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The thyroid and Covid-19

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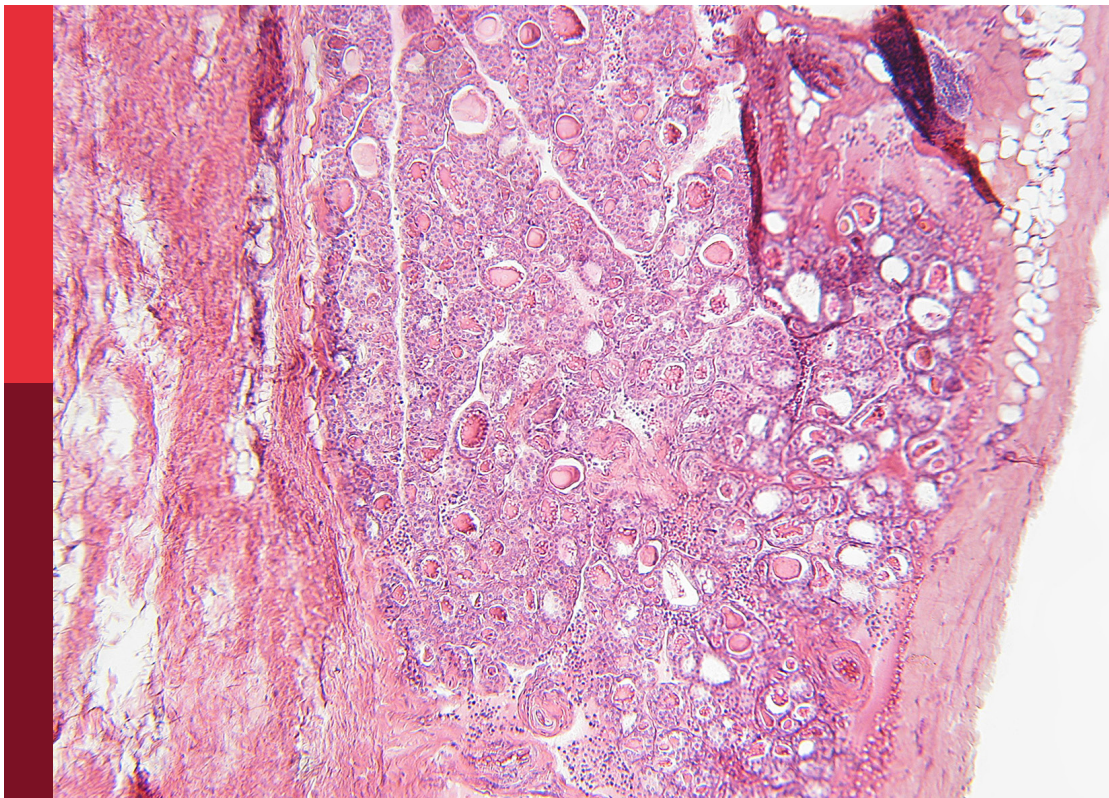

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The thyroid and Covid-19

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Editorial: New insights in thyroid and Covid-19

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Editorial on the Research Topic
The thyroid and Covid-19

The Coronaviruses Disease 2019 (Covid-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is perhaps the most dramatic threat to human health since the Spanish flu in 1918. Almost 700 million cases and more than 6 million deaths have been reported worldwide by November 20, 2022 (1). The lung is the main affected organ, and the most critical clinical presentation has been characterized by interstitial pneumonia, acute respiratory distress syndrome, multiple organ failure, and death (2).

Multiple endocrine organs, such as the pituitary, pancreas, adrenal, gonads, and thyroid gland, have also been affected (3). Detrimental effects on thyroid function have been reported in patients with and without pre-existing thyroid disease. Nonthyroidal illness syndrome (NTIS), subacute thyroiditis (SAT), Hashimoto's thyroiditis, and Graves' disease have been the most frequent thyroid dysfunctions associated with Covid-19 (4). Two major pathophysiological models have been implicated, a direct effect by virus attack causing follicular cells damage and an indirect effect caused by an immune-inflammatory abnormal response to the virus (5). Most recently, thyroid autoimmune diseases have also been reported following Covid-19 vaccination (6).

Thus, thyroid disorders associated with Covid-19 infection or vaccination have emerged as a new focus of research in thyroidology, which has motivated a particular Research Topic by Frontiers Endocrinology. We (the editorial team) invited potential collaborators worldwide from different disciplines to submit their recent research on Covid-19 and thyroid diseases. Eleven articles involving 83 authors were accepted for publication following at least two peer reviews. The published articles fit in Covid-19 and thyroid function- and autoimmunity, analyzing different themes of clinical interest, such as SAT, NTIS, hypothyroidism, pregnancy, vaccination, and autoimmune-inflammatory response.

In a systematic review including 19 studies (17 case reports and 2 case series), Trimboli et al. found that the size and quality of published data are poor and that the clinical presentation of Covid-19-related SAT is like the classic forms, being usually milder and not requiring any specific treatment.

NTIS was addressed in four studies. Zou et al. studied 149 Covid-19 patients and found that NTIS patients (27.5%) had more robust inflammatory responses, such as higher levels of C-reactive protein and erythrocyte sedimentation rate compared to those with non-NTIS. In addition, NTIS was an independent risk factor for Covid-19 severity. Wang et al. showed similar findings in a retrospective study comparing Covid-19 patients with non-Covid-19 patients. They also observed that thyroid dysfunction recovers gradually and spontaneously and tended to be associated with longer viral nucleic acid cleaning time, suggesting a direct effect of the SARS-CoV-2 virus on the gland. Lui et al. included 541 patients without known thyroid disorders with Covid-19; 15.4% had abnormal thyroid function, NTIS being the most frequent. TSH and FT3 levels independently correlated with lymphocyte counts and SARS-Cov-2 viral load. The authors also found that patients who had both lymphopenia and NTIS were more likely to deteriorate than those who had only one alone and those without lymphopenia or NTIS. Finally, in another study including 174 hospitalized patients with Covid-19, Swiatkowska-Stodulska et al. found that FT3 measured at admission was an independent predictor of unfavorable endpoints such as death, mechanical ventilation, vasopressor infusion, and prolonged hospital stay. All these data suggest an essential role of an abnormal immune- and inflammatory response in the pathogenesis of NTIS in Covid-19 patients and its correlation with Covid-19 severity.

Different features of thyroid function were explored in the other three studies. Gerwen et al. did not find any association between pre-existing hypothyroidism with increased risk of hospitalization, mechanical ventilation, or death, indicating that no additional precautions or specific recommendations are needed for patients with hypothyroidism. On the other hand, a retrospective cohort study by Lin et al. showed that pregnant women in their first trimester during the Covid-19 outbreak in Shanghai were at increased risk of having isolated hypothyroxinemia, emphasizing the importance of monitoring thyroid function in pregnant women during and after Covid-19. In addition, Yang and Xu found that postoperative thyroid patients tended to have more mental health problems and less psychological support during the Covid-19 pandemic. Fröhlich and Wahl. presented the readers with a narrative overview of several themes, such as thyroid hormones- and their metabolite's actions, thyroid dysfunctions in Covid-19, and the potential use of L-T3 and their metabolites in the treatment of severely ill Covid-19 patients.

Finally, two interesting articles addressed the link between Covid-19, thyroid autoimmunity, and vaccination. Lui et al. observed that interferon therapy for Covid-19 was associated with modest increases in thyroid peroxidase (TPO) antibody titers and incidence. Furthermore, incident anti-TPO positivity was more likely to be related to abnormal thyroid function during convalescence, suggesting that clinicians monitor thyroid function and anti-thyroid antibodies among interferon-treated Covid-19 patients. As a complement to the work of Lui et al., an interestingly combined retrospective and prospective study by Huang et al. demonstrated that thyrotropin receptor antibody (TRAB) serum levels of Graves' disease patients decreased less after inactivated SARS-Cov-2 vaccination and showed an upward trend. Not surprisingly, FT3 and FT4 levels were consistent with it. These data provide evidence for clinicians monitoring TRAB- and thyroid hormone levels after inactivated SARS-Cov-2 vaccination.

Most of the studies in this Research Topic of Frontiers in Endocrinology focus on the thyroid dysfunctions associated with Covid-19, having further advanced the evidence for clinicians in managing adults and pregnant women with- and without pre-existent thyroid dysfunctions. Additionally, new insights into the potential effects of vaccination on thyroid autoimmunity were highlighted.

Author contributions

JS wrote de manuscript. GB, CN, and MC critically reviewed and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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ORIGINAL RESEARCH
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Outcomes of Patients With Hypothyroidism and COVID-19: A Retrospective Cohort Study

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Coronavirus diseases (COVID-19) is associated with high rates of morbidity and mortality and worse outcomes have been reported for various morbidities. The impact of pre-existing hypothyroidism on COVID-19 outcomes remains unknown. The aim of the present study was to identify a possible association between hypothyroidism and outcomes related to COVID-19 including hospitalization, need for mechanical ventilation, and all-cause mortality. All patients with a laboratory confirmed COVID-19 diagnosis in March 2020 in a large New York City health system were reviewed. Of the 3703 COVID-19 positive patients included in present study, 251 patients (6.8%) had pre-existing hypothyroidism and received thyroid hormone therapy. Hypothyroidism was not associated with increased risk of hospitalization [Adjusted Odds Ratio (OR_{adj}): 1.23 (95% Confidence Interval (CI): 0.88- 1.70)], mechanical ventilation [OR_{adj}: 1.17 (95% CI: 0.81–1.69)] nor death [OR_{adj}: 1.07 (95% CI: 0.75–1.54)]. This study provides insight into the role of hypothyroidism on the outcomes of COVID-19 positive patients, indicating that no additional precautions or consultations are needed. However, future research into the potential complications of COVID-19 on the thyroid gland and function is warranted.

Keywords: COVID-19, hypothyroidism, survival, outcomes, epidemiology, cohort

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INTRODUCTION

Coronavirus disease (COVID-19), the disease caused by severe acute coronavirus 2 (SARS-CoV-2), has spread dramatically worldwide and is associated with high rates of morbidity and mortality (1, 2). The clinical presentation ranges from an asymptomatic infection to severe viral pneumonia with acute respiratory failure, sepsis and death (3). Older age, male gender and the presence of multiple comorbidities have been identified as the main risk factors for more severe disease and worse outcomes (3, 4). There is some epidemiological evidence that certain comorbidities are associated with worse outcomes. However, the impact of hypothyroidism on outcome in COVID-19 positive patients remains unknown.

It has been established that angiotensin-converting enzyme-2 (ACE2) is the functional host receptor for SARS-CoV-2, ACE2 is expressed in various cells in different organs, including the thyroid gland (5, 6). Data on thyroid function or thyroid pathology in COVID-19 patients is not yet available although destruction of follicular cells was seen in an autopsy study of SARS infected

patients published in 2007 (7). Currently, the American Thyroid Association (ATA) does not have specific recommendations for patients with hypothyroidism but underlines the importance of minimizing the spread of COVID-19 and advises patients with underlying hypothyroidism to continue taking their medication as prescribed (8).

The aims of this study are therefore to investigate the association between pre-existing hypothyroidism and COVID-19 related outcomes, including hospitalization, need for mechanical ventilation and all-cause mortality.

MATERIALS AND METHODS

Study Population

All patients with a positive result on a reverse-transcriptase-polymerase-chain-reaction (RT-PCR) SARS-CoV-2 assay of a nasopharyngeal swab specimen and therefore diagnosed with laboratory confirmed COVID-19 between March 1, 2020 and April 1, 2020 were identified via the electronic medical record of a large New York City health system ($n = 4{,}343$). Both hospitalized and ambulatory patients were included. Patients were excluded if they were <18 years old ($n = 55$) or had insufficient clinical documentation available or accessible, including confidential patient records ($n = 585$), resulting in a final study population of 3703 COVID-19 positive patients. This study was approved by the Program for the Protection of Human Subjects (PPHS) of the Icahn School of Medicine at Mount Sinai.

Data Collection

The medical records of all patients were retrospectively reviewed and data relevant to our study was collected and securely stored using Research Electronic Data Capture software (REDCap, Vanderbilt University). COVID-19 patients were identified as having hypothyroidism as a comorbidity when (1) the significant medical history in the medical record mentioned "hypothyroidism" (ICD-9 code 244, 245.2 or ICD-10 code E02, E03, E06.3) or (2) the term hypothyroidism was found within the clinical notes, combined with receiving thyroid hormone therapy before the COVID-19 related hospital visit/ admission. Data was collected on age, sex, race, smoking status, and body mass index (BMI) with the cut-offs used for normal weight (<25 kg/m^2), overweight (25–30 kg/m^2) and obese (>30 kg/m^2), as proposed by the Center for Disease Control and Prevention (CDC) (9). Data on comorbidities was collected and translated into a categorical variable on the number of comorbidities. Survival time was calculated as the time (days) between a positive result on a RT-PCR SARS-CoV-2 assay of a nasopharyngeal swab specimen and last follow-up. Our primary predictor of interest was the presence of hypothyroidism. Data on the primary outcomes of interest was collected up to May 13, 2020 and included hospital admission, need for invasive mechanical ventilation (i.e., intubation), and all-cause mortality.

Statistical Analysis

Demographic and clinical characteristics were compared between the hypothyroidism group and the no hypothyroidism group using two-sided *t*-test for age and chi^2 tests for the categorical variables. Adjusted analysis was performed using multivariable logistic regression to calculate the odds of hospitalization between the hypothyroidism and the no hypothyroidism group, adjusting for age, sex, race, BMI, smoking status and number of comorbidities. Multivariable logistic regression was also used to calculate the odds of mechanical ventilation and death between the hypothyroidism and the no hypothyroidism group within the group of hospitalized patients.

We additionally applied propensity score matching methods to deal with potential bias due to non-random treatment allocation among COVID-19 positive patients with and without hypothyroidism (10). Propensity scores were calculated using a logistic regression model adjusting for age, sex, race, BMI, smoking status, and number of comorbidities. A one-to-three matching technique was utilized by matching one patient from the hypothyroidism group to three patients from the no hypothyroidism group based on their propensity score values, using the Greedy matching technique (10). Event analysis for the outcome hospitalization was determined. Within the group of hospitalized patients, we again matched one patient from the hypothyroidism group to three patients from the no hypothyroidism group based on their propensity score values and ran event analysis for the outcome mechanical ventilation and all-cause mortality. We additionally performed time-to-event analysis for all-cause mortality. Patients with a survival time exceeding 50 days were censored to avoid a small number of patients at risk. The results of time-to-event analysis were expressed as a Kaplan-Meier curve with significance indicated using log-rank test. Cox proportional hazard model of all-cause mortality with robust sandwich variance estimates of standard errors was performed and expressed as hazard ratio. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Our study population consisted of 3703 COVID-19 positive patients, of which 251 patients (6.8%) had pre-existing hypothyroidism. Of the 251 patients with pre-existing hypothyroidism, 22 patients (8.8%) had Hashimoto's disease. Patients in the hypothyroidism group were significantly older, more frequently female and Non-Hispanic White and had significantly more other comorbidities (**Table 1**); 68.1% of the COVID-19 positive patients with hypothyroidism needed hospitalization.

Hospitalization

Hypothyroidism was not associated with increased risk of hospitalization [Adjusted Odds Ratio (OR_{adj}): 1.23 (95% Confidence Interval (CI): 0.88–1.70)] (**Table 2**). Propensity score matching yielded 241 patients with hypothyroidism and 723 patients without hypothyroidism with balanced variables between the groups (**Supplementary Table 1**). The risk of hospitalization was not statistically significantly different between the matched groups [OR: 0.76 (95% CI: 0.58–1.00)] (**Table 2**).

TABLE 1 | Demographic and clinical characteristics of the study population by hypothyroidism status ($n = 3,703$).

	Hypothyroidism ($n = 251$) *n* (%)	Without hypothyroidism ($n = 3,452$) *n* (%)	*p*-value
Age (years ± SD)	65.0 (± 16.9)	56.4 (±18.2)	<0.001
Female	173 (68.9)	1,481 (42.9)	<0.001
Race			<0.001
NHW	114 (45.4)	899 (26.0)	
NHB	39 (15.5)	953 (27.6)	
Other/ unknown	98 (39.0)	1,600 (46.4)	
Smoking			<0.001
Never	153 (61)	1,991 (57.7)	
Former	72 (28.7)	641 (18.6)	
Current	7 (2.8)	186 (5.4)	
Unknown	19 (7.6)	634 (18.4)	
BMI			<0.001
<25	74 (29.5)	771 (22.3)	
25-30	75 (29.9)	981 (28.4)	
> 30	87 (34.7)	985 (28.5)	
Unknown	15 (6.0)	715 (20.7)	
Number of Comorbidities*			<0.001
0	55 (21.9)	1,305 (37.8)	
1	52 (20.7)	786 (22.8)	
2	40 (15.9)	575 (16.7)	
>2	104 (41.4)	786 (22.8)	
Hospital admission	171 (68.1)	1,844 (53.4)	<0.001

NHB, Non-Hispanic Black; NHW, Non-Hispanic White; SD, standard deviation.
**Comorbidities include hypertension, coronary artery disease, atrial fibrillation, congestive heart failure, peripheral vascular disease, cerebrovascular accident/ transient ischemic attack, dementia, diabetes, chronic kidney disease stage III or greater, malignancy (including all types of cancer as well as lymphoma and leukemia), asthma, chronic obstructive pulmonary disease and prior venous thromboembolism.*

Mechanical Ventilation and Mortality in Hospitalized Patients

In the group of hospitalized patients ($n = 2,015$), hypothyroidism was not associated with an increased risk of mechanical ventilation [OR_{adj}: 1.17 (95% CI: 0.81–1.69)] or death [OR_{adj}: 1.07 (95% CI: 0.75–1.54)] (**Table 2**). Propensity score matching yielded 158 hospitalized patients with hypothyroidism and 474 hospitalized patients without hypothyroidism with balanced variables between the groups (**Supplementary Table 2**). Hypothyroidism was not associated with increased risk of mechanical ventilation [OR: 0.85 (95% CI: 0.58–1.25)] or death [OR: 1.04 (95% CI: 0.71–1.52)] (**Table 2**). There was no statistically significant difference in survival (p= 0.898) between the two groups [HR: 0.98 (95% CI: 0.72–1.34)] (**Figure 1**).

TABLE 2 | Association between hypothyroidism and COVID-19 outcomes.

	Adjusted analysis* OR_{adj} (95% CI)	Propensity matched analysis OR (95% CI)
Hospitalization	1.23 (0.88–1.70)	0.76 (0.58–1.00)
Mechanical ventilation#	1.17 (0.81–1.69)	0.85 (0.58–1.25)
Death#	1.07 (0.75–1.54)	1.04 (0.71–1.52)

**Adjusted for age, sex, race, BMI, smoking status, number of comorbidities.*
#Only included hospitalized patients.

DISCUSSION

This large, retrospective cohort study showed that hypothyroidism is not associated with increased risk of COVID-19 related hospitalization or a worse outcome, including death. The current recommendations by the ATA for patients with hypothyroidism are therefore accurate and no additional precautions are needed for patients suffering from hypothyroidism.

From published literature, we know that well-managed hypothyroidism is not associated with increased infection risk although there is some evidence that susceptibility to infection might increase in patients with poorly controlled hypothyroidism (11, 12). Therefore, the treatment of thyroid disorders in COVID-19 positive patients remains of significant importance amid the ongoing pandemic. The unprecedented strain on medical resources and implementation of social distancing measures pose a fundamental challenge to the treatment of patients with chronic conditions, including hypothyroidism. Hypothyroidism requires management with thyroid hormone replacement therapy and active monitoring through regular biochemical testing to ensure that therapeutic levels are maintained (13). Iatrogenic overtreatment with thyroid hormone supplementation is a known risk factor for the development of thyrotoxicosis (14). A joint statement by the British Thyroid Association and the Society for Endocrinology (BTA/SfE) as well as the ATA regarding the COVID-19 pandemic therefore strongly advise that patients with thyroid disease continue taking their thyroid medications as prescribed in order to reduce any risk of thyroid dysregulation (15) that could lead to a more severe COVID-19 outcome.

Besides pre-existing hypothyroidism as potential focus of interest, there is some evidence that endocrinological disruption and destruction of thyroid tissue may be a complication of COVID-19, even in patients without pre-existing endocrinological conditions (7, 16–19). It is known that SARS-CoV, the virus responsible for the SARS outbreak in 2003, as well as SARS-CoV-2 use ACE2 to enter human cells (20, 21). ACE2 is expressed by various cells in the body, including the thyroid gland (5, 6). Destruction of thyroid gland tissue and temporary or permanent thyroid dysfunction should therefore be subject of future studies.

This study was a retrospective study using data collected from the electronic medical records of ambulatory and hospitalized COVID-19 positive patients. A limitation of this study includes that the study population consisted solely of patients within the New York metropolitan area therefore potentially limiting the generalizability of the results. Although assessing the outcomes of patients with hypothyroidism due to Hashimoto's disease

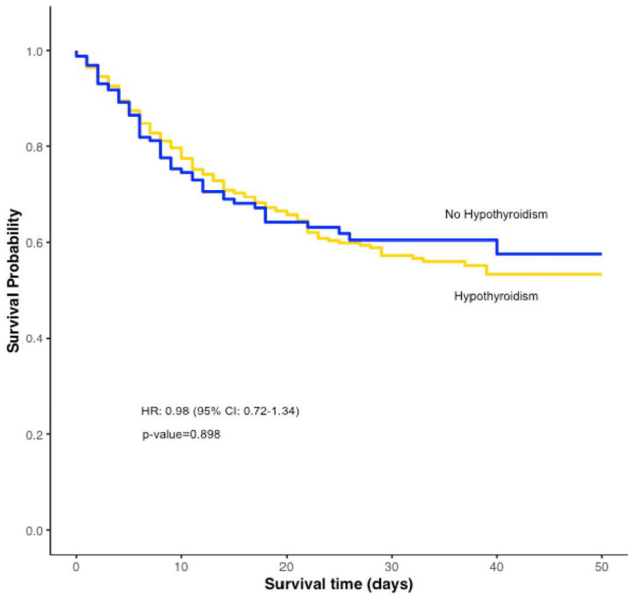

FIGURE 1 | Overall survival in the propensity matched hypothyroidism- no hypothyroidism cohort.

compared to patients with hypothyroidism due to other causes would be clinically relevant, a subgroup analysis was not feasible because of too low number of patients with Hashimoto's disease in our study cohort. The increased patient volume and reduced consultation time per patient associated with the increased influx of patients during the pandemic potentially resulted in missing data, especially for ambulatory patients, on certain covariates including race, BMI, and smoking status. Furthermore, we were unable to collect mortality data for the patients who were still hospitalized at the time of data collection, therefore potentially introducing bias. However, we estimate that little bias was introduced because only 1.3% of the study population was still hospitalized at the time of data collection.

To our knowledge, this is the first study investigating the COVID-19 associated outcomes in patients with pre-existing hypothyroidism in a population drawn from the first and largest COVID-19 epicenter in the US, with sufficiently long follow-up to rigorously assess whether pre-existing hypothyroidism is a risk factor for worse outcomes.

In conclusion, hypothyroidism is not a risk factor associated with worse outcomes in COVID-19 positive patients, therefore no additional precautions or consultations are needed. However, future research into the potential complications of COVID-19 on the thyroid gland and function is warranted.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the Program for the Protection of Human Subjects (PPHS) of the Icahn School of Medicine at Mount Sinai.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to the conception or design of the work (CS, MG, MA, CL, DT, EG), or the acquisition, analysis, or interpretation of data, or the creation of new software used in the work (MG, MA, CL, JB, LN, DT, CS, EG), or have drafted the work or substantively revised it (MG, MA, CL, CS), and have approved the submitted version (plus any substantially modified version that involves the author's contribution to the study [all authors]), and to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00565/full#supplementary-material

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Euthyroid Sick Syndrome in Patients With COVID-19

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Background: Coronavirus disease 2019 (COVID-19) has been shown to affect almost every organ throughout the body. However, it is not clear whether the thyroid gland is impaired in COVID-19 patients. Euthyroid sick syndrome (ESS) is usually associated with the disease severity and deterioration prognosis in critical illness. In this study, the thyroid function of COVID-19 patients was assessed and factors associated with outcomes were analyzed to determine the potential predictive value of ESS.

Methods: Clinical and laboratory data of COVID-19 patients with or without ESS in Changsha, China, were collected and analyzed on admission. Kaplan-Meier curve and cox regression model were utilized to determine the correlation between ESS and the endpoints. Subsequently, a receiver operating characteristic (ROC) curve was plotted to evaluate the predictive performances of FT3 and C-reactive protein (CRP) in the disease severity.

Results: Forty-one (27.52%) cases of COVID-19 patients diagnosed with ESS. ESS patients had higher proportions of fever, shortness of breath, hypertension, diabetes, and severe events than those of non-ESS patients. The levels of erythrocyte sedimentation rate and C-reactive protein, and the positive rate of procalcitonin were significantly higher, whereas the lymphocyte count was apparently lower in ESS patients than in non-ESS patients. The regression analysis showed that ESS was significantly associated with the disease severity of COVID-19 (HR = 2.515, 95% CI: 1.050–6.026, $P = 0.039$). The areas under the curve (AUCs) for predicting the severe disease were [0.809 (95% CI 0.727–0.892), $P < 0.001$] and [0.792 (95% CI 0.689–0.895), $P < 0.001$] for FT3 and CRP, respectively.

Conclusion: ESS was significantly associated with the disease severity and inflammatory parameters in COVID-19 patients.

Keywords: Coronavirus Disease 2019 (COVID-19), euthyroid sick syndrome, thyroid function, disease severity, C-reactive protein

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease which causes severe respiratory illness. It was first reported in Wuhan, China, in December 2019 (1–3). It has spread widely around the world and been declared a pandemic by the World Health Organization (WHO) on March 11, 2020. As of August 28, over 24,000,000 cases and 800,000 deaths have been identified worldwide (4). The etiological agent of COVID-19 has been confirmed as a novel coronavirus, initially named 2019-nCov but now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (5).

COVID-19 can impair almost any structure in the human body from the brain to the toes, including lungs, liver, heart, kidney, brain, gut, etc. (6). The thyroid gland is a neuroendocrine organ which plays an important role in regulating immunity and metabolism. Whether the thyroid gland is affected by SARS-CoV-2 remains unclear. An identity match of more than 85% has been identified between SARS-CoV-2 and a bat SARS-like CoV genome published previously (7). A study composed of 48 SARS patients found that 93.7% patients had a low serum level of triiodothyronine (T3) (8). During acute illness, changes in the serum levels of thyroid hormones have been depicted which represent a condition known as euthyroid sick syndrome (ESS). ESS is characterized by a decreased level of serum T3 and/or thyroxine (T4) without an increased secretion of thyroid-stimulating hormone (TSH) (9). ESS is the physiologic adaptation and pathologic response to acute disease, occurring in the fasting state in healthy individuals, as well as in the context of infection, trauma, myocardial infarction, and malignancy (10). Previous studies suggested that thyroid hormone levels, especially low serum levels of free T3 (FT3), are usually associated with the disease severity and deterioration prognosis in critical illness (11, 12).

In this study, we assessed the thyroid function in patients with COVID-19 and analyzed the related factors to determine the potential value of ESS in predicting the disease severity. We found that COVID-19 patients with ESS had a higher risk of severe events than non-ESS patients. Besides, ESS was an independent risk factor for the severe event in COVID-19 patients. ESS also displayed a potent correlation with the inflammatory parameters.

METHODS

Study Design and Participants

This was a retrospective cohort study. The study was approved by the institutional ethics board of the second Xiangya Hospital of Central South University (No. 2020001). Laboratory-confirmed COVID-19 patients (aged 19–84 years old) admitted to Public Health Treatment Center of Changsha, China from January 17 to March 14, 2020, were enrolled. Patients with underlying primary thyroid disease, history of chemotherapy or radiotherapy in the last 6 months, suspicion of underlying hypothalamic or pituitary disease were excluded. ESS was described as serum FT3 <2.3 pg/ml with low or normal levels of TSH. Patients were divided into two groups according to serum FT3 values: ESS group and non-ESS group.

Data Collection

Two members of our team carefully gathered and individually reviewed the medical records of patients. Detailed information on demographic data, clinical symptoms, underlying comorbidities, medical history, laboratory parameters, and chest computed tomographic (CT) scans were checked and extracted. Blood samples were drawn from the patients on their first day of the hospitalization.

Endpoints

The primary endpoint of our study was the severe event. The second endpoints were the rates of noninvasive and invasive ventilation, mortality, virus shedding time, and length of hospital stay. We used one of the following criteria to determine the severe event of COVID-19: 1) respiratory rate ≥30/min; 2) oxygen saturation ≤93%; 3) PaO2/FiO2 ≤300 mmHg; 4) progression of lung lesions exceeding 50% within 24–48 h; 5) mechanical ventilation was implemented; 6) shock; 7) intensive care unit admission (13). The virus shedding time was defined as the time from illness onset (the day of diagnosis for asymptomatic patients) to the first negative samples without any positive sample thereafter.

Statistical Analysis

Continuous variables in our study were expressed as the median with inter quartile range (IQR) for data not following normal distribution and analyzed using the Mann-Whitney U test. Categorical data were described using frequencies and percentages. The $\chi 2$ test or Fisher's exact test was used to compare the differences of the categorical variables. The Kaplan-Meier (KM) curve and cox proportional hazard regression models were applied to determine the association between ESS and the endpoints, with the hazards ratio (HR) and 95% confidence interval (95% CI) being reported. Finally, a receiver operating characteristic (ROC) curve was established to evaluate the predictive performances of FT3 and C-reactive protein (CRP) in the disease severity of COVID-19.

RESULTS

A total of 149 COVID-19 patients were enrolled in this study, of whom 41 (27.52%) cases were diagnosed with ESS. Out of these, 14 (34.15%) were males. The median age was 58 (IQR: 50–66) years old. The most common symptoms in the ESS group were fever [39 (95.12%)], fatigue [18 (43.90%)], cough [36 (87.80%)], shortness of breath [25 (60.98%)], expectoration [20 (48.78%)], and anorexia [21 (51.22%)]. Hypertension [10 (24.39%)] and diabetes [7 (17.07%)] were the most common comorbidities (**Table 1**).

Compared with non-ESS patients, patients with ESS were older [58 years (IQR: 50–66) *vs* 41 years (IQR: 31–56.5), $P < 0.001$) and exhibited a female dominance (65.85 *vs* 47.22%, $P = 0.042$).

TABLE 1 | Baseline characteristics of patients with ESS and non-ESS.

	ESS group (n = 41)	Non-ESS group (n = 108)	All patients (n = 149)	*P* value
Gender (male/female)	14/27	57/51	71/78	0.042*
Age, y, M (IQR)	58 (50, 66)	41 (31, 56.5)	47 (36, 61.5)	<0.001*
Symptoms				
Fever (n, %)	39 (95.12)	73 (67.59)	112 (75.17)	0.001*
Fatigue (n, %)	18 (43.90)	43 (39.81)	61 (40.94)	0.650
Cough (n, %)	36 (87.80)	88 (81.48)	124 (83.22)	0.356
Shortness of breath (n, %)	25 (60.98)	26 (24.07)	51 (34.23)	<0.001*
Expectoration (n, %)	20 (48.78)	49 (45.37)	69 (46.31)	0.709
Hemoptysis (n, %)	3 (7.32)	2 (1.85)	5 (3.36)	0.128
Pharyngalgia (n, %)	6 (14.63)	21 (19.44)	27 (18.12)	0.496
Vomiting (n, %)	4 (9.76)	12 (11.11)	16 (10.74)	1.000
Diarrhea (n, %)	8 (19.51)	24 (22.22)	32 (21.48)	0.719
Abdominal pain (n, %)	3 (7.32)	2 (1.85)	5 (3.36)	0.128
Nausea (n, %)	5 (12.20)	12 (11.11)	17 (11.41)	1.000
Anorexia (n, %)	21 (51.22)	47 (43.52)	68 (45.64)	0.399
Myalgia (n, %)	3 (7.32)	11 (10.19)	14 (9.40)	0.825
Chill (n, %)	8 (19.51)	12 (11.11)	20 (13.42)	0.179
Dizziness (n, %)	7 (17.07)	12 (11.11)	19 (12.75)	0.330
Headache (n, %)	7 (17.07)	16 (14.81)	23 (15.44)	0.733
Comorbidities				
Hypertension (n, %)	10 (24.39)	7 (6.48)	17 (11.41)	0.005*
Cardiovascular (n, %)	3 (7.32)	4 (3.70)	7 (4.70)	0.619
Diabetes (n, %)	7 (17.07)	3 (2.78)	10 (6.71)	0.006*
Chronic liver disease (n, %)	1 (2.44)	6 (5.56)	7 (4.70)	0.712

**means a significant difference; ESS, euthyroid sick syndrome; M, median; IQR, inter quartile range; y, years.*

Furthermore, patients with ESS tended to have a higher prevalence of fever and shortness of breath compared to non-ESS patients (95.12 *vs* 67.59%, $P = 0.001$; 60.98 *vs* 24.07%, $P < 0.001$, respectively). The occurrences of hypertension and diabetes were higher in ESS patients than those in non-ESS patients (24.39 *vs* 6.48%, $P = 0.005$; 17.07 *vs* 2.78%, $P = 0.006$, respectively) (**Table 1**).

Patients with ESS had significantly lower levels of T4 (median 106.30 *vs* 121.98 nmol/L, $P < 0.001$), and free T4 (FT4) (median 14.47 *vs* 16.08 pmol/L, $P = 0.026$) than non-ESS patients, but no significant difference was detected with respect to TSH. The levels of erythrocyte sedimentation rate (median 66.5 *vs* 35 mm/h, $P < 0.001$) and CRP (median 38.99 *vs* 11.26 mg/L, $P < 0.001$) were significantly higher, whereas the lymphocyte count (0.91 *vs* 1.27 $\times 10^9$/L, $P < 0.001$) was obviously lower in ESS patients than those of non-ESS patients. Additionally, patients with ESS had a higher positive rate of procalcitonin compared with non-ESS patients (53.66 *vs* 29.63%, P = 0.006) (**Table 2**). COVID-19 patients with ESS were linked with stronger inflammatory responses, characterized by higher levels of inflammatory markers.

The associations between ESS and the outcomes of COVID-19 patients were presented in **Table 3**. COVID-19 patients with ESS had a significantly higher prevalence of severe events (36.59 *vs* 10.19%, $P < 0.001$). Nonetheless, no significant effects of ESS were found on the rates of noninvasive (2.44 *vs* 1.85%, $P = 1.000$) and invasive ventilation (2.44 *vs* 0.93%, $P = 0.476$), mortality (0.00 *vs* 0.93%, $P = 1.000$), virus shedding time [19 days (IQR: 14–23.5) *vs* 17 days (IQR: 13–24), $P = 0.670$], and length of hospital stay [17 days (IQR:12–25) *vs* 15 days (IQR: 11–23), $P = 0.345$]. As observed from the KM curve, patients with ESS had a significantly higher risk of severe events compared with those without ESS (log-rank $P < 0.001$, **Figure 1**). The results of cox regression analysis showed that both ESS (HR = 2.515, 95% CI: 1.050–6.026, $P = 0.039$), and CRP (HR = 1.031, 95% CI: 1.015–1.046, $P < 0.001$) were independent risk factors for the disease severity (**Table 4**).

Considering that CRP was one of the most reported risk factors of the disease severity of COVID-19 patients (14–17), ROC curve was conducted to determine the predictive performances of FT3 and CRP in the disease severity. The areas under the curve (AUCs) for FT3 and CRP predicting the severe disease were [0.809 (95% CI 0.727–0.892), $P < 0.001$] and [0.792 (95% CI 0.689–0.895), $P < 0.001$] (**Figure 2**), respectively.

DISCUSSION

In the present study, we identified 41 (27.52%) COVID-19 patients with ESS. We compared the clinical characteristics between ESS and non-ESS patients and analyzed factors related to the disease severity of patients with COVID-19. The results demonstrated that ESS patients had a higher prevalence of severe events than non-ESS patients, which was consistent with the severity of symptoms and elevated inflammatory markers. The cox regression model suggested that ESS is an independent risk factor for the severity of COVID-19.

Previous studies demonstrated that a decreased level of FT3 was a prognostic indicator of critical disease, which was associated with all-cause mortality, especially in the intensive

TABLE 2 | Laboratory findings of patients with ESS and non-ESS.

	ESS group (n = 41)	Non-ESS group (n = 108)	All patients (n = 149)	*P* value
WBC, $\times10^9$/L, M (IQR)	4.62 (3.38, 6.15)	4.67 (3.68. 5.98)	4.63 (3.60, 6.03)	0.708
Lymphocyte count, $\times10^9$/L, M (IQR)	0.91 (0.59, 1.13)	1.27 (0.91, 1.70)	1.13 (0.83, 1.52)	<0.001*
T3, nmol/L, M (IQR)	1.01 (0.81, 1.10)	1.47 (1.35, 1.68)	1.39 (1.11, 1.59)	<0.001*
T4, nmol/L, M (IQR)	106.30 (89.38, 119.09)	121.98 (107.78, 136.13)	118.80 (100.67, 131.15)	<0.001*
FT4, pmol/L, M (IQR)	14.47 (12.72, 17.18)	16.08 (13.43, 18.91)	15.80 (13.35, 18.11)	0.026*
TSH, uIU/ml, M (IQR)	1.36 (1.01, 2.28)	1.74 (1.13, 2.71)	1.63 (1.07, 2.56)	0.065
ESR, mm/h, M (IQR)	66.5 (43, 82.5)	35 (15, 60)	46 (21.5, 68.5)	<0.001*
CRP, mg/L, M (IQR)	38.99 (19.05, 60.96)	11.26 (2.92, 22.88)	16.07 (4.14, 34.07)	<0.001*
PCT ≥0.05 ng/ml, (n, %)	22 (53.66)	32 (29.63)	54 (36.24)	0.006*
ALT, U/L, M (IQR)	18.95 (13.37, 28.75)	19.72 (14.11, 26.41)	19.08 (13.75, 26.44)	0.973
AST, U/L, M (IQR)	25.05 (21.31, 34.82)	23.84 (18.85, 29.19)	23.97 (19.38, 30.47)	0.098
Tbil, μmol/L, M (IQR)	11.14 (7.73, 14.59)	10.29 (8.20, 14.82)	10.60 (8.08, 14.68)	0.784
SCr, mmol/L, M (IQR)	54.91 (37.67, 65.55)	50.51 (41.05, 65.66)	51.11 (40.74, 65.43)	0.883
CK, U/L, M (IQR)	75.90 (50.10, 139.10)	68.25 (44.38, 105.63)	68.70 (45.40, 111.40)	0.321
CK-MB, M (IQR)	11.00 (6.35, 13.95)	9.50 (6.10, 12.30)	10.00 (6.10, 12.99)	0.320
Chest CT positive rate (n, %)	41 (100.00)	101 (93.52)	142 (95.30)	0.216

**means a significant difference; ESS, euthyroid sick syndrome; M, median; IQR, inter quartile range; WBC, white blood cell count; T3, triiodothyronine; T4, thyroxine; FT4, free thyroxine; TSH, stimulating hormone; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PCT, procalcitonin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; SCr, serum creatinine; CK, creatine kinase; CK-MB, creatine kinase isoenzyme.*

TABLE 3 | Outcomes of patients with ESS and non-ESS.

	ESS group (n = 41)	Non-ESS group (n = 108)	All patients(n = 149)	*P* value
Severe event (n, %)	15 (36.59)	11 (10.19)	26 (17.45)	<0.001*
Noninvasive ventilator (n, %)	1 (2.44)	2 (1.85)	3 (2.01)	1.000
Invasive ventilator (n, %)	1 (2.44)	1 (0.93)	2 (1.34)	0.476
Mortality (n, %)	0 (0.00)	1 (0.93)	1 (0.67)	1.000
Virus shedding time (days, IQR)	19 (14, 23.5)	17 (13, 24)	17.50 (13, 24)	0.670
Length of hospital stay (days, IQR)	17 (12, 25)	15 (11, 23)	15.5 (11, 24.75)	0.345

**means a significant difference; ESS, euthyroid sick syndrome; IQR, inter quartile range.*

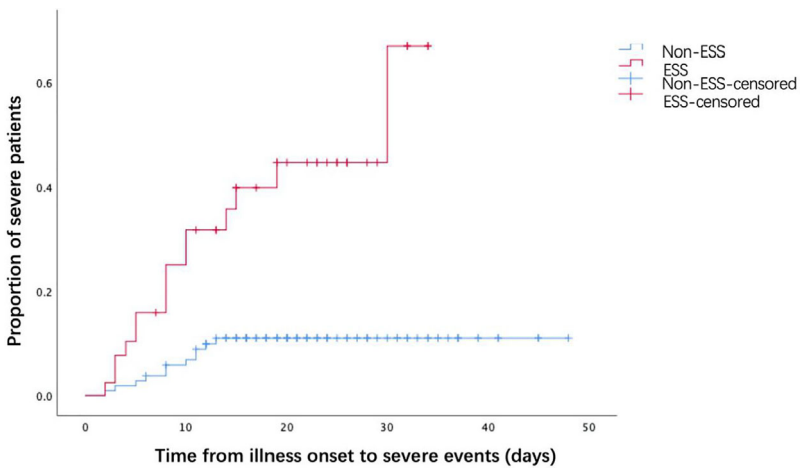

FIGURE 1 | The time-dependent risk of reaching to the severe events.

care unit (18). A study consisting of 503 patients diagnosed with community-acquired pneumonia reported that ESS is an independent risk factor for the 30-day mortality (19). The pathophysiological process of ESS involved in the induction of type III deiodinase and decrease of type I deiodinase, which resulted in the increased conversion of T4 to rT3 instead of T3 (9). Furthermore, the HPT axis was suppressed under pathological conditions, contributing to the decreased secretion of TSH (20). This pathophysiological mechanism also involved the binding of thyroid hormone to plasma protein, transport of thyroid hormone in peripheral tissues, and the hormone receptor activity (10).

TABLE 4 | Association of ESS and disease severity in the cox regression model.

	Beta	SE	Wald	*P* value	Hazard ratio	95.0% CI	
						Lower	Upper
ESS	0.922	0.446	4.280	0.039*	2.515	1.050	6.026
Gender	0.313	0.456	0.472	0.492	1.368	0.559	3.345
Age	0.006	0.017	0.147	0.701	1.006	0.974	1.040
Hypertension	0.417	0.549	0.576	0.448	1.517	0.517	4.447
Cardiovascular disease	-0.777	0.579	1.800	0.180	0.460	0.148	1.431
CRP	0.030	0.008	15.246	<0.001	1.031	1.015	1.046

**means a significant difference; ESS, euthyroid sick syndrome; CRP, C-reactive protein.*

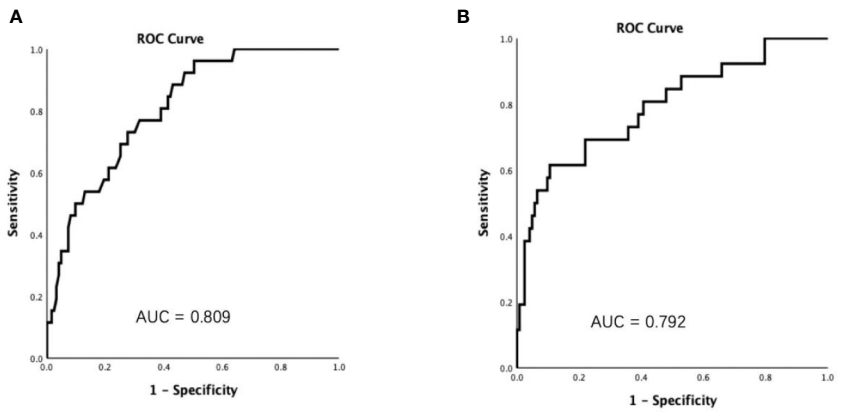

FIGURE 2 | Receiver operating characteristic (ROC) curves showed that the areas under the curve (AUCs) were 0.809 and 0.792 for FT3 **(A)** and C reactive protein **(B)**, respectively.

In COVID-19 patients, ESS may be directly caused by infection of thyroid cells with SARS-CoV-2. Despite the fact that no article on the involvement of the thyroid gland in COVID-19 patients has been published, varying degrees of damage to the thyroid gland were confirmed in SARS patients back in 2007 (21). Meanwhile, the patients with COVID-19 had lower serum TSH and TT3 levels than healthy controls and non-COVID-19 pneumonia patients (19). Angiotensin-converting enzyme (ACE) 2, an important receptor in the pathogenesis of COVID-19, was demonstrated to be expressed in the thyroid tissues (22). Hence, the thyroid gland may be one of the targets damaged by SARS-CoV-2. Withal, the mechanism for this hypothesis remains unknown. Further investigation is necessary to determine whether ACE2 has an impact on the thyroid function in patients with COVID-19. Second, cytokine storms were very common in COVID-19 patients, especially in severe cases, characterized by the uncontrolled and excessive release of inflammatory mediators resulting in overwhelming systemic inflammation and even multiple organ dysfunction (23). In our research, the inflammatory responses seemed to be stronger in patients with ESS, with higher levels of CRP and erythrocyte sedimentation rate as well as a higher positive rate of procalcitonin. It is well known that cytokines are the key molecules involved in coordinating the hormone, immune, and inflammatory responses to stressful stimuli (24). In the previous study, patients admitted to the intensive care unit elicited lower serum concentrations of T4, FT4, T3, FT3, and TSH, while the serum levels of inflammatory cytokines including IL-1β and TNF-α were markedly elevated (25). The increase of inflammatory cytokines can result in suppression of central TSH and 5'-deiodinases activity. Third, our results indicated that ESS patients were more likely to have the symptom of fever compared with non-ESS patients. The invasion of SARS-CoV-2 causing hyperthermia can lead to down-regulation of 5'-deiodinases activity, resulting in decreases in T3 levels. Meanwhile, the disease related negative nitrogen balance and body consumption, can also lead to a decline in the serum levels of thyroid hormone transport proteins, inhibiting T4 transport in T3-producing tissues. Fourth, the effects of drugs on the thyroid function should not be ignored. Glucocorticoids and dopamine can inhibit the secretion of TSH by the pituitary and the intake of T4 by peripheral tissues. Likewise, amiodarone and beta-adrenergic blocking agents can suppress deiodinase activity and consequently T3 production, contributing to a decrease in serum T3 levels. Also, non-steroidal anti-inflammatory drugs (NSAIDs) are capable of transiently increasing free thyroid hormone levels by preventing their binding to plasma transport proteins (26). Given that the thyroid functions of our

patients were assessed on admission, further studies including the effects of drugs on the thyroid function are imperative to demonstrate the impact of COVID-19 pharmacotherapy on the development of ESS.

Our work above had some limitations. First, this study was a retrospective study, and the thyroid function was accessed only at admission. Second, the sample size was too small and only a few patients required the mechanical ventilation. Based on this fact, the assessment of the correlation between ESS and the ventilation requirement performed in this study may be biased. Third, the levels of glucocorticoid, rT3 and the pituitary function were unknown, which are needed to exclude the effects of other factors on the functions of various endocrine glands.

In summary, more than 25% COVID-19 patients were diagnosed with ESS in our research. ESS is an independent risk factor for the disease severity of COVID-19. COVID-19 patients with ESS had stronger inflammatory responses, with higher levels of CRP and erythrocyte sedimentation rate as well as a higher positive rate of procalcitonin. Further investigation with a large sample size and an appropriate study design are needed to demonstrate the impact of ESS in COVID-19 patients.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because: The data used in this paper are from public health treatment center, which can only be obtained with the approval of relevant institutions. Requests to access the datasets should be directed to YZ, zhongyanjun@csu.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by: The study was approved by the institutional ethics board of the Second Xiangya Hospital of Central South University (No. 2020001). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

RZ and CW were involved in study design, interpreting data, statistical analysis, creating tables and figures, and writing of the manuscript. SZ, GWa, QZ, BY, YW, and HD were involved in collecting data. YZ, SW, and GWu were involved in interpreting data, statistical analysis, and designed the research, supervised the work. All authors contributed to the article and approved the submitted version.

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Thyroid Hormone Changes in Early Pregnancy Along With the COVID-19 Pandemic

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Purpose: COVID-19 (Coronavirus Disease 2019) was first reported in December 2019 and quickly swept across China and around the world. Levels of anxiety and depression were increased among pregnant women during this infectious pandemic. Thyroid function is altered during stressful experiences, and any abnormality during early pregnancy may significantly affect fetal development and pregnancy outcomes. This study aimed to determine whether the COVID-19 pandemic induces thyroid hormone changes in early pregnant women.

Methods: This study comprised two groups of pregnant women in Shanghai in their first trimester – those pregnant women before the COVID-19 outbreak from January 20, 2019, to March 31, 2019 (Group 1) and those pregnant during the COVID-19 outbreak from January 20, 2020, to March 31, 2020 (Group 2). All women were included if they had early pregnancy thyrotropin (TSH), free triiodothyronine (FT3), free thyroxine (FT4), total triiodothyronine (TT3), and total thyroxine (TT4) concentrations, thyroid peroxidase (TPO) antibody or thyroglobulin antibody (TgAb) available and did not have a history of thyroid diseases or received thyroid treatment before or during pregnancy. We used propensity score matching to form a cohort in which patients had similar baseline characteristics.

Results: Among 3338 eligible pregnant women, 727 women in Group 1 and 727 in Group 2 had similar propensity scores and were included in the analyses. Pregnant women in Group 2 had significantly higher FT3 (5.7 vs. 5.2 pmol/L, P<0.001) and lower FT4 (12.8 vs. 13.2 pmol/L, P<0.001) concentrations compared with those in Group 1. Pregnant women in Group 2 were more likely to develop isolated hypothyroxinemia (11.6% vs. 6.9%, OR, 1.75 [95% CI, 1.20–2.53], *P*=0.003) than those in Group 1 but had a significantly lower risk of TgAb positivity (12.0% vs. 19.0%, OR, 0.58 [95% CI, 0.43–0.78], *P*<0.001).

Conclusion: Pregnant women in their first trimester in Shanghai during the COVID-19 outbreak were at an increased risk of having higher FT3 concentrations, lower FT4 concentrations, and isolated hypothyroxinemia. The association between thyroid hormones, pregnancy outcomes, and the COVID-19 outbreak should be explored further.

Keywords: thyroid, early pregnancy, COVID-19, anxiety, depression

INTRODUCTION

COVID-19 (Coronavirus Disease 2019), caused by SARS-Cov-2 (Severe Acute Respiratory Syndrome Coronavirus 2) virus, is a highly infectious disease with a significant mortality rate and limited effective treatment (1). The outbreak was first reported in December 2019 in Wuhan, China, and since then, the number of cases has continued to escalate exponentially worldwide (2). The severity of COVID-19 was underestimated before it was officially confirmed as a type B infectious disease by the National Health Council, and the nation took heightened action to fight against it on January 20, 2020 (3). Since then, the number of diagnoses and deaths has grown rapidly internationally with significant epidemic prevention work implemented (4). Due to the uncertainty and low predictability of COVID-19, depression and anxiety symptoms have been common among the general population (5–7).

Pregnant women are also affected by the COVID-19 pandemic due to safety concerns for their fetuses (8, 9). In a case-control study, anxiety levels among pregnant women during the SARS outbreak were significantly higher than levels pre-SARS outbreak (8). A recent cross-sectional study conducted in pregnant women during the COVID-19 pandemic found that half of the participants indicated that the COVID-19 outbreak had serious psychological effects, with two-thirds reporting higher than normal levels of stress (9). Almost half of the women expressed high anxiety due to concerning potential vertical transmission of this disease (9). The negative psychological impact caused by COVID-19 was especially highlighted among women in their early pregnancies (9). This kind of stress and anxiety may have a significant physiological impact on pregnant women.

Thyroid hormones regulate various physiological processes related to pregnancy, such as the development and function of the placenta, fetal growth, and the expression of neuropeptides at the onset of labor (10–12). Overt thyroid diseases, such as hyperthyroidism and hypothyroidism, occur in approximately 0.5% and 0.05% of pregnant women, respectively and are associated with adverse outcomes for pregnant women and their fetuses (13, 14). Compared with overt thyroid diseases, thyroid dysfunctions such as subclinical hyperthyroidism and isolated hypothyroxinemia and thyroid autoimmune diseases have higher incidences (14) and have been associated with an increased risk for preeclampsia, spontaneous abortion, preterm delivery, low birth weight, and intrauterine growth retardation (IUGR) (15). Thus, the timely detection and treatment of pregnant women with thyroid dysfunction is clinically important (16). Few studies have focused on the physiological and psychological well-being of pregnant women during an infectious disease outbreak to understand the development of subclinical diseases that also need clinical management. The purpose of this study was to examine whether the COVID-19 outbreak was independently associated with thyroid hormone fluctuation and an increased risk of thyroid dysfunction in early pregnancy.

MATERIALS AND METHODS

Sample Collection

This retrospective cohort study was conducted in the International Peace Maternity and Child Health Hospital (IPMCH), which is a university-affiliated hospital and accounts for approximately 20% of births in Shanghai, China. As a part of standard antenatal care, all pregnant women underwent thyroid assessment in their first trimester and were divided into two groups. On January 20, 2020, COVID-19 was officially classified as a Type B infectious disease by the National Health Commission and thus defined as the start date of public awareness. Those pregnant women who underwent thyroid assessment between January 20, 2019, and March 31, 2019, were classified as the pre–COVID-19 epidemic group (Group 1), and those pregnant women screened between January 20, 2020, and March 31, 2020, were classified as the during-COVID-19 epidemic group (Group 2). A total of 3038 women in Group 1 and 2082 women in Group 2 were enrolled, all of whom had thyrotropin (TSH), free thyroxine (FT4), free triiodothyronine (FT3), TPOAb or TgAb concentrations available and had no COVID-19 symptoms such as fever, cough, and myalgia symptoms (1). Women were excluded if they were not in their first trimester, underwent *in vitro* fertilization, had twin pregnancies, or had a history of thyroid disease or TPOAb positivity. Ethics approval was obtained by the institutional review board (No. GKLW2019-51).

Exposure and Outcomes

When pregnant women come to the hospital for prenatal care, all data, including sociodemographic and clinical data, were collected by doctors and nurses and recorded in the electronic medical file. Thyroid hormones, including TSH, FT3, FT4, TT3, TT4, TPOAb, and TgAb, were measured by taking fasting blood samples from the cubital vein and centrifugation within 6 hours to separate the serum, which was then detected with the Architect i2000 immunoassay (Abbott, Chicago, USA) during the whole study period. The lower limits of detection and the intra- and interassay coefficients of variation were 0.0038 mIU/L and 1.6% and 3.59%, respectively, for TSH; 1.54 pmol/L and 2.97% and 4.03%, respectively, for FT3; 0.6200 pmol/L and 1.9% and 4.01%, respectively, for FT4; 0.38 nmol/L and 2.3% and 4.07%, respectively, for TT3; 0.62 pmol/L and 1.9% and 4.01%, respectively, for TT4; 0.5 IU/ml and 10% and 10%, respectively, for TPOAb; and 0.31 IU/ml and 20% and 20%, respectively, for TgAb.

Outcomes included thyroid function measurements (thyrotropin (TSH), free triiodothyronine (FT3), free thyroxine (FT4), total triiodothyronine (TT3), and total thyroxine (TT4) concentrations, thyroid peroxidase (TPO) antibody and thyroglobulin antibody (TgAb)). Subclinical thyroid diseases were defined according to the cohort-specific 2.5th and 97.5th population percentiles for TSH, FT3, FT4, TT3 and TT4 after exclusion of TPO antibody-positive women (17). TPO antibodies ≥5.61 IU/ml were considered positive. TgAbs ≥4.11 IU/ml were considered positive. Subclinical hyperthyroidism was defined as a TSH concentration below the 2.5th percentile and an FT4 concentration within the normal range (2.5th–97.5th percentile). Subclinical hypothyroidism was defined as a TSH concentration above

the 97.5th percentile and an FT4 concentration within the normal range. Overt hyperthyroidism was defined as a TSH concentration below the 2.5th percentile and an FT4 concentration above the 97.5th percentile. Overt hypothyroidism was defined as a TSH concentration above the 97.5th percentile and an FT4 concentration below the 2.5th percentile. Isolated hypothyroxinemia was defined as an FT4 concentration below the 2.5th percentile and a TSH concentration within the normal range. Elevated T3 was defined as a TT3 concentration above the 97.5th percentile. Low T3 was defined as a TT3 concentration below the 2.5th percentile.

Sociodemographic Outcomes

Maternal age, educational level, gravida, and body mass index (BMI) were collected in addition to smoking and drinking status, gestational weeks, and previous history of thyroid disease. We also collected paternal age and education. Maternal and paternal age was reported at the time of thyroid function tests and was categorized as 18 to 29, 30 to 39 and > 39 years. Maternal and paternal educational levels were defined as the years of education after graduation from primary school and were categorized as < 6 years (low), 6 to 10 years (middle) and > 10 years (high). Gravida was defined as the total number of pregnancies, including live births, stillbirths and abortions. BMI was calculated as weight in kilograms divided by height in meters squared and categorized as low weight (<18.5), normal weight (18.5–23.9), overweight (24.0–27.9), and obese (≥28.0). Maternal smoking was self-reported and categorized as nonsmoker, past smoker or current smoker. Maternal drinking was similarly categorized. Weeks gestation was established based on measurement of the fetal crown–rump length (CRL) (18). A previous history of thyroid disease was defined as self-reported hyperthyroidism, hypothyroidism, thyroid nodules that underwent thyroidectomy, and thyroid autoimmune diseases.

Statistical Analysis

Considering the difference in baseline characteristics between the two groups of participants (**Table 1**), we used propensity score matching to regroup a cohort of 1454 first trimester pregnant women with similar baseline characteristics. The propensity score is the conditional probability of having a specific exposure level (Group 1 and Group 2) under a given set of baseline covariates (19, 20). A multivariate logistic regression model was used to estimate propensity scores, and all baseline characteristics listed in **Table 1** were included as covariates, using a 1:1 matching protocol with no replacement for matching with a caliper width equal to 0.01 and evaluating the P values of all baseline covariates before and after matching to assess whether they are balanced. Standard differences of less than 5% indicate a relatively small imbalance. All variables in the matched cohort are without missing data.

Descriptive analysis of normally distributed continuous variables is expressed as the mean and standard deviation (SD). For nonnormally distributed variables, we use the median and interquartile range (IQR); categorical variables are expressed in proportion and percentage. We used the Mann-Whitney U test to compare the median difference of continuous variables and the chi-square test to compare the difference in proportion. To perform a univariate analysis of the association between thyroid dysfunction and all other factors, a chi-square test was used (if the number in the cell was less than 5, Fisher's two-sided exact test was used). In a multivariate logistic regression model, logistic regression analysis was performed on the relationship between (subclinical) thyroid diseases and all those variables with a P value <0.1 in univariate analysis. Sensitivity analysis and subgroup analysis were used to evaluate the difference in thyroid hormones and (subclinical) thyroid diseases in each subgroup between the matched Group 1 and Group 2 to identify potential bias. A two-sided P value of less than 0.05 was considered statistically significant. All statistical tests were conducted using SPSS version 24.0 and R version 3.6 (*Packages forestplot, ggplot2*).

RESULTS

Sample Characteristics

After exclusion, a total of 3338 pregnant women participated in the study, with 2012 in Group 1 and 1326 in Group 2 (**Figure 1**). **Table 1** shows the comparisons of the baseline characteristics between the two groups before and after propensity-score matching. The majority of women overall were between 30 and 39 years old (n =1832, 54.9%), nondrinkers (n =2834, 97.7%), and nonsmokers (n =2885, 99.5%). Compared with Group 1, Group 2 was more likely to be older (31 vs 30 years old, P<0.001), earlier in pregnancy (i.e., lower weeks gestation) (12.1 vs 12.7 weeks, P<0.001) and less likely to be a current drinker (1.4% vs. 2.7%, P=0.03). With propensity score matching, 727 women exposed to the COVID-19 outbreak (Group 2) were matched with 727 women in Group 1. After matching, the P values of all variables were greater than 0.05, indicating that no significant differences existed between the two groups (**Table 1**).

Thyroid Hormone Concentrations Among Early Pregnant Women

Women in Group 2 who were exposed to the COVID-19 outbreak had significantly higher FT3 (5.7 vs. 5.2 pmol/L, P<0.001) and lower FT4 (12.8 vs. 13.2 pmol/L, P<0.001) concentrations than those in Group 1. There were no significant differences between the two groups in the concentrations of TSH (1.32 vs. 1.28 mIU/L, P=0.45), TT3 (2.0 vs. 2.0 nmol/L, P=0.73) and TT4 (140.2 vs. 140.4 nmol/L, P=0.56) (**Table 2**) (**Figure S1**).

Risk of Thyroid Diseases Among General Early Pregnant Women

Univariate analysis found that factors associated with subclinical thyroid disease included weeks gestational, gravida, maternal BMI, paternal age and educational levels, and cohort group (Group 1 vs Group 2) (**Table S1**). To examine the risk of developing subclinical thyroid disease, logistic regression was performed with results suggesting that exposure to the COVID-19 outbreak increased a woman's risk of isolated hypothyroxinemia (11.6% vs. 6.9%, OR, 1.75 [95% CI, 1.20–2.54], *P*=0.003) and decreased their risk of TgAb positivity (12.0% vs. 19.0%, OR, 0.58 [95% CI, 0.43–0.78], *P*<0.001)

TABLE 1 | Baseline characteristics before and after propensity-score matching.

Assessment characteristics	Before matching			After matching		
	Group 1N = 2,012	Group 2N = 1,326	P value	Group 1 N = 727	Group 2 N = 727	P value
Maternal Demographics						
Age, median (Q1–Q3) in years	30 (28–32)	31 (28–33)	<0.001	30 (28–33)	30 (28–33)	0.52
Age distribution						
18–29	937/2,012 (46.6)	499/1,326 (37.6)	<0.001	297 (40.9)	289 (39.8)	0.52
30–39	1,046/2,012 (52.0)	786/1,326 (59.3)		417 (57.4)	420 (57.8)	
>39	29/2,012 (1.4)	41/1,326 (3.1)		13 (1.8)	18 (2.5)	
Weeks gestational at blood sampling, median (95% range)	12.7 (12.3–13.1)	12.1 (11.4–12.9)	<0.001	12.4 (12.0–13.0)	12.4 (12.0–12.9)	0.78
Body mass index, median (Q1-Q3), kg/m^2	20.8 (19.3–22.7)	20.9 (19.3-–22.9)	0.51	20.9 (19.3-–22.9)	20.9 (19.3-–23.0)	0.91
Body mass index distribution						
Underweight	251/1,937 (13.0)	126/982 (12.8)	0.15	95 (13.1)	99 (13.6)	0.74
Normal	1391/1,937 (71.8)	700/982 (71.3)		514 (70.7)	508 (69.9)	
Overweight	244/1,937 (12.6)	115/982 (11.7)		95 (13.1)	87 (12.0)	
Obesity	51/1,937 (2.6)	41/982 (4.2)		23 (3.2)	33 (4.5)	
Gravida, median (Q1–Q3)	1 (1–2)	2 (1–2)	0.08	2 (1–2)	1 (1–2)	0.50
Smoking status						
Nonsmoker or past smoker	1,916/1,923 (99.6)	969/977 (99.2)	0.11	725 (99.7)	722 (99.3)	0.45
Current smoker	7/1923 (0.5)	8/977 (0.8)		2 (0.3)	5 (0.7)	
Drinking status						
Nondrinker or past drinker	1,871/1,923 (97.3)	963/977 (98.6)	0.03	713 (98.1)	716 (98.5)	0.55
Current drinker	52/1,923 (2.7)	14/977 (1.4)		14 (1.9)	11 (1.5)	
Educational level						
Low	140/1,935 (7.2)	74/981 (7.5)	0.11	41 (5.6)	52 (7.2)	0.64
Middle	1,411/1,935 (72.9)	678/981 (69.1)		530 (72.9)	517 (71.1)	
High	384/1,935 (19.8)	229/981 (23.3)		156 (21.5)	158 (21.7)	
Paternal Demographics						
Age, median (Q1–Q3) in years	32 (29–35)	32 (30–36)	<0.001	32 (30–35)	32 (30–35)	0.90
Age distribution						
18–29	540/1,885 (28.6)	202/948 (21.3)	<0.001	163 (22.4)	161 (22.2)	0.96
30–39	1,222/1,885 (64.8)	669/948 (70.6)		505 (69.5)	510 (70.2)	
>39	123/1,885 (6.5)	77/948 (8.1)		59 (8.1)	56 (7.7)	
Educational level						
Low	297/3,211 (9.2)	40/454 (8.8)	0.19	44 (6.1)	52 (7.2)	0.96
Middle	2,216/3,211 (69.0)	301/454 (66.3)		509 (70.0)	494 (68.0)	
High	698/3,211 (21.7)	113/454 (24.9)		174 (23.9)	181 (24.9)	

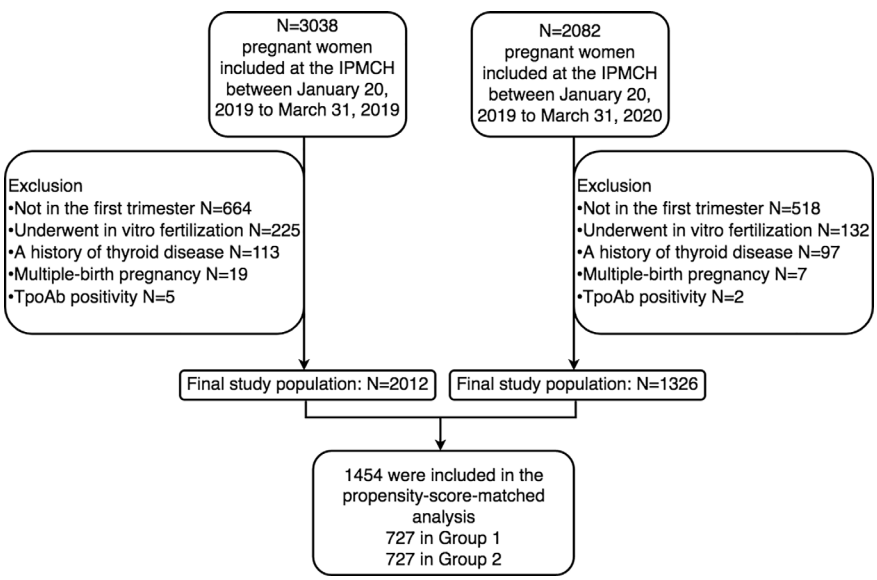

FIGURE 1 | Flowchart illustrating study population selection and data availability.

(**Table 3**). Conversely, there was no significant difference between the two groups in the risk of subclinical hyperthyroidism (3.0% vs. 1.9%, OR, 1.60 [95% CI, 0.81–3.15], *P*=0.18), subclinical hypothyroidism (2.6% vs. 4.4%, OR, 0.58 [95% CI, 0.33–1.04], *P*=0.07) and overt hyperthyroidism (0.3% vs. 0.6%, OR, 0.51 [95% CI, 0.09–2.78], *P*=0.43) (**Table 3**) (**Figure S2**).

Sensitivity Analysis

Figure 2 shows the results of the FT3 subgroup analysis, which indicated that women in early pregnancy exposed to the COVID-19 outbreak who were overweight or obese preconceptionally had a higher FT3 concentration (overweight: 6 vs 5.2 pmol/L, P<0.001, obesity: 5.9 vs. 5.5, P=0.006) (**Figure 2A**, **Table S2**). Furthermore, exposure to the COVID-19 outbreak could elevate FT3 concentration in all subgroups except women over 40 years old (**Figures 2B, D, E**, **Table S2**). A lower maternal education level was associated with higher FT3 concentrations (low vs. middle vs. high: 5.6 vs. 5.4 vs. 5.3 pmol/L, P=0.03) when pooled data from two groups.

Figure 3 shows the results of the FT4 subgroup analysis. Women in early pregnancy exposed to the COVID-19 outbreak who were overweight or obese preconceptionally had a lower FT4 concentration (overweight: 12.5 vs 12.8 pmol/L, P=0.23, obesity: 11.8 vs. 12.8, P=0.03) (**Figure 3A**, **Table S3**). Those who were less than 30 years old and between 30 and 39 years old had significantly lower FT4 concentrations (< 30: 13.1 vs. 13.3 pmol/L, P=0.008, 30–39: 12.8 vs 13.1 pmol/L, P<0.001) (**Figure 3B**, **Table S3**), and women between 30 and 39 had lower FT4 concentrations (12.9 vs. 13.2 pmol/L, P=0.001) in comparison to those under 30 years of age. Furthermore, the FT4 concentration difference was greater between Group 1 and Group 2 among those between 30 and 39 years old compared to women less than 30 years old (−0.3 vs −0.2 pmol/L, P<0.001) (**Figure 3B**, **Table S3**). Women exposed to the COVID-19 outbreak at all education levels had lower FT4 concentrations than Group 1 (12.8 vs. 13.1 pmol/L, P<0.001), although a significant difference was only found at the middle level (**Figure 3C**, **Table S3**). A higher maternal education level was associated with lower FT4 concentrations (low vs. middle vs. high: 12.75 vs. 13.0 vs. 13.2 pmol/L, P=0.01) when pooled data from two groups. The lower concentration of FT4 in Group 2 than in Group 1 was not significant among women with husbands over 40 years old (12.4 vs 12.9 pmol/L, P=0.07) or in low (12.8 vs 12.8 pmol/L, P=0.27) or high (13.0 vs 13.2 pmol/L, P=0.14) educational levels, but it was significant among those whose husbands were under the age of 40 (under 30: 12.9 vs 13.4 pmol/L, P=0.03, 30 to 39: 12.9 vs 13.2 pmol/L, P=0.002) or had a higher educational level (12.8 vs 13.2 pmol/L, P<0.001) (**Figures 3D, E**, **Table S3**).

Figure 4 shows the results of the isolated hypothyroxinemia subgroup analysis. An increased risk of isolated hypothyroxinemia was found in all subgroups except women over 40 years old or those with an underweight BMI. Women in early pregnancy exposed to the COVID-19 outbreak between 30 and 39 years old had a higher risk of isolated hypothyroxinemia (OR, 1.81 vs. 1.76, P<0.001) in comparison to those under 30 years of age. Women who were overweight or obese preconceptionally had a higher risk of developing isolated hypothyroxinemia in comparison to normal or underweight women exposed to the COVID-19 outbreak (OR 1.92 vs. 1.87 vs. 0.63).

Figure 5 shows the results of the TgAb positivity subgroup analysis, which indicated that the risk of TgAb positivity among all subgroups was lower in Group 2 than in Group 1 except at the paternal high educational level. Interestingly, older paternal age was associated with a lower prevalence rate of TgAb positivity in early pregnant women (Group 1: 18–29 vs 29–39 vs >39: 21.1% vs 18.6% vs 16.1%, Group 2: 18–29 vs 29–39 vs >39: 12.5% vs 11.9% vs 10.7%).

TABLE 2 | Thyroid hormone concentrations in the propensityscore-matched cohort.

	Group 1	Group 2	P value
TSH, mIU/L (median, 95% range)	1.28 (0.03–4.15)	1.32 (0.02–3.9)	0.45
Free thyroxine 3 (FT3), pmol/L (median,95% range)	5.2 (4.2–6.6)	5.7 (4.3–7.4)	<0.001
Free thyroxine 4 (FT4), pmol/L (median,95% range)	13.2 (11–16.5)	12.8 (10.7–16.9)	<0.001
Total thyroxine 3 (TT3), nmol/L (median,95% range)	2.0 (1.4–2.8)	2.0 (1.5–2.7)	0.73
Total thyroxine 4 (TT4), nmol/L (median,95% range)	140.4 (100.2–197.3)	140.2 (100.3–192.3)	0.56

TABLE 3 | Risk of thyroid diseases in the propensity score-matched cohort.

(Subclinical) Thyroid diseases	Group 1n(%)	Group 2n(%)	Odds Ratio (95%)	Adjusted Odds Ratio (95% CI)	P value
Subclinical hyperthyroidism	14 (1.9)	22 (3.0)	1.59 (0.81–3.13)	1.60 (0.81–3.15)a	0.18
Subclinical hypothyroidism	32 (4.4)	19 (2.6)	0.58 (0.33–1.04)	0.58 (0.33–1.04)	0.07
Overt hyperthyroidism	4 (0.6)	2 (0.3)	0.50 (0.10–2.73)	0.51 (0.10–2.78)b	0.43
Isolated Hypothyroxinemia	50 (6.9)	84 (11.6)	1.77 (1.23–2.55)	1.75 (1.20–2.54)c	0.003
Thyroglobulin antibody positivity	138 (19.0)	87 (12.0)	0.58 (0.43–0.78)	0.58 (0.43–0.78)	<0.001
Elevated T3	50 (6.9)	35 (4.8)	0.69 (0.44–1.07)	0.37 (0.07–1.91)d	0.23
Low T3	5 (0.7)	2 (0.3)	0.40 (0.08–2.06)	0.67 (0.43–1.05)e	0.08

The table shows the number and proportion of pregnant women with (subclinical) thyroid disease during early pregnancy in Group 1 and Group 2. The adjusted odds ratio was calculated in the multivariate logistic regression model by including all confounding factors with a P value <0.1 in univariate analysis. a: Paternal age distribution is included as cofounding factor in logistic regression model; b: Gravida is included as cofounding factor in logistic regression model; c: Maternal BMI is included as cofounding factor in logistic regression model; d: BMI distribution is included as cofounding factor in logistic regression model e: Maternal BMI, paternal educational level, maternal age and age distribution, weeks gestational are included as cofounding factor in logistic regression model.

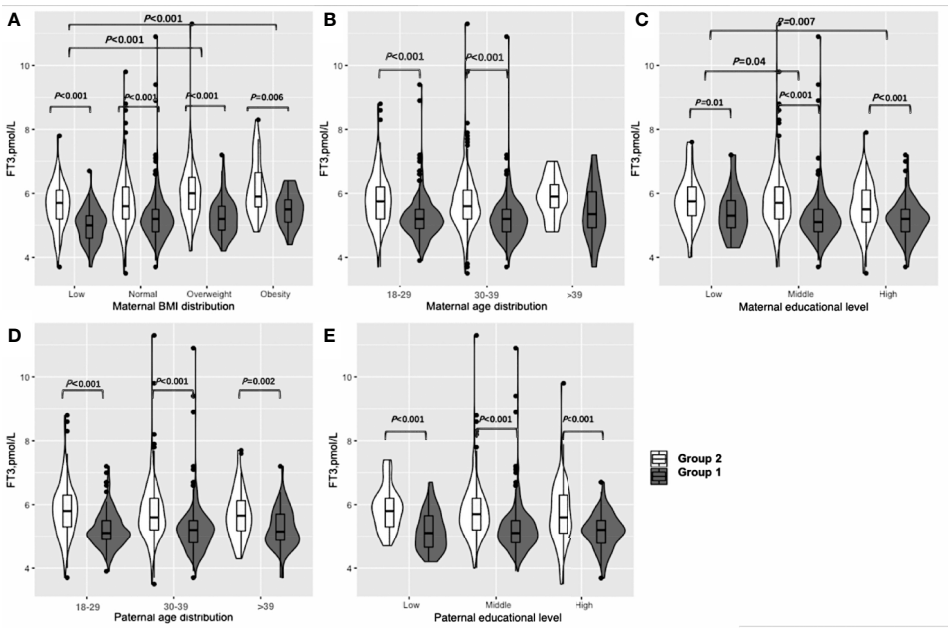

FIGURE 2 | Subgroup analysis of FT3 concentration in the Propensity-Score-Matched Cohort. Figure shows the FT3 concentration in different subgroup of Group 1 and Group 2. The violin diagram is a combination of box plot and density plot. The thick black bar in the middle of the boxplot represents the median and quartile range, and the black line extending up and down represents the 95% confidence interval. The black dots on the black line indicate data values that exceed the 95% confidence interval. The outer contour indicates the distribution of the data. **(A)** Maternal BMI distribution. **(B)** Maternal age distribution. **(C)** Maternal educational level. **(D)** Paternal age distribution. (E) Paternal educational level.

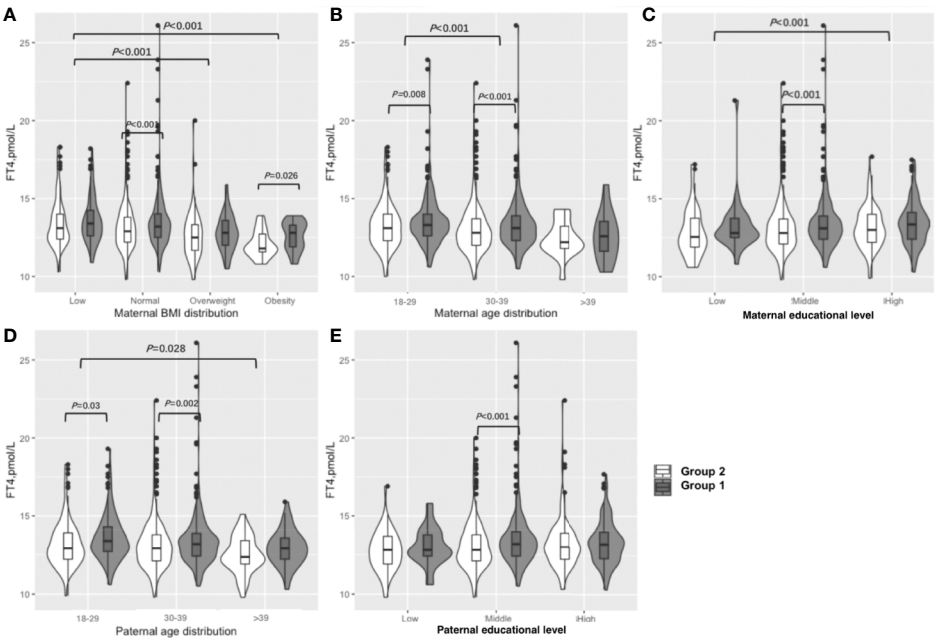

FIGURE 3 | Subgroup analysis of FT4 concentration in the Propensity-Score-Matched Cohort. Figure shows the FT4 concentration in different subgroup of Group 1 and Group 2. The violin diagram is a combination of box plot and density plot. The thick black bar in the middle of the boxplot represents the median and quartile range, and the black line extending up and down represents the 95% confidence interval. The black dots on the black line indicate data values that exceed the 95% confidence interval. The outer contour indicates the distribution of the data. **(A)** Maternal BMI distribution. **(B)** Maternal age distribution. **(C)** Maternal educational level. **(D)** Paternal age distribution. **(E)** Paternal educational level.

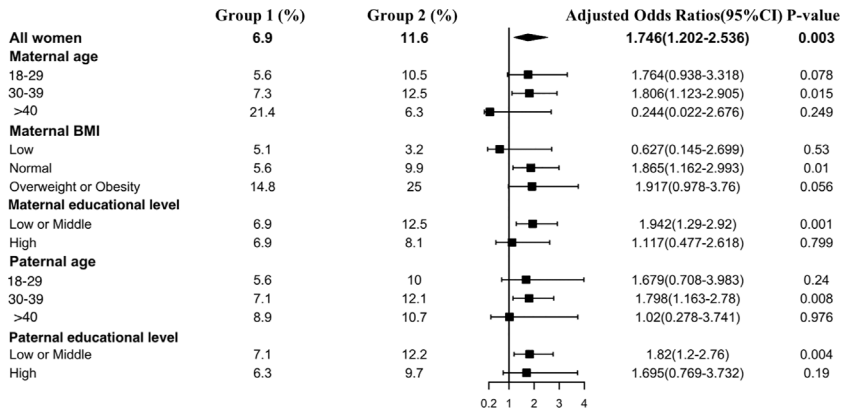

FIGURE 4 | Subgroup analysis of the risk of isolated hypothyroxinemia in the Propensity-Score-Matched Cohort. Figure shows the proportion of pregnant women with isolated hypothyroxinemia in each subgroup of Group 1 and Group 2 (expressed as the number of pregnant women with isolated hypothyroxinemia / the total number of women in this subgroup *100%). The forest chart in the fourth column describes the adjusted odds ratio in each subgroup. The diamond represents the overall adjustment odds ratio. The position of the small rectangle below indicates the point estimate of the adjustment odds ratio of each subgroup. The solid line range indicates the 95% confidence interval of the adjustment risk ratio of each subgroup. Adjusted odds ratio is calculated in the multivariate logistic regression model by including all confounding factors with a P value < 0.1 in univariate analysis.

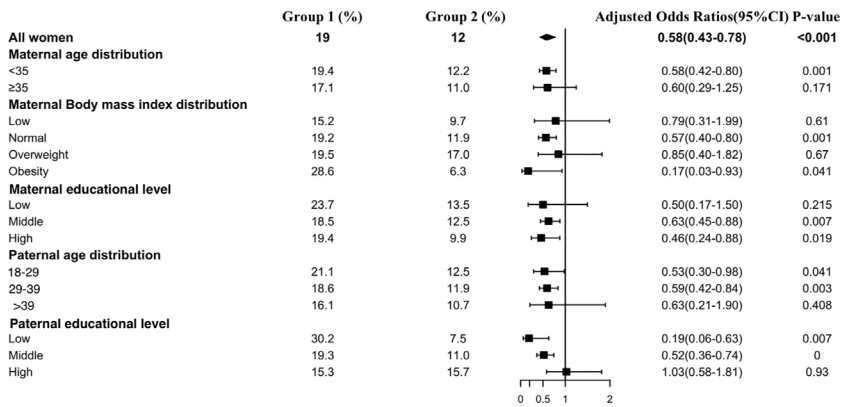

FIGURE 5 | Subgroup analysis of the risk of TgAb positivity in the Propensity-Score-Matched Cohort. Figure shows the proportion of pregnant women with TgAb positivity in each subgroup of Group 1 and Group 2 (expressed as the number of pregnant women with TgAb positivity / the total number of women in this subgroup *100%). The forest chart in the fourth column describes the adjusted odds ratio in each subgroup. The diamond represents the overall adjustment odds ratio. The position of the small rectangle below indicates the point estimate of the adjustment odds ratio of each subgroup. The solid line range indicates the 95% confidence interval of the adjustment risk ratio of each subgroup. Adjusted odds ratio is calculated in the multivariate logistic regression model by including all confounding factors with a P value < 0.1 in univariate analysis.

After excluding TgAb-positive women, there were 589 in group 1 and 640 in group 2. The results were similar to the population without exclusion. Women in Group 2 who were exposed to the COVID-19 outbreak had significantly higher FT3 (5.7 vs. 5.2 pmol/L, P<0.001) and lower FT4 (12.9 vs. 13.2 pmol/L, P<0.001) concentrations than those in Group 1. There were no significant differences between the two groups in the concentrations of TSH (1.31 vs. 1.24 mIU/L, P=0.37), TT3 (2.0 vs. 2.0 nmol/L, P=0.54) and TT4 (140.9 vs. 141.1 nmol/L, P=0.33) (**Table S4**). In addition, TgAb-negative women exposed to the COVID-19 outbreak had an increased risk of isolated hypothyroxinemia (11.1% vs. 6.5%, OR, 1.77 [95% CI, 1.16–2.69], P=0.008). No significant difference was found in the risk of subclinical hyperthyroidism (3.1% vs. 1.5%, OR, 2.08 [95% CI, 0.94–4.60], P=0.07) or subclinical hypothyroidism (2.5% vs. 3.9%, OR, 0.63 [95% CI, 0.33–1.21], P=0.16) (**Table S5**).

For TgAb-positive women, there were 138 in Group 1 and 87 in Group 2. Those women in Group 2 who were exposed to the COVID-19 outbreak had significantly higher FT3 (5.6 vs. 5.0 pmol/L, P<0.001) and lower FT4 (12.6 vs. 13.2 pmol/L, P=0.03) concentrations than those in Group 1. There were no significant differences between the two groups in the concentrations of TSH (1.47 vs. 1.39 mIU/L, P=0.74), TT3 (2.0 vs. 2.0 nmol/L, P=0.73) and TT4 (136.1 vs. 139.7 nmol/L, P=0.44) (**Table S6**). The COVID-19 outbreak increased the risk of isolated hypothyroxinemia, although there was no significant

difference (14.9% vs. 8.7%, OR, 1.93 [95% CI, 0.80–4.64], P=0.14). No significant difference was found in the risk of subclinical hyperthyroidism (2.3% vs. 3.6%, OR, 0.63 [95% CI, 0.12–3.30], P=0.58) or subclinical hypothyroidism (3.4% vs. 6.5%, OR, 0.33 [95% CI, 0.08–1.40], P=0.13) (**Table S7**).

DISCUSSION

This study found that women in early pregnancy who were exposed to the COVID-19 outbreak had a higher concentration of FT3 and a lower concentration of FT4 in comparison to those not exposed to this pandemic. Furthermore, we found an increased risk of isolated hypothyroxinemia and a lower risk of TgAb positivity among early pregnant women exposed to the COVID-19 outbreak who did not have any previous thyroid diseases after propensity score matching and adjusting for potential confounders, including maternal and paternal age and educational level, gravida status, maternal smoking and drinking status, maternal BMI and weeks gestational.

Infectious disease pandemics will naturally increase anxiety and fear among individuals in society (4). Pregnant women are vulnerable due to additional safety concerns for their fetuses because they also worry that vertical transmission will damage the health of the fetus in addition to their own health (9). A substantial portion of pregnant women overestimated their risk of COVID-19 infection during the outbreak, leading to even higher rates of depression and anxiety than what is typically expected during the antenatal period (9). The depression rate in pregnant women is positively correlated with the number of newly confirmed cases, the number of suspicious cases and the number of deaths per day (21). It is well documented that antenatal depression and anxiety are associated with poor pregnancy outcomes and have negative influences on child development (22–24).

During pregnancy, thyroid hormones of pregnant women undergo significant physiological changes. The concentration of FT4 increases in the early stages of pregnancy and maintains this level until delivery and then rapidly declines postnatally (17). Conversely, FT3 changes little during pregnancy (17). In early pregnancy, the increase in FT4 levels is due to increased placental production of human chorionic gonadotropin (hCG), which leads to a reduction in TSH (11). Although the fetal thyroid begins to develop around the 5th or 6th week of pregnancy, the fetus cannot synthesize thyroid hormone by itself during early pregnancy (25). Thus, the fetal thyroid hormones required for normal neurological development are completely maternally derived. Alterations in maternal FT4 concentration due to significant stress, such as a COVID-19 outbreak, may increase the risk of adverse pregnancy outcomes.

It is currently known that isolated hypothyroxinemia is associated with a higher risk of preterm and very preterm birth (26) and neurocognitive dysplasia in offspring (27, 28). Previous studies have shown a relationship between thyroid antibody positivity and concomitant anxiety or depression disorder, where individuals with autoimmune thyroiditis exhibit an increased chance of developing depression and anxiety (29). However, the relationship between thyroid autoantibodies and preterm delivery is controversial. A prospective cohort study of 120 pregnant women in western European cities reported that those with normal thyroid function but positive TPOAb or TgAb had a significantly increased preterm birth rate (16% vs. 8%, p <0.005) (30). Conversely, another prospective study of 1179 women in Japan did not find an increased risk of preterm birth among TgAb-positive women. The preterm birth rates in the study group and the control group were both extremely low (3% vs. 3.1%), which the researchers suggested was the result of ethnic differences (31). Another prospective study of 10062 pregnant women reported that the risk of preterm birth did not increase among women who were positive for TPOAb and/or TgAb in early pregnancy, although premature rupture of membranes significantly increased (32). Importantly, thyroid autoantibodies are associated with the suboptimal development of offspring. One study evaluated the neurocognitive ability of 43 5.5-year-old children who were born at term. Children with TgAb-positive mothers had lower sensory performance and exercise scores, and those with TgAb-positive umbilical cord blood also had lower sensory performance scores (33). Although we do not know the pregnancy outcomes of women exposed to the COVID-19 outbreak who had isolated hypothyroxinemia or TgAb positivity in our study until writing this article, we will follow up on their pregnancy outcomes and the health status of their offspring.

The relationships between mood alteration and thyroid function were first recognized in a seminal review (34). Later, a meta-analysis of 20 studies found that individuals with anxiety were significantly more likely to have thyroid disease and that anxiety was inversely related to TSH levels (35). Several cross-sectional studies have shown that those with thyroid dysfunction have an increased risk of anxiety or depression (36–38). Therefore, previous studies have mostly focused on the relationship between depression or anxiety and TSH concentration, overt hyperthyroidism or overt hypothyroidism. The relationship between mental disorders and isolated hypothyroxinemia, FT3 or FT4 has not been explored. In this study, a higher concentration of FT3, a lower concentration of FT4, a higher risk of isolated hypothyroxinemia and a nonsignificantly higher concentration of TSH were found in pregnant women exposed to the COVID-19 outbreak. We speculate that this is because anxiety due to the COVID-19 outbreak amplifies the TSH enhancement of FT4 to FT3 conversion (39). This hypothesis requires further study.

In the current study, we found some interesting results in the sensitivity analysis. BMI was a determinant of thyroid function during pregnancy. Consistent with previous research (40, 41), we found that higher maternal BMI preconceptually was associated with higher FT3 concentrations, lower FT4 concentrations, and an increased risk for isolated hypothyroxinemia and TgAb positivity. The correlation between BMI and thyroid function has been reported previously and is hypothesized to be due to changes in energy balance caused by increased heat production in obese women (42). Why were the higher concentration of FT3, lower concentration of FT4 and higher risk of isolated hypothyroxinemia in women > age 40 exposed to COVID -19 outbreak not significantly different? First, the sample of pregnant

women older than 40 years is relatively small. Furthermore, advanced maternal age was associated with lower FT3 (43). The enhancement of TSH on the conversion of T3 to T4 reaches its peak at the age of 30 to 40 years (44). Its enhancement begins to weaken after 40 (44). This can also explain why the risk of FT4 and isolated hypothyroxinemia is more significant in women aged 30 to 39 years in this study than in women < age 30. We also found that the higher maternal and paternal education level, the less significant the difference between the COVID-19 exposure and the nonexposure group, that is, the lower FT3 concentration, the higher FT4 concentration, and the lower risk of isolated hypothyroxinemia. A study that evaluated the depression and anxiety symptoms of pregnant women under COVID-19 may explain it. It showed that the higher the maternal education level, the less likely it is to report anxiety and depression because a higher education level often means higher income, more comprehensive knowledge of COVID-19, access to good medical resources and better prevention measures (21). Therefore, the thyroid function of pregnant women with higher education levels is less affected. The effect of paternal age on maternal FT3 and FT4 concentrations and the risk of isolated hypothyroxinemia is similar to that of maternal education level and age. This may be because people tend to choose those who are close to their age as their spouse. This can be proven in this study. The correlation coefficient between paternal age and maternal age was 0.72 ($P<0.001$). We also found that the main conclusions in TgAb-positive or TgAb-negative women are similar to the whole population. However, for TgAb-positive women, there was no significant difference in the increased risk of isolated hypothyroxinemia due to the COVID-19 outbreak. The small sample size of TgAb-positive women could partly explain this finding.

There are some limitations in our study. First, we did not demonstrate the mental health status of participants exposed to the COVID-19 outbreak. However, another study by our team, discussed above, evaluated the depression and anxiety symptoms of pregnant women in 25 hospitals in China before and at the time of the epidemic. It has been fully demonstrated that participants in this article are also more likely to experience symptoms of anxiety and depression under the pressure of the epidemic (21). Further, this study was completed within 2 months of the COVID-19 outbreak, and thus, only short-term thyroid responses were examined; long-term follow-up is required to examine subacute thyroid dysfunction, pregnancy outcomes, and postpregnancy thyroid function. Thyroid hormone changes were observed in pregnant women, and whether there is the same change in nonpregnant women and the general population is worthy of further research. In addition, although we have taken the confounding factors that affect thyroid hormone levels into account as comprehensively as possible, there are possibly some other unmeasured confounders causing bias in the propensity-score-matched cohort.

The current study provides evidence that exposure to extreme stress, such as the COVID-19 outbreak, can alter thyroid function in early pregnant women. When a pregnant woman is exposed to emergency or highly stressful events, the thyroid function of pregnant women deserves concern. However, whether this monitoring and treatment would lead to safe and effective results needs further research.

CONCLUSION

This is the first study to examine alterations in thyroid functioning among pregnant women in their first trimester who are exposed to extreme stress, such as the COVID-19 outbreak. We found that exposure to the COVID-19 outbreak was independently associated with a higher FT3 concentration, a lower FT4 concentration, a higher risk of isolated hypothyroxinemia and a lower risk of TgAb positivity in pregnant women during the first trimester in Shanghai. Based on our study, we can infer that anxiety induced by COVID-19 during early pregnancy significantly influenced maternal thyroid function. Timely thyroid function tests and psychological intervention programs are recommended to be considered for pregnant women during emergencies or extremely stressful events and warrant additional research. Furthermore, the association between pregnancy outcomes and COVID-19 outbreaks requires further exploration.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The International Peace Maternity and Child Health Hospital institutional review board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by TL, CZ, HZ, YW, and LC. The first draft of manuscript was written by TL. Review and editing were performed by C-LD, HH, and YTW. All authors commented on previous versions of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.606723/full#supplementary-material

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Thyroid Function Abnormalities in COVID-19 Patients

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Purpose: The novel coronavirus COVID-19, has caused a worldwide pandemic, impairing several human organs and systems. Whether COVID-19 affects human thyroid function remains unknown.

Methods: Eighty-four hospitalized COVID-19 patients in the First Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China) were retrospectively enrolled in this study, among which 22 cases had complete records of thyroid hormones. In addition, 91 other patients with pneumonia and 807 healthy subjects were included as controls.

Results: We found that levels of total triiodothyronine (TT3) and thyroid stimulating hormone (TSH) were lower in COVID-19 patients than healthy group ($p < 0.001$). Besides, TSH level in COVID-19 patients was obviously lower than non-COVID-19 patients ($p < 0.001$). Within the group of COVID-19, 61.9% (52/84) patients presented with thyroid function abnormalities and the proportion of thyroid dysfunction was higher in severe cases than mild/moderate cases (74.6 vs. 23.8%, $p < 0.001$). Patients with thyroid dysfunction tended to have longer viral nucleic acid cleaning time (14.1 ± 9.4 vs. 10.6 ± 8.3 days, $p = 0.088$). To note, thyroid dysfunction was also associated with decreased lymphocytes ($p < 0.001$) and increased CRP ($p = 0.002$). The correlation between TT3 and TSH level seemed to be positive rather than negative in the early stage, and gradually turned to be negatively related over time.

Conclusion: Thyroid function abnormalities are common in COVID-19 patients, especially in severe cases. This might be partially explained by nonthyroidal illness syndrome.

Keywords: COVID-19, thyroid function, abnormality, thyroid stimulating hormone, pathogenesis

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread worldwide and led to the declaration of Public Health Emergency of International Concern by the World Health Organization (WHO) (1, 2). As of June 14, 2020, a total of 7,844,978 cases have been confirmed worldwide, among them, 428,045 people have died of COVID-19. Patients infected with COVID-19 display mainly symptoms similar with pneumonia such as fever, fatigue, cough, shortness of breath (3, 4). And many patients have symptoms outside of the respiratory system including poor appetite, diarrhea, nausea, vomiting and palpitation (3, 4). The main management of COVID-19 infection is supportive, and acute respiratory distress syndrome (ARDS) induced respiratory failure is the leading cause of mortality (3, 5–7).

Severe and complex effects on several human organs and systems including respiratory, immune, digestive, circulatory, hepatic, renal, and hematological systems have been reported in COVID-19 patients (3–6). Whether COVID-19 affects human thyroid function remains unknown. Previously, thyroid dysfunction was identified in patients with severe acute respiratory syndrome (SARS) caused by a different strain of coronavirus (8). Therefore, COVID-19 may also influence the function of thyroid. Recently, Brancatella et al. reported the first case of subacute thyroiditis after SARS-CoV-2 infection (9). Both reports therefore indicate that the thyroid gland may also be a target organ of SARS-CoV-2. Investigating thyroid function in COVID-19 patients might help to uncover the pathogenesis of SARS-CoV-2 and provide effective information for clinical practice.

In the present study, 84 hospitalized COVID-19 patients were enrolled retrospectively from the First Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). In addition, 91 other patients with pneumonia and 807 healthy subjects were included as controls. The thyroid function in COVID-19 was compared with that in pneumonia patients, and its relationship with disease severity, viral nucleic acid cleaning time, auto-antibodies, leukocytes, inflammatory biomarkers and cytokines was also investigated. Furthermore, the nature history of thyroid function during patients' recovery were also studied to depict the development of thyroid dysfunction causing by SARS-CoV-2.

MATERIALS AND METHODS

Participants

Ninety-six hospitalized patients from the First Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China), who were definitively diagnosed as COVID-19 according to WHO interim guidance (10), participated. They represented all COVID-19 patients admitted to our hospital in the period from January 22nd to March 16th, 2020, and diagnosed by the positive of nucleic acid of SARS-CoV-2 *via* nasal pharyngeal swab or phlegm. Within two days of admission, 85 of the 96 patients were assessed for thyroid function. One patient was excluded due to pregnancy leaving a total of 84 patients enrolled. In addition, 91 non-COVID-19 pneumonia patients from the Department of Respiratory Diseases or the ICU who were infected by bacteria, fungus, and virus, and 807 healthy subjects who underwent annual routine physical checkup in our Health Management Center were included as controls. Since the examination of thyroid function is included in the panel of annual routine physical checkup, the thyroid hormone levels of these healthy subjects are available. Non-COVID-19 pneumonia cases were matched for age, gender, and disease severity with COVID-19 patients.

Clinical classification of COVID-19 was based on the Handbook of COVID-19 Prevention and Treatment (11), and cases were judged as severe if they met any of the following criteria: 1) respiratory rate over 30 breaths/min; 2) oxygen saturation ≤93% at a rest state; and 3) arterial partial pressure of oxygen (PaO2)/oxygen concentration (FiO2) ≤300 mmHg. In additions, patients with >50% lesions progression within 24 to 48 h in lung imaging were also regarded as severe cases. Patients who presented with classical respiratory tract symptoms but did not reach the criteria of "Severe" were classified as "Moderate" while those with no or mild symptoms without CT pneumonia manifestation were classified as "Mild". All cases provided informed consent. The research was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine.

Data Collection

Thyroid hormones including triiodothyronine (TT3), thyroxine (TT4), and thyroid stimulating hormone (TSH) were successfully collected in all studied groups. In COVID-19 patients, we also have 22 patients with complete records of thyroid hormones including TT3, TT4, free triiodothyronine (fT3), free thyroxine (fT4) and TSH. All COVID-19 patients had record of TT3, TT4, and TSH at admission. We also collected the clinicopathologic characteristics of age, gender, disease severity, and lab data of inflammatory biomarker (leukocytes, C-reactive protein, procalcitonin), inflammatory cytokines (interleukin-6, interleukin-10, tumor necrosis factor-α, interferon-γ), auto-antibodies (thyroglobulin antibody, thyroid peroxidase antibody), and viral nucleic acid cleaning time of patients. The viral nucleic acid cleaning time was defined as the period from diagnosed as positive to negative of nucleic acid of SARS-CoV-2 *via* nasal pharyngeal swab or phlegm, and the negative result was repeated twice with interval of one day.

Research Procedures and Statistical Analysis

Between COVID-19 patients with complete and incomplete records of thyroid hormones, no statistic difference was found in rate of thyroid dysfunction, disease severity or viral nucleic acid cleaning time (**Table S1**). Then we compared age, gender, and thyroid function among COVID-19, non-COVID-19

pneumonia patients and healthy subjects. The associations between thyroid function and disease severity, inflammatory biomarker (leukocytes, C-reactive protein, procalcitonin), inflammatory cytokines (interleukin-6, interleukin-10, tumor necrosis factor-α, interferon-γ), auto-antibodies (thyroglobulin antibody, thyroid peroxidase antibody), and viral nucleic acid cleaning time were analyzed. We also analyzed the dynamic changes of thyroid function during patients' recovery period.

Statistical analysis was conducted by SPSS (version 21.0) (SPSS Inc., Chicago, IL, USA) and R language (Version 3.6.3). Pearson chi-square test and analysis of variance (ANOVA) were used to analyze the characteristics and thyroid function among COVID-19, non-COVID-19 pneumonia patients and healthy subjects (False Discovery Rate (FDR) correction was used for multiple comparison), and to establish the factors associated with dysfunction of thyroid. Pearson correlation was performed in the correlation analysis. In addition, a polynomial regression curve was fitted between time after hospitalization and TSH or TT3 levels. For all analyses, $p < 0.05$ was regarded as statistically significant.

RESULTS

Characteristics of Participants

The COVID-19 patients had a mean age of 57.3 ± 14.5 years old and 63% (53/84) were male. They did not differ statistically from non-COVID-19 pneumonia patients or healthy subjects in age or gender (**Table 1**). Since the COVID-19 group and non-COVID-19 pneumonia group were matched on disease severity, we didn't find any difference on clinical classification between the two groups (**Table 1**). Patients having thyroid dysfunction were those with any abnormality in TT3, TT4, or TSH. Based on quantification of thyroid hormones, a total of 52 (52/84, 61.9%) COVID-19 patients have thyroid dysfunction. Among thyroid dysfunction cases, two patients had decreased TSH accompanied by slightly enhanced TT4 while the remained 50 patients presented with lower levels of TT4, TT3, or TSH (**Figure S1**).

Thyroid Dysfunction in COVID-19 Patients

When compared with healthy subjects, the levels of TT3 and TSH were significantly lower in COVID-19 patients ($p < 0.001$), while no significant difference was found in TT4 ($p = 0.391$) (**Table 1**). We then focused our research on TT3 and TSH. Next, we investigated the thyroid function alterations between COVID-19 and non-COVID-19 pneumonia patients. The level of TT3 in COVID-19 patients (1.02 ± 0.32 nmol/L) was similar with that in non-COVID-19 patients (0.92 ± 0.38 nmol/L) ($p = 0.59$) (**Table 1**). However, the TSH level was significantly lower in COVID-19 cases (0.62 ± 0.62 mIU/L vs. 1.07 ± 0.94 mIU/L, $p < 0.001$) (**Table 1**). TSH secreted by adenohypophysis normally drives the output of thyroid hormones and will be inhibited by enhanced thyroid hormones (particularly fT3) in terms of the negative feedback loop of pituitary-thyroid axis. However, our correlation analysis revealed that the levels of TT3 and TSH were positively correlated, instead of negatively related, in COVID-19 patients ($R = 0.575$, $p < 0.001$) (**Figure 1**). Correlation analysis showed that fT3 was in line with TT3 ($p < 0.001$) (**Figure S2**).

Analysis of Clinical Values for Thyroid Dysfunction in COVID-19 Patients

COVID-19 patients were further divided into a thyroid dysfunction subgroup and a normal subgroup according to TT3, TT4, and TSH levels. No obvious difference was found in age and sex between these two subgroups (**Table 2**). Abnormal thyroid function was more commonly detected in severe cases than mild/moderate cases (74.6 vs. 23.8%, $p < 0.001$) (**Table 2**). Interestingly, thyroid dysfunction tended to be associated with longer viral nucleic acid cleaning time (14.13 ± 9.39 vs. 10.56 ± 8.29 days, $p = 0.088$). Additionally, we identified increased levels of leucocytes ($p < 0.001$), neutrophils ($p < 0.001$), CRP ($p = 0.002$) and PCT ($p = 0.054$), and decreased level of lymphocytes ($p < 0.001$) in thyroid dysfunction group. Meanwhile, we did not find any significance in levels of auto-antibodies (thyroglobulin

TABLE 1 | Comparison of clinical features among COVID-19, non-COVID-19 pneumonia patients and healthy subjects.

Characteristics	COVID-19 patients (N = 84)	Non-COVID-19 pneumonia patients (N = 91)	Healthy subjects (N = 807)	*p* value	
	Mean ± SD or n (%)	Mean ± SD or n (%)	Mean ± SD or n (%)	COVID-19 vs. Non-COVID-19	COVID-19 vs. Healthy subjects
Mean age (yrs)	57.3 ± 14.5	60.1 ± 16.7	57.7 ± 13.0	0.472	0.782
Gender					
Male	53 (63.1%)	60 (65.9%)	474 (58.7%)	0.695	0.695
Female	31 (36.9%)	31 (34.1%)	333 (41.3%)		
Clinical classifications					
Mild and moderate	21 (25.0%)	24 (26.4%)	–	0.835	–
Severe and critical	63 (75.0%)	67 (73.6%)	–		–
Thyroid Function					
TT4 (nmol/L)	99.04 ± 25.96	82.28 ± 26.47	97.25 ± 17.13	***0.000***	0.391
TT3 (nmol/L)	1.02 ± 0.32	0.92 ± 0.38	1.59 ± 0.24	0.059	***0.000***
TSH (mIU/L)	0.62 ± 0.62	1.07 ± 0.94	1.55 ± 0.94	***0.001***	***0.000***

TT4, total thyroxine or tetraiodothyronine, normal range 62.68–150.84 nmol/L; TT3, total triiodothyronine normal range 0.89–2.44 nmol/L; TSH, thyroid-stimulating hormone, normal range 0.35–4.94 nmol/L.

p values were False Discovery Rate (FDR)-corrected. p value in bold was regarded as statistically significant.

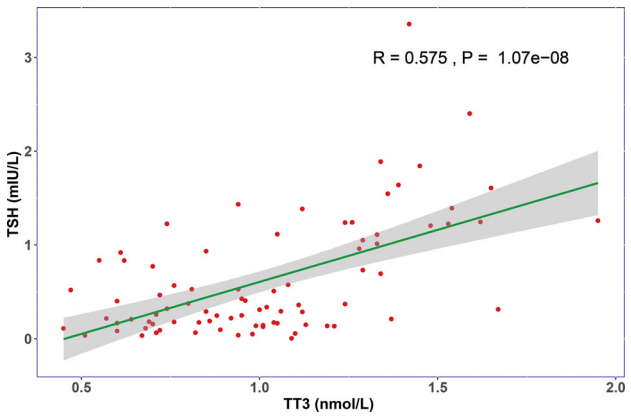

FIGURE 1 | The relationship of TT3 and TSH levels on admission. The TT3 and TSH levels in COVID-19 patients are positively correlated (R = 0.575, P < 0.001).

antibody, thyroid peroxidase antibody) and cytokines (IL-6, IL-10, TNF-α, IFN-γ) (**Table 2**).

The Natural History of Thyroid Dysfunction in COVID-19 Patients

In order to examine the natural history of thyroid dysfunction induced by SARS-CoV-2, we analyzed seven patients with records of thyroid function during their recovery period. All seven patients had lower than normal range of TSH levels on admission, and none of them was treated by glucocorticoid or thyroxine. We observed that the levels of TT3 and TSH increased gradually within 2 months after hospitalization (**Figure 2**). At Day 30, all the patients' TT3 and TSH levels recovered to normal without any thyroid hormone replacement (**Figure 2**). We further found the correlation between TT3 and TSH levels seem to shift from a positive pattern to a negative pattern overtime, indicating a recovery of the pituitary-thyroid axis. However, given limited patient number, the p-values did not reach statistical significance (**Figure 3**).

DISCUSSION

COVID-19 is an infectious illness that has caused a pandemic worldwide. As a novel type of disease with high infectivity and mortality, the pathophysiology of COVID-19 has not been fully studied. A number of studies have reported severe and complex effects of COVID-19 in several human organs and systems including respiratory, immune, digestive, circulatory, hepatic, renal, and hematological systems (6). However, whether COVID-19 affects human thyroid function remains unknown. Here, we report the influence of COVID-19 on thyroid function. We found that COVID-19 patients presented with lower levels of

TABLE 2 | Clinical characteristics and selected laboratory abnormalities of COVID-19 patients with and without thyroid dysfunction.

	Thyroid dysfunction* (N = 52) Mean ± SD or n (%)	Normal (N = 32) Mean ± SD or n (%)	P value
Gender			
Male	35 (66.0%)	18 (34.0%)	0.308
Female	17 (54.8%)	14 (45.2%)	
Clinical classifications on admission			
Mild and moderate	5 (23.8%)	16 (76.2%)	***0.000***
Severe and critical	47 (74.6%)	16 (25.4%)	
Viral nucleic acid cleaning time (days)	14.1 ± 9.4	10.6 ± 8.3	0.088
Thyroid auto-antibodies			
TPOAb (IU/ml, normal range 0–5.61)	23.95 ± 38.12	24.71 ± 57.08	0.945
TGAb (IU/ml, normal range 0–4.11)	35.05 ± 142.01	20.98 ± 47.31	0.613
Cytokines			
IL-6 (pg/ml; normal range 0–6.61)	59.27 ± 98.24	51.99 ± 95.17	0.748
IL-10 (pg/ml; normal range 0–2.31)	8.15 ± 10.92	6.11 ± 7.58	0.378
TNF-α (pg/ml; normal range 0–33.27)	69.14 ± 259.33	31.27 ± 38.23	0.438
IFN-γ (pg/ml; normal range 0–20.06)	32.92 ± 73.10	30.92 ± 47.39	0.895
Blood routine tests			
Leucocytes ($x10^9$/L; normal range 4–10)	8.71 ± 5.33	4.92 ± 1.64	***0.000***
Neutrophils ($x10^9$/L; normal range 2–7)	7.97 ± 5.30	3.40 ± 1.51	***0.000***
Lymphocytes ($x10^9$/L; normal range 0.8–4)	0.62 ± 0.33	1.09 ± 0.41	***0.000***
Platelets ($x10^9$/L; normal range 83–303)	189.13 ± 71.13	209.38 ± 75.93	0.221
Haemoglobin (g/L; normal range: male 131–172, female 113–151)	132.50 ± 17.14	137.16 ± 15.69	0.216
Infection-related biomarkers			
Procalcitonin (ng/L; normal range 0–0.5)	0.22 ± 0.46	0.06 ± 0.45	0.054
C reactive protein (mg/ml; normal range 0–8)	38.14 ± 37.01	15.60 ± 17.73	***0.002***
Blood biochemistry			
Globulin (g/L; normal range 20–40)	29.34 ± 5.85	27.82 ± 4.16	0.204

TPOAb, thyroid peroxidase antibody; TGAb, thyroglobulin antibody; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor α; IFN-γ, interferon-γ.
p value in bold was regarded as statistically significant.
**Thyroid dysfunction indicates any abnormalities in the levels of TT4, TT3, or TSH.*

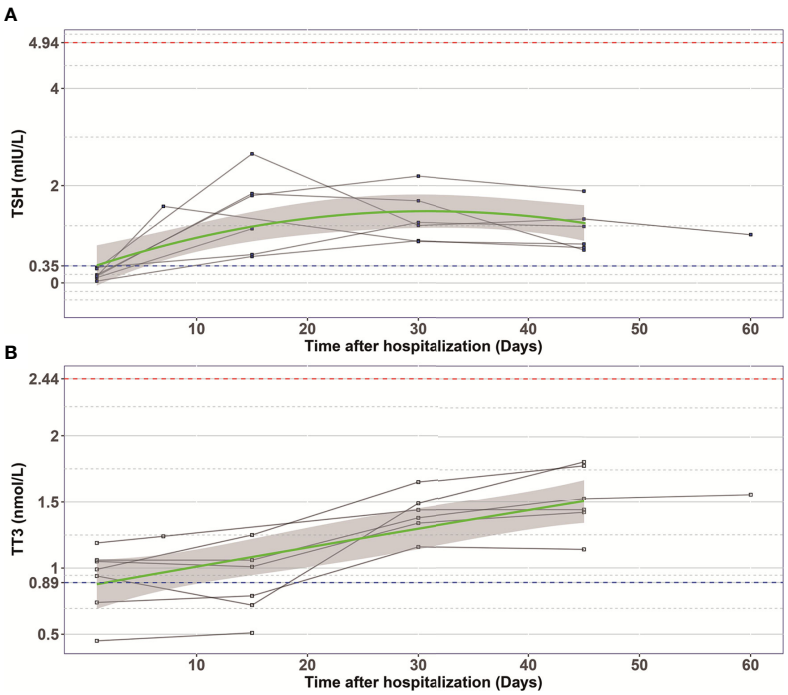

FIGURE 2 | The changes of TSH **(A)** and TT3 **(B)** levels during hospitalization in COVID-19 patients with abnormal TSH level on admission. Every polyline represents the variation trend of TSH or TT3 level of one patient. Dashed blue lines, the lower limit of normal TSH (0.35 mIU/L) and TT3 (0.89 nmol/L) value; Dashed red lines, the upper limit of normal TSH (4.94 mIU/L) and TT3 (2.44 nmol/L) value; Green curves represent the fitting of data; Grey shaded areas, depict the 95% confidence band for the fitted curve.

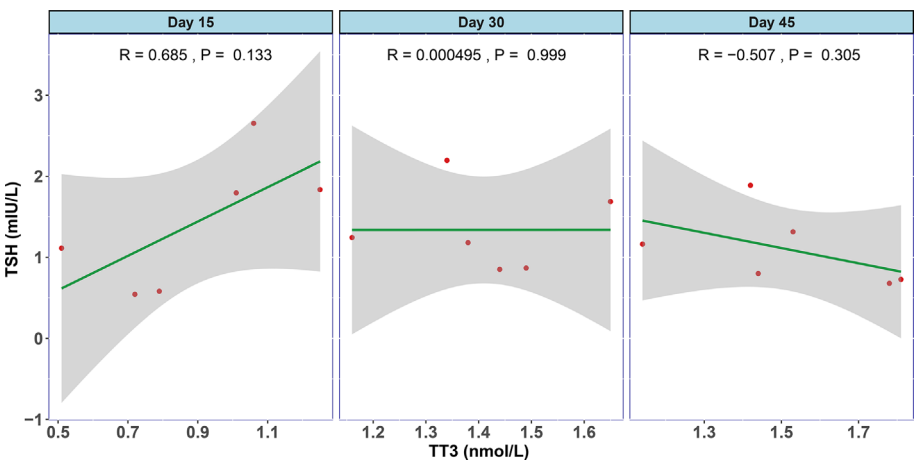

FIGURE 3 | The association between TT3 and TSH levels in different disease stages of COVID-19. The levels of TT3 and TSH tend to be positively correlated in the early stage (Day 15) and turn towards negatively correlated in Day 45, but the p values are not significant.

TT3 and TSH than healthy subjects while their TSH levels were considerably lower than non-COVID-19 pneumonia patients. We also observed that thyroid dysfunction in COVID-19 patients may recover without thyroid hormone replacement within 30 days. This seems to mimic the pattern seen in patients with non-thyroidal illness (NTI).

Nonthyroidal illness syndrome presents as abnormal thyroid function in serious diseases other than thyroid disorders, including infection, cancer, cardiovascular and gastrointestinal disease, burn, and trauma (12). It is well established that NTI is a consequence of an acute phase response to severe systemic illness or macronutrient restriction and usually presents as decreased plasma T3 level, or low

or normal T4 and TSH levels (12, 13). The phenomenon of decreased T3 and TSH in COVID-19 patients was consistent with NTI. In COVID-19 patients, a profile of cytokines, such as IL-2, IL-6, IL-7, INF-γ, and TNF-α, is associated with disease severity and mortality of patients (3, 5, 7, 14). Our results also showed that thyroid dysfunction was associated with increased inflammation biomarkers including CRP and leucocytes, indicating inflammatory reaction played an important role in thyroid dysfunction of COVID-19. Therefore, serious infection in COVID-19 is a primary cause of NTI.

Furthermore, even though the disease severity was matched, we still found the TSH level of COVID-19 patients was significantly lower than that in non-COVID-19 pneumonia patients. This suggests thyroid function abnormalities in COVID-19 patients cannot be totally explained by NTI, possibly because of the attack of SARS CoV-2 virus. The wide distribution of COVID-19 nucleic acid in respiratory tract, saliva, feces, and breastmilk indicates that direct viral attack to the target cells may be an alternative reason (15–17). Angiotensin-converting enzyme 2 (ACE2) is a receptor providing the main entry site for SARS-CoV to invade human cells, and this in turn facilitates direct damage of virus through the course of infection (18, 19). Li et al. recently reported that ACE2 was highly expressed in the thyroid (20), suggesting that the thyroid gland may be a potential target for direct attack of COVID-19. Our study showed that thyroid dysfunction tended to be associated with viral nucleic acid cleaning time, indicating virus infection and replication may account for the abnormal thyroid hormones. However, our study also showed that disease severity, which may influence the viral nucleic acid cleaning time, was associated with thyroid dysfunction, thus the true relationship of thyroid function and viral nucleic acid cleaning time need to be further studied.

In patients with SARS caused by another strain of coronavirus (8), severe pathologic injury in follicular epithelial cells with follicular distortion and collapse was found in thyroid glands (21). After investigating the endocrine cells in the pituitary gland of five SARS patients, Wei et al. found that TSH positive cells were significantly decreased (22), indicating thyroid epithelial cells, as well as endocrine cells of adenohypophysis, may be attacked and damaged by coronavirus. Thus, we speculated that COVID-19 may have similar pathogenesis as SARS, explaining why the TSH level in COVID-19 patients was significantly lower than non-COVID-19 patients. In the present study, we also noticed 7 patients, who had lower than normal levels of TSH and TT3 on admission, with normalization by Day 30. Furthermore, the malfunctional feedback between TT3 and TSH returned to work overtime, indicating a recovery of the pituitary-thyroid axis abnormalities as well. A recent case report of thyroiditis after SARS-CoV-2 infection came up by Brancatella et al. confirmed this hypothesis. That case displayed thyroid dysfunction followed by a triphasic course including thyrotoxicosis, hypothyroidism, and euthyroidism, and then recovered to normal in one month (9).

There are several limitations which might cause potential bias. The study is single centered, with limited sample size, which may lead to bias of the study. Also, the study was conducted retrospectively with little attention paid to thyroid function during treatment of COVID-19, most patients had not dynamically monitored the thyroid function. Furthermore, only 22 patients had complete thyroid function including fT3 and fT4. Also, COVID-19 patients were admitted to hospital at different disease stages with different severity, and most patients with mild symptom did not have thyroid function tests, which may also cause bias to an extent. Thus, more patients from multiple centers should be analyzed, and complete record and dynamic changes of thyroid function should be concerned and investigated prospectively in the future studies.

In conclusion, the current study demonstrated that thyroid function abnormalities were common in COVID-19 patients, especially in severe cases. The thyroid dysfunction seems to dynamically change within the course of disease and recover gradually and spontaneously. While this may be partially explained by non-thyroidal illness syndrome, it is also possible that the thyroid gland is a direct target of the SARS CoV-2 virus.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WW, XS, YD, and WF designed the study, analyzed the data and wrote the paper. JS, ZC, HZ, KX, QN, and XX collected data and performed the study. LT and YQ designed the study, supervised the whole process, and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.623792/full#supplementary-material

Supplementary Figure 1 | The distribution of thyroid hormones abnormalities in COVID-19 patient.

Supplementary Figure 2 | The respective association between fT3 and TT3 levels in COVID-19 patients on admission. Correlation analysis shows that fT3 is positively related with TT3 ($R = 0.668$, $P < 0.01$).

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REVIEW
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Physiological Role and Use of Thyroid Hormone Metabolites - Potential Utility in COVID-19 Patients

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Thyroxine and triiodothyronine (T3) are classical thyroid hormones and with relatively well-understood actions. In contrast, the physiological role of thyroid hormone metabolites, also circulating in the blood, is less well characterized. These molecules, namely, reverse triiodothyronine, 3,5-diiodothyronine, 3-iodothyronamine, tetraiodoacetic acid and triiodoacetic acid, mediate both agonistic (thyromimetic) and antagonistic actions additional to the effects of the classical thyroid hormones. Here, we provide an overview of the main factors influencing thyroid hormone action, and then go on to describe the main effects of the metabolites and their potential use in medicine. One section addresses thyroid hormone levels in corona virus disease 19 (COVID-19). It appears that i) the more potently-acting molecules T3 and triiodoacetic acid have shorter half-lives than the less potent antagonists 3-iodothyronamine and tetraiodoacetic acid; ii) reverse T3 and 3,5-diiodothyronine may serve as indicators for metabolic dysregulation and disease, and iii) Nanotetrac may be a promising candidate for treating cancer, and resmetirom and VK2809 for steatohepatitis. Further, the use of L-T3 in the treatment of severely ill COVID-19 patients is critically discussed.

Keywords: triiodothyronine, non-thyroidal illness syndrome, COVID-19, 3,5-diiodothyronine, tetraiodoacetic acid, triiodoacetic acid, thyromimetics

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INTRODUCTION

Thyroid hormones (TH) are endocrine hormones that influence nearly all cells of the human body. Deficiency and excess, hypothyroidism and hyperthyroidism, demonstrate the action of TH on fetal development, lipid and carbohydrate metabolism, growth, cardiovascular, central nervous, and reproductive systems. TH action is determined by the level of circulating hormones and their metabolites, serum binding (distribution) proteins, cellular transporters, type and amount of deiodinases (D), and expression of receptors (**Figure 1**). Treatment with endocrine hormones is usually restricted to supplementation in case of insufficient endogenous production (e.g. in autoimmune diseases) or decreased target sensitivity (e.g. insulin resistance). TH may however also be candidates to treat specific conditions, like obesity, diabetes mellitus type 2, and metabolic syndrome. TH analogues with antagonistic effects may be helpful to support recovery from illness. Biological issues like lack of selectivity of TH action and finding suitable pharmaceutical formulations pose problems for treatment. Numerous recent studies focused on the role of pre-existing morbidities

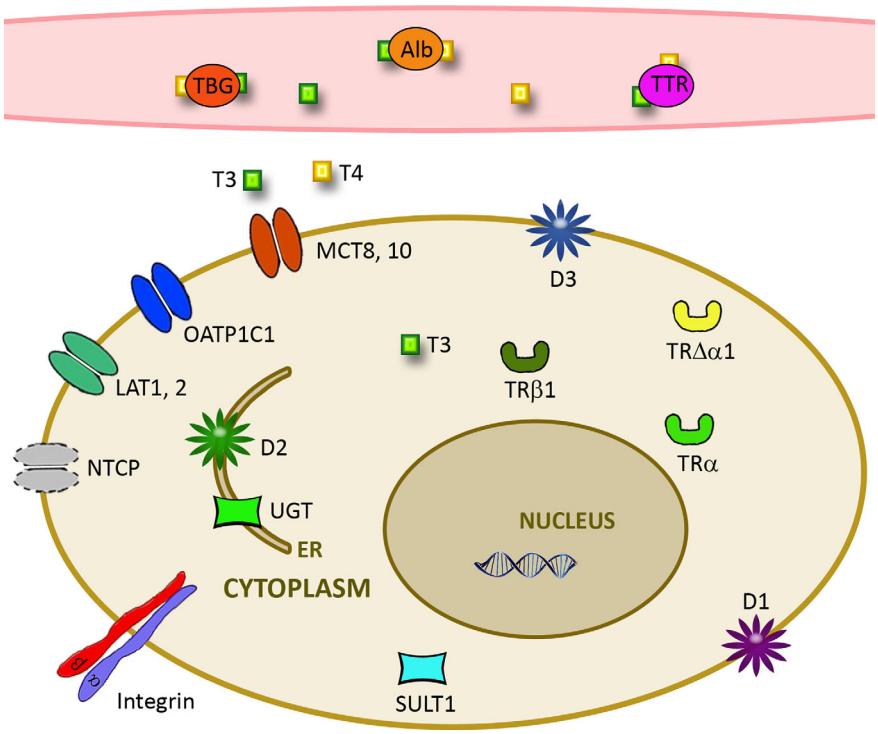

FIGURE 1 | Important parameters for cellular effects of thyroid hormones. Free TH levels are influenced by the amount of distribution proteins such as albumin (Alb), thyroxine-binding globulin (TBG), and transthyretin (TTR). Cellular uptake is determined by expression of transporters like L-amino acid transporter (LAT), monocarboxylate transporter (MCT), Na+/taurocholate cotransporting polypeptide (NTCP), and organic anion transporting polypeptide (OATP). Deiodinases D1-3 together with Sulfotransferases (SULT1) and UDP-glucuronosyltransferases (UGT) determine action on the cellular level. Cellular effects are further influenced by the level of αvβ3 integrin and truncated and nuclear receptors TRα and TRβ.

for risk of infection with Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) and severity of corona virus disease 2019 (COVID-19). Although thyroid dysfunction appears to play no important role as risk factor for contracting COVID-19 disease, alterations in TH levels were observed in hospitalized patients.

This review starts with an overview of production, transport, uptake, cellular metabolism and action of the classical representatives T3 and T4 and proceeds with a description of the biologically active TH metabolites. Although all of these aspects are covered by excellent reviews, this more practically oriented description of the different aspects influencing TH action has been included to illustrate the variety of factors that affect TH action and point out differences between the metabolites. One section is devoted to the role of TH in COVID-19. Finally, potential medical applications of TH metabolites and problems in the pharmacological formulation of these agents are addressed.

BLOOD TRANSPORT, METABOLISATION, CELLULAR UPTAKE, AND CELLULAR EFFECTS OF TH

Structurally, TH belong to the amine hormones, which are derivates of amino acids such as tyrosine, tryptophan, and histidine. They include catecholamines (epinephrine and norepinephrine), melatonin, and histamine. The molecules generally are highly water-soluble, bind to membrane receptors and act *via* G-proteins, adenylcyclase, c-AMP, and protein kinases. TH differ from the general rule in the way that they are lipophilic and cause effects *via* both nuclear and membrane receptors (1).

Proteins influencing the cellular action of classical TH are shown in **Figure 1** and described in the following sections.

Production and Blood Levels

Production of TH by the thyroid is 85 µg/day for T4 and 6.5 µg/day for T3. The majority of the estimated total amount of 30 µg T3/day is produced outside the thyroid parenchyma *via* T4 deiodination, mainly by deiodinases. TH levels show circadian rhythmicity with peak values of T4 from 8-12 am and lowest levels from 10 pm-3 am. T3 levels are highest from 7 am-1 pm and lowest from 11 pm-3 am (2). Levels are linked to those of thyroid-stimulating hormone (TSH), which precede them by around 6 h (peak at 2-4 am and nadir at 4-8 pm) (3). These data suggest a link of TH levels to metabolism and arousal. Overall, fluctuations of T4 and T3 levels in serum are not prominent, while tissue concentrations of T3 can vary dramatically (4). Regulation of TH levels through the hypophysis - pituitary gland – thyroid (HPT) axis is the main mechanism of TH

secretion. The mechanism is described in several reviews dedicated to this topic [e.g. (5, 6)] and will not be addressed here. Age and sex influence TH levels and free TH but not TSH concentrations decrease in men with age, while in women the free TH levels remain constant but TSH level increase in an age-dependent manner (7). Decreased monocarboxylate transporter (MCT)8 expression and decreased deiodinase (D)1 activity in aged livers increase TH receptor (TR)β protein and shift T3 activity from liver to kidney (8). Reports of a correlation of low fT4 and longevity lead to the hypothesis that a slower metabolism with reduced production of oxygen radicals results in reduced cell damage and longer life. Prominent changes were seen during pregnancy when T4 levels increased sharply between week 6-9 and more slowly thereafter, resulting in stable values between week 20-27 of gestation. Increases in TH during pregnancy are accompanied by increase of thyroxine binding globulin (TBG) levels in blood due to longer half-life of the protein. fT4 and fT3 levels, the important parameters for TH action in the tissue, remain in the normal range (9). TH are important for fetal development and more detailed information on pregnancy-related changes in TH levels is available elsewhere [e.g. (10, 11)].

Transport in Blood by TH Distributor Proteins

TH are transported in blood bound to transport proteins. For T4 the binding is 75% to TBG, 20% to transthyretin (TTR, prealbumin), and 5% to serum albumin. Apolipoprotein B and apolipoprotein A1-containing lipoproteins, contribute with 3% of T4 and 6% of T3 to TH transport. In the rare case of familial dysalbuminemic hyperthyroxinemia and hypertriiodothyroninemia higher binding of T4 and T3 may occur (12). The affected individuals have higher T4 and T3 levels but do not have any symptoms because fT4 and fT4 are in the normal range. Only a small fraction of 0.03% of T4 and 0.3% of T3 is circulating in free form in the blood (13). The main source of all distributor proteins is the liver but choroid plexus and retinal pigment epithelium are additional sources for TTR. Albumin and TTR are produced in syncytiotrophoblast cells of the placenta during pregnancy (14). Affinity of T4 to distributor proteins is higher than that of T3 and binding affinity for T4 and T3 increases in the order albumin<TTR<TBG (15). In contrast to what was initially hypothesized, limited solubility of T4 is not the reason for the need for distributor protein binding because solubility of T4 is higher than fT4 levels. The difference is impressive as the maximum solubility of T4 at pH 7.4 is 2.3 μM, while concentration of free T4 in human blood is only 24 pM. According to the current hypothesis, binding to distributor proteins serves as a buffer for T4 and provides a more even tissue distribution of the TH. Of the three most important proteins albumin, TTR, and TBG, TTR is responsible for most of the delivery because TBG binds T4 too tightly to allow release. Binding affinity of TH to albumin, on the other hand, is too weak and the protein is not efficient for distribution. The importance of TTR is corroborated by the fact that its absence of TTR is the only distributor protein pathology not compatible with human life, while absence of albumin and TBG deficiency does not create major symptoms. TTR is not only a binding protein for TH, it has many other biological functions (16). Only in mammals, TTR has higher affinity for T4 than for T3. Although intracellular T3 cannot be determined by blood analysis, measurements of T4, T3, and TSH are the commonly used parameters to assess thyroid function (https://www.thyroid.org/thyroid-function-tests/).

Membrane Transporters

Entry into cells can be passive based on the lipophilicity of the molecules but also by transporters. The amphipathic nature due to the lipophilic aromatic rings and hydrophilic amino acid side chains may be the reason why thyroid hormones are suboptimal candidates for passive diffusion. The most important transporters of TH are MCT8, MCT10 and organic anion transporting polypeptide (OATP) 1C1. Further, L-amino acid transporters (LAT) 1 and 2, multidrug resistance-associated proteins (MDP), Na+/taurocholate co-transporting peptide (NTCP) 1 and fatty acid translocase (FAT) are involved (17). MCT10 transports T3 better than MCT8, while for T4 transport MCT8 is better than MCT10 (18). MCTs are the only exclusive TH transporters and are abundantly expressed in liver, intestine, kidney, and placenta. Both can transport TH into and out of cells but the relevance of export is unclear. OATP1C1 preferentially transports T4 and rT3 over T3, and is expressed mainly in the brain (4). LAT1 transports several TH metabolites in the order 3,3'-T2>T3~rT3>T4. The presence of multiple membrane transporters appears particularly relevant in Allan-Herndon-Dudley Syndrome (AHDS), where decreased numbers of oligodendrocytes in the brain correlates with TH due to mutation of the MCT8 and contributes to the pathology (19). As OATP1C1 is not highly expressed in the pre- and perinatal human brain, MCT10, LAT1 and LAT2 are the most likely candidates for the basal TH supply in these patients.

Importers and exporters in the nuclear membrane regulate the transport of TR from cytoplasm to nucleus (20). Mutation of the transporter proteins of TRα (importins α1, β1, and 7) and of TRβ (importin α1/β1 heterodimer) may play a role in TH resistance. Exportins 4, 5, 7, and calreticulin/exportin 1 complex are mainly involved in export from the nucleus.

Deiodinases

There are 3 enzymes acting as deiodinases with different specificities, cellular localization, and organ distribution. The important effect of TH regulation by deiodinases on cellular levels is best illustrated by the reciprocal actions of D2 and D3 regarding energy expenditure (21). c-AMP induction of D2 expression in brown adipose tissue upon cold exposure increases energy expenditure, while hypoxia-inducible factor (HIF)-1α–dependent induction of D3 in myocardium and brain ischemia decreases expenditure. If the energy sparing is exaggerated, activation of D3 can lead to non-thyroidal illness syndrome (NTIS), also termed euthyroid sick syndrome. NTIS is characterized by elevated rT3 and low total T3 and fT3. TSH and fT4 can be normal but mortality increases steeply when both

parameters, as in critically ill patients, decrease [(22), see also in the section *Reverse rT3*]. The condition is viewed as a protection mechanism to reduce energy expenditure that does not require treatment, while other groups recommend treatment with thyrotropin-releasing hormone (TRH), TSH, or T3 and T4.

All three deiodinases are selenoproteins but have different specificities. D1 is localized at the plasma membrane and exists in two forms, and can increase and decrease T3 levels. D2 localized in the endoplasmic reticulum provides T3 for receptor activation in the tissue, and D3 at the plasma membrane metabolizes T4 to rT3 and degrades T3 to T2 (23). Due to the fast binding to ubiquitin with subsequent proteasomal degradation, D2 has a much shorter half-life (30 min) than D1 and D3 (12 h). D1 catalyses 5'-deiodination (D1_1) as outer ring deiodinase (ORD) and converts T4 to T3 and rT3 to 3',5'-T2 (24). Substrate affinity decreases in the order rT3>>T4≈T3. The D1 form for 5-deiodination (D1_2), also known as inner ring deiodinase (IRD), converts T4 to rT3 and T3 to 3,3'-T2. D1_1 has a greater affinity to T3 than to T4 and inactivation of T3 prevails over conversion of T4 to rT3. The high affinity to rT3 and sulfated thyrothyronines led to the assumption that the main function of D1 is to recover iodide from inactive compounds. In addition to Se deficiency, illness, specific drugs, cadmium, mercury or lead intoxication, and stress shift the balance to rT3 generation. D1 enzymes are abundant in liver, kidney and skeletal muscle (25). D2 catalyses the same reaction as D1_1 and is located in brain, pituitary gland, and brown adipose tissue. The affinity to T4 is greater than for rT3. A variety of signals, bile acids, flavonols, chemical chaperones, insulin, and peroxisome proliferator activated receptor (PPAR)-γ, induce D2 expression, while endoplasmic reticulum stress and liver X receptor/retinoid X receptor (LXR/RXR) activation dampen the D2 pathway. D3, with substrate affinity T4>T3 and function for T3 degradation, is similar in activity to D1_2 and found in the central nervous system. From its localization at the plasma membrane D3 may migrate to the nuclear membrane in ischemia, where it inactivates T4 and T3 (26).

Effects of polymorphisms of deiodinases on thyroid metabolism have been studied, but with controversial results. Carriers of the D_{1b}-G/T(rs12095080) allele in D1 had higher T3 and T3/rT3 ratio, while carriers of D_{1a}-G/T(rs11206244) allele had increases in fT4 and rT3 and decreases in T3 and T3/rT3 ratio. The affected individuals, however, presented no symptoms of thyroid dysfunction (4). An indication of interference with thyroid function has been reported in carriers of Thr92Ala polymorphism in D2, which need higher L-T4 concentrations to achieve euthyroidism and show delayed T3 secretion in response to TSH.

Regulation of TH on the cellular level includes conjugation. Sulfotransferases are located in the cytoplasm particularly of liver, kidney, and brain. Several members of the sulfotransferase (SULT) 1 family, namely SULT1A1, SULT1A2, SULT1A3, SULT1B1, and SULT1C2 catalyze conjugation with velocity 3,3'-T2> T3~ rT3> T4 (24). Glucuronidation of TH is performed by members of the Uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferase (UGT) 1A family, located in the endoplasmic reticulum. Tetrac and Triac are more rapidly glucuronidated in the liver than T4 and T3.

Mechanisms of Cellular Action by TH

In some mammalian species, a cytosolic T3-binding μ-crystallin was identified. According to rodent studies, ketimine reductase μ-crystallin, which is the human homologue, may mediate the intranuclear transport of T3, but allosteric regulation of enzymatic activity of the reductase by T3 also appears possible (27). Non-genomic regulation was discovered later than genomic regulation. Several reviews are available for detailed information on the complex and variable regulation of TH action [e.g. (28–30)]. Diversity exists on the receptor level because due to multiple splicing, multiple nuclear TRs, designated α1-α3 and β1-β3, and the truncated forms Δα1, Δβ2, and Δβ3 can be generated (31). The α2 and α3 isoforms and all truncated TRs are non-T3 binding proteins and function as antagonists of TH signaling. As a general rule, TRβ1 is mainly expressed in tissues linked to regulation of metabolism, while TRα1 is the major isoform in the heart. TRβ1 is highly expressed in liver, TRβ2 is particularly relevant for brain, retina, and inner ear, and TRβ3 is expressed in rodent tissues but not in humans (32). Action of nuclear receptors on transcription can be briefly described as follows (**Figure 2**). TRs form homo- and heterodimers with retinoid X receptors (RXR). Unliganded TRs bind to TH response element sequences in T3 target genes and mediate transcriptional repression. Co-repressor proteins (nuclear receptor co-repressor protein (NCoR)/homolog silencing mediator of retinoid) and TR are recruited to the RXR-TR heterodimer in the absence of T3 and inhibit target gene expression. The proteins contain three repressor domains and two receptor interacting domains (28). The receptor domains form a large repressor complex by interaction with different types of histone deacetylases (HDAC 1, 3) and transducin (beta)-like 1 (TBL-1). T3 binding causes a change in conformation with displacement of co-repressors and dissociation of the complex. Recruitment of co-activators results in interaction with RXR-TR heterodimer and transcription. Steroid receptor coactivator-1 (SRC-1) interacts with cyclic AMP (cAMP) response element binding protein (CREB) response element-binding protein (CBP) and Adenovirus early region 1A binding protein p300 (p300) through its activation domain 1 (AD1), with histone acetyltransferases through its AD2 and with ATP-dependent chromatin remodeling complex through its AD3 (33). The formation of such a coactivator complex results in chromatin remodeling and bridges the hormone-activated receptors with the general transcription machinery for transcriptional activation of their specific target genes. More information on the mechanism is available elsewhere [e.g. (28, 34)].

Non-genomic action of TH is initiated at the plasma membrane, in the cytoplasm (TRα or TRβ) or in intracellular organelles (e.g. mitochondria) (30) (**Figure 2**). Integrin αvβ3 represents one of the 24 integrin family members with an Arg-Gly-Asp (RGD) domain, which mediate interaction with extracellular matrix proteins, such as osteopontin, fibronectin,

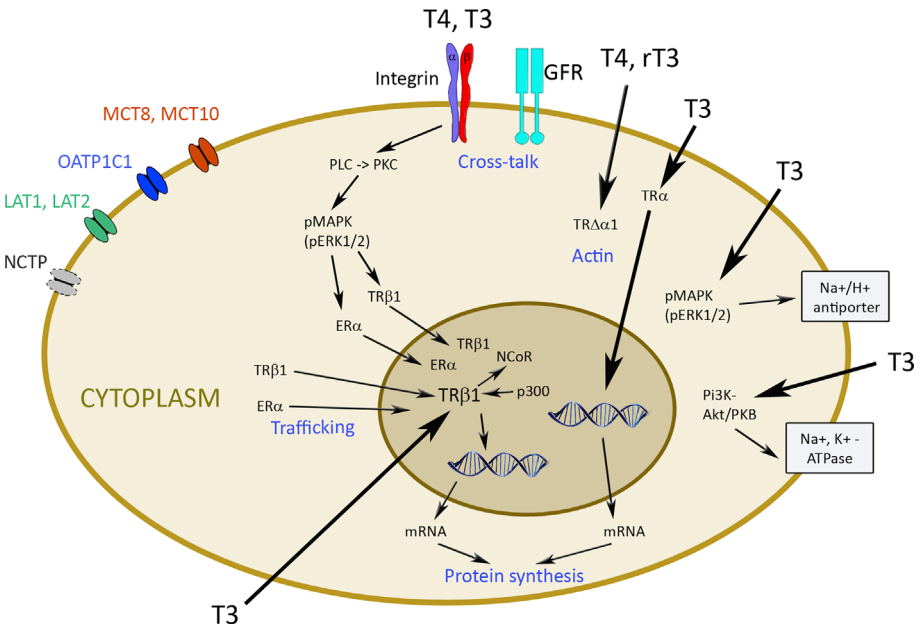

FIGURE 2 | Transporters and mechanisms of genomic and non-genomic actions of thyroid hormones. TH enters cells by diffusion or *via* transporters like monocarboxylate transporter MCT8 and MCT10, organic anion transporting polypeptide (OATP)1C1, L-amino acid transporter (LAT) 1 and 2, and Na+/taurocholate cotransporting polypeptide (NCTP). Hormones inside the cell can bind to thyroid hormone transporter (TR) α and β1 and induce genomic changes *via* transcription. Nuclear receptor co-repressor protein (NCoR) and Adenovirus early region 1A binding protein p300 (p300) are involved in this process. Non-genomic regulation occurs by binding to integrin αvβ3 with activation of phospholipase C (PLC), protein kinase C (PKC), phosphorylated mitogen-activated protein kinase (pMAPK), and extracellular signal-regulated kinase (ERK) to induce trafficking of the phosphorylated TRβ1 and estrogen receptor (ER) into the nucleus. Cross-talk of αvβ3 integrin with growth factor receptors (GFR) is particularly important for cancer cells. T4 and rT3 can also act *via* TRΔα1 on actin, and T3 through pMAPK/ERK1/2 on Na^+/H^+ antiporter and *via* phosphatidyl-inositol 3 kinase (PI3K) and Akt/protein kinase B (PKB) on Na^+/K^+-ATPase.

and vitronectin. The binding site of TH is distinct from this site and consists of the two domains S1 and S2. Binding to S1 is specific to T3 and S2 accepts T4 and, with lower affinity, T3 (28).

There is cross-talk between αvβ3 integrin and growth factor receptors [e.g. vascular endothelial growth factor (VEGF), endothelial growth factor (EGF), transforming growth factor β (TGFβ), insulin growth factor (IGF) 1) and basic fibroblast growth factor (bFGF)], which can be modulated by activation of extracellular signal-regulated kinase (ERK)1/2 (35, 36). Actions of αvβ3 integrin are mediated by two binding domains (28). Binding of TH to the S2 domain activates phospholipase C (PLC) and protein kinase Cα (PKCα), which leads to phosphorylation of TRβ, estrogen receptor (ER), signal transducer and activator of transcription 1 (STAT1), and p35. The phosphorylated TRβ1 translocates into the nucleus. Upon binding of T3 to the S1 domain, phosphatidyl-inositol 3 kinase (PI3K)/Akt/protein kinase B (PKB) pathway *via* Src kinase is regulated and Src kinase activation induces TRα1 translocation from the cytoplasm to the nucleus. The non-genomic regulation ensures independent function of the cytoplasmic and nuclear receptors (37, 38). TRΔα1 is involved in the maintenance of the actin cytoskeleton by T4 and rT3, and the p30 TRα1 peptide at the plasma membrane regulates T3-induced proliferation of non-malignant cells *via* ERK1/2 and Akt pathways. Binding of T3 in the cytoplasm to TRβ1 activates the PI3K/Akt/mTOR pathway, which then activates Na^+/K^+-ATPase. The T3-TRβ1 complex stimulates the Na^+/H^+ exchanger at the plasma membrane *via* MAPK/ERK 1/2 (28).

Due to the numerous interactions between non-genomic and classical TR signaling, Flamant et al. suggested a new classification system with four types of TH signaling based on involvement of TR and extent of interaction with DNA (39). Type I describes TR-dependent signaling with direct binding to DNA and can occur as monomer, homodimer or heterodimer (usually with retinoid X receptor, RXR) binding to response elements, TR binding to enhancer elements, and as a heterodimer with other partners, such as retinoic acid receptors. Type II is TR-dependent signaling with indirect DNA binding, where TRs interact with a number of chromatin-associated proteins. In type III, signaling is TR-dependent but no binding to DNA occurs. Interaction of cytoplasmic TR with kinases associated to the plasma membrane is the mechanism, and p30 protein translated from an internal codon of TRα1 is one example. In type IV signaling of TH no TR are involved and binding of TH to αvβ3 integrin regulates kinases, influences actin polymerization, and allosterically regulates metabolic enzymes.

RELATIONSHIP OF SARS-COV-2 AND THYROID

Influence of COVID-19 on TH Levels

Based on a report of co-morbidities in COVID-19 patients, thyroid dysfunction is not a predisposing parameter for the disease. About 0.5% of the 7,162 patients reported had thyroid disease as a co-morbidity (40), which corresponds to a lower incidence than in the general adult population of the United States of America. Another study reported incidence similar to the general population (41). Both studies concurred that hypothyroidism is not a risk factor associated with worse outcome in COVID-19-positive patients (42) but thyroid disorders were linked to higher mortality of COVID-19 infected patients (43).

A variable percentage of COVID-19 patients was affected by thyroid dysregulation and different pattern of abnormalities were seen (**Table 1**). It has to be taken into account that not all studies used the same control groups and that the focus of the studies were different. While in one set of studies COVID-19 specific effects on the TH levels should be studied, in others the role of TH levels in the severity of the disease should be identified. In three studies, only a minority of COVID-19 patients presented with abnormal thyroid values (44, 45, 47). Inflammation markers (CRP, erythrocyte sedimentation, lactate dehydrogenase levels) correlated with reduced TSH and fT3 levels. Further, patients with low T3 had a higher risk for deterioration (44). Decreased TSH and T3 levels, isolated decreased TSH levels, decreased TSH and increased T4 levels, and decreased TSH and fT4 were reported. In the studies, where more than one third of COVID-19 patients had abnormal TH levels, decreased TSH and T3 was seen more often than decreased TSH in combination with increased T4 levels. In the group of the severely ill COVID-19 patients TSH and total T3 were reduced and the extent of T3 reduction correlated with the severity of the disease (48). It was further reported that decreases in T3 levels were more pronounced in the COVID-19 patients than in other critically ill patients. Lower TSH and total T3 levels in COVID-19 patients compared to patients hospitalized for non-COVID-19 pneumonia were also reported in two other studies (49, 51). Decreases in total T3 and TSH levels were correlated to increases in pro-inflammatory cytokines, such as interleukin (IL)-2, IL-6, IL-7, interferon (INF)-γ, and tumor necrosis factor (TNF)-α. T3 predicted all-cause mortality and IL-6 and C-reactive protein (CRP) were negatively correlated with fT3 levels. TSH and fT3 levels were also significantly decreased in deceased patients compared to recovered patients (52). Of note, differences in T4 levels were not statistically different in these groups. In the study published by Malik et al., by contrast, severely ill COVID-19 patients had increased T4, TSH, IL-6 and procalcitonin levels (50). The preprint evaluated a patient collective, where 85.7% of the controls (hospitalized non-COVID-19 patients) showed abnormal TH levels and where the incidence of hyperthyroidism with 4.5% was considerably higher than in Europe (0.7%) and the United States of America (0.5%). It may be assumed that the reported results from this single center study cannot be generalized.

Different mechanisms have been postulated to explain the observed alterations of TH levels in COVID-19 patients. Elevated IL-6 levels are typical for severe COVID-19 infections, while the concurrent increase in anti-inflammatory IL-10 is interpreted as response to overwhelming systemic inflammation (53). This interleukin has also be identified as key cytokine in NTIS and was identified as strong independent predictors of mortality in NTIS (54). FT3 levels in NTIS patients were negatively correlated to IL-10 levels but not associated with IL-6 levels. There is reason to assume that severely ill COVID-19 patients suffer from NTIS because IL-6 levels, which are generally increased in this condition, were identified as prognostic parameter for severity of COVID-19 infection (55).

The thyroid may, however, also be a target of the virus because expression of angiotensinogen converting enzyme 2

TABLE 1 | Overview of studies on prevalence and relevance of thyroid dysregulation in COVID-19.

Number of COVID-19 patients in the study	Patients with abnormal TH values (%)	Focus of the study	Type of alterations in TH levels; control groups	Reference
191	13.1	Specificity of TH level changes for COVID-19	TSH decreased (5.2%); fT3 decreased (5.2%); COVID-19 *vs* non-COVID-19 hospitalized patients	(44)
334	13.7	Specificity of TH level changes for COVID-19	TSH and fT4 decreased; COVID-19 *vs* non-COVID-19 hospitalized patients	(45)
52	21	Role of TH levels for the severity of COVID-19	TSH decreased and T4 increased (15%); critically ill COVID-19 *vs* critically ill non-COVID-19 patients	(46)
60	35	Role of TH levels for the severity of COVID-19	TSH and T3 decreased (18.3%); TSH decreased and T4 increased (9.1%); critically ill *vs* non-severe COVID-19	(47)
50	58	Specificity of TH level changes for COVID-19	TSH and total T3 decreased and T4 normal; COVID-19 *vs* non-COVID-19 pneumonia and healthy individuals	(48)
84	61.9	Specificity of TH level changes for COVID-19	TSH and total T3 decreased; COVID-19 *vs* non-COVID-19 pneumonia and healthy individuals	(49)
48	75	Specificity of TH level changes for COVID-19	TSH and total T4 increased, total T3 decreased; COVID-19 pneumonia *vs* non-COVID-19 pneumonia	(50)
100	na	Role of TH levels for the severity of COVID-19	TSH and fT3 decreased; critically ill *vs* to non-severe COVID-19	(51)
113	na	Role of TH levels for the severity of COVID-19	TSH and fT3 decreased: deceased COVID-19 *vs* survivors	(52)

(ACE2) and the transmembrane protease serine 2 (TMPS2) is high (56). To compensate the lack of information about biological effects of SARS-CoV-2, data obtained from the closest related member of this virus family, SARS-CoV, are often used for estimating SARS-CoV-2 effects. Although no virus RNA was found in thyroid tissue, data from five autopsies of SARS-CoV patients showed apoptosis in follicular and interfollicular cells (57). This suggests that destructive thyroiditis may occur in COVID-19 patients. Subacute thyroiditis (SAT) can be caused by viral infections and manifests itself by thyrotoxicosis, followed by hypothyroidism and return to the euthyroid condition (58). The classical form is characterized by painful swelling of the thyroid and this was confirmed in few case reports of COVID-19 patients. Atypical thyroiditis, which does not manifest with pain and swelling, was seen in 15% of COVID-19 patients and could also explain an increase in T4 (46). To reveal a potential effect of the SARS-CoV-2 virus on the thyroid, thyrotoxicosis was evaluated in patients admitted to the high intensive care unit (HICU) in 2019 compared to 2020. In 2020, 15% of the patients had thyrotoxicity compared to 1% in 2019. By contrast, the rate of pre-existing thyroid disorders was lower in 2020 than in 2019, making thyroid disorders as risk factor for severe COVID-19 unlikely. Elevated T4 levels were seen in COVID-19 patients, but not the elevated T3 levels typically seen in viral SAT (46).

In addition to a direct attack on the thyrocytes, the virus may trigger destruction of the thyroid indirectly *via* cytokines (59). The authors observed thyrotoxicosis in 20% of COVID-19 patients admitted to the hospital. These patients presented with low TSH, increased T4 and normal fT3 levels in combination with high IL-6 levels. As has been shown in cancer patients treated with TNF-α and healthy individual after IV injection with IL-6, pro-inflammatory cytokines decrease T3 levels (60). Cellular studies indicated action on various steps of T3 synthesis, TNF-α further decreased synthesis of TSH. Changes of TH levels induced by the cytokines were similar to the pattern seen in NTIS.

Injury of TSH-producing cells of the pituitary gland as reason for the decreased TSH levels is also suspected (48). This theory is supported by the observation that number and intensity of the staining with anti-TSH antibody was decreased in pituitary glands of deceased COVID-19 patients. Decreased TSH levels may result from direct virus attack on TSH-producing cells in the pituitary gland or by action of cytokines (49).

Further explanation for the abnormal TH levels would be treatment with glucocorticoids (48). Glucocorticoids over a wide range of concentrations decrease TSH levels and inhibit the conversion of T4 to T3, while stimulating the conversion of T4 to rT3 (61). The induced pattern resembles NTIS. Since about 50% of COVID-19 patients are treated with glucocorticoids, this effect needs to be taken into account (60).

In summary, NTIS, elevated IL-6 and TNF-α levels, glucocorticoid treatment and hypophysitis may cause a similar pattern of decreased TSH levels, slightly reduced T4 and T3 levels. SAT and atypical thyroiditis, by contrast, would be characterized by transiently elevated T4 and T3 levels. After recovery normalization of TH levels without treatment has been reported for infections with the SARS-CoV and SARS-CoV-2, which occurs after both thyroiditis (SAT or atypical) and NTIS (50, 59, 62). The rate of hypothyroidism in 7% of patients after infection with SARS-CoV is in the same order as for SAT (62, 63). Indication of rT3 levels may help in differential diagnosis but this parameter is not routinely being determined.

It should also be mentioned that heparin, a prophylactic anticoagulation for hospitalized COVID-19 patients in hospital, can interfere with the measurement of free TH levels (60). The activity of lipoprotein lipase in blood samples is increased by heparin and non-esterized fatty acids are generated during sample storage. These acids displace T3 and T4 from their binding proteins and cause artificially high levels of the free hormones.

Common Targets of SARS-CoV-2 and TH

Effects of SARS-CoV-2

Primary targets of SARS-CoV-2 are pneumocytes, immune cells, and vascular endothelial cells. Although most patients with COVID-19 manifest fever and respiratory tract symptoms, SARS-CoV-2 infection may also cause extra-respiratory symptoms, including cardiac, gastrointestinal, hepatic, renal, neurological, olfactory, gustatory, ocular, cutaneous and haematological system. Independent from the primary manifestation, death is generally associated with elevated levels of cytokines, interleukin IL-6, IL-1, and tumor necrosis factor (TNF-α), and COVID-19-associated coagulopathy (CAC) (64). Thrombotic complications were seen in one third of patients infected with SARS-CoV-2. Incidence of venous thromboembolism in COVID-19 patients was high, affecting 69% of patients admitted in the Intense Care Unit (ICU), and much higher than in other patients with acute respiratory distress syndrome (ARDS) (53). It is proposed that in the early phase of the disease, hypercoagulation predominates, and anticoagulation e.g. by heparin may be helpful. CAC presents specific differences to common coagulopathies like sepsis-induced coagulopathy and disseminated intravascular coagulation. Typical features of CAC are increased D-dimer levels, elevated inflammatory cytokines but minor changes in prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count (65). D-dimer elevation was the most common finding and associated with severity and prognosis (66). Incidence of thrombocytopenia was seen only in 11% of SARS-CoV-2 infections and lower than for SARS-CoV infections (53). Blood levels of (vWF) and factors of the complement system are also increased. Increased release of van Willebrand factor (vWF) and FVIII, elevated expression of P-selectin by endothelial cells and damage of the glycocalyx of the endothelial cells has been reported in COVID-19 patients. These findings suggest endothelial dysfunction due to infection of the cells with the virus. Endothelial dysfunction affects regulation of vascular tonus, endothelial permeability, cell adhesion, and anticoagulation. The release of nitric oxide is particularly important for prevention of leukocyte and platelet adhesion, inflammation, migration of immune cells, smooth muscle cell proliferation and suppression of apoptosis.

Effects of TH

Circulating TH levels may improve or worsen the clinical situation based on their action on clotting, endothelial function and immune system. It has been reported that manifest hypothyroidism was linked to bleeding and hyperthyroidism to venous thromboembolism and that fT4 levels were associated with increased FVII, fibrinogen, and vWF levels (67). It is hypothesized that T4 acted by increased transcription of coagulation proteins by activation of TRβ. On the other hand correlated fT3 levels with FIX activity, but neither fT4, fT3 nor TSH with fibrinogen, antithrombin III (ATIII), tissue plasminogen activator (t-PA), plasminogen activator inhibitor 1 (PAI-1) or vWF (68). The association of hyperthyroidism with hypercoagulable states and moderate-to-severe hypothyroidism with hypocoagulable states appear to be caused by the respective fT4 levels and not associated with fT3 levels (69). Blood clots formed in hyperthyroid individuals showed a much denser fibrin network and were more resistant to fibrinolysis (70).

Increased rT3 levels found in NTIS, on the other hand, may promote hypocoagulable states in the patients because rT3 inhibits collagen-induced platelet aggregation. T4, on the other hand, promotes platelet aggregation and degranulation, and T3 appears to cause no effects in platelets (71). The lack of action of T3 on platelets can be explained by the fact that platelets are anucleate and do not have nuclear receptor proteins. Expression of αvβ3 integrin on platelets is lower than that of other integrins, and T4, in addition to αvβ3 integrin binding, may support pathologic platelet aggregation by regulation of CX3CL1 (Fractalkine) (71, 72). Fractalkine induces platelet adherence to collagen.

TH can further increase the interaction of platelets and endothelium through the αvβ3 integrin-adjacent receptor VEGF, increasing angiogenesis (70). Potential mechanisms include increased amount of CD31 (PECAM-1) or increased αvβ3 integrin/PECAM-1 binding. Oral addition of L-T4 to euthyroid individuals resulted in elevated expression of several coagulation proteins. TH status appears to play a role for the development of atherosclerosis, and subclinical hypothyroidism was found to be correlated with endothelial dysfunction, where increased TSH levels were hypothesized as mechanism of action (73). Application of L-T4 to women with subclinical hypothyroidism did not reduce intima-media thickness (74). L-T3, on the other hand, induced expression of endothelial nitric oxide synthase (eNOS) in human umbilical vein endothelial cells in the presence of IL-1β and acted vasoprotective in hypertensive rats by reducing ROS levels (75, 76). The proposed mechanism here is T3 binding to integrin αvβ3 followed by PI3K/Akt signaling and increased eNOS production (77).

The importance of T3 and T4 levels and the T3/T4 ratio in the euthyroid condition for the immune system has been studied by Hodkinson et al. (78). Higher T3 levels were associated with higher complement levels, increased phagocytic activity of monocytes, elevated natural killer (NK) cell counts, higher percentage expression of IL-6, and higher monocyte counts. Higher T4 levels were correlated to higher complement C3 and C4, C-reactive protein concentrations, neutrophil counts, and percentage expression of T memory cells (78). In combination with other studies, the following differences between T3 and T4 were identified. T3 in physiological concentrations increased NK cell activity and interferon (IFN)-γ responses on NK activity. Further, T3 stimulated maturation, functional activation, viability, and antigen cross presentation-allostimulatory capacity boosting antigen-specific cytotoxic T cell responses of dendritic cells (DCs) (79). T4 was unable to induce effects in DCs because only T3 was taken up by the cells. Similarly, NK cell activity was not increased, when T4 was applied to isolated lymphocytes. T4, 3,5-T2, and T3 increased respiratory burst activity, reactive oxygen species (ROS) levels, myeloperoxidase and NADPH oxidase activity of neutrophilic granulocytes, as well as ROS levels and phagocytosis of macrophages. Bacterial killing and pro-inflammatory response may be increased or decreased. In addition, T4 inhibited secretion of migration inhibitory factor (MIF) by macrophages, while no changes in IL-6 and TNF-α levels were detected. T4 increases expression of VEGF, ICAM-1, E-selectin, IL-6, and TNF-α in human umbilical vein endothelial cells (HUVEC) (80). It is suggested that inhibition of IL-6 signaling induced by T3 has potent regulatory functions during infection and inflammation, and decreased intracellular concentrations of T3 resulted in impaired polarization of macrophages into pro-inflammatory M1 type (81, 82). T3 stimulates immune reactions *via* action on lymphocytes and monocytes and supraphysiological levels of T3 decreased replication of vesicular stomatitis virus (83) and supplementation with T3 increased the number of resident (potentially beneficial) peritoneal macrophages in mice with endotoxemia (84). TRβ1 was identified as the major player mediating T3 effects on macrophages. Decreased T3 levels in NTIS most likely impair immune cell function, particularly action of the specific immune system leading to insufficient protection against pathogens, e.g. viruses. Predictive data on the reaction of the human immune system *in vivo* are difficult to obtain because evaluation *in vitro* cannot represent its complexity and species-specificity of the immune system limits the value of animal studies.

BIOLOGICAL EFFECTS OF METABOLISATION PRODUCTS OF TH

Detection of Metabolisation Products

In addition to T4 and T3, various TH metabolites circulate in blood, some of which mediate biological effects. Exact levels are not known due to cross-reactivity of TH metabolites in conventional immunoassays, and it is also clear that metabolites found in plasma do not reflect tissue concentrations (85). Uncertainties of exact levels are not restricted to TH metabolites but exist also for T3, fT4 and fT3 levels. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the reference method for exact TH quantification but shows a general trend for overestimation, particularly of free TH (86). Based on LC-MS/MS data, 48% percent of patients were classified as hypothyroid compared to 11% by immunoassay. In another study, in a cohort of 40 patients diagnosed with subclinical hypothyroidism (normal fT4 and increased TSH levels in conventional immunoassay), 65% had fT4 and fT3 levels below the reference level. A major reason

for the better performance of LC-MS/MS in the determination of fT4 and fT3 is the removal of TH binding proteins during sample preparation.

Important TH metabolites that circulate in blood include the iodinated derivates of the phenolic amino acid thyronine similar to T4 and T3, namely reverse T3 (rT3), 3,3'-diiodothyronine (3,3'-T2), and 3,5 – diiodothyronine (3,5-T2), but also tetraiodoacetic acid (Tetrac), triiodoacetic acid (Triac), 3-iodothyronamine (3-T1AM), thyronamine (T0AM), and the conjugated TH metabolites, TH sulfate, and glucuronide. T4 deiodination leads to rT3, and T4, T3, and rT3 deiodination to 3,3'-T2 and 3,5-T2, respectively. 3-T1AM and T0AM are formed by T4 and T3 deiodination and amino acid decarboxylation (**Figure 3**). Tetrac and Triac are produced by oxidative deamination and decarboxylation of T4 and T3.

Reverse T3

The role of rT3 is receiving increasing interest because high levels are seen in NTIS, in contrast to low T3 and T4 levels. The TH metabolite is also increased in starvation, surgery, bone marrow transplantation, heart attack, coronary bypass grafting, and chronic dieting (87). Centenarians exhibit significantly lower TSH levels together with slightly higher rT3 levels than aged controls (7). rT3 binds to TBG with 40% higher affinity than T4. Also TTR and albumin plus several other serum proteins, in particular high density lipoproteins bind rT3 but contribution to overall transport is minimal.

Upon binding of rT3 to D1 or D2, biologically inactive 3,3-T2 is formed. Drugs like dexamethasone, propyl thiouracil, iopanic acid and sodium ipodate, amiodarone, and propranolol decrease T3 and increase rT3 levels (25) rT3 influences various cellular events, e.g. mitosis, cell migration and invasion, by conversion of soluble actin to fibrous actin. rT3 has a role in glial-mediated neuronal guidance during mammalian brain development but when rT3 is lacking, no prominent symptoms arise (85). Increases in rT3 are seen in physiological and pathological conditions. Serum T3 was significantly lower and serum rT3 significantly higher in diabetic patients prior to treatment as

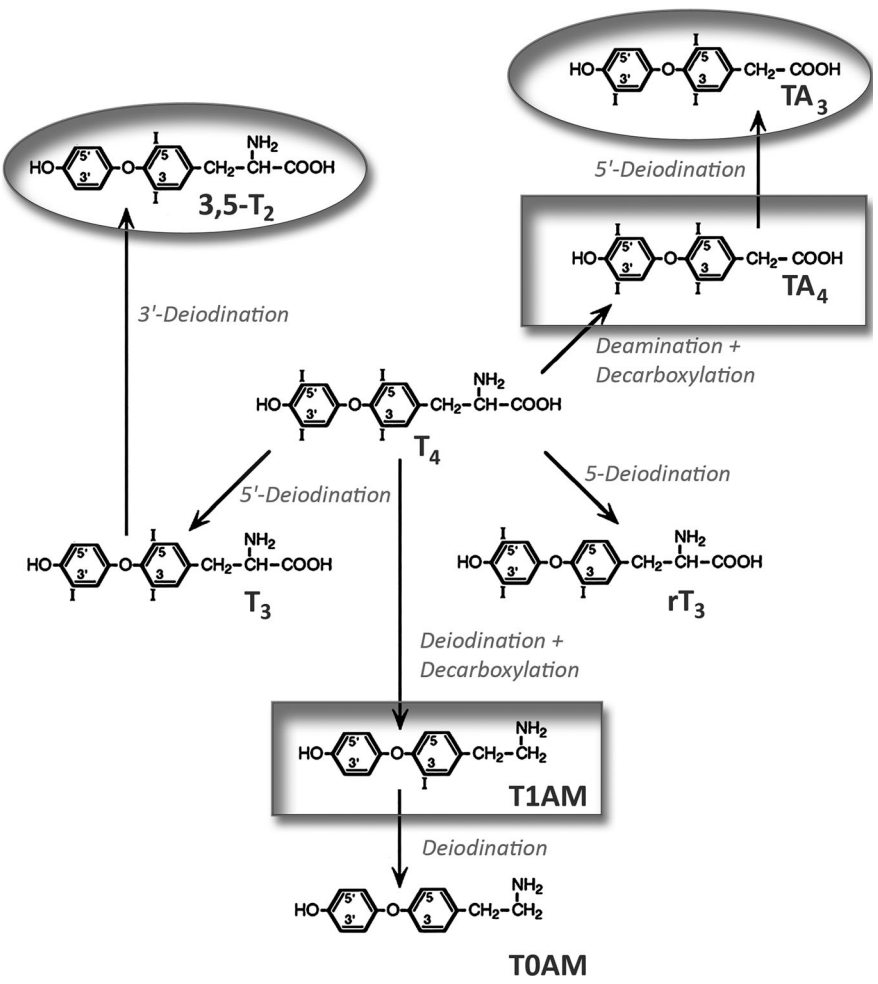

FIGURE 3 | Generation of biologically active thyroid hormone metabolites from thyroxine. Circles mark agonists and boxes antagonistically acting metabolites. 3,5-T2, 3,5-diiodothyronine; T1AM, 3-iodothyronamine; rT_3, reverse triiodothyronine; TA_4, tetraiodoacetic acid; T0AM, thyronamine; TA_3, triiodoacetic acid.

compared to normal subjects (88). Both T3 and rT3 normalized in 20 patients studied when adequate metabolic control was achieved as reflected by normalization of HbA1. Increased levels of rT3 also occurred in patients with myocardial infarction, hepatitis, or hepatic cirrhosis (89). On the other hand, insulin resistance was linked to an increased T3/rT3 ratio (90). All data suggest that the T3/rT3 ratio is decreased when metabolism is dysregulated, a notion supported by the finding that stress (induced by sleep deprivation, cold exposure, examination) and elevated cortisol levels (even if still in the normal range) are linked to raised rT3 levels (25). It appears that a rise in rT3 indicates short-term changes in metabolism rather than being a marker for chronic alterations because after 3 weeks of caloric restriction, T4 and rT3 levels returned to normal. The theory of rT3 being a marker for acute metabolic dysregulation is also compatible with the observed decrease in rT3 in hibernating bears. In this physiological long-term state, the bears showed a hypothyroid condition with decreased rT3 levels (91). Species-specificity, on the other hand, could also be the reason for the different reaction.

According to a generally accepted hypothesis, rT3 levels increase in specific conditions to conserve energy and to protect cells against insufficient T4, which could be converted to T3. rT3 is hypothesized to act as an energy-saving mechanism and as compensation for the decreased T3 levels (92). Upon dieting, T3 levels can decrease to 50% and after prolonged dieting not return to normal levels upon normal food intake. Conversely, in alternative day fasting normal T3 and rT3 levels are restored after a meal (93). During starvation more than the normal rate of 40% of T4 is converted to rT3 and less than the normal 60% to T3 with the effect of a changed rT3/T3 ratio. Increase of rT3 levels in dieting is mainly due to decreased elimination of rT3 during fasting, while decrease of rT3 after re-feeding is caused by decreased production. D1 was more sensitive to starvation than D2 and D3, which is consistent with the finding that changes in T3 affect mainly peripheral tissues, not the brain. This regulation serves the purpose of maintaining function of the central nervous system. Carbohydrate levels are important regulators in rT3 and T3 levels in low caloric intake between 360-1200 cal/day (94).

The most dramatic manifestation, where increased rT3 levels are seen, is NTIS. Also in this situation, the changes are interpreted as protection mechanism to reduce energy expenditure that does not require treatment, while other groups recommend treatment with thyrotropin-releasing hormone (TRH), TSH, or T3 and T4. In patients with NTIS, half-life of rT3 was short (3h) and levels mainly influenced by liver deiodinases (95). Davis et al. do not exclude the possibility that increased rT3 levels in NTIS contribute to hypercoagulation because rT3 acts similar to T4 on αvβ3 integrin (96). rT3 levels were negatively correlated to D1 in liver and positively to D3 in liver and muscle. The proposed mechanism of decreased action of D1 is downregulation by inflammatory cytokines TNF-α, interferon α, and IL-6. IL-6 plays a key role in the regulation of TH levels by acting on central and peripheral levels (**Figure 4**). IL-6 is increased in NTIS and levels inversely correlated (r=0.56) with T3 levels and positively correlated (r=0.78) with rT3 levels (97). Low T3 and T4 levels are detected extremely frequently in patients in intensive care, namely in 70% and 50%, respectively. Processes contributing to the findings in NTIS include IL-6 induced decrease of TBG and TTR, decreased T4/T3 tissue uptake, increased or unchanged transporter expression, decreased D1 and increased D3 expression in liver and increased D2 in muscle (98). Changes in acute and prolonged disease differ in the way that pulsatile TSH surge is present, TRH mRNA and T4 levels normal, T3 low and rT3 elevated in the acute, while TSH surge is absent, TRH mRNA and T4 levels low, T3 very low, and rT3 normal in chronic NTIS.

Circulating rT3 levels are also increased in cancer patients (96). rT3 increases proliferation of cancer cells *via* αvβ3 integrin signaling, which is highly expressed in such cells. It is supposed that cancer cells use this metabolite for proliferation instead of T3, which at physiological concentrations does not promote proliferation. This is in line with the finding that suppression of TH synthesis by methimazole and substitution by T3 in terminal patients induced stabilization or regression of the disease (71). Another, very rare, manifestation of increased rT3 levels is consumptive hypothyroidism seen in hemangioma (99).

3,5 Diiodothyronine (3,5-T2)

Of the several diiodothyronines, biological effects have been identified for 3,5-T2, while other diiodothyronines have higher serum levels (3,3'-T2: 1-8 ng/dl; 3',5'-T2: 1.5-9 ng/dl) but no effect on metabolism (100). 3,5-T2 has blood levels of 0.2-0.75 ng/dl and low binding to TBG (101). Other studies reported up to 15 ng/dL, and blood levels have to be interpreted with caution because interference between diiodothyronines and T3 occurs (85). Inter-individual differences in 3,5-T2 levels have been reported, which are not linked to T3 levels. In NTIS, 3,5-T2 correlated with rT3 and may result from either increased production from T3 or decreased degradation. The 3,5-T2 levels increased by 30% in NTIS and may be seen as a balance to the increased rT3, that has opposite effects on metabolism (102). It is assumed that increased 3,5-T2 levels in otherwise healthy individuals may be an indication of an underlying disease. 3,5-T2 can bind to the T3 receptor and induce TSH suppression and alter expression of classical T3-regulated genes in liver and other tissues. 3,5-T2 increases mitochondrial oxygen consumption in rats rapidly (103). Increased 3,5-T2 levels in disease have been interpreted as compensation for low cellular T3 levels (104). Survival in chronically cold-exposed animals is improved due to activation of thermogenesis, stimulation of β-oxidation, and up-regulation of F(0)-F(1)-ATP synthase. In HepG2 hepatocytes, 3,5-T2 blocked proteolytic cleavage of SREBP 1, which resulted in decreased fatty acid synthase expression. Administration to humans rapidly increases resting metabolic rate. Upon chronic administration (28 d) body weight decreased and metabolic rate increased in a study of just two participants. Although T3- and 3,5-T2-induced effects are inhibited by propranolol, application of deiodinases did not influence the effects and these were more rapid than T3-induced events, suggesting independent mechanisms of T3 and 3,5-T2 actions. T3 stimulates fatty acid synthesis (FAS, fatty acid synthase) and β-oxidation (ACC, acetyl-CoA carboxylase) while 3,5-T2 stimulates only β-oxidation (105). Decrease of FAS is regulated by SREBP-1 at the non-genomic level.

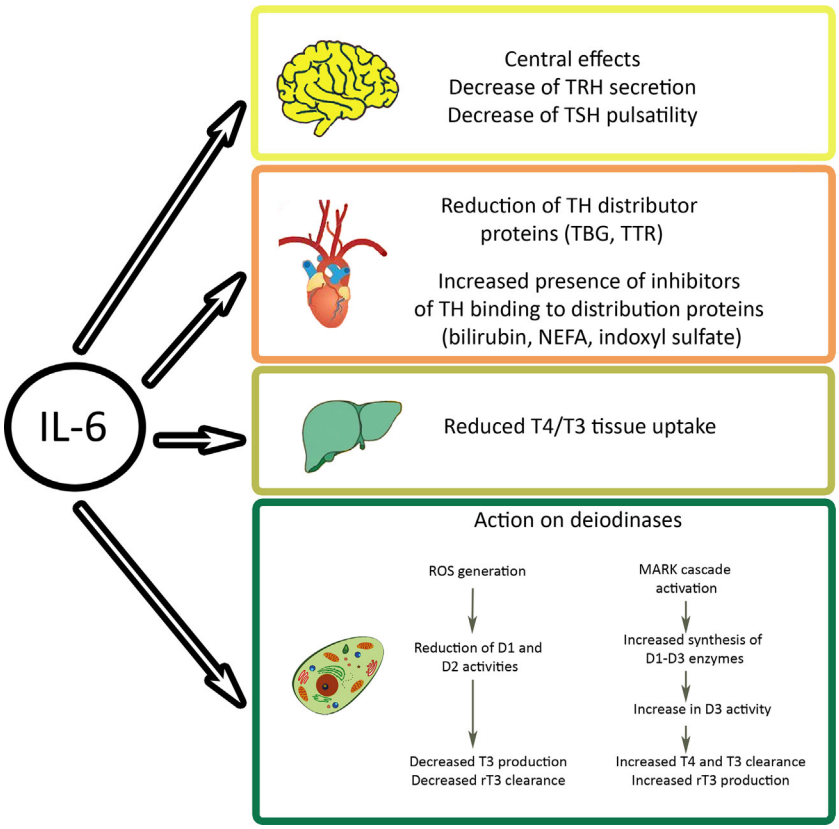

FIGURE 4 | Effects of interleukin (IL)-6 on cellular thyroid hormone levels. The inflammatory cytokine acts on hypothalamus and pituitary gland of the brain, on levels of free hormones, on transporters, and on cellular deiodinases. Indoxyl sulfate is a metabolite of tryptophan that accumulates in kidney damage. NEFA, non-esterified fatty acids; TBG, thyroxine binding globulin; TRH, thyrotropin releasing hormone; TSH, thyroid stimulating hormone; TTR, transthyretin.

3,5-T2 stimulated mitochondrial oxidative metabolism of fatty acids and reduced SREBP-1. The latter effect was linked to the pro-apoptotic effects of T2, suggesting potential application as an anti-cancer agent. Other effects of 3,5-T2 include activation of protein kinases Akt, p38, PKC-δ, ERK, ATP-activated protein kinase (AMPK) and silent mating-type information regulation 2 homolog (sirtuin, SIRT)1, a nuclear deacetylase that enhances mitochondrial activity.

Thyronamines

Thyronamines are decarboxylated and deiodinated metabolites of thyroid hormones with 3-iodothyronamine (3-T1AM) and thyronamine (T0AM) as the most important representatives in humans. Blood levels are 0.6-2.3 ng/dl but intracellular levels can be 20 times higher than T3 and twice as high as T4 (106). 3-T1AM is produced by ornithine carboxylase and is very stable. It is observed up to 6 days after oral application of L-T4. 3-T1AM accumulates in skeletal muscle, myocardium>liver>adipose tissue. 3-T1AM and T0AM differ from T3 and T4 regarding their effects and their binding to serum proteins in the way that the highly lipophilic molecules are transported by apolipoprotein B100 instead of the common TH distributor proteins. As a general rule, the action of 3'3,5-T3 is roughly equal to 3,5-T2, while T1AM acts as an antagonist. T3 has a stronger action on body temperature and insulin secretion than 3,5-T2, while the opposite applies to insulin sensitivity, heart rate and contractility (107). Upon activation of 3-T1AM, metabolic rate decreased and metabolism was shifted from carbohydrate to lipid with elevated H_2O_2 production. Sirtuin increased expression of SIRT4 and SIRT6-dependent genes. 3-T1AM activates trace amine-associated receptor 1 (TAAR 1), adrenergic receptors, and serotonin 1β receptors and reduces metabolic rate and increases lipid utilization, resembling the pattern of sleep or hibernation. 3-T1AM has also neuromodulatory effects, which are, according to animal experiments, partly mediated by inhibition of dopamine and norepinephrine reuptake and transport into synaptic vesicles. 3-T1AM and T0AM cause similar effects (e.g. hypothermia, negative chronotropy, reduction of respiratory coefficient). More details are given by Köhrle (85).

Thyroacetic Acids (Tetraiodoacetic and Triiodoacetic Acid)

Thyroacetic acids circulate at relatively high levels in blood and are formed by desamination of iodothyronines. Tetraiodoacetic acid (Tetrac) is a physiological metabolite of T4, a ligand of αvβ3 integrin, and a precursor of triiodoacetic acid (Triac). Levels are increased upon fasting as with T3 and appear to serve the same

purpose, i.e. the shunting away of T4 from T3 (108). Half-lives of Triac at 6h and Tetrac at 3-4d are related to the half-lives of T4 (7d) and T3 (1d), respectively. Deiodinases D1 and D2 can convert Tetrac to Triac, and D1 and D3 can deiodinate Triac with higher affinity than T3. The hydrophilic molecules have little binding to the distributor proteins, and are transported exclusively by TTR. Cellular uptake of Triac takes place by tissue-specific transporters different from MCT8, MCT10 and OATP. Triac is excreted in the urine after conjugation to glucuronic acid. Tetrac and Triac are much better substrates for glucuronidation than T4 and T3 and are, as sulfates and glucuronates, better substrates for deiodination in liver and kidney than T3 and T4 (109).

Tetrac blocks T4 at the binding site of the αvβ3 integrin and acts *via* MAPK/ERK signaling in competition with T4. The binding disrupts cross-talk with adjacent growth factor receptors like VEGF, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and EGF (110). Tetrac appears to be able to disrupt this cross-talk and through this mechanism block migration, angiogenesis and tissue invasion of cancer cells (111). Tetrac can also lower DNA repair, drug resistance and radioresistance by blocking P-glycoprotein (P-gp) and multidrug resistance protein (MRP). By its action on the Na^+/H^+ antiporter, the pH shifts away from the optimum of the P-gp pump and the intracellular pH becomes more acidic. In cancer cells with inherent radioresistance due to changed conformation of the receptor, Tetrac can reduce the effect (96). In addition to prevention of angioneogenesis in cancer, Tetrac can reduce fibrosis in hepatic fibroblasts (35). The increased Tetrac levels in patients with Graves' disease have to be discussed in the light of the greater risk of these patients for cancer (112) and the promoting effect of hyperthyroidism on cancer progression (36, 113). It may be speculated that conversion of T4 to Tetrac in cancer patients is lower than the 20% reported in normal individuals (114). In this way the effects of the other TH may outbalance the anti-tumor effects of Tetrac.

Similar to rT3, Triac levels are increased in fasting and in NTIS. However, in contrast to rT3, Triac acts as a thyromimetic, affecting the liver, adipose tissue, bone, and brain but not the heart. Triac binds to TRα1 with similar binding affinity as T3 and to TRβ with six-fold higher affinity but has limited potency due to its short half-life (115). Triac causes pituitary suppression of TSH, and effects on bone, kidney, liver and body weight similar to T3. Triac has suppressive function on TSH and decreases leptin secretion by adipocytes in this condition. This is surprising because, when blood T3 levels are low, increased TSH is expected. Triac is special because the metabolite acts as a thyromimetic in hypothyroid conditions and increases metabolism. In the euthyroid condition, metabolic rate is reduced. The applied doses were 4-30 times lower than L-T4 and 15-100 times lower than L-T3 but much higher than needed for TSH suppression.

MEDICAL USES OF IODOTHYRONINES, THYROAMINES, AND IODOACETATES

Medical uses may consist in functioning as biomarkers or as candidates for treatment.

Use of L-T4 and L-T3

Thyroxine (T4) is one of the top 10 medically-prescribed drugs worldwide, with the most common indication being hypothyroidism. At first glance, supplementation of TH appears less challenging than application of insulin in diabetes mellitus, where food intake, physical activity and stress change hormone requirement (116). Levels of T3 have a diurnal rhythm with peak round 4 am and a nadir between 3-5 pm but actual differences in T3 are low (117). T4 levels are constant mainly due to the long half-life of T4 of about 7 days, which eliminates the need to adapt for circadian changes. On the other hand, identification of the ideal personalized dose may pose problems. Requirements of L-T4 appear to be influenced by residual thyroid function because the small amounts of co-secreted T3 upon normal T4 secretion have regulatory function on the HPT axis (118).

Doses for L-T4 supplementation can be calculated based on body weight or body mass index (BMI) with and without inclusion of additional factors (e.g. patient sex). Generally, both a TSH-based estimate and a body weight-based estimate yield similar initial estimates of dose requirement (119). Type of formulation, co-medication (iron, calcium carbonate, -citrate, -acetate, vitamin C, phenytoin, rifampin) and nutrients (soja products, milk, coffee, grape fruit, papaya), co-existing conditions (pregnancy, renal failure, hepatic disease), deiodinase expression and peripheral conversion, dosing (lean/obese), drug distribution (circulation, plasma proteins), drug absorption (food intake, timing gastrointestinal tract motility), patient compliance, co-morbidities (malabsorption, surgery), and disease stage (stable, progressing, residual thyroid tissue) cause variable TH levels upon administration of the same amount of L-T4 (120). Conversely, chronic conditions like diabetes mellitus, cardiac disease, hepatic disease, osteoporosis do not have a pronounced influence on L-T4 requirement. Some general rules, the reduced requirement of aged individuals and postmenopausal women should also be taken into account. A list of medical conditions, food ingredients, and drugs that interfere with L-T4 supplementation is available elsewhere (121). If patients complain of hypothyroid symptoms despite L-T4 therapy, a panel of other diseases, which could cause symptoms similar to TH deficiency, has to be excluded. These conditions include: diabetes mellitus, adrenal insufficiency, hypopituitarism, celiac disease, pernicious anemia, anemia, multiple myeloma, chronic kidney damage, chronic liver disease, and congestive heart failure (117). Similarly, B12 deficiency, folate, vitamin D, and iron deficiency, obesity, hypercalcemia, electrolyte imbalance, treatment with β-blockers, statins and opiates, stress, lack of sleep, alcohol excess, sleep apnea, chronic fatigue syndrome, co-poisoning, depression, polymyalgia rheumatica, and fibromyalgia can mimic inadequate L-T4 supplementation. Reasons for insufficient treatment like lack of compliance or change of L-T4 formulation should also be considered. The different existing formulations were regarded as bioequivalent and products, in theory, should be interchangeable (122). Bioequivalence of two products means that 90% confidence interval of the ratio of the log-transformed exposure measure, area under the curve (AUC) and maximal plasma concentration (Cmax), falls within 80-125%. The method is not undisputed because only T4 levels are used for the

evaluation and conventional immunoassays may overestimate TH levels. Furthermore, the method cannot distinguish dose differences of up to 33% (e.g. 400 *vs* 600 μg) and doses that differ by 12.5% (400 *vs* 450 μg). There is uncertainty if products are really bioequivalent and, therefore, change between products is not recommended because changes of 12.5 μg may have dramatic effects on a drug like L-T4, which has a narrow therapeutic window. In gastrointestinal diseases such as celiac disease, gastritis, or lactose intolerance, that decrease absorbance, liquid formulations may work better than tablets. However, even when euthyroidism according to standard readout parameters for TSH and fT4 is achieved, ~15% of patients experience some level of psychological impairment. These patients express the wish for alternative treatment, often the combination of L-T4 with L-T3 or the prescription of desiccated porcine thyroids. The D-enantiomer of L-T4 dextrothyroxine (D-T4) has been tested in clinical trials for its antihyperlipidemic effects. Although D-T4 lowered serum cholesterol in the Coronary Drug Project (1966 and 1975), groups were discontinued early because of increased mortality, probably due to tachycardia (123).

The question whether combined L-T3 + L-T4 supplementation is superior to L-T4 monotherapy has been under debate for many years and numerous reviews have been dedicated to this question (e.g (4, 124, 125).). Patients under replacement therapy have lower T3 and higher T4 levels compared to control individuals. When L-T4 was dosed to reach T3 levels of the controls, TSH was lowered or suppressed (126). Only the combination of L-T3 + L-T4 normalized TSH, serum and tissue T4 and T3 concentrations. In another study including patients with elective total thyroidectomy, L-T4 monotherapy was able to bring serum T3 back to the same presurgical levels without suppressing TSH but with elevated fT4 levels. Also better quality of life under combination therapy compared to L-T4 monotherapy has been reported (127). Improved effects by L-T3 + L-T4 combination may be explained by placebo effects, differential signaling in neurons and positive effects by action on serotonin and catecholamines (120). A systematic review of ten randomized controlled trials comparing combination therapy *vs* monotherapy found no statistically significant differences in biochemical markers, mood states, and adverse effects. Commercially available products like Armour Thyroid, Nature-Throid, Bio-Throid, WP-Thyroid, Westhiod, and NP-Throid consist of desiccated porcine thyroid extracts. Although containing both TH may be advantageous, one major criticism is the species-specific relation of T4:T3, of 4:1 in pigs and 14:1 in humans. Combination therapy may only be adequate in patients bearing the Thr92Ala D2 (rs225014) polymorphism. This genetic polymorphism may affect half-life of the protein and could hypothetically disrupt TH signaling in D2-expressing tissues. Nevertheless, human studies, so far, did not find lower TSH levels, changes in fT4 and fT3, metabolic syndrome etc. in carriers of this polymorphism (119). Patients with MCT10 (rs17606253) polymorphism, on the other hand, preferred the combination therapy to L-T4 monotherapy (128). This led to the recommendation to restrict the combination therapy to populations with such polymorphisms. T3 supplementation is adequate for patients with TR mutations, where TRβ mutation hyperthyroid symptoms are frequent upon L-T4 therapy. They manifest as tachycardia because heart effects are dominated by TRα signaling and when the L-T4 dose, which is adjusted to the dysfunctional TRβ is too high (129). In patients with TRα mutations, low T4/T3 ratio is typical and symptoms of hypothyroidism with variable manifestations are seen. The consensus report on evidence-based use of L-T3/L-T4 combinations in treating hypothyroidism reported that there is dissatisfaction with the existing standard of care and that "a new well-designed adequately powered clinical trial of combination therapy" is needed because not all studies met the current standard requirements (130).

Treatment of heart diseases may be another indication for use of TH. This may appear surprising as arrhythmia and mortality are linked to overdoses of L-T4. However, in heart disease, tissue levels of deiodinases are altered and less T3 is being produced. In hypothyroidism MCT10 expression in the heart is increased and deiodinase expression thus changed to produce more T3. The opposite is observed in hyperthyroidism showing that the heart is capable to adapt to abnormal TH levels. A combination of L-T4 + L-T3 is suggested because supplementation with either of the TH alone did not restore T3 in rats (131). L-T3 alone has positive effects in myocardial infarction and protects hypothyroid rats against arrhythmia. L-T3 and L-T4 showed beneficial effects in patients with heart failure (132). Small clinical trials reported promising effects of L-T3 administration in patients with acute myocardial infarction, cardiac surgery and transplantation.

TH interact with catecholamines (epinephrine and norepinephrine) in that infusion of T3 before trauma in pigs increased epinephrine levels and decreased T3 and T4 levels (133). The resultant hypothyroid state decreased responsiveness to α- and β-adrenergic agonists. Similar effects occur also during brain death and provide the theoretical basis for the use of TH in preparation of organ transplantation. It is assumed that loss of TH, cortisol, antidiuretic hormone (ADH) and insulin leads to reduction in perfusion, inhibition of mitochondrial function and organ failure. Therefore, the United Network for Organ Sharing (UNOS) Critical Pathway for the Organ Donor recommends hormone replacement with TH, vasopressin, methylprednisolone and insulin; both L-T3 and L-T4 may be used. According to a large retrospective analysis L-T3 or L-T4 therapy produced more transplantable hearts, lungs, kidneys, pancreases, and intestines, while the number of transplantable livers was not increased (134). Positive effects include increased chronotropy and upregulation of ATPase-rich α heavy chain isoenzyme that results in increased inotropy. In combination with the increased smooth muscle relaxation and vasodilation, diastolic pressure is lowered and resistance to left ventricular ejection reduced (135). In general, disparate results were reported for heart transplantation. The studies differed in the use of TH (either alone or in combination with other hormonal therapies) in methodology and biological readouts. For instance, donor administration of L-T4 improved survival of the graft recipient. The recipients who received L-T4 prior to the transplantation had a survival advantage over those who did not and results were better when L-T4 was applied to the donor before the declaration of brain death (136). Early L-T4 therapy was

associated with an increase in solid organ procurement rate with odds ratio of 1.9 (137).

Application of L-T3 alone is commonly restricted to specific situations such as preparation of patients with advanced thyroid cancer for nuclear imaging, for primary ablation or whole body scan, if recombinant TSH is not available. Further, clinical data indicate that application of L-T3 to patients in the terminal phase of lung cancer, pancreatic cancer, mesothelioma, soft tissue cancer, and glioblastoma can prolong survival (138). The interventional lowering of fT4 by application of L-T3 alone or in combination with methimazole is better tolerated than withdrawal of TH by methimazole alone. Twenty percent of the patients exceeded the 20% expected 1-year survival upon L-T3 treatment (139). The reported prolongation of survival may be caused by reduction of the promoting effects of T4 on tumor proliferation, coagulation, and angiogenesis. Additional indications appear possible as L-T3 in septic rats prevented the consumption of coagulation inhibitors like ATIII (140).

Efficacy of L-T3 in critically ill COVID-19 patients is under investigation. A phase II clinical trial (ClinicalTrials.gov Identifier: NCT04348513) including patients diagnosed with pulmonary infection due to COVID-19 in intensive care requiring mechanical respiratory support was started. The trial will study the effect of intravenous high dose L-T3 for enhancing recovery of critically ill COVID-19 patients. In this trial, T3 treatment is started with a bolus injection, followed by maintenance dose of 0.113 g/kg/h for 38h and 0.057 g/kg/h for up to 30d (141). Successful weaning from ventilation was defined as primary outcome. The treatment was initiated under the assumption that thyroid dysregulation in severely ill COVID-19 patients shows the pattern of NTIS. Although a correlation between low T3 and diseases severity has been established, replacement with TH in NTIS in general is not suggested (142). Application of L-T3, instead of L-T4, was chosen based on the following findings; i) T3, in contrast to T4, does not induce hypercoagulation (71), ii) T3 stimulates secretion of the anti-inflammatory IL-10 and strengthens specific immune response by action on DCs (79), and iii) T3 prevents action of T4 on integrin αvβ3. T4 may be facilitating virus uptake because it increases expression of the genes for the specific integrin monomers αv and β3 and increases internalization of the integrin (143). In other words, fT4, in contrast to T3, may increase the number of binding sites for the virus on the target cell surface. Finally, endothelial dysfunction, induced by increased TSH level, may improve because high T3/T4 levels will reduce TSH secretion of the anterior pituitary gland (144). In the light of later studies, which reported heterogenous pattern of thyroid dysfunction with decreased TSH levels in combination with decreased T3 or increased T4 levels (**Table 1**), systemic administration of L-T3 appears debatable. The differentiation between decreased TSH values as indication for NTIS in prolonged disease or as thyrotoxicosis is very important because 32% of thyrotoxic COVID-19 patients developed atrial fibrillation (59). Atrial fibrillation is not typical for viral SAT but one of the most common arrhythmias caused by SARS-CoV-2-induced myocardial injury (58). It was hypothesized that the SARS-CoV-2 virus showed specific cardiotoxicity but a more recent study suggests that patients with pre-existing heart disease may be more likely to contract the disease (145). In hyperthyroidism, T3 more than T4, may cause atrial fibrillation (146). It is, therefore, possible that a combination of L-T3 administration and COVID-19 induced myocardial damage leads to an increase in adverse cardiac effects. The determination of physiological parameters and troponin I levels performed in the NCT04348513 trial are suitable to identify such effects. Another phase II trial will study effects of locally administered T3 to reduce pulmonary edema in COVID-19 pneumonia (ClinicalTrials.gov Identifier: NCT04725110), based on beneficial effects of intratracheally administered modified Triostat® (liothyronine) to rats on alveolar fluid clearance (147). Primary outcome is the change of the extravascular lung water index. Kidney function (glomerular filtration rate and creatinine levels) but not cardiac function will be monitored. Although local action on the Na/K ATPase to stimulate alveolar clearance is the primary mode of action (148), the lipophilic molecule can also cross the alveolar barriers and reach the systemic circulation, resulting in increases of circulating T3 levels.

L-T3 is more difficult to dose correctly than L-T4 because absorption is fast and half-life is short. Absorption leads to 40% increase in T3 blood levels, which is markedly higher than the normal daily fluctuation of 5-10%. The high T3 levels represent a risk factor for cardiovascular events and hip fractures (149). Slow release formulations, such as metal coordinated poly-zinc-liothyonine, may be a solution because stable levels of circulating T3 were obtained in rats. T3 from the poly-zinc complex is slowly absorbed. Over a time span of 8 days levels gradually increased but were still in the reference range. Alternatively, T3 sulfate may be applied, which has to be activated in the liver. Oral administration of T3 as sulphate may also be an option because endogenous desulphatases can slowly produce T3 (150).

Adequate supplementation with L-T4 is monitored by levels of TSH, fT4, fT3 and fT4/fT3 ratio. It is agreed that fT3 is a much better biochemical marker for euthyroidism than TSH but determination of fT3 levels is often inaccurate (118). A recent meta-analysis reported that fT4 levels were better correlated to clinical symptoms than TSH levels (151). There is no general recommendation by the European Thyroid Association to determine rT3 levels, except in infantile hepatic hemangiomatosis (IHH). This condition is also termed consumptive hypothyroidism and has to be differentiated from congenital hypothyroidism because treatments differ (152). Consumptive hypothyroidism in adults due to hemangioma or hemangioendothelioma has only been reported in a few case studies (99). Although both diseases need supplementation with TH, patients with IHH receive 22–70 μg/kg/day L-T4 or L-T3 compared to 5–10 μg/kg/day L-T4 or L-T3 in congenital hypothyroidism. For combinations of L-T4 and L-T3, lower doses may be required (153). The reason for the thigh doses in IHH is the high expression of D3 in the hemangioma tissue. It has been postulated that IHH may originate from placental angioblasts and arise from embolization of placental endothelial cells. The liver carries the highest risk for hemangioma development as it is the first

organ to be perfused with the incoming blood from the placenta (154).

Use of 3,5 T2

3,5 T2 is an interesting candidate as hypolipidemic drug and for cancer therapy. 3,5-T2 has a suitable action profile for targeting steatosis because it acts independently of T3. Anti-steatotic effects of T3 and 3,5-T2 are caused *via* different mechanisms. 3,5-T2 decreases lipogenesis and increases β-oxidation, while T3 only increases β-oxidation. TSH suppression and cardiac effects were observed. In summary, studies differed in dosage of 3,5 T2 (10-100 μg/kg), treatment regime (single versus chronic) and application route (sc or ip). High fat-induced obesity with co-administration or treatment with 3,5-T2 did not show anti-steatotic effects in all studies (155). Accumulation of 3,5-T2 in the liver was seen only upon application of this molecule, while no accumulation of 3,5-T2 was observed when T3 was applied. The effect was specific for hepatocytes and did not occur in cardiomyocytes. 3,5-T2 increased glucose consumption but did not increase inotropy and chronotropy. In skeletal muscle ATP kinase activation and increased GLUT4 expression induced a switch from fast-twitch (white fibres) to slow-twitch (red fibres) typical for glycolytic phenotype. In animal studies no clear evidence was obtained that the anti-steatotic effects occur at concentrations that do not suppress the HPT-axis or cause adverse effects on the heart. Similarly, in a 4-week trial with the 3,5-T2-mimetic analogue TRC-150094 no increase in insulin sensitivity, decrease of fatty acids in serum or in intrahepatic triglycerides was observed (156). Based on this data the use of 3,5-T2 for treatment of metabolic syndrome and for reduction of body weight is discouraged. This applies also for use outside of the medical setting, where individuals who wish to lose weight or boost their energy use 3,5-T2 as a dietary supplement. These over-the-counter products contain variable (50-300 μg/pill) and often not stated amounts of 3,5-T2 (157).

Other molecules having the same profile as 3,5-T2 have been designed to act preferentially on TRβ receptors, expressed in liver and brain, and to avoid cardiac adverse effects induced by binding to TRα. Some of these molecules were designed based on the finding that the binding pocket of TRβ is more flexible than that of TRα (32). GC-1 (sobetirome) and KB-2115 (eprotirome) had a 10- and 20-fold higher TRβ/TRα ratio than T3, respectively. KB07811 (VK2809) is a prodrug converted to the active compound in the liver, and MGL-3196 (resmetirom) has a 30-fold higher TRβ/TRα ratio than T3. Studies for the indication of hypercholesterolemia were successful but stopped because unexpected cartilage defects in canine bones were seen in preclinical testing (158). For application in non-alcoholic fatty liver disease, however, MGL-3196 has been tested in clinical trials (159), and results are encouraging. Similarly, also VK2809 reduced liver fat content in clinical trials. Another indication for sobetirome is treatment of X-linked adrenoleukodystrophy (ALD), where the protocol of the planned clinical trial is currently being revised (32). The disease is caused by mutation of very long fatty acid transporters located in the peroxisomal membrane encoded by the *ABCD1* gene.

Use of Thyroamines

3-T1AM has several biological effects in animals, where it increases plasma glucose, induces carbohydrate oxidation, gluconeogenesis, ketogenesis and decreases body weight in obese mice (160). In addition to the endocrine effects, 3-T1AM favors learning in animals by action on TAAR1 (161). The molecule, however, has a very short half-life because sulfatation and glucuronidation are so fast that very frequent doses are needed to obtain the desired effect. By its negatively inotropic and chronotropic action it can prevent myocardial lesions caused by ischemia. It binds to transient receptor potential cation channel subfamily melastatin member (TRMP) 8 that blunts function of the Ca-channel Transient Receptor Potential Vanilloid (TRPV4) expressed by tumor cells and activated by heat, capsaicin, binding of growth factors and cytokine secretion. Hypothermic effects, modulation of glucose and insulin secretion, and cardiac effects suggest potential application in myocardial infarction and brain ischemia (85). Effects of 3-T1AM on feeding behaviour, learning, anti-amnestic response, protection against β-amyloid toxicity, and anticonvulsant effects, observed in rodents, present interesting applications but have not been studied in humans so far (32).

Use of Thyroacetic Acids

The two thyroacetic acids differ markedly in their action, Triac as a thyromimetic and Tetrac as a TH antagonist. The advantage of Triac from the pharmaceutical perspective is the fact that it accesses the brain by bypassing MCT8 (85). On the other hand, Triac is very rapidly conjugated to glucuronic acid (~1500 and ~200 times faster than T3 and T4, respectively) and needs frequent application.

The main off-label application of Triac is treatment of patients with TRβ mutations (115). AHDS may be another indication for Triac to prevent the local hypothyroidism in the brain due to MCT8 mutation. Following promising results in animal models, a clinical trial in children harboring mutations in MCT8 has been initiated. Triac can be purchased over the counter in several European countries as a dietary supplement (trade name: Tiratricol) to reduce weight. Anti-angiogenetic effects may occur but were never reported by users. The FDA issued an official warning not to use Triac-containing supplements because Triac markedly reduced bone density and may induce thyrotoxic hypokalemic periodic paralysis (162).

Tetrac, when taken up into cells acts as a weak thyromimetic. In order to cause effects mainly be αvβ3 integrin signaling to improve anti-tumor action, increase of the extracellular action of Tetrac is important. For pharmaceutical application in cancer, Tetrac was bound to 200 nm poly(lactic-co-glycolic acid) (PLGA) nanoparticles (Nanotetrac) and the binding resulted in increased antiangiogenic efficacy *in vitro* (71). After the uptake, nanoparticles were located in the cytoplasm and Tetrac in the nucleus of cancer cells. Action was caused by regulation of cytokines in combination with maturation of endothelial cells, stabilization of vessels, and inhibition of neo-angiogenesis. *In vivo*, Tetrac antagonized T3 and T4 effects regarding differentiation of mesenchymal stem cells (MSCs) into cancer-

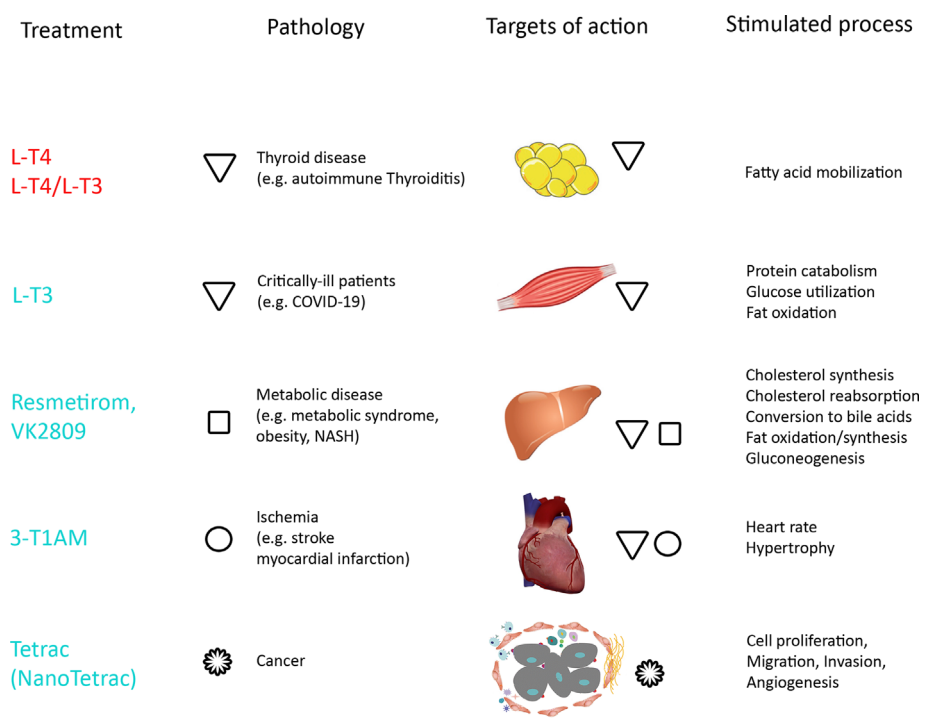

FIGURE 5 | Overview of treatments, pathologies and targets of action with stimulated processes by TH. Tissues relevant for the review are shown and other target tissues like bone, brain, kidney, gastrointestinal tract not represented. Established treatments are written in red, compounds/applications in clinical trials in turquoise. Pathologies and affected tissues are marked by symbols for the compounds (triangles for T3 and T4, squares for Resmetirom and VK2809, circles for 3-T1AM, and rosettes for Tetrac).

associated fibroblasts, angiogenesis and recruitment of MSCs to tumor cells. In animal models Nanotetrac was 10-times more efficient in inhibiting tumor growth than the original molecule (163). Tetrac was granted Orphan Drug status by the US FDA to be used in thyroid cancer treatment (164).

CONCLUSIONS

The action of TH is more complex than that of other endocrine amine hormones. Endocrine action of catecholamines for example has many targets to increase blood pressure, heart rate, glucogenolysis, glucagon secretion, and decrease insulin secretion and lipolysis but acts only by the two hormones epinephrine and norepinephrine (165). In contrast, five metabolites have agonistic and antagonistic effects to the classical TH T4 and T3.

A comparison of biologically active TH metabolites shows that i) the more potent acting T3 and Triac appear to have shorter half-lives than less potent antagonists 3T1AM and Tetrac. Ii) rT3 and 3,5-T2 may serve as indicators for metabolic dysregulation and disease, and iii) Nanotetrac may be a promising candidate for treatment of cancer and MGL-3196 for steatohepatitis, obesity, and metabolic syndrome (**Figure 5**). Use of L-T3 or combinations of L-T3 and L-T4 appear not to act better than L-T4 in common indications (e.g. hypothyroidism or thyroidectomy).

The observed alterations of TH levels in COVID-19 may result from a combination of thyrotoxic effects and NTIS. Outcome of local and systemic administration of L-T3 in critically ill patients potentially may provide further insight into the still unclear relationship between SARS-CoV-2 and thyroid metabolism.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.587518/full#supplementary-material

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SYSTEMATIC REVIEW
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COVID-19-Associated Subacute Thyroiditis: Evidence-Based Data From a Systematic Review

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Subacute thyroiditis (SAT) is a thyroid disease of viral or post-viral origin. Whether SAT represents a complication of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still unclear. Our aim was to systematically review the literature to 1) explore the size of the literature about SAT in COVID-19 and 2) evaluate the clinical characteristics of SAT. PubMed/MEDLINE, Embase, and Scopus were searched until April 20, 2021. Original papers, case reports, and case series reporting SAT in COVID-19 patients were included. Authors and their country, journal, year of publication, COVID-19 and SAT clinical presentation, thyroid function, therapy, and follow-up data were extracted. Nineteen papers (17 case reports and 2 case series) were included, describing 27 patients, 74.1% females, aged 18 to 69 years. COVID-19 was diagnosed by nasopharyngeal swab in 66.7% cases and required hospitalization in 11.1%. In 83.3% cases, SAT occurred after COVID-19. Neck pain was present in 92.6% cases and fever in 74.1%. Median TSH, fT3, and fT4 were 0.01 mU/l, 10.79 pmol/l, and 27.2 pmol/l, respectively. C-reactive-protein and erythrocyte sedimentation rate were elevated in 96% of cases. Typical ultrasonographic characteristics of SAT were observed in 83.3% of cases. Steroids were the most frequent SAT therapy. Complete remission of SAT was recorded in most cases. In conclusion, the size and quality of published data of SAT in COVID-19 patients are poor, with only case reports and case series being available. SAT clinical presentation in COVID-19 patients seems to be similar to what is generally expected.

Keywords: subacute thyroiditis (SAT), subacute thyroiditis de Quervain, SARS-CoV-2, COVID-19, thyroid

INTRODUCTION

Subacute thyroiditis (SAT) is a self-limited thyroid disease of viral or post-viral origin. SAT, also known as de Quervain thyroiditis, is typically characterized by a triphasic clinical course of thyrotoxicosis, hypothyroidism, and return to normal thyroid function. From a clinical point of view, SAT presents with neck pain typically with radiation to the ears and a wide spectrum of systemic symptoms, which include fever, asthenia, and malaise. In the initial phase, many patients also present clinical and/or biochemical manifestation of mild-moderate thyrotoxicosis, such as tremor and palpitations (1). Thyroid follicles are infiltrated, resulting in disrupted basement membrane and rupture of the follicles. This injury is thought to be the result of cytolytic T-cell recognition of viral and cell antigens (2).

While persistent hypothyroidism is a rare event, high circulating levels of inflammatory markers, such as C-reactive protein (CRP), and, more specifically, erythrocyte sedimentation rate (ESR) represent the most frequent biochemical finding at presentation (3, 4).

Several respiratory viruses, including coxsackievirus (5), mumps (6), Epstein–Barr virus (7), cytomegalovirus (8), and influenza virus (9), were reported to be associated with SAT development (10). However, owing to the fact that specific antiviral treatment is not required in most cases, diagnostic evaluations aimed at identifying the etiological viruses are not routinely performed in these patients. Thus, the viral or post-viral origin of SAT is supported by both direct and indirect evidences. Epidemiological data showed an overlap of seasonal outbreaks of infectious diseases and SAT outbreaks (6, 11). Indeed, high titers of virus-specific antibodies or positive virus swabs were found in patients harboring SAT and an association between presence of antibodies to specific viruses and SAT was observed (5, 6, 12). On the other hand, virus culture from thyroid tissue as well as viral RNA identification from thyroid cytological samples yielded conflicting results (13–15). At present, it is unclear whether follicle damage in SAT is caused by direct viral infection of the gland or by the host's immunological response to the viral infection.

With the beginning of the coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a thyroid impact was considered due to the potential of SARS-CoV-2 to cause multiorgan effects. Among the thyroid SARS-CoV-2 complications, SAT was early reported by some case report articles (16), and the question whether SAT might be an underestimated SARS-CoV-2 manifestation was raised (17). Subsequently, several original articles, editorials, and reviews were published on the thyroid sequelae experienced by patients with COVID-19 (18–22). More recently, a systematic review found that COVID-19 patients can develop thyroid dysfunction, frequently non-thyroidal illness syndrome, when hospitalized in an intensive care unit. Furthermore, several data supported the notion that having a thyroid disease would not increase the risk for SARS-CoV-2 infection, and thyroid patients do not need a COVID-19-adapted follow-up (23). According to the available data summarized in review articles, whether SAT can represent a COVID-19 complication remains an open question. Specifically, the prevalence of COVID-19-related SAT and the similarity of its clinical presentation to usual SAT remain unknown.

Therefore, the present study was conceived to summarize the published data about the association between COVID-19 and SAT. The literature was systematically reviewed to retrieve the largest number of original papers, case reports, and case series articles reporting SAT in patients diagnosed with SARS-CoV-2. The aims of the present study were to 1) explore the size and quality of the literature about SAT in COVID-19 and 2) evaluate the clinical characteristics of SAT in these patients.

Abbreviations: COVID-19, Coronavirus Disease 2019; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SAT, subacute thyroiditis; T3, triiodothyronine; T4, thyroxine; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroperoxidase antibodies; TSH, thyroid-stimulating hormone.

MATERIALS AND METHODS

Review Conduction

The systematic review was conducted according to the PRISMA statement (24), and the checklist is reported as supplemental file (**Supplemental File 1**).

Search Strategy

A comprehensive computer literature search of the PubMed/MEDLINE, Embase, and Scopus databases was conducted to find published articles on the topic of our review. The search algorithm was created based on combinations of specific terms: ("De Quervain thyroiditis" OR "subacute thyroiditis") AND ("SARS-CoV-2" OR COVID OR COVID-19 OR coronavirus). A beginning date limit was not used, and the search was updated until April 20, 2021, without language restrictions. To identify additional studies and expand our search, the references of the retrieved articles were also screened.

The authors declare that the study selection was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Study Selection

Studies or subsets of studies that report data on the detection of SAT in patients with previous or concurrent occurrence of COVID-19 were eligible for inclusion. The main exclusion criteria were a) articles not within the field of interest of this review; b) review articles, editorials, or comments; c) articles that did not provide clear study characteristics or reports that had overlapping patient data; and d) cases in which the SAT diagnosis was not clearly rendered. Two authors (LC, LS) independently reviewed the titles and abstracts of the retrieved articles, applying the inclusion and abovementioned exclusion criteria. Then, the same two researchers independently reviewed

the full text of the articles to determine their final inclusion. Disagreements were solved in a final mutual meeting involving also other two authors (PT, MR). **Figure 1** illustrates the research strategy and flow of articles.

Data Extraction

For each included study, information was extracted concerning reference data (authors, journal, year of publication, and country of origin). Number of patients evaluated, clinical data regarding COVID-19 presentation and SAT presentation, biochemical evaluation of thyroid function parameters, therapy for subacute thyroiditis, and long-term follow-up data were also extracted.

Each case report and case series were carefully evaluated to verify that no patient was included in more than one study.

RESULTS

Retrieved Articles

Using the above search strategy, 32 records were initially found. Among these, 14 were excluded because they did not fit with the study aim while the remaining 18 (16, 17, 25–41) were included in the systematic review. Another paper (40), not included in the initial pool of retrieved records, was added to the systematic review because it was known by the authors. Finally, 19 articles, consisting of 17 case reports and 2 case series (with four and six SAT cases), were included. Remarkably, no original articles including large sample size were found. **Figure 1** illustrates the flow of articles.

General Features of Included Articles

The 19 articles included in the systematic review included a total number of 27 SAT in patients diagnosed with COVID-19. The 19 studies were published from May 21, 2020 (16), to April 14, 2021 (41), by authors from 10 countries: seven cases from Italy, seven from Iran, four from the United States of America, two from Spain, two form India, one from Mexico, one from the Philippines, one from Singapore, one from Turkey, and one from the United Kingdom. The main demographic and clinical characteristics (regarding COVID-19 and SAT presentation) of each included patient are summarized in **Table 1**.

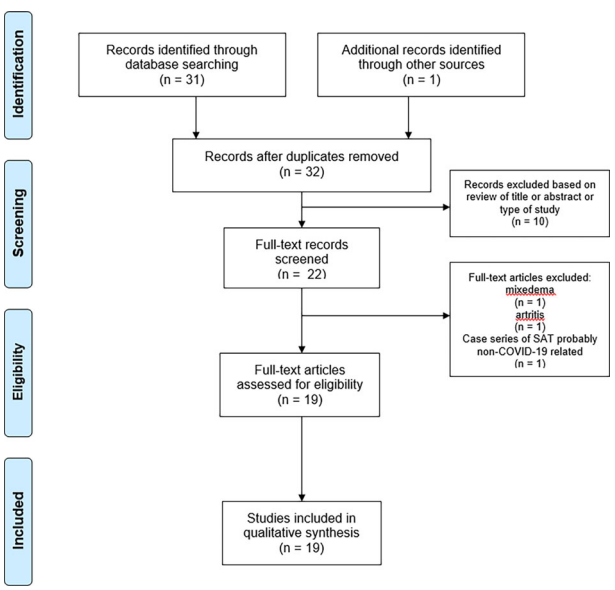

FIGURE 1 | Search strategy and flow of articles.

Demographic Features of Patients

Twenty (74.1%) patients were females and seven (25.9%) males. Patients' age at SAT occurrence ranged from 18 to 69 years (median 37.5, IQR 33–46). No patient was previously diagnosed with thyroid disease.

COVID-19 Diagnosis and Presentation

COVID-19 diagnosis was rendered through real-time polymerase chain reaction (RT-PCR) on nasopharyngeal swab in 18 cases (66.7%), and by positivity of specific IgG in eight cases (29.6%). In one case, COVID-19 was suspected based on the presence of its typical symptoms (anosmia, dysgeusia) with no direct evidence of SARS-CoV-2 infection. COVID-19 was asymptomatic in 3 cases (11.1%), with mild upper respiratory symptoms in 21 cases (77.7%), and with pneumonia requiring hospitalization in 3 cases (11.1%). SARS-CoV-2-related peculiar symptoms (i.e., anosmia or dysgeusia) were reported in 5 (18.5%) out of 27 cases.

Time Interval Between SAT Occurrence and COVID-19 Diagnosis

The timing of SAT diagnosis with respect to COVID-19 was described in 24 cases:

In 20 (83.3%) cases, SAT occurred after COVID-19 onset, after a median of 30 (IQR 16–32) days. In three (12.5%) cases, SAT and COVID-19 were synchronous. In one (4.2%) patient (N # 21), the onset of SAT preceded by 47 days the molecular confirmation of COVID-19. This latter case should be briefly overviewed. At SAT diagnosis, the patient did not have fever or respiratory symptoms but a right lower lobe pneumonia was found on a chest radiography. Unfortunately, the reverse transcription-polymerase chain reaction for SARS-CoV-2 using nasopharyngeal and oropharyngeal swabs was performed only after several weeks after the onset of SAT symptoms and found positive. Although the possibility that SARS-CoV-2 infection was already present at the onset of SAT cannot be ascertained, the authors suggested that SAT might occur also in patients with confirmed COVID-19 without respiratory manifestations.

SAT Clinical Presentation

Neck pain was present in 25 (92.6%) cases; notably, one of the two patients without neck pain was taking opioid drugs since a recent surgical intervention (N. #13). General symptomatology (i.e., asthenia and malaise) was described in 23 (85.2%) patients. Fever was recorded in 20 (74.1%) patients. Palpitations were experienced by 22 (81.5%) patients, and one presented a new-onset episode of atrial fibrillation.

TABLE 1 | Description of the anagraphic characteristics and clinical presentation of COVID-19 and SAT of the 27 included patients.

Year	N.	Reference	Country	Age	Gender	Preexisting Thyroid Disease	COVID-19 Diagnosis	Clinical presentation of COVID-19	Onset after COVID-19 (days)	General	Neck pain	Fever	Palpitations	Therapy
2020	1	(32)	Spain	46	F	NO	Positive IgG	Asymptomatic		YES	YES	YES	NO	Prednisone (40 mg/day as the starting dose, gradually tapered)
2020	2	(25)	Turkey	41	F	NO	RT-PCR	Asymptomatic	0	NO	YES	YES	NO	Prednisolone 16 mg daily
2020	3	(16)	Italy	18	F	NO	RT-PCR	Mild upper respiratory symptoms (rhinorrhea and cough)	19	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2020	4	(17)	Italy	38	F	NO	RT-PCR	Mild upper respiratory symptoms	16	YES	YES	YES	YES[c]	Prednisone (25 mg/day as the starting dose, gradually tapered)
2020	5	(17)	Italy	29	F	NO	Positive IgG	Rhinorrhea	30	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered), propranolol 40 mg/day
2020	6	(17)	Italy	29	F	NO	RT-PCR	Fever, cough, rhinorrhea, anosmia	36	YES	YES	NO	YES	Ibuprofen 600 mg/day
2020	7	(17)	Italy	46	F	NO	RT-PR	Fever, cough, rhinorrhea, anosmia, asthenia	20	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2020	8	(28)	Mexico	37	F	NO	RT-PCR	Odynophagia and anosmia	30	YES	YES	NO	NO	No treatment (still thyrotoxic after 1 month)
2020	9	(39)	India	58	M	NO	RT-PCR	Fever	0	NO	YES	YES	YES	Prednisolone (30 mg/day as the starting dose, gradually tapered), propranolol 40 mg/day
2021	10	(29)	USA	37	M	NO	RT-PCR	Productive cough, fever, chills, dyspnea	30	YES	YES	NO	YES	Aspirin, propranolol
2021	11	(37)	Iran	33	M	NO	RT-PCR	Interstitial pneumonia	8	YES	YES	YES	YES	Dexamethasone 4 mg every 8 h for 5 days, then oral prednisone 25 mg daily with tapering
2021	12	(34)	UK	57	F	NO	Likely diagnosis[a]	Mild upper respiratory symptoms, anosmia	60	YES	YES	NO	YES	Ibuprofen 200 mg three times per day and paracetamol 1 g three times per day
2020	13	(38)	Italy	69	F	NO	RT-PCR	Interstitial pneumonia	5	YES	NO[b]	NO	YES	First methimazole, then shifted to methylprednisolone 40 mg/die for 3 days, then prednisone 25 mg/die progressively tapered
2021	14	(36)	USA	41	F	NO	RT-PCR	Fever, cough, and coryza	14	YES	YES	YES	YES	Ibuprofen 600 mg every 6 h and prednisone 40 mg daily.
2021	15	(41)	USA	67	M	NO	RT-PCR	Heart failure and interstitial pneumonia	0	YES	NO	YES	YES	Methimazole for 1 month, after no recovery shifted to prednisone
2020	16	(27)	Singapore	34	M	NO	RT-PCR	Fever, mild upper respiratory symptoms, anosmia	3	NO	YES	NO	YES	Prednisolone (20 mg/day as starting dose, gradually tapered)
2020	17	(33)	USA	29	F	NO	RT-PCR	Mild upper respiratory symptoms	49	YES	YES	YES	YES	Prednisone (40 mg/day as the starting dose, gradually tapered), atenolol 50 mg/die

(Continued)

TABLE 1 | Continued

Year	N.	Reference	Country	Age	Gender	Preexisting Thyroid Disease	COVID-19 Diagnosis	Clinical presentation of COVID-19	Onset after COVID-19 (days)	General	Neck pain	Fever	Palpitations	Therapy
2020	18	(30)	Spain	28	F	NO	RT-PCR	Diarrhea, abdominal pain	14	YES	YES	YES	YES	Aspirin 500 mg and propranolol 40 mg every 6 hours
2020	19	(26)	Italy	43	F	NO	RT-PCR	Fever, mild upper respiratory symptoms	45	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2021	20	(40)	India	29	F	NO	RT-PCR	Fever, cough, and other flu-like symptoms	45	NO	YES	YES	YES	Indomethacin 25 mg and propanol 40 mg thrice daily
2020	21	(31)	Philippines	47	F	NO	RT-PCR	Lobar pneumonia	-47	YES	YES	NO	NO	Mefenamic acid, later shifted to celecoxib
2021	22	(35)	Iran	37	F	NO	Positive IgG	Myalgia for a few days	30	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2021	23	(35)	Iran	35	M	NO	Positive IgG	Asymptomatic		YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2021	24	(35)	Iran	41	F	NO	Positive IgG	Low-grade fever and mild myalgia for few days	30	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2021	25	(35)	Iran	52	M	NO	Positive IgG	Low-grade fever, dry cough, and mild myalgia for few days	30	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2021	26	(35)	Iran	34	F	NO	Positive IgG	Asymptomatic		YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)
2021	27	(35)	Iran	26	F	NO	Positive IgG	Self-limited dry cough for 1 week	30	YES	YES	YES	YES	Prednisone (25 mg/day as the starting dose, gradually tapered)

RT-PCR, real-time polymerase chain reaction; COVID-19, coronavirus disease 2019; SAT, subacute thyroiditis.

[a]*No direct demonstration of SARS-CoV-2.*

[b]*While on morphine for back surgery.*

[c]*Atrial fibrillation.*

Thyroid Laboratory Tests and Inflammation Markers

Serum assessment was quite heterogeneous among the 19 studies. Available data are summarized in **Table 2**.

Thyroid function tests were performed in all patients but one and showed overt thyrotoxicosis in all cases. Particularly, median TSH (available in 26 out of 27 cases) was 0.01 mU/l (IQR 0.008–0.07), median free-T4 (23 cases) was 27.2 pmol/l (IQR 22.4-31.1) with a median increase of 1.27 times (IQR 1.17–1.83) the upper limit of the reference range, and median-free T3 (15 cases) was 10.79 pmol/l (IQR 8.5–19.1) with a median increase of 2.11 times (IQR 1.67–2.80) of the upper limit of the reference range. Total T4 was measured in three (11.1%) patients ranging from 13.5 to 23.1 μg/dl. Total T3 was measured in eight (29.6%) patients with a median value of 2.15 ng/ml (IQR 1.865–2.49).

Thyroid autoantibodies were measured in 18 (66.6%) of cases. Indeed, anti-thyroglobulin antibodies (TgAb) and anti-thyroperoxydase antibodies (TPOAb) can be detected in some non-autoimmune thyroid diseases, such as SAT (42). Although some studies reported the *de novo* appearance of TgAb in up to 25%–50% of SAT patients, and, to a lesser extent, TPOAb, this positivity is usually transient and most patients do not develop autoimmune sequelae (43). Moreover, anti TSH-receptor antibody (TRAb) testing is performed in some patients in the thyrotoxic phase of SAT to exclude the presence of Graves' disease.

Positive TgAb tests were found in 3 out of 11 patients in whom they were measured. Positive TPOAb tests were found in 3 out of 16 patients. TRAb measurement was performed in 15 patients with negative results in all cases. Thyroglobulin was measured in five (18.5%) patients and was always elevated.

SAT-specific inflammation markers, such as CRP and ESR, were performed in 25 patients and were high in 24 (96%).

Thyroid-Specific Imaging

The results of thyroid-specific imaging performed for the included patients are summarized in **Table 3**. Thyroid ultrasound was performed in 24 cases (88.9%). The typical ultrasonographic characteristics of SAT (patchy hypoechogenic areas with reduced vascularization) were observed in 20 cases,

TABLE 2 | Biochemical evaluation of thyroid function parameters, thyroglobulin, thyroid autoantibodies, and inflammatory markers in the 27 included patients.

Year	N.	Ref	TSH	TSH ref range	FT3	FT3 ref range	FT4	FT4 ref range	Tg	TgAb	TPOAb	TRAb	CRP/ESR
2020	1	(32)	0.11	0.5–4.78 mIU/ml	N/A	N/A	2.18	0.89–1.76 ng/dl	N/A	N/A	Slightly positive	N/A	High
2020	2	(25)	<0.008	N/A	7.7	3.1–6.8 pmol/l	25.7	12–21 pmol/l	N/A	0	0	0	High
2020	3	(16)	<0.04	0.5–4.1 mIU/l	8.7	4.6–8.4 pmol/l	27.2	11–23 nmol/l	5.6	120 UI/ml (positive)	0	0	High
2020	4	(17)	0.1	0.4–4.5 mIU/ml	8.0	2.3 to 4.2 pmol/l	29.3	6–16 pmol/l	75.3	0	0	0	High
2020	5	(17)	< 0.01	0.4–4.5 mIU/ml	8.9	2.3 to 4.2 pmol/l	31.8	6–16 pmol/l	80	38 (positive)	0	0	High
2020	6	(17)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2020	7	(17)	<0.01	0.4–4.5 mIU/ml	6.9	2.3 to 4.2 pmol/l	27.8	6–16 pmol/l	N/A	N/A	N/A	0	Normal
2020	8	(28)	<0.001	N/A	211[a]	80–150 ng/dl[a]	1.6	0.7–1.48 ng/dl	N/A	0	0	N/A	High
2020	9	(39)	<0.005	0.27–4.2 mIU/l	2.88[a]	0.80–2.0 ng/ml[a]	20.11[b]	5.10–14.1 μg/dl[b]	N/A	N/A	N/A	N/A	High
2021	10	(29)	0.01	0.4–4.5 μIU/ml	202[a]	80–150 ng/dl[a]	2.3	0.6–1.3 ng/dl	N/A	N/A	0	0	High
2021	11	(37)	<0.001	N/A	236[a]	75–195 ng/dl [a]	23.1[b]	4–11 μg/dl[b]	N/A	N/A	0	0	High
2021	12	(34)	0.1	0.27–4.2 mU/l	N/A	N/A	21.2	12–22 pmol/l	N/A	6.61 U/ml (positive)	71.8 UI/ml (positive)	0	High
2020	13	(38)	0.08	0.27–4.2 mU/l	5.5	2–4.4 pg/ml	24.6	0.3–17 pg/ml	187	N/A	N/A	N/A	N/A
2021	14	(36)	<0.008	0.7–4.20 mIU/l	3.39[a]	1.232–3.08 nmol/l[a]	60.63	11.61–23.22 pmol/l	N/A	N/A	96.71 (positive)	0	High
2021	15	(41)	0.029	0.27–4.2 μIU/ml	1.2[a]	0.80–2.0 ng/ml[a]	2.1	0.8–1.7 ng/dl	N/A	0	0	0	High
2020	16	(27)	<0.01	0.65–3.70 mU/l	13.4	3.2–5.3 pmol/l	41.8	8.8–14.4 pmol/l	N/A	N/A	0	0	High
2020	17	(33)	0.01	mU/l	374[a]	80–150 ng/l[a]	4.4	0.6–1.3 ng/l	N/A	N/A	0	0	High
2020	18	(30)	<0.001	0.38–5.33 mU/l	N/A	N/A	37.5	7.0–16.0 pmol/l	N/A	0	0	0	High
2020	19	(26)	0.006	0.27–4.2 mU/l	7.03	1.71–3.71 pg/ml	2.69	0.7–1.48 ng/dl	188	0	0	0	High
2021	20	(40)	0.007	N/A	5.05[a]	ng/ml[a]	7.77	ng/dl	N/A	0	0	0	High
2020	21	(31)	0.05	0.47–4.68 μIU/ml	1.4[a]	0.97–1.69 ng/ml [a]	1.68	0.78–2.19 pg/ml	N/A	0	0	0	High
2021	22	(35)	<0.01	0.4–4.0 mU/l	25.4	3.1–6.8 pmol/l	2.3	12–21 pmol/l	N/A	N/A	N/A	N/A	High
2021	23	(35)	0.12	0.4–4.0 mU/l	19.3	3.1–6.8 pmol/l	24.7	12–21 pmol/l	N/A	N/A	N/A	N/A	High
2021	24	(35)	<0.01	0.4–4.0 mU/l	23.7	3.1–6.8 pmol/l	21.9	12–21 pmol/l	N/A	N/A	N/A	N/A	High
2021	25	(35)	0.17	0.4–4.0 mU/l	21.6	3.1–6.8 pmol/l	26.7	12–21 pmol/l	N/A	N/A	N/A	N/A	High
2021	26	(35)	0.23	0.4–4.0 mU/l	18.1	3.1–6.8 pmol/l	18.4	12–21 pmol/l	N/A	N/A	N/A	N/A	High
2021	27	(35)	0.07	0.4–4.0 mU/l	18.9	3.1–6.8 pmol/l	19.5	12–21 pmol/l	N/A	N/A	N/A	N/A	High

N/A, not available; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroperoxydase antibodies; TRAb, anti-TSH-receptor antibodies; CRP/ESR, C-reactive protein/erythrocyte sedimentation rate.

[a]*Total T3.*

[b]*Total T4.*

TABLE 3 | Results of thyroid-specific imaging in the 27 included patients.

Year	N.	Ref	Ultrasound	US typical for SAT	Scintigraphy
2020	1	(32)	Enlarged thyroid with heterogeneous echotexture	NO	Reduced uptake
2020	2	(25)	Increased vascularity, heterogeneous parenchyma	NO	N/A
2020	3	(16)	Multiple, diffuse hypoechoic areas	YES	N/A
2020	4	(17)	Enlarged thyroid gland with multiple hypoechoic areas and absent vascularization at color Doppler	YES	N/A
2020	5	(17)	Increased thyroid volume with bilateral diffuse hypoechoic areas and absent vascularization at color Doppler ultrasonography	YES	No uptake
2020	6	(17)	Increased thyroid volume (25 ml) with bilateral diffuse hypoechoic areas	YES	N/A
2020	7	(17)	Increased thyroid volume (18 ml) with bilateral diffuse hypoechoic areas and absent to mild vascularization at color Doppler ultrasonography	YES	N/A
2020	8	(28)	N/A		No uptake
2020	9	(39)	Diffuse bilateral enlargement of thyroid with hypoechogenicity and increased vascularity on color Doppler	YES	Reduced uptake
2021	10	(29)	Diffusely heterogeneous echotexture	NO	N/A
2021	11	(37)	Bilateral ill-defined hypoechoic areas	YES	N/A
2021	12	(34)	Patchy areas of variably-reduced parenchymal echogenicity bilaterally	YES	Reduced uptake
2020	13	(38)	Enlarged hypoechoic thyroid, decreased vascularity	YES	No uptake
2021	14	(36)	Heterogeneous thyroid gland with bilateral patchy ill-defined hypoechoic areas	YES	N/A
2021	15	(41)	Mildly enlarged thyroid gland with no increased vascularity and 5-mm bilateral cysts	NO	N/A
2020	16	(27)	Enlarged thyroid gland with heterogeneous echotexture; hypoechoic areas with ill-defined margins corresponding to the hard regions palpable. Reduced blood flow in both lobes	YES	N/A
2020	17	(33)	N/A		N/A
2020	18	(30)	N/A		No uptake
2020	19	(26)	Diffusely enlarged and hypoechogenic thyroid gland.	YES	Reduced uptake
2021	20	(40)	Enlarged heterogeneously hypoechoic left lobe of thyroid and isthmus with normal vascularity, and bulky right lobe of thyroid with few ill-defined hypoechoic areas which suggestive of thyroiditis.	YES	No uptake
2020	21	(31)	Slightly enlarged right thyroid lobe, with ill-defined hypoechogenicity and normal vascularity in both lobes	YES	N/A
2021	22	(35)	Hypoechoic areas	YES	N/A
2021	23	(35)	Hypoechoic areas	YES	N/A
2021	24	(35)	Hypoechoic areas	YES	N/A
2021	25	(35)	Hypoechoic areas	YES	N/A
2021	26	(35)	Hypoechoic areas	YES	N/A
2021	27	(35)	Hypoechoic areas	YES	N/A

N/A, not available; US, ultrasound.

while in the remaining cases non-specific patterns were described. Thyroid ^{99m}Tc-pertechnetate scintigraphy was performed in nine (33.3%) patients, with the uptake being either reduced (four cases) or absent (five cases).

SAT Treatment

Steroidal therapy was the most frequently administered therapy for SAT: the most used agent was prednisone (in 13 cases), but less frequently dexamethasone (in one case), prednisolone (in two cases), and methylprednisolone (in one case) were used. The median of prednisone equivalents administered per day was 25 mg (IQR 25–35). Seven patients received non-steroidal anti-inflammatory agents (including aspirin, indomethacin, and mefenamic acid). These therapies were often paired with beta-blockers (most frequently propranolol). Two patients with thyrotoxicosis were initially treated with anti-thyroid drugs and subsequently switched to steroid therapy.

SAT Outcome

A complete resolution of symptoms and thyrotoxicosis was recorded in most cases, with the exception of four patients (14.8%) in whom an evolution toward subclinical hypothyroidism was witnessed and another one who developed overt hypothyroidism.

Risk of Bias

Data extracted from the articles were almost complete in all the above outcomes except that regarding the laboratory tests. In fact, unfortunately, the antibody profile was reported in less than 50% of cases with a significant risk of bias due to missing results. On the contrary, the risk of bias was negligible in all the other outcomes.

Summary of Findings

The synthesis of results was made according to PRISMA statement (24). As reported in **Table 4**, based on the data found in the literature, the certainty of evidence was moderate in all outcomes except that of laboratory tests, which was lower.

DISCUSSION

SAT is generally secondary to upper respiratory tract infections by several viruses (3, 10); thus, SAT might represent a potential

TABLE 4 | Summary of findings about COVID-19-associated SAT.

Outcome	Participants (n)	Certainty of the evidence	Comments
Gender	27	Moderate	SAT more often recurs in women
Presentation of COVID-19	27	Moderate	In patients with SAT COVID-19 usually presents with mild upper respiratory symptoms
Time interval between SAT and COVID-19	24	Moderate	SAT typically manifests 3–60 days after COVID-19
SAT clinical presentation	27	Moderate	COVID-19-associated SAT usually manifests as classical SAT (i.e., neck pain, asthenia and malaise, palpitations, fever)
Thyroid laboratory tests and inflammation markers	Variable	Low to moderate	Overt thyrotoxicosis is present in all cases of SAT; CRP and ESR, and thyroglobulin are typically elevated
Thyroid US	24	Moderate	The ultrasonographic characteristics of classical SAT (patchy hypoechogenic areas with reduced vascularization) are observed in COVID-19-associated SAT
SAT treatment	27	Moderate	Steroidal therapy was the most frequently administered therapy
SAT outcome	27	Moderate	A complete resolution of symptoms and euthyroidism is reached in most cases

SAT, subacute thyroiditis; US, ultrasound; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
GRADE Working Group grades of evidence.
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect.

complication of SARS-CoV-2 infection. The fact that SARS-CoV-2 recognizes angiotensin-converting enzyme 2 (ACE-2) as its cellular entry receptor (44) and the recent demonstration of ACE-2 expression in follicular thyroid cells would further support this possibility (45–47).

Here we conceived a systematic review to achieve more solid evidence about the relationship between SAT and COVID-19. Particularly, we aimed to evaluate the size and quality of the published literature on this topic, and the clinical characteristics of SAT in this setting of patients.

Regarding the first objective, we found 19 studies reporting a total of 27 patients with SAT. Surprisingly, we did not find original articles with large sample size although the first case report was published 1 year ago (16). The 17 case reports (16, 25–34, 36–41) and the 2 case series (17, 35) described carefully the history of patients and their SAT. In this context, it should be highlighted that two recent systematic reviews on a similar topic included 21 (48) and 17 (49) SAT cases, respectively. Our systematic review, conducted 3 months later and conceived with two specific aims, found 27 cases. The fact that the number of reported SAT cases in literature did not exponentially increase in a time frame during which COVID-19 cases significantly increased worldwide indirectly confirms that SAT is a rare complication of COVID-19. Furthermore, because of the lack of large-sample studies, both size and quality of the literature about SAT in COVID-19 patients have to be considered poor. From this point of view, taking up the pertinent question raised by Brancatella et al. (17) of whether SAT is an underestimated manifestation of SARS-CoV-2, the issue remains unsolved. A most reasonable answer would be that SAT represents, at best, a rare complication of COVID-19.

Considering the second aim of the present systematic review, SAT occurred generally after COVID-19. Its clinical presentation appears to be similar to "classic" forms of SAT, encompassing neck pain, asthenia/malaise, fever, and palpitations. Under a biochemical point of view, the reported patients always presented with thyrotoxicosis, usually with elevated serum inflammation markers. When available, the ultrasonographic/scintigraphic features of these patients were generally typical of virus-related thyroiditis. In addition, most patients were treated with steroids with complete resolution of symptoms. It should be noted that the median initial dose of prednisone employed in the reported patients (25 mg/day) is lower than the one recommended by the most recent guidelines (40 mg daily) (50). In general, patients with post-COVID-19 SAT presented a moderate-mild form of the disease, in terms of both clinical and biochemical presentation, with no peculiar clinical features.

The COVID-19 presentation and severity in the 27 patients with SAT deserve to be discussed. First, some specific and peculiar symptoms of COVID-19, such as anosmia and/or dysgeusia, were reported in a minority of SAT patients (19%). It is worth underlining that the above symptoms received great informative emphasis, making it unlikely that they were left unrecognized. Second, most patients experienced a pauci- or asymptomatic COVID-19 disease, with only three patients requiring hospitalization, and that their median age was 37 years, much younger than the typical COVID-19 hospitalized patients. Moreover, the female-to-male ratio was high, this being the opposite of what happens in a case series of hospitalized COVID-19 patients (51). In this context, it should be highlighted that in a recent study by Trimboli et al. (52) aimed at searching for an association between SAT and SARS-CoV-2 in COVID-19-specific symptoms and contact tracing data, it was found that among the 10 included SAT patients, none had positive SARS-CoV-2 diagnostic tests, and only one case had a contact with people who were diagnosed with SARS-CoV-2.

To date, several large series studies as well as several reviews have evaluated the thyroid impact in COVID-19 hospitalized/discharged patients (18–22). Surprisingly, even if these large case series (18–20, 23) were specifically aimed at describing the

thyroidal repercussions of COVID-19, no occurrence of SAT was reported, allowing the following speculation. It could be that, among the hospitalized COVID-19 patients, the typical symptoms of SAT were misdiagnosed in severe COVID-19 disease or masked by the routine use of high-dose corticosteroids currently employed in critical COVID-19 patients (53). This hypothesis would hold particularly true only for the so-called "second wave" of the pandemic, since corticosteroids were contraindicated by most national guidelines in the early phase of the pandemic (54). It could be thus hypothesized that SAT would be a typical complication of less severe forms of COVID-19 occurring in community-dwelling, young female subjects that are less frequently included in clinical studies, while hospitalized patients would more often experience non-thyroidal illness syndrome due to the hyperinflammatory state typical of severe COVID-19 (55–57). In this context, a systematic review by Ruggeri et al. (58) provided a comprehensive review of all COVID-19-related inflammatory disorders. This paper highlighted, from a slightly different point of view, that thyroid dysfunction is frequently observed in COVID-19 patients, regardless of the underlying thyroid disease, and physicians should be aware of its possible occurrence. The fact that no extreme increases in the number of SAT cases occurred in the last months, even in areas greatly affected by COVID-19 (59), could be due to the fact that social distancing measures and mask use reduced the diffusion of other SAT-causative viruses, similarly to what happened with the H1N1 virus (60).

The here reviewed evidence has several limitations: even if the clues in favor of a SARS-CoV-2-induced SAT exist, the size of published cases is poor, with only case reports and case series being available. Also, our review process was greatly limited by the nature of the available literature and by the lack of uniformity in part of the data, mainly the laboratory tests performed.

These results can give evidence-based information to help clinicians who encounter cases of COVID-19-related SAT in everyday practice. In particular, available literature suggests that these forms of SAT are rather mild and do not require any specific treatment when compared with "classic" SAT forms. Nevertheless, this complication does not appear to be particularly frequent in COVID-19 patients, especially in those who require hospitalization. More data regarding larger series of patients with a more uniform evaluation of thyroid function parameters will be required to draw more firm conclusions on the real incidence of SAT in COVID-19 patients. Moreover, long-term follow-up data will indicate if patients who experience COVID-19-related SAT are at risk for long-term thyroid sequelae.

In conclusion, the size of published data of SAT in COVID-19 patients is poor and the SAT clinical presentation in COVID-19 patients appears overall similar from that generally expected. According to these evidence-based data, SAT cannot be considered as a direct or frequent complication of SARS-CoV-2. However, since the rapid worldwide diffusion of SARS-CoV-2 and its variants, the present findings might change in the next future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

PT, LCr, and MR designed and conceptualized the study, analyzed the data, and drafted the manuscript for intellectual content; all the co-authors interpreted the data and revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.707726/full#supplementary-material

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The Impact of Interferon Beta-1b Therapy on Thyroid Function and Autoimmunity Among COVID-19 Survivors

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Background: Some studies have indicated that interferon (IFN) may be valuable in COVID-19. We aimed to evaluate the impact of short-term IFN on incident thyroid dysfunction and autoimmunity among COVID-19 survivors.

Methods: We included consecutive adults without known thyroid disorder admitted to Queen Mary Hospital for COVID-19 from July 2020 to January 2021 who had thyroid function tests (TFTs) and anti-thyroid antibodies measured both on admission and at three months.

Results: 226 patients were included (median age 55.0 years; 49.6% men): 135 were IFN-treated. There tended to be more abnormal TFTs upon reassessment in IFN-treated patients (8.1% *vs* 2.2%, p=0.080). 179 patients (65.4% IFN-treated) had a complete reassessment of anti-thyroid antibodies. There were significant increases in titres of both anti-thyroid peroxidase antibodies (anti-TPO: baseline 29.21 units [IQR: 14.97 – 67.14] *vs* reassessment 34.30 units [IQR: 18.82 – 94.65], p<0.001) and anti-thyroglobulin antibodies (anti-Tg: baseline 8.23 units [IQR: 5.40 – 18.44] *vs* reassessment 9.14 units [IQR: 6.83 – 17.17], p=0.001) in the IFN-treated group but not IFN-naïve group. IFN treatment (standardised beta 0.245, p=0.001) was independently associated with changes in anti-TPO titre. Of the 143 patients negative for anti-TPO at baseline, 8 became anti-TPO positive upon reassessment (seven IFN-treated; one IFN-naïve). Incident anti-TPO positivity was more likely to be associated with abnormal TFTs upon reassessment (phi 0.188, p=0.025).

Conclusion: IFN for COVID-19 was associated with modest increases in anti-thyroid antibody titres, and a trend of more incident anti-TPO positivity and abnormal TFTs during convalescence. Our findings suggest that clinicians monitor the thyroid function and

anti-thyroid antibodies among IFN-treated COVID-19 survivors, and call for further follow-up studies regarding the clinical significance of these changes.

Keywords: COVID-19, SARS-CoV-2, thyroid function tests, autoimmunity, interferon beta-1b, thyroid gland

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected more than 190 million people worldwide and caused more than 4 million deaths (1). Various therapeutic options are evaluated for COVID-19. Some studies have indicated that interferon may be valuable in COVID-19. Interferon beta-1b has been shown to shorten the duration of viral shedding, alleviate symptoms, reduce cytokine responses, and may reduce mortality and intensive care unit admission (2, 3).

Interferon beta-1b is an example of drug repurposing to fight against COVID-19 (4). Indeed, interferon beta-1b is a well-established therapy for multiple sclerosis (5). As a maintenance therapy used in a chronic setting, its long-term safety has been evaluated. Incident thyroid dysfunction and autoimmunity have been recognised in chronic interferon beta-1b therapy among patients with multiple sclerosis in retrospective and prospective, monocentric and multicentric studies (6). In contrast to the long-term treatment with interferon beta-1b in multiple sclerosis in terms of years, interferon beta-1b is given for a much shorter duration (typically a few days) in the context of acute COVID-19. Nonetheless, given the concerns of thyroid dysfunction and autoimmunity with interferon beta-1b in multiple sclerosis, it is prudent to investigate whether short-term interferon beta-1b therapy in acute COVID-19 is associated with thyroid dysfunction and autoimmunity. This will inform our clinical practice in the management of COVID-19 patients.

Hence, we carried out this prospective study of COVID-19 survivors to evaluate the impact of interferon beta-1b therapy on thyroid function and autoimmunity.

METHODS

The public health ordinance in Hong Kong required all patients tested positive for COVID-19 to be admitted to the hospital, including those detected on contact tracing and the Universal Community Testing Programme, regardless of symptoms (7). Our institution is one of the major centres in Hong Kong receiving confirmed COVID-19 patients. Consecutive adult patients (aged ≥18 years) admitted to our institution for COVID-19 between 21 July 2020 and 20 January 2021 were prospectively recruited. The presence of SARS-CoV-2 was confirmed in all patients by reverse transcription-polymerase chain reaction (RT-PCR) from the nasopharyngeal swab (NPS) or deep throat saliva (DTS), using the LightMix SarbecoV E-gene assay (TIB Molbiol, Berlin, Germany), which targeted the envelope protein (E) gene of SARS-CoV-2 (7, 8). Exclusion criteria were (i) history of thyroid, hypothalamic or pituitary disorders; (ii) use of anti-thyroid drugs or thyroid hormone replacement; and (iii) use of medications with potential impact on thyroid function, including systemic steroid, amiodarone, heparin and dopamine, before admission. Each patient had baseline blood tests taken within 24 hours after admission before starting COVID-19 treatments.

Serum thyroid-stimulating hormone (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) were measured with immunoassays ADVIA Centaur® TSH3-Ultra, FT4 and FT3 assays, respectively (Siemens Healthcare Diagnostics Inc., Erlangen, Germany). The reference ranges for TSH, fT4, and fT3 were 0.35–4.8 mIU/L, 12–23 pmol/L and 3.2–6.5 pmol/L, respectively. Anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase (anti-TPO) antibody titres were measured with QUANTA Lite® Thyroid T and TPO enzyme-linked immunosorbent assay, respectively (Inova Diagnostics, San Diego, CA, USA). Positive anti-Tg and anti-TPO was defined by >100 World Health Organization (WHO) units (thereafter 'units'), as specified by the manufacturer. Basic haematology and biochemistry panel, glycated haemoglobin (HbA1c) and inflammatory markers (C-reactive protein [CRP], erythrocyte sedimentation rate) were measured. Abnormal laboratory parameters were defined according to their respective reference ranges (7).

Demographics and significant comorbidities were recorded. Obesity was defined by the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code 278.0. Diabetes was defined by a known diagnosis of diabetes or HbA1c ≥6.5% on admission. Charlson comorbidity index was calculated for each patient. COVID-19-related symptoms were evaluated with a standard checklist. Respiratory rate, baseline oxygen saturation by pulse oximetry, and oxygen requirement on admission were captured. Cycle threshold (Ct) values were obtained from the qualitative LightMix SarbecoV E-gene assay (TIB Molbiol, Berlin, Germany) performed on specimens from NPS or DTS (whichever was lower) on admission. The Ct value represents the number of cycles required for a gene target or a PCR product to be detected. While viral loads were not directly measured with a dedicated quantitative RT-PCR assay in this analysis, studies have shown a good correlation between Ct values and SARS-CoV-2 viral loads (9, 10), such that the lower the Ct values, the higher the viral loads.COVID-19 severity was classified according to the 'Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition)' published by the Chinese National Health Commission (11). Patients' clinical outcomes were captured. For patients treated for COVID-19, one or more of the following were given: clofazimine (12), ribavirin, interferon beta-1b, or remdesivir (2). Dexamethasone (13) and subcutaneous low-molecular-

weight heparin (LMWH) (14) were added at physicians' discretion as clinically indicated. Interferon beta-1b was given once daily subcutaneously at a dose of 16 million IU. The decision to use interferon beta-1b was not influenced by the baseline thyroid function and antibody levels. The duration of interferon beta-1b therapy was recorded.

Follow-up visits were arranged around three months from admission to reassess thyroid function tests (TFTs) and anti-thyroid antibodies. Patients who had TFTs reassessed were included in the current study.

The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Written consent has been obtained from each patient or subject after fully explaining the purpose and nature of all procedures used.

All statistical analyses were performed with IBM® SPSS® version 26. Two-sided p-values <0.05 were considered statistically significant. Data were presented as median with interquartile range (IQR) or number with percentage as appropriate. Between-group comparisons were performed with the t-test or Mann-Whitney U test for continuous variables as appropriate and Chi-square or Fisher's exact tests for categorical variables as appropriate. Within-group comparisons were performed with paired t-test or Wilcoxon signed-rank test for continuous variables and McNemar's test for categorical variables. Phi-coefficient was used to assess the relationship between two dichotomous categorical variables. Multivariable linear regression analysis was used to identify the independent determinants of changes in anti-TPO titre. All variables with statistical significance (p<0.05) in the univariate analysis were included in the multivariable regression analysis. Values not normally distributed were logarithmically transformed before analyses.

RESULTS

Baseline Characteristics of the Cohort

A total of 226 patients were included, with their baseline characteristics summarised in **Table 1**. Their median age was 55.0 years, with no sex preponderance. Hypertension, diabetes and obesity were the most common comorbidities. The majority had non-severe acute COVID-19. 59.7% of the cohort received interferon beta-1b, and 16.4% received dexamethasone. The median duration of interferon beta-1b therapy was 5 days (range: 1 – 15 days; 65.9% received 5 days of interferon). Only 2.2% of the cohort required intensive care unit admission.

Evolution of TFTs in the Cohort

Reassessment TFTs were performed at a median interval of 90 days (IQR: 68 – 105) after acute COVID-19. Out of the 46 patients who had abnormal TFTs upon admission, 38 (82.6%) recovered during convalescence. The evolution of thyroid function in the cohort is summarised in **Figure 1**. Four remained in subclinical thyrotoxicosis, defined by low TSH (i.e. <0.35 mIU/L) with normal fT4 and fT3 (i.e. fT4 and fT3 within their respective reference ranges). One had persistently low fT3 on reassessment, as he was admitted for fluid overload and clinically ill at the time of reassessment. One patient who initially had low fT3 developed T3-toxicosis (i.e. suppressed TSH <0.01 mIU/L with normal fT4 but elevated fT3 levels) at three months, followed by spontaneous resolution another three months later, suggestive of painless thyroiditis. Two patients had persistent subclinical hypothyroidism with positive anti-TPO, likely pre-existing Hashimoto's thyroiditis diagnosed upon admission for COVID-19.

Among the 180 patients who had normal TFTs at baseline, 5 had abnormal TFTs upon reassessment: one patient had subclinical hypothyroidism; two patients had isolated mildly elevated fT4 levels; one had isolated mildly elevated fT3 levels; the remaining patient had isolated mildly low fT4 level.

Among all 226 patients, 135 were treated with interferon beta-1b. The comparison of the patients who were and were not treated with interferon is summarised in **Table 1**. Interferon-treated patients had higher SARS-CoV-2 viral load and more lymphopenia on admission. The length of stay of interferon-treated patients was longer. As ribavirin was given in combination with interferon, the interferon-treated patients were more likely to be treated with ribavirin. There tended to be more abnormal TFTs upon reassessment among interferon-treated patients (interferon-treated 11/135 [8.1%] *vs* interferon-naïve 2/91 [2.2%], p=0.080). Subgroup analysis of patients with normal TFTs at baseline (n=180) showed numerically more abnormal TFTs upon reassessment among interferon-exposed patients compared to those interferon-naïve (interferon-treated 4/104 [3.8%] *vs* interferon-naïve 1/76 [1.3%], p=0.308).

Impact of Interferon beta-1b on Anti-Thyroid Antibodies

The impact of interferon beta-1b on anti-thyroid antibodies was evaluated in 179 patients who had complete anti-TPO and anti-Tg results available at baseline and reassessment (**Figure 2**). One hundred seventeen patients were treated with interferon beta-1b, while 62 patients were interferon-naïve. There was no difference in age, sex, baseline COVID-19 severity, and baseline anti-TPO and anti-Tg positivity between interferon-treated and interferon-naïve patients. We first analysed the changes in anti-TPO and anti-Tg titres according to interferon exposure. (**Figure 3**) Among interferon-treated patients, both anti-TPO and anti-Tg titres showed statistically significant, but modest, increases upon reassessment. Anti-TPO titres increased from 29.21 units (IQR: 14.97 – 67.14) to 34.30 units (IQR: 18.82 – 94.65) (p<0.001). Anti-Tg titres increased from 8.23 units (IQR: 5.40 – 18.44) to 9.14 units (IQR: 6.83 – 17.17) (p<0.001). On the other hand, among interferon-naïve patients, titres of anti-TPO (baseline: 33.14 units [IQR: 19.77 – 79.12] *vs* reassessment: 27.80 units [IQR: 18.80 – 81.73], p=0.228) and anti-Tg (baseline: 9.76 units [IQR: 6.60 – 15.85] *vs* reassessment: 9.96 units [IQR: 6.92 – 15.89], p=0.908) did not significantly change upon reassessment. Changes in anti-TPO titres among the interferon-treated group were significantly different from the interferon-naïve group (interferon-treated: 3.75 units [IQR: -1.91 to 11.96] *vs*

TABLE 1 | Baseline characteristics of the cohort (n = 226).

	All	Interferon-treated	Interferon-naïve	P value
Number	226	135	91	—
Baseline characteristics				
Age (years)	55.0 (41.8 – 63.0)	56.0 (42.0 – 64.0)	54.0 (39.0 – 62.0)	0.297
Male	112 (49.6%)	66 (48.9%)	46 (50.5%)	0.807
Smoking	28/191 (14.7%)	18/112 (16.1%)	10/79 (12.7%)	0.511
Drinking	40/185 (21.6%)	21/106 (19.8%)	19/79 (24.1%)	0.488
Abnormal TFTs on admission	46 (20.4%)	31 (23.0%)	15 (16.5%)	0.235
Baseline anti-TPO positive	45 (19.9%)	26 (19.3%)	19 (20.9%)	0.765
Baseline anti-Tg positive	22 (9.7%)	16 (11.9%)	6 (6.6%)	0.191
COVID-19 severity				0.293
Mild	155 (68.6%)	97 (71.9%)	58 (63.7%)	
Moderate	61 (27.0%)	32 (23.7%)	29 (31.9%)	
Severe	10 (4.4%)	6 (4.4%)	4 (4.4%)	
SARS-CoV-2 PCR Ct value	24.10 (18.67 – 29.45)	21.77 (17.49 – 26.90)	28.10 (19.95 – 32.19)	**<0.001**
Lymphopenia	99 (43.8%)	67 (49.6%)	32 (35.2%)	**0.032**
CRP (mg/dL)	1.05 (0.31–2.83)	1.00 (0.31 – 2.82)	1.04 (0.31 – 3.06)	0.444
ESR (mm/hr)	40 (23 – 62)	39 (21 – 55)	46 (24 – 71)	0.068
Comorbidities				
Charlson comorbidity index				0.772
0	173 (76.5%)	104 (77.0%)	69 (75.8%)	
1	30 (13.3%)	18 (13.3%)	12 (13.2%)	
≥2	23 (10.2%)	13 (9.6%)	10 (11.0%)	
Hypertension	54 (23.9%)	33 (24.4%)	21 (23.1%)	0.813
Diabetes mellitus	32 (14.2%)	20 (14.8%)	12 (13.2%)	0.731
Obesity	17 (7.5%)	9 (6.7%)	8 (8.8%)	0.553
Malignancy	15 (6.6%)	7 (5.2%)	8 (8.8%)	0.286
CAD or heart failure	9 (4.0%)	5 (3.7%)	4 (4.4%)	0.999
Pulmonary disease	9 (4.0%)	5 (3.7%)	4 (4.4%)	0.999
Stroke or TIA	4 (1.8%)	3 (2.2%)	1 (1.1%)	0.650
Clinical course				
Length of hospitalisation (days)	8 (6–12)	9 (7 – 13)	6 (2 – 11)	**<0.001**
Oxygen requirement	27 (11.9%)	17 (12.6%)	10 (11.0%)	0.715
Intensive care unit admission	5 (2.2%)	4 (3.0%)	1 (1.1%)	0.651
Treatment				
Interferon beta-1b	135 (59.7%)	135 (100%)	0 (0%)	**<0.001**
Ribavirin	98 (43.4%)	98 (72.6%)	0 (0%)	**<0.001**
Remdesivir	54 (23.9%)	29 (21.5%)	25 (27.5%)	0.300
Dexamethasone	37 (16.4%)	25 (18.5%)	12 (13.2%)	0.288
Clofazimine	5 (2.2%)	4 (3.0%)	1 (1.1%)	0.651
SC LMWH	5 (2.2%)	4 (3.0%)	1 (1.1%)	0.651

Data presented as median with interquartile range or number with percentage as appropriate.

TFT, thyroid function test; anti-TPO, anti-thyroid peroxidase antibody; anti-Tg, anti-thyroglobulin antibody; COVID-19, coronavirus disease 2019; PCR Ct value, polymerase chain reaction cycle threshold value; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CAD, coronary artery disease; TIA, transient ischaemic attack; SC LMWH, subcutaneous low molecular weight heparin, Values in bold represent statistical significance.

interferon-naïve: -2.63 units [IQR: -8.95 to 6.45], p=0.001). In contrast, the changes in anti-Tg titres were not significantly different between the two groups (p=0.294).

We further studied the factors associated with changes in anti-TPO titres upon reassessment. Among the continuous variables, age, baseline CRP or ESR, SARS-CoV-2 PCR Ct values, or length of hospitalisation did not correlate with changes in anti-TPO titres. Among the categorical variables, only interferon beta-1b treatment was significantly associated with changes in anti-TPO titres (p=0.001), but not sex, smoking/drinking, abnormal TFTs on admission, baseline COVID-19 severity, baseline anti-TPO positivity, baseline lymphopenia or comorbidities. Multivariable linear regression analysis showed that interferon beta-1b treatment (standardised beta 0.245, p=0.001) was a positive independent determinant of changes in anti-TPO titres.

To investigate the impact of interferon treatment on incident anti-TPO positivity, we analysed the 143 patients who were anti-TPO negative at baseline: 94 were interferon-treated, and 49 were interferon-naïve. Eight patients became anti-TPO positive upon reassessment (**Table 2**). There were more events of incident anti-TPO positivity in the interferon-treated group than in the interferon-naïve group, although not reaching statistical significance due to the small number of events (interferon-treated 7/94 [7.4%] *vs* interferon-naïve 1/49 [2.0%], p=0.264). Interestingly, with incident anti-TPO positivity, it was more likely to observe abnormal TFTs upon reassessment (phi 0.188, p=0.025).

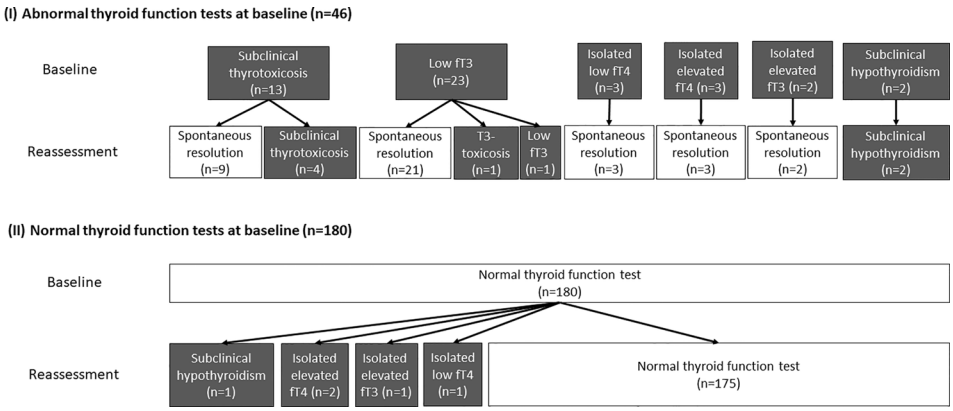

FIGURE 1 | The evolution of the thyroid function of all 226 patients (grey boxes represent abnormal thyroid function while white boxes represent normal thyroid function).

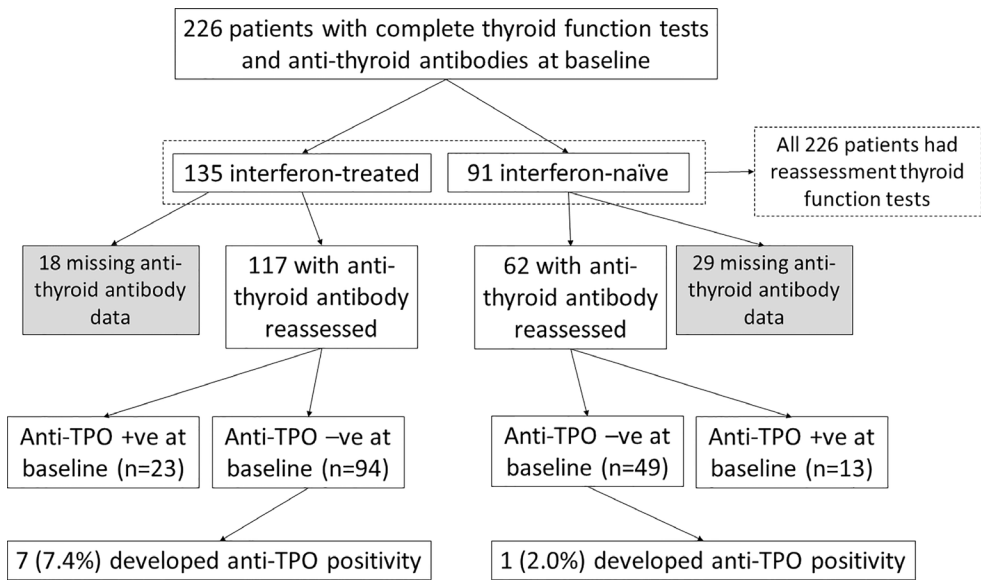

FIGURE 2 | Flow diagram of the study.

DISCUSSION

We provided the first systematic analysis of the impact of COVID-19 treatment on the evolution of thyroid function and autoimmunity in patients with COVID-19. Our main finding was that interferon beta-1b treatment for COVID-19, even for such a short duration of a few days, could induce modest increases in anti-thyroid antibody titres and be associated with more incident anti-TPO positivity upon reassessment at three months. Furthermore, abnormal TFTs upon reassessment was more likely in interferon-treated patients and patients having incident anti-TPO positivity. Our findings would support the need for thyroid function and antibody monitoring in interferon-treated COVID-19 patients, and call for further follow-up studies regarding the clinical significance of these changes.

The interrelationship between thyroid and COVID-19 has become more evident with concerted efforts from research groups in basic science and clinical thyroidology (15, 16). With the expanding therapeutic armamentarium for COVID-19, the impact of COVID-19 treatment on the thyroid should be evaluated. Our current study focused on interferon beta-1b because of the concern about incident thyroid dysfunction and autoimmunity with its chronic use in multiple sclerosis. Some studies have indicated that interferon may be valuable in COVID-19, especially when initiated in the early stage of

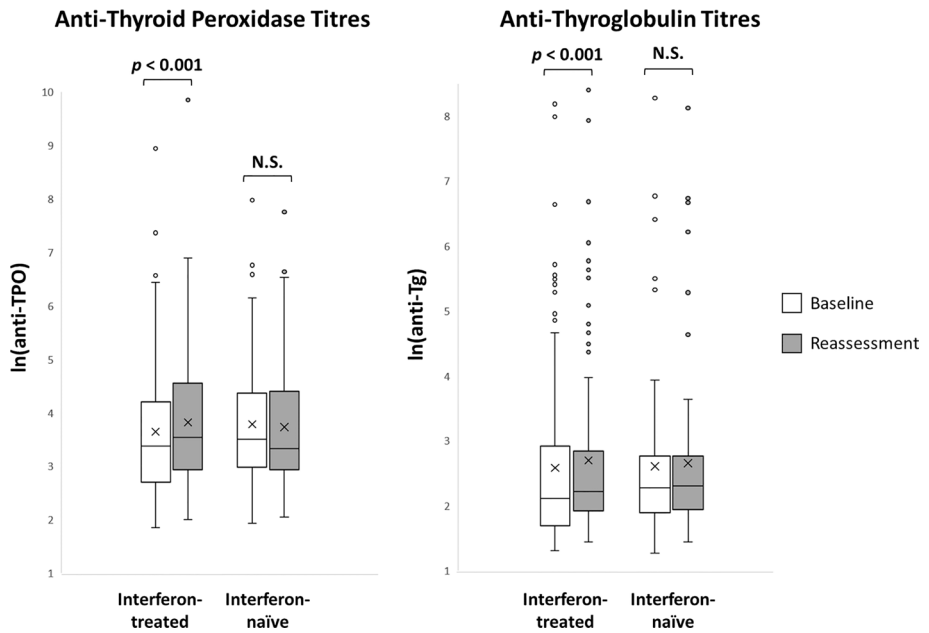

FIGURE 3 | Anti-thyroid antibody titres at baseline and reassessment according to interferon exposure (n = 179). N.S., not significant.

TABLE 2 | The thyroid function and antibody profile of patients who developed incident anti-TPO positivity upon reassessment.

Patient number	Sex/ Age	On admission					COVID-19 treatment	Reassessment					
		TSH[a]	fT4[a]	fT3[a]	Anti-TPO[b]	Anti-Tg[b]		Days	TSH[a]	fT4[a]	fT3[a]	Anti-TPO[b]	Anti-Tg[b]
1	M/55	1.6	15	3.3	89.28	21.07	REM	71	3.7	14	3.7	**197.9**	38.01
2	M/58	0.55	16	**2.9**	64.92	**2957.1**	IFN+RIB+DEX	102	**0.02**	21	**7.2**	**100.12**	**2793.9**
3	F/67	1.3	18	N/A	45.45	4.31	IFN+RIB	89	1.7	17	5.3	**148.21**	6.58
4	F/61	0.61	19	4.1	44.36	5.10	IFN+RIB	109	0.45	18	4.7	**129.69**	5.62
5	F/61	1.2	17	3.5	6.85	4.90	IFN+RIB	93	2.8	20	5.0	**141.24**	8.31
6	M/21	1.1	17	4.4	99.85	6.62	IFN+RIB	105	0.47	19	5.2	**128.51**	9.67
7	M/51	2.2	16	4.6	97.06	4.84	IFN+RIB	90	1.7	20	5.6	**122.37**	7.31
8	M/45	1.6	13	4.9	82.88	8.97	IFN+REM +DEX	28	1.6	**11**	5.1	**108.09**	6.23

M, male; F, female; TSH, thyroid-stimulating hormone (mIU/L); fT4, free thyroxine (pmol/L); fT3, free triiodothyronine (pmol/L); anti-TPO, anti-thyroid peroxidase antibody (units); anti-Tg, anti-thyroglobulin antibody (units); COVID-19, coronavirus disease 2019; IFN, interferon beta-1b; RIB, ribavirin; REM, remdesivir; DEX, dexamethasone; N/A, not available.
Age expressed in years.
[a]*Reference ranges: TSH 0.35 – 4.8 mIU/L, fT4 12–23 pmol/L, fT3 3.2–6.5 pmol/L.*
[b]*Positive anti-TPO defined by >100 units; positive anti-Tg defined by >100 units.*
Values out of reference ranges are in bold.

viraemia. Hung et al. have demonstrated early initiation of triple therapy (interferon beta-1b as the backbone) within one week from symptom onset to be effective (17). Similarly, a trial in Toronto evaluating peginterferon lambda treatment within one week from symptom onset has demonstrated efficacy in viral clearance (18). Although interferon use in the multinational SOLIDARITY trial has demonstrated no significant benefits in overall mortality, initiation of ventilation, and duration of hospital stay among hospitalised COVID-19 patients, the negative results could be explained by the probably late initiation of treatment (19). Antiviral agents are likely to be most effective during the early stage of viraemia, which is before the inflammatory pulmonary phase requiring hospitalisation (20). Hence, the treatments in the SOLIDARITY trial may not work in this late inflammatory pulmonary phase of the COVID-19. Furthermore, the patient characteristics in the SOLIDARITY trial were heterogeneous, and information on SARS-CoV-2 viral load was not available. As one of the potential treatment options of COVID-19, it is therefore clinically relevant to understand the impact of interferon treatment on the thyroid.

Earlier longitudinal studies of the impact of interferon beta-1b treatment among patients with multiple sclerosis reported up to 33% thyroid dysfunction and 20% thyroid autoimmunity, especially during the first year of treatment (21, 22). However,

another study suggested only a random non-significant change in thyroid function, which did not correlate with thyroid autoimmunity (6). A subsequent larger cohort study of 106 patients with multiple sclerosis, with a much longer follow-up for up to 7 years, provided a clearer picture. Up to a quarter of patients developed incident thyroid dysfunction and autoimmunity, mainly during the first year of treatment with interferon beta (1a or 1b), supporting the need for thyroid function and antibody monitoring, especially during the first year (23). Among these, some developed permanent hypothyroidism. Moreover, pre-existing or incident thyroid autoimmunity was predictive of incident thyroid dysfunction (23). A further Italian long-term follow-up study evaluating various disease-modifying agents for multiple sclerosis confirmed the direct role of interferon therapy on the thyroid, showing a rate of around 10% for thyroid dysfunction and autoimmunity on treatment (24). Proposed mechanisms of the impact of interferon therapy on the thyroid include a direct inhibitory effect of interferon on iodine organification, especially in patients who develop hypothyroidism without antibody production (21); and the autoimmune reaction or immune system dysregulation associated with chronic interferon exposure (24). Compared to chronic use in multiple sclerosis, we have evaluated the impact of much shorter treatment duration (median of 5 days) and a more intense dosing regimen (16 million IU/day), in contrast to the usual regimen of 8 million IU every other day as a chronic therapy in multiple sclerosis. Intriguingly, even with such a short duration of interferon therapy, we still observed a 5.6% rate of incident anti-TPO positivity at three months, which correlated with a higher chance of abnormal TFTs during convalescence. Moreover, we noted a trend towards more abnormal TFTs upon reassessment in interferon-treated patients, although most of these were not clinically overt. Despite a modest magnitude of anti-TPO titre elevation among patients who developed incident anti-TPO positivity, further follow-up is warranted for potential subsequent thyroid dysfunction as the occurrence of anti-TPO can precede thyroid dysfunction (25). Hence, our findings would suggest the need for TFT and anti-thyroid antibody monitoring, especially in COVID-19 patients treated with interferon beta-1b. Further follow-up of these patients on a longer-term will elucidate whether this phenomenon is transient or permanent.

In our cohort, most interferon-treated patients were also treated with ribavirin, and none were treated with ribavirin alone. It could be difficult to tell whether interferon beta-1b alone or the interferon-ribavirin combination accounted for the increase in anti-thyroid antibody titres. Ribavirin is commonly used together with interferon in treating hepatitis C virus (HCV) infection. Ribavirin induces the production of T helper 1 (Th1) cytokines in the immune response against HCV. Combining ribavirin with interferon, therefore, stimulates the immune system response and eradicates HCV from the body. The Th1-like immune response involved has been shown to be a factor in the development and maintenance of organ-specific autoimmune diseases. Hence, ribavirin may be associated with the occurrence of autoimmunity (26). Nonetheless, a previous study has shown that adding ribavirin to interferon-alpha therapy in patients with HCV-related chronic hepatitis did not modify the anti-thyroid antibody pattern (27).

Studying the patterns of thyroid function and autoimmunity in the interferon-naïve group could facilitate our understanding of the potential of SARS-CoV-2 in triggering autoimmunity (28). In our cohort, we did not observe significant increases in anti-TPO and anti-Tg titres among the interferon-naïve group. There was only one patient having incident anti-TPO positivity among the 62 interferon-naïve patients. From our current prospective longitudinal observational study, there is no convincing evidence of COVID-19 triggering autoimmunity suggested by existing case reports of patients who developed Graves' disease and Hashimoto thyroiditis following the diagnosis of COVID-19 (29). Nevertheless, SARS-CoV-2 infection may cause destructive thyroiditis (30), which may lead to subsequent development of autoimmunity, exemplified by cases of Graves' disease (31) and Hashimoto thyroiditis (32) a few months after the initial episode of subacute thyroiditis (believed to be of viral origin). We have yet to observe these phenomena in our cohort, possibly related to the relatively short follow-up duration. Most patients with subclinical thyrotoxicosis in the acute COVID-19 in our cohort spontaneously normalised at three months. A longer-term follow-up of this cohort may shed light on this postulated sequela.

The strength of our study was the systematic reassessment of both thyroid function and anti-thyroid antibodies stratified by interferon exposure, showing an increase in anti-TPO and anti-Tg titres and incident anti-TPO positivity in the interferon-treated group, despite the anti-TPO and anti-Tg assays being semi-quantitative. The fact that the COVID-19 severity was non-severe for most patients in our cohort means that our results may be generalisable to COVID-19 patients at large. There are certain limitations in this study. Firstly, the sample size was relatively small, and there were missing data regarding anti-thyroid antibodies upon reassessment. Nevertheless, baseline characteristics including age, sex, COVID-19 severity, SARS-CoV-2 viral load and abnormal TFTs were comparable between those with and without anti-thyroid antibodies upon reassessment. Secondly, SARS-CoV-2 viral loads were represented by Ct values. Despite a good correlation (9, 10), direct quantitative measurements of viral loads would have been preferable if available. Thirdly, obesity was defined by the ICD-9-CM diagnostic code in our study as a categorical variable, instead of body mass index as a continuous variable, and was likely to be underreported. Fourthly, high-resolution computed tomography was done at the physicians' discretion. Thus, the detection of imaging features of pneumonia in our cohort might be less sensitive. Fifthly, thyroid imaging was not available to assess the potential impact of interferon therapy or SARS-CoV-2 on the thyroid beyond TFT and anti-thyroid antibodies. Finally, the duration of follow-up was relatively short in the current study. The longer-term impact of interferon beta-1b will be informed with the continuation of follow-up of this cohort.

CONCLUSION

Interferon beta-1b treatment, even when used in the short term for COVID-19, could induce modest increases in anti-thyroid antibody titres. It was also associated with incident anti-TPO positivity during convalescence, correlating with a higher likelihood of abnormal TFTs during convalescence. If short courses of interferon treatment become a standard therapy for COVID-19, the modest changes detected thus far may indicate a phenomenon of clinical importance. As for now, our findings suggest that clinicians monitor the thyroid function and anti-thyroid antibodies among interferon-treated COVID-19 survivors, and call for further follow-up studies regarding the clinical significance of these changes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DL wrote the manuscript. DL, IH, CHL, AL, AT, PP, TH, CC, CYL, and WC researched the data. DL and CF performed statistical analyses. IH, CHL, AL, KKT, CWL, WC, YW, KL, and KCT critically reviewed and edited the manuscript. KCT initiated and supervised the study, is the guarantor of this work, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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The Independent Association of TSH and Free Triiodothyronine Levels With Lymphocyte Counts Among COVID-19 Patients

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Background: Both lymphopenia and thyroid dysfunction are commonly observed among COVID-19 patients. Whether thyroid function independently correlates with lymphocyte counts (LYM) remains to be elucidated.

Methods: We included consecutive adults without known thyroid disorder admitted to Queen Mary Hospital for COVID-19 from July 2020 to April 2021 who had thyroid-stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3) and LYM measured on admission.

Results: A total of 541 patients were included. Median LYM was 1.22 x 10^9/L, with 36.0% of the cohort lymphopenic. 83 patients (15.4%) had abnormal thyroid function tests (TFTs), mostly non-thyroidal illness syndrome (NTIS). Patients with lymphopenia had lower TSH, fT4 and fT3 levels than those without. Multivariable stepwise linear regression analysis revealed that both TSH (standardized beta 0.160, p<0.001) and fT3 (standardized beta 0.094, p=0.023), but not fT4, remained independently correlated with LYM, in addition to age, SARS-CoV-2 viral load, C-reactive protein levels, coagulation profile, sodium levels and more severe clinical presentations. Among the 40 patients who had reassessment of TFTs and LYM after discharge, at a median of 9 days from admission, there were significant increases in TSH (p=0.031), fT3 (p<0.001) and LYM (p<0.001). Furthermore, patients who had both lymphopenia and NTIS were more likely to deteriorate compared to those who only had either one alone, and those without lymphopenia or NTIS (p for trend <0.001).

Conclusion: TSH and fT3 levels showed independent positive correlations with LYM among COVID-19 patients, supporting the interaction between the hypothalamic-pituitary-thyroid axis and immune system in COVID-19.

Keywords: COVID-19, SARS-CoV-2, thyroid function tests, lymphopenia, lymphocytes, euthyroid sick syndromes

INTRODUCTION

Lymphopenia is a common hematologic finding in coronavirus disease 2019 (COVID-19) (1), carrying prognostic implication in view of its association with disease severity and mortality (2). On the other hand, thyroid involvement by COVID-19 is increasingly recognized since the first report of subacute thyroiditis after COVID-19 (3). Data from larger cohorts of COVID-19 patients have enabled better delineation of the patterns of thyroid dysfunction, which include thyroiditis and non-thyroidal illness (NTIS) (4). NTIS, characterized by low free triiodothyronine (fT3) levels, also carries prognostic implication in COVID-19 (5–7). Furthermore, patients with more severe illness were reported to have concomitant low thyroid-stimulating hormone (TSH) levels (6, 7). These highlight the clinical relevance of lymphocyte counts, TSH and thyroid hormones in the course of COVID-19. Studies have suggested potential effects of TSH and thyroid hormones on the immune system, including the lymphocyte population (8). For example, animal studies have suggested the potential role of TSH in improving lymphocyte proliferation (9); circulating thyroid hormone levels are positively associated with immunological reactivity among healthy individuals, such as maintenance of the lymphocyte subpopulations (10).

In COVID-19, postulated mechanisms for lymphopenia include: (i) the direct effect of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on the apoptosis of lymphocytes, bone marrow impairment and thymic suppression; (ii) cytokine-induced apoptosis of lymphocytes; and (iii) metabolic and biochemical derangements (such as lactic acidosis) (11) which may influence the production, survival and function of lymphocytes (2). In fact, all these postulated mechanisms may also lead to the disturbances in the hypothalamic-pituitary-thyroid axis, previously reported in COVID-19 patients (7, 12, 13). COVID-19 may also involve multiple extrapulmonary systems, as SARS-CoV-2 entry receptor – angiotensin converting enzyme 2 (ACE2) – expression is found in a wide variety of human tissues (14). A recent study suggested potential associations between TSH/thyroid hormones and lymphopenia in a Dutch cohort of COVID-19 patients, showing that patients with severe lymphopenia had lower TSH, free thyroxine (fT4) and fT3 levels and higher levels of inflammatory markers, similar to findings in patients with bacterial sepsis (15). Whether this association is an epiphenomenon confounded by the presence of all the above discussed factors remains to be elucidated. Hence, we carried out this study to investigate whether an independent association exists between TSH/thyroid hormones and lymphopenia in COVID-19 patients, which may shed light onto the interaction of TSH/thyroid hormones with the immune system in the clinical course of COVID-19.

METHODS

Public health ordinance in Hong Kong required all patients tested positive for COVID-19 be admitted to hospital (16), including those detected on contact tracing and Universal Community Testing Programme (17), regardless of symptoms. Our institution, Queen Mary Hospital, is one of the major centers in Hong Kong receiving confirmed COVID-19 patients. Consecutive adult patients (aged ≥18 years) admitted to Queen Mary Hospital for COVID-19 between 21 July 2020 and 20 April 2021 were prospectively recruited (12, 18, 19). The presence of SARS-CoV-2 was confirmed in all patients by RT-PCR from the nasopharyngeal swab (NPS) and/or deep throat saliva (DTS), using the LightMix SarbecoV E-gene assay (TIB Molbiol, Berlin, Germany) which targeted the envelope protein (E) gene of SARS-CoV-2 as we described previously (20). Patients were excluded if they (i) had history of thyroid, pituitary or hypothalamic disorders; (ii) were on anti-thyroid drugs or thyroid hormone replacement; (iii) were on medications with potential impact on thyroid function including systemic steroid, amiodarone, heparin and dopamine; or (iv) had active hematologic or solid malignancies. Each patient had blood tests within 24 hours after admission, before the initiation of COVID-19 treatments.

Serum TSH, fT4 and fT3 were measured with immunoassays ADVIA Centaur® TSH3-Ultra, FT4 and FT3 assays respectively (Siemens Healthcare Diagnostics Inc., USA). The reference ranges for TSH, fT4 and fT3 were 0.35–4.8 mIU/L, 12–23 pmol/L and 3.2–6.5 pmol/L, respectively. Anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase (anti-TPO) antibody titers were measured with QUANTA Lite® Thyroid T and TPO enzyme-linked immunosorbent assay respectively (Inova Diagnostics, USA). Positive anti-Tg and anti-TPO was defined by >100 units. Anti-TSH receptor antibody (anti-TSHR) titer was measured with Anti-TSH Receptor (TRAb) Fast ELISA (IgG) test kit (EUROIMMUN Medizinische Labordiagnostika AG, Germany), using porcine TSHR. Anti-TSHR was considered positive if >1 IU/L. NTIS was defined by low fT3 with normal/low TSH (21).

Basic hematology and biochemistry panel, glycated hemoglobin (HbA1c) and C-reactive protein (CRP) were measured. Lymphopenia was defined according to the laboratory reference range, i.e. if absolute lymphocyte count $<1.06\times10^9$/L. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in all individuals (22). Abnormalities in the hematological and biochemical parameters were defined by their respective laboratory reference ranges.

Demographics and major comorbidities were recorded. Obesity was defined by the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code 278.0. Diabetes was defined by a known diagnosis of diabetes or HbA1c ≥6.5% on admission. COVID-19-related symptoms were evaluated with a standard checklist. Respiratory rate, baseline oxygen saturation by pulse oximetry, and oxygen requirement on admission were captured. Chest x-ray was performed in each patient on admission. Cycle threshold (Ct) values were obtained from the qualitative LightMix SarbecoV E-gene assay (TIB Molbiol, Berlin, Germany) performed on specimens from NPS and/or DTS

(whichever was lower) on admission. The Ct value represents the number of cycles required for a gene target or a PCR product to be detected. While viral loads were not directly measured with a dedicated quantitative RT-PCR assay in this analysis, studies have shown a good correlation between Ct values and SARS-CoV-2 viral loads (23, 24), such that the lower the Ct values, the higher the viral loads.

COVID-19 severity was classified into mild, moderate, severe and critical according to the 'Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition)' published by the Chinese National Health Commission (NHC) (25). Each patient's clinical outcomes were captured. Severe COVID-19 outcomes were defined by a composite of new-onset oxygen requirements, intubation and mechanical ventilation, intensive care unit (ICU) admission and death.

In the early phase of this study, reassessment blood tests including thyroid function tests (TFTs) and lymphocyte counts were arranged around 1–2 weeks after discharge. Due to the subsequent significant increase in the case load of COVID-19 patients, the early reassessment was discontinued. Hence, only a subset of patients had reassessment of TFTs and lymphocyte counts early after discharge.

The study followed the principles in the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. All participants gave informed consent.

All statistical analyses were performed with IBM® SPSS® version 26. Two-sided p-values <0.05 were considered statistically significant. Data were presented as median with interquartile range (IQR), or number with percentage as appropriate. Data not conforming to normal distributions were logarithmically transformed before analyses. Between-group comparisons were performed with t-test for continuous variables, and Chi-square or Fisher's exact test for categorical variables as appropriate. Pearson correlation was used to analyze the univariate correlation between clinical variables and lymphocyte counts. Multivariable stepwise linear regression analysis was used to identify the independent variables associated with lymphocyte counts. Multivariable stepwise logistic regression analysis was used to identify the independent variables associated with severe COVID-19 outcomes. All variables with statistical significance in the univariate analysis were included in the multivariable regression analysis.

Several sensitivity analyses were performed in the evaluation of the associations between TFTs and lymphocyte counts: (i) repeating the analyses after excluding patients with overt/subclinical hypothyroidism and overt thyrotoxicosis; and (ii) evaluating the correlation between TSH and lymphocyte counts in the subgroup of patients with low fT3.

RESULTS

In total, 541 patients were included in this analysis. Median age was 50 years (IQR: 36 – 63) and 245 (45.3%) were men. Their baseline characteristics are summarized in **Table 1**. Hypertension (21.1%) and diabetes (16.1%) were the most common comorbidities. Most patients (n=380, 70.2%) were symptomatic at presentation: cough, fever and sore throat were the most common symptoms. Most patients had non-severe disease on presentation, only 3.1% of the cohort required supplementary oxygen on admission. Only 1.5% of the cohort carried the SARS-CoV-2 variants, including mutations N501Y, L452R and E484K. The median lymphocyte count on admission was 1.22 x 10^9/L (IQR: 0.90 – 1.68), with 36.0% of the cohort being lymphopenic.

We compared patients with normal lymphocyte count to those with lymphopenia (**Table 1**). Of note, patients with lymphopenia were older than those with normal lymphocyte count. Hence, subsequent comparisons were corrected for age. Patients with lymphopenia had lower TSH, fT4 and fT3 levels than those with normal lymphocyte count. There tended to be more men having lymphopenia in this cohort, although the difference did not reach statistical significance (age-adjusted p=0.076). Patients with lymphopenia were more likely symptomatic on presentation and had lower SARS-CoV-2 Ct value (i.e. higher viral load) upon admission. They had worse profiles of acute phase reactants (higher CRP and lower albumin levels), worse coagulation profile and lower serum sodium levels. They were more likely to require supplementary oxygen on admission. **Figure 1** shows the distribution of albumin levels, prothrombin time and sodium levels in the group with normal lymphocyte count and that with lymphopenia.

Abnormal TFTs, falling largely into three categories, were observed in 83 patients (15.3% of the cohort), in line with the findings described in our previous publication (12). (i) Seven patients likely had pre-existing thyroid dysfunction: one patient had overt thyrotoxicosis (TSH <0.01 mIU/L, fT4 51 pmol/L, fT3 15 pmol/L) with positive anti-TPO and anti-Tg, his anti-TSHR titer was elevated at 3.6 IU/L, likely representing co-existing Graves' disease diagnosed upon admission for acute COVID-19; six patients had subclinical hypothyroidism – three of them positive for anti-TPO. (ii) Forty-five patients had abnormal fT3 levels: 41 patients had low fT3 compatible with NTIS [38 had isolated low fT3, 2 had concomitant low TSH, and one had concomitant mildly raised fT4 (24 pmol/L)]; 4 patients had elevated fT3 where assay interference could not be totally excluded [2 had isolated high fT3 (6.6 – 6.7 pmol/L); one had mildly elevated fT4 (25 pmol/L) and fT3 (6.6 pmol/L); one patient had mildly elevated TSH (5.6 mIU/L), normal fT4 (17 pmol/L) and mildly elevated fT3 (7.0 pmol/L)]. (iii) 31 patients were considered to have thyroid dysfunction compatible with different phases of thyroiditis: 25 patients had a biochemical picture compatible with subclinical thyrotoxicosis, i.e. isolated low TSH with normal fT4 and fT3; 2 patients had isolated low fT4 (11 pmol/L); 4 patients had isolated elevated fT4 (24 pmol/L).

Variables Associated With Lymphocyte Counts

We studied the correlations of TSH, thyroid hormones and other clinical variables with the lymphocyte counts. (**Table 2**). TSH, fT4 and fT3 levels positively correlated with the lymphocyte counts.

TABLE 1 | Baseline characteristics of the cohort.

	All	Normal Lymphocyte Count	Lymphopenia	Age-Adjusted P value
Number	541	346	195	—
Age (years)	50.0 (36.0 – 63.0)	46.0 (34.0 – 61.0)[a]	57.0 (42.0 – 66.0)[a]	—
Male	245 (45.3%)	148 (42.8%)	97 (49.7%)	0.076
Thyroid function test				
TSH (mIU/L)	1.20 (0.78 – 1.70)	1.30 (0.91 – 1.80)	1.00 (0.61 – 1.50)	**<0.001**
fT4 (pmol/L)	17.0 (15.0 – 19.0)	18.0 (16.0 – 19.0)	17.0 (15.0 – 18.0)	**0.025**
fT3 (pmol/L)	4.2 (3.7 – 4.8)	4.4 (4.0 – 4.9)	3.9 (3.4 – 4.4)	**<0.001**
Comorbidities				
Hypertension	114 (21.1%)	64 (18.5%)	50 (25.6%)	0.463
Diabetes	87 (16.1%)	51 (14.7%)	36 (18.5%)	0.376
Obesity	26 (4.8%)	16 (4.6%)	10 (5.1%)	0.955
IHD/CHF	22 (4.1%)	14 (4.0%)	8 (4.1%)	0.160
Stroke/TIA	13 (2.4%)	4 (1.2%)	9 (4.6%)	0.153
Cancer	17 (3.1%)	7 (2.0%)	10 (5.1%)	0.281
Symptomatic presentation	380 (70.2%)	218 (63.0%)	162 (83.1%)	**<0.001**
Fever	180 (33.3%)	95 (27.5%)	85 (43.6%)	**<0.001**
Myalgia	58 (10.7%)	37 (10.7%)	21 (10.8%)	0.966
Malaise	69 (12.8%)	37 (10.7%)	32 (16.4%)	0.079
Rhinorrhoea	66 (12.2%)	43 (12.4%)	23 (11.8%)	0.903
Cough	218 (40.3%)	126 (36.4%)	92 (47.2%)	0.088
Dyspnoea	33 (6.1%)	17 (4.9%)	16 (8.2%)	0.256
Sore throat	135 (25.0%)	79 (22.8%)	56 (28.7%)	0.064
Headache	56 (10.4%)	32 (9.2%)	24 (12.3%)	0.102
Nausea/vomiting	19 (3.5%)	14 (4.0%)	5 (2.6%)	0.384
Diarrhoea	59 (10.9%)	43 (12.4%)	16 (8.2%)	0.088
Anosmia/ageusia	63 (11.6%)	44 (12.7%)	19 (9.7%)	0.617
Symptom count ≥3	163 (30.1%)	99 (28.6%)	64 (32.8%)	0.320
Viral load				
Ct value at baseline	24.76 (18.01 – 31.20)	27.50 (19.01 – 33.00)	21.00 (16.70 – 26.32)	**<0.001**
Acute phase reactants				
C-reactive protein (mg/dL)	0.57 (0.31 – 2.05)	0.39 (0.31 – 1.39)	1.06 (0.31 – 3.14)	**<0.001**
Albumin (g/L)	42.0 (40.0 – 45.0)	43 (41 – 46)	42 (39 – 44)	**0.040**
Coagulation profile				
Platelet (x 10^9/L)	217 (174 – 266)	236 (190 – 284)	191 (156 – 225)	**<0.001**
Prothrombin time (s)	11.7 (11.4 – 12.1)	11.6 (11.3 – 12.0)	11.9 (11.6 – 12.3)	**<0.001**
Biochemical parameters				
Sodium (mmol/L)	140 (138 – 141)	140 (138 – 141)	139 (137 – 140)	**<0.001**
Potassium (mmol/L)	3.7 (3.4 – 4.0)	3.8 (3.5 – 4.0)	3.7 (3.4 – 4.0)	0.054
Urea (umol/L)	3.9 (3.1 – 4.8)	3.9 (3.0 – 4.7)	4.1 (3.2 – 5.0)	0.739
eGFR (mL/min)	96 (82 – 109)	98 (88 – 112)	91.2 (75.4 – 103.0)	0.237
ALT (U/L)	25 (17 – 39)	26 (18 – 40)	22 (17 – 35)	0.278
AST (U/L)	27 (21 – 37)	27 (21 – 35)	28 (22 – 40)	0.078
LDH (U/L)	212 (179 – 263)	212 (180 – 260)	211 (179 – 267)	0.525
Creatine kinase (U/L)	98 (67 – 155)	95 (66 – 149)	108 (69 – 160)	0.176
Troponin T (ng/L)	5.71 (3.78 – 8.42)	5.56 (3.61 – 7.72)	6.23 (4.31 – 9.65)	0.354
Oxygen requirement on admission	17 (3.1%)	5 (1.4%)	12 (6.2%)	**0.016**

Data are presented as median (interquartile range) and number (percentage) as appropriate.

Values in bold represent statistical significance.

[a]*p < 0.001 in the comparison of age among patients with normal lymphocyte counts and lymphopenia.*

TSH, thyroid stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; IHD, ischaemic heart disease; CHF, congestive heart failure; TIA, transient ischaemic attack; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

Clinical parameters having positive correlations with the lymphocyte counts included Ct value, albumin, platelet, sodium and eGFR, while those having inverse correlations with the lymphocyte counts included age, CRP, prothrombin time (PT), urea, aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK).

Regarding categorical variables, lymphocyte counts were lower among patients with hypertension (p=0.006), malignancy (p=0.014), and elevated Troponin T (p=0.009). Lymphocyte counts did not differ according to the presence of diabetes (p=0.234), obesity (p=0.234), ischemic heart disease/heart failure (p=0.158) or lung disease (p=0.119). There was a trend towards lower lymphocyte counts among men, although the difference was not statistically significant [1.16 x 10^9/L (IQR: 0.85 – 1.61) in men vs 1.25 x 10^9/L (IQR: 0.94 – 1.71) in women, p=0.086]. However, patients requiring supplementary oxygen on admission, or those symptomatic on presentation had lower lymphocyte counts (both p<0.001). Lymphocyte counts did not differ when classified according to symptom burden (<3 vs ≥3 symptom counts) (p=0.128).

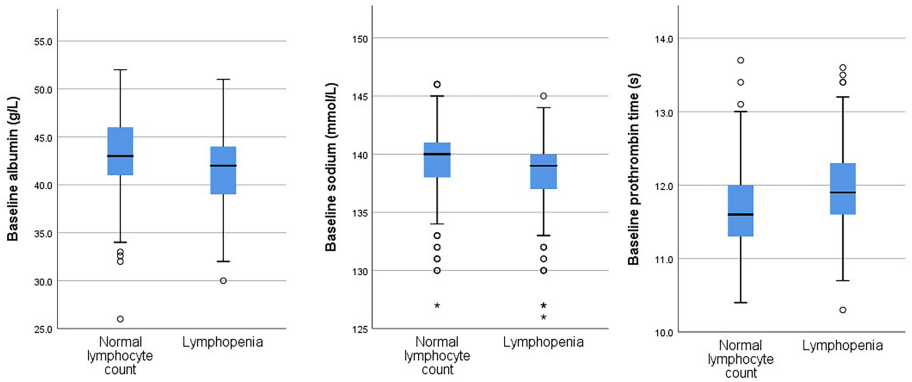

FIGURE 1 | Distributions of values of baseline albumin levels, sodium levels and prothrombin times in the groups with and without lymphopenia. An extreme outlier is indicated by an asterisk.

TABLE 2 | Pearson correlation of clinical parameters with lymphocyte counts.

	Crude r	P value
TSH (mIU/L)[a]	0.231	**<0.001**
fT4 (pmol/L)	0.146	**0.001**
fT3 (pmol/L)	0.338	**<0.001**
Age (years)	-0.310	**<0.001**
Viral load		
Cycle threshold value at baseline[a]	0.378	**<0.001**
Acute phase reactants		
C-reactive protein (mg/dL)[a]	-0.308	**<0.001**
Albumin (g/L)	0.231	**<0.001**
Coagulation profile		
Platelet (x 10^9/L)[a]	0.406	**<0.001**
Prothrombin time (s)	-0.199	**<0.001**
Biochemical parameters		
Sodium (mmol/L)	0.298	**<0.001**
Potassium (mmol/L)	0.047	0.277
Urea (umol/L)[a]	-0.180	**<0.001**
eGFR[a]	0.318	**<0.001**
AST (U/L)[a]	-0.153	**<0.001**
ALT (U/L)[a]	0.048	0.266
LDH (U/L)[a]	-0.110	**0.011**
Creatine kinase (U/L)[a]	-0.136	**0.001**

Data are presented as median (interquartile range) and number (percentage) as appropriate.
Values in bold represent statistical significance.
TSH, thyroid stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.
[a]*logarithmically transformed before analysis.*

TSH and fT3 Levels Were Independently Associated With Lymphocyte Counts

In the multivariable stepwise linear regression analysis (**Table 3**), among the components of TFTs, TSH (standardized beta 0.160, p<0.001) and fT3 (standardized beta 0.094, p=0.023), but not fT4, remained independently and positively correlated with lymphocyte counts. Other independent variables associated with lower lymphocyte counts included: older age, lower Ct value, higher CRP, worse coagulation profile (lower platelet and higher PT), lower sodium levels and more severe clinical presentations (symptomatic presentation and oxygen requirement on admission). On the other hand, LDH levels positively correlated with lymphocyte counts. Further inclusion of sex did not modify the independent correlation of TSH and fT3 with lymphocyte counts. Sex was not an independent determinant of lymphocyte counts.

TABLE 3 | Independent determinants of lymphocyte counts on multivariable stepwise linear regression analysis.

	Standardized Beta	P value
Thyroid stimulating hormone (mIU/L)[a]	0.160	<0.001
Free triiodothyronine (pmol/L)	0.094	0.023
Age (years)	-0.132	0.001
Cycle threshold value[a]	0.208	<0.001
C-reactive protein (mg/dL)[a]	-0.165	<0.001
Platelet (x 10^9/L)[a]	0.226	<0.001
Prothrombin time (s)	-0.090	0.010
Sodium (mmol/L)	0.085	0.026
Lactate dehydrogenase (U/L)[a]	0.134	0.002
Oxygen requirement on admission	-0.103	0.005
Symptomatic presentation	-0.093	0.011

Model included thyroid stimulating hormone, free thyroxine, free triiodothyronine, age, cycle threshold value, C-reactive protein, albumin, platelet, prothrombin time, sodium, urea, estimated glomerular filtration rate, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, elevated troponin T, hypertension, malignancy, supplementary oxygen on admission, symptomatic presentation.
[a]*logarithmically transformed before analysis.*

Sensitivity Analyses

We repeated the analyses after exclusion of patients with possibly pre-existing thyroid disorders – one patient with overt thyrotoxicosis and 6 patients with subclinical hypothyroidism. Similar results were obtained: both TSH (standardized beta 0.138, p<0.001) and fT3 (standardized beta 0.126, p=0.006) remained independently associated with lymphocyte counts in the multivariable stepwise linear regression analysis.

We evaluated the correlation between TSH and lymphocyte counts among the subgroup of 41 patients with NTIS, characterized by low fT3. TSH still showed a significant positive correlation with lymphocyte counts (r = 0.344, p=0.032).

Recovery of TFTs and Lymphocyte Counts

A subgroup of patients received reassessment of lymphocyte counts and TFTs in 1 – 2 weeks' time: 40 patients had reassessment after a median of 9 days (IQR: 4 – 15). Paired comparisons (**Table 4**) showed similar trends of improvement for TSH (p=0.031), fT3 (p<0.001) and lymphocyte counts (p<0.001), while fT4 showed no significant changes (p=0.186). Of 18 patients (45.0%) with lymphopenia on admission for COVID-19, 8 remained lymphopenic upon reassessment. Hence, 10 of 18 patients (55.6%) recovered. On the other hand, of 10 patients (25.0%) had abnormal TFTs on admission for COVID-19, 2 remained abnormal upon reassessment. Hence, 8 of 10 patients (80.0%) with abnormal TFTs had recovered.

Prognostic Implications of NTIS and Lymphopenia in COVID-19

Among all 541 patients, 42 (7.8%) had severe COVID-19 outcomes. When classified according to presence of lymphopenia and NTIS, there was a significant increasing trend of likelihood of severe COVID-19 outcomes with increasing number of abnormalities (p<0.001): 16 out of 332 patients (4.8%) with normal lymphocyte count and no NTIS; 17 out of 184 patients (9.2%) with either lymphopenia or NTIS; and 9 out of 25 patients (36.0%) with both lymphopenia and NTIS.

We further investigated whether NTIS or lymphopenia carried independent prognostic implications in COVID-19. The comparison between patients who did and did not develop severe COVID-19 outcomes is summarized in **Table 5**. Patients who developed severe COVID-19 outcomes were older, more likely to be men and more likely to have pre-existing cardiometabolic comorbidities. Apart from NTIS and lymphopenia, differences were observed in a range of biomarkers: a more adverse profile was noted in patients who developed severe COVID-19 outcomes. To examine whether NTIS or lymphopenia were independently associated with severe COVID-19 outcomes, we employed multivariable stepwise logistic regression analysis. In the final model of the multivariable logistic regression (**Table 6**), NTIS (adjusted OR 3.64, p=0.005) joined other known risk factors of severe COVID-19 outcomes (male, comorbidities, higher viral loads and higher inflammatory index) to be the independent variables associated with severe COVID-19 outcomes, while lymphopenia was no longer an independent predictor.

TABLE 5 | Comparison between patients who did and did not develop severe COVID-19 outcomes.

	Patients without Severe COVID-19 Outcomes	Patients with Severe COVID-19 Outcomes	P value
Number	499 (92.2%)	42 (7.8%)	—
NTIS	28 (5.6%)	11 (26.2%)	<0.001
Lymphopenia	171 (34.3%)	24 (57.1%)	0.003
Age >50 years	233 (46.7%)	32 (76.2%)	<0.001
Male	217 (43.5%)	28 (66.7%)	0.004
Comorbidities			
Hypertension	98 (19.6%)	16 (38.1%)	0.005
Diabetes	73 (14.6%)	14 (33.3%)	0.002
Obesity	21 (4.2%)	5 (11.9%)	0.025
IHD/CHF	17 (3.4%)	5 (11.9%)	0.007
Stroke/TIA	9 (1.8%)	4 (9.5%)	0.013
Cancer	15 (3.0%)	2 (4.8%)	0.634
Symptomatic presentation	343 (68.7%)	37 (88.1%)	0.008
Ct value <25	247 (49.5%)	32 (76.2%)	<0.001
Elevated CRP	204 (40.9%)	34 (81.0%)	<0.001
Hypoalbuminaemia	65 (13.0%)	15 (35.7%)	<0.001
Thrombocytopenia	99 (19.8%)	12 (28.6%)	0.178
Elevated PT	2 (0.4%)	0 (0%)	0.999
Hyponatraemia	53 (10.6%)	13 (31.0%)	<0.001
Hypokalaemia	173 (34.7%)	12 (28.6%)	0.424
Elevated urea	15 (3.0%)	6 (14.3%)	<0.001
eGFR <60 mL/min	18 (3.6%)	5 (11.9%)	0.010
Elevated ALT	72 (14.4%)	9 (21.4%)	0.222
Elevated AST	117 (23.4%)	20 (47.6%)	0.001
Elevated LDH	165 (33.1%)	27 (64.3%)	<0.001
Elevated CK	52 (10.4%)	10 (23.8%)	0.009
Elevated troponin T	41 (8.2%)	9 (21.4%)	0.005

NTIS, non-thyroidal illness syndrome; IHD, ischaemic heart disease; CHF, congestive heart failure; TIA, transient ischaemic attack; Ct, cycle threshold; CRP, C-reactive protein; PT, prothrombin time; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase.

DISCUSSION

Our study showed that in COVID-19, both TSH and fT3 positively correlated with lymphocyte counts independent of demographics, comorbidities, viral load, inflammatory markers and organ dysfunction. Most of the abnormal TFTs and lymphopenia recovered soon after acute COVID-19. These findings would suggest possible interactions between the hypothalamic-pituitary-thyroid axis and the immune system in

TABLE 4 | Thyroid function tests and lymphocyte counts of patients who had reassessment around 1–2 weeks after acute COVID-19 (n=40).

	Baseline	Reassessment	P value
Lymphocyte count (x 10^9/L)[a]	1.14 (0.80 – 1.41)	1.57 (1.30 – 2.10)	**<0.001**
Thyroid-stimulating hormone (mIU/L)[a]	1.20 (0.61 – 1.60)	1.43 (0.83 – 1.78)	**0.031**
Free thyroxine (pmol/L)	17.0 (14.0 – 20.0)	17.5 (16.3 – 19.5)	0.186
Free triiodothyronine (pmol/L)	4.1 (3.7 – 4.8)	4.9 (4.5 – 5.2)	**<0.001**

Data presented as median (interquartile range).
Values in bold represent statistical significance.
[a]*logarithmically transformed before analyses.*

TABLE 6 | Variables associated with severe COVID-19 outcomes in the final model of the multivariable stepwise logistic regression analysis.

Variables	Adjusted OR (95% CI)	P-value
NTIS	3.64 (1.49 – 8.91)	**0.005**
Male (vs female)	2.20 (1.05 – 4.61)	**0.037**
IHD/CHF	3.23 (0.91 – 11.5)	0.070
Stroke/TIA	5.49 (1.32 – 22.9)	**0.019**
Ct value <25	3.34 (1.47 – 7.58)	**0.004**
Elevated CRP	3.70 (1.51 – 9.10)	**0.004**
Hypoalbuminaemia	2.12 (0.92 – 4.89)	0.078
Elevated creatine kinase	2.28 (0.95 – 5.45)	0.065

NTIS, non-thyroidal illness syndrome; IHD, ischaemic heart disease; CHF, congestive heart failure; TIA, transient ischaemic attack; Ct, cycle threshold; CRP, C-reactive protein. The model included NTIS, lymphopenia, age >50 years, male, hypertension, diabetes, obesity, IHD/CHF, stroke/TIA, symptomatic presentation, Ct value <25, hypoalbuminaemia, hyponatraemia, elevated urea, estimated glomerular filtration rate <60 mL/min, elevated aspartate aminotransferase, elevated lactate dehydrogenase, elevated creatine kinase and elevated troponin T.
Values in bold represent statistical significance.

COVID-19. COVID-19 patients who had both NTIS (characterized by low fT3) and lymphopenia were more likely to have severe COVID-19 outcomes compared to those who only had either one of NTIS or lymphopenia. Furthermore, NTIS, but not lymphopenia, was an independent predictor of severe outcomes in COVID-19, suggesting thyroid function to be one of the better markers of COVID-19 severity.

Independent Associations of TSH and fT3 With Lymphocyte Counts

In this cohort of patients with predominantly non-severe COVID-19, we reported a 36.0% prevalence of lymphopenia, consistent with the rates of lymphopenia among non-severe COVID-19 patients described in the literature (varying from 1% to 80%) (2). We observed an independent positive correlation of TSH and fT3 levels with lymphocyte counts in this study. While both thyroid function and lymphocyte counts may simply be markers of illness, the fact that a significant association between thyroid function and lymphocyte counts remained after adjusting for a range of COVID-19-related parameters may suggest a potential interaction between hypothalamic-pituitary-thyroid axis and the immune system. *In vitro* incubation of T-lymphoma mouse cell line with thyroid hormones for 24 to 72 hours showed increased proliferation, mediated by protein kinase C and involved activation of inducible nitric oxide synthase (8). Hypothyroidism in humans and experimentally-induced hypothyroidism in rats have been shown to be associated with diminished thymic activity, effects that were reversed with thyroid hormone replacement (26). Reversal of propylthiouracil-induced hypothyroidism in mice with T3 replacement led to recovery of lymphocyte proliferative ability (27). Nonetheless, these results should be applied to our study with caution as TFTs in experimentally-induced hypothyroidism were characterized by low fT4 and fT3 but high TSH, in contrast to the low fT3 and low TSH concerning our study. In addition to thyroid hormones, TSH has also been shown to interact with the immune system. TSH receptors are found on the surface of B and T lymphocytes. In murine model, there was improvement in the proliferative capacity and natural killer cell activity of spleen lymphocytes by TSH (9). A study of athyreotic patients due to total thyroidectomy for differentiated thyroid cancer showed that administration of recombinant human TSH led to a significant rise in the percentage of natural killer T cells and B lymphocytes in their peripheral blood. This showed a potential direct impact of TSH on immune cells, independent of thyroid hormone action (28). Indeed, in our subgroup analysis, TSH still positively correlated with lymphocyte counts among patients with low fT3, further highlighting the independent association between TSH and lymphocyte counts.

Of note, fT4 levels did not show an independent correlation with lymphocyte count in the multivariable linear regression model. It may be because T3 is the active form of thyroid hormone converted from T4. On the other hand, it may reflect that in NTIS, fT3 and TSH drop earlier than fT4. Interestingly, a recent meta-analysis pooling 58 studies of correlations between thyroid function and clinical parameters indicates that fT4 seems to correlate with clinical parameters better than TSH and fT3 (29). There are also suggestions in the meta-analysis that correlations of TSH and fT3 with clinical parameters may be confounded by reverse causation. In our current association study, elements of reverse causality in the correlation of TSH and fT3 with lymphocyte counts could not be entirely excluded. This issue can be better answered by interventional studies on the benefits of thyroid hormone replacement in the context of lymphopenia in COVID-19 (30). Furthermore, considering the strength of the effects of TSH and fT3 on lymphocyte counts in the multivariable model, it is likely that thyroid function is only one of the many contributors to lymphopenia in COVID-19, rather than playing a dominant role, given the evidence of expression of the entry receptor of SARS-CoV-2, ACE2, in various human tissues (14).

Other Independent Determinants of Lymphocyte Counts in COVID-19

Our study revealed multiple independent determinants of lymphocyte counts in COVID-19 (2). Lymphocyte counts decline with age, consistent with other studies (31), which may be related to thymic involution leading to changes in the overall immune competence (32). Secondly, SARS-CoV-2 PCR Ct values positively correlated with lymphocyte counts, meaning that a higher SARS-CoV-2 viral load is associated with lymphopenia. This suggested a viral-specific mechanism of lymphopenia. Indeed, expression of ACE2, the entry receptor for SARS-CoV-2, has been found in lymphocytes. Hence, there may be a direct cytotoxic effect from SARS-CoV-2 (1, 33). Thirdly, higher levels of inflammatory markers such as CRP were associated with lymphopenia. Interleukin 6 (IL-6) is known to induce gene expression and release of CRP from the liver and from immune cells (34). IL-6 is highly expressed during viral infection, and can cause apoptosis of lymphocytes (35). Fourthly, the positive correlation between platelet and lymphocyte counts suggested a possible element of infection of the bone marrow resulting in abnormal hematopoiesis (36). Fifthly, the association of lower platelet counts, increasing PT and oxygen requirement with lower lymphocyte counts could be explained by the COVID-19-related cytokine storm leading to disseminated

intravascular coagulopathy and acute respiratory distress syndrome (37). These proinflammatory cytokines can suppress the lymphocyte proliferation. Lastly, the association between hyponatremia and lymphopenia could be explained by the increase in proinflammatory cytokines in COVID-19. IL-6 may provide a common link between hyponatremia and lymphopenia, as IL-6 can lead to lymphopenia and has been shown to be inversely correlated with sodium levels in COVID-19 in an Italian study (38). In that study, hyponatremia improved after administration of tocilizumab, an IL-6 receptor antagonist (38). IL-6 may play a pathogenic role in causing electrolyte disturbance by inducing non-osmotic release of vasopressin (39).

Thyroid Function and Lymphocyte Counts With Recovery From COVID-19

The trajectories of thyroid function recovery suggest that COVID-19 is the cause of the thyroid abnormalities. A recent study which evaluated a Dutch cohort of COVID-19 patients reported the comparison of TSH, thyroid hormones and inflammatory markers between 17 patients with severe lymphopenia and 18 patients without lymphopenia (15). In line with their findings, we found that patients with lymphopenia had lower TSH, fT4 and fT3, and higher CRP levels. Furthermore, we revealed the independent association of TSH and fT3 with lymphocyte counts even after adjusting for the levels of acute phase reactants. Interestingly, in that Dutch cohort, among the 15 COVID-19 patients who underwent reassessment blood tests 1 week later, 12 of them showed recovery of lymphocyte counts approaching normal ranges, whereas thyroid hormones did not significantly change, especially T3 levels remaining relatively low. The authors thus concluded that the results argued either for different kinetics of recovery of lymphopenia and thyroid function, or against a direct causal relationship between lymphopenia and thyroid function abnormalities. Among our 40 patients who underwent reassessment of TFTs and lymphocyte counts, we observed a parallel recovery in TSH and fT3 with lymphocyte counts. The differences between our results and those from the Dutch cohort could be explained by the milder spectrum of COVID-19 in our cohort, and the differences in inclusion criteria in the Dutch cohort. Our results could support a different kinetics in the recovery of thyroid function and lymphopenia, such that in milder cases, thyroid function and lymphopenia may recover in parallel. Nonetheless, our results may still be consistent with a possible direct interaction between the hypothalamic-pituitary-thyroid axis and the immune system. Given that this is an association of our study, whether thyroid hormone replacement is beneficial in the context of lymphopenia remains to be elucidated in ongoing studies in COVID-19 (30).

Prognostic Implication of Thyroid Function

Our study revealed that patients with NTIS, but not lymphopenia, would have worse COVID-19 outcomes. Among COVID-19 patients with NTIS, low TSH also held prognostic significance. SARS-CoV-2 infection and its associated inflammation can lead to both lymphopenia and NTIS, so thyroid function and lymphopenia may merely reflect COVID-19 severity, where our results might suggest that thyroid function is among the better markers of COVID-19 severity, instead of lymphopenia. On the other hand, pre-clinical studies have demonstrated the influence of thyroid hormones and TSH on lymphocyte counts. As lymphopenia is believed to be a defective immune response to the virus (40), such influence on lymphocyte count may contribute to the prognostic significance of NTIS and low TSH in the context of NTIS.

Male sex has been described in different populations to be associated with worse COVID-19 outcomes including mortality (41). Hence, it might be expected to observe a male predominance among the group with lymphopenia, a marker of COVID-19 severity. In line with this, in our study, we observed a trend towards more men having lymphopenia, and lymphocyte counts tended to be lower in men than in women. Moreover, male sex was among the five prognostic factors for severe COVID-19. Nonetheless, as only total lymphocyte counts were measured in our study, further details about lymphocyte subsets were not available. Some studies have demonstrated differences in the patterns of lymphocyte subsets which may explain the worse prognosis of COVID-19 among men (42). This may explain the lack of significant sex bias observed in the current study.

Our study shed light onto the interaction between TSH/thyroid hormones and the immune system. It also offered a potential explanation for the prognostic role of NTIS in COVID-19. Our results were generated from a relatively large cohort of complete thyroid function assessment, thus allowing adjustments for multiple potential confounders. Nevertheless, our study should be interpreted bearing the following limitations. Firstly, this is an observational study of associations between TFT and lymphocyte count, which do not prove causality. Secondly, TFTs were only reassessed one week after the initial TFTs on admission. Further studies with more frequent TFT monitoring during the course of illness can delineate the kinetics of TFT and lymphocyte count recovery with a higher resolution. Thirdly, SARS-CoV-2 viral loads were represented by Ct values. Despite a good correlation (23, 24), direct quantitative measurements of viral loads would have been preferable if available. Fourthly, obesity was defined by the ICD-9-CM diagnostic code in our study as a categorical variable, instead of body mass index as a continuous variable, and was likely to be underreported. Fifthly, high-resolution computed tomography was done at the physicians' discretion. Thus, the detection of imaging features of pneumonia in our cohort might be less sensitive. Last but not least, only total lymphocyte counts were measured in this study. Further details about lymphocyte subsets were not available, which may provide more insights into the interrelationship between the hypothalamic-pituitary-thyroid axis and the immune system.

CONCLUSION

TSH and fT3 levels showed independent positive correlations with lymphocyte counts among COVID-19 patients. There was a parallel recovery in TFTs and lymphocyte count around 1 week after acute illness. These results suggested potential interactions between the

hypothalamic-pituitary-thyroid axis and the immune system. Furthermore, patients with both lymphopenia and NTIS had the worst clinical course of acute COVID-19, supporting the potential prognostic role of thyroid hormones in COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DL wrote the manuscript. DL, CHL, WC, AL, AT, PP, TH, CC, and CYL researched the data. DL and CF performed statistical analyses. CHL, WC, AL, KKT, CWL, KCT, YW, IH, and KL critically reviewed and edited the manuscript. KL initiated and supervised the study, is the guarantor of this work, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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The Effect of Inactivated SARS-CoV-2 Vaccines on TRAB in Graves' Disease

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Background: The ongoing coronavirus disease 2019 (COVID-19) pandemic has forced the development of vaccines. Reports have suggested that vaccines play a role in inducing autoimmune diseases (AIDs). Scattered cases have reported that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines may promote thyroid disease, including Graves' disease (GD). However, the effect of inactivated SARS-CoV-2 vaccine on GD remains unclear. The aim of the present study was to investigate the response of thyrotropin receptor antibody (TRAB) to inactivated SARS-COV-2 vaccines.

Methods: We conducted a retrospective study to observe the differences in thyroid function and TRAB trends between pre-vaccination (n=412) and post-vaccination (n=231) groups at an interval of 2 months. We then retrospectively observed the differences in serum thyroid function and TRAB levels at 3 months before (n=280), 1 month before (n=294), 1 month after (n=306), and 3 months after (n=250) vaccination. Subsequently, 173 GD patients who were not vaccinated with inactivated SARS-COV-2 vaccines were selected for a prospective study. Thyroid function and TRAB assessment were performed before 3 and 1 months and 1 and 3 months after the first dose of vaccination and were then compared by repeated measures ANOVA to explore their dynamic changes.

Results: A retrospective study preliminarily observed that the trend of TRAB post-vaccination was opposite of that pre-vaccination (p=0.000), serum TRAB levels decreased before vaccination and increased after vaccination. In this prospective study, repeated measures ANOVA indicated significant differences in serum FT3 (p=0.000), FT4 (p=0.000), TSH (p=0.000), and TRAB (p=0.000) levels at different time points before and after vaccination. Serum TRAB levels showed dynamic changes that decreased significantly at 1 month before vaccination (p=0.000), no significant differences at 1 month after vaccination (p=0.583), and reflected an upward trend at 3 months after vaccination (p=0.034). Serum FT3 and FT4 levels showed similar trends to serum TRAB levels before and after vaccination. Instead, the serum TSH levels showed a continuous upward trend over time.

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Conclusion: Based on the results obtained in both retrospective and prospective studies, we concluded that serum TRAB levels decreased less after inactivated SARS-CoV-2 vaccination and showed an upward trend, which may be related to humoral immunity induced by vaccination.

Keywords: inactivated SARS-CoV-2 vaccine, Graves' disease (GD), thyrotropin receptor antibody (TRAB), autoimmune disease (AID), autoimmune thyroid disease (AITD)

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected more than 20 million individuals and caused more than 5 million deaths worldwide by December 12, 2021. COVID-19 can cause both pulmonary and systemic inflammation, potentially leading to multi-organ dysfunction. Thyroid diseases, including thyrotoxicosis, hypothyroidism, and non-thyroid disease syndromes, can also be caused by COVID-19 (1). To date, there is no specific treatment for SARS-CoV-2, and vaccination is a basic and effective way to prevent the spread of this virus. Currently, the types of vaccines in use include inactivated virus vaccines, live attenuated virus vaccines, nucleic acid vaccines, recombinant viral vector vaccines, and recombinant subunit vaccines (2). In China, more than 1 billion people have been vaccinated with inactivated SARS-CoV-2, and mass vaccination continues.

Graves' disease (GD) is an organ-specific autoimmune disease (AID) which is characterized by thyrotropin receptor antibody (TRAB). Genetic factors account for 80% of the risk of developing GD, whereas the other 20% are related to environmental risk factors (3). For example, autoimmune thyroid disease (AITD) is a common side effect of alemtuzumab therapy in patients with multiple sclerosis (4). These factors contribute to the onset of GD in genetically susceptible individuals by breaking down the mechanisms that lead to immune tolerance. The immunopathogenesis of GD is complex, and TRAB is the ultimate cause of hyperthyroidism (5). It binds to the thyroid-stimulating hormone (TSH) receptor on the surface of thyroid follicular cells, resulting in persistent and uncontrolled thyroid stimulation, leading to abnormal overproduction of thyroid hormones and hyperthyroidism.

Vaccines have long been suspected to play a role in inducing AIDs (6). There have been isolated case reports of arthritis, vasculitis, and central or peripheral nervous system symptoms following vaccination. Although these cases tend to be very infrequent, there have been reports of AID after SARS-CoV-2 vaccination, including AITD. SARS-CoV-2 vaccines-induced thyroid disease is not a single report. Recently, Alberto et al. and Zettinig et al. successively reported four cases of GD with positive TRAB induced by SARS-Cov2 RNA vaccination, which met the diagnostic criteria for autoimmune/inflammatory syndrome induced by adjuvants (ASIA) (7, 8). Another study reported that three women developed anterior neck pain after inactivated SARS-CoV-2 vaccine and were diagnosed with subacute thyroiditis, which is also thought to be a phenomenon of ASIA (9). Furthermore, one woman with a history of controlled GD developed ocular symptoms and signs after the mRNA COVID-19 vaccine, which, combined with elevated thyroid-stimulating immunoglobulin and orbital imaging, was consistent with a diagnosis of active Graves ophthalmopathy (GO) (10). Among the outpatients in our hospital, there were also cases of AITD induced by inactivated SARS-CoV-2 vaccines, including GD and Hashimoto's thyroiditis (HT), which mainly manifested as hyperthyroidism, such as a swollen neck, palpitations, and weight loss (this case report is in the process of publication). In the aforementioned reports, there were both newly diagnosed and recurrent or aggravated cases.

Until now, little was known about the effect of inactivated SARS-CoV-2 vaccines on GD. As a marker of diagnosis and evaluation of treatment and remission, TRAB was the entry point of the present study. This study aimed to explore the response of TRABs in GD after inactivated SARS-CoV-2 vaccines in retrospective and prospective studies to further investigate the factors that may modulate these responses.

SUBJECTS AND METHODS

Study Population

These retrospective studies were performed to observe the effects of vaccination on thyroid function before and after vaccination. We included all GD patients in the Endocrinology Department of the Second Affiliated Hospital of Fujian Medical University from January to August 2021. The patients included conformed to the diagnostic and treatment criteria of GD of the European Thyroid Association (ETA) (2018). Patients with GO, GD after I131 or surgical treatment, thyroid cancer, pregnant and suckling period females, or other autoimmune diseases were excluded (**Figure 1**). All enrolled patients were treated with methimazole (MMI) combined with levothyroxine (L-T4) to avoid drug-induced hypothyroidism according to the ATD, which is the first-line treatment for GD. All of them were biochemically consistent with hyperthyroidism and were TRAB-positive, and treatment regimens remained unchanged during the study period. According to the time point of the first vaccination, the included population was divided into pre-vaccination (n=412) and post-vaccination (n=231) groups. Pre-vaccination referred to patients with GD who had not yet been vaccinated with inactivated SARS-CoV-2 vaccine. Post-vaccination, the GD was vaccinated with inactivated SARS-CoV-2 vaccine. We collected data on thyroid function and TRAB levels, which were measured

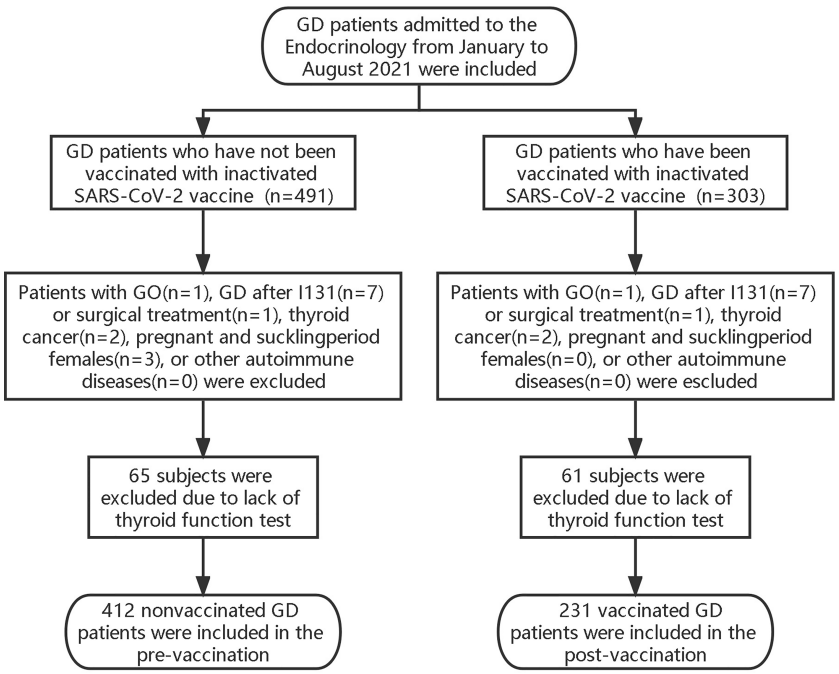

FIGURE 1 | Flow charts of retrospective study population.

at 2-month intervals within a specified time through the clinical system.

We then retrospectively observed the differences in serum thyroid function and TRAB levels at different times before and after vaccination. We included all GD patients who received the first dose of inactivated SARS-CoV-2 vaccine in the Endocrinology Department of the Second Affiliated Hospital of Fujian Medical University from January to October 2021 (n=482). Patients with GO (n=1), GD after I131 (n=8) or surgical treatment (n=1), thyroid cancer (n=2), pregnant and suckling period females (n=0), or other autoimmune diseases (n=0) were excluded. Thyroid function and TRAB levels of the included GD patients were reviewed 3 months before, 1 month before, 1 month after, and 3 months after vaccination according to the time point of the first vaccination. After excluding patients with missing checklists at all the above time points (n=156), 314 patients were included and divided into four groups according to time points: pre-vaccination -3 month and -1 month and post-vaccination +1 month and +3 months. Due to the absence of follow-up at some time points, the cases in each group were as follows: pre-vaccination -3 months (n=280) and -1 month (n=294) and post-vaccination +1 month (n=306) and +3 months (n=250).

Subsequently, we conducted a prospective study on GD patients who were admitted to the Endocrinology Department of our hospital between March and May 2021 and had not been vaccinated. The enrolled population also met the diagnostic and treatment criteria of GD in ETA (2018), and the treatment regimen followed first-line treatment, which remained unchanged during the study period. According to their willingness and the control of hyperthyroidism, they are advised to receive inactivated SARS-COV-2 vaccines. The exclusion criteria were as follows: 1. GO; 2. patients who needed to change treatment due to illness during the test; 3. GD after I131 or surgical treatment; 4. thyroid cancer; 5. pregnant and suckling period females; 6. GD accompanied by serious medical diseases, liver and kidney dysfunction, or granulocytopenia; and 7. other autoimmune diseases. Finally, we enrolled 173 GD patients who had not received inactivated SARS-COV-2 vaccines. All subjects provided informed consent to participate in the study, which was approved by the local ethical committee.

Vaccination

We reviewed the vaccination information in the Fujian Health Code to confirm and collect the type and date of vaccination and vaccine manufacturers of the participants to ensure the accuracy of information collection. They were also asked about their discomfort after the vaccination. The vaccine manufacturers of the enrolled patients included SINOVAC, Beijing Bio, and Chengdu Bio, which produced inactivated SARS-COV-2 vaccines.

Assays

Venous blood samples were collected before 3 and 1 months and 1 and 3 months after the first dose of inactivated SARS-COV-2 vaccines. Serum free triiodothyronine 3 (FT3), free thyroxine 4 (FT4), thyroid-stimulating hormone (TSH), and TRAB levels were measured using a competitive electrochemiluminescence immunoassay (ECLIA) according to the manufacturer's

instructions (Roche COBAS-E601). Normal ranges of these parameters were as follows: FT3 (3.1-6.8pmol/L), FT4 (12.0-22.0pmol/L), TSH (0.27-4.20mIU/L), TRAB (0.00-1.75IU/L). The samples were analyzed in routine clinical laboratories at the Second Affiliated Hospital of Fujian Medical University.

Statistical Analysis

All analyses were performed using Statistical Package for the Social Sciences software version 23 (SPSS Inc., Chicago, IL). Continuous variables were normally distributed and shown as mean ± standard deviation ($\bar{x} \pm s$) and irregularly distributed data were expressed as medium (interquartile range). The Kolmogorov-Smirnov test was used for variables with skewed distributions. We used Blom's formula to transform the skewed distribution into a normal distribution. The paired t-test and *post hoc* one-way analysis of variance (ANOVA) were used to assess the statistical significance of differences among the groups. Repeated measures ANOVA was performed to compare dynamic changes in thyroid function and TRAB levels in this prospective study. Statistical significance was set at $p < 0.05$.

RESULTS

The Change Trend of TRAB in Post-Vaccination Was Opposite of That in Pre-Vaccination

In this retrospective study, we reviewed changes in thyroid function and TRAB in populations at different stages of vaccination, including pre-vaccination (n=412) and post-vaccination (n=231), to determine the effect of inactivated SARS-CoV-2 vaccines on TRAB. The baseline clinical data of the two groups were analyzed statistically to exclude other influencing factors after vaccination for GD. No significant differences were observed between the groups in terms of sex, age, medication, or other clinical characteristics. Serum FT3 (p=0.000), FT4 (p=0.000), and TRAB (p=0.000) levels were significantly lower after 2 months than before pre-vaccination. There were no differences in thyroid function and TRAB between the 2-month post-vaccination intervals. The t-test showed that the TRAB change was statistically different between the two groups (p=0.000). In contrast to the pre-vaccination values, the TRAB change trend post-vaccination (1.290IU/L vs. 0.060IU/L) (p=0.000) was the opposite (**Table 1** and **Figure 2**).

The Changes in Serum TRAB Levels Decreased Before Vaccination and Increased After Vaccination

To detect changes in serum thyroid function and TRAB levels at different time points before and after vaccination, we compared thyroid function and TRAB levels at 3 months, 1 month before and 1 month, 3 months after the first dose of vaccine. The baseline clinical data of the groups were analyzed statistically to exclude other influencing factors after vaccination for GD. No significant differences were observed between the groups in terms of sex, age, medication, and other clinical characteristics. The ANOVA analysis showed that there was a statistically significant difference in TRAB among the groups (p=0.019). The change trend of TRAB decreased before vaccination (5.880IU/L vs. 4.275IU/L) (p=0.009) and increased after vaccination (4.345IU/L vs. 4.475IU/L) (p=0.509). The change trends of FT3 and FT4 were both similar to those of TRAB, which decreased before vaccination (FT3: 5.220 pmol/L vs. 4.905 pmol/L, p=0.002; FT4: 16.415 pmol/L vs. 16.050 pmol/L, p=0.164) and increased after vaccination (FT3: 4.860 pmol/L vs. 4.990 pmol/L, p=0.247; FT4: 16.375 pmol/L vs. 16.840 pmol/L, p=0.271). The difference was that TSH levels continued to rise (0.218 mIU/L vs. 0.548 mIU/L vs. 0.817 mIU/L vs. 1.070 mIU/L) (p=0.000) (**Table 2**).

Baseline Characteristics of GD in Prospective Study

To further investigate the relationship between vaccination and serum TRAB levels, 173 GD who had received inactivated SARS-COV-2 vaccines were enrolled in a prospective study to compare the dynamic changes of serum TRAB levels before and at 3 and 1 months after vaccination. The baseline characteristics and clinical parameters of GD in this prospective study are summarized in **Table 3**. The mean age of the subjects in this

TABLE 1 | Comparison of clinical characteristics and thyroid function between groups.

	Pre-vaccination (n = 412)			Post-vaccination (n = 231)			P
Gender (female%)	77.910			76.190			0.618
Age (year)	39.020 ± 11.298			38.830 ± 10.895			0.941
Duration (month)	12.067 (5.433,20.725)			15.167 (9.300,25.600)			0.000
Thiamazole (mg)	15.127± 4.902			14.946 ± 5.563			0.844
Letrox (ug)	54.854 ± 36.674			56.522 ± 37.830			0.585
	2 Months Before	2 Months After	P	2 Months Before	2 Months After	P	–
FT3 (pmol/L)	5.295 (4.490,7.540)	4.765 (4.110,5.790)	0.000	4.770 (4.210,5.810)	4.950 (4.490,5.750)	0.756	–
FT4 (pmol/L)	16.490 (13.400,21.678)	15.765 (12.913,19.380)	0.000	16.050 (13.320,19.410)	16.740 (14.380,20.120)	0.246	–
TSH (mIU/L)	0.134 (0.005,1.758)	0.524 (0.006,2.170)	0.308	0.815 (0.017,2.850)	1.300 (0.035,3.170)	0.419	–
TRAB (IU/L)	6.300 (3.075,13.145)	4.455 (2.313,9.893)	0.000	4.450 (2.260,10.930)	4.470 (2.410,10.340)	0.237	–
TRAB Change (IU/L)	1.290 (0.440,2.770)			0.060 (-0.600,1.010)			0.000

Data are presented as mean±standard error (x±s) or median (interquartile range). Categorical outcomes were shown as absolute and relative prevalence of complications (%). FT3 free triiodothyronine 3, FT4 free thyroxine 4, TSH thyroid-stimulating hormone, TRAB thyrotropin receptor antibody.

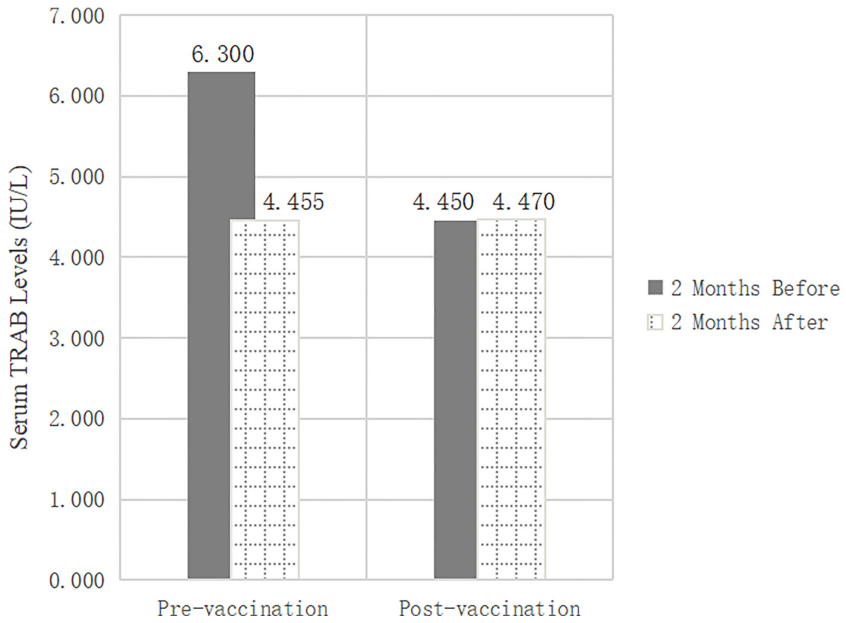

FIGURE 2 | Changes of TRAB levels and amplitude between pre-vaccination and post-vaccination groups.

study was 39.168 years, and 73.988% were women. The subjects had a median disease duration of 16.333 months, and their medication was MMI 14.957 mg and LT-4 58.960 μg on average. The baseline median serum FT3, FT4, TSH, and TRAB levels were 5.230 pmol/L, 15.930 pmol/L, 0.375 mIU/L, and 5.450 IU/L, respectively.

Serum TRAB Levels Decreased Slightly After Vaccination and Showed an Upward Trend

Repeated measures ANOVA indicated significant differences in serum FT3 (p=0.000), FT4 (p=0.000), TSH (p=0.000), and TRAB (p=0.000) levels at different time points before and after vaccination. Serum TRAB levels showed dynamic changes that decreased significantly at 1 month before vaccination (5.450IU/L vs. 3.950 IU/L) (p=0.000), no significant differences at 1 month after vaccination (3.95 0IU/L vs. 3.700 IU/L) (p=0.583), and a slight change and reflected an upward trend at 3 months after vaccination (3.700 IU/L vs. 4.100 IU/L) (p=0.034) (**Table 4** and **Figure 3**). Serum FT3 and FT4 levels showed similar trends to serum TRAB levels before and after vaccination. However, the slight differences were that FT3 and FT4 showed an upward trend at 1 month after vaccination, although there was no difference in the change of FT3 (4.630pmol/L vs. 4.740 pmol/L) (p=0.095) and FT4 (15.490 pmol/L vs. 16.220 pmol/L) (p=0.068) 1 month before and after vaccination, and the

TABLE 2 | Variation in thyroid function at different time points before and after vaccination.

	Pre-vaccination		Post-vaccination		P	*Post Hoc*
	3 Months Before (n = 280)	1 Month Before (n = 294)	1 Month After (n = 306)	3 Months After (n = 250)		
Gender (female%)	79.286	76.871	77.124	76.400	0.857	–
Age (year)	40.181 ±10.913	40.252 ±10.838	40.101 ±10.886	39.606±10.678	0.904	–
Duration (month)	11.150 (4.467,21.050)	13.917 (6.508,23.075)	15.283 (8.500,25.600)	18.367 (10.292,29.233)	0.000	–
Thiamazole (mg)	15.384±4.667	15.145±4.901	15.204±4.852	15.350±4.476	0.955	–
Letrox (ug)	53.214 ±37.309	52.976 ±38.665	51.797 ±38.476	53.200±37.555	0.968	–
FT3 (pmol/L)	5.220 (4.468,7.108)	4.905 (4.220,6.000)	4.860 (4.268,5.950)	4.990 (4.485,5.878)	0.006	0.002[a] 0.997[b] 0.247[c]
FT4 (pmol/L)	16.415 (13.313,21.478)	16.050 (13.300,19.413)	16.375 (13.410,20.118)	16.840 (14.465,19.915)	0.361	0.164[a] 0.999[b] 0.271[c]
TSH (mIU/L)	0.218 (0.005,2.018)	0.548 (0.006,2.283)	0.817 (0.012,2.595)	1.070 (0.017,3.133)	0.000	0.169[a] 0.047[b] 0.318[c]
TRAB (IU/L)	5.880 (2.708,13.070)	4.275 (2.203,10.310)	4.345 (2.288,10.110)	4.475 (2.453,9.853)	0.019	0.009[a] 0.865[b] 0.509[c]

Data are presented as mean±standard error (x±s) or median (interquartile range). Categorical outcomes were shown as absolute and relative prevalence of complications (%). FT3 free triiodothyronine 3, FT4 free thyroxine 4, TSH thyroid-stimulating hormone, TRAB thyrotropin receptor antibody.

[a]*3 Months Before versus 1 Month Before.*

[b]*1 Month Before versus 1 Month After.*

[c]*1 Month After versus 3 Months After.*

TABLE 3 | Demographic and clinical characteristics of enrolled patients (n = 173).

Gender (female%)	73.988
Age (year)	39.168 ±10.713
Duration (month)	16.333 (9.617,26.309)
Thiamazole (mg)	14.957±5.293
Letrox (ug)	58.960 ±37.246
FT3 (pmol/L)	5.230 (4.500,6.570)
FT4 (pmol/L)	15.930 (13.530,20.300)
TSH (mIU/L)	0.375 (0.005,2.020)
TRAB (IU/L)	5.450 (2.555,11.350)

Data are presented as mean±standard error (x±s) or median (interquartile range). Categorical outcomes were shown as absolute and relative prevalence of complications (%). FT3 free triiodothyronine 3, FT4 free thyroxine 4, TSH thyroid-stimulating hormone, TRAB thyrotropin receptor antibody.

upward trend was more significant 3 months after vaccination (FT3: 4.740 pmol/L vs. 5.020 pmol/L, p=0.001; FT4: 16.220 pmol/L vs. 16.610 pmol/L, p=0.012). Instead, serum TSH levels showed a continuous upward trend over time (0.375 mIU/L vs. 0.948 mIU/L vs. 1.110 mIU/L vs. 1.420 mIU/L) (p=0.000).

DISCUSSION

In the present study, we combined retrospective and prospective studies to investigate the effect of inactivated SARS-CoV-2 vaccines on TRAB in patients with GD. The results of inter-group comparison and repeated measures ANOVA indicated that serum TRAB levels decreased less after inactivated SARS-CoV-2 vaccination and showed an upward trend. Similarly, the serum FT3 and FT4 levels increased after vaccination. To the best of our knowledge, this is the first comparative assessment of serum TRAB levels with inactivated SARS-CoV-2 vaccines in GD since the COVID-19 outbreak.

SARS-CoV-2 is spreading rapidly worldwide with high numbers of confirmed cases and fatality rates and limited treatment options. Widespread vaccination against COVID-19 is a crucial tool to control the pandemic. In China, most citizens are vaccinated with inactivated SARS-CoV-2. Inactivated vaccines are a mature technology with highly efficient proliferation and high genetic stability and are widely used for the prevention and control of emerging infectious diseases (11). Inactivated SARS-CoV-2 vaccines are made by taking live viral samples from multiple patients and replicating them in Vero cells of the African green monkey cell line, which is susceptible to infection (12). Then, the strain with the best proliferation and lowest mutagenesis rate is isolated, further proliferated, inactivated, and absorbed onto aluminum hydroxide to generate (13). In preclinical studies, inactivated SARS-CoV-2 vaccines provided complete protection against SARS-CoV-2 infection by triggering effective humoral immune responses and inducing SARS-CoV-2-specific neutralizing antibodies in serrated animals and nonhuman primates (14). Furthermore, vaccines have been found to elicit a rapid humoral response in healthy individuals to be tolerable and immunogenic (15).

Adjuvants are compounds added to vaccines to enhance immunogenicity, which could lead to practical advantages, including dose-sparing and inducing a more rapid, strong, and long-lasting immune response (16, 17). Aluminum compounds are the most widely used adjuvants for human vaccines. Aluminum traps soluble antigens, interacts with dendritic cells, enhances antigen presentation and complement and eosinophil activation, promotes an influx of neutrophils, enhances the secretion of pro-inflammatory cytokines and chemokines, and reduces immunopathology, elevating protective immunity levels to the threat of homologous viruses (18). While everything has two sides, the more effective it is, the higher is the risk. However, adjuvants are not completely free of side effects. In genetically predisposed individuals, adjuvants may induce ASIA by disrupting the host's immunological balance through molecular simulations, triggering polyclonal activation of B lymphocytes, or other similar etiological mechanisms (19). Adjuvants can trigger generalized autoimmune reactions, resulting in multiple autoantibodies, and contribute to the development of autoimmune diseases including AITD, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), Guillain Barre syndrome (GBS), and multiple sclerosis (18).

GD is the most common cause of hyperthyroidism. Under normal conditions, TSH receptors (TSHRs) located on the surface of thyroid cells bind to TSH, which activates adenylate cyclase and phosphoinositol-dependent signaling pathways to produce thyroid hormones (20). Hyperthyroidism is caused by the growth and reproduction of thyroid cells and persistent and uncontrolled thyroid stimulation resulting from the interaction

TABLE 4 | Variation in thyroid function of prospective subjects (n = 173).

	Pre-vaccination		Post-vaccination		P	Pairwise Comparison
	3 Months Before	1 Month Before	1 Month After	3 Months After		
FT3 (pmol/L)	5.230 (4.500,6.570)	4.630 (4.095,5.515)	4.740 (4.235,5.760)	5.020 (4.530,5.735)	0.000	0.000[a] 0.095[b] 0.001[c]
FT4 (pmol/L)	15.930 (13.530,20.300)	15.490 (12.910,18.760)	16.220 (13.540,19.145)	16.610 (14.660,19.400)	0.000	0.006[a] 0.068[b] 0.012[c]
TSH (mIU/L)	0.375 (0.005,2.020)	0.948 (0.017,2.700)	1.110 (0.042,2.765)	1.420 (0.204,3.225)	0.000	0.002[a] 0.064[b] 0.138[c]
TRAB (IU/L)	5.450 (2.555,11.350)	3.950 (2.080,8.780)	3.700 (2.000,7.835)	4.100 (2.360,8.965)	0.000	0.000[a] 0.583[b] 0.034[c]

Data are presented as median (interquartile range). Categorical outcomes were shown as absolute and relative prevalence of complications (%). FT3 free triiodothyronine 3, FT4 free thyroxine 4, TSH thyroid-stimulating hormone, TRAB thyrotropin receptor antibody.

[a]*3 Months Before versus 1 Month Before.*

[b]*1 Month Before versus 1 Month After.*

[c]*1 Month After versus 3 Months After.*

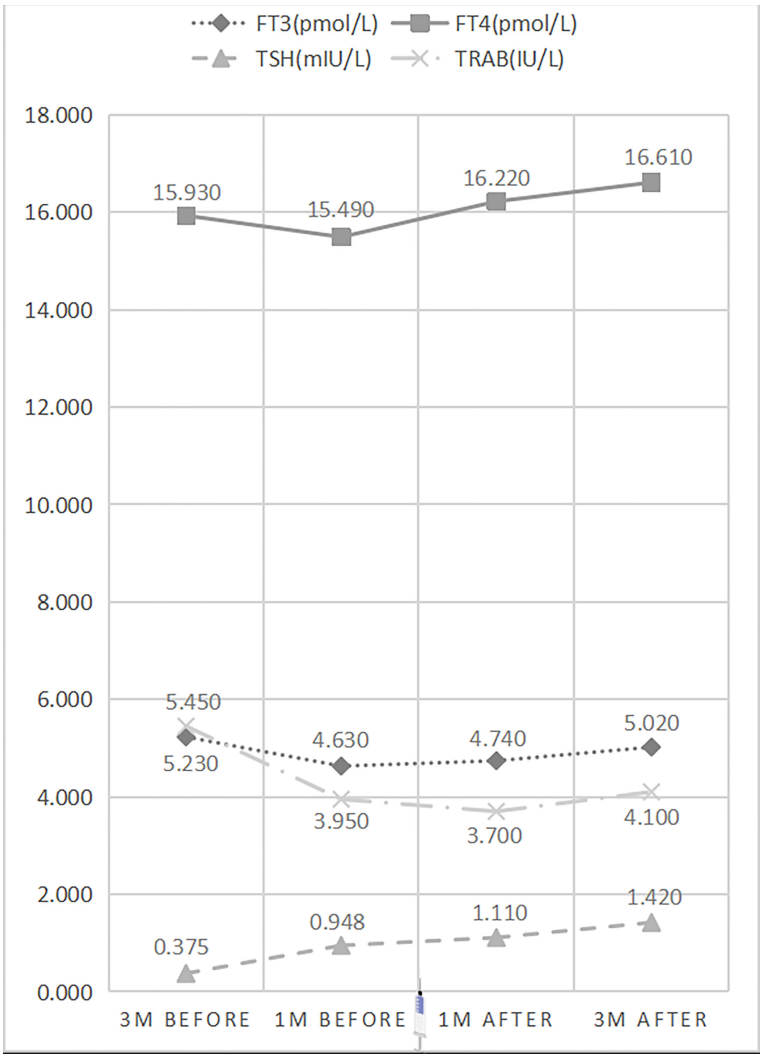

FIGURE 3 | Line charts of variation in thyroid function of prospective subjects.

of TRAB with TSHR in genetically predisposed individuals with GD. The pathogenesis of GD involves the destruction of thyroid immune tolerance, with the most pathogenic antibody being TRAB, and the immune mechanism is complex. A large amount of evidence has shown that the active phase of GD is associated with an immune prevalence of the Th1 immune response, whereas the inactive or later phases of GD are associated with a switch from Th1 to Th2 immune prevalence (21). It has been speculated that the mechanism of immune tolerance disruption is the maladjustment of autoreactive B cells that switch to plasma cells that produce pathogenic immunoglobulin G (20). Moreover, increasing the stimulating effect of Th2 cells on B cells promotes the production of more TRABs, which is considered another mechanism of GD occurrence (20).

TRABs were explored as an entry point of this study as an important indicator to evaluate treatment efficacy and recurrence. We conducted a retrospective study to observe the differences in TRAB trends between pre-vaccination and post-vaccination. Before vaccination, serum thyroid function and TRAB levels decreased significantly at intervals of 2 months under standard treatment, indicating an effective treatment. In contrast to pre-vaccination, no significant differences were found in serum thyroid function and TRAB levels post-vaccination, which indicated that there was no improvement in thyroid function and TRAB after vaccination under the same treatment regimen. Simultaneously, we compared serum TRAB levels at specified time points before and after vaccination. The results showed that the TRAB increased after vaccination, which also supports the previous prediction. The differences in the duration of the retrospective study were due to the overlap among subgroups of GD enrolled during the research, with the course of disease apparently longer post-vaccination than before.

Generally, serum TRAB levels should decline over time with standard treatment, which was not observed in our study. However, an unexpected increase in TRAB levels was observed after vaccination. The expected trend of TRAB titer is to decrease before vaccination, which contrasts with the TRAB titer plateau immediately after vaccination (+1-month time point) and the subsequent inverted and unexpected trend of TRAB increase at +3-months time-point after vaccination. To further explore the dynamic changes in TRAB before and after vaccination, we expanded a prospective study that measured the levels of serum thyroid function and TRAB at 3 and 1 months before and after vaccination in GD and then performed repeated measurement ANOVA. Lifestyle and treatment of the enrolled subjects were unaltered during the study. Consistent with the results of this retrospective study, there were significant differences in serum FT3, FT4, and TRAB levels at different time points before and after vaccination. Serum TRAB levels showed dynamic changes that decreased significantly at 1 month before vaccination, showed no significant differences at 1 month after vaccination, and changed slightly and reflected an upward trend at 3 months after vaccination. Serum FT3 and FT4 levels showed trends similar to those of serum TRAB levels before and after vaccination, but the nuances were that their upward trend moved forward and appeared 1 month after vaccination. Surprisingly, the changes in TSH after vaccination 1-3 months were inconsistent with those in FT3 and FT4, showing a continuous upward trend. We speculate that this phenomenon may be related to the sensitivity of TSH, which usually changes earlier than FT3 and FT4 levels. It may be that thyroid hormone is about to decline at the time point, but we have not captured their decreased levels due to the short follow-up time.

Based on the results obtained in both retrospective and prospective studies, we concluded that serum TRAB levels decreased less after vaccination and showed an upward trend. Thinking along the lines above, the association between inactivated SARS-CoV-2 vaccines and serum TRAB levels may be related to humoral immunity. After vaccination, antibodies were generated through humoral immunity, which could stimulate B cells and promote the synthesis of TRABs, changing the original declining trend of TRABs. Another explanation may be that adjuvants added to inactivated vaccines may disrupt the host immune balance and stimulate B cell cloning, affecting the original trend of TRAB.

Our study has two limitations. First, the current study was conducted in a single-center cohort of patients, with the possibility of selection bias. Second, the short follow-up period in our study resulted in unclear trends in serum TRAB levels 3 months after vaccination. The follow-up period should be extended to validate the time point of the peak of its increase.

The data presented here demonstrate that inactivated SARS-CoV-2 vaccines may affect TRAB trends in GD patients. Nevertheless, we must emphasize that the initial goal of vaccination is to protect the population from infection and reduce infection and mortality, and inactivated SARS-CoV-2 vaccines are not contraindicated in patients with GD (22). COVID-19 has led to millions of disabilities and deaths worldwide, especially in men, the elderly, and those with previous health problems; therefore, we believe that the risks of COVID-19 outweigh the minor risks of the vaccine in these populations. However, autoimmune diseases, especially GD, predominantly affect young women, who have a significantly reduced risk of severe Covid-19 disease. Thus, a careful analysis of the risk/benefit ratio should be continuously applied and revised according to the new scientific data that are produced daily. The results of this study provide evidence for clinical management and clinicians should be aware that TRAB levels may stop declining after vaccination.

CONCLUSION

Taken together, based on retrospective and prospective studies, the data presented here demonstrate that serum TRAB levels decreased less after inactivated SARS-CoV-2 vaccination and showed an upward trend, and FT3 and FT4 were consistent with it. This may be related to humoral immunity induced by vaccination. This finding suggests that humoral immunity induced by inactivated SARS-CoV-2 vaccine may affect autoimmunity. The advantages and disadvantages of vaccination should be weighed according to the applicable population. Clinicians should be aware that TRAB levels may stop declining following vaccination.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Second affiliated Hospital of Fujian Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors planned the concept of this report and wrote and revised the final manuscript. All authors contributed to the article and approved the submitted version.

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Mental Health in Postoperative Thyroid Patients During the COVID-19 Pandemic

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Background: Little is known about mental health in patients after thyroid surgery during the peak of the COVID-19 pandemic in China. This study aimed to assess the mental health of postoperative thyroid patients and to explore potential factors associated with psychological symptoms.

Methods: In this study, we surveyed 241 patients who underwent thyroid surgery at Peking Union Medical College Hospital. Insomnia, anxiety, depression, and posttraumatic stress symptoms (PTSS) were measured using the Insomnia Severity Index (ISI), Generalized Anxiety Disorder Questionnaire (GAD-7), Patient Health Questionnaire (PHQ-9), and Impact of Event Scale-Revised (IES-R), respectively.

Results: A significant proportion of postoperative patients reported experiencing insomnia, anxiety, depression, and PTSS. Patients that were older, single/divorced/widowed, and less educated; had lower income and poor general health; had undergone surgery within the past six months; had disrupted follow-up, and; searched social media for COVID-19-related information were associated with worse mental health.

Conclusions: During the COVID-19 pandemic, postoperative thyroid patients tended to develop mental health problems and have less psychological support, emphasizing the importance of patient education and psychological interventions.

Keywords: thyroid surgery, mental health, COVID-19, anxiety, depression

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INTRODUCTION

With the coronavirus disease 2019 (COVID-19) pandemic outbreak at the end of 2019, the disease rapidly spread worldwide. In the initial period, China was the most impacted by this pandemic, and there was an accompanying epidemic of anxiety and depression nationwide (1–3). People experienced numerous inconveniences due to the implementation of home quarantine policies (4, 5). Additionally, hospitals admitted large numbers of COVID-19 patients, and the shortage of

frontline medical staff resulted in limited follow-up for postoperative patients (6, 7). Postoperative thyroid patients require close monitoring and treatment, and the majority of these patients are also cancer patients, who may be more vulnerable to negative emotions that could lead to psychological problems (8–10).

Several studies have explored anxiety, depression, and quality of life during the COVID-19 pandemic in a variety of populations, including the general population (1, 2, 11), health care workers (12–14), medical students (15, 16), cancer patients (17–19), and postoperative surgical patients (20, 21). The majority of these study populations experienced negative emotional states during the pandemic; however, to date, no studies have focused on the mental health of patients after thyroid surgery (22). While thyroid cancer is one of the more inert cancers with a relatively good prognosis, a diagnosis of thyroid nodules, especially thyroid cancer, would affect a patient's postoperative psychological status. And several studies have reported similar or greater mental burden in thyroid patients (23, 24). The prevalence of thyroid cancer has increased every year, as has the volume of thyroid surgery (25–27), and the quality of life for postoperative thyroid patients has been of great concern to researchers (28, 29). However, during the COVID-19 pandemic, postoperative thyroid patients did not receive attention commensurate with their numbers.

The current study employed 4 validated psychological scales to assess symptoms of insomnia, anxiety, depression, and posttraumatic stress symptoms (PTSS) in postoperative thyroid patients during the peak of the COVID-19 pandemic in China and to investigate factors associated with these psychological symptoms. The goal of our study was to report the mental health status of postoperative thyroid patients and provide a reference for conducting thyroid surgery and perioperative patient education during the COVID-19 outbreak based on the potential influencing factors.

MATERIALS AND METHODS

Setting and Participants

From 2020.02.04 to 2020.02.18, when the COVID-19 epidemic was at its peak in China, we identified and contacted patients who had undergone thyroid surgery at Peking Union Medical College Hospital and were receiving regular follow-up in the outpatient clinic. An online questionnaire was sent to them *via* the contact information in the medical record system, and data were collected twice a day. The questionnaire was set to allow submission only after all questions were answered. According to the inclusion criteria, patients had to be 18-70 years of age, have undergone thyroid surgery, have had no surgical complications (e.g., permanent hypoparathyroidism, recurrent laryngeal nerve palsy, etc.) during hospitalization and follow-up, and can ensure the accuracy of the information. The presence of other malignancies or a history of diagnosed psychological disorders, either self-reported by patients or confirmed through the electronic medical records by the researchers, were used as exclusion criteria.

The study was approved by the Ethics Committee of Peking Union Medical College Hospital.

Demographic and Clinical Characteristics

Patients' sociodemographic characteristics, such as age, sex, employment status, marital status, education level, annual family income, and knowledge of their condition, were recorded from answers to the questionnaire, as well as whether their follow-up in the outpatient clinic which was usually once every 1 month to 3 months was disrupted by the COVID-19 pandemic and whether they searched for COVID-19-related news and medical information through social media. The clinical features of the patients, including comorbidities, time since surgery, type of surgery, and pathological results, were obtained by accessing the hospital's electronic medical record system.

Applied Questionnaires

Insomnia Severity Index (ISI): The 7-item ISI questionnaire is widely used to assess the symptoms and severity of insomnia (30); each question is scored from 0 to 4, for a total score of 28. Participants who scored 0-7 are classified as having no insomnia, 8-14 indicates mild insomnia, 15-21 indicates moderate insomnia and 22-28 indicates severe insomnia.

Generalized Anxiety Disorder Questionnaire (GAD-7): The GAD questionnaire consists of 7 questions measuring anxiety symptoms that are scored on a scale of 0 ("not at all") to 3 ("nearly every day") (31) for a total score of 21. A score of 0-4 indicates no anxiety, 5-9 indicates mild anxiety, 10-14 indicates moderate anxiety, and a score of more than 15 indicates severe anxiety.

Patient Health Questionnaire (PHQ-9): The PHQ is a questionnaire consisting of 9 items used to assess depression symptoms that are each scored from 0 ("not at all") to 3 ("nearly every day"), for a total score of 27 (32). A score of 0-4 indicates no depression, 5-9 indicates mild depression, 10-14 indicates moderate depression, and a score of more than 15 indicates severe depression.

Impact of Events Scale-Revised (IES-R): The 22-item IES-R questionnaire is used to assess PTSS symptoms (33); each question is scored from 0 ("not at all") to 4 ("extremely"), for a total score of 88. A score of 0-23 indicates no PTSS, 24-32 indicates mild PTSS, 33-36 indicates moderate PTSS and 37-88 indicates severe PTSS.

All questionnaires measured mental health symptoms within the past two weeks.

Statistical Analysis

Categorical data are expressed as rates, and continuous data are expressed as the median (range) or mean (SD). Independent samples t-tests, one-way ANOVAs, and Kruskal-Wallis tests were used to analyze the association between the categorical variables and scores on each psychological symptom (i.e., insomnia, anxiety, depression, and PTSS), and Pearson correlation analyses were used to analyze the association between the continuous variables and psychological symptom

scores. Independent variables identified as significant in the bivariate analysis were included in a multivariable linear regression model to analyze further the independent factors influencing each psychological symptom.

All analyses were conducted in SPSS (version 26.0; IBM) and GraphPad Prism (version 9.1.1), with a two-tailed p-value of ≤0.05 considered statistically significant.

RESULTS

Patient Characteristics

Of the 248 postoperative patients who provided informed consent, seven were excluded: two could not ensure the accuracy of their information and five had a history of psychological disorders; thus, 241 patients were included in the study. The demographic and clinical characteristics of the patients are shown in **Table 1**. The median age of these patients was 4l years (range: 23-67 years), with 78.8% female (n=190), 72.2% employed or full-time students (n=174), 85.5% married (n=206), 76.8% possessing a college degree or higher education (n=185), 71.8% with an annual household income >60,000 RMB (n=173), and 81.7% in good general health with no comorbidities (n=197).

Of the 241 patients, the majority underwent total thyroidectomy (70%, n=170) and had malignant pathology results (90.9%, n=219). The time since surgery was roughly evenly split, with 32.4%, 36.9%, and 30.7% of patients reporting that <6 months, 6-11 months, and >12 months had elapsed since surgery, respectively. The vast majority of patients felt that they had a complete or basic understanding of their condition (94.9%, n=228), and 61.0% (n=147) felt that their condition was not serious. During the peak period of the pandemic, 41.9% (n=101) of patients reported disruptions to their follow-up and treatment, and 77.6% of patients (n=187) used social media to search for news, medical information, and guidance related to COVID-19 while at home.

TABLE 1 | Baseline characteristics of patients.

Characteristic	n (%)
Age, year [median (range)]	41 (23-67)
Sex	
Male	51 (21.1)
Female	190 (78.8)
Employment status	
Employed or full-time student	174 (72.2)
Unemployed	32 (13.3)
Retired	35 (14.5)
Marital status	
Married	206 (85.5)
Single, divorced, or widowed	35 (14.5)
Highest level of education	
High school or below	56 (23.2)
College or higher	185 (76.8)
Annual family income, RMB[1]	
≦60,000	68 (28.2)
>60,000	173 (71.8)
Number of comorbidities	
0	197 (81.7)
1	32 (13.3)
2	9 (3.7)
3	3 (1.2)
Time since surgery, month	
<6	78 (32.4)
6-11	89 (36.9)
≧12	74 (30.7)
Type of surgery	
Total thyroidectomy	170 (70.5)
Unilateral lobectomy	71 (29.5)
Pathology	
Benign	22 (9.1)
Malignant	219 (90.9)
Lymph node metastasis (N=219)	
Yes	91 (37.8)
No	128 (53.1)
Understand their condition	
Complete or basic understanding	228 (94.9)
Partial understanding	13 (5.4)
Self-identification of the severity	
Very serious or somewhat serious	94 (39.0)
Not too serious or not serious	147 (61.0)
Usual follow-up or treatment disrupted	
No	140 (58.1)
Yes	101 (41.9)
Social media information	
No	54 (22.4)
Yes	187 (77.6)

[1] *1 RMB is equivalent to 0.16 USD.*

Mental Health Outcomes

Table 2 displays the results of the questionnaire corresponding to the 4 psychological symptoms, with median scores of 4 (IQR: 0-9) on the ISI, 3 (IQR: 0.5-7) on the GAD-7, 2 (IQR: 0-6.5) on the PHQ-9 and 10 (IQR: 2-22) on the IES-R, respectively. Of the 241 patients, those with symptoms of insomnia, anxiety, depression, and PTSS accounted for 32.0% (n=77), 39.4% (n=95), 33.6% (n=81), and 21.2% (n=51), respectively, with the majority of patients experiencing mild psychological symptoms and fewer experiencing moderate and severe symptoms; in contrast, there were more patients with severe PTSS than those with moderate PTSS according to the IES-R.

Factors Related to Mental Health

Table 3 shows the association between demographic factors, clinical characteristics, and independent variables related to COVID-19 and mental health status. Bivariate analysis revealed that in postoperative thyroid patients, older patients had higher levels of PTSS during the peak period of COVID-19 (p=0.047), and single/divorced/widowed patients had higher levels of depression and PTSS than married patients (p=0.034 and p=0.022, respectively). Patients with a college degree or higher education had lower levels of insomnia, anxiety, and PTSS than patients with less-than-high school education (p=0.010, p=0.034, and p=0.012, respectively), and patients with a higher annual family income had lower levels of insomnia, depression, and PTSS (p=0.009, p=0.035, and p=0.036, respectively). The number of comorbidities was associated with every mental health dimension, and there was also a significant association between time since surgery and insomnia as well as depression.

TABLE 2 | Overview of insomnia, anxiety, depression and PTSS.

		n (241)	%
ISI	Median (IQR) 4 (0-9)		
	Normal	164	68.0
	Mild insomnia	61	25.3
	Moderate insomnia	14	5.8
	Severe insomnia	2	0.8
GAD-7	Median (IQR) 3(0.5-7)		
	Normal	146	60.6
	Mild anxiety	70	29.0
	Moderate anxiety	19	7.9
	Severe anxiety	6	2.5
PHQ-9	Median (IQR) 2 (0-6.5)		
	Normal	160	66.4
	Mild depression	56	23.2
	Moderate depression	15	6.2
	Severe depression	10	4.1
IES-R	Median (IQR) 10 (2-22)		
	Normal	190	78.8
	Mild PTSS	27	11.2
	Moderate PTSS	8	3.3
	Severe PTSS	16	6.6

ISI, Insomnia Severity Index; GAD-7, Generalized Anxiety Disorder Questionnaire; PHQ-9, Patient Health Questionnaire; IES-R, Impact of Events Scale-Revised.

Surprisingly, patients that had surgery 6-11 months prior had the lowest levels of psychological symptoms of insomnia and depression, as shown in **Figure 1**. Patients who had undergone thyroid surgery within the past 6 months or more than 12 months ago had higher levels of insomnia and depression, and similar trends were found for anxiety and PTSS symptoms, although these were not significant. Insomnia and depression levels were significantly higher when patients knew less about their condition (p=0.022 and p=0.004, respectively). Patients whose routine follow-up was disrupted and those who searched social media for COVID-19-related information had higher levels of anxiety and PTSS than patients with non-disrupted follow-up (p=0.006 for GAD-7 scores, p=0.023 for IES-R scores) and those with less exposure to social media (p=0.008 for GAD-7 scores, p<0.001 for IES-R scores).

Based on the results of the bivariate analysis, nine variables – age, marital status, highest level of education, annual family income, number of comorbidities, time since surgery, understanding their condition, usual follow-up or treatment disrupted, and social media information – were included in the multivariate linear regression model. **Figure 2** shows the distribution of baseline characteristics of these variables in distinct degrees of psychological symptoms. The results of the multivariable linear regression are presented in **Table 4**. Time since surgery was an independent factor related to insomnia [odds ratios (ORs), -1.96 (95% CI, -3.58 to -0.34)]; marital status [ORs, 2.07 (95% CI, 0.55 to 3.60)] and number of comorbidities [ORs, -2.16 (95% CI, -3.69 to -0.63)] were independent factors related to anxiety; marital status [ORs, 2.22 (95% CI, 0.42 to 4,02)], time since surgery [ORs, -1.84 (95% CI, -3.30 to -0.38)], and knowledge of their condition [ORs, 3.50 (95% CI, 0.79 to 6.20)] were independent factors affecting depression; and age [ORs, 0.19 (95% CI, 0.01 to 0.37)], marital status [ORs, 7.06 (95% CI, 2.32 to 11.80)], number of comorbidities [ORs, 9.59 (95% CI, 1.16 to 18.03)] and use of social media for COVID-19 information [ORs, 6.28 (95% CI, 2.37 to 10.19)] were independent factors influencing PTSS. The degree of influence of these factors were displayed in **Figure 3**.

DISCUSSION

The psychological and emotional issues in numerous populations during the COVID-19 pandemic have received increasing attention (11–21). While psychological symptoms such as insomnia, anxiety, depression, and reduced quality of life after thyroid surgery have been reported by patients (34–36), the mental health of postoperative thyroid patients during the pandemic and the factors affecting it remain relatively unknown (37). To our knowledge, this current study is the first investigating mental health and influencing factors during the peak of the COVID-19 pandemic.

Our study found that a significant proportion of postoperative thyroid patients experienced insomnia, anxiety, depression, and PTSS, mostly to a mild degree. Age, marital status, education level, annual family income, number of comorbidities, time since surgery, the patients' knowledge of their condition, disruption to follow-up during the COVID-19 pandemic, and coping strategies were associated with one or more of the psychological symptoms of insomnia, anxiety, depression, and PTSS.

Overall, we found that during the COVID-19 pandemic, postoperative thyroid patients reported more symptoms of insomnia, anxiety, depression, and PTSS than the normal population and worse psychological status than postoperative thyroid patients before COVID-19 (1, 11, 36); however, these patients had better mental health than postoperative patients with other types of cancers (18).

Consistent with previous studies, we found that single/divorced/widowed patients were at a higher risk of psychological symptoms than married since they received more emotional support (36, 38). Higher education levels and higher annual family income were associated with lower psychological symptoms, consistent with the literature (11, 36), possibly because patients with higher education levels usually have more comprehensive information about both their condition and the COVID-19 pandemic. Moreover, higher income suggests higher risk tolerance. Together, these factors may support patients in coping with negative emotions and prevent the development of psychological disorders.

As shown in **Table 1**, almost a third of the patients underwent thyroid surgery more than 12 months before enrollment, and we set time since surgery as a variable for analysis. Notably, we found that it was an independent factor related to insomnia and depression and had nonsignificant but similar associations with anxiety and PTSS scores. Interestingly, patients who had undergone surgery within the past 6 months reported the most severe psychological symptoms, followed by those who underwent surgery more than 12 months ago, while patients who had surgery between 6 and 12 months ago had the best

TABLE 3 | Bivariate analysis of insomnia, anxiety, depression, and PTSS scores.

	ISI		GAD-7		PHQ-9		IES-R	
	Mean (SD)	***p*-value**	**Mean (SD)**	***p*-value**	**Mean (SD)**	***p*-value**	**Mean (SD)**	***p*-value**
Sex		0.689		0.355		0.707		0.266
Male	5.45 (5.91)		3.65 (3.46)		3.69 (4.55)		11.80 (12.44)	
Female	5.11 (5.35)		4.27 (4.44)		3.98 (5.04)		14.14 (13.49)	
Employment status		0.150		0.166		0.397		0.176
Employed or full-time student	4.78 (5.30)		3.82 (3.69)		3.67 (4.54)		12.81 (12.34)	
Unemployed	5.78 (5.54)		5.06 (5.44)		4.88 (5.49)		14.09 (13.32)	
Retired	6.63 (6.03)		4.89 (5.42)		4.29 (6.13)		17.37 (17.03)	
Marital status		0.089		0.061		**0.034**		**0.022**
Married	4.93 (5.37)		3.87 (4.00)		3.56 (4.59)		12.84 (13.14)	
Single, divorced, or widowed	6.63 (5.81)		5.69 (5.32)		6.00 (6.30)		18.37 (13.32)	
Highest level of education		**0.010**		**0.034**		0.110		**0.012**
High school or below	6.82 (5.21)		5.36 (5.06)		4.84 (5.02)		17.93 (14.66)	
College or higher	4.68 (5.45)		3.77 (3.91)		3.64 (4.88)		12.35 (12.59)	
Annual family income, RMB[1]		**0.009**		0.099		**0.035**		**0.036**
≦60,000	6.63 (5.99)		4.97 (5.25)		5.18 (6.22)		16.50 (15.08)	
>60,000	4.61 (5.15)		3.81 (3.75)		3.42 (4.24)		12.52 (12.37)	
Number of comorbidities		**0.012**		**0.002**		**0.009**		**0.007**
0	5.23 (5.46)		4.29 (4.41)		3.97 (5.03)		13.62 (13.49)	
1	3.53 (5.23)		2.09 (2.13)		2.38 (3.85)		9.69 (10.32)	
2	8.00 (4.85)		6.33 (2.74)		6.56 (3.40)		24.11 (9.56)	
3	10.67 (3.06)		9.33 (5.69)		9.00 (7.00)		26.00 (18.68)	
Time since surgery, month		**0.010**		0.055		**0.017**		0.202
<6	6.09 (5.90)		4.79 (4.42)		4.95 (5.36)		15.03 (13.46)	
6-11	4.02 (5.19)		3.45 (4.18)		2.90 (4.57)		11.89 (12.89)	
≧12	5.61 (5.13)		4.27 (4.10)		4.05 (4.69)		14.30 (13.50)	
Type of surgery		0.674		0.299		0.231		0.846
Total thyroidectomy	5.08 (5.35)		3.95 (4.13)		3.67 (4.75)		13.54 (12.92)	
Unilateral lobectomy	5.41 (5.76)		4.58 (4.52)		4.51 (5.32)		13.90 (14.19)	
Pathology		0.651		0.794		0.815		0.935
Benign	5.68 (5.54)		4.36 (4.87)		3.68 (4.50)		13.86 (14.58)	
Malignant	5.13 (5.47)		4.11 (4.20)		3.94 (4.98)		13.62 (13.18)	
Lymph node metastasis (N=219)		0.815		0.779		0.987		0.392
Yes	5.23 (5.80)		4.21 (4.63)		3.93 (5.41)		14.53 (14.58)	
No	5.05 (5.24)		4.05 (3.88)		3.95 (4.67)		12.98 (12.10)	
Understand their condition		**0.022**		0.175		**0.004**		0.083
Complete or basic understanding	4.99 (5.33)		4.05 (4.25)		3.70 (4.77)		13.29 (13.05)	
Partial understanding	8.54 (6.91)		5.69 (4.15)		7.69 (6.20)		19.85 (16.19)	
Self-identification of the severity		0.576		0.350		0.491		0.602
Very serious or somewhat serious	5.43 (5.58)		4.46 (4.68)		4.19 (5.41)		14.20 (13.74)	
Not too serious or not serious	5.02 (5.41)		3.93 (3.96)		3.74 (4.61)		13.29 (13.01)	
Usual follow-up or treatment disrupted		0.056		**0.006**		0.323		**0.023**
No	4.61 (5.12)		3.50 (4.00)		3.65 (5.03)		11.99 (12.47)	
Yes	5.97 (5.84)		5.02 (4.44)		4.29 (4.79)		15.93 (14.07)	
Social media information		0.145		**0.008**		0.340		**<0.001**
No	4.22 (5.52)		2.80 (3.91)		3.35 (5.34)		7.91 (10.85)	
Yes	5.45 (5.42)		4.52 (4.28)		4.08 (4.81)		15.30 (13.48)	
	r		r		r		r	
Age, year	0.099	0.126	0.107	**0.099**	0.006	0.930	0.128	**0.047**

[1] *1 RMB is equivalent to 0.16 USD*

The bold entries indicate variables with p value less than 0.05.

mental health. Chen et al. have shown that symptoms of anxiety and depression in patients become milder within 1 year after surgery (39), similar to our results. A possible explanation is that patients' lives gradually return to normal after surgery. However, our results are novel in that patient mental health symptoms worsened again 1 year after surgery; this unanticipated result could stem from an increase in fear of recurrence after 1 year had elapsed (40–42).

According to the multivariable linear regression model, we found a significant association between the number of comorbidities and all 4 mental health symptoms: having 2 comorbidities was an independent risk factor for PTSS. Surprisingly, however, patients with one comorbidity reported the lowest scores on the psychological scales, i.e., had better mental health than that patients without comorbidities. This result has not been previously reported and is contrary to our expectations; therefore, it should be treated with caution. It is difficult to explain this observation, but it might be related to fatigue due to comorbidity (43). Another possible explanation is that patients with one comorbidity tend to be more conscious and well informed about their physical health and therefore experienced fewer mood

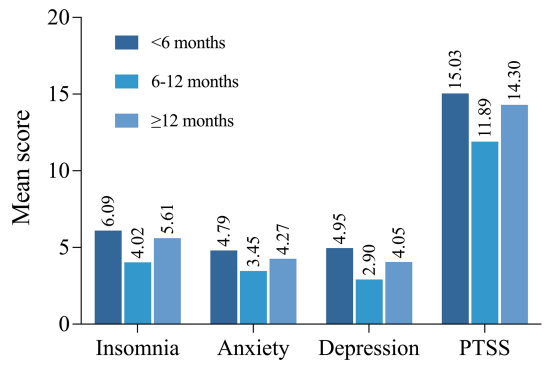

FIGURE 1 | Comparison of the mean scores of insomnia, anxiety, depression and PTSS among patients at different time since surgery.

fluctuations due to the COVID-19 pandemic than patients without comorbidities, whereas the poor mental health of patients with two or more comorbidities may be due to their poorer general condition (44).

Another interesting finding was that proactive search of COVID-19-related information and medical guidance from social media was associated with higher levels of anxiety and PTSS and was an independent risk factor for PTSS. This is consistent with the findings of previous studies (45–48). Potentially, information overload on social media makes it difficult for people with insufficient expertise to distinguish between truths and falsehoods. Especially for patients who are worried about their condition and those who cannot visit hospitals due to the pandemic, information overload increases their anxiety and PTSS about COVID-19 (17, 45, 49).

Our study is the first to report on the mental health of postoperative thyroid patients during the peak of the COVID-19 pandemic and the potential factors that influenced mental health. However, some possible limitations should be noted. First, we did not enroll a control group, although we made comparisons to previous studies. Second, this was a single-center study, and our patients were all from nearby areas; the hospital was far from Wuhan, the center of the epidemic in China. In addition, there was no specific preoperative mental assessment to determine the potential impact of thyroid function on their psychological status, which may influence postoperative mental health.

According to our findings, patients who were older, single/divorced/widowed, less educated, had lower annual income, in poor general health, had undergone surgery within the past six months, had disrupted access to postoperative follow-up, and actively searched social media for COVID-19-related information were more likely to develop mental health problems. These findings suggest that older, single/divorced/widowed patients should be encouraged to reach out more to friends and seek emotional support. Additionally, patients with lower levels of education,

TABLE 4 | Multivariable linear regression analysis of insomnia, anxiety, depression and PTSS scores.

	ISI	GAD-7	PHQ-9	IES-R
	OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]
Age	0.055 [-0.02, 0.13]	0.06 [-0.01, 0.12]	0.02 [-0.06, 0.09]	**0.19 [0.01, 0.37]**
Marital status				
Married				
Single, divorced, or widowed	1.73 [-0.27, 3.72]	**2.07 [0.55, 3.60]**	**2.22 [0.42, 4.02]**	**7.06 [2.32, 11.80]**
Highest level of education				
High school or below				
College or higher	-0.80 [-2.69, 1.09]	-0.65 [-2.10, 0.80]	-0.02 [-1.72, 1.68]	-2.31 [-6.80, 2.18]
Annual family income, RMB				
≦60,000				
>60,000	-1.28 [-2.96, 0.41]	-0.35 [-1.64, 0.94]	-1.37 [-2.89, 0.15]	-1.33 [-5.33, 2.68]
Number of comorbidities				
0				
1	-1.72 [-3.72, 0.28]	**-2.16 [-3.69, -0.63]**	-1.39 [-3.20, 0.41]	-3.79 [-8.54, 0.97]
2	2.17 [-1.38, 5.73]	1.68 [-1.03, 4.40]	2.24 [-0.96, 5.44]	**9.59 [1.16, 18.03]**
3	2.39 [-3.83, 8.61]	2.35 [-2.40, 7.10]	3.08 [-2.52, 8.68]	3.29 [-11.47, 18.05]
Time since surgery, month				
<6	ref	ref	ref	ref
6-11	**-1.96 [-3.58, -0.34]**	-1.16 [-2.40, 0.08]	**-1.84 [-3.30, -0.38]**	-2.24 [-6.09, 1.61]
≧12	-0.01 [-1.70, 1.69]	0.06 [-1.36, 1.23]	-0.48 [-2.01, 1.04]	0.77 [-3.25, 4.79]
Understand their condition				
Complete or basic understanding	ref	ref	ref	ref
Partial understanding	2.67 [-0.33, 5.68]	0.86 [-1.44, 3.16]	**3.50 [0.79, 6.20]**	4.40 [-2.74, 11.52]
Usual follow-up or treatment disrupted				
No	ref	ref	ref	ref
Yes	0.85 [-0.56, 2.25]	1.06 [-0.01, 2.13]	0.16 [-1.10, 1.43]	1.98 [-1.35, 5.32]
Social media information				
No	ref	ref	ref	ref
Yes	0.61 [-1.04, 2.26]	1.18 [-0.08, 2.44]	0.42 [-1.06, 1.90]	**6.28 [2.37, 10.19]**

The bold entries indicate variables with p value less than 0.05.

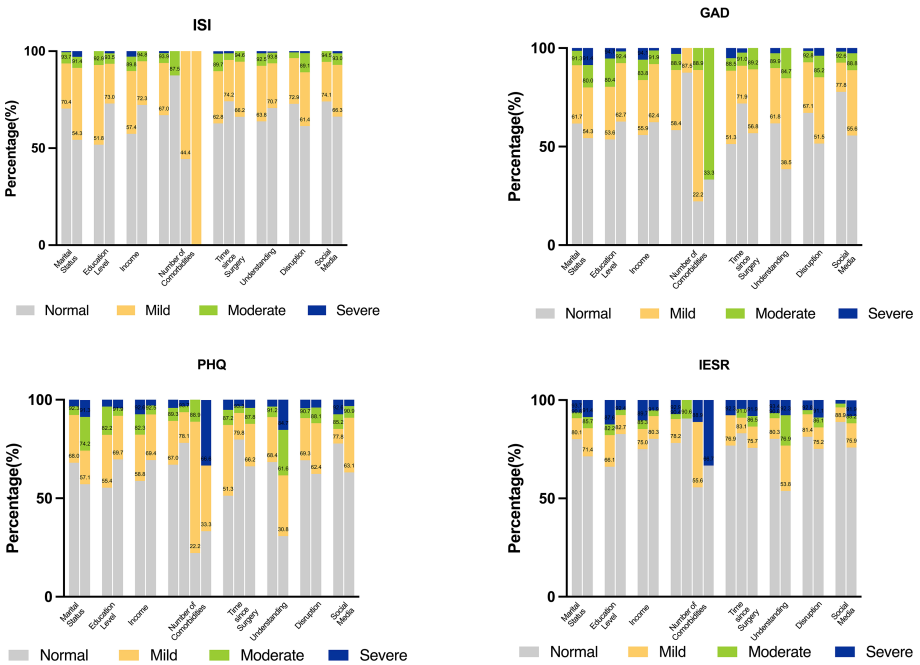

FIGURE 2 | Stacked bar blots to identify the distribution of baseline characteristics in distinct degrees of psychological symptom. (Interpretations: Marital status: Married vs. Single/divorced/widowed; Education level: High school or below vs. College or higher; Income: ≦60,000 vs.>60,000; Number of comorbidities: 0 vs. 1 vs. 2 vs. 3; Time since surgery: <6 vs. 6-11 vs. ≧12; Understanding: Complete or basic vs. Partial; Disruption: No vs. Yes; Social Media: No vs. Yes).

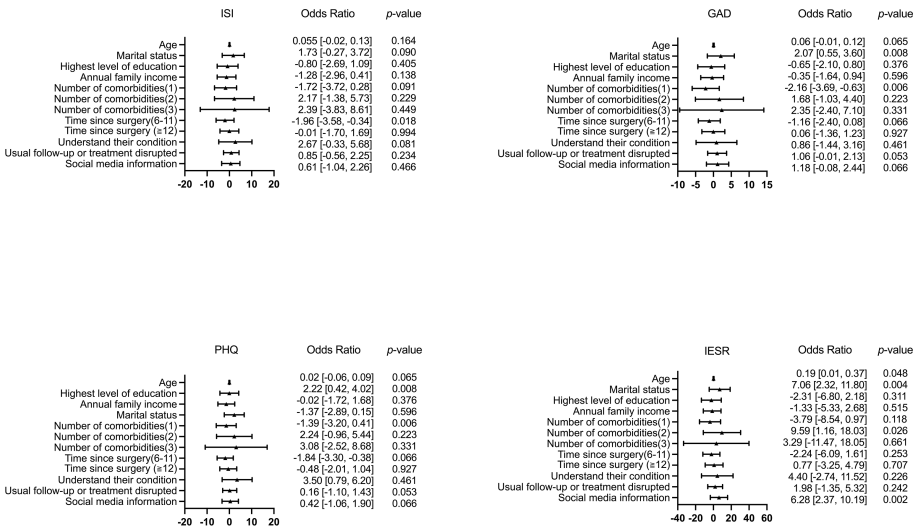

FIGURE 3 | Forest plots to identify independent affecting factors for insomnia, anxiety, depression, and PTSS.

lower income, more comorbidities, and those who have undergone surgery within the past 6 months are at higher risk of developing mental health problems and need to be promptly identified to provide more psychological support and intervention.

In addition, the mental health of patients might be improved by enhancing patient education and improving their understanding of their condition. Providing postoperative patients with medical care during the COVID-19 pandemic, such as educating patients to correctly identify medical information on social media, providing psychological counseling services for patients during special periods, offering special online consultation section, and organizing inter-patient support to alleviate patients' anxiety and PTSS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

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AUTHOR CONTRIBUTIONS

SY data analysis, data interpretation, original manuscript drafting; XX: study concept and design, data collection, data interpretation, original manuscript drafting and editing. All authors contributed to the article and approved the submitted version.

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Thyroid Function, Inflammatory Response, and Glucocorticoids in COVID-19

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The ongoing COVID-19 pandemic calls for extensive research on various medical topics. Since the beginning of the pandemic, multiple studies investigated the impact of SARS CoV-2 on thyroid function. However, crucial data, such as trend progression over time or influence of commonly used drugs, might still be missing. We checked the thyroid function in 174 patients with PCR-confirmed COVID-19. Our research covered three separate time points of hospitalization (days 1, 4, and 10). We did not exclude patients treated with glucocorticoids but, instead, compared them with patients not treated with steroids. We correlated the results of thyroid function tests with markers of systemic inflammation. We checked if abnormal thyroid function can predict unfavorable outcomes defined as combined primary endpoint and/or secondary endpoints; the combined primary endpoint was the occurrence of death, mechanical ventilation, non-invasive ventilation, vasopressor infusion, or prolonged hospital stay, and the secondary endpoint was any of the listed events. In general, 80.46% of evaluated patients displayed abnormalities in thyroid function tests over at least one time point throughout the observation. We noticed a high prevalence of features typical for thyroid dysfunction in non-thyroidal illness (NTI). Free triiodothyronine (fT3) concentration was significantly lower in the group requiring glucocorticoids. Patients displaying abnormal thyroid function were statistically more likely to meet the predefined combined primary endpoint. We found that fT3 measured at admission could be perceived as an independent predictor of endpoint completion for all analyzed groups. Thyroid involvement is common in COVID-19. Our study supports the idea of thyroid function abnormalities being important clinical tools and allowing early recognition of possible detrimental outcomes of the disease.

Keywords: thyroid, COVID-19, coronavirus disease 2019, SARS CoV-2, free triiodothyronine, interleukin-6, glucocorticoids, TSH

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INTRODUCTION

Over the past two years of the COVID-19 pandemic, the thyroid function in the context of COVID-19 quickly gained wide attention of researchers. As early as in the mid-2020, first studies on the topic were published. Chen and colleagues, the authors of one of the first articles, noticed that thyrotropin (TSH) and total triiodothyronine (T3) in COVID-19 patients were significantly lower than in their

counterparts who either suffered from non-COVID-19 pneumonia or were healthy (1). Subsequent studies confirmed the increased occurrence of features typical for thyroid dysfunction in nonthyroidal illness (NTI); however, the abnormalities were rather mild and rarely required specific treatment (2). A large prospective study conducted by Beltrão et al. concluded that early evaluation of simple biomarkers such as free triiodothyronine (fT3) and reverse triiodothyronine (rT3) in patients with moderate to severe COVID-19 can be helpful in assessment of the overall prognosis (3). A systematic review of 1237 patients enrolled into 7 studies performed by Giovanella et al. proved thyroid dysfunction in COVID-19 to be common, ranging between 13 and 64% of cases, and once again called attention to the positive correlation between the disease severity and abnormal thyroid function (4). Extensive reviews of COVID-19-related thyroid pathologies performed by Murugan and Alzahrani further highlighted the depth and prevalence of observed anomalies, calling for a careful evaluation of potentially affected individuals, with special regards for, amongst others, NTI, autoimmune thyroiditis, and subacute thyroiditis (5, 6). Data pointing out high incidence of new onset of hyperthyroidism (up to 20% of examined cases) in COVID-19 was presented (7). Currently, it is believed that observed thyroid pathologies arise not only from the well-known phenomena such as the thyroid dysfunction in NTI, but also from the direct action of SARS CoV-2 on thyrocytes and thyrotrope pituitary cells (6, 8–13).

Most of the available studies deliver the results of thyroid function tests assessed over a single time point and often correlate them with markers of inflammation (2, 14, 15). In our project, the tests were carried out over three separate time points throughout the hospital stay. Also, we aimed at providing information about possible relationships between the thyroid function and the occurrence of unfavorable endpoints (prolonged hospital stay, non-invasive or mechanical ventilation, infusion of vasoactive amines, and death) and about potential links between the concentration of hypothalamus-pituitary-thyroid (HPT) hormones and inflammatory markers.

MATERIALS AND METHODS

We designed a multiple cross-sectional study which recruited 180 adult patients (≥18 years old) with PCR-confirmed COVID-19 (nasopharyngeal swabs). All patients required hospital stay at the 7 Navy Hospital in Gdańsk, Poland, which served as our recruitment center between the 14 February 2021 and the 1 December 2021. 6 patients were excluded from the final analysis due to essential data missing which left us with 174 patients for the final analysis.

We gathered information about the patients' metrical and anthropometric data, as well as previous medical history. Prospectively, we evaluated oxygen supplementation, glucocorticoid use, vital parameters, the occurrence of unfavorable events (death, mechanical and/or non-invasive ventilation, vasopressor use), length of hospitalization, and laboratory tests (TSH, total thyroxine [T4], free thyroxine [fT4], fT3, rT3, anti-thyroperoxidase antibodies [anti-TPO abs], anti-thyroglobulin antibodies [anti-TG abs], leukocyte count [LEU], neutrocyte count [NEU], lymphocyte count [LYMPH], interleukin 6 [IL-6], C-reactive protein [CRP]).

Laboratory assays were performed using venous blood collected between 6 AM and 8 AM. There were three separate dates for blood collection, later referred to as the time points: day 1, 4, and 10 of the hospital stay. The blood collection could be moved up to 24 hours, however, we highly encouraged timely procedures. On all three dates, we assessed TSH, T4, fT4, fT3, rT3, CRP, IL-6, LEU, NEU, and LYMPH. In addition, on day 1 we checked anti-TG abs and anti-TPO abs.

Laboratory tests were performed by commercial laboratories. Due to technical abilities at the time of recruitment, rT3 measurement was performed in two separate laboratories. Information on laboratory methods is available in **Table 1**.

Some of the recruited patients required glucocorticoids (GCs) for COVID-19 or chronic diseases. Exogenous GC use modifies the endocrine function, therefore, for the sake of proper data analysis, we separated the patients who received GCs (the glucocorticoid group – GCG; N = 93) from those who did not (the no-glucocorticoid group – NGCG; N = 81). The GCs were either dexamethasone or methylprednisolone. Patients were labeled as the GCG if they received parenteral and/or oral GCs at least once during their stay and GCs were administered at least one day before the blood was collected.

We conducted the study in accordance with the Declaration of Helsinki. Our project was approved by the Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk (permissions NKBBN/373/2020, NKBBN/373-96/2021, NKBBN/373-184/2021). Written consent was obtained by the recruiters. As some patients arrived in bad general condition due to COVID-19 and/or concomitant diseases, they were unable to give written consent. In such cases, recruiters made a clear remark on the consent form. All recruited patients maintained their consent to participation. Inclusion criteria consisted of PCR-confirmed COVID-19, age of at least 18 years, and consent to participation. There were no exclusion criteria other than dissent to participation. The study was registered at ClinicalTrials.gov (NCT05070091).

STATISTICAL ANALYSIS

The Centre of Biostatistics and Bioinformatics Analysis, the Medical University of Gdańsk in Gdańsk, Poland performed the analysis. The analysis was performed using the R environment, with additional use of MS Excel. The threshold of significance was defined as $p<0.05$.

Quantitative data was evaluated using the Shapiro-Wilk W test and, based on the distribution pattern, was presented as either arithmetic mean with standard deviation (normal distribution) or as median with interquartile range (distribution deviating from normal). To describe qualitative data, absolute numbers and percentages were used.

TABLE 1 | Detailed technical information on used laboratory assays.

Parameter	Reference norm with units	Laboratory	Analytical method	Analyzer
1. TSH	0,27-4,2 µIU/ml	Diagnostyka Laboratoria, Gdańsk, Poland	Electrochemiluminescence assay	Cobas 8000, Roche
2. fT4	12-22 pmol/l	Diagnostyka Laboratoria, Gdańsk, Poland	Electrochemiluminescence assay	Cobas 8000, Roche
3. fT3	3,1-6,8 pmol/l	Diagnostyka Laboratoria, Gdańsk, Poland	Electrochemiluminescence assay	Cobas 6000, Roche
4. rT3	i. <0,95 ng/ml ii. 0,1-0,35 µg/l	i. Central Clinical Laboratory, University Clinical Centre, Gdańsk, Poland ii. Cerba International, Barcelona, Spain	i. Chemiluminescence immunoassay ii. Chemiluminescence immunoassay	i. Maglumi 800, Snibe ii. Maglumi 800, Snibe
5. T4	5,13-14,10 µg/dl	Diagnostyka Laboratoria, Gdańsk, Poland	Electrochemiluminescence assay	Cobas, Roche
6. anti-TPO abs	<9 IU/ml	Diagnostyka Laboratoria, Gdańsk, Poland	Electrochemiluminescence assay	Cobas 8000, Roche
7. anti-TG abs	<10 IU/ml	Diagnostyka Laboratoria, Gdańsk, Poland	Electrochemiluminescence assay	Cobas 8000, Roche
8. CRP	0-5 mg/l	Diagnostyka Laboratoria, Gdańsk, Poland	Immunoturbidimetric assay	Cobas 6000, Roche
9. IL-6	<5.9 pg/ml	Central Clinical Laboratory, University Clinical Centre, Gdańsk, Poland	Chemiluminescence immunoassay	Immulite XP, Siemens
10. LEU	females: 3.98-10.04x10^3/µl males: 4.23-9.07x10^3/µl	Diagnostyka Laboratoria, Gdańsk, Poland	*Fluorescence flow cytometry*	XT-4000i, Sysmex
11. NEU	2-7x10^3/µl	Diagnostyka Laboratoria, Gdańsk, Poland	*Fluorescence flow cytometry*	XT-4000i, Sysmex
12. LYMPH	1-3x10^3/µl	Diagnostyka Laboratoria, Gdańsk, Poland	*Fluorescence flow cytometry*	XT-4000i, Sysmex

The Spearman's test for rank correlation was applied to correlate the available variables, with the Spearman's r_{SP} correlation coefficient was used to check the strength of correlations. By applying the coefficient of determination R^2, the proportion of variation explained by correlation was calculated. The groups were compared using the Mann-Whitney U test. Associations between variables were checked using the Cramer's phi, the Yule's Q, and the Kendall's tau tests.

The changes in hormone levels over time were determined by the repeated measures ANOVA (rmAOV). The results were later validated using the Mauchly's sphericity test and, sometimes, adjusted using the Greenhouse-Geisser correction. The Student's t test was introduced for *post-hoc* multiple comparisons, with the FDR correction by Benjamini and Hochberg used whenever necessary.

Logistic regression and ROC curve assessment were used to check if baseline fT3 can be seen as an early predictor of unfavorable endpoints. The relationship between thyroid dysfunction and endpoints was validated by calculating the odds ratio (OR) with a 95% confidence interval. OR was measured using the Fisher's exact test and logistic regression.

Analyses covering rT3 were carried out separately depending on the laboratory site.

RESULTS

The final analysis included 174 patients: 93 from the NGCG and 81 from the GCG. In the NGCG, 12 patients had a history of hypothyroidism and 11 received levothyroxine (LT4); one patient required anti-thyroid treatment due to hyperthyroidism. In the GCG, 6 patients had a history of hypothyroidism and 4 required LT4; one patient used thyrostatics for hyperthyroidism. All patients with a history of thyroid dysfunction were clinically euthyroid at the time of recruitment. 7 patients (4.0%) required GCs for chronic conditions other than COVID-19 (4 – dexamethasone, and 3 – methylprednisolone); in this group, GCs were maintained as usual.

The mean age of recruited patients was 66.77 ±15.06 years, age range 18-94 years. 84 out of 174 patients (48.3%) were women. Half of the patients (87 out of 174 – 50.0%) required hospital stay lasting 10 days or longer. 102 (58.62%) patients suffered from arterial hypertension, 53 (30.46%) – type 2 diabetes, 17 (9.77%) – heart failure, 23 (13.22%) – atrial fibrillation, 30 (17.24%) – coronary artery disease, 18 (10.34%) – asthma and/or chronic obstructive pulmonary disease, 26 (14.94%) – chronic kidney disease, 17 (9.77%) required hemodialysis, 11 (6.32%) had a history of stroke, 3 (1.72%) – pulmonary embolism, and 14 (8.05%) – active neoplasia. BMI of 139 patients was analyzed – mean BMI equaled 27.57 ± 5.25 kg/m^2, with 53 (38.1%) individuals being overweight, and 39 (28.1%) – obese. Supplemental oxygen was introduced in 100 out of 173 subjects (57.8%). Over the period of observation, 30 patients (17.2%) died, 12 (6.9%) needed NIV/CPAP and/or HFNO, 3 (1.7%) – ventilator, and 6 (3.5%) – vasopressor infusion.

We assessed the thyroid function of patients in three predefined time points. The normal range was based on the recommendations provided by the laboratory kits' producers (**Table 1**). The parameters we considered were TSH, T4, fT4, fT3, and rT3, with an addition of anti-TG and anti-TPO abs. The results were labeled abnormal if any of the parameters deviated from the laboratory norms. In some patients, the clinical and/or laboratory data were incomplete which is reflected by the number of studied cases (N).

140 out of 174 patients (80.46%) displayed abnormal thyroid function in at least one time point throughout the observation. In

time point #1, abnormal thyroid function was present in 33 out of 56 (58.9%) patients from the GCG and in 67 out of 118 (56.8%) patients from the NGCG. In time point #2, thyroid dysfunction was present in 51 out of 74 (68.9%) patients from the GCG and in 48 out 83 (57.8%) patients from the NGCG. In time point #3, thyroid function was abnormal in 49 out of 63 (77.8%) GCG patients and in 33 out of 46 (71.7%) NGCG patients (**Table 2**).

Next, we focused on the connection between the thyroid function and the GC use: we found statistically significant differences between the GCG and the NGCG. TSH concentration was lower in the GCG as compared with the NGCG in all three time points (time point #1: $p<0.01$; time point #2: $p<0.0001$; time point #3: $p<0.05$) – **Figure 1**. Levels of fT3 were significantly lower in the GCG than in the NGCG in time points #2 and #3 (time point #2: $p=0.001$, time point #3: $p<0.0001$) – **Figure 2**. We observed a statistically relevant decrease in T4 concentration in the GCG as compared with the NGCG only in time point #3 ($p<0.05$). There were no relevant differences in fT4 and rT3 levels between the GCG and the NGCG in any of the time points (**Tables 3**–**5**).

As the next step of our analysis, we checked the relationship between the thyroid function and the occurrence of unfavorable clinical outcomes defined as the endpoints. The combined primary endpoint was a composite of hospitalization ≥10 days, mechanical ventilation, non- invasive ventilation (CPAP/NIV) and/or high-flow nasal oxygen device (HFNO), vasopressor use, death. The secondary endpoints were defined as any of the conditions listed above. 95 out of 140 (67.86%) patients with abnormal thyroid function and 12 out of 34 (35.29%) patients with normal thyroid function met the combined primary endpoint.

Patients displaying abnormal thyroid function were statistically more likely to meet the combined primary endpoint (OR=3.9, $p=0.0001$); among the secondary endpoints, statistical significance was found for prolonged hospitalization (OR=5.1, $p<0.0005$). When the confounders, such as age, sex, and BMI, were considered, the ORs were even higher (OR=5.3, $p=0.001$ and OR=6.6, $p=0.001$, consecutively). No significant relationship between the thyroid function and secondary endpoints other than prolonged hospital stay was found.

We checked if the baseline fT3 concentration can predict the endpoints. The analysis was performed for the entire studied population, as well as for the GCG and the NGCG separately. We found that fT3 measured at admission might predict the occurrence of endpoints in all analyzed groups (the general population: regression coefficient -1.067, $p=0.0000$; the GCG: regression coefficient -1.286, $p=0.0010$; the NGCG: regression coefficient -0.936, $p=0.0051$). Statistical significance was found regardless of the confounders (age, sex, BMI). Negative values of the regression coefficients suggested that the higher the fT3 concentration, the lower the risk of unfavorable endpoints.

Next, possible correlations between thyroid function and markers of generalized inflammation were assessed. For the first time point, significant negative correlations were found between TSH and CRP ($r=-0.169$, $p=0.030$), fT3 and CRP ($r=-0.398$, $p=0.000$), fT3 and IL-6 ($r=-0.262$, $p=0.001$), fT3 and LEU ($r=-0.237$, $p=0.002$), T4 and IL-6 ($r=-0.182$, $p=0.022$), T4 and LEU ($r=-0.189$, $p=0.016$), and rT3 and IL-6 ($r=-0.170$, $p=0.032$). For the second time point, there was a statistically important positive correlation between TSH and IL-6 ($r=0.209$, $p=0.011$) and negative correlation for fT3 and CRP ($r=-0.304$, $p=0.000$), fT3 and LEU ($r=-0.195$, $p=0.017$), and T4 and IL-6 ($r=-0.179$, $p=0.029$). For the third time point, there was a positive correlation between TSH and CRP ($r=0.263$, $p=0.006$), TSH and IL-6 ($r=0.349$, $p=0.000$), and a negative correlation between TSH and LEU ($r=-0.396$, $p=0.000$), fT3 and LEU ($r=-0.327$, $p=0.001$), and fT4 and CRP ($r=-0.202$, $p=0.038$). Laboratory results are displayed in **Tables 3**–**5**.

We verified if the presence of anti-thyroid antibodies can take its toll on the thyroid function in COVID-19. The presence of anti-TPO abs was confirmed in 13 out of 164 (7.9%) patients and anti-TG abs – in 12 out of 165 (7.3%). TSH levels in patients with positive anti-TG and anti-TPO abs were checked over all three time points. There were considerable differences in TSH levels between the groups with positive and negative autoantibodies, but only for the first time point ($p<0.05$). At the same time, TSH concentration in patients with anti-TG abs was significantly lower over all three time points (#1: $p<0.0001$; #2: $p<0.005$; #3: $p<0.005$). As we assessed the relationship between the presence of anti-thyroid antibodies and the endpoint completion, we found no significant results.

DISCUSSION

Most articles on thyroid function in COVID-19 involve HPT parameters assessed over a single time point (15–19). The majority of authors decided to exclude patients treated with GCs as GCs can influence the HPT axis (14, 19, 20). In our project, we decided not only to evaluate hormonal parameters, but to seek for potential trends appearing over the course of prolonged observation (up to 10 days post-admission). We chose

TABLE 2 | Pattern of thyroid function abnormalities throughout the observation time.

Type of abnormalities	NGCG			GCG		
	Time point #1 (number/percentage of cases)	Time point #2 (number/percentage of cases)	Time point #3 (number/percentage of cases)	Time point #1 (number/percentage of cases)	Time point #2 (number/percentage of cases)	Time point #3 (number/percentage of cases)
Total	67/56.78%	48/57.83%	33/71.74%	33/58.93%	51/68.92%	49/77.78%
Thyroid dysfunction in non-thyroidal illness	51/43.22%	32/38.55%	20/43.48%	29/51.78%	40/54.05%	42/66.67%
Unspecified/mixed	16/13.56%	16/19.28%	13/28.26%	4/7.14%	11/14.86%	7/11.11%

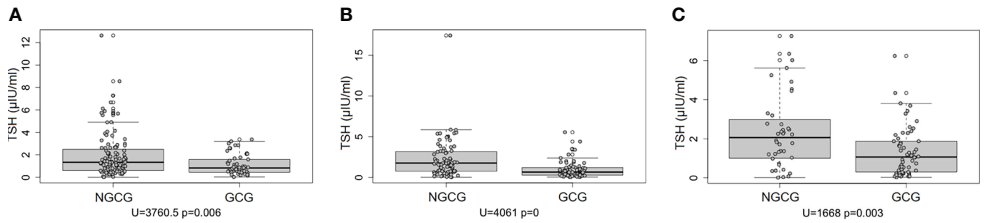

FIGURE 1 | Concentration of TSH over three consecutive time points among patients not treated with glucocorticoids (NGCG) and treated with glucocorticoids (GCG). **(A)** time point #1; **(B)** time point #2; **(C)** time point #3.

to include patients treated with GCs and compare them with their counterparts who did not receive GCs. We evaluated the thyroid function abnormalities and their potential effect on the clinical outcomes.

The available data shows that between 13% and 78% of COVID-19 patients display abnormal thyroid function and the degree of dysfunction differs based on the disease severity (7, 15, 20, 21). This is in accordance with our own research which documented thyroid function pathology, defined as typical laboratory abnormalities within at least one assessed parameter, in more than 80% of all recruited patients over the period of observation. The percentage of affected individuals raised over time, with more than 70% patients with abnormal thyroid function over the third time point of follow-up. These findings support the observations made by Campi and colleagues who decided to record hormonal trends over an extended period (18).

The hormonal pattern we noticed was characteristic for NTI and the results were comparable between the GCG and the NGCG (NTI criteria used: low fT3 and/or low fT4, and/or low T4, and/or elevated rT3, and/or low TSH). However, some of the observed abnormalities, such as decreased TSH and fT3, especially within the GCG, could be explained not only by the NTI, but the use of exogenous GCs as well. The thyroid function abnormalities in NTI, also referred to as euthyroid sick syndrome or low triiodothyronine syndrome, are a complex topic. The pathogenesis might involve excessive generalized immune response and cytokine storm, direct cytotoxic effect of SARS CoV-2 on pituitary and thyroid cells (9–12), altered activity of deiodinases (especially if the concomitant disease is severe), changes in concentrations of carrier proteins for thyroid hormones, changes of thyroid hormone carriers activity (9), endogenous dopamine release, and non-neoplastic hypercortisolemia (18, 20, 22).

Unfavorable clinical prognosis is common in patients with NTI and COVID-19 who did not receive GCs (9, 15, 21, 23). Our project demonstrated that low fT3 can precede unfavorable clinical outcomes both in patients treated and not treated with GCs. As Campi and colleagues offered a longer follow-up, their data revealed that the thyroid profile tends to reverse back to normal in survivors but remains abnormal in those who eventually die (18).

Our analysis proved that HPT dysfunction might predict the occurrence of the combined primary endpoint, and among the secondary endpoints, there is a statistically significant chance for the patients with abnormal thyroid function to require a prolonged hospitalization. A relationship between thyroid function pathology and the clinical course of COVID-19 was confirmed by other authors (14, 16, 23). Zhang and colleagues in their evaluation of COVID-19 patients with abnormal thyroid function described an increased incidence of severe form of the disease, requiring antibiotic treatment, GCs, high-flow oxygenation, non-invasive ventilation, or invasive ventilation. Low triiodothyronine syndrome was the most prevalent clinical entity in the studied group (16).

To fully assess the thyroid function, we evaluated not only the tropic and peripheral hormones, but checked the anti-thyroid antibody titers and the potential relationship between their presence and the disease severity. COVID-19 patients with positive anti-TG abs displayed significantly lower concentration of TSH in all three preset time points as compared with their counterparts with negative antibodies; however, patients with positive autoantibodies did not show a higher likelihood of meeting the endpoints. Lui and colleagues assessed the anti-thyroid antibodies in COVID-19 during hospital stay and three

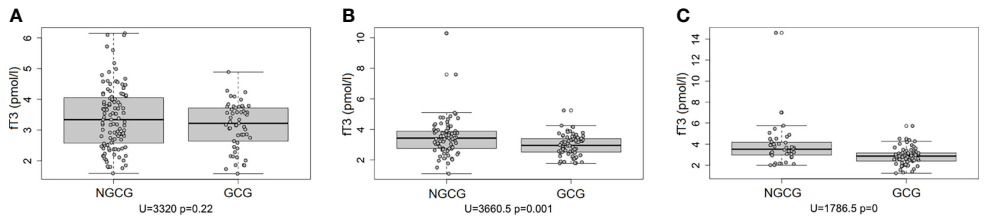

FIGURE 2 | Concentration of fT3 over three consecutive time points among patients not treated with glucocorticoids (NGCG) and treated with glucocorticoids (GCG). **(A)** time point #1; **(B)** time point #2; **(C)** time point #3.

TABLE 3 | Chosen clinical and laboratory parameters – time point #1.

Characteristics	Time point #1											
	NGCG						GCG					
	N	Mean ± SD	LQ	Medium	UQ	Range	N	Mean ± SD	LQ	Medium	UQ	Range
Age (years)	118	67.229 ± 15.134	58.000	69.000	79.000	18.00-94.00	56	65.804 ± 14.984	56.750	68.000	75.750	31.00-90.00
BMI (kg/m^2)	95	26.815 ± 5.146	23.620	25.760	29.530	17.33-40.89	44	29.213 ± 5.156	25.620	27.895	31.328	19.53-44.08
Oxygen demand (l/min.)	117	2.517 ± 5.237	0.000	0.000	4.000	0.00-30.00	56	10.562 ± 15.958	3.000	6.000	10.000	0.00-75.00
Hospital stay (days)	118	12.890 ± 9.135	7.000	10.000	18.000	1.00-50.00	56	10.429 ± 6.126	6.000	9.000	14.250	1.00-30.00
TSH (μIU/ml)	112	1.916 ± 1.9699	0.619	1.330	2.428	0.005-12.62	53	1.117 ± 0.900	0.444	0.827	1.590	0.025-3.37
fT3 (pmol/l)	112	3.389 ± 1.017	2.582	3.340	4.043	1.590-6.15	53	3.145 ± 0.756	2.640	3.220	3.720	1.580-4.89
fT4 (pmol/l)	112	15.357 ± 3.352	13.180	14.620	16.900	9.660-27.07	53	15.132 ± 3.384	13.440	14.700	17.540	4.880-23.05
T4 (μg/dl)	110	7.570 ± 1.791	6.385	7.520	8.595	4.240-13.00	52	7.575 ± 2.321	5.885	7.185	9.045	2.380-14.5-
rT3 – laboratory #1 (μg/l = ng/ml)	37	0.219 ± 0.052	0.190	0.210	0.240	0.130-0.35	12	0.202 ± 0.041	0.170	0.190	0.220	0.160-0.29
rT3 – laboratory #2 (ng/ml = μg/l)	72	0.652 ± 0.239	0.478	0.605	0.755	0.270-1.35	40	0.605 ± 0.315	0.465	0.555	0.685	0.080-1.90
anti-TG abs (IU/ml)	112	75.477 ± 395.412	3.200	3.200	11.800	3.200-4000.00	53	12.136 ± 24.652	3.200	3.200	11.600	3.200-141.00
anti-TPO abs (IU/ml)	112	21.841 ± 75.617	3.000	3.000	12.425	3.000-589.00	52	17.151 ± 51.859	3.000	3.000	12.000	3.000-346.00
IL-6 (pg/ml)	111	35.888 ± 47.938	9.390	19.400	41.650	1.410-267.00	53	233.631 ± 1368.543	7.210	20.000	59.100	1.41-10,000.00
CRP (mg/l)	112	61.400 ± 67.871	9.875	39.700	84.775	0.700-320.10	53	103.50 ± 100.612	21.700	78.100	139.900	1.00-427.70
LEU (^10^3/μl)	112	6.786 ± 3.578	4.375	5.845	8.152	1.790-26.16	53	6.706 ± 3.823	3.920	5.590	8.280	1.90-21.22
NEU (^10^3/μl)	112	4.640 ± 3.241	2.570	3.560	5.610	1.220-21.36	53	5.29 ± 3.670	3.120	4.390	6.520	1.08-20.24
LYMPH (^10^3/μl)	112	1.332 ± 0.652	0.858	1.260	1.755	0.260-3.56	53	0.898 ± 0.499	0.650	0.800	1.030	0.17-2.78

TABLE 4 | Chosen clinical and laboratory parameters – time point #2.

Characteristics	Time point #2											
	NGCG						GCG					
	N	Mean ± SD	LQ	Medium	UQ	Range	N	Mean ± SD	LQ	Medium	UQ	Range
Age (years)	83	68.157 ± 14.374	60.000	70.000	78.500	18.00-94.00	74	65.459 ± 15.664	56.000	67.000	78.000	31.00-94.00
BMI (kg/m^2)	67	26.533 ± 4.877	23.775	25.950	28.360	17.40-40.82	58	28.752 ± 5.501	24.828	27.815	31.250	17.33-44.08
Oxygen demand (l/min.)	82	2.293 ± 6.787	0.000	0.000	0.000	0.00-40.00	73	8.493 ± 11.168	0.000	5.000	11.000	0.00-60.00
Hospital stay (days)	83	13.458 ± 9.793	7.000	10.000	18.500	2.00-50.00	74	12.730 ± 5.836	8.000	12.000	16.000	4.00-30.00
TSH (μIU/ml)	80	2.252 ± 2.365	0.787	1.420	2.770	0.032-17.42	70	0.964± 1.030	0.289	0.656	1.202	0.051-5.55
fT3 (pmol/l)	79	3.506 ± 1.235	2.755	3.430	3.895	1.100-10.29	70	2.964 ± 0.683	2.520	2.955	3.400	1.780-5.24
fT4 (pmol/l)	80	15.709 ± 3.480	13.453	15.380	17.355	9.870-31.69	70	16.117± 3.499	13.518	15.830	18.677	7.050-24.43
T4 (μg/dl)	78	7.828 ± 1.683	6.628	7.745	8.900	4.690-13.60	70	7.813 ± 2.194	6.418	7.730	8.968	3.370-16.00
rT3 – laboratory #1 (μg/l = ng/ml)	18	0.198 ± 0.068	0.150	0.180	0.232	0.120-0.35	16	0.213 ± 0.057	0.180	0.205	0.222	0.140-0.38
rT3 – laboratory #2 (ng/ml = μg/l)	56	0.726 ± 0.319	0.528	0.635	0.843	0.330-2.24	50	0.645± 0.230	0.472	0.620	0.775	0.270-1.34
IL-6 (pg/ml)	78	41.296 ± 62.231	8.975	21.000	48.275	1.41-399.00	70	39.765 ± 167.153	3.990	7.920	18.100	1.41-1374.00
CRP (mg/l)	79	52.347 ± 60.984	9.150	36.300	66.000	0.70-281.80	70	40.333 ± 41.274	13.875	24.750	57.425	0.80-171.30
LEU (^10^3/μl)	80	6.576 ± 3.230	4.355	5.615	8.035	0.56-18.25	69	7.460 ± 2.861	5.200	7.500	10.090	2.39-13.32
NEU (^10^3/μl)	80	4.392 ± 2.997	2.375	3.250	5.862	0.25-14.31	69	5.658 ± 2.522	3.450	5.880	7.270	1.33-11.66
LYMPH (^10^3/μl)	80	1.331 ± 0.676	0.880	1.200	1.765	0.12-3.92	69	1.040 ± 0.585	0.670	0.920	1.230	0.35-3.42

months after the discharge and their follow-up did not confirm a long-standing relationship between the presence of autoantibodies at the baseline and worse clinical outcomes but, quite on the contrary, patients with positive autoantibodies took shorter to recover from symptomatic COVID-19 (23)

Available studies confirmed a link between thyroid function and inflammatory markers, such as ferritin, fibrinogen, erythrocyte sedimentation rate, interleukin-8, interleukin-15, or LYMPH (14, 20, 24). Typically, the more severe the infection was, the more pronounced the abnormalities of thyroid function and the degree of inflammation were. In our material, we noticed multiple various correlations between the results of thyroid function tests and inflammatory markers, with CRP, LEU, and IL-6 being emphasized the most. The variety of observed abnormalities highlights the complex background of the process and most likely arises from different physiopathological processes. We paid special

TABLE 5 | Chosen clinical and laboratory parameters – time point #3.

Characteristics	Time point #3											
	NGCG						GCG					
	N	Mean ± SD	LQ	Medium	UQ	Range	N	Mean ± SD	LQ	Medium	UQ	Range
Age (years)	46	68.435 ± 14.842	61.500	70.000	75.000	18.00-94.00	63	68.365 ± 14.544	61.500	69.000	80.000	31.00-94.00
BMI (kg/m^2)	38	26.054 ± 4.267	22.868	25.735	28.263	19.10-38.30	49	29.302 ± 5.980	24.690	27.940	32.030	17.33-44.08
Oxygen demand (l/min.)	41	1.976 ± 5.303	0.000	0.000	0.000	0.00-30.00	61	4.984 ± 9.856	0.000	0.000	6.000	0.00 -52.00
Hospital stay (days)	46	15.565 ± 8.400	9.000	13.500	20.000	4.00-41.00	63	15.952 ± 8.261	10.000	14.000	19.000	6.00- 50.00
TSH (μIU/ml)	40	2.344 ± 1.886	1.017	2.060	2.875	0.006-7.26	62	1.311 ± 1.239	0.314	1.065	1.850	0.024-6.24
fT3 (pmol/l)	40	3.863 ± 2.055	2.982	3.515	4.162	2.000-14.59	62	2.845 ± 0.833	2.405	2.865	3.165	1.230-5.73
fT4 (pmol/l)	40	16.401 ± 5.558	13.662	15.570	17.320	9.290-44.12	62	16.319 ± 3.989	13.625	16.595	19.125	6.710-25.38
T4 (μg/dl)	40	8.338 ± 2.462	7.312	7.880	9.032	4.200-17.40	61	7.158 ± 2.056	5.730	7.250	8.260	2.910-12.62
rT3 – laboratory #1 (μg/l = ng/ml)	11	0.184 ± 0.043	0.155	0.170	0.200	0.140-0.26	14	0.197 ± 0.052	0.162	0.190	0.230	0.120-0.31
rT3 – laboratory #2 (ng/ml = μg/l)	26	0.919 ± 0.547	0.650	0.815	1.070	0.320-3.27	45	0.734 ± 0.835	0.460	0.560	0.720	0.110-5.88
IL-6 (pg/ml)	40	47.343 ± 115.409	8.705	16.900	29.275	1.41-704.00	61	140.251 ± 917.906	3.530	8.130	22.200	1.41-7182.00
CRP (mg/l)	40	47.150 ± 54.222	8.550	32.800	58.500	0.80-280.30	62	37.098 ± 67.678	3.450	7.900	30.700	0.80-342.60
LEU (^10^3/μl)	40	6.558 ± 2.678	4.920	5.770	7.195	1.92-16.96	62	10.039 ± 5.770	6.478	9.230	11.925	3.09-39.01
NEU (^10^3/μl)	40	4.224 ± 2.734	2.638	3.385	4.970	0.68-15.84	62	7.702 ± 5.548	4.395	6.585	9.047	1.36-35.97
LYMPH (^10^3/μl)	40	1.352 ± 0.621	0.865	1.290	1.768	0.43-3.19	62	1.287 ± 0.756	0.747	1.185	1.585	0.28-4.46

attention to IL-6 known as a potential trigger of cytokine storm, which is often the underlying cause of a severe disease (18, 22, 25, 26) and might influence the thyroid function tests (22). The reverse correlation between the basic thyroid function parameters, such as TSH or fT3, and inflammatory markers was reported before (18), and additional studies confirmed that thyroid dysfunction seemed more pronounced in severe states (19). While low fT3 in most cases can be viewed as a manifestation of NTI, especially with fT4 and TSH decreased and/or within the normal limits, and without clinical signs and symptoms of thyroidopathy, the physiopathological background of decreased TSH may be more complex. We agree with other authors that multiple explanations of this phenomenon in COVID-19 should be considered (18, 22, 27). For instance, Croce et al. in their review of thyroid function in COVID-19 explained that multiple proinflammatory cytokines can influence the HPT, eventually leading to NTI and a drop in peripheral thyroid hormones and/or TSH (22). Other plausible explanations of reduced TSH concentrations include the development of typical or atypical subacute thyroiditis, the inhibitory effect of systemic GCs on the pituitary, or thyrotoxicosis.

As described above, the topic of thyroid function in COVID-19 can be complex. Assessment of the thyroid panel early during hospitalization – as early as on day one – can benefit the patients and allow an early recognition of potential warning signs. Thus, a simple laboratory profile of basic parameters such as TSH, fT3, and fT4 could be implemented into routine diagnostics, with additional clinical value added by repeated testing in different time-points. NTI is common in COVID-19 and, often, the more pronounced it is, the poorer the prognosis is. The abnormalities typical for NTI should reverse once the patient recovers. Nonetheless, some patients might suffer from subacute thyroiditis, hypothyroidism, or thyrotoxicosis. In such cases, clinical signs and symptoms typical for suspected disorders should be assessed together with laboratory (full thyroid profile, anti-thyroid antibodies, sedimentation rate, CRP) and radiological (thyroid ultrasound) work-up, and disease-specific treatment should follow once a diagnosis is concluded.

Strengths of our research are: i. prolonged observation covering three separate time points; ii. inclusion of patients treated with GC who were underrepresented in previous studies; iii. wide range of assessed parameters, including rT3 and IL-6; iv. evaluation of relationship between endpoint completion and thyroid hormone levels; v. unified approach to patient recruitment, blood collection, and follow-up.

However, our study also has its weaknesses: i. recruitment in only one center; ii. ethnically uniform studied group; iii. division of rT3 assessment between two separate laboratories; iv. lack of follow-up focused on detailed clinical signs and symptoms typical for thyroid disorders; v. lack of post-discharge follow-up.

SUMMARY

Our research proves an important role of endocrine function in COVID-19. Thyroid function markers, especially fT3, are important for the diagnostic process and allow early recognition of unfavorable outcomes. There is a close relationship between thyroid function and the degree of systemic inflammation in COVID-19 patients. Simple hormonal tests like fT3 could predict the hospitalization outcomes, especially in patients with severe disease. Our analysis supplements and confirms the data on observed HPT abnormalities in COVID-19.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk. The patients/participants provided their written informed consent to participate in this study. The Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk waived the requirement of written consent in patients unable to to provide it due to bad general state caused by COVID-19 and/or concomitant diseases.

AUTHOR CONTRIBUTIONS

RŚ-S – manuscript concept and preparation, results interpretation, manuscript revision, literature collection and review, project supervision. AB – manuscript concept and preparation, database creation, results interpretation, literature collection and review. ER – patient recruitment, data collection. All authors contributed to the article and approved the submitted version.

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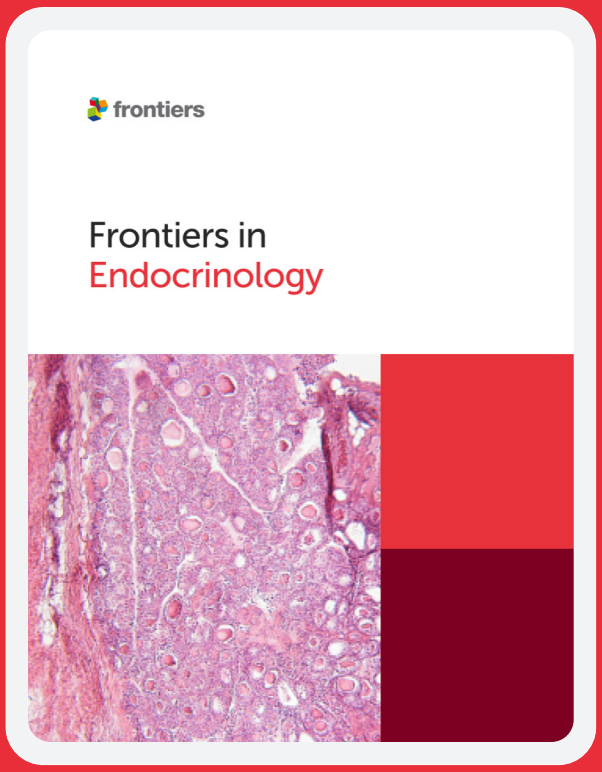

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