Thyroid Dysfunction in Relation to Immune Profile, Disease Status and Outcome in 191 Patients with COVID-19

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Abstract

Objective: SARS-CoV-2-related thyroiditis is increasingly recognized. The role of thyroid autoimmunity and SARS-CoV-2 viral load in SARS-CoV-2-related thyroid dysfunction is unclear. We evaluated the thyroid function of a cohort of COVID-19 patients, in relation to their clinical features, biochemical, immunological and inflammatory markers.

Methods: Consecutive adult patients, without known thyroid disorders, admitted to Queen Mary Hospital for COVID-19 from 21 July to 21 August, 2020 were included. Serum levels of thyroid-stimulating hormone (TSH), free thyroxine, free triiodothyronine (fT3) and anti-thyroid antibodies were measured on admission.

Results: Among 191 patients with COVID-19 (mean age 53.5 ± 17.2 years; 51.8% male), 84.3% were mild, 12.6% were moderate, and 3.1% were severe. 13.1% had abnormal thyroid function. Ten patients had isolated low TSH, suggestive of subclinical thyrotoxicosis due to thyroiditis, although the contribution of autoimmunity was likely in two of them. Autoimmune thyroiditis probably also contributed to subclinical hypothyroidism in another patient. Ten patients had isolated low fT3, likely representing non-thyroidal illness syndrome. Lower SARS-Cov-2 PCR cycle threshold values and elevated C-reactive protein were independently associated with occurrence of low TSH (p=0.030) and low fT3 (p=0.007) respectively. A decreasing trend of fT3 with increasing COVID-19 severity (p=0.032) was found. Patients with low fT3 had more adverse COVID-19-related outcomes.

Conclusion: Around 15% of patients with mild to moderate COVID-19 had thyroid dysfunction. There may be a direct effect of SARS-CoV-2 on thyroid function, potentially leading to exacerbation of pre-existing autoimmune thyroid disease. Low fT3, associated with systemic inflammation, may have a prognostic significance.

Keywords: COVID-19; SARS-CoV-2; thyroid function tests; thyroiditis; euthyroid sick syndromes; thyroid gland

Introduction

Since the first cases of coronavirus disease 2019 (COVID-19) due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were reported in China in late 2019, COVID-19 has spread rapidly worldwide, and a global pandemic was declared by the World Health Organization in March 2020 (1). Angiotensin-converting enzyme 2 (ACE2), the functional receptor for SARS-CoV-2, plays a role in the pathogenesis of COVID-19. ACE2 expression is present in many endocrine organs, including the endocrine pancreas and thyroid gland (2). The first case report in May 2020 of subacute thyroiditis after COVID-19 provided the suggestion of a possible link (3). Since then, thyroid dysfunction, especially thyrotoxicosis due to thyroiditis, has been described in several COVID-19 patient cohorts.

The earliest cohort of 50 Chinese patients with at least moderate COVID-19 showed 56% of patients had low thyroid-stimulating hormone (TSH) levels, with their TSH and total triiodothyronine (T3) levels lower than healthy controls and non-COVID-19 pneumonia patients. However, most thyroid function tests (TFTs) were checked during steroid treatment, potentially confounding these findings (4). Later, an Italian cohort of 287 COVID-19 patients with severe disease reported 10.8% of overt thyrotoxicosis. A higher IL-6 level was associated with higher odds of thyrotoxicosis, suggesting a relationship between systemic inflammation and thyrotoxicosis (5). A contemporary Italian study showed a higher incidence of SARS-CoV-2 related atypical thyroiditis among COVID-19 patients requiring intensive care, compared with non-COVID-19 patients requiring intensive care and COVID-19 patients not requiring intensive care (6). Importantly, this study was the first to provide imaging evidence of SARS-CoV-2 induced thyroiditis with combined thyroid ultrasound and scintigraphy scans. In these cohorts, complete TFTs (including TSH, free thyroxine [fT4] and free T3 [fT3]) and

anti-thyroid antibodies were selectively done, and SARS-CoV-2 viral loads were not reported. Uncertainties remained regarding thyroid dysfunction among COVID-19 patients with mild to moderate severity, and the clinical risk factors predicting thyroid dysfunction, including SARS-CoV-2 viral load and anti-thyroid antibody positivity.

