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Risk factors for endocrine complications in transfusion-dependent thalassemia patients on chelation therapy with deferasirox: a risk assessment study from a multicentre nation-wide cohort

Running title: Endocrine complications in iron overload

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Abstract

Transfusion-dependent patients typically develop iron-induced cardiomyopathy, liver disease, and endocrine complications. We aimed to estimate the incidence of endocrine disorders in transfusion-dependent thalassemia (TDT) patients during long-term iron-chelation therapy with deferasirox (DFX). We developed a multicentre follow-up study of 426 TDT patients treated with once-daily DFX for a median duration of 8 years, up to 18.5 years. At baseline, 118, 121, and 187 patients had 0, 1, or ≥2 endocrine diseases respectively. 104 additional endocrine diseases were developed during the follow-up. The overall risk of developing a new endocrine complication within 5 years was 9.7% (95%CI=6.3–13.1). Multiple Cox regression analysis identified 3 key predictors: age showed a positive log-linear effect (adjusted HR for 50% increase=1.2, 95%CI=1.1–1.3, P=0.005), the serum concentration of thyrotropin (TSH) showed a positive linear effect (adjusted HR for 1 mIU/L increase=1.3, 95%CI=1.1–1.4, P<0.001) regardless the kind of disease incident, while the number of previous endocrine diseases showed a negative linear effect: the more the diseases at baseline the less the chance of developing a new one (adjusted HR for unit increase=0.5, 95%CI=0.4–0.7, P<0.001). Age and TSH had similar effect sizes across categories of baseline diseases. Adding levothyroxine administration as a covariate, the estimates did not change. Although in DFX-treated TDT patients the risk of developing an endocrine complication is generally lower than the previously reported risk, there is considerable risk variation and the burden of these complications remains high. We developed a simple risk score chart enabling clinicians to estimate their patients' risk. Future research will look at increasing the amount of variation explained from our model and testing further clinical and laboratory predictors, including the assessment of direct endocrine MRI.

Key-words: blood transfusion; iron overload; iron chelation; deferasirox; endocrine function.

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Contributions: MC, SP, and AIL designed the study. Each author collected the data from his/her own centre and takes responsibility for the accuracy of the data provided. AIL carried out the statistical analysis. MC and AIL drafted the manuscript. All authors contributed to the interpretation of the data and approved the manuscript. The centres in Naples, Genoa, Milan, and Padua are part of the European Reference Network on rare haematological diseases (ERN-EuroBloodNet). All centres involved in the study are part of the Italian Society for Thalassemia and Hemoglobinopathies (SITE) and paediatric centres are part of the Italian Association of Paediatric Haematology and Oncology.

Introduction

Transfusion-induced iron overload in thalassemia patients typically results in iron-induced cardiomyopathy, liver disease, and endocrine complications. However, those three phenomena have been studied to different extents.

In transfusion-dependent thalassemia (TDT) patients, mortality due to cardiovascular and hepatic complications has markedly declined during the last decades.[1–3] The development of magnetic resonance imaging techniques (MRI), specifically designed to quantify myocardial and hepatic iron concentration, measuring heart T2* and liver iron concentration (LIC), has enabled the design of clinical trials evaluating the efficacy of iron chelators in targeting specific iron overload.[4] Moreover, new anti-hepatitis C drugs have remarkably reduced the complications linked to hepatitis C infection, which used to dramatically deteriorate liver iron overload.[5]

However, in spite of the outstanding advances in the care of cardiovascular and hepatic complications due to blood transfusions, the management of endocrine complications has been left behind and, nowadays, they are the most frequent and the most resource-draining complications in TDT patients.[3] In addition, serological testing fails to identify high-risk groups and, once occurred, these complications are often irreversible. While MRI imaging of endocrine glands offers promise in detecting preclinical disease, it has not reached the level of validation required for routine clinical use .[6]

The once-daily oral iron chelator deferasirox was shown to be effective in chelating iron from the heart and the liver, with preservation of the heart function,[7–9] and with reversal of the hepatic fibrosis.[10] While the effective control of heart and liver siderosis remains the primary goal in the management of TDT patients, observational data suggest that iron loading in endocrine organs may precede myocardial involvement and there is now substantial evidence on the role of iron overload in endocrine morbidity.[11–14] Nonetheless, there have been small studies on endocrine disorders in TDT patients during chelation therapy with deferasirox[15,16] and the data are still scarce, although deferasirox is now the most prescribed drug for iron chelation in TDT patients.[17]

The aim of this study was to assess the incidence of endocrine diseases including hypothyroidism, hypoparathyroidism, glucose metabolisms disorders, hypogonadism, and metabolic bone disease in patients suffering from TDT who are on treatment with the drug deferasirox.

Methods

In this multicentre study, TDT patients from 21 hospitals located in 21 cities and 19 regions of Italy were assessed for eligibility to be recruited in the cohort.

We considered the following endocrine conditions:

1. Hypothyroidism (Overt: TSH >10 microU/ml and low FT4; subclinical: TSH 5–10 microU/ml and normal FT4);
2. Hypoparathyroidism (low PTH and calcium and high phosphorus);
3. Hypogonadism (Hypogonadotropic hypogonadism, in adult female: amenorrhea, low estradiol levels and low or normal LH/FSH levels; in adult male: low testosterone levels, clinical signs or symptoms consistent with hypogonadism and low/normal LH/FSH. Testosterone reference ranges vary according to patients' age at the time of biochemical assessment. In general, they were regarded as normal if >3.5 ng/mL and unequivocally pathological <2.3 ng/mL. Additional data [clinical features, free testosterone] were taken into account for values between 2.3 and 3.5 ng/mL. Hypergonadotropic hypogonadism, in adult female: amenorrhea and raised FSH [> 30 U/L] with undetectable estradiol; in adult male: raised gonadotropins with low total testosterone and clinical signs consistent with hypogonadism);
4. Pubertal disturbances (Delayed puberty: lack of breast budding [Tanner stage 2] in girls by the age of 13 and testicular volume <4 mL in boys by the of 14; Arrested puberty: lack of pubertal progression over a year or more);
5. Disorders of glucose metabolism (Diabetes: fasting plasma glucose ≥126 mg/dL or 2-h plasma glucose (2-h PG) value during a 75-g oral glucose tolerance test (OGTT) >200 mg/dL; Impaired Fasting Glucose (IFG): fasting glucose between 100 and 125 mg/dl; Impaired Glucose Tolerance: 2-h PG during 75-g OGTT levels between 140 and 199 mg/dL);
6. Bone metabolism disorder (Osteoporosis: Bone Mineral Density T score ≤ -2.5 and Z score value ≤ -2; Osteopenia: T score value – 1.01/ - 2.5 and Z score value – 1.01/ -2. In childhood, osteoporosis was defined by either the association of at least 2 pathological fractures by the age of 10 years/ 3 by the age of 19 and Z score ≤ -2 or by the finding of at least one vertebral crush, in the absence of high-energy trauma or local disease, irrespectively of the BMD recorded; low bone mineral density was defined as the finding of BMD Z score ≤ -2, in the absence of the above-mentioned additional criteria for osteoporosis).

According to standardized protocols[18], laboratory tests for detection of endocrine disorders were performed every year in patients with no endocrine complications and more frequently (every 3-6 months) in patients with endocrine disorders, as per consolidated clinical practice and according to the endocrinologists' prescription. Routine laboratory tests, such as glycaemia and serum electrolytes, were assessed every 1-3 months in occasion of pre-transfusion cross-match testing. Weight, height and Tanner stage were assessed every 6 months in patients <18 years of age.

The study protocol was approved by Ethical committees and institutional review boards of all the participating centres and was conducted in accordance with the Declaration of Helsinki and ICH guidelines for good clinical practice. All patients provided written informed consent.

A detailed description of all methods used is available online in the Online Supplementary Methods.

