Review Article

Thyroid Disorders in Homozygous β-Thalassemia: Current Knowledge, Emerging Issues and Open Problems

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Abstract. Changes in thyroid function and thyroid function tests occur in patients with β-thalassemia major (TM). The frequency of hypothyroidism in TM patients ranges from 4% to 29 % in different reports. The wide variation has been attributed to several factors such as patients' genotype, age, ethnic heterogeneity, treatment protocols of transfusions and chelation, and varying compliance to treatment. Hypothyroidism is the result of primary gland failure or insufficient thyroid gland stimulation by the hypothalamus or pituitary gland. The main laboratory parameters of thyroid function are the assessments of serum thyroid-stimulating hormone (TSH) and serum free thyroxine (FT4). It is of primary importance to interpret these measurements within the context of the laboratory-specific normative range for each test. An elevated serum TSH level with a standard range of serum FT4 level is consistent with subclinical hypothyroidism. A low serum FT4 level with a low, or inappropriately normal, serum TSH level is consistent with secondary hypothyroidism. Doctors caring for TM patients most commonly encounter subjects with subclinical primary hypothyroidism in the second decade of life. Several aspects remain to be elucidated as the frequency of thyroid cancer and the possible existence of a relationship between thyroid dysfunction, on one hand, cardiovascular diseases, components of metabolic syndrome (insulin resistance) and hypercoagulable state, on the other hand. Further studies are needed to explain these emerging issues. Following a brief description of thyroid hormone regulation, production and actions, this article is conceptually divided into two parts; the first reports the spectrum of thyroid disease occurring in patients with TM, and the second part focuses on the emerging issues and the open problems in TM patients with thyroid disorders.

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Introduction. In recent years our knowledge of hypothyroidism in children, adolescents and young adults with homozygous β-thalassemias (β-thal) has increased. Regarding clinical phenotype β-thal are classically classified as: major (TM), intermedia (TI), and minor (β-thal trait). Based on the need of transfusions for survival, homozygous patients are further characterized as Transfusion-Dependent Thala-ssemia (TDT), as are all patients with TM, and Non-Transfusion Dependent Thalassemia (NTDT) as are, with few exceptions, the TI patients. Patients with TDT require regular, lifelong blood transfusions for survival, starting before the age of 2 years (the "classic form" of TM).[1-4]

The etiology of thyroid disorders in these patients is substantially different from that of the general population because transfusional iron overload in TDT and increased iron uptake from the gastrointestinal tract in TI, are implicated in over 90% of morbidity and mortality in patients with b-thal. Therefore, the knowledge of risk factors influencing the development of hypothyroidism is a critical component of long-term monitoring and treatment of patients affected by TM.

Following a brief description of thyroid hormones regulation, production, and actions, this article is conceptually divided into two parts: the spectrum of thyroid disease occurring in patients with TM, and the emerging issues and the open problems in TM patients with thyroid disorders.

Regulation of Thyroid Hormones Production and Physiologic Actions. The thyroid gland is regulated by thyrotropin-releasing hormone (TRH) and thyroid stimulating hormone (TSH).[5] Thyroid-stimulating hormone (TSH) is a glycoprotein hormone secreted by the anterior pituitary. It usually exhibits a diurnal variation with a peak shortly after midnight and a nadir in the late afternoon. At the peak of this variation, the TSH can be double the value at the nadir.[6-8] Many signals from peripheral tissues can indirectly affect TRH secretion. These include gonadal hormones, leptin, and factors related to feeding, cold or sleep.[9-11]

Thyroxine (T4) and triiodothyronine (T3) are produced by the thyroid gland. The formation of thyroid hormones depends on an exogenous supply of iodine. About 100 μg of iodide is required daily to generate sufficient quantities of thyroid hormone. A healthy individual produces approximately 90 to 100 μg of T_4 and 30 to 35 μg of T_3 from the thyroid gland daily. An estimated 80% of the T_3 produced daily in humans is derived from peripheral metabolism (5'-monodeiodination) of T_4, with only about 20% secreted directly from the thyroid gland itself. T_4 and T_3 circulate bound primarily to carrier proteins. T_4 binds strongly to thyroxine-binding globulin (TBG, ~ 75 percent) and weakly to thyroxine-binding prealbumin (TBPA ~ 20 percent) and albumin (~ 5 percent). T_3 binds tightly to TBG and weakly to albumin, with little binding to TBPA. Although T4 is produced in greater amounts, T3 is the biologically active form.[12,13]

Thyroid hormones are key regulators of metabolism and development and are known to have pleiotropic effects in many different organs. Thyroid hormones affect normal growth and development (particularly in bone and central nervous system), help regulation of lipids (adipose tissue) and protein breakdown in muscle, increase absorption of carbohydrates from the intestine and increase dissociation of O2 from hemoglobin acting on RBC 2,3-diphosphoglycerate (DPG).[12,13]

Thyroid hormone regulates virtually every anatomic and physiologic component of the cardiovascular system. The major effects of thyroid hormones on the heart are mediated by triiodothyronine (T_3). T_3 generally increases the force and rate of systolic contraction and diastolic relaxation[14], decreases vascular resistance, including coronary vascular tone, and increases coronary arteriolar angiogenesis.[14] Thyroid hormones act in the liver, white adipose tissue, skeletal muscle, and pancreas, influence plasma glucose levels, insulin sensitivity, and carbohydrate metabolism.[15]

Prevalence of Thyroid Disorders in Thalassemia Major.
1. Primary Hypothyroidism. The advent of more precise diagnostic techniques, which enable different aspects of thyroid function assessment, showed that hypothyroidism is a graded phenomenon. Therefore, several definitions have been used to define different aspects of impaired thyroid function including in TDT patients. The following grades have been identified: 1) Sub-biochemical hypothyroidism consists of an exaggerated TSH response to TRH test in the presence of normal TSH and FT4; 2) Sub-clinical hypothyroidism is a combination of high TSH (> 4.2 mIU/L and <10 mIU/L) with normal FT4 levels; 3) Overt (clinical) hypothyroidism is a combination of high TSH (TSH >10 mIU/L) with low FT4.

The frequency of primary hypothyroidism in TDT patients, in different reports, ranges from 4% to 29 %, based on the level of FT4/T4 and TSH.[16,17] In general, subclinical hypothyroidism is more common compared to overt hypothyroidism.This wide variation has been attributed to several factors such as patients' genotype age, ethnic groups, differences in treatment protocols of transfusion and chelation with marked variations
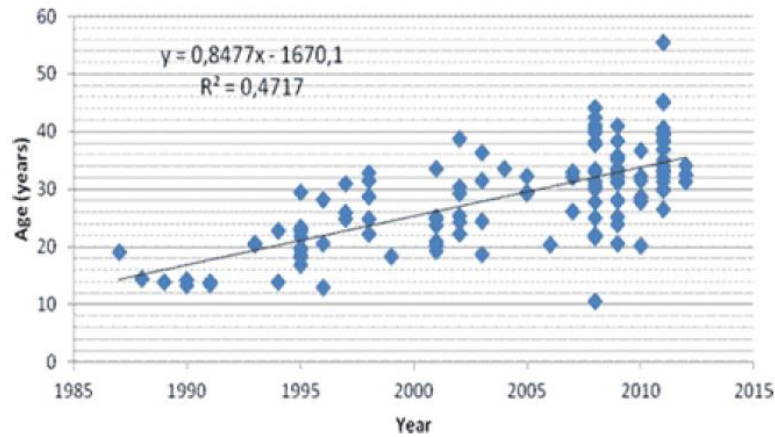
Figure 1. Correlation between the age at diagnosis of primary and secondary hypothyroidism vs. the year of diagnosis (R2 =0.47). From: Delaporta P, Karantza M, Boiu S, Stokidis K, Petropoulou T, Papasotiriou I, Kattamis C, Kattamis A. Thyroid Function in Greek Patients with Thalassemia Major. Blood. 2012;120: Abs. 5176).

in compliance and efficiency.[17-24]

A lower prevalence of hypothyroidism is found among patients with lower iron load, as measured by ferritin levels.[21,25] An increased frequency of hypothyroidism was reported by Belhoul et al. [25] in splenectomised TM patients (26% versus 4.5% of non-splenectomised thalassemic patients). In non-splenectomised thalassemics, the spleen might represent a reservoir of iron excess and might have a potential scavenging effect on free iron fractions, including non-transferrin bound iron.[26]

However, further studies are needed to confirm this hypothesis that should include evaluations of factors involved in iron redistribution in TDT patients.[27]

Thyroid failure usually starts in the second, and increases gradually in the third and fourth decades of life in patients who started early subcutaneous chelation therapy with desferrioxamine (**Figure 1**); in patients starting late iron chelation therapy, or with poor compliance to treatment, dysfunction of thyroid failure starts earlier.[27-31] Therefore, an assessment of thyroid function is generally recommended after the age of 10 years.[17]

2. Central Hypothyroidism (CH). The thyroid gland appears to fail before the central components of the pituitary-thyroid axis which seems to be less susceptible to iron-induced damage.[4,32]

The diagnosis of central hypothyroidism (CH) is difficult from a clinical and biochemical perspective. It is based on low circulating levels of FT_4 in the presence of low to normal TSH concentrations.

Tatò et al.[33] found an inadequate response of the free α-subunit to TRH stimulation tests in 14 euthyroid TM patients (8 females and 6 males, aged 15-24 years), suggesting a central involvement.

De Sanctis et al.[34] performed a cross-sectional analysis on an extensive database using the clinical records of their TM patients to explore the prevalence of CH in prepubertal (<11 years: 25 patients; 13 males)

peripubertal (between 11 and 16 years: 9 patients; 3 males), and pubertal TM subjects (>16 years: 305 patients; 164 males). CH was present in 26 (7.6%) TM patients. Their mean age was 29.9 ± 8.4 years, 14 (53.8%) were males, and 12 (46.1%) were females. The prevalence of CH, characterized by low FT4 with low/normal TSH levels was 6% in patients with a chronological age below 21 years and 7.9% in those above 21 years.

Similar results have been reported by Delaporta et al.[35] (**Table 1**), while higher percentages were reported in Iranian[22] (16% in 114 patients, with a mean age of 20.9 ±7.8 years) and Qatari patients (76.4% in 48 children and adolescents, up to the age of 18 years).[36]

In the general population, CH is about 1000-fold rarer than primary hypothyroidism.[37] In contrast with primary hypothyroidism, low FT4 with low/normal TSH levels are the biochemical hallmarks of overt forms of CH, while the milder defects, characterized by FT4 levels still within the normal range, could remain undiagnosed.[37] To support the diagnosis of CH, a reduction of FT4 larger than 20% vs.the initial FT4 levels has been suggested in patients with different pituitary diseases followed over several years.[38] This cut-off was set on the basis of a 10% variation over time of T4 levels in healthy individuals.[39]

In summary, significant advancement has been made in recent years in diagnosing CH in TM patients,

