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Long COVID in Patients with Mild to Moderate Disease: Do Thyroid Function and Autoimmunity Play a Role?

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Corresponding Author:	Karen SL Lam, MD The University of Hong Kong --- Select One --- HONG KONG
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	The University of Hong Kong
Corresponding Author's Secondary Institution:	
First Author:	David Tak Wai Lui
First Author Secondary Information:	
Order of Authors:	David Tak Wai Lui
	Chi Ho Lee
	Wing Sun CHOW
	Alan Lee
	Anthony Raymond Tam
	Polly Pang
	Tip Yin Ho
	Carol Ho-yi FONG
	Chun Yiu Law
	Eunice Leung
	Kelvin To
	Kathryn Choon-beng TAN
	Yu Cho WOO
	Ching Wan Lam
	Ivan Hung
	Karen SL Lam, MD
Order of Authors Secondary Information:	
Abstract:	Objective: Long COVID (LC) is an emerging global health issue. Fatigue is a common feature. Whether thyroid function and autoimmunity play a role is uncertain. We aimed to evaluate the prevalence and predictors of LC and the potential role of thyroid function and autoimmunity in LC. Methods: We included consecutive adults without known thyroid disorder, admitted to a major COVID-19 centre for confirmed COVID-19 from July to December 2020 who

	had thyroid function tests (TFTs) and anti-thyroid antibodies measured on admission and at follow-up. LC was defined by the presence or persistence of symptoms upon follow-up. Results: In total, 204 patients (median age: 55.0 years; 46.6% men) were reassessed at a median of 89 days (IQR: 69–99) after acute COVID-19. Forty-one (20.1%) had LC. Female (adjusted odds ratio [aOR] 2.48, p=0.018) and SARS-CoV-2 PCR cycle threshold value <25 on admission (aOR 2.84, p=0.012) independently predicted the occurrence of LC. Upon follow-up, most abnormal TFTs in acute COVID-19 resolved, and incident thyroid dysfunction was rare. Nonetheless, we observed incident anti-TPO (anti-thyroid peroxidase) positivity. While baseline or follow-up TFTs were not associated with the occurrence of LC, among 172 patients symptomatic in acute COVID-19, symptom resolution was more likely in those with positive anti-TPO upon follow-up (p=0.043). Conclusion: LC is common among COVID-19 survivors, with female and those with higher viral load in acute COVID-19 particularly vulnerable. The observation of incident anti-TPO positivity warrants further follