RESEARCH

Thyroid function in COVID-19 and the association with cytokine levels and mortality

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Abstract

The hypothalamic–pituitary–thyroid hormone axis might be affected in COVID-19, but existing studies have shown varying results. It has been hypothesized that hyperinflammation, as reflected by the secretion of cytokines, might induce thyroid dysfunction among patients with COVID-19. We explored thyroid hormone involvement in the acute phase of symptomatic COVID-19 and its possible associations with cytokine levels and mortality risk. This was a single-center study of 116 consecutive patients hospitalized for moderate-to-severe COVID-19 disease. Serum concentrations of thyroid-stimulating hormone (TSH), free thyroxine (T_4), and 45 cytokines/chemokines were measured in all patients within 3 days of admission. Data were extracted retrospectively through a manual review of health records. At admission, 95 (81.9%) were euthyroid; while 21 (18.1%) had biochemically thyroid dysfunction including subclinical thyrotoxicosis ($n = 11$), overt thyrotoxicosis ($n = 2$), hypothyroidism ($n = 1$), non-thyroidal illness ($n = 2$), and normal TSH but high free T_4 ($n = 5$). TSH levels were inversely correlated with IL-8 ($r_s = –0.248$), IL-10 ($r_s = –0.253$), IL-15 ($r_s = –0.213$), IP-10 ($r_s = –0.334$), and GM-CSF ($r_s = –0.254$). Moreover, IL-8 levels, IP-10, and GM-CSF were significantly higher in patients with serum TSH < 0.4 mIU/L. Lastly, a two-fold increment of IL-8 and IL-10 was associated with significantly higher odds of having TSH < 0.4 mIU/L (odds ratio 1.86 (1.11–3.10) and 1.78 (1.03–3.06)). Serum TSH was not associated with 30- or 90-day mortality. In conclusion, this study suggests that fluctuations of TSH levels in patients with COVID-19 may be influenced by circulating IL-8, IL-10, IL-15, IP-10, and GM-CSF as previously described in autoimmune thyroid diseases.

Key Words

- COVID-19
- thyroid function
- thyroid gland
- SARS-CoV-2
- mortality
- cytokines
- IL-8
- IL-10

Endocrine Connections (2021) **10**, 1234–1242

Introduction

The clinical spectrum of coronavirus disease 2019 (COVID-19) varies from asymptomatic or mild conditions to severe pulmonary involvement with pneumonia and respiratory failure, multisystemic involvement, and even death. Less commonly, extrapulmonary systems are affected – including the endocrine system (1). Following case reports of thyroiditis associated with COVID-19, it has been hypothesized that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) might induce alterations in the hormonal secretion from the thyroid gland (2, 3, 4). Studies have shown varying degrees of thyroid dysfunction among patients with COVID-19 infection with 5–20% being hyperthyroid and up to 5% being hypothyroid (5, 6, 7, 8, 9).

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https://doi.org/10.1530/EC-21-0301

Different theories have been proposed for the alterations in thyroid function status seen among patients with COVID-19. For one, SARS-CoV-2 enters cells by engaging the angiotensin-converting enzyme 2 (ACE-2) receptor. ACE-2 is expressed by most human tissues, including the thyroid gland. Hence, SARS-CoV-2 might affect the thyroid gland directly by inducing destructive thyroiditis. Furthermore, it has been suggested that the hyperinflammation associated with moderate to severe COVID-19 may cause thyroid dysfunction as the state mimics the immune activation that accompanies inflammatory thyroid disease (10). A range of different cytokines have been linked to destructive thyroiditis (11, 12), as well as Graves' disease (GD) and Graves' orbitopathy (13, 14, 15), and recent studies have reported inverse correlations between thyroid stimulating hormone (TSH) and interleukin 6 (IL-6) in patients with COVID-19 (6, 9, 16).

Thus, this study aimed to evaluate thyroid hormone involvement in the clinical course and survival of patients admitted with moderate to severe COVID-19 infection by exploring (1) the difference in clinical characteristics and mortality between patients with and without decreased serum TSH concentration, respectively, (2) the serum concentration of TSH as a predictor of mortality, and (3) the correlation between serum concentrations of TSH and a variety of cytokines.

