over the course of time, and in 11 cases (40.74%) the nodules began to grow again.

The therapeutic protocol in the case of the 45 patients with thyroidal papillary carcinoma included a surgical treatment, post-surgery ablation therapy and hormonal suppression therapy. The surgical treatment of these patients consisted in complete thyroidectomy for a number of 39 patients (86.67%) and quasi-total thyroidectomy in 6 cases (13.33%). TSH dosing every 3 months revealed the fact that of the 37 patients who followed a suppression treatment only with levothyroxine, the dose was efficient and enough to keep TSH cut-offs $\leq 0.1 \mu U/l/ml$ in 26 patients (70.27%). In 11 of the patients (29.73%) the thyroxine dose was adjusted by 25 micrograms. In the 8 patients who had received suppression therapy with levothyroxine and triiodothyronine, the TSH evaluation every 3 months of treatment indicated cut-offs $\leq 0.1\mu U/l/ml$ for a number of 6 patients (75%), and in 2 patients (25%) it was needed a supplementation by 25 µg of the levothyroxine dose. The 6 months’ evaluation emphasised in all the 8 patients TSH cut-offs TSH $\leq 0.1 \mu U/l/ml$.

Tiroglobulin evaluation prior to the surgical intervention in all the 45 patients with thyroidal papillary carcinoma indicated normal levels in all patients. 3 months after the surgery there were established the tiroglobulin levels which were undetectable ($< 0.1 \text{ ng/ml}$) for a number of 29 cases (64.45%) and it had detectable cut-offs, but within the normal interval in 16 patients (35.55%).

In all the 4 cases of thyroidal medullar carcinoma a wide surgical intervention was practiced: it was performed complete bilateral thyroidectomy accompanied by the dissection and removal of upper, lower and tracheoesophageal cervical ganglions. 6 months after the surgical intervention I evaluated the TSH level to monitor the efficiency of the suppression and hormonal substitution treatment and the base-line level of serum calcitonin. In 2 patients the calcitonin cut-off decreased below 100 pg/ml, and in 2 patients the cut-offs were kept over 100 pg/ml, a sign of residual disease or of remittance and it was decided the introduction into the therapeutic scheme the administration of somatostatin analogus (LAR octreotide). The subsequent evolution of the 4 patients with thyroidal medullar carcinoma was favourable under the administrated treatment and no decease was recorded over the period of study development.

In both cases of thyroidal anaplastic carcinoma the surgical intervention was radical and consisted in complete thyroidectomy with ganglionary removal. It was performed an external cervical radiotherapy and it was introduced a thyroidal substitution therapy with levothyroxine in doses of 150 µg daily. The treatment scheme in these cases was completed by chemotherapy: it was used the doxorubicin combination with cisplatin. The doxorubicin dose was 60mg/m² administrated every 3 weeks. 6 months after the surgery it was recorded the decease of the 76 years old patient, and 12 months after the surgery the 71 years old patient deceased.

CHAPTER XI
HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY AND INFLUENCE UPON THE THERAPEUTIC CONDUCT IN PATIENTS WITH BENIGN AND MALIGNANT THYROIDAL NODULAR FORMATIONS

In this chapter there have been analysed the histopathologic and immunohistochemical modifications for a lot of 165 patients with thyroidal nodular formations in which a surgical intervention was performed. Based on the achieved results it has been undoubtedly established the benign or malignant character of the thyroidal nodules, as well as the prognostic of the malignant forms. At the
end of the chapter, the results achieved by aspiration puncture with a fine needle in these patients, were compared with those achieved following the post-surgery anatomopathologic examination.

CHAPTER XII
CONCLUSIONS

1. The achieved study indicated first of all a greater spread of thyroidal nodules in female patients (90.43%) as compared with the male patients (men and women report is 9.4/1) and within the age group 45-54 years old for both sexes.

2. Malignant nodules were identified in 16.36% of the patients, indicating a significantly higher spread of the benign thyroidal nodules.

3. For the malignant nodular formations it has been found a frequency peak in young ages 15-24 years old and 25-34 years old. From the perspective of the morphologic type of thyroidal carcinoma, the following types were set a diagnosis in our study: papillary carcinoma, follicular carcinoma, medullar carcinoma and anaplastic carcinoma. The most frequently met form was that of thyroidal papillary carcinoma (84.91%).

4. The greatest part of thyroidal nodules was associated with the normal TSH levels. Of the total of patients with thyroidal nodular formations 79.93% showed euthyroid sick syndrome, 14.82% had hypothyroidism and 5.25% of cases were set the diagnosis of hyperthyroidism. All patients with malignant thyroidal nodules showed euthyroid sick syndrome.