Statistical analysis

Data were cleaned before the analysis: we checked all variables for missing, illogic or implausible values, also through cross-checks with related variables (e.g. chronologic orders). Continuous variables were checked for abnormal distributions and outliers. We used Cox-regression to fit survival analyses with follow-up days as the underlying time variable. Survival time was measured as the number of days passed from the beginning of the treatment regimen with the drug deferasirox to the first of either the diagnosis of the first new endocrine disease, side effect due to deferasirox leading to therapy suspension, death, or censoring. We centred the covariates before interaction analyses. We adopted two strategies for the development of the multiple models: we started with the covariates having higher biological plausibility of effect, or we started with those with lower P value from at the bivariate stage. The two approaches reached the same final model. The assumption of proportional hazards was checked by using the Schoenfeld residuals test. We computed the proportion of variation explained by the models (adjusted R^2) using the Royston method with bootstrap confidence intervals (5000 replications).[19] We derived the predicted probabilities of developing a new endocrine disease within 5 years and 1 year by using the margins command in Stata v.14. Two patients were excluded from the analysis as they already had all possible 5 endocrine diseases at baseline.

Results

Out of 426 patients enrolled, accounting for 3517 person-years, 104 participants developed at least one new endocrine disease after a mean and median follow-up time of 8 years (range: 1 month–18.5 years). The mean iron intake at baseline was 0.28 +/- 0.08 mg/kg/day (range: 0.14–0.49) and at the end of study was 0.26 +/- 0.12 mg/kg/day (range: 0.16–0.50). The mean hemoglobin level was 9.8 +/- 0.68 g/dl (range: 9.4–10.6) indicating the majority of patients had good control of their chronic anaemia.

No deaths were recorded. Overall, 18 (4%) patients experienced adverse events (AEs) that determined temporary or permanent drug discontinuation. The most frequent AEs were related to gastrointestinal intolerance (epigastralgia, heartburn, abdominal pain; n=8) and increased transaminases (n=8). Increased in serum creatinine (n=1) and Lichen planus (n=1) were reported as other AEs which caused drug interruption. In 9 (2%) cases the drug was discontinued because of treatment failure, reported as increase in serum ferritin (n=6), cardiac T2* (n=2), LIC (n=1). In one case the treatment failure was reported along with gastrointestinal intolerance.

Table 1 shows a cross-tabulation between the number of endocrine diseases at baseline and the number of new endocrine diseases occurred during the follow-up. The 75.6% of the total sample (322/426) did not develop any new endocrine disease during the follow-up (95%CI= 71.2%–79.6%). Out of the 104 (24.4%) with newly diagnosed endocrinopathies, 84.6% developed only one (95%CI= 76.2%–90.9%). Out of 118 patients with no endocrine diseases at baseline, 43 (36.4%) developed at least one endocrine disease during the follow-up (95%CI= 27.8%–45.8%). Out of 121 patients having one endocrine diseases at baseline already, 34 (28.1%) developed at least one additional endocrine disease during the follow-up (95%CI= 20.3%–37.0%).

Among the 118 patients with no endocrine diseases at baseline, bone metabolism disorders occurred the most (17.8% [95%CI=11.4–25.9]), followed by hypogonadism (12.7% [95%CI=7.3–20.1]). Those two conditions were also the most prevalent ones in patients with one disease a baseline (80.2% [95%CI=71.9%–86.9%] and 11.6% [95%CI=6.5%–18.7%] respectively) and were those that most likely occurred as additional diseases during the follow-up.

Figure 1 shows the overall crude risks for all 104 first incidents, by incident type and age group. It appears that most of the new incidents occurred after the age of 20, with a new spike between 35 and 45. As for paediatric

patients, the increase seems to be starting after the age of 12. No cases of insulin dependent (ID) diabetes were reported in patients with no endocrine disorders at baseline (Figure 2). Kaplan-Meier survival probability curves with age as the underlying time variable are reported in the online supplementary files.

Tables 2A and 2B show a description of the sample by prevalent endocrine diseases at baseline and by incident endocrine diseases during the follow-up. Age, TSH, and low BMD were associated with both prevalent and incident disorders. Among the markers of iron overload, ferritin and T2* were associated with prevalent but not with incident disorders, whereas LIC was not associated with any of them. During the follow-up, iron overload test results either decreased by a small extent or remained stable over time, while the SDs of those differences was more than three times their means. The number of prevalent endocrine disorders was inversely associated with the incidence of a new disorder. Only 11 patients (2.6%) had a side-effect related to the deferasirox (gastrointestinal upset).

Tables 3A and 3B show the results from the multiple Cox regression models. In both models, the adjusted hazard rate of developing a new endocrine disorder decreased by about 50% for each prevalent endocrine disease at baseline ($P < 0.001$) and increased by about 25% for each mIU/L of TSH at baseline ($P<0.001$). The two models differ in the way in which the variable age was treated. In model 3A we treated age as a log-linear variable whereas in model 3B we treated age as a linear variable, but in that case, we also included a binary indicator for paediatric/adult patient and an interaction term between age and the indicator as well. The latter model showed a higher adjusted R^2 (0.25 vs 0.22) although that difference was not significant (95%CIs 0.19–0.42 vs 0.10–0.37). According to model 3A, each 50% increase in age was associated with an increase of about 18% in the hazard of an incident new disease ($P=0.005$) after having adjusted for TSH and number of previous endocrine conditions. Kaplan-Meier survival probability curves are reported in the appendix.

Table 4A shows the 5-year risk predictions according to levels of age, TSH, and number of endocrine diseases at baseline, based on estimates from model 3B. On average, the whole cohort of patients had a risk of 9.7% (95%CI=6.3%–13.1) of developing an additional endocrine disease within 5 years from the start of therapy with the drug deferasirox. However, there was considerable variation according the baseline conditions. For example, an average 14-year-old patient with a TSH of 3 mIU/L who already suffered from one endocrine disorder had a risk of developing another disorder within 5 years of about a 10%, whereas a 35-year-old patient with a TSH of 5 mIU/L and no disease at baseline had a risk of 50%. Table 4B shows the 1-year risk predictions according to

levels of age, TSH, and number of endocrine diseases at baseline, based on estimates from model 3B. The overall 1y-risk was 1.1% (95%CI= 0.6%–1.7%).

Fifty-five patients were on therapy with levothyroxine at the beginning of the follow-up. We carried out a sensitivity analysis by running the same analysis on patients who were and were not on levothyroxine separately to see if levothyroxine modified the estimates from the final models. In patients who were (n=55) and were not (n=371) on levothyroxine, the adjusted HR for 1 mIU/L increase in TSH was 1.26 (95%CI=1.02–1.55, P=0.032) and 1.29 (95%CI=1.07–1.56, P=0.006) respectively. The estimates from the other predictors did not change either. Therefore, TSH was a predictor of additional endocrine disease incidence regardless of levothyroxine administration. In addition, we conducted stratified analyses after splitting the sample at the median follow-up time, or at the age of 16, or at 0/1+ prevalent endocrine diseases at baseline. The results from those subgroup analyses were similar to the main one.

Given that chronic iron overload is supposed to be the main driver of endocrine complications due to blood transfusions, we have not only used the baseline markers of iron overload (ferritin, LIC, and T2*) in our predictive models, but we have also tested the latest available measures and the difference between initial and final measures. In no cases those markers had any effect on the incidence of endocrine complications. TSH was not correlated with any marker of iron overload (Spearman's rhos <0.07, P>0.18).

Discussion

Endocrine complications remain the most common and resource-consuming disorders secondary to iron overload in TDT patients. In historical cohorts, disturbances of sexual axis affected 80% of patients, while bone metabolism disorders and short stature were reported in up to 60% and 50% of overall study population, respectively. Prevalence of hypothyroidism and diabetes ranged from 6 to 14%, while hypoparathyroidism was reported up to 25%.[18]

In our long-term cohort study of patient affected by TDT treated with the iron-chelating drug deferasirox, the risk of developing an endocrine complication is generally lower than the previously reported risk, but there is considerable risk variation, according to several parameters such as patient’s age, number of endocrine

complications already present before the start of the therapy, and TSH serum concentration. We developed a simple risk chart enabling clinicians to derive an approximate estimate of their patients' risk.