Methods

This was a single-center study including patients hospitalized for COVID-19 at the Department of Infectious Diseases at Copenhagen University Hospital – Amager and Hvidovre Hospital, Hvidovre, Denmark, between 16 March and 19 May 2020 as previously described (17).

Inclusion criteria for this study were (i) presence of SARS-CoV-2 confirmed by RT polymerase chain-reaction from either naso-/oropharyngeal swab, sputum or endotracheal aspirate, (ii) age ≥ 18 years, and (iii) COVID-19 illness requiring hospitalization. Patients were excluded if they had previous thyroid illness of any kind or if they were in glucocorticoid treatment at admission.

Data collection

Data were retrospectively obtained through a manual review of health records. Extracted data included demographic variables, comorbidity, and diagnostic values at admission including vital parameters (temperature, respiratory rate, peripheral oxygen saturation), biochemical parameters (C-reactive protein (CRP) and lactate dehydrogenase (LDH), white blood differential counts), findings of the initial chest radiograph, and clinical outcome. Baseline oxygen requirements were ascertained as the highest level of respiratory support within 24 h from admission. BMI was calculated as weight (kg) divided by squared height (m).

Biochemical analyses

Blood was drawn from the patients between 07:00 and 09:00 h within the first 3 days of admission. Serum was stored at –80°C until analysis.

TSH and free thyroxine (T_4) were measured by electro-chemiluminescence immunoassays (Elecsys® TSH and FT4III on the e801 Module, Cobas 8000 platform, Roche Diagnostics GmbH). Limits of quantification were 0.005 mIU/L and 1.3 pmol/L, respectively, and the coefficients of variation were 2% at 4 mIU/L for TSH and 6% at 10 pmol/L for free T_4. Both analyses were accredited by the Danish Accreditation Fund for medical examination according to the international standard DS/EN ISO 15189.

Definitions

Thyroid dysfunction was classified into four different categories based on serum concentrations of TSH and free T_4: overt thyrotoxicosis (TSH < 0.4 mIU/L and free T_4 > 22.0 pmol/L); subclinical thyrotoxicosis (TSH < 0.4 mIU/L and 12.0 ≤ free T_4 ≤ 22.0 pmol/L); overt hypothyroidism (TSH > 4.8 mIU/L and free T_4 < 12.0 pmol/L); subclinical hypothyroidism (TSH > 4.8 mIU/L and 12.0 ≤ free T_4 ≤ 22.0 pmol/L) ; or non-thyroidal illness (TSH < 4.8 mIU/L and free T_4 < 12.0 pmol/L). TSH values in the reference range (0.4–4.8 mIU/L) were classified as euthyroid.

Cytokines were analyzed by magnetic fluorescently labeled microsphere beads as a part of a suspension array system (45-plex Fixed Panel (LKTM014), R&D Systems) on a BioPlex 200 instrument (Bio-Rad) with minor modifications and according to the manufacturer's instructions. The lower and upper limits of quantification were defined as the lowest/highest measurable standard ± three times the standard deviation (S.D.). Values below or above these limits were assigned a value of 10% lower or higher than the limit of quantification.

Cytokines included monocyte chemoattractant protein-1 (CCL2/JE/MCP1); macrofage inflammatory protein-1-α (CCL3/MIP1A); MIP1B (CCL4/MIP1B); regulated upon activation normal T-cell expressed, and

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presumably secreted (CCL5/RANTES); eotaxin (CCL11); MIP3A (CCL20/MIP3A); MIP3B (CCL19/MIP3B); TNF ligand superfamily member 5 (CD40 ligand/TNFSF5); fractalkine (CX3CL1); growth regulated oncogene-α (CXCL1/GROA/KC/CINC-1); GROB (CXCL2/MIP2/CINC3); interferon-inducible protein 10 (CXCL10/IP10/CRG2); EGF; fibroblast growth factor (FGF basic/FGF2/bFGF); Fms-like tyrosine kinase-3 ligand (Flt-3 ligand/FLT3L); granulocyte colony stimulating factor (G-CSF/CSF3); granulocyte-macrophage colony stimulating factor (GM-CSF/CSF2); granule enzyme B (Granzyme B); interferon-α2 (IFNA2); IFNB; IFNG; interleukin-1α (IL1A/IL-1F1); IL1B (IL-1 beta/IL-1F2); IL-1 receptor antagonist (IL-1ra/IL-1F3); IL2; IL3; IL4; IL5; IL6; IL7; IL8 (CXCL8); IL10; IL12p70; IL13; IL15; IL17 (IL-17A); IL17E (IL25); IL33; programmed death-1 ligand 1 (PD-L1/B7-H1); platelet-derived growth factor-AA (PDGF-AA); PDGF-AB/BB; transforming growth factor-α (TGFA); tumor necrosis factor-α (TNFA); TNF-related apoptosis inducing ligand (TRAIL/TNFSF10); and vascular endothelial growth factor (VEGF).

