Cardiovascular morbidity and mortality in patients with Resistance to Thyroid Hormone β (RTHβ): a linked record study

Supplementary Appendix

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SUPPLEMENTARY METHODS

Modified Charlson Comorbidity Scores

Comorbidity scores were derived using a modification of the Charlson Comorbidity Index (CCI) (1). Individual scores were calculated as the sum of weighted scores for each of 17 disease conditions identified from hospital admission data using ICD-10 codes for secondary diagnosis fields (DIAG2-DIAG14) (2, 3) (Supplementary table 1).

Laboratory assays

The assay principles and reference ranges for thyroid function tests (FT4, FT3, and TSH) are presented in Supplementary table 2.

Study design and power considerations

Due to the low prevalence of RTHβ our study was designed as a cohort study with age-and-sex matched controls from the background population in the Secure Anonymised Information Linkage (SAIL) databank. Our primary endpoint was all-cause mortality with individual cardiovascular events and major adverse cardiovascular events as secondary outputs. Our sample size calculation was based on an estimated hazard ratio for mortality or major adverse cardiovascular events of 2·5 in an untreated hyperthyroid population, from published data in patients with Graves' hyperthyroidism (4). We modelled the range of sample sizes required for patients and controls at different patient-to-control ratios in order to achieve a two-sided α of 0·05 and power (ß) of 0·8 (supplementary figure 1). Since the control population were available on the SAIL databank without additional resource requirements, we opted for a 50:1 ratio of background population vs patients (supplementary figure 1). As has been shown, power can be improved by increasing matching ratios above 5:1 when the exposure or outcome prevalence is low among control subjects (5). Thus, our sample size of 55 patients and 2750 controls was adequately powered to demonstrate a hazard ratio of 2·5 for our primary outcomes.

Restricted Cubic Spline Regression

We modelled a potential non-linear relationship between the baseline FT4 concentration and mortality or MACE using restricted cubic spline regressions. Cubic splines allow flexible smooth transformations of the relationship between a quantitative covariate and an outcome (6, 7). We used the *mkspline* command in Stata to set 4 equally-spaced knots at percentiles 5, 35, 65 and 95 according to the recommendation

by Harrell (6). Varying the positions of the knots did not significantly influence our estimates. The reference values were set at 20 pmol/L. Predicted hazard ratios (HR) were derived from Cox regression models adjusted for age, gender, year of diagnosis, and comorbidity. We used the *xblc* post-estimation package in Stata to plot the regression between FT4 and log HR for mortality/MACE. P values for non-linearity were obtained using likelihood ratio tests.

Missing data

All datasets were complete for age, sex, comorbidity, and genetic mutation. FT4 and TSH values were missing for ≤5 patients, with FT3 measurements missing in 6 patients. Patients with at least one missing thyroid test, i.e., either FT4, FT3, or TSH (n=6, 11%) did not differ from the rest of the RTHβ population in terms of age, sex, comorbidity, or mortality or cardiovascular outcomes (data suppressed for privacy restrictions). A logistic regression model showed that missing data was not associated with age (odds ratio, 95% confidence interval [OR 95%CI] 1.03, 0.98-1.10) sex (OR 95%CI 0.38, 0.07-2.19) comorbidity (OR 95%CI 0.18, 0.12-2.76), mortality (OR 95%CI 1.26, 0.13-12.71), or MACE (OR 95%CI 2.62, 0.37-18.51). Thus we assumed that the data was missing at random (8) and addressed this in sensitivity analysis using multiple variable imputation by chained equations. We generated 10 imputed datasets with 100 iterations and fitted Cox proportional models within each dataset, after which estimates were pooled according to Rubin's rules (9).

SAIL databank privacy policy

In line with data privacy regulations, the SAIL databank operates a statistical disclosure control policy with the purpose of limiting the risk of participant identification. This includes minimum set thresholds for data display. SAIL prohibits the reporting of cells containing counts of ≤5 or the reporting of data that would enable counts of ≤5 to be derived from combining information across multiple cells. In compliance with this policy, we did not display risk tables for the survival analysis since small counts could be derived from combining information across multiple risk tables for individual outcomes.

