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ORIGINAL ARTICLE

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Diagnostic and therapeutic approach to multiple endocrine neoplasia type 2B in pediatric patients

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Abstract Multiple endocrine neoplasia (MEN) 2B is a hereditary syndrome including medullary thyroid carcinoma (MTC), pheochromocytoma, gastrointestinal (GI) disorders, marfanoid facies, and multiple ganglioneuromas. MTC is the major cause of mortality, and often appears during the 1st decade of life. RET proto-oncogene mutations are responsible for MEN 2B. Other RET mutations cause MEN 2A syndrome, familial MTC, or Hirschsprung's disease. We studied three MEN 2B patients with the aim of delineating the best diagnostic and therapeutic protocol. The gold standards for diagnosis are histochemical study of the rectal mucosa and molecular analysis of RET, which in familial cases detects MEN 2B at a preclinical stage so that early total prophylactic thyroidectomy can be performed. In non-familial cases, the diagnosis can be suggested by the presence of GI symptoms, ganglioneuromas, and/or the typical facies. The intestinal innervation pattern, analyzed with the acetylcholinesterase technique, is pathognomonic for MEN 2B. In our protocol a rectal biopsy is, therefore, the first measure. The surgical treatment of MEN 2B is total thyroidectomy with cervical lymphadenectomy of the central compartment of the neck. When possible, this intervention should be performed prophylactically before 1 year of age.

Keywords Multiple endocrine neoplasia 2B · Medullary thyroid carcinoma · RET proto-oncogene · Thyroidectomy · Hirschsprung's disease

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Introduction

Multiple endocrine neoplasia (MEN) 2B is a rare autosomal-dominant hereditary syndrome characterized by the association of medullary thyroid carcinoma (MTC) and pheochromocytoma (PC) with gastrointestinal (GI) symptoms, marfanoid facies, and multiple ganglioneuromas [17]. MTC is present in 100% of MEN 2B cases, and often appears in the 1st decade of life [9]. MTC is the main cause of death in patients not receiving early or prophylactic treatment. Surgical treatment of MTC is the only therapy that has proved effective in cases with a localized tumor.

Molecular analysis of the RET gene has changed the history of this syndrome, as it allows the identification of MEN 2B mutations in asymptomatic patients and makes it possible to perform a prophylactic thyroidectomy in children. RET mutations can also be responsible for MEN 2A syndrome (MTC, PC, and hyperparathyroidism) or familial MTC (FMTC). The same RET gene is causative for Hirschsprung's disease (HD) in a variable percentage of patients [12, 15], and HD can be associated with MEN 2. Molecular analysis has provided an important contribution to understanding the Ret protein functions and correlating the genotype and phenotype in Ret mutation carriers.

This paper presents three cases of MEN 2B with the aims of underlining the importance of early diagnosis and treatment and designing a protocol meeting these requirements.

Materials and methods

We reviewed MEN 2B patients treated in our institution. The preoperative work-up, type of intervention, and follow-up are presented for each patient.

One was operated upon in 1980, when neither molecular nor histochemical diagnosis was available; the others were operated upon in 1995 and in 1997, respectively. Genetic analysis of the RET proto-oncogene, allowing the molecular diagnosis of MEN 2, has been available at Gaslini Institute since 1994. The analysis was performed in MEN 2A and 2B familial cases, subjects presenting with sporadic MTC or PC, in patients with HD.

Exons 10, 11, 13, 14, 15, and 16 of the RET proto-oncogene were analyzed for the detection of point mutations. In MEN 2A/FMTC cases, the analysis was first conducted in exons 10 and 11 by denaturing gradient gel electrophoresis (DGGE) and subsequently in the remaining exons [13, 14, 15, and 16] if alterations had not been previously identified. In MEN 2B patients, exon 16 was primarily screened since more than 90% of cases present the M918T mutation [6]. Finally, all HD patients were screened for mutations in exon 10, since some families presenting with the association of HD and FMTC/MEN2A have been reported to segregate one of the mutations affecting the cysteine residues in exon 10 [6]. The analysis of the same exons was also carried out in sporadic PC patients.

DGGE analysis was performed as previously reported [8]. Sequencing of the altered polymerase chain reaction (PCR) products was performed directly using dye terminator chemistry (Dye terminator cycle sequencing kit, ABI Prism, Perkin Elmer, Norwalk, CT) following the user's manual instructions. Electrophoresis of the cycle-sequencing products was carried out in an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA) and results were analyzed using appropriate software.

In all the patients, basal plasma calcitonin (CT) was measured. Values lower than 14 pg/ml and 19 pg/ml were considered normal in females and males, respectively [7]. The pentagastrin test (PGT) was performed in one case by bolus infusion of 0.5 μg/kg pentagastrin in 5 ml of 0.9% NaCl. Plasma CT was measured before and 1.5 and 5 min after the infusion of the bolus. Stimulated CT values were considered normal when lower than 30 pg/ml in females and 110 pg/ml in males [7, 20].

In two patients histochemical study of intestinal innervation by the acetylcholinesterase (AChE) activity technique described by Karnovsky and Roots [11] was performed. The specimen was acquired by rectal suction biopsies in one patients and a sigmoid segment proximal to an aganglionic portion during pull-through for associated HD in the other.

Resected thyroids were weighed, measured, fixed in formalin, and divided into three parts: the right and left lobe and isthmus. Each part was divided by transverse serial sections and embedded in toto. Histologic sections were obtained from specimens embedded in paraffin using the semiserial section technique. The sections were stained alternatively with hematoxylin-eosin and histochemical reactions for thyrocalcitonin (BioGenex, prediluted, polyclonal), chromogranin A (BioGenex, prediluted, clone LK2H10), and thyroglobulin (BioGenex 1:10, clone 2H11). For histochemical reactions, routine procedures for antigen unmasking were used (treatment in microwave oven in citrate buffer, pH 6, 10 mM). Dako Envison peroxidase was the system of detection used.

In cases 1 and 2 the tumor nodules detected macroscopically were excised in toto and tested with the same histochemical reactions.

Results

Case 1 A female who had been treated in other institutions since the age of 5 years for GI symptoms presented at the age of 13 years with a lateral cervical and a thyroid mass (no uptake on scintigraphy), marfanoid facies, and mucocutaneous neuromas. A clinical diagnosis of MEN 2B was made. Neither histochemical studies nor molecular analysis was available at that time. The latero-cervical mass was removed; histology showed lymph-node metastases of a MTC. The patient had a very high plasma CT level (20,000 pg/ml). She underwent a total thyroidectomy with bilateral cervical lymphadenectomy. Histology showed four thyroid nodules, the largest 2 cm in diameter, all representing MTCs.

After surgery, small bilateral cervical masses and high CT levels (about 4,000–10,000 pg/ml) persisted, and the patient underwent local radiotherapy. One of the lesions was biopsied and showed fibrous tissue. Subsequently, CT and carcinoembryonic antigen (CEA) levels further increased (to 80,000 pg/ml and 57 ng/ml, respectively) in the absence of metastases on bone scintigraphy and chest and abdominal computerized tomography (CT). She had frequent episodes of abdominal distension and meteorism. Three years after surgery, bone scintigraphy (multiple areas of hyperactivity: cranial, facial, sacral, dorsal), abdominal ultrasonography (US), and CT (multiple hepatic lesions) were positive, and treatment with cisplatin was started. The patient died 4 years later (7 years after surgery) of diffuse metastases.