Type of thyroid dysfunction	Total (n=364)
Primary % (n)	17.6 (64)
Secondary % (n)	17.3 (63)
Thyroiditis % (n)	1.6 (6)
Thyroid cancer % (n)	0.8 (3)
Normal thyroid function % (n)	62.6 (228)

Table 1. Prevalence of thyroid dysfunctions and thyroid cancer in 364 Greek TM patients (mean age 33.0±9.9 years, 180 females and 184 males). From Delaporta P, Karantza M, Boiu S, Stokidis K, Petropoulou T, Papasotiriou I, Kattamis C, Kattamis A. Blood 2012;120: Abs. 5176, modified.

thus increasing the clinical awareness of this complication. Both the hypothalamus and pituitary gland appear to be affected by iron overload, and this can explain the defective TSH secretion in response to low FT4 in thalassemic patients. The deposition of iron in the pituitary gland and its deleterious effects on pituitary size and function has been reported in many studies and reviews.[40-42] Nevertheless, there is a rising impression that the frequency of CH is underestimated because only a few studies have been reported in the current literature. Furthermore, the presence of a mild rise of TSH levels associated with a borderline low FT4 represents a further clinical challenge for the diagnosis and treatment of the mixed forms of hypothyroidism (De Sanctis V, personal observations). In patients with CH an assessment of other pituitary hormone deficiencies may be required.

Clinical Manifestations and other Diagnostic Parameters. The severity of the clinical manifestations generally reflects the degree of thyroid dysfunction and time needed for the development of hypothyroidism. The clinical presentation of patients with subclinical hypothyroidism may be subtle, without any symptoms, and may be detected merely during routine screening of thyroid function. Patients with primary hypothyroidism may present with short stature, delayed puberty, fatigue, cold intolerance, weight gain, constipation, and dry skin.[43] In TM patients with clinical hypothyroidism, cardiac failure and pericardial effusion have been reported.[43]

The clinical manifestations of CH are usually milder than those observed in primary hypothyroidism.

Although there is no significant relationship between gender and thyroid dysfunction, a higher incidence of thyroid dysfunction has been reported in female patients with subclinical hypothyroidism.[44] It has been also reported that thalassemic patients with primary hypothyroidism have more frequent endocrine complications, including insulin dependent diabetes mellitus (79%), hypoparathyroidism (65%), and failure of puberty (37%).[21]

In one of our papers, the first and most common endocrine complication in TM patients was hypogonadotropic hypogonadism (36.3%; diagnosed at the age of 16 years in females and 18 years in males) followed by subclinical hypothyroidism (18.1%) at a mean age of 20.2 years (range 12-32 years), insulin-dependent diabetes mellitus (36.3%) at a mean age of 22.5 years (range 12-35 years), and secondary amenorrhea (27.2%) at a mean age of 36.3 years (range 34-38 years).[45]

There is very little evidence for the presence of autoimmune thyroiditis. In the Delaporta et al. study[35] the reported prevalence in 364 TM patients was 1.6% (**Table 1**). However, no comparison data were reported in the Greek control population. Interestingly, the prevalence of anti-thyroid antibodies (ATA) is significantly lower (9.2%) in TM women than that found in age-matched euthyroid women (20.0%). This suggests that iron overload may inhibit rather than trigger thyroid autoimmunity.[46] In another study serum ferritin levels were found to be significantly higher in ATA positive compared to ATA negative patients (4,870 ±1,665 ng/mL versus 2,922 ± 2,773 ng/mL; p: < 0.0001), which advocates potential iron-mediated tissue damage rather than a primary autoimmune process of the thyroid gland.[47] Nevertheless, more well-designed studies are needed to confirm these preliminary observations.

Thyroid ultrasonography may show different echo patterns. Pitrolo et al.[48] observed reduced echogenicity in 47% of TM patients and a diffuse spotty echogenicity in 33% of them, indicative of thyroid dysfunction. Filosa et al.[49] reported features of dyshomogeneity of the parenchyma with different degrees of severity.

Despite the limitations of serum or plasma ferritin (SF) for the estimation of iron stores in patients with iron overload, this indirect parameter remains essential in monitoring iron overload. Assays of SF are available worldwide, relatively well standardized, and not expensive. In the absence of confounding factors, such as inflammation, vitamin C deficiency, oxidative stress, hepatocyte dysfunction, and increased cell death, SF levels correlate with the size of cellular iron stores.[50] Currently, tissue iron can be detected by nuclear magnetic resonance (MRI) imaging. This technique has been used to assess myocardial, spleen, pituitary, adrenal, pancreas and liver iron content in patients with known or suspected iron overload disorders.[51]

Pathophysiology. Thyroid dysfunction appears to be primarily due to the toxicity of the excess unbound iron within cells or in plasma, generating reactive oxygen species, leading to lipid peroxidation, that under conditions of iron overload, leads to the generation of both unsaturated (malondialdehyde and hydroxynonenal) and saturated (hexanal) aldehydes. Both have been implicated in cellular dysfunction, cytotoxicity and cell death.[16,17]

Certain tissues are particularly susceptible to excess iron incorporation in the presence of Non-Transferrin-Bound Iron (NTBI). TRH stimulates TSH β promoter activity by two distinct mechanisms involving calcium influx through L type Ca^{2+} channels (LTCCs) and protein kinase C.[52,53] The most recent evidence suggests that LTCCs are the front-runners for mediating NTBI transport in iron overload conditions. LTCCs are moderately abundant in thyrotropes that appear to be at the greatest risk in iron overload. In addition, protein kinase C is regulated by iron with the possible deleterious effect of excess iron on its function.[53,54]