Condition	ICD-10 diagnosis codes	Score
Acute myocardial infarction	I21, I22, I252	1
Congestive heart failure	I50	1
Peripheral vascular disease	I71, I790, I739, R02, Z958, Z959	1
Cerebral vascular disease	I60, I61, I62, I63, I65, I66, G450, G451, G452, G458, G459, G46, I64, G454, I670, I671, I672, I674, I675, I676, I677, I678, I679, I681, I682, I688, I69	1
Dementia	F00, F01, F02, F051	1
Pulmonary disease	J40, J41, J42, J44, J43, J45, J46, J47, J67, J44, J60, J61, J62, J63, J66, J64, J65	1
Connective tissue disease	M32, M34, M332, M053, M058, M059, M060, M063, M069, M050, M052, M051, M353	1
Peptic ulcer disease	K25, K26, K27, K28	1
Liver disease	K702, K703, K73, K717, K740, K742, K746, K743, K744, K745	1
Diabetes	E109, E119, E139, E149, E101, E111, E131, E141, E105, E115, E135, E145	1
Diabetes with complications	E102, E112, E132, E142, E103, E113, E133, E143, E104, E114, E134, E144	2
Hemiplegia or paraplegia	G81, G041, G820, G821, G822	2
Renal disease	N03, N052, N053, N054, N055, N056, N072, N073, N074, N01, N18, N19, N25	2
Cancer	C0, C1, C2, C3, C40, C41, C43, C45, C46, C47, C48, C49, C5, C6, C70, C71, C72, C73, C74, C75, C76, C81, C82, C83, C84, C85, C883, C887, C889, C900, C901, C91, C92, C93, C940, C941, C942, C943, C945, C947, C95, C96	2
Metastatic cancer	C77, C78, C79, C80	3
Severe liver disease	K729, K766, K767, K721	3
HIV	B20, B21, B22, B23, B24	6

Supplementary Table 1: Modified Charlson Comorbidity Scores

Reference (2, 3)

Laboratory	Assay method, platform	Reference Intervals		
		FT4, pmol/L	FT3, pmol/L	TSH, mU/L
Laboratory 1				
1998 - 2011	CCIA, ADVIA Centaur, Bayer[1]	9·8–23·1	2·22 – 5·35	0·35 – 5·50
2011 - 2018	CMIA, Abbott ARCHITECT[2]	9·0 –19·1*	2·60 – 5·70	0·30 – 4·40
2019 –2021	CMIA, Abbott ARCHITECT[2]	8·9–17·3	2·40 – 6·00	0·30 – 4·48
Laboratory 2				
1998 - 2007	CCIA, IMMULITE 2000, DPC[3]	10·3–24·5	2·30 – 6·29	0·40 - 4·00
2007 - 2013	ECLIA, Roche E-170[4]	10·3–24·5	3·10 – 6·80	0·40 - 4·00
2014 –2021	ECLIA, Roche	11·0–25·0	3·10 – 6·80	0·27 – 4·20
Laboratory 3				
1995 – 2011	CCIA, ADVIA Centaur, Bayer[1]	10·0-25·0	2·22 – 5·35	0·35-5·50**
2011- 2012	CCIA, ADVIA Centaur, Bayer[1]	10·0-25·0	2·22 – 5·35	0·35-5·50
2012- 2013	ECLIA, Roche E-170[4]	10·3–24·5	3·10 – 6·80	0·40 - 4·00
2014 –2021	ELECSYS, Roche	11·0–25·0	3·1 – 6·8	0·27 – 4·20
Laboratory 4				
1998-2006	CCIA, IMMULITE 2000, DPC[3]	11·5–22·7	2·30 – 6·29	0·40 - 4·00
2006-2013	CMIA, Abbott ARCHITECT[2]	9·0–19·1	2·60 – 5·70	0·30 - 4·40
Laboratory 5				
1998—2000	MEIA, Abbott Axsym[2]	9·0–19·1	2·60 – 5·70	0·30 - 4·40
2000—2006	CCIA, ADVIA Centaur, Bayer[1]	9·8–23·1	2·22 – 5·35	0·35 - 5·50
2007—2013	CMIA, Abbott ARCHITECT[2]	9·0–19·1	2·60 – 5·70	0·30 - 4·40