5. In our study 31.6% of patients showed increased cut-offs of the anti tyrosine-peroxidase and antithyroglobulin antibodies levels and among these most of them were women.

6. In all 17 patients with benign thyroidal nodular formations with hyperthyroidism, the TSH level normalised within 6 months under treatment with thiamazole and carbimazol.

7. The treatment with synthesis and beta blocking antithyroid agents, in patients with benign thyroidal nodules and hyperthyroidism determined the disappearance of subjective adrenergic onsets (tremor, throbbing, sweat), after 6 months of administration, in over 80% of patients.

8. In 75% of patients with modified glycaemic cut-offs, from the lot of patients with benign nodules that have associated hyperthyroidism, the synthesis antithyroid agents treatment, the hypogluic diet and the oral hypoglicemiant agents administration determined normalisation of glycaemic cut-offs within a 3 month’s period.

9. In patients with benign thyroidal nodules with hyperthyroidism who indicated modifications of the total cholesterol cut-offs and triglycerides I noticed that synthesis antithyroid agents doses normalised these parameters after 3 months of administration.

10. In the lot of patients with benign nodular formations who had modifications of the calcium and magnesium cut-offs, association of calcium preparations, vitamin D and magnesium in the specific therapy determined the normalisation of calcaemia and magnesium in the blood cut-offs after 3 months.

11. During the administration of ATS therapy no modifications of haemoleucogram and leucocitary formula were recorded.

12. In the lot of patients with benign nodules and hypothyroidism, the intensity of lipidic metabolism modifications was correlated with the intensity of the thyroidal hormones deficiency.

13. Association of the hypolipemiant treatment, with fibrates and statins, at the treatment with levothyroxine, in patients with increased values of the total cholesterol, LDH-cholesterol, triglycerides, lipemia, achieved normalisation of these parameters in 80% of cases, in 6 months.

15. The thyroidal echography and scintigraphy, as well as the thyroidal puncture examination with fine needle, provided us important data for the diagnosis of the thyroidal nodular formations on which the subsequent therapeutic attitude was based.
16. Data achieved in our study suggest that thyroglobulin represents a useful biomarker to estimate healing in patients with differentiated thyroidal carcinomas, as well as in signalling appearance of relapse or distance metastases.
17. Only the 4 cases of thyroidal medullar carcinoma (7.55% of overall cases of thyroidal carcinomas in the study and 1.24 % of overall cases in the study) indicated increased cut-offs of the calcitonin biomarker. We consider that the calcitonin routine determination in all patients with thyroidal nodular formations is justified for the diagnosis of thyroidal medullar carcinoma.
18. In patients with thyroidal medullar carcinoma, calcitonin cut-offs increased following treatment are correlated with the relapse and presence of metastases.
19. In patients with thyroidal medullar carcinoma it was noticed that the LAR octreotide administration (somatostatin analogue) in doses at 20 mg once in 4 weeks has improved cut-offs of biomarkers: cromogranin A, serotonin, acid 5-HIAA, without decreasing the calcitonin cut-offs.
20. In the case of benign nodular formations associated with normal thyroidal function, levothyroxine administrated in suppression doses can reduce nodules size, but, after the treatment interruption, in a significant percentage of cases (40.74 %) nodules size began to grow again.
21. In patients with thyroidal medullar carcinoma, the immunohistochemical examination indicated additional aspects as compared to the anatomopathological examination, aspects that were useful in establishing the subsequent therapeutic conduct (administration of somatostatin analogues).
22. All patients with malignant nodular formations were submitted to surgical intervention having curative purpose, the selection procedure being complete thyroidectomy, practiced in 88.68% of cases, and for the rest it was chosen a quasi-total thyroidectomy.
23. The achieved results indicated that there is no statistically significant difference between malignancy and the size of the thyroidal nodules, either for sole nodules or multinodular goiters.
24. In the case of patients with operated malignant thyroidal nodules, the substitution treatment was performed with levothyroxine in doses of 100-150µg/daily for 84.91% of cases and in the rest of the cases it was used the association between levothyroxine 150 µg/daily and triiodotironin 50µg/daily.
25. The clinical study reveals that the thyroidal nodular disease it is a pathology in which the evolution of thyroidal nodules can be benign, but in small proportion can be malign, and from the hormonal function point of view the evolution can be toward euthyroidism, hypothyroidism or hypothyroidism.
26. Regarding therapeutic management in thyroidal nodular disease, the clinical study permits the settled of complex and differential therapeutical conduct of the patients with thyroidal nodules, as well as the evolution of the prognostic biomarkers under specific treatment in euthyroidism, hipo or hyperthyroidism.
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