Case 2 A 15-year-old girl presented with mild constipation alternating with diarrhea. Clinical observation showed marfanoid facies and multiple neuromas on the tongue. On contrast enema, the colon had a reduced caliber without haustra; some diverticula were present in the descending and sigmoid colon. A rectal biopsy analyzed by AChE showed submucosal plexus hyperplasia with submucosal ganglioneuromatosis and fibromatosis (Fig. 1); giant and ectopic ganglia were present in the muscularis mucosae and lamina propria. Two thyroid nodules were detected. The diagnosis of MEN 2B was made on the basis of the histochemical picture. The molecular data (mutation M918T of RET exon 16) confirmed the diagnosis, total thyroidectomy was performed. Histology showed two MTCs (Fig. 2). Six years after the operation, the patient is healthy and has not had recurrence of tumors in the thyroid or other sites.

Case 3 A 3-year-old female with gastrointestinal (GI) disorders characterized by constipation alternating with diarrhea since the first weeks of life presented with a radiologic picture of megacolon proximal to a short, narrowed distal segment. The AChE assay showed increased cholinergic fibers in the lamina propria and absent ganglia in the lower portion of the rectum. The patient was operated upon for HD using the Duhamel technique. The postoperative histochemical study of the resected proximal sigmoid colon showed intestinal ganglioneuromatosis and fibromatosis typical of MEN 2B. Screening for RET mutations showed a typical MEN 2B mutation (M918T), and the PGT was positive with a CT level of 240 pg/ml after stimulation at 5 min, whereas basal CT was normal (8.8 pg/ml). The patient under-