Supplementary Table 2: Thyroid hormone assay reference ranges

For all assays the coefficient of variation was<10%; CCIA, competitive chemiluminescent immunoassay, CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; MEIA, Microparticle Enzyme Immunoassay; 1. Bayer Diagnostics, Newbury, UK; 2. Abbott Diagnostics, Maidenhead, Berks, UK; 3. Diagnostics Product Corporation, Llanberis, Wales; 4. Roche Diagnostics GmbH, Mannheim, Germany, *from January to December 2013 the reference range for this assay was changed to 9·2—21·0 pmol/L, **from September 2009 to April 2011, reference range for this assay was 0·3-6·0 mU/L

	Numbers (%)		All		Male		Female		
	Controls N=2750	RTHβ N=55	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value	P-int
Crude model									
MACE	252 (9)	14 (25)	2.85 (1.56, 5.21)	0.001	3.70 (1.55, 8.82)	0.003	2.46 (1.10, 5.48)	0.03	0.66
Atrial fibrillation	42 (2)	7 (13)	8.67 (3.51, 21.42)	<0.001	20.13 (7.03, 57.66)	<0.001	3.64 (0.76, 17.38)	0.11	0.10
Heart failure	36 (1)	<5	5.29 (1.91, 14.61)	<0.001	12.24 (3.08, 48.69)	<0.001	3.17 (0.78, 12.88)	0.11	0.19
AMI	110 (4)	<5	1.79 (0.67, 4.75)	0.24	1.92 (0.51, 7.19)	0.33	1.66 (0.41, 6.65)	0.48	0.91
Stroke	56 (2)	<5	1.60 (0.36, 7.12)	0.54	2.82 (0.35, 22.48)	0.33	1.09 (0.13, 8.88)	0.93	0.57
Adjusted model									
MACE	252 (9)	14 (25)	2.65 (1.43, 4.90)	0.002	3.47 (1.40, 8.61)	0.007	2.24 (0.98, 5.12)	0.06	0.68
Atrial fibrillation	42 (2)	7 (13)	8.31 (3.37, 20.51)	<0.001	21.87 (7.61, 62.82)	<0.001	3.41 (0.82, 16.14)	0.12	0.08
Heart failure	36 (1)	<5	5.21 (1.88, 14.49)	0.002	11.06 (2.38, 51.51)	<0.001	3.10 (0.75, 12.77)	0.12	0.19
AMI	110 (4)	<5	1.61 (0.58, 4.44)	0.36	1.84 (0.47, 7.21)	0.38	1.42 (0.33, 6.04)	0.63	0.92
Stroke	56 (2)	<5	1,58 (0.36, 6,96)	0.55	2.83 (0.35, 22.80)	0.33	1.07 (0.13, 8.47)	0.95	0.57

Supplementary Table 3: Sub-hazard ratios for cardiovascular events stratified by sex incorporating non-cardiac death as competing risk.

Sub hazard ratios (HR) and 95% Confidence intervals (95%CI) were derived from Competing risk regression models according to the Fine and Gray sub-distribution hazard model with non-cardiac death as a competing risk. Adjusted models adjusted for baseline age. MACE, major adverse cardiovascular events, P-int, P value for the interaction with sex. Numbers (%) are the number of events (%) in the group, Small counts (< 5) are suppressed due to privacy restrictions.