Hence, we evaluated the thyroid function, clinical features, biochemical, immunological and inflammatory markers in a cohort of COVID-19 patients with mild to moderate severity. We aimed to characterize the abnormal TFTs, and examine the clinical parameters associated with abnormal TFTs and their potential prognostic significance.

Materials and Methods

Consecutive adult patients (aged ≥18 years) admitted to Queen Mary Hospital for COVID-19 between 21 July 2020 and 21 August 2020 were prospectively recruited. 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. Patients were excluded if they (i) had a history of thyroid disorders; (ii) were on anti-thyroid drugs and/or thyroid hormone replacement; and (iii) were on medications with potential impact on thyroid function including systemic steroid, amiodarone, heparin and dopamine.

Each patient had blood tests upon admission before the initiation of treatments of COVID-19. Serum TSH, fT4 and fT3 were measured with immunoassays. Serum TSH was measured with ADVIA Centaur® TSH3-Ultra assay (Siemens Healthcare Diagnostics Inc., USA). Serum fT4 was measured

with ADVIA Centaur® FT4 assay (Siemens Healthcare Diagnostics Inc., USA). Serum fT3 were measured with ADVIA Centaur® FT3 assay (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. fT3/fT4 ratio was calculated as an indirect index of deiodinase converting T4 to T3 (7). In the context of thyrotoxicosis, fT3/fT4 ratio of <0.3 has been reported to be more likely thyroiditis than Graves' disease (8). Anti-thyroglobulin antibody (anti-Tg) titer was measured with QUANTA Lite® Thyroid T enzyme-linked immunosorbent assay (ELISA) (Inova Diagnostics, USA). Anti-thyroid peroxidase antibody (anti-TPO) titer was measured with QUANTA Lite® TPO ELISA (Inova Diagnostics, USA). Anti-TSH receptor antibody (anti-TSHR) titer was measured with the Anti-TSH Receptor (TRAb) Fast ELISA (IgG) test kit (EUROIMMUN Medizinische Labordiagnostika AG, Germany), using porcine TSHR. Positive anti-Tg and anti-TPO was defined by >101 units. Basic hematology and biochemistry panel, and inflammatory markers (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]) were measured.

Demographics and major comorbidities were recorded. COVID-19-related symptoms were evaluated with a standard checklist. Respiratory rate, baseline oxygen saturation by pulse oximetry (SpO2), 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) collected on the same day of TFT collection. 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 that Ct values and SARS-CoV-2 viral loads correlated well (9–10), such that the lower the Ct values, the higher the viral loads. Clinical outcomes, including mortality, length of hospitalization,

dexamethasone and/or supplementary oxygen requirements, were captured among those discharged or died by the time of manuscript preparation.

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 (NHC) (11). Mild disease was defined by mild clinical symptoms without manifestations of pneumonia on imaging. Moderate disease was defined by fever and respiratory symptoms, and manifestations of pneumonia on imaging. Severe disease was defined by any of the following: respiratory rate ≥30/min, SpO2 ≤93% at rest, and >50% progression in 48 hours on imaging. Critical disease was defined by respiratory failure requiring mechanical ventilation, shock, and intensive care unit admission.

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.

Data were presented as mean±SD, median with interquartile range (IQR), or number with percentage as appropriate. Between-group comparisons were performed with t-test and Mann-Whitney U test for continuous variables, and Chi-square or Fisher's exact tests for categorical variables as appropriate. For data not normally distributed, as determined by the Kolmogorov-Smirnov test, natural logarithmic transformation was applied before analyses. Multivariable logistic regression analysis was used to identify the variables independently associated with low TSH and low fT3 respectively. Multivariable linear regression analysis was used to identify the independent variable associated with fT3/fT4 ratio. Variables with statistical significance in the univariate analysis

were included in multivariable regression analysis. Two-sided p-values <0.05 were considered statistically significant. All statistical analyses were performed with IBM® SPSS® version 26.