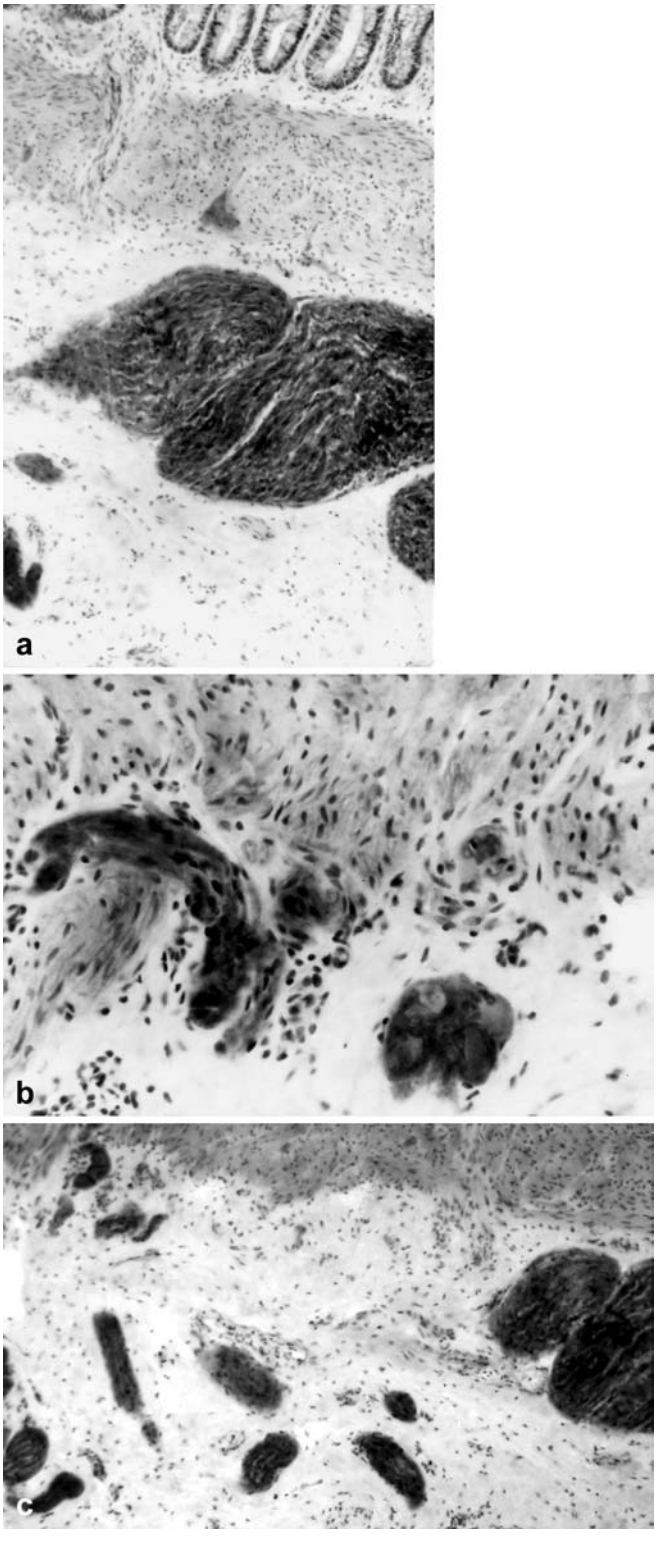

Fig. 1A–C. AChE activity in rectal suction biopsies of MEN 2B. **A** Submucosal neurofibromatosis (H & E × 100). **B**, **C** Submucosal hyperplasia with heterotopic ganglia in muscularis mucosae × 250 × 100

went total thyroidectomy at 3 years of age. Histology showed C-cell hyperplasia with 'in situ' MTC (Figs. 3 and 4). Three years after surgery, she is in good health.

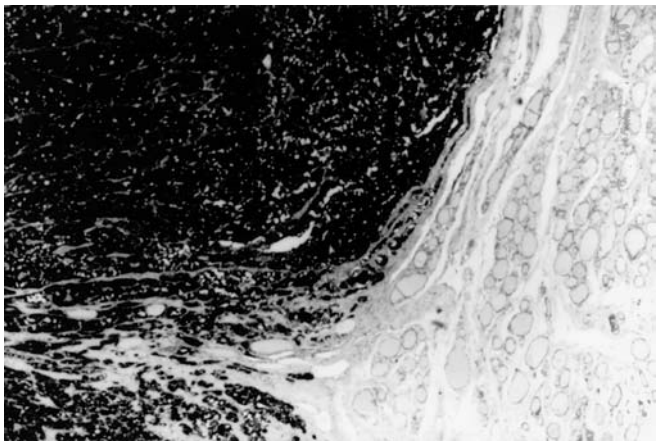

Fig. 2. Medullary thyroid cMTC associated with MEN 2B: invasive tumor seen on left (antithyrocalcitonin reaction, × 50)

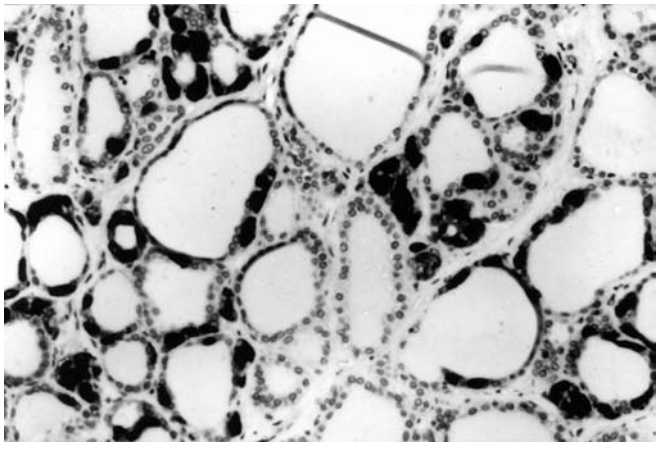

Fig. 3. C-cell hyperplasia associated with MEN 2B: C-cells form circumferential proliferation around follicule cells (antithyrocalcitonin reaction, × 250)