Apart from iron overload, other factors responsible for organ damage have been recognized, including chronic hypoxia due to anaemia[55], that may potentiate the toxicity of iron deposition in endocrine glands, and hepatitis C virus (HCV) infection.[4,16,17]

Many patients with thalassemia have been infected with hepatitis C virus (HCV) through blood transfusion before the introduction of screening of blood donors in 1992. HCV genotype 1b infection was the most frequent in Italy. In cohorts of adult TDT patients epidemiological studies, the proportion of patients with genotype 1b infection often exceeds 50%. Chronic HCV infection is associated with a high risk of developing cirrhosis, hepatocellular carcinoma and liver failure if left untreated.[56] Chronic hepatitis C infection may also lead to subclinical hypothyroidism due to the direct cytopathic effect of HCV on thyroid cells or with the use of interferon.[57] Liver disease is also associated with an increase in inflammatory cytokines, which negatively affect the hypothalamus -pituitary- thyroid axis, leading to suppressed TSH levels in some patients.[58] In the light of eradication of HCV in the thalassemia population with direct-acting antiviral drugs, the prevalence of thyroid dysfunctions could be ameliorated. However long-term studies are needed to confirm this assumption.

The Long-Term Natural History of Thyroid Function in Thalassemia. Longitudinal studies have shown worsening of thyroid function in thalassemic patients with advancing age. However, the progression is variable, and it may take years to progress to overt hypothyroidism.

Landau et al.[32] studied the course of thyroid disease in TM patients in a 15-year longitudinal study. The authors found that more than 30% of TM patients had an abnormal response to TRH and 14% changed from normal to overt hypothyroidism.

Zervas et al.[19] reported that approximately 1 of 5 β-thal patients with average thyroid hormone values showed an exaggerated TSH response to the TRH test. In another study, an exaggerated TSH response to TRH test was found in 8 out of 24 TM patients (33.3%). TSH peak values, after TRH test, positively correlated with ferritin levels, liver enzymes (ALT), and compliance index to chelation therapy. Three out of 8 patients (37.5%) developed subclinical or overt hypothyroidism from 3 to 11 years later. [59] Similar results were also observed in 25% of the patients (27 females and 23 males, mean age 25.7 ± 1.4) by Filosa et al. during 12 years of follow-up.[48]

Soliman et al.[37] documented a slowly progressive decrease of FT4 over a 12 year-period, associated with a corresponding slow decrease of basal TSH. These findings indicate a central component of hypothyroidism. In support of these observations, Hashemi et al.[60] reported a higher incidence of

secondary hypothyroidism (12%) compared to primary hypothyroidism (2%) in their thalassemic patients.

In conclusion, as survival rates of patients with TM are continuously improving, there is a steadily growing need for regular follow-up and surveillance strategies of thyroid function. TRH stimulation test may be a useful means of early diagnosis of thyroid dysfunction. An exaggerated TSH response to TRH test is frequently found in patients with TM and iron overload, and may evolve into subclinical or clinical hypothyroidism. A slowly progressive decrease of FT4 and basal TSH has been observed in young adult subjects, indicating CH. Early diagnosis and treatment of these complications are essential to ensure a good quality of life and to reduce late morbidity and mortality.

Risk Factors for the Development of Thyroid Disorders in Addition to Iron Overload. The etiology of thyroid disorders in TM patients is substantially different from that in the general population; transfusional iron overload and increased iron uptake from the gastrointestinal tract, as a result of ineffective erythropoiesis accompanied by anemia and hypoxia, are implicated in over 90% of morbidity and mortality in patients with β-thal.[1-4] Therefore, the knowledge of risk factors influencing the development of hypothyroidism represents a critical component of long-term monitoring and treatment of patients affected by TM.

1. Iron overload. The association between iron overload and hypothyroidism was studied by Belhoul et al.[25] in 382 TM patients treated with regular transfusions and desferrioxamine (DFO) at the Thalassemia Center in Dubai (UAE). The mean age of patients was 15.4 ± 7.6 years, with an equal sex distribution. On multivariate logistic regression analysis, patients with a serum ferritin level >2,500 ng/mL were 3.53 times (95% CI 1.09-11.40) more likely to have diabetes mellitus (DM), 3.25 times (95% CI 1.07-10.90) more likely to have hypothyroidism, 3.27 times (95% CI 1.27-8.39) more likely to have hypoparathyroidism, and 2.75 times (95% CI 1.38-5.49) more likely to have hypogonadism compared to patients with a serum ferritin level ≤ 1,000 ng/mL. Splenectomized patients with serum ferritin levels ≤ 2,500 ng/mL had comparably high rates of all endocrinopathies as patients with serum ferritin levels > 2,500 ng/mL.

In the Gamberini et al. study[21] the main risk factors associated with endocrine complications in 273 patients with TM, were high serum ferritin levels, poor compliance with DFO therapy, early onset of transfusion therapy (only for hypogonadism) and splenectomy (only for hypothyroidism). Serum ferritin levels of ~ 2,000 ng/mL were found to correlate with

hypogonadism, and levels of 3,000 ng/mL for hypothyroidism [primary hypothyroidism (80%) and central (20%)], hypoparathyroidism and DM.