Results

191 COVID-19 patients were included: 99 men (51.8%) and 92 women (48.2%). The mean age was 53.5 ± 17.2 years. Hypertension was present in 52 patients (27.2%), diabetes in 25 patients (13.1%), coronary artery disease or heart failure in 12 patients (6.3%), history of stroke or transient ischemic attack in 8 patients (4.2%), history of or current malignancy in 8 patients (4.2%) and pulmonary diseases in 6 patients (3.1%). The mean Ct value was 26.0 ± 6.9. Most patients had mild disease on admission (n=161, 84.3%), while 24 had moderate disease (12.6%) and 6 had severe disease (3.1%). None was critically ill on admission, i.e. all patients were initially admitted to the non-intensive care unit. Most patients were symptomatic (n=144, 75.4%). The most common symptoms were fever (n=96, 66.7%), cough (n=84, 58.3%), sore throat (n=59, 41.0%), diarrhea (n=37, 25.7%), and anosmia and/or ageusia (n=30, 20.8%). None of the patients presented with overt symptoms of thyroiditis such as neck pain or signs and symptoms of thyrotoxicosis. 21 patients (11.0%) presented with tachycardia.

Regarding the thyroid status, TSH was measured in all 191 patients, fT4 in 188 patients (98.4%) and fT3 in 178 patients (93.2%). Anti-TPO and anti-Tg were available in 188 patients (98.4%), whereas anti-TSHR was available in 183 patients (95.8%).

The median TSH of the cohort was 1.20 mIU/L (IQR: 0.78–1.70). Twelve patients (6.3%) had abnormal TSH, all had concomitant fT4 and fT3 measured. TSH was mildly decreased (0.10–0.34 mIU/L) in 8 patients, where 7 showed an isolated decrease in TSH and one was associated with normal fT4 and low fT3 of 2.1pmol/L. TSH was lower than 0.10 mIU/L in another 3 patients: their fT4 and fT3 were normal. **Table 1** listed the TFTs and anti-thyroid antibody titers of the 11 patients with subnormal TSH (patient number 1–11). Among these patients, the fT4 levels were on the higher end of the reference range (16–22 pmol/L) with fT3/fT4 ratios mostly <0.3, in keeping with thyroiditis (8). However, in two of these 11 patients, suppressed TSH levels were found together with high normal fT4 and fT3 as well as elevated anti-TSHR (normal ≤1 IU/L), and one was also positive for anti-TPO, raising the possibility of subclinical hyperthyroidism due to Graves' disease. One patient had raised TSH level compatible with subclinical hypothyroidism (TSH 11 mIU/L, normal fT4 12 pmol/L and fT3 3.9 pmol/L). She had a highly elevated anti-TPO titer of 18719 units and mildly elevated anti-Tg titer of 177 units, also suggesting an autoimmune cause for her subclinical hypothyroidism. (**Table 1**; patient number 12)

The mean fT4 of the cohort was 18.1 ± 2.4 pmol/L. fT4 levels were abnormal only in 3 patients. (**Table 1**; patient number 13–15) All of them had a borderline raised fT4 of 24 pmol/L. Their TSH levels were normal. One of them had a lowish fT3 level of 3.0 pmol/L.

The mean fT3 of the cohort was 4.12 ± 0.71 pmol/L. fT3 levels were abnormal in 12 patients (6.7%), all were low. Isolated low fT3 was observed in 10 of them, i.e. normal TSH and fT4 levels. One patient had borderline raised fT4 of 24 pmol/L, and the other patient had mildly decreased TSH of 0.21 mIU/L.

Overall, abnormal TFTs (defined by TSH, fT4 or fT3 out of reference ranges) were observed in 25 patients (13.1%). Fourteen patients (7.3%) had features of thyrotoxicosis, defined as low TSH and/or raised fT4. Interestingly, from our cohort, we observed 10 patients with isolated low TSH levels and another 10 patients with isolated low fT3 levels.

When classified according to the Chinese NHC clinical severity (11), median TSH in patients with mild, moderate, and severe diseases were 1.20 mIU/L (IQR 0.80–1.60), 1.10 mIU/L (IQR 0.74–2.43), and 0.98 mIU/L (IQR 0.80–1.15) respectively. Although TSH levels appeared to be decreasing, the difference was not statistically significant (p=0.409). fT4 levels did not differ across disease severity (p=0.642). On the other hand, a significant decreasing trend was observed for fT3 levels with increasing disease severity (mild: 4.18 ± 0.71 pmol/L, moderate: 3.83 ± 0.57 pmol/L, severe: 3.68 ± 0.80 pmol/L; p=0.032).