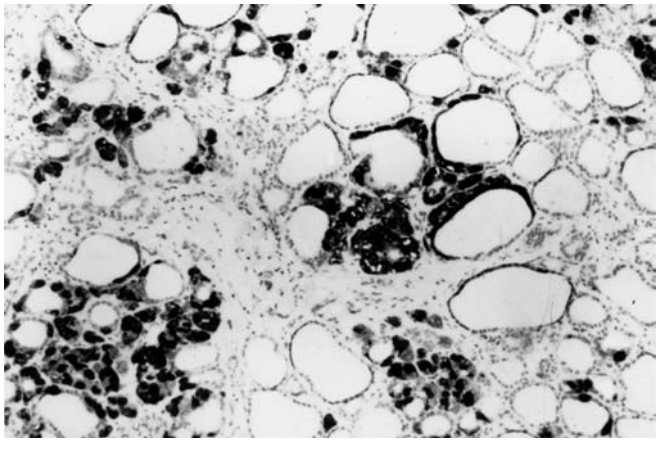

Fig. 4. C-cell hyperplasia with early medullary thyroid carcinoma associated with MEN 2B: C-cells not only proliferate circumferentially around follicle cells, but also in interstitium, forming small nodules (antithyrocalcitonin reaction, × 100)

Discussion

The prognosis in patients with MEN 2B depends on early diagnosis and surgical treatment. According to the literature, MTC occurs in 100% of cases of MEN 2B [9] and is very aggressive. When it becomes clinically manifest, it can be too late for curative surgery. Metastases are present at surgery in 45% of MEN 2B patients with clinical or biochemical evidence of MTC [18]. In our three cases, the patient who died had MTC with lymph-node metastases at 13 years of age, but had already shown signs and symptoms of MEN 2B at 5 years.

In agreement with the literature [13], in our patients the first clinical signs of MEN 2B affected the GI system (Fig. 5) and were associated in the two older patients with typical marfanoid facies and multiple ganglioneuromas. Marfanoid facies are not easy to identify in the first years of life, and ganglioneuromas are not evident at that time, but can be found if searched for carefully. GI symptoms of MEN 2B generally include constipation alone or alternating with diarrhea. These signs generally appear very early and are sometimes already present at birth, but rarely suggest the diagnosis of MEN 2B. In children with constipation or stipsis alternating with diarrhea, the presence of ganglioneuromas on the tongue and oral mucosa, as well as the typical facies of MEN 2B and a family history of MTC or PC should be determined. In suspected cases, a rectal biopsy has to be performed.