A liver iron concentration (LIC) cut-off point of $\geq$ 6 mg Fe/g dry weight (d.w.) was found to be the best threshold for discriminating the presence and absence of endocrine/bone morbidity (hypothyroidism, osteoporosis, or hypogonadism), in NTDT patients, with a risk factor of 4.05 times higher compared to TI patients with a LIC < 6 mg Fe/g d.w.[61]

2. Amiodarone-induced hyper-hypothyroidism. Amiodarone is an efficient antiarrhythmic agent often used in clinical practice, despite its potentially serious side effects. Although the mechanisms of action of amiodarone on the thyroid gland and thyroid hormone metabolism are poorly understood, the structural similarity of amiodarone to thyroid hormones may play a role in causing thyroid dysfunction. A 100 mg tablet contains an amount of iodine that is 250-times higher than the recommended daily iodine requirement.

Amiodarone-induced thyroid dysfunction includes amiodarone-induced thyrotoxicosis (AIT, with an incidence of ~3% to 9%)[61-66] and amiodarone-induced hypothyroidism (AIH, with an incidence of 15%-20%),[63] both of which may develop in a normal thyroid gland or in settings of a pre-existing thyroid disease.[61-65]

Amiodarone-induced thyrotoxicosis is challenging to treat, as patients are iodine saturated and therefore cannot undergo radioiodine ablation.[61-63] Thyroidectomy remains a valuable option for AIT management, particularly for patients with suboptimal response to medical therapy and high risk for cardiac complications.[67]

A higher prevalence of overt hypothyroidism (22.7%) as compared to controls (4.1%, p: 0.02) was found in TM patients 3 months after starting amiodarone, while the prevalence of subclinical hypothyroidism was similar in amiodarone-treated (18.2%) and untreated (15%) TM patients.[63]

Overt hypothyroidism resolved spontaneously after amiodarone withdrawal in one case, while the remaining TM patients were maintained euthyroid on amiodarone by L-thyroxine administration. After 21-47 months of amiodarone therapy, three patients (13.6%) developed AIT (2 overt and< 1 subclinical), which remitted shortly after amiodarone withdrawal. No case of AIT was observed in TM controls (p= 0.012 vs. amiodarone-treated patients).[68]

In conclusion, clinicians should keep in mind the possibility of development of thyroid disorders in patients on treatment with amiodarone even after several years of use. Although it is difficult to decipher the specific factors contributing to the successful management in patients with AIT, Kotwal et al.[67] suggested that the outcome of these patients is most likely derived from the coordinated efforts of endocrinologists, thyroid surgeons, cardiologists, anesthesiologists, and members of all of collaborating teams.

Emerging Issues.
1. Thyroid cancer. Parallel to the significant amelioration of the main clinical features of the disease, achieved on efficient treatment, adult patients suffer from treatment-related complications that affect the heart, liver, bones and endocrine glands, requiring specialized health care by specialists. The possibility of occurrence of other diseases such as malignancies, considered rare in the past, are currently increasing with prolonged survival opening new scenarios in oncoming years. The most commonly reported cancers are hepatocellular carcinoma (HCC),[69] hematologic malignancies,[70.71] and thyroid cancer.[73-75]

From 2000 to 2011, in a single Thalassaemia Unit following 195 TM patients, 11 carcinomas were diagnosed: 4 of the liver, 1 of the lung, 1 of the adrenal gland and 5 of the thyroid gland. The mean patients' age was 42.6 years.[72] A prevalence of 0.8% of thyroid cancer was reported by Delaporta et al.[35] in 364 TM patients. In a recent multicenter survey, the prevalence of thyroid papillary and follicular carcinoma was 0.41%.[68] The highest prevalence rates were registered in Greece and Italy (1.3% and 1.57%, respectively), followed by Oman (1.0%).[75]

In the general population, the risk of harboring thyroid cancer is highest in women, in certain inherited genetic abnormalities (Cowden's disease, Gardner's syndrome, Carney complex, type I medullary thyroid cancer, or familial adenomatous polyposis), low iodine diets, after radiation exposure, and to endocrine disrupters.

In thalassemia, other factors have to be taken into consideration. Iron overload and hepatitis C (HCV) infection have potential carcinogenic effects. Iron overload can promote the growth of some cancer cells probably through the activation of ribonucleotide reductase and may promote the formation of mutagenic hydroxyl radicals. In addition, iron excess diminishes host defenses through inhibition of the activity of CD4 lymphocytes and the suppression of the tumoricidal action of macrophages can enhance host cell production of viral nucleic acids which may be involved in the development of human tumors.[72] Several clinical, epidemiologic studies have suggested the oncogenetic role for HCV. HCV is an RNA virus that cannot be integrated into the host genome and could exert its oncogenic potential through indirect mechanisms, with the contribution of potential genetic or environmental factors.[72,76] If confirmed by further clinical and epidemiologic studies, thyroid cancer should be included among the serious complications of iron overload and/or chronic HCV infection.

In conclusion, the occurrence of thyroid malignancies in adult thalassemic patients is an emerging concern for physicians, that requires the need for an annual thyroid ultrasound surveillance. According to the European Thyroid Association guidelines at least one of the following thyroid ultrasound features has a high suspicion of malignancy: irregular shape, irregular margins, microcalcifications (<1-mm, most often round calcification), marked hypoechogenicity.[77]

2. Hypothyroidism and the heart. The role of hormones and growth factors in modulating cardiovascular functions are well known.[78-81] It has been reported that thyroid hormone action on cardiomyocyte regulates myocardial contractility, diastolic, and systolic function. Moreover, thyroid hormones also exert profound effects on the heart and cardiovascular hemodynamics. Thyroid hormone deficiency results in low heart rate and weakening of myocardial contraction and relaxation, with prolonged systolic and early diastolic times.[82,83] Typical electrocardiographic changes that can be seen in hypothyroidism include sinus bradycardia, prolonged QTc, low voltage, and the rarely atrioventricular block.