Ferritin, LIC and cardiac T2* are considered as markers of iron overload, but the correlation between those markers and risk of endocrine complications is controversial,[20] since many studies have shown no correlation,[21–23] confirming our results. This disconnection with iron overload parameters has been observed also in chronic metabolic syndromes, although substantial evidence shows that the clinical course of these disorders is affected by iron overload.[24] The different mechanisms of iron uptake and accumulation among different organs may be responsible of that phenomenon. The iron accumulates in the liver due to transferrin-mediated mechanisms and LIC has inadequate ability to predict that risk in extrahepatic organs.[20] The endocrine glands and the heart, instead, develop pathologic iron overload exclusively through uptake of non-transferrin bound iron (NTBI). The mechanism by which this uptake occurs is controversial too, but L-type calcium must play a role as they are present in large numbers in cardiomyocytes, pancreatic beta cells, in various cell types of the anterior pituitary gland (including gonadotrophs, thyrotrophs, and corticoptrophs), and in the parathyroid-hormone-producing cells of the parathyroid gland.[25] Although NTBI composes a very small fraction of body iron, it produces oxidative stress and organ damage.[26] While elevated LIC increases patients' risk of iron overload complications, there is not a LIC threshold below which cardiac and endocrine iron accumulation does not occur.[27] The explanation of this paradox is that many chronically transfused patients have fully-saturated transferrin, regardless of their LIC,[28] and, as heart and endocrine glands exclusively accumulate NTBI, it is possible for them to be in positive iron balance even if the total body iron balance (LIC) is neutral or negative.[27] Patients who miss chelator doses expose their extrahepatic organs to unrestricted uptake of labile iron species.[29]

Previous studies reported a correlation between cardiac T2* and manifest endocrinopathies.[21,22] However, those studies concerned patients with severe iron overload, with T2* <20 msec, while our sample had average ferritin <1000 ng/ml, LIC <5mg/dw, and T2* >30 msec which are considered as the acceptable target levels to reach during iron-chelating therapy.[18] It has been shown that the iron overload of endocrine glands comes before that of the heart, although both phenomena are mediated by NTBI.[11] However, endocrine organs have superior reserve capacity and the clinical manifestations concerning them may appear years after a silent iron accumulation.[12] When iron overload continues, due to lack of patient compliance or to inadequate dose of iron-binding therapy, the heart starts showing signs of overload, which can be identified through MRI-T2*.[20] Therefore, cardiac T2* is not an early indicator of iron overload. We have not found a correlation between T2*

and endocrine complications in our cohort of patients, as the vast majority of our patients had an acceptable iron balance. Abnormal cardiac T2* is an excellent marker of NTBI control, but it is insensitive because exposure must be severe and quite prolonged. As a result, abnormal cardiac T2* has a very high positive predictive value for endocrine iron deposition. However, once the heart has been successfully de-ironed, endocrine glands typically retain moderate iron deposition. Finally, even when the endocrine glands have been successfully de-ironed, their functional reserve has been destroyed.[4,11,12,20-22]

As there is considerable variation in the risk of endocrinopathies in patients without signs of heart and liver overload,[17,21,22,30–32] and because those kinds of endocrinopathies, when manifest, are irreversible[17,21,22,30–32], further clinical and laboratory predictors in addition to MRI imaging of endocrine glands are needed to prevent endocrine complications.

We proposed our risk chart on the model 3B, in which the association between risk of complication and age was considered as being linear, while adding a binary marker of adulthood and an interaction parameter between age and adulthood. We preferred this model to the simpler model 3A (log-linear age alone) because it had a slightly better R^2 and mostly because adults and children affected by TDT are usually treated in separate health care centres and as a matter of fact they define two separate categories. We have developed the largest analysis on endocrine complications in TDT patients ever developed so far and this is the first study providing clear benchmarks for patients' management. However, the predictive power of our risk chart must be improved and validated.

It is plausible that a diagnosis of an endocrinopathy produces a warning effect that is similar to that observed after an abnormal cardiac T2*, which makes patients have better compliance and clinicians increase iron-chelating dose.[33] That may explain why previous endocrinopathies were associated with lower incidence of new ones in our sample. Another reason may be that the therapy for an endocrine disease ameliorates the function of other endocrine axes. This has been previously shown for bone metabolism disorders, metabolic syndrome, and glucose and lipid metabolism disorders after treatment for hypothyroidism and hypogonadism.[34–36] Furthermore, endocrine glands are not equally vulnerable to the iron toxicity, and patients with more endocrinopathies have already wiped out the most endangered endocrine glands.

All our patients were on regular iron-chelation therapy and had acceptable levels of iron load. Therefore, the markers of iron overload were expected to be stable over time or to have minor fluctuations. However, iron

overload increased for same patients. If that phenomenon was due to scarce patient compliance and if compliance was associated with our explanatory variables, our estimates may be biased. However, also iron overload measures taken contemporarily with disease incidence have shown no effect, as well as their deltas. A lack of compliance could have been assumed if at least the latest tests assessing iron overload were associated with higher risk.

Heightened TSH has been associated with endothelial dysfunction,[37] defined as a diminished bioavailability of nitric oxide (NO) and/or an increase in vasoconstrictive factors such as endothelin (ET-1). That condition has been well documented in thalassemia patients and is associated with cardiac, hepatic and endocrine clinical complications.[32,38] Endothelial dysfunction in TDT is a progressive process, starting from childhood, and recent studies found significantly higher plasma levels of asymmetric dimethylarginine (ADMA), a novel risk marker of cardiovascular disease implicated in the pathogenesis of endothelial dysfunction, in very young TDT children.[39] So, increased TSH may be an early expression of systemic endothelial dysfunction in TDT which is considered an independent risk factor of future complications.[32] TSH appears the best marker of systemic endocrine gland dysfunction, as its measurement is very accurate and widely used in clinical practice,[40] differently from the several limits in the assessment of other pituitary hormones, as GH, ACTH, LH/FSH.[41] Furthermore, production of TSH is the last affected by the progressive damage of pituitary gland in TDT patients which impairs firstly GH secretion, followed by LH/FSH and ACTH.[42] For all these reasons, TSH may be the sentinel for endocrine gland dysfunction. Along with TSH, age is also associated with endothelial dysfunction, which could be the main driver of endocrine and cardiovascular risk.[32] These observations pave the way for the early identification of clinical complications in other metabolic diseases, which have been reported greatly affected by iron overload.[24]

The variation in risk of complications that our best model could explain was insufficient (25%). Therefore, there must be factors other than those we considered that have some effect on the incidence on endocrine complications. These may include NTBI, transferrin saturation, smoking, other markers of endothelial dysfunction, pancreas and pituitary R2* which we have not considered. Furthermore, the different chelation history among the study cohort (older patients treated with subcutaneous deferoxamine for longer compared to younger patients treated with oral deferasirox for longer) creates an inherent age effect to be taken into consideration.

The apparent increase in endocrine complications after the age of 12 is certainly related to different factors, such as the current inability to recognize hypogonadism prior to puberty; the effect of hypogonadism on bone metabolism due to the impact of steroids on bone mineralization; the delay between the start of iron damage in the gland tissues and the onset of overt clinical complications, e.g diabetes.[4,11-13] Furthermore, adolescence is also marked by less physical activity and more adverse body habitus and nutrition that worsen insulin sensitivity. For all these reasons, children are not protected by iron damage in endocrine glands and conversely,

they require a more aggressive prophylaxis to avoid pituitary and pancreatic iron accumulation which will be clinically manifest only different years later, when the functional reserve has been destroyed. Different chelation goals (such as transferrin desaturation and the use of direct endocrine imaging) and alternative chelation strategies are necessary to better protect endocrine glands.