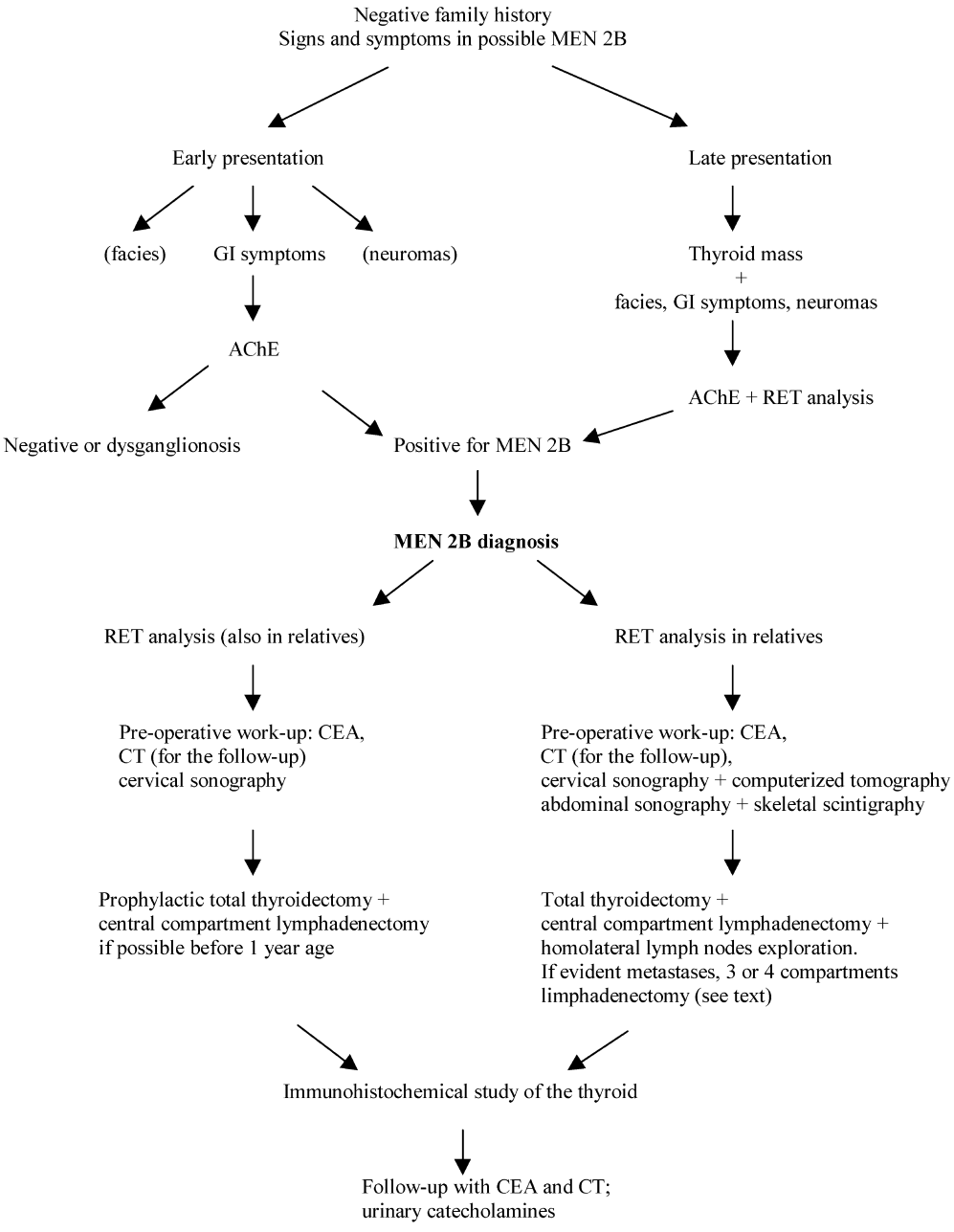

Fig. 5. Algorithm for diagnosis and treatment of MEN 2B

In two of our patients, MEN 2B was diagnosed by AChE enzyme-histochemical study [11]. In both cases the pathognomonic picture of MEN 2B was observed: submucosal-plexus hyperplasia with giant ganglia (ganglioneuromatosis), submucosal fibromatosis, and ectopic ganglia. In our opinion, a rectal biopsy should be performed first, as it allows diagnosis at an early disease stage. RET analysis is essential to confirm the diagnosis, and has to be extended to include relatives. All carriers of MEN 2B mutations should undergo a total thyroidectomy. On the basis of our experience and the literature [10, 13, 18, 21, 22], a prophylactic thyroidectomy is justified within the 1st year of life in patients with a genetic diagnosis of MEN 2B.

The presentation of MEN 2B with a thyroid mass can occur in cases with delayed diagnosis. In these patients neuromas, the typical facies, and GI symptoms are usually present. The diagnosis must be confirmed as soon as possible by rectal biopsy and molecular analysis in order to perform a total thyroidectomy and lymphadenectomy. Fine-needle aspiration of the mass is not advisable in our opinion because the result does not change the treatment, which in every case is surgical.

In the preoperative work-up we include cervical US and measurement of the biochemical MTC markers CT and CEA, which are useful for follow-up [7]. If a thyroid mass is present, it is advisable to perform CT, abdominal US, and skeletal scintigraphy to search for lymph-node, hepatic, or bone metastases.

CT after stimulation (PGT) was measured only in one of our patients and was positive. At surgery, she had 'in situ' MTC and C-cell hyperplasia.

In the past, the PGT was aimed at identifying MTC at a preclinical stage in subjects at risk. Today, it has been replaced by molecular genetic analysis, which is much safer. In pediatric patients, the PGT can be poorly tolerated and give false-negative results. When positive, it can indicate the presence of a carcinoma or C-cell hyperplasia [2, 7]. For these reasons, in our opinion this test is no longer indicated in the diagnosis of MEN 2B, whereas evaluation of basal plasma CT can play a role in follow-up to monitor possible recurrence.