Ten years ago, in a long-term follow-up study, De Sanctis et al.[44] reported that cardiac involvement was present in ~50% of TM patients with subclinical hypothyroidism and moderate/severe iron overload. Patients mean age was 15.7 ± 3.5 years (range 9-22 years). A positive direct correlation was observed between the following variables: TSH and serum ferritin, thyroglobulin and basal TSH, basal TSH and peak levels after TRH stimulation test. During four years follow-up, 16.6% died from heart failure and arrhythmia. In patients with hypothyroidism, the changes in cardiovascular function responded to replacement therapy with L-thyroxine and efficient chelation regimen.

Recently, a retrospective cohort study was performed to evaluate, in a large historical cohort of 957 TM patients who underwent cardiovascular magnetic resonance (CMR) for myocardial iron overload (MIO) assessment, whether hypothyroidism was associated with a higher risk of heart complications (heart failure, arrhythmias, and pulmonary hypertension).[84] The authors identified 115 (12%), hypothyroid patients. Hypothyroid and non-hypothyroid patients had comparable MIO, but hypothyroid patients showed significantly lower biventricular stroke volume index, ejection fraction and left ventricular cardiac index. Accordingly, the prevalence of overall heart dysfunction (LV, RV or both) was higher in hypothyroid patients (43.5% vs 33.5%; p: 0.0314). Hypothyroid patients had a significant higher frequency of heart failure (19.1% vs 9.1%; p: 0.003) and arrhythmias (11.3% vs 4.3%; p:

0.003). These data confirm the link between thyroid function and heart diseases also in TM patients and stress the need to prevent hypothyroidism in this population.[84]

In conclusion, hypothyroidism seems to increase the risk for heart failure, arrhythmias and heart dysfunction in TM patients; thus in TM patients, a sequential assessment of thyroid function and effective iron chelation therapy are recommended to prevent thyroid dysfunction and significant myocardial dysfunction.

Prevention of Endocrine Complications. Efficient treatment with iron chelating drugs of patients with TM is considered the standard care that leads to improvement of morbidity and of survival.[4] To date, there are 3 significant classes of iron chelators: hexadentate (Deferoxamine [DFO], Desferal®, Novartis Pharma AG,Basel, Switzerland); bidentate (Deferiprone, [DFP] Ferriprox®, Apotex Inc., Toronto, ON, Canada), and tridentate (Deferasirox [DFX], Exjade® and Jadenu®, Novartis Pharma AG, Basel, Switzerland).[85]

In 2008, a longitudinal study in TM patients followed at Ferrara Centre, showed that the incidence of hypothyroidism, diabetes mellitus, and hypoparathyroidism declined during treatment with DFO given subcutaneously SC (**Figure 2**).[21] Similar results were reported by Farmaki et al.[86-89] The authors showed that regular and intensive combined chelation therapy with DFO and DFP improved the thyroid function in TM patients with iron overload. The time needed to reverse hypothyroidism with combined chelation therapy varied according to the patient's age and iron load status. After 6.5 consecutive years of therapy with DFX (up to 10 years) in 86 patients with TM, no new cases of hypothyroidism or diabetes occurred.[90]

In brief, iron overload-induced hypothyroidism may respond to adequate iron chelation therapy promoting prevention or/and reversal of the disease and other associated comorbidities. Irrespective of iron chelation agent, adherence to iron chelation therapy is essential in order to achieve prevention of thyroid dysfunctions. Our experience in two groups of TM patients is reported in **figure 3**. One group had very good compliance to chelation therapy with DFO, given s.c. for 6 to 7 times a week, and another group with absent or weak compliance (DFO therapy from 2 to 3 times a week). No endocrine complications were observed in the first group of patients (De Sanctis V, personal observations).

Treatment.
1. Primary hypothyroidism. In patients with a TSH >10 mUI/L, thyroxine therapy (L-T4) is considered reasonable due to the systemic adverse effects of primary hypothyroidism (**Figure 4**). This is especially
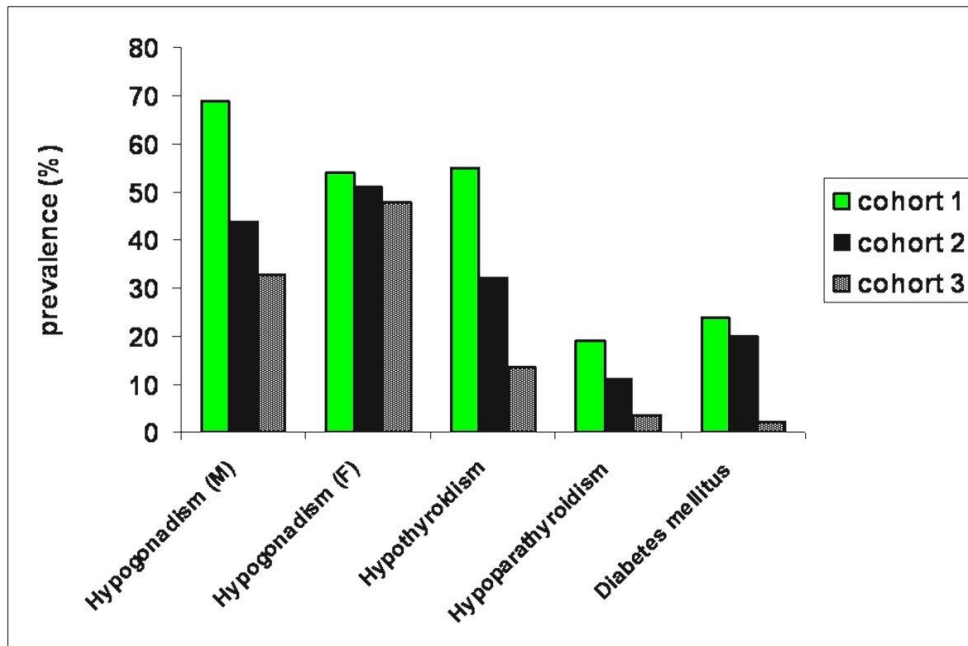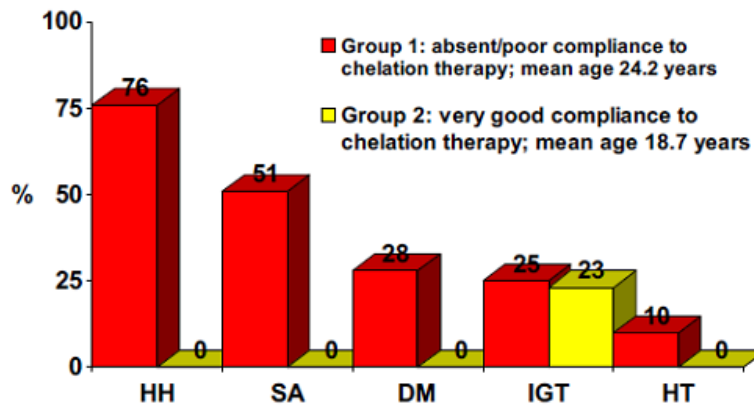
Figure 2. Prevalences of endocrine complications in 3 groups of 273 β-thalassemia major patients followed at the Thalassemia Centre of Ferrara. Cohorts 1 and 2 started late chelation therapy with desferrioxamine given s.c. (mean age: 16.7 ± 2.8 years and 9.1± 2.9 years, respectively) and Cohort 3 started early therapy (mean age: 2.8 ± 1.0 years) (From Ref. 21, modified).

| Group 1 | Serum ferritin level (± SD) 5,597 ± 3,445 ng/mL |
| Group 2 | Serum ferritin level (± SD) 976 ± 321 ng/mL |

| HH = Hypogonadotropic hypogonadism |
| SA = Secondary amenorrhea |
| DM = Insulin dependent diabetes mellitus |
| IGT = Impaired glucose tolerance test |
| HT = Primary hypothyroidism |

Figure 3. Percentage of endocrine complications in patients with β-thalassemia major in relation to the compliance with long-term iron chelation therapy with desferrioxamine (De Sanctis V, personal observations).

true in patients with iron overload. L-T4 monotherapy remains the treatment of choice due to its long half-life and the convenience of a single daily dose, and the assumption that T4 is converted mainly to T3 as needed.

The initial L-T4 dosage may range from 12.5 µg/daily to a full replacement dose based on the age, weight, cardiac status and severity, and duration of hypothyroidism of the patient. Adjustment of the dose can be made based on clinical and laboratory data.

Close monitoring is required to avoid overtreatment because L-T4 may cause arrhythmias and accelerated bone loss.[91, 92]

In patients with CH, monitoring of therapy should be based on serum FT4 levels instead of serum TSH levels, and the sample should be collected prior to ingesting the morning dose of thyroxine. In patients with co-existent hypocortisolism, glucocorticoid replacement should be initiated prior to L-T4 replacement.
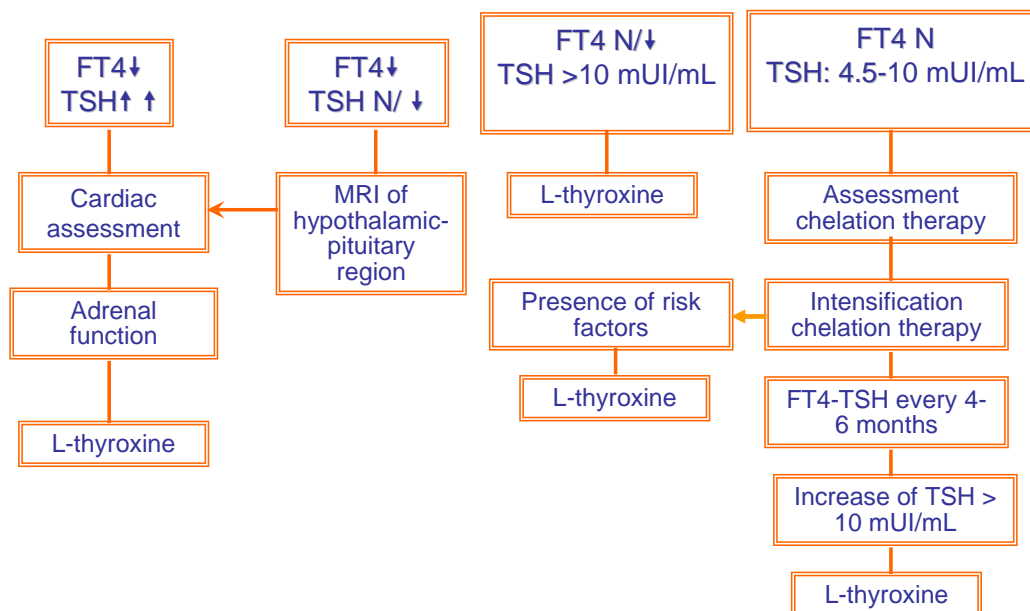
Figure 4. When to consider treatment of hypothyroidism. Abbreviations: MRI: Magnetic Resonance Imaging; N: normal thyroid-stimulating hormone (TSH) level.