We compared patients having normal TSH with those having subnormal TSH (**Table 2**). Regarding symptomatology, while more patients with subnormal TSH presented with fever (p=0.030), the proportion of patients who presented with sore throat (p=0.741), diarrhea (p=0.129) or tachycardia (p=0.349) did not differ between the patients with subnormal TSH and those with normal TSH. Levels of inflammatory markers and anti-thyroid antibody positivity were comparable between the groups. More patients who presented with fever had subnormal TSH (p=0.030). Moreover, patients with subnormal TSH had a lower Ct value (p=0.011). Multivariate logistic regression analysis revealed that a lower Ct value (p=0.030), but not the presence of fever (p=0.084), was independently associated with subnormal TSH.

Table 3 showed the comparison between patients with normal fT3 and low fT3. Patients with low fT3 also had lower fT4 and fT3/fT4 ratio than those with normal fT3. Although there was no difference in the proportion of symptomatic patients, more febrile patients had low fT3. Higher levels of inflammatory markers (CRP and ESR) and indices of tissue injury such as aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were associated with low fT3. Neither anti-thyroid antibody positivity nor the viral load was associated with low fT3. Multivariable stepwise logistic regression analysis revealed that only higher CRP was independently associated with low fT3 (p=0.007).

We further studied the correlation between fT3/fT4 ratio with clinical parameters. Age (r = -0.322, p<0.001), AST (r = -0.152, p=0.044), LDH (r = -0.172, p=0.022), CRP (r = -0.407, p<0.001) and ESR (r = -0.478, p<0.001), and all showed significant inverse correlation with fT3/fT4 ratio. fT3/fT4 ratio was lower among those with fever (p<0.001). Multivariable linear regression analysis (**Table 4**) showed that only ESR remained independently associated with fT3/fT4 ratio.

At the time of manuscript preparation, clinical outcomes of 184 patients (96.3%) were available, where two patients died (1.1%). 16 patients (8.7%) deteriorated clinically (defined as worsening in ≥1 category of clinical severity according to the Chinese NHC guideline). 20 patients (10.9%) required dexamethasone and/or supplementary oxygen for COVID-19 during hospitalization. Two patients (1.1%) required intensive care unit admission. The median length of stay was 8 days (IQR 6–11). The proportion of patients requiring dexamethasone and/or supplementary oxygen did not differ between those with normal TSH and subnormal TSH (p=0.368). Length of stay was also comparable between patients with normal and subnormal TSH (p=0.127). However, compared with those with normal fT3, more patients with low fT3 required dexamethasone and/or supplementary oxygen

(5/12 [41.7%] vs 12/159 [7.5%], p=0.003) and prolonged hospital stay (≥14 days) (5/12 [41.7%] vs 20/157 [12.7%], p=0.018). Importantly, patients with low fT3 had a higher chance of deterioration in clinical severity compared to those with normal fT3 (6/12 [50.0%] vs 8/159 [5.0%], p<0.001), whereas abnormality in TSH was not associated with deterioration in clinical severity (p=0.280). There was no significant difference between non-ICU and ICU groups in terms of TSH (non-ICU: 1.15 [IQR 0.77-1.70] vs ICU: 1.05 mIU/L, p=0.751), fT4 (non-ICU: 18.2±2.4 vs ICU: 16.0±2.8 pmol/L, p=0.212) and fT3 (non-ICU: 4.1±0.7 vs ICU: 3.5±0.8 pmol/L, p=0.202) levels.

At the time of manuscript preparation, among the 25 patients with abnormal thyroid function during hospitalization, 10 patients (40%) had been re-evaluated after hospital discharge, after a median of 28 days (IQR: 21–47) from the admission thyroid function test. The results are summarized in **Table 5**.

Patient number 16–18 had isolated low fT3 compatible with non-thyroidal illness syndrome (NTIS), where 2 of them recovered and one had persistent isolated low fT3.

Patient number 4, 8, 10–14 had admission TFT compatible with thyroiditis. Patient number 4 likely had Graves' disease with anti-TSHR 9.9 IU/L. His thyroid function subsequently normalized, in keeping with recovery from an additional thyroidal insult due to the viral disease, leading to an increased release of thyroid hormones, on top of his pre-existing Graves' disease. Patient number 12 had persistent subclinical hypothyroidism and required initiation of thyroxine replacement. With the high anti-TPO titer, this likely represented pre-existing Hashimoto's thyroiditis diagnosed during hospitalization for COVID-19. The rest of the patients' thyroid function profiles were compatible with thyroiditis at various stages of evolution.

Discussions

Our study presented a comprehensive TFT (TSH, fT4 and fT3) and anti-thyroid antibody profile among a cohort of 191 COVID-19 patients. We showed a 13.1% rate of abnormal TFTs among a cohort of predominantly mild to moderate COVID-19 patients. Two distinct groups of patients were identified, 10 having isolated low TSH (likely representing thyroiditis) and another 10 having isolated low fT3 (likely representing NTIS). Lower Ct values were independently associated with the presence of low TSH. Also, the incidence of thyroid abnormalities suggestive of thyroiditis in our cohort was similar to that found in cohorts of patients with more severe disease. These may suggest a potential direct viral effect on the thyroid gland if confirmed by studies using dedicated quantitative RT-PCR assays to measure SARS-CoV-2 viral loads. On the other hand, a higher CRP level was independently associated with low fT3. fT3 also showed a decreasing trend with worsening clinical severity of COVID-19. Together with the independent inverse correlation between ESR and fT3/fT4 ratio, these suggested the effect of systemic inflammation on the deiodinase activity, producing a TFT picture classically described in NTIS. Besides, low fT3 may have prognostic significance as it was associated with multiple COVID-19-related adverse events including clinical deterioration, requirement of dexamethasone and/or supplementary oxygen, and prolonged hospital stay.

SARS-CoV-2 related thyroiditis has been reported to occur concurrently with COVID-19 presentation (5–6,12–13) or following resolution of COVID-19 (12–13). The different timing suggested both direct viral and post-viral manifestations of COVID-19. In the Italian cohort of severe COVID-19 patients, higher IL-6 levels were independently associated with the occurrence of thyrotoxicosis, suggesting an inflammatory pathophysiology for the SARS-CoV-2 related thyroiditis (5). However, it remains to be elucidated whether the viral load of SARS-CoV-2 and presence of anti-thyroid antibodies are

associated with the occurrence of thyroid dysfunction. Muller *et al* (6) reported data regarding anti-thyroid antibodies (anti-TPO, anti-Tg, anti-TSHR) in the 9 patients attending follow-up around 2 months after COVID-19 related thyroid dysfunction. They were all negative except for one patient who was diagnosed to have autoimmune thyroiditis, likely pre-existing and diagnosed during the hospitalization for COVID-19. Thus, thyroid dysfunction appears not to be mediated by autoimmunity. In our cohort, we observed 10 patients with subnormal TSH, high normal fT4 and normal fT3, likely biochemical picture of subclinical thyrotoxicosis rather than NTIS as low TSH in the context of NTIS is typically associated with low fT4 when the non-thyroidal illness is severe. In contrast, the fT4 levels of these patients with subnormal TSH were all on the higher end of the reference range. The fT3/fT4 ratios were mostly <0.3, in keeping with thyroiditis (8). It was noted, however, that two of these patients had suppressed TSH levels associated with high normal fT4 and fT3, as well as elevated anti-TSHR and, in one of them, positivity for anti-TPO. (**Table 1**; patient number 4–5) These likely represent subclinical hyperthyroidism due to Graves' disease. Another patient with subclinical hypothyroidism also had a remarkably high anti-TPO antibody titer suggestive of autoimmune thyroiditis. (**Table 1**; patient number 12) All these three patients had their anti-thyroid antibodies checked upon admission. Autoimmune processes, especially the production of antibodies, take time to develop. Hence, in keeping with the study by Muller *et al* (6), thyroid dysfunction in our patients was probably not mediated by autoimmunity triggered by their SARS-CoV-2 infection. On the other hand, infections represent an environmental trigger of subsequent autoimmune thyroid diseases and thyroid dysfunction (16). It is known that in the months or years following subacute viral thyroiditis there is a higher incidence of thyroid autoimmunity (17) and hypothyroidism (18). Whether this is also applicable to patients after thyroiditis induced by SARS-CoV-2 will be answered by follow-up studies of these COVID-19 patients. Interestingly, a lower Ct value was the only independent variable associated with low TSH, rather than inflammatory markers and thyroid autoimmunity. As the SARS-CoV-2 receptor ACE2 is highly expressed in the thyroid gland (2), future studies accurately measuring the viral load by dedicated quantitative RT-PCR, with follow-

up, as well as cytological and histological correlations, are important to verify the mechanism of thyroid damage by SARS-CoV-2. We also showed that more patients with low TSH had fever, in line with the reports of patients with SARS-CoV-2-related thyroiditis presenting with fever (10–13). Most of these cases reported were anti-thyroid antibody negative. Thus far, most case reports of thyroiditis were diagnosed when patients presented with typical symptoms of thyroiditis. Our study had identified the potential clinical features associated with an increased likelihood of thyroiditis which should alert clinicians of the indication to check TFT in their COVID-19 patients, especially in the context of lower Ct values (e.g. in the low 20s) and fever.

We also identified a subgroup of patients with isolated fT3 (i.e. with normal TSH and fT4), described in the literature among patients requiring intensive care, or with severe illness, chronic diseases, acute illness or stress such as surgery (19). In the Italian cohort of COVID-19 patients requiring intensive care who developed thyroiditis (6), low TSH and normal or elevated fT4 but accompanied by low fT3 was observed, suggesting the presence of a component of NTIS. In this study, we showed that NTIS could also be present in patients with mild to moderate COVID-19 not requiring intensive care. We further demonstrated that patients with low fT3 had a lower fT3/fT4 ratio, an indirect index of deiodinase activity converting T4 to T3 (7), higher indices of tissue injury (AST and LDH), and higher inflammatory markers (CRP and ESR), but Ct values were not different. The independent inverse correlation of fT3/fT4 ratio with ESR further supported the role of systemic inflammation, which in turn is associated with systemic tissue injury, in causing reduced deiodinase activity. This results in decreased conversion of T4 to T3, leading to low fT3. Consistent with reports of the prognostic role of low fT3 in conditions including cardiovascular and liver diseases (20–23), we also observed that COVID-19 patients with low fT3 had more adverse outcomes. Indeed, a previous retrospective study showed that COVID-19 patients who died had lower fT3 on admission compared with the survivors (24). This warrants a longitudinal follow-up of COVID-19 patients with isolated low fT3 for further potential prognostic implications.

Our study analyzed the TFT and anti-thyroid antibody profile together with inflammatory markers and Ct values, the surrogate marker of SARS-CoV-2 viral loads, among COVID-19 patients, which shed light on their patterns of thyroid dysfunction, and the associated clinical predictors and prognostic implications. There are certain limitations, however. First, we did not have control groups of healthy individuals and non-COVID-19 pneumonia patients. Second, Ct values obtained from the qualitative RT-PCR assay of SARS-CoV-2 were used as the surrogate of the viral load, which should have been measured directly with a dedicated quantitative RT-PCR assay. Third, as all COVID-19 patients were treated in isolation facilities, patients with low TSH did not have thyroid ultrasonography and radionuclide scans to delineate the etiologies of subclinical thyrotoxicosis. Nonetheless, as discussed above, the patterns of thyroid dysfunction among patients with low TSH likely reflected thyroiditis. Third, 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, as we have planned for reassessment TFT at week 12 from discharge as part of the clinical management. Hence, at the time of manuscript preparation, only some patients with abnormal TFT had been recalled earlier for reassessment TFT.

Conclusions

We demonstrated 13.2% of thyroid dysfunction among patients with predominantly mild to moderate COVID-19. Lower Ct values obtained from qualitative SARS-CoV-2 RT-PCR assays were associated with low TSH, while more systemic inflammation was associated with low fT3 and fT3/fT4 ratios. Both low TSH and low fT3 occurred more frequently among patients presented with fever. Low fT3 may have prognostic significance, being associated with more adverse COVID-19-related outcomes. Clinicians should be vigilant about the possible presence of thyroid dysfunction among COVID-19 patients, especially in the context of fever, lower Ct values and inflammatory markers.

Authors' Contribution: DTWL wrote the manuscript. DTWL, CHL, WSC, ACHL, ART, CYL and EKHL researched the data. DTWL and CHYF performed statistical analyses. CHL, WSC, ACHL, KKWT, KCBT, YCW, CWL, IFNH and KSLL critically reviewed and edited the manuscript. KSLL initiated and supervised the study, is the guarantor of this work and as such had 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.