	Numbers (%)		All		Male		Female		
	Controls N=2750	RTHβ N=55	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value	P-int
Crude model									
All-cause mortality	308 (11)	12 (22)	2.75 (1.54, 4.91)	0.001	3.82 (1.39, 10.47)	0.009	2.47 (1.21, 5.00)	0.01	0.39
MACE	252 (9)	14 (25)	3.31 (1.93, 5.67)	<0.001	4.17 (1.82, 9.54)	0.001	3.04 (1.49, 6.21)	0.002	0.51
Atrial fibrillation	42 (2)	7 (13)	9.77 (4.38, 21.77)	<0.001	24.80 (8.79, 70.00)	<0.001	4.20 (0.99, 17.74)	0.05	0.05
Heart failure	36 (1)	<5	6.29 (2.23, 17.69)	<0.001	13.65 (2.90, 64.33)	0.001	3.91 (0.93, 16.45)	0.06	0.19
AMI	110 (4)	<5	1.93 (0.71, 5.22)	0.20	2.04 (0.50, 8.39)	0.32	1.86 (0.45, 7.61)	0.39	0.89
Stroke	56 (2)	<5	1.76 (0.43, 7.24)	0.43	2.99 (0.40, 22.40)	0.29	1.26 (0.17, 9.19)	0.82	0.55
Adjusted model									
All-cause mortality	308 (11)	12 (22)	2.75 (1.54, 4.90)	0.001	3.65 (1.33, 10.02)	0.012	2.46 (1.21, 5.00)	0.01	0.39
MACE	252 (9)	14 (25)	3.13 (1.83, 5.37)	<0.001	3.90 (1.70, 8.94)	0.001	2.86 (1.40, 5.83)	0.004	0.52
Atrial fibrillation	42 (2)	7 (13)	9.44 (4.23, 21.06)	<0.001	25.32 (8.84, 72.52)	<0.001	3.97 (0.94, 16.80)	0.06	0.05
Heart failure	36 (1)	<5	6.14 (2.18, 17.27)	0.001	12.77 (2.70, 60.44)	0.001	3.74 (0.89, 15.72)	0.07	0.18
AMI	110 (4)	<5	1.77 (0.65, 4.81)	0.26	1.95 (0.47, 8.04)	0.35	1.64 (0.40, 6.74)	0.49	0.87
Stroke	56 (2)	<5	1.69 (0.41, 6.94)	0.47	2.97 (0.40, 22.30)	0.29	1.20 (0.16, 8.74)	0.86	0.55

Supplementary Table 4: Hazard ratios for mortality and cardiovascular events stratified by sex.

Hazard ratios (HR) and 95% Confidence intervals (95%CI) were derived from Cox regression models. Adjusted models are adjusted for baseline age. MACE, major adverse cardiovascular events, P-int, P value for the interaction with sex. Numbers (%) are the number of events (%) in the group, Small counts (< 5) are suppressed due to privacy restrictions.