Statistics

Data processing and statistical analysis were performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). The study population was characterized using descriptive statistics. Categorical variables were reported as frequencies with percentages. Continuous variables were presented as medians with interquartile range (IQR) and $\log_2$-transformation was performed, when relevant. Comparison of baseline characteristics and cytokine levels between the group with TSH levels < 0.4 mIU/L and the group with TSH levels ≥ 0.4 mIU/L was performed using χ^2-test, Fisher's exact test, and Mann–Whitney *U*-test, as appropriate.

Correlation between continuous variables was computed as Spearman correlation coefficient. Logistic regression analysis was used to evaluate the crude and adjusted association between TSH and 30- and 90-day mortality. Non-Gaussian distributed variables were $\log_2$-transformed to achieve normal distribution prior to analysis. Receiver operating characteristics (ROC) analysis with area under the curve (AUC) was performed to evaluate the discriminatory ability of TSH as a biomarker for 30-day mortality.

Ethics

The study was approved by the Regional Data Protection Center (P-2020-260) and by the Regional Committee on Health Research Ethics (H-20040649). Individual consent to store samples in the biobank was waived by the Committee.

Results

A total of 129 patients with COVID-19 were identified. Six were excluded due to known hypothyroidism (all were treated with L-thyroxine at admission), and seven were excluded due to glucocorticoid treatment at admission, yielding a total of 116 eligible patients. No other patients had known thyroid illnesses or received any other thyroid modulating drugs. Demographics, clinical, and biochemical characteristics of the total population, and patients with TSH levels above, or equal to, or below 0.4 mIU/L are provided in Table 1. The population was mostly older, predominantly female and had moderate to severe disease as indicated by the frequent presence of a pulmonary infiltrate and requirement of high flow or low flow oxygen support at admission (81%). Hypertension was the most frequent comorbidity, followed by diabetes and chronic pulmonary disease. One-third had a BMI above 30 kg/m^2. By 30 and 90 days after admission, 24 and 29% had died, respectively.

In total, 95 (81.9%) patients were euthyroid, while 21 patients (18.1%) had biochemically thyroid dysfunction. The latter included subclinical thyrotoxicosis in 11 patients (9.5%), overt thyrotoxicosis in 2 patients (1.7%), overt hypothyroidism in 1 patient (0.8%), non-thyroidal illness in 2 patients (1.7%), and 5 patients (4.3%) with free T_4 > 22 pmol/L, but TSH concentrations within the reference range (probably interference in the T_4 assay) (Table 2). One patient with a TSH below 0.4 mIU/L presented with a sore throat.

Characteristics according to serum concentrations of TSH

No statistically significant differences were found in sex, age, or comorbidities according to normal or low levels of TSH. Neither were there any differences in respiratory rate, peripheral oxygen saturation, pulmonary infiltrate on X-ray, days of symptoms before admission, baseline oxygen requirement, admission to intensive care unit nor need of mechanical ventilation between the two groups. Patients in the group with serum TSH levels ≥ 0.4 mIU/L had significantly lower free T_4 levels compared to the group with TSH < 0.4 mIU/L (median 16 IQR (15–18) pmol/L vs 19 (19–21) pmol/L, $P = 0.0003$).

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Table 1 Baseline characteristics, admission values, and mortality within 30 and 90 days in patients hospitalized with COVID-19. Patients are grouped according to thyroid-stimulating hormone (TSH) concentrations.

	TSH ≥ 0.4 mIU/L (*n* = 103)	**TSH < 0.4 mIU/L** (*n* = 13)	**Total** (*n* = 116)	***P*-value**
Age, median (IQR)	71 (58–80)	75 (74–81)	72 (59–80)	0.31
Sex (%)				
Females, *n* (%)	66 (64)	6 (46)	72 (62)	0.34
BMI, median (IQR)	28 (25–32)	31 (28–35)	29 (25–32)	0.11
BMI (%)				
<30	52 (50)	5 (38)	57 (49)	
≥30	35 (34)	6 (46)	41 (35)	0.56
Serum TSH, median (IQR)	1.15 (0.79–1.58)	0.25 (0.21–0.31)	1.04 (0.60–1.45)	**0.0004**
Serum TSH, *n* (%)				
Low (<0.4 mIU/L)	–	13 (100)	13 (11)	
Normal (0.4–4.80 mIU/L)	102 (99)	–	102 (88)	
High (>4.80 mIU/L)	1 (1)	–	1 (1)	**0.0004**
Free T_4, median (IQR) pmol/L	17 (15–18)	19 (19–21)	17 (15–18)	**0.0003**
Free T_4, *n* (%)				
Low (<12 pmol/L)	3 (3)	0 (0)	3 (3)	
Normal (12–22 pmol/L)	95 (92)	11 (85)	106 (91)	
High (>22 mol/L)	5 (5)	2 (15)	7 (6)	0.26
CRP, median (IQR) mg/L	87 (49–151)	98 (56–141)	93 (50–150)	0.86
Blood lymphocyte count, median (IQR) 10^9/L	1.02 (0.7–1.32)	1.13 (0.75–1.28)	1.02 (0.75–1.29)	0.94
Plasma LDH, median (IQR) U/L	332 (244–430)	434 (278–462)	332 (250–455)	0.38
Respiratory rate at admission, median (IQR), b.p.m	20 (18–26)	24 (19–28)	21 (18–28)	0.33
Peripheral oxygen saturation at admission, median (IQR), %	95 (93–97)	96 (91–98)	95 (93–97)	0.72
Infiltrate on x-ray at admission (%)	86 (83)	12 (92)	98 (84)	0.67
Days with symptoms before admission, median (IQR)	7 (5–11)	6 (6–10)	7 (5–10)	0.92
Oxygen requirements at admission, *n* (%)				
No oxygen requirement	21 (20%)	1 (8%)	22 (19%)	
Low flow oxygen requirement	44 (43%)	7 (54%)	54 (44%)	
High flow oxygen requirement	38 (37%)	5 (38%)	43 (37%)	
Mechanical ventilation	0	0	0	0.52
ICU admission during hospitalization, *n* (%)	17 (17%)	3 (23%)	20 (17%)	0.84
Mechanical ventilation during hospitalization, *n* (%)	16 (16%)	3 (23%)	19 (16%)	0.77
Hypertension, *n* (%)	46 (45)	7 (54)	53 (46)	0.74
Diabetes, *n* (%)	33 (32)	5 (38)	38 (33)	0.88
Astma, *n* (%)	11 (11)	1 (8)	12 (10)	1.00
COPD, *n* (%)	8 (8)	1 (8)	9 (8)	1.00
30 days mortality, *n* (%)	23 (22)	5 (38)	28 (24)	0.35
90 days mortality, *n* (%)	28 (27)	6 (46)	34 (29)	0.27

COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; Free T4, free thyroxine; ICU, intensive care unit; IL-6, interleukin-6; IQR, inter-quartile range; LDH, lactate dehydrogenase; TSH, thyroid-stimulating hormone.
Bold indicates statistical significance.

Numeric correlations of TSH and biomarkers (CRP, LDH, cortisol, and blood lymphocyte and neutrophil count) and clinical factors (respiratory rate, temperature, and days with symptoms) were weak. Among the cytokines, IL-8 (r_s = –0.248, P = 0.008), IL-10 (r_s = –0.253, P = 0.007), IL-15 (r_s = –0.213, P = 0.02), IP-10 (r_s = –0.334, P = 0.0003), and GM-CSF (r_s = –0.254, P = 0.007) were inversely correlated to TSH (Fig. 1).

Cytokine levels among patients with TSH concentrations <0.4 and ≥0.4 mIU/L are listed in Table 3. Thirty-eight of 45 cytokines were detectable in serum. IL-3, IL-4, IL-5, IL-17A, IL-17E, IFN-β, and FGF-basic were all below the limit of detection. Of the remaining, IL-8, GM-CSF, and IP10 were statistically significantly higher among patients with TSH concentrations < 0.4 mIU/L. IL-10 and IL-15 were both borderline statistically significant (P = 0.05 and P = 0.05, respectively) with higher levels among patients with TSH < 0.4 mIU/L.

In adjusted logistic regression analysis (adjusted for age and sex), a doubling of IL-8 levels was associated with an

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Table 2 Distribution of 119 patients hospitalized for COVID-19 according to their combined serum concentrations of thyroid-stimulating hormone (TSH) and free thyroxine (free T_4).

	Free T_4		
	<12.0 pmol/L	12.0 pmol/L ≤ free T_4 ≤ 22.0 pmol/L	>22 pmol/L
TSH			
<0.4 mIU/L	0	11	2
0.4–4.8 mIU/L	2	95	5
>4.80 mIU/L	1	0	0

T4, thyroxine; TSH, thyroid-stimulating hormone.

increased odds ratio (OR) of 1.86 of having TSH levels <0.4 mIU/L (OR 1.86 (1.11–3.10), $P=0.02$). A doubling of IL-10 was associated with a 1.78 higher OR (OR 1.78 (1.03–3.06), $P=0.04$).

TSH and mortality

A total of 28 patients (24%) in the population died within 30 days after admission; hereof 5 patients (38%) in the group with serum TSH levels <0.4 mIU/L, and 23 patients (22%) in the group with serum TSH levels ≥0.4 mIU/L. No significant difference in mortality was found between the two groups. Neither TSH in the whole cohort nor in the group with TSH levels <0.4 mIU/L was associated with 30- and 90-day mortality in crude and adjusted logistic regression models (adjusted for age, sex, and IL-6).

TSH was a poor discriminator of 30-day mortality by ROC analysis (AUC = 0.54; 95% CI: 0.41–0.67) compared to IL-6 (AUC = 0.78, 95% CI: 0.67–0.88).

Free T_4 and non-thyroidal disease severity

No correlation between free T_4 levels and disease severity, as measured by 30- and 90-day mortality, ICU admission, and mechanical ventilation during hospitalization, was found (data not shown).

Discussion

In this observational study of 116 consecutively admitted patients with COVID-19 infection, we studied the baseline thyroid function (TSH and free T_4 concentrations) and found that most patients were euthyroid at admission. Two patients (1.7%) had biochemical overt thyrotoxicosis. Subclinical thyrotoxicosis was found in 9.5% of patients which is comparable to – although in the high end of – the general population at any given time (18). Lastly, 1.7% of patients were found to have non-thyroidal illness syndrome. In this study, IL-8, IL-10, IL-15, IP-10, and GM-CSF were significantly inversely correlated with TSH concentrations, although the correlation was moderate to weak (–0.213 to –0.334), furthermore, a two-fold increment of IL-8 and IL-10 was associated with an OR of having TSH < 0.4 mIU/L of 1.86 and 1.76, respectively. IL-8, IP-10,

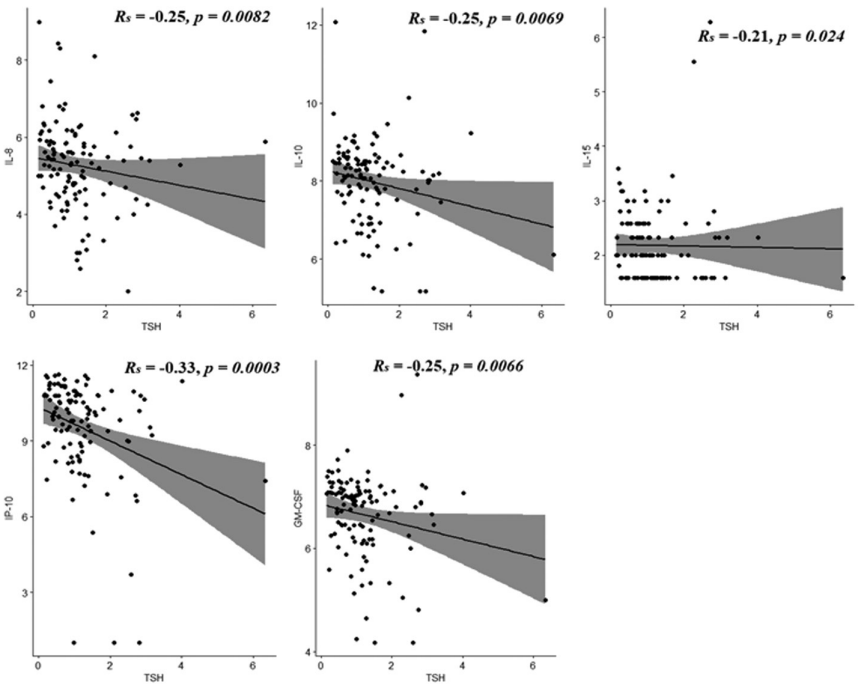

Figure 1
Spearman correlation plot between thyroid-stimulating hormone (TSH) and log_2-transformed interleukin (IL)-8, IL-10, IL-15, interferon-inducible protein (IP)-10, and granulocyte-macrophage colony-stimulating factor (GM-CSF), respectively (top panel from left: IL-8, IL-10, IL-15, bottom panel from left: IP-10, GM-CSF).

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https://doi.org/10.1530/EC-21-0301

Table 3 Serum concentrations of cytokines (pg/mL) among patients with thyroid-stimulating hormone (TSH) concentrations ≥0.4 and <0.4 mIU/L.

	TSH ≥ 0.4 mIU/L (*n* = 103)	**TSH < 0.4 mIU/L** (*n* = 13)	***P*-value**
IL-1α, median (IQR)	16 (12–20)	15 (10–18)	0.575
IL-1β, median (IQR)	9.5 (7–12)	8 (6–10)	0.120
IL-1ra, median (IQR)	2843 (1689–5146)	3262 (2017–4634)	0,857
IL-2, median (IQR)	4 (3–5)	4 (3–5)	0.973
IL-6, median (IQR)	76 (37–166)	91 (71–171)	0.204
IL-7, median (IQR)	12 (9–16)	13 (9–21)	0.678
IL-8, median (IQR)	39 (23–56)	61 (39–79)	**0.011**
IL-10, median (IQR)	277 (173–364)	357 (294–423)	**0.052**
IL-12, median (IQR)	22 (22–22)	22 (22–22)	0.366
IL-15, median (IQR)	4 (3–5)	5 (4–9)	**0.053**
IL-33, median (IQR)	17 (13–22)	21 (17–22)	0.116
IFN-α, median (IQR)	7 (7–7)	7 (7–7)	0.586
IFN-γ, median (IQR)	4 (4–13)	4 (4–6)	0.942
TNF-α, median (IQR)	13 (13–18)	14 (13–25)	0.143
CD40, median (IQR)	4.8 (3–7)	4.5 (3–6)	0.398
MCP-1, median (IQR)	765 (570–1093)	881 (769–1850)	0.086
MIP-1α, median (IQR)	5 (5–11)	11 (5–24)	0.182
MIP-1β, median (IQR)	409 (350–470)	436 (353–537)	0.333
MIP-3α, median (IQR)	21 (14–35)	29 (14–56)	0.434
MIP-3β, median (IQR)	203 (149–363)	293 (179–381)	0.187
RANTES, median (IQR)	30295 (20468–50512)	40530 (33809–52323)	0.166
Eotaxin, median (IQR)	222 (170–270)	283 (192–347)	0.235
GRO-α, median (IQR)	220 (157–344)	216 (207–301)	0.580
GRO-β, median (IQR)	1663 (1239–2668)	2280 (1789–2689)	0.131
IP-10, median (IQR)	957 (439–1760)	1760 (1004–2359)	**0.042**
EGF, median (IQR)	214 (147–298)	221 (183–300)	0.722
Flt-3 ligand, median (IQR)	125 (99–149)	130 (101–153)	0.532
G-CSF, median (IQR)	62 (49–76)	54 (49–77)	0.480
GM-CSF, median (IQR)	115 (74–134)	136 (133–156)	**0.019**
Granzyme B, median (IQR)	15 (6–22)	19 (5–39)	0.381
PD-L1, median (IQR)	185 (133–235)	304 (121–338)	0.268
PDGF-AA, median (IQR)	5170 (5170–5170)	5170 (5170–5170)	0.846
PDGF-AB/BB, median (IQR)	3181 (2091–4852)	2698 (2142–4093)	0.369
TRAIL, median (IQR)	32 (23–51)	29 (23–33)	0.381
VEGF, median (IQR)	419 (295–650)	452 (347–614)	0.172

CD, cluster differentiation; FGF basic, fibroblast growth factor; Flt-3 ligand, Fms-like tyrosine kinase-3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; Granzyme B, granule enzyme B; GRO-α, growth regulated oncogene-α; GRO-β, growth regulated oncogene-β; IFN, interferon; IL, interleukin; IP-10, interferon-inducible protein 10; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; PDGF-AA, platelet-derived growth factor-AA; PDGF-AB/BB, platelet-derived growth factor-AB/BB; PD-L1, programmed death-1 ligand 1; RANTES, regulated upon activation normal T-cell expressed, and presumably secreted; TGF-α, transforming growth factor-α; TNF, ligand superfamily member 5; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis inducing ligand; TSH, thyroid-stimulating hormone; VEGF, vascular endothelial growth factor.

and GM-CSF were significantly higher among patients with TSH concentrations below 0.4 mIU/L. Importantly, we found no association between mortality and, respectively, serum TSH concentrations or serum TSH <0.4 mIU/L.

Several studies are comparable to this study. Khoo *et al.* found that 5.6% had subclinical thyrotoxicosis and that none had biochemical overt thyrotoxicosis (5). Furthermore, despite decreased levels of TSH in their population of patients with COVID-19 as compared to a control group of patients without COVID-19, they found no difference in the rate of thyrotoxicosis or hypothyroidism. Comparable to our results, the THYRCOV study reported subclinical thyrotoxicosis in 9.4% of patients (6). However, 10.8% had overt thyrotoxicosis compared to the observed 1.7% in our study. Lui *et al.* reported subclinical thyrotoxicosis in 5.7% of patients and no patients with overt thyrotoxicosis in their population (7). Lastly, Campi *et al.* also reported 10.4% with transient levels of TSH below the lower cut-off at admission, all of them with normal free T_4 concentrations. Additionally, they reported that 23.5% of patients had transiently low TSH concentrations during hospitalization, but that TSH levels among patients

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that survived returned to normal before discharge (9). Epidemiology of thyroid diseases is dependent on iodine intake, and iodine intake differs among living areas and populations, which makes it challenging to compare the occurrence of thyroid diseases (18, 19). Both insufficient and excessive intake affect the thyroid gland. To some extent, this may explain the contrasting results. Another explanation may be that immunoassays for free thyroid hormones can be affected by alterations in serum binding proteins that occur in many physiological and disease states. Studies have shown substantial differences in free T_3 and free T_4 measurements among different immunoassay methods (20).

In this study, no association between having suppressed TSH levels and mortality was found. Contrary to our results, Zhang and coworkers showed that fatal outcome and a poor clinical outcome were associated with thyroid dysfunction, in general (8). This included overt thyrotoxicosis, subclinical thyrotoxicosis, subclinical hypothyroidism, overt hypothyroidism, and euthyroid sick syndrome. However, patients were only included if they had a positive sample of SARS-CoV-2 and a standard measure of TSH and free T_3 or free T_4, which could cause a selection bias. Most commonly, thyroid function is not a part of standard laboratory tests among patients admitted to the hospital, hence these patients might have been severely ill beforehand. Furthermore, they measured free T_3 as an equivalent to free T_4, which is a non-standard measure for thyroid function.

SARS-CoV-2 may like other viruses directly affect thyroid tissue and lead to subacute thyroiditis (de Quervain thyroiditis). It has been suggested that SARS-CoV-2 may induce destructive thyroiditis through interaction and entry via thyroid gland surface ACE-2 receptors. Only one patient in our population with TSH < 0.4 mIU/L presented with a sore throat, and no one complained of pain in the neck before admission. Besides, previous studies have shown that up to 10% of the background population in Denmark have subclinical thyrotoxicosis at any given time which is comparable to the results in this study (18). Therefore, we do not find any clear association of SARS-CoV-2-induced thyroiditis in these study results, which corresponds well with previous work (9).

It has been proposed that TSH levels are altered during hyperinflammation associated with increase of IL-6, which is abundant in severe COVID-19 disease (6, 9). In this study, we did not find any significant correlation between TSH and IL-6 but a significant correlation between TSH concentrations and, respectively, IL-8, IL-10, IL-15, IP-10, and GM-CSF. Furthermore, levels of IL-8, IP-10, and GM-CSF were significantly higher among patients with TSH concentrations <0.4 mIU/L. Many different cytokines have been linked to autoimmune thyroid diseases, such as IL-8 and IP-10, which in prior studies have been reported in Graves' disease and Graves' orbitopathy (13, 14, 21, 22, 23). In fact, studies have suggested that IP-10 could be a good biomarker for disease severity in autoimmune thyroid disease (24). Although levels of IL-15, IP-10, and GM-CSF were higher among patients with serum TSH < 0.4 mIU/L, only IL-8, and IL-10 were associated with higher odds of having TSH levels <0.4 mIU/L, which leads to think that these two cytokines could be the most prominent on the influence on the HPT axis alterations seen in patients hospitalized with COVID-19.

Predominantly, this study population had altered TSH concentrations without changes in thyroid hormones, which proposes that the effect of hyperinflammation on the HPT axis is rather central than peripheral. This study suggests that the alterations of TSH may be due to a combination of suppressed TSH in the background population at any given time (18) and that the hyperinflammation seen in patients hospitalized for COVID-19 – and especially circulating IL-8 and IL-10 – may play a role at a non-thyroidal stage. Despite this, former studies have reported that TSH levels return to normal (5, 9), and since we found no association between mortality and thyroid dysfunction, routine measurements of thyroid function status at the admission of hospitalized patients with COVID-19 have limited value.

Strengths of this study included first and foremost (1) that the analysis included a broad range of cytokines; (2) that thyroid dysfunction was classified through standardized assessment of TSH and free T_4 in all patients as part of the study; (3) that all TSH and free T_4 were measured simultaneously and with state of the art methods; and (4) that the comprehensive data collection allowed us to exclude patients with either a chronic thyroid dysfunction or a medication that might affect the hypothalamic–pituitary–thyroid axis. The main pitfall of this study is the lack of follow-up data on TSH and free T_4 as well as on cytokines. Other limitations included: (1) the single-center design; (2) the collection of data retrospectively, (3) the relatively small sample size. However, few studies investigating the effect of thyrotoxicosis in COVID-19 have yet been published; (4) the lack of an appropriate control group; and (5) the lack of measurements of T_3 and autoantibodies, which could have been helpful in further enlightening the presence of euthyroid sick syndrome and autoimmunity and destructive thyroiditis, respectively.

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In conclusion, thyroid dysfunction was not a frequent finding in our population of patients admitted to the hospital with moderate to severe COVID-19. In this study, TSH levels were not associated with mortality. Although no clear cause-effect relationship can be drawn from this study, our study results suggest that IL-8, IL-10, IL-15, IP-10, and GM-CSF may play a role in the decrease of TSH among patients with COVID-19. To the best of our knowledge, this is the first study to link other cytokines than IL-6 to thyroid dysfunction in patients with COVID-19.

Declaration of interest

T B reports grants from Novo Nordisk Foundation, grants from Simonsen Foundation, grants and personal fees from GSK, grants and personal fees from Pfizer, personal fees from Boehringer Ingelheim, grants and personal fees from Gilead, personal fees from MSD, grants from Lundbeck Foundation, grants from Kai Hansen Foundation, personal fees from Pentabase ApS, grants from Erik and Susanna Olesen's Charitable Fund, outside the submitted work. A J received unrestricted research grants from Novo Nordisk and Ferring and received speaker fees from Novo Nordisk, Ferring, IPSEN, Takeda and Pfizer, outside the submitted work. All other authors report no conflict of interests.

Funding

The study was partly funded by Holbaek Hospital's Research Foundation and R&D Systems provided a discount on the reagents used in this study.

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Received in final form 17 August 2021
Accepted 1 September 2021
Accepted Manuscript published online 1 September 2021