Data Availability: Datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Table 1. Thyroid function profiles of the patients with abnormal thyroid function (excluding patients with isolated low fT3)

Patient number	Age (years)	Sex	TSH (mIU/L)	fT4 (pmol/L)	fT3 (pmol/L)	fT3/fT4 ratio	Anti-TPO titer (units)	Anti-Tg titer (units)	Anti-TSHR titer (IU/L)
1	41	M	**0.26**	22	3.4	0.155	-ve	-ve	0.8
2	73	M	**0.20**	16	3.8	0.238	-ve	-ve	0.4
3	64	F	**0.22**	19	3.6	0.189	-ve	-ve	0.6
4	60	M	**<0.01**	22	5.2	0.236	**628**	-ve	**9.9**
5	30	F	**<0.01**	21	6.5	0.310	-ve	-ve	**2.2**
6	42	F	**0.14**	21	3.9	0.186	-ve	-ve	0.1
7	91	F	**0.21**	16	**2.1**	0.131	-ve	-ve	0.6
8	52	F	**0.24**	17	3.2	0.188	-ve	-ve	0.5
9	40	F	**0.30**	17	3.3	0.194	-ve	-ve	0.4
10	53	M	**0.06**	16	3.8	0.238	-ve	-ve	0.9
11	59	M	**0.31**	20	5.4	0.270	-ve	-ve	**1.2**
12	75	F	**11**	12	3.9	0.325	**18719**	**177**	N/A
13	52	F	2.6	**24**	4.0	0.167	-ve	**258**	0.6
14	68	M	1.1	**24**	**3.0**	0.125	-ve	-ve	**1.2**
15	66	M	2.8	**24**	4.4	0.183	-ve	-ve	**1.3**

Abbreviations: M, male; F, female; TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; anti-TPO, anti-thyroid peroxidase; anti-Tg, anti-thyroglobulin; anti-TSHR, anti-TSH receptor; -ve, negative; N/A, not available.

Values in bold represent abnormal values.

Table 2. Comparison between patients with normal TSH and those with subnormal TSH

	Normal TSH (n=179)	Subnormal TSH (n=11)	P-value
TSH (mIU/L)	1.20 (0.88–1.70)	0.21 (0.06–0.26)	**<0.001**
fT4 (pmol/L)*	18.1 ± 2.4	18.8 ± 2.5	0.329
fT3 (pmol/L)**	4.12 ± 0.67	4.02 ± 1.22	0.782
fT3/fT4 ratio	0.229 ± 0.041	0.212 ± 0.052	0.197
Age (years)	53.2 ± 17.3	55.0 ± 17.2	0.742
Male	94 (52.5%)	5 (45.4%)	0.649
COVID-19 severity			0.447
Mild	151 (84.3%)	10 (90.9%)	
Moderate	22 (12.2%)	1 (9.1%)	
Severe	6 (3.4%)	0 (0%)	
Symptomatic	133 (74.3%)	10 (90.9%)	0.298
Baseline oxygen saturation (%)	98 (97–99)	97 (95–99)	0.737
Oxygen required on admission (%)	6 (3.4)	0 (0)	0.999
Fever	86 (48.0%)	9 (81.8%)	**0.030**
Anti-Tg/TPO positivity	41 (22.0%)	1 (9.1%)	0.461
Creatinine (umol/L)	72 (58-85)	67 (56-86)	0.724
AST (U/L)	26 (21–38)	27 (22–33)	0.694
Creatine kinase (U/L)	90 (65–135)	105 (46–187)	0.817

Lactate dehydrogenase (U/L)	227 (188–268)	209 (174–249)	0.365
CRP (mg/dL)	0.46 (0.31–1.71)	1.13 (0.31–2.10)	0.272
ESR (mm/hr)	35.0 (20.0–56.0)	34.0 (23.0–49.0)	0.807
SARS-CoV-2 PCR Ct value	26.31 ± 6.84	20.89 ± 5.43	**0.011**

*Normal TSH, n=176; subnormal TSH, n=11; **normal TSH, n=166; subnormal TSH, n=11.

Data are presented as mean±SD, median (IQR), number (%) as appropriate.

Abbreviations: TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; anti-TPO, anti-thyroid peroxidase; anti-Tg, anti-thyroglobulin; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Ct, cycle threshold.

Interval from symptom-onset to thyroid function testing, and comorbidities, were comparable between the two groups.