Certain medications, supplements, and even some foods may affect the L-T4 absorption, such as iron supplements, aluminum hydroxide, which is found in some antacids, and calcium supplements. Therefore, the physician must make the appropriate adjustments in L-T4 dosage in the face of absorption variability and drug interactions.[93]

2. Subclinical hypothyroidism (average FT4 and increased TSH). Current guidelines do not recommend routine thyroid hormone substitution in subjects with normal FT4 levels and a TSH between 4.5 and 10 mUI/L.[94] However, the term subclinical hypothyroidism implies that patients should be asymptomatic (although symptoms are difficult to assess), especially in patients with chronic disease. Thyroid function tests on a 4-6 months interval are recommended to monitor treatment based mainly on serum TSH level (**Figure 4**).[94]

Special attention has to be paid to patients with clinical features or laboratory findings of reduced growth velocity, short stature, delayed puberty, cardiac failure, arrhythmias, or iron overload. A recovery of subclinical hypothyroidism has been observed in some iron overloaded TM patients after intensive iron chelation therapy.[86-89] In individual patients, a trial of thyroid hormone substitution, for several months, may be considered based on a combination of age, patient's personal history, complaints, and presence of risk factors.[90]

In pregnancy or in women trying to conceive, a mildly increased serum TSH should always be treated as mild thyroid failure which is associated with adverse outcomes for both mother and foetus.[95]

The management of thyroid disease during pregnancy has been reviewed in the guidelines of several societies, including the American Thyroid Association (ATA) and the Endocrine Society and the European Thyroid Association (ETA).[96-99] Due to the physiologic changes in TSH levels during pregnancy, the ATA guidelines recommend using trimester-specific reference ranges for TSH.[96] If these reference ranges are not available in the laboratory, the following reference ranges can be used: first trimester, 0.1 to 2.5 mIU/L; second trimester, 0.2 to 3.0 mIU/L; third trimester, 0.3 to 3.0 mIU/L.[100] Also, different reference ranges for TSH in the first trimester have been reported for different populations.[101] Therefore, the correct interpretation of thyroid function tests requires knowledge of a woman's gestational week and the appropriate population-based reference interval.

Open Problems. Currently available data do not lead to definitive conclusions concerning the treatment of subclinical thyroid disease in TM patients. In the general population, possible consequences of subclinical hypothyroidism include cardiac dysfunction, atherosclerosis, elevated total and LDL cholesterol, and progression to clinical hypothyroidism.[19,32,37,59] Several other aspects remain to be elucidated such as the frequency of thyroid cancer and the existence of a relationship between thyroid dysfunction, cardiovascular diseases, components of metabolic syndrome (insulin resistance) [14,15,102] and coagulation disorders.[103,104] Therefore, further studies are needed to explain these emerging issues.

Conclusions. Hypothyroidism is a clinical disorder commonly encountered in iron-overloaded patients

with thalassemia and is defined as failure of the thyroid gland to produce sufficient thyroid hormone to meet the metabolic demands of the body. The etiology of thyroid disorders in thalassemia patients is substantially different from that of the general population. Therefore, the knowledge of risk factors influencing the development of hypothyroidism is a critical component for long-term monitoring and treatment of TM patients.

Hypothyroidism is the result of primary gland failure or insufficient thyroid gland stimulation by the hypothalamus or pituitary gland. The prevalence of HT increases with age (after the second/third decade of life), although in developing countries or in patients with severe iron overload it may occur in the first decade of life.[28] The identification of risk factors influencing the development of hypothyroidism is a critical component of long-term monitoring and treatment of TM patients, according to the international guidelines.[94]

Early diagnosis and treatment of these complications are essential to ensure a good quality of life and to reduce late morbidity and mortality. In patients with CH or TSH >10 mU/L, thyroxine therapy is recommended. A reversal of subclinical hypothyroidism or improvement of primary hypothyroidism has been observed in some iron overloaded TM patients after intensive iron chelation. Therefore, periodic assessment of iron overload and follow-up to improve adherence to chelation therapy and patients' satisfaction should be strongly considered

in order to improve the quality of life (QoL) and life expectancy of patients.

The incidence of thyroid cancer detection has increased by 4.5% per year over the last 10 years, faster than for any other cancer.[105] The US Preventive Services Task Force (USPSTF) does not recommend screening for thyroid cancer in asymptomatic adult persons. It does not apply to persons who experience hoarseness, pain, difficulty swallowing, or other throat symptoms or persons who have lumps, swelling, asymmetry of the neck, or other reasons for a neck examination.

It also does not apply to persons at increased risk of thyroid cancer because of a history of exposure to ionizing radiation (eg, medical treatment or radiation fallout), particularly persons with a diet low in iodine, an inherited genetic syndrome associated with thyroid cancer (eg, familial adenomatous polyposis), first-degree relative with a history of thyroid cancer [106] or iron overload. The emerging issue of thyroid cancer in adult TM patients indicates the need for preventive measures, yearly thyroid US surveillance and careful follow-up. Recently Chen et al.[107], based on the Thyroid Imaging Reporting and Data System (TI-RADS), built a new model using a combination of ultrasound patterns including margin, shape, echogenic foci, echogenicity and nodule halo sign with age to differentiate benign and malignant thyroid nodules, which had high sensitivity and specificity.

Finally, additional studies are required to determine the association between iron overload, oxidative stress, HCV infection, and thyroid carcinomas.

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