In conclusion, although in DFX-treated TDT patients the risk of developing an endocrine complication is generally lower than the previously reported risk, there is considerable risk variation and the burden of these complications remains high. This is the first study providing a practical tool for physicians to identify patients at higher risk of developing endocrine complications. Future research will look at increasing the amount of variation explained from our model and testing further clinical and laboratory predictors, including the assessment of direct endocrine MRI.

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Tables

Table 1. Cross-tabulation of number of conditions at baseline and number of conditions occurred during the follow-up.

Table 2A. Sample description stratified by number of endocrine diseases at baseline.

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Table 3A. Risk factors for developing a new endocrine disease during the follow-up: results from the simplest multiple Cox regression model.

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Table 4A. Predicted risk chart for developing a new endocrine disease within 5 years, in percentages. The overall 5y-risk was 9.7%

Table 4B. Predicted risk chart for developing a new endocrine disease within 1 year, in percentages. The overall 1y-risk was 1.1%

Table 1. Cross-tabulation of number of conditions at baseline and number of conditions occurred during the follow-up.

No of endocrine diseases at baseline	No of new endocrine diseases occurred				Total
	0	1	2	3	
0	75 (63.6%) (23.3%)	32 (27.1%) (36.4%)	10 (8.5%) (66.7%)	1 (0.9%) (100.0%)	118 (100%) (27.7%)
1	87 (71.9%) (27.0%)	30 (24.8%) (34.1%)	4 (3.3%) (26.7%)	0 (0.0%) (0.0%)	121 (100%) (28.4%)
2	86 (80.4%) (26.7%)	21 (19.6%) (23.9%)	0 (0.0%) (0.0%)	0 (0.0%) (0.0%)	107 (100%) (25.1%)
3	59 (90.8%) (18.3%)	5 (7.7%) (5.7%)	1 (1.5%) (6.7%)	n/a	65 (100%) (15.3%)
4	15 (100.0%) (4.7%)	0 (0.0%)	n/a	n/a	15 (100%) (3.5%)
Total	322 (75.6%) (100%)	88 (20.7%) (100%)	15 (3.5%) (100%)	1 (0.2%) (100%)	426 (100%) (100%)

Table 2A. Sample description stratified by number of endocrine diseases at baseline.

Factors and categories*	Number of endocrine diseases at baseline			P Value
	0	1	2+	
	N=118	N=121	N=187	
Age (years)	9.1 (5.4-23.2)	28.9 (18.6-36.7)	34.7 (29.6-39.7)	<0.001
Paediatric patient (<16 y.o.)	68.6% (81/118)	17.4% (21/121)	3.7% (7/187)	<0.001
Age if child (n=109)	6.9 (3.7)	10.9 (3.5)	10.9 (3.9)	<0.001
Age if adult (n=317)	31.3 (11.9)	32.0 (10.5)	35.6 (7.3)	0.001
Gender male	50.8% (60/118)	49.6% (60/121)	38.5% (72/187)	0.054
Splenectomised	23.7% (28/118)	47.9% (58/121)	75.4% (141/187)	<0.001
Used drugs other than DFX in the past	27.1% (32/118)	18.2% (22/121)	36.4% (68/187)	0.002
Heart disease	4.4% (5/114)	7.8% (9/116)	19.5% (34/174)	<0.001
Thyroid disorder	0.0% (0/118)	6.6% (8/121)	35.8% (67/187)	
Parathyroid disorder	0.0% (0/118)	1.7% (2/121)	7.0% (13/187)	
Gonadal disorder	0.0% (0/118)	11.6% (14/121)	88.2% (165/187)	
Glucose metabolism disorder	0.0% (0/118)	0.0% (0/121)	26.7% (50/187)	
Bone metabolism disorder	0.0% (0/118)	80.2% (97/121)	93.0% (174/187)	
Ferritin (ng/ml)	1342.1 (×/2.0)	937.2 (×/2.3)	844.9 (×/2.2)	<0.001
Ferritin >2000 (ng/ml)	28.4% (29/102)	18.3% (21/115)	15.0% (26/173)	0.023
LIC (mg Fe/g dry weight)	4.9 (×/2.4)	4.0 (×/2.4)	3.9 (×/2.2)	0.18
LIC				0.34
<3	28.6% (20/70)	43.2% (41/95)	37.4% (55/147)	
3-	38.6% (27/70)	27.4% (26/95)	38.1% (56/147)	
7-	22.9% (16/70)	22.1% (21/95)	19.7% (29/147)	
15+	10.0% (7/70)	7.4% (7/95)	4.8% (7/147)	
Heart T2* (msec)	36.0 (11.3)	35.7 (9.4)	30.5 (12.0)	<0.001
EF (%)	64.0 (6.6)	62.9 (5.8)	64.4 (6.8)	0.33
TSH (mIU/L)	2.5 (×/1.5)	2.1 (×/1.6)	2.1 (×/2.1)	0.061
On levotyroxine	0.0% (0/104)	5.2% (6/115)	27.8% (49/176)	<0.001
FT4 (pmol/L)	14.8 (3.3)	14.2 (2.9)	14.5 (4.6)	0.54
TSH index	2.8 (0.7)	2.6 (0.7)	2.7 (0.9)	0.39
PTH (pg/mL)	25.0 (×/1.6)	27.3 (×/1.8)	21.9 (×/2.3)	0.19
Glycaemia (mg/dL)	84.5 (×/1.1)	84.2 (×/1.1)	95.1 (×/1.3)	<0.001
Calcium (mg/dL)	9.4 (9.1-9.7)	9.3 (8.9-9.6)	9.3 (9.0-9.7)	0.25
Phosphorus (mg/dL)	4.3 (3.5-5.0)	4.1 (3.5-4.7)	4.0 (3.4-4.5)	0.17
BMD femur (g/cm2)	0.9 (0.8-1.0)	0.7 (0.6-0.9)	0.7 (0.6-0.8)	<0.001
BMD femur (z score)	-0.4 (1.5)	-1.5 (1.1)	-2.0 (1.0)	<0.001
BMD femur (t score)	-0.5 (1.5)	-1.6 (0.9)	-2.1 (1.0)	<0.001
BMD L1-L4 (g/cm2)	1.0 (0.9-1.1)	0.8 (0.7-0.9)	0.8 (0.7-0.9)	<0.001
BMD L1-L4 (z score)	-0.8 (-1.4--0.3)	-2.1 (-2.9--1.3)	-2.5 (-3.2--1.9)	<0.001
BMD L1-L4 (t score)	-0.8 (-1.4--0.3)	-2.2 (-3.0--1.3)	-2.8 (-3.4--2.0)	<0.001
New endocrine disease occurred	36.4% (43/118)	28.1% (34/121)	14.4% (27/187)	<0.001
Thyroid disorder occurred	5.1% (6/118)	8.8% (10/113)	5.0% (6/120)	0.39
Parathyroid disorder occurred	0.8% (1/118)	1.7% (2/119)	4.0% (7/174)	0.18
Gonadal disorder occurred	12.7% (15/118)	12.1% (13/107)	18.2% (4/22)	0.74
Glucose metabolism disorder occurred	3.4% (4/118)	2.5% (3/121)	5.8% (8/137)	0.36
Bone metabolism disorder occurred	24.6% (29/118)	41.7% (10/24)	23.1% (3/13)	0.22
Side effect occurred	0.8% (1/118)	4.1% (5/121)	2.7% (5/187)	0.28
Δ Ferritin †	-476.6 (1519.6)	-310.8 (1419.9)	-430.6 (1103.1)	0.62
Δ LIC †	-1.4 (5.2)	-1.6 (6.6)	-1.8 (6.6)	0.93
Δ T2star †	0.7 (13.8)	1.9 (11.5)	5.9 (12.8)	0.008
Δ TSH †	-0.1 (1.4)	0.1 (1.5)	0.5 (5.1)	0.41
Δ TSH index †	-0.0 (0.8)	0.0 (0.8)	0.2 (0.9)	0.092
Δ BMD femur (g/cm2)†	-0.0 (0.1)	0.0 (0.3)	0.0 (0.2)	0.74
Δ BMD L1-L4 (g/cm2)†	-0.2 (0.7)	0.0 (0.1)	0.0 (0.2)	0.007

For normally-distributed variables, data are presented as mean (SD) with P value from ANOVA. For log-normal variables, data are presented as geometric mean (×/geometric SD) with P value from ANOVA on logged values. For continuous variables with other types of distributions, data are presented as median (IQR) with P value from Kruskal-Wallis test. For categorical variables, data are presented as % (n/total) with P value from Pearson's chi-squared test.

*Measured at baseline, unless otherwise specified. †Intra-individual difference between measures taken at the end and at the beginning of the follow-up.

Table 2B. Sample description stratified by categories of outcome measure.

Factors and categories*	New incident		P Value
	No	Yes	
	N=322	N=104	
Age (years)	30.5 (18.2-38.3)	27.0 (11.8-35.4)	0.029
Paediatric patient (<16 y.o.)	23.3% (75/322)	32.7% (34/104)	0.056
Age if child (n=109)	7.5 (4.0)	8.9 (3.7)	0.082
Age if adult (n=317)	34.2 (8.6)	33.2 (11.1)	0.42
Gender male	43.2% (139/322)	51.0% (53/104)	0.16
Splenectomised	54.7% (176/322)	49.0% (51/104)	0.32
Used drugs other than DFX in the past	28.9% (93/322)	27.9% (29/104)	0.84
Heart disease	10.7% (33/309)	15.8% (15/95)	0.18
Thyroid disorder	19.9% (64/322)	10.6% (11/104)	0.030
Parathyroid disorder	3.1% (10/322)	4.8% (5/104)	0.41
Gonadal disorder	46.3% (149/322)	28.8% (30/104)	0.002
Glucose metabolism disorder	14.0% (45/322)	4.8% (5/104)	0.012
Bone metabolism disorder	70.8% (228/322)	41.3% (43/104)	<0.001
No. of endocrine diseases at baseline			<0.001
0	23.3% (75/322)	41.3% (43/104)	
1	27.0% (87/322)	32.7% (34/104)	
2+	49.7% (160/322)	26.0% (27/104)	
Ferritin (ng/ml)	979.3 (×/2.2)	995.8 (×/2.4)	0.86
Ferritin >2000 (ng/ml)	18.5% (55/297)	22.6% (21/93)	0.39
LIC (mg Fe/g dry weight)	4.1 (×/2.4)	4.2 (×/2.1)	0.85
LIC			0.51
<3	37.3% (90/241)	36.6% (26/71)	
3-	33.2% (80/241)	40.8% (29/71)	
7-	22.8% (55/241)	15.5% (11/71)	
15+	6.6% (16/241)	7.0% (5/71)	
Heart T2* (msec)	32.9 (10.7)	34.1 (13.5)	0.46
EF (%)	64.0 (6.0)	63.2 (7.9)	0.41
TSH (mIU/L)	2.1 (×/1.9)	2.5 (×/1.6)	0.019
On levotyroxine	15.8% (47/298)	8.2% (8/97)	0.063
FT4 (pmol/L)	14.5 (4.0)	14.5 (3.2)	0.92
TSH index	2.7 (0.8)	2.8 (0.8)	0.24
PTH (pg/mL)	25.1 (×/2.0)	21.8 (×/2.1)	0.26
Glycaemia (mg/dL)	89.3 (×/1.3)	86.8 (×/1.2)	0.29
Calcium (mg/dL)	9.3 (9.0-9.6)	9.3 (9.0-9.8)	0.41
Phosphorus (mg/dL)	4.1 (3.5-4.7)	4.0 (3.4-4.7)	0.54
BMD femur (g/cm2)	0.7 (0.6-0.8)	0.8 (0.7-0.9)	0.050
BMD femur (z score)	-1.7 (1.1)	-1.5 (1.3)	0.32
BMD femur (t score)	-1.9 (1.1)	-1.5 (1.3)	0.079
BMD L1-L4 (g/cm2)	0.8 (0.7-0.9)	0.9 (0.8-1.0)	0.019
BMD L1-L4 (z score)	-2.3 (-3.0--1.6)	-1.8 (-2.6--1.0)	0.020
BMD L1-L4 (t score)	-2.6 (-3.3--1.7)	-1.8 (-2.8--1.0)	0.003
Side effect	2.8% (9/322)	1.9% (2/104)	0.63
Δ Ferritin †	-405.3 (1263.5)	-413.4 (1481.1)	0.96
Δ LIC †	-1.7 (6.6)	-1.7 (4.9)	0.97
Δ T2star †	3.7 (12.1)	3.4 (14.7)	0.85
Δ TSH †	0.2 (4.0)	0.3 (1.9)	0.89
Δ TSH index †	0.1 (0.8)	0.2 (0.9)	0.29
Δ BMD femur (g/cm2)†	0.0 (0.2)	-0.0 (0.2)	0.23
Δ BMD L1-L4 (g/cm2)†	0.0 (0.2)	-0.1 (0.5)	0.009

For normally-distributed variables, data are presented as mean (SD) with P value from ANOVA. For log-normal variables, data are presented as geometric mean (×/geometric SD) with P value from ANOVA on logged values. For continuous variables with other types of distributions, data are presented as median (IQR) with P value from Kruskal-Wallis test. For categorical variables, data are presented as % (n/total) with P value from Pearson's chi-squared test.

*Measured at baseline, unless otherwise specified. †Intra-individual difference between measures taken at the end and at the beginning of the follow-up.

Table 3A. Risk factors for developing a new endocrine disease during the follow-up: results from the simplest multiple Cox regression model.

Variable at the beginning of follow-up	Mutually-adjusted hazard ratio	(95%CI)	P
Diseases at baseline (1 increase)	0.53	(0.43 0.66)	<0.001
TSH (1 mIU/L increase)	1.25	(1.13 1.38)	<0.001
Age (50% increase)	1.18	(1.05 1.33)	0.005

Table 3B. Risk factors for developing a new endocrine disease during the follow-up: results from the multiple Cox regression model showing the highest adjusted R^2, which was used to draw the risk charts.

Variable at the beginning of follow-up	Mutually-adjusted hazard ratio	(95%CI)	P
Diseases at baseline (1 increase)	0.54	(0.43 0.67)	<0.001
TSH (1 mIU/L increase)	1.26	(1.15 1.39)	<0.001
Age (5-year increase)	1.12	(1.00 1.26)	0.053
Child vs Adult	6.70	(1.32 34.02)	0.022
Interaction Age*Child	1.59	(1.02 2.47)	0.041

Table 4A. Predicted risk chart for developing a new endocrine disease within 5 years, in percentages. The overall 5y-risk was 9.7%

	Diseases at baseline = 0			Diseases at baseline = 1			Diseases at baseline = 2			Diseases at baseline = 3		
Age	**TSH=1**	**TSH=3**	**TSH=5**	**TSH=1**	**TSH=3**	**TSH=5**	**TSH=1**	**TSH=3**	**TSH=5**	**TSH=1**	**TSH=3**	**TSH=5**
1	6.3	9.9	15.2	3.4	5.4	8.5	1.9	3.0	4.7	1.0	1.6	2.5
2	6.5	10.2	15.8	3.6	5.6	8.8	1.9	3.1	4.8	1.0	1.7	2.6
4	7.1	11.1	17.1	3.9	6.1	9.6	2.1	3.3	5.3	1.1	1.8	2.9
6	7.7	12.0	18.5	4.2	6.6	10.4	2.3	3.6	5.7	1.2	2.0	3.1
8	8.4	13.1	20.1	4.6	7.3	11.4	2.5	4.0	6.3	1.4	2.2	3.4
10	9.3	14.4	21.9	5.1	8.0	12.5	2.8	4.4	6.9	1.5	2.4	3.8
12	10.3	15.8	24.0	5.7	8.9	13.8	3.1	4.9	7.6	1.7	2.6	4.2
14	11.4	17.5	26.3	6.3	9.9	15.2	3.5	5.4	8.5	1.9	3.0	4.7
16	12.8	19.4	28.9	7.1	11.0	16.9	3.9	6.1	9.6	2.1	3.3	5.3
18	14.3	21.6	31.6	8.0	12.4	18.9	4.4	6.9	10.8	2.4	3.8	6.0
20	16.1	23.9	34.5	9.1	14.0	21.0	5.1	7.9	12.2	2.8	4.3	6.8
25	21.5	30.5	41.5	12.7	18.9	27.3	7.2	11.1	16.6	4.0	6.2	9.6
30	27.8	36.9	46.9	17.7	25.0	33.8	10.5	15.6	22.4	6.0	9.2	13.8
35	33.5	41.4	50.2	23.6	31.0	38.7	15.2	21.3	28.5	9.1	13.4	19.2
40	37.2	43.9	52.7	29.1	35.1	41.5	20.8	27.1	33.2	13.5	18.9	25.0
45	39.0	45.9	55.2	32.5	37.2	43.3	26.2	31.0	35.5	19.0	24.4	29.5
50	40.5	47.9	57.9	34.0	38.6	45.2	29.5	32.8	36.8	24.2	28.3	31.6

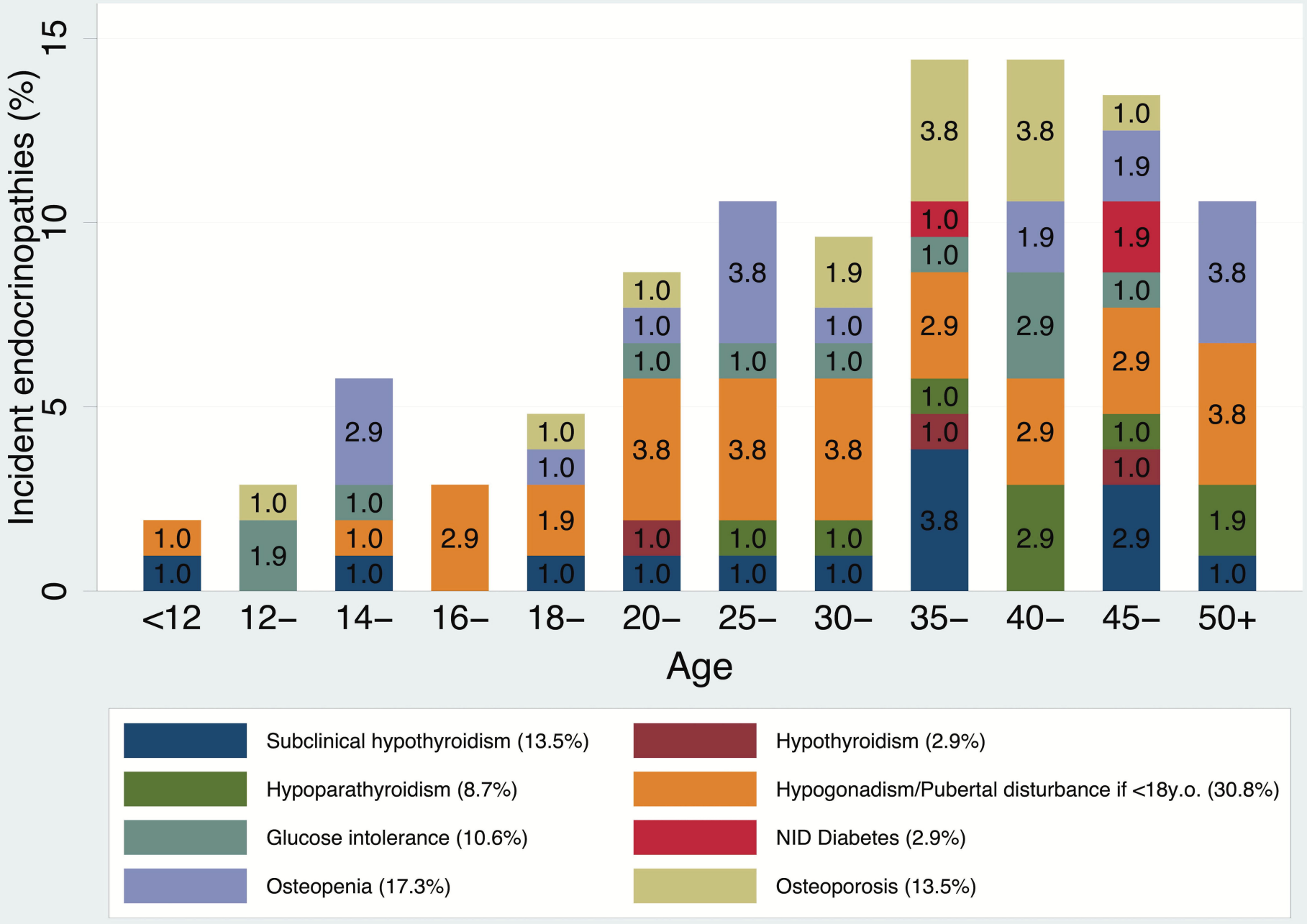
Table 4B. Predicted risk chart for developing a new endocrine disease within 1 year, in percentages. The overall 1y-risk was 1.1%

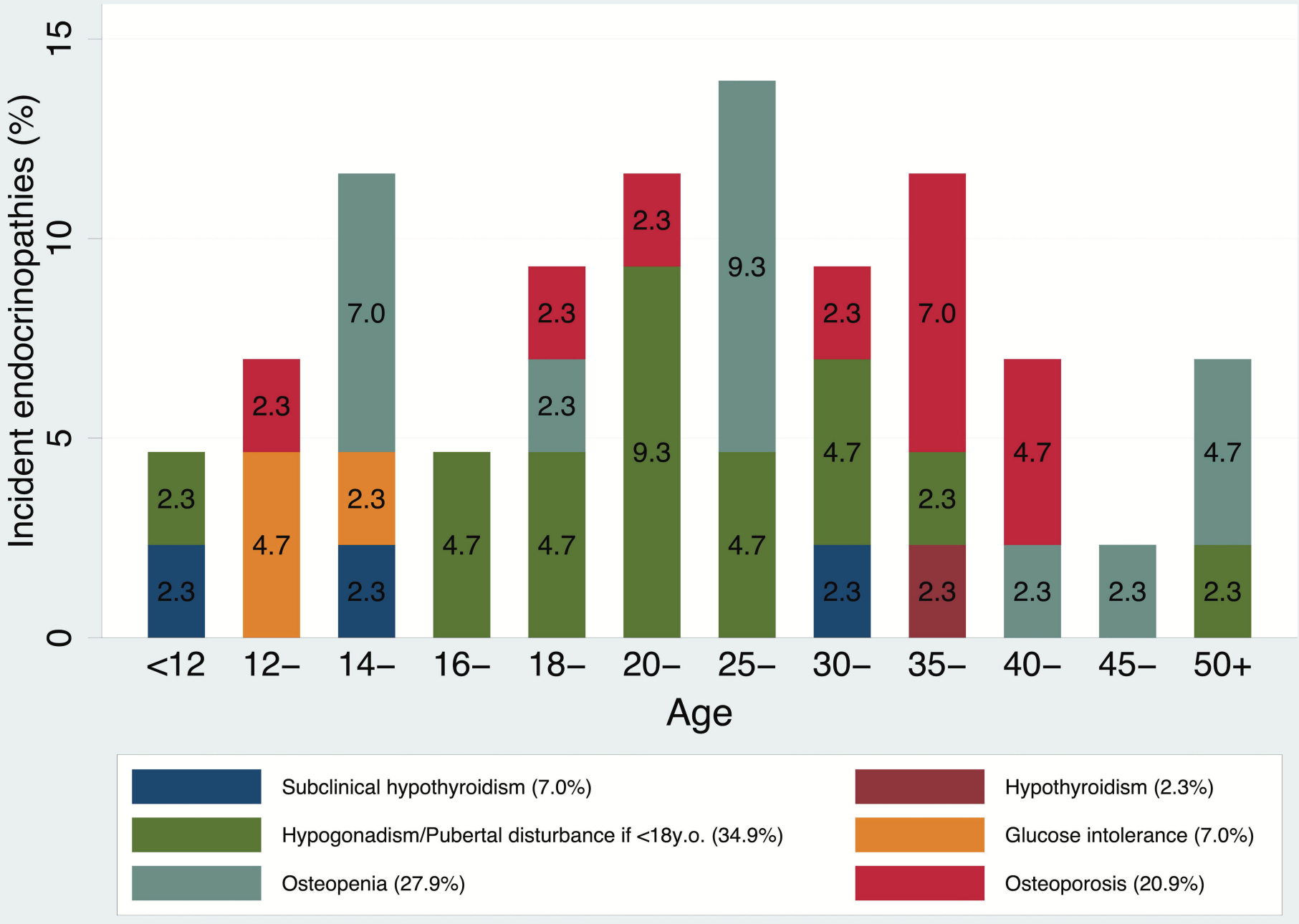
	Diseases at baseline = 0			Diseases at baseline = 1			Diseases at baseline = 2			Diseases at baseline = 3		
Age	**TSH=1**	**TSH=3**	**TSH=5**	**TSH=1**	**TSH=3**	**TSH=5**	**TSH=1**	**TSH=3**	**TSH=5**	**TSH=1**	**TSH=3**	**TSH=5**
1	0.6	1.0	1.6	0.3	0.5	0.9	0.2	0.3	0.5	0.1	0.2	0.3
2	0.7	1.1	1.7	0.4	0.6	0.9	0.2	0.3	0.5	0.1	0.2	0.3
4	0.7	1.1	1.8	0.4	0.6	1.0	0.2	0.3	0.5	0.1	0.2	0.3
6	0.8	1.3	2.0	0.4	0.7	1.1	0.2	0.4	0.6	0.1	0.2	0.3
8	0.9	1.4	2.2	0.5	0.7	1.2	0.3	0.4	0.6	0.1	0.2	0.3
10	1.0	1.5	2.4	0.5	0.8	1.3	0.3	0.4	0.7	0.1	0.2	0.4
12	1.1	1.7	2.7	0.6	0.9	1.5	0.3	0.5	0.8	0.2	0.3	0.4
14	1.2	1.9	3.0	0.6	1.0	1.6	0.3	0.6	0.9	0.2	0.3	0.5
16	1.4	2.1	3.4	0.7	1.2	1.8	0.4	0.6	1.0	0.2	0.3	0.5
18	1.5	2.4	3.9	0.8	1.3	2.1	0.4	0.7	1.1	0.2	0.4	0.6
20	1.8	2.8	4.4	1.0	1.5	2.4	0.5	0.8	1.3	0.3	0.4	0.7
25	2.6	4.1	6.3	1.4	2.2	3.5	0.8	1.2	1.9	0.4	0.6	1.0
30	3.9	6.1	9.3	2.1	3.4	5.3	1.2	1.8	2.9	0.6	1.0	1.6
35	6.1	9.2	13.6	3.4	5.2	8.0	1.9	2.9	4.5	1.0	1.6	2.5
40	9.3	13.6	19.1	5.4	8.2	12.1	3.0	4.7	7.1	1.7	2.6	4.1
45	14.0	19.1	24.6	8.5	12.4	17.4	4.9	7.5	11.0	2.8	4.3	6.5
50	19.5	24.4	28.4	13.0	17.8	22.8	7.9	11.6	16.1	4.6	7.0	10.3

Figure Legends

Figure 1. Overall crude risks for all 104 first incidents (n=426), by incident type and age group.

Figure 2. Crude risks for all 43 first incidents in patients with no endocrinopathies at baseline (n=118), by incident type and age group.


Incident endocrinopathies (%)
0
5
10
15
<12
12–
14–
16–
18–
20–
25–
30–
35–
40–
45–
50+
Age
Subclinical hypothyroidism (13.5%)
Hypothyroidism (2.9%)
Hypoparathyroidism (8.7%)
Hypogonadism/Pubertal disturbance if <18y.o. (30.8%)
Glucose intolerance (10.6%)
NID Diabetes (2.9%)
Osteopenia (17.3%)
Osteoporosis (13.5%)

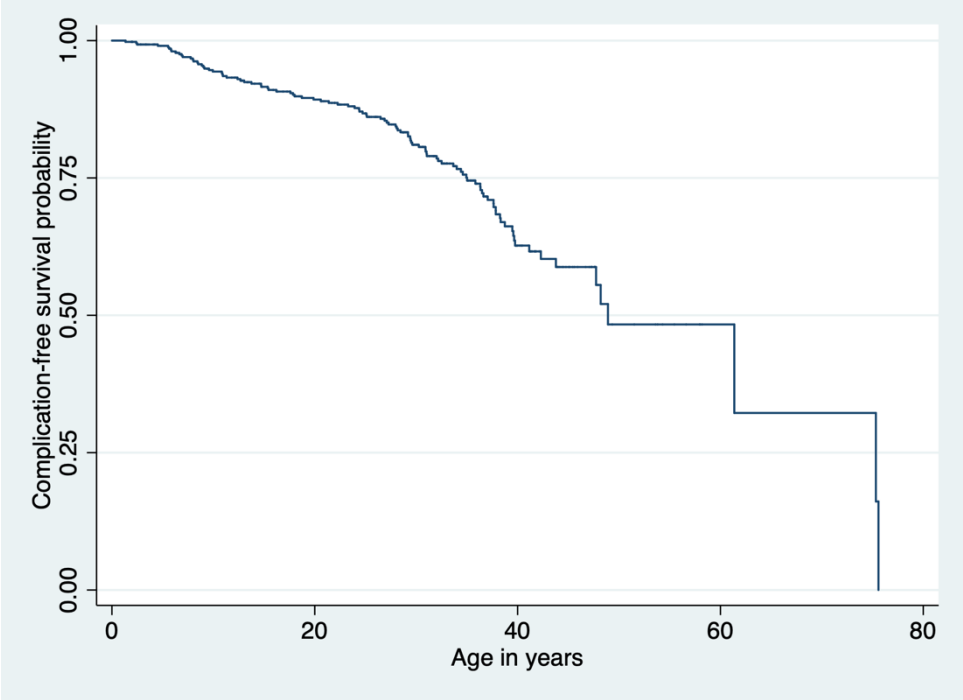

Incident endocrinopathies (%)
0
5
10
15
2.3
4.7
2.3
2.3
7.0
2.3
4.7
2.3
2.3
4.7
2.3
9.3
2.3
4.7
9.3
2.3
2.3
4.7
7.0
2.3
4.7
2.3
2.3
4.7
<12
12–
14–
16–
18–
20–
25–
30–
35–
40–
45–
50+
Age
Subclinical hypothyroidism (7.0%)
Hypogonadism/Pubertal disturbance if <18y.o. (34.9%)
Osteopenia (27.9%)
Hypothyroidism (2.3%)
Glucose intolerance (7.0%)
Osteoporosis (20.9%)