Table 3. Comparison between patients with normal fT3 and low fT3

Variables	Normal fT3 (n=166)	Low fT3 (n=12)	P-value
TSH (mIU/L)	1.20 (0.81–1.70)	0.92 (0.55–1.50)	0.133
fT4 (pmol/L)	18.3 ± 2.3	16.1 ± 3.1	**0.002**
fT3 (pmol/L)	4.21 ± 0.63	2.79 ± 0.29	**<0.001**
fT3/fT4 ratio	0.232 ± 0.041	0.178 ± 0.034	**<0.001**
Age (years)	52.7 ± 17.3	60.4 ± 13.5	0.132
Male	85 (51.2%)	8 (66.7%)	0.300
COVID-19 severity			0.434
Mild	141 (84.9%)	9 (75.0%)	
Moderate	20 (12.0%)	2 (16.7%)	
Severe	5 (3.0%)	1 (8.3%)	
Symptomatic	124 (74.7%)	11 (91.7%)	0.298
Baseline oxygen saturation (%)	98 (97–99)	98 (95–98)	0.419
Oxygen required on admission (%)	5 (3.0)	1 (8.3)	0.346
Fever	78 (47.0%)	10 (83.3%)	**0.015**
Anti-Tg/TPO positivity	36 (21.7%)	3 (25.0%)	0.730
Creatinine (umol/L)	71 (56–84)	84 (70–93)	0.065
AST (U/L)	26 (21–38)	32 (26–53)	**0.042**
Creatine kinase (U/L)	93 (65–137)	143 (97–168)	0.055
Lactate dehydrogenase (U/L)	223 (185–262)	299 (257–333)	**<0.001**
CRP (mg/dL)	0.43 (0.31–1.51)	6.29 (1.93–9.48)	**<0.001**

ESR (mm/hr)	34.0 (20.0–53.5)	73.0 (39.3–97.0)	**0.005**
SARS-CoV-2 PCR Ct value	26.03 ± 7.04	25.12 ± 4.69	0.542

Data are presented as mean±SD, median (IQR), number (%) as appropriate.

Abbreviations: TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; anti-TPO, anti-thyroid peroxidase; anti-Tg, anti-thyroglobulin; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Ct, cycle threshold.

Interval from symptom-onset to thyroid function testing, and comorbidities, were comparable between the two groups.

Table 4. Multivariable linear regression analysis of the association between fT3/fT4 ratio and clinical variables

Variables	Standardized beta	p-value
Age (years)	-0.128	0.097
Fever (yes / no)	-0.099	0.181
AST (U/L)*	-0.012	0.888
LDH (U/L)*	0.084	0.349
CRP (mg/dL)*	-0.183	0.062
ESR (mm/hr)*	-0.310	**<0.001**

*Variables are log-transformed before analysis

Abbreviations: AST, aspartate aminotransferase; LDH, lactate dehydrogenase; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 5. Patients with abnormal thyroid function test on admission and had re-evaluation after discharge

Patient number	Age (years)	Sex	TSH (mIU/L)	fT4 (pmol/L)	fT3 (pmol/L)	Anti-TPO titer (units)	Anti-Tg titer (units)	Anti-TSHR titer (IU/L)	Days from admission TFT	TSH (mIU/L)	fT4 (pmol/L)	fT3 (pmol/L)
4	60	M	**<0.01**	22	5.2	**628**	-ve	**9.9**	46	0.86	15	5.0
8	52	F	**0.24**	17	3.2	-ve	-ve	0.5	29	**0.28**	20	5.0
10	53	M	**0.06**	16	3.8	-ve	-ve	0.9	27	0.70	21	3.7
11	59	M	**0.31**	20	5.4	-ve	-ve	**1.2**	25	**0.07**	20	6.4
12	75	F	**11**	12	3.9	**18719**	**177**	N/A	47	**10**	12	N/A
13	52	F	2.6	**24**	4.0	-ve	**258**	0.6	21	1.8	17	4.4
14	68	M	1.1	**24**	**3.0**	-ve	-ve	**1.2**	11	1.5	21	N/A
16	74	M	0.60	16	**3.0**	**113**	-ve	**1.2**	50	1.7	17	4.0
17	59	M	0.83	15	**2.3**	-ve	-ve	0.4	52	1.4	12	**2.1**
18	45	M	1.6	14	**3.1**	65	-ve	0.2	15	1.3	18	4.7

Abbreviations: M, male; F, female; TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; anti-TPO, anti-thyroid peroxidase; anti-Tg, anti-thyroglobulin; anti-TSHR, anti-TSH receptor; -ve, negative; N/A, not available; TFT, thyroid function test

Values in bold represent abnormal values.