		Mortality				MACE			
	N (%)	CHR (95% CI)[a]	P-value	AHR (95% CI)[b]	P-value	CHR (95% CI)	P-value	AHR (95% CI)	P-value
Age	55	1·07 (1·03–1·10)	<0·001	1·06 (1·02, 1·10)	0·003	1·05 (1·02–1·08)	<0·001	1·06 (1·02, 1·10)	0·002
Sex									
Male	22 (40)	Ref		Ref		Ref		Ref	
Female	33 (60)	1·18 (0·36–3·93)	0·79	1·17 (0·33, 4·14)	0·81	0·81 (0·33–2·01)	0·65	0·86 (0·24, 3·06)	0.82
Comorbidity									
Charlson 0	38 (87)	Ref		Ref		Ref		Ref	
Charlson ≥1	7 (13)	1·53 (1·11–2·12)	0·01	1·27 (0·85, 1·89)	0·24	1·40 (1·08–1·82)	0·012	1·52 (0·95, 3·06)	0.09
Thyroid hormones[c]									
FT4	53	3·40 (1·48–8·16)	0·004	3·41 (1·32, 8·82)	0·01	2·04 (1·10, 3·79)	0·024	1·93 (1·03, 3·63)	0·02
FT3	49	1·12 (0·97–1·29)	0·12	1·17 (0·99, 1·37)	0·06	1·07 (0·95, 1·21)	0·28	1·08 (0·95, 1·24)	0·25
TSH	53	1·10 (0·34–3·59)	0·87	1·18 (0·28, 4·89)	0·82	0·70 (0·25, 1·95)	0·50	0·51 (0·17, 1·59)	0·25
Mutation cluster									
Cluster 1	10 (15)	Ref		Ref		Ref		Ref	
Cluster 2	18 (34)	2·52 (0·28–22·84)	0·41	1·60 (0·51, 50·35)	0·76	1·90 (0·39–9·25)	0·43	2·26 (0·29, 17·46)	0·44
Cluster 3	12 (23)	0·73 (0·06–8·44)	0·80	2·10 (0·10, 41·37)	0·63	0·90 (0·16–5·12)	0·90	3·07 (0·43, 21·69)	0·23
Cluster 4	15 (28)	1·26 (0·13–11·80)	0·84	2·60 (0·15, 46·24)	0·53	1·05 (0·20–5·47)	0·96	1·23 (0·18, 8·86)	0·83
Diagnosis year[d]									
1st tertile	19 (35)	Ref		Ref		Ref		Ref	
2nd tertile	18 (33)	0·69 (0·20–2·39)	0·55	1·07 (0·15, 7·65)	0·95	0·90 (0·33–2·47)	0·84	1·35 (0·30, 6·00)	0·72
3rd tertile	18 (33)	0·32 (0·36–2·85)	0·31	0·58 (0·05, 6·05)	0·65	0·48 (0·12–1·85)	0·28	0·52 (0·86, 3·11)	0·48

Supplementary Table 5: Mortality and MACE outcomes in RTHβ by baseline characteristics with thyroid hormones analysed as multiples of the upper reference limits

a, CHR, crude hazard ratio, b, AHR, adjusted hazard ratio. Adjusted models were corrected for age, sex, comorbidity, and diagnosis year. In addition, thyroid hormones were adjusted for laboratory site. c, Thyroid hormones were available for 53 (FT4, TSH) and 49 (FT3) patients respectively. Nomenclature of mutation clusters

corresponds to previously defined hotspots in the TRβ hormone binding domain (clusters 1-3) and an additional amino acid position (cluster 4), outside the established hotspots. Age and thyroid hormones were treated as continuous variables and HR are as per year of age and per pmol/L (FT4, FT3) and mU/L (TSH), respectively. d, Diagnosis year was divided into tertiles from the earliest to the most recent diagnosis.

		Mortality				MACE			
	N	CHR (95% CI)[a]	P-value	AHR (95% CI)[b]	P-value	CHR (95% CI)	P-value	AHR (95% CI)	P-value
Thyroid hormones									
FT4	53	1.06 (1.02-1.11)	0.003	1.07 (1.02-1.12)	0.007	1·03 (1·00–1·06)	0·04	1.04 (1.00-1.07)	0.03
FT3	49	1.09 (0.93, 1.27)	0.30	1.16 (0.99-1.38)	0.06	1·04 (0·92–1·16)	0·54	1.09 (0.96-1.24)	0.17
TSH	53	1.03 (0.83-1.30)	0.76	1.01 (0.81-1.25)	0.96	0·96 (0·80–1·15)	0·66	0.90 (0.75-1.08)	0.24

Supplementary Table 6: Mortality and MACE outcomes in RTHβ depending on baseline thyroid function at the time of diagnosis by missing imputation method

a, CHR, crude hazard ratio, b, AHR, adjusted hazard ratio. Adjusted models were corrected for age, sex, comorbidity, and diagnosis year, and laboratory site. c, HR are as per pmol/L (FT4, FT3) and mU/L (TSH), respectively.