Members of families with MEN 2B patients have to be screened for the M918T mutation. None of our patients had a family history of MEN 2B. Even in the presence of a family history, the genetic analysis should always be associated with histochemical studies of rectal biopsy, as this allows rapid diagnosis (1 day).

An interesting aspect of MEN 2 is the association with HD. It is well-known that RET mutations can be causative for both HD and MEN 2 [3]. In particular, in MEN 2A the most frequent RET mutation (85%) affects codon 634 of exon 11, while in MEN 2B codon 918 of exon 16 is almost always involved. In HD, RET mutations can affect any portion of the gene. The most frequent mutations found in patients with the association of HD and MEN 2A/FMTC involve codons 609, 618, and 620 of exon 10 [1, 5, 14, 19]. In HD patients, molecular analysis of standard MEN 2A/FMTC mutations is therefore recommended to identify that subpopulation of patients carrying mutations with a potential oncologic risk.

From 1994 to date, 420 HD patients were analyzed for mutations in exon 10 of RET at the Genetic Laboratory of the Gaslini Institute. In 3 patients a mutation in this exon was found. In addition, in 1 patient with the association of HD with MEN 2B (case 3), a mutation in exon 16 (M918T) was detected. This was the first case of HD associated with MEN 2B published in the literature [16]. In the 420 HD patients studied, 4 mutations with oncologic risks were found (1%).

The Ret protein is a tyrosine kinase receptor that plays an important role in the activation of signaling pathways through phosphorylation of key tyrosine residues in response to different ligands. In MEN 2A and 2B, functioning RET mutations result in constitutive activation of the tyrosine-kinase receptor, with subsequent phosphorylation and overtransmission of the signal by different downstream pathways. These latter can be specifically activated by the different mutations, which result in a large spectrum of possible phenotypes (MEN 2A, MEN 2B, FMTC, with different degrees of penetrance and expressivity). In contrast, RET mutations associated with HD are inactivating, loss-of-function mutations resulting in a reduction of the amount of functional RET protein on the cell surface. Mutations found in patients with HD and MEN 2 are able to activate the signaling pathways, as in isolated MEN 2, but the mutated isoform is unable to translocate to the cell surface. The result of activation in the thyroid and adrenal glands is tumorigenesis, while the decrease of functional protein on the cell surface causes the HD phenotype [6].

In agreement with the literature [18], we believe that a central-compartment cervical lymphadenectomy should be performed during thyroidectomy for MEN 2B. Homolateral lymph-node exploration (2 compartments) has to be performed in cases with macroscopic evidence of carcinoma at surgery, and bilateral lymphadenectomy (3 compartments) in the presence of evident lymph-node metastases. If mediastinal lymph nodes are metastatic on CT, the lymphadenectomy has to be extended to the mediastinum (4 compartments) [4].

Although autotransplantation of parathyroid glands in the forearm is often performed in pediatric patients [10, 18], we prefer to preserve them in their primary site in order to avoid the trauma and mechanical stress that occur frequently in children's upper limbs.

MEN 2B patients have to be followed yearly with measurement of CEA and CT for possible MTC relapse (more strictly in the 1st year after surgery) and urinary metanephrines and fractionated catecholamines (epinephrine, norepinephrine, dopamine) to monitor the possible development of PC.

In conclusion, early diagnosis and treatment of patients with the MEN 2B syndrome are essential to survival. The rarity of this syndrome can cause delayed diagnosis. MEN 2B is characterized by early clinical

signs as nonspecific GI disorders and, later, development of the typical facies and the presence of ganglioneuromas. These signs, which anticipate tumor development by many years, should suggest the diagnosis, which is based on rectal biopsy and genetic analysis. Our diagnostic algorithm of MEN 2B (Fig. 5) seems to offer the best life expectancy to affected patients.

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