Supplemental Material

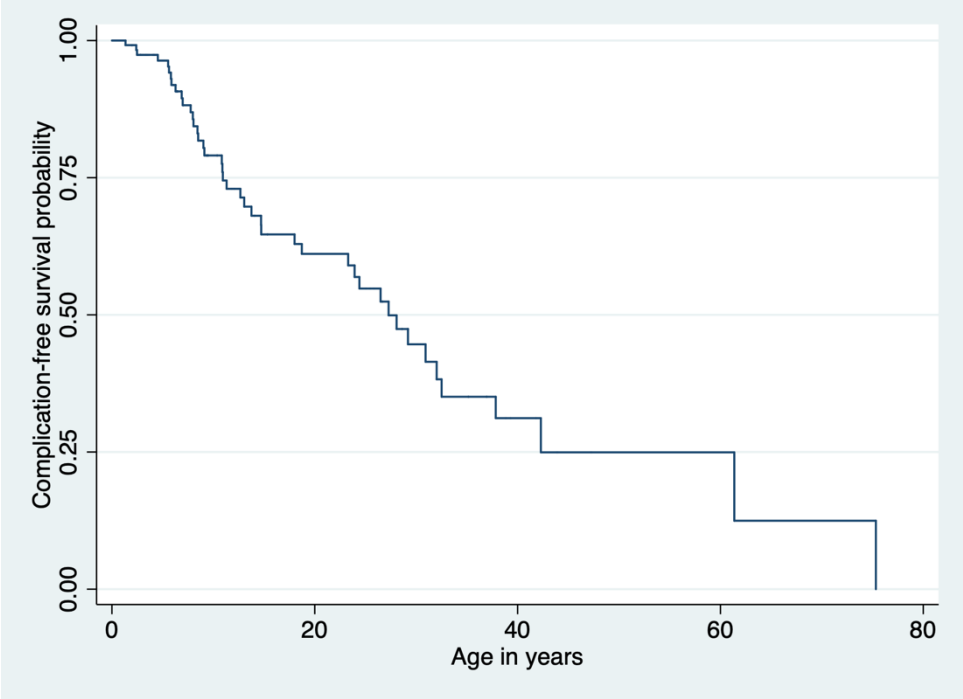
Online Supplementary Methods

Standardized protocols for the management of the disease were shared and discussed during meetings hold with clinicians from each centre. All consecutive patients visited at the participating sites since September 2009 were recruited into the cohort provided the inclusion criteria were met: affected by TDT and assigned to long- term deferasirox monotherapy. All patients included in the study had to maintain the same chelator during the observation in order to avoid biases related to the change of chelator drug. In the event of patient death, the collection was performed until the last available assessment. In the event of deferasirox discontinuation, endocrine data were collected until the time of discontinuation, along with reasons for discontinuation, such as adverse events, therapy failure, poor compliance, medical decision, etc. For those patients who started deferasirox therapy prior to 2009 and were still on the same chelation therapy at the study enrolment, the observation time was extended to the start of the deferasirox therapy. All centres followed standardized protocols, also for the transfusion program,[18] maintaining pre-transfusion haemoglobin above 9-10.5 g/dl or higher levels for children or patients with cardiac complications (≥ 10.5 g/dl). Some variables were routinely measured in adults, such as estradiol, testosterone, LH, and FSH, BMD.

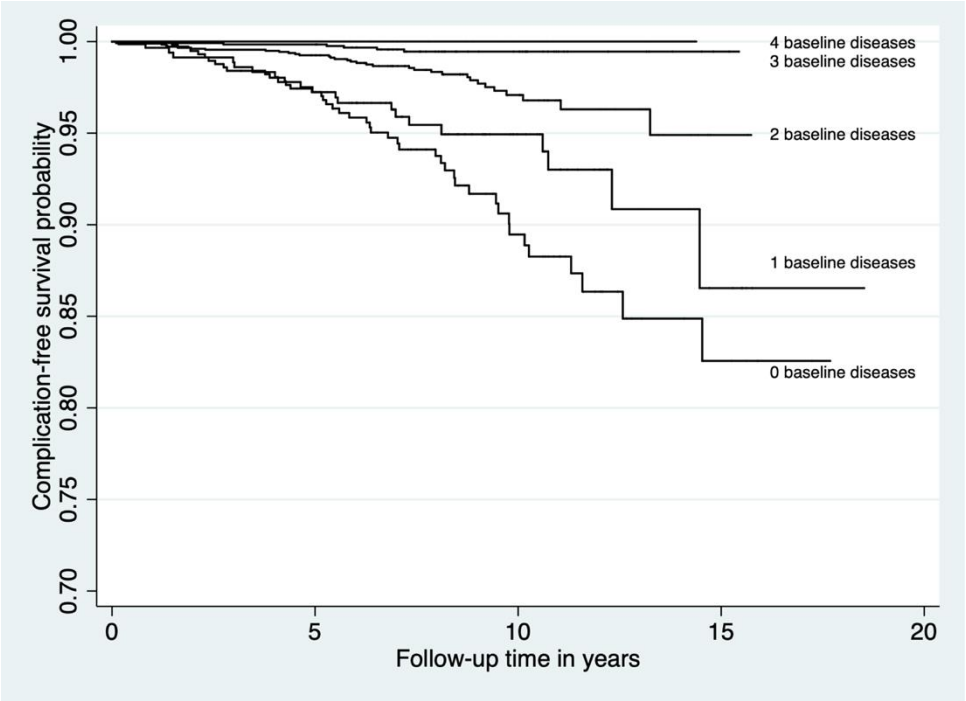
Supplemental Material 1. Crude survival curve for all patients (n=426, events=104), with age as the underlying time variable.



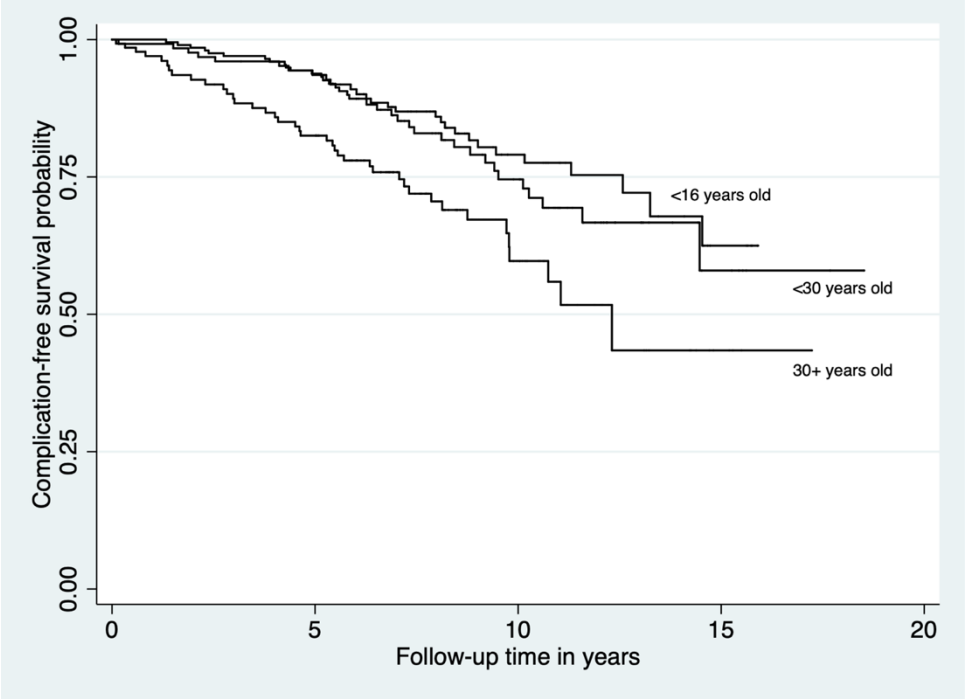
Supplemental Material 2. Crude survival curve for patients with no endocrinopathies at baseline (n=118, events=43), with age as the underlying time variable.



Supplemental Material 3. Survival curves by number of endocrinopathies at baseline, adjusted for age and TSH.



Supplemental Material 4. Survival curves by age group, adjusted for TSH and number of endocrine diseases at baseline.



Supplemental Material 5. Survival curves by levels of TSH, adjusted for age and number of endocrine conditions at baseline.