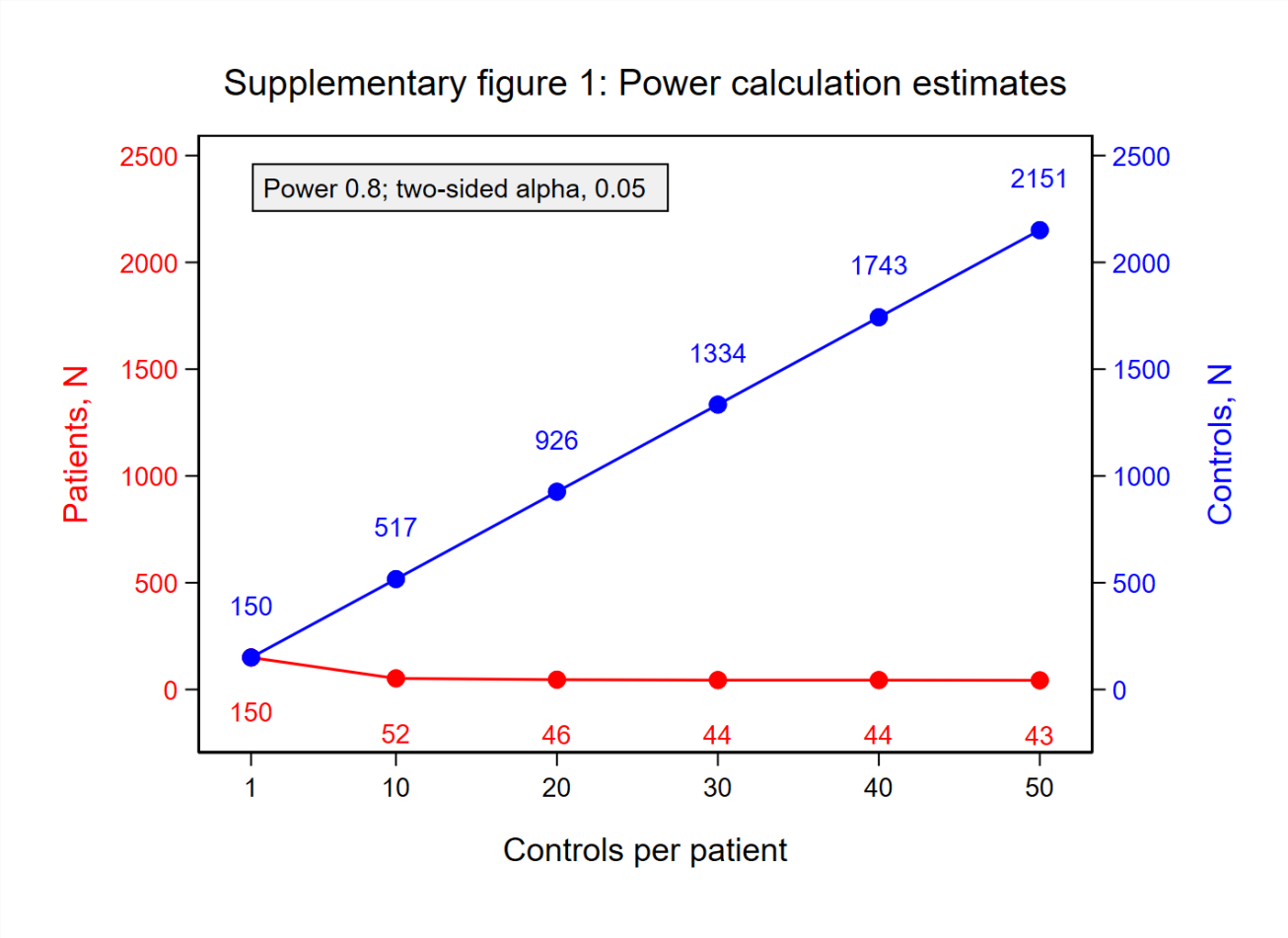
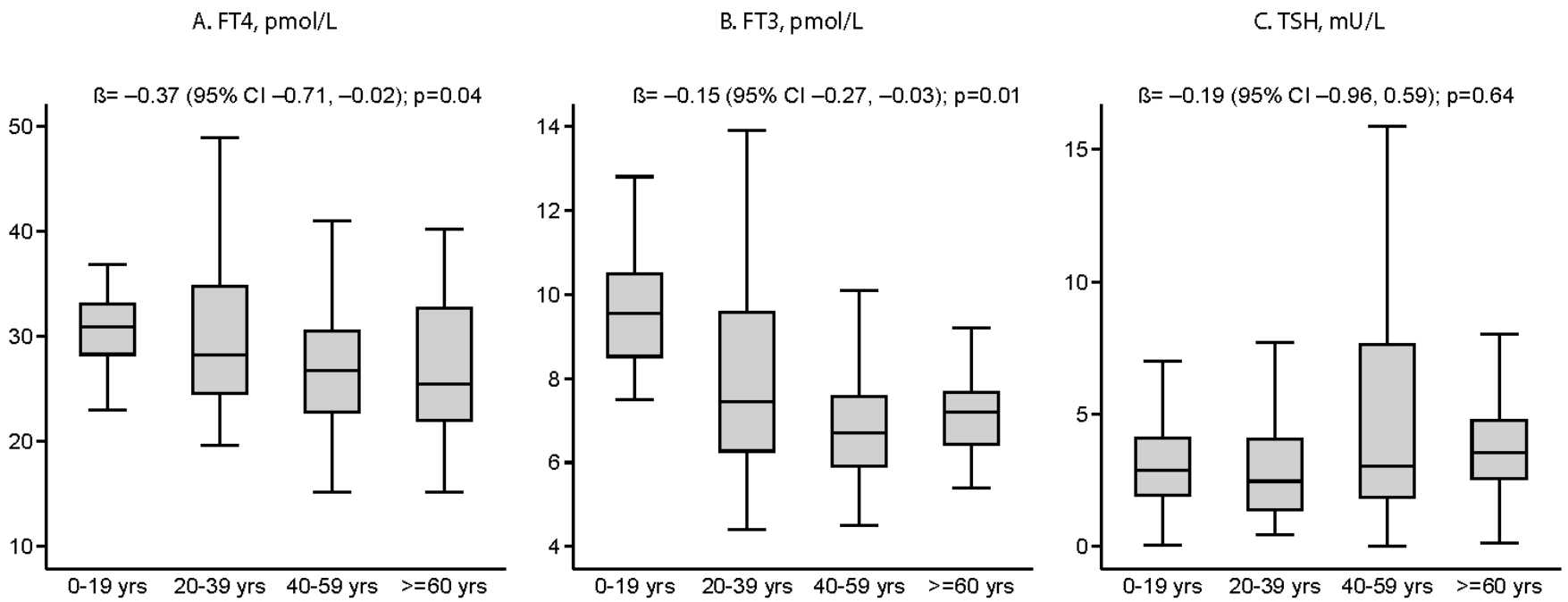


Figure shows estimated number of patients (red labels) and controls (blue labels) needed at various controls per patient ratios to demonstrate a hazard ratio of 2.5 for mortality or MACE with a two-sided α of 0.05 and power (ß) of 0.8.



Supplementary Figure 2: Thyroid hormone levels by age-group

Box plots illustrate median and inter-quartile range for thyroid hormone levels (FT4, FT3, TSH) by age-group using all available data. ß coefficients (95% confidence intervals) are estimates of the change in median hormone level per year and are derived from quantile mixed-effects models with age (years) as a fixed effect and subject as a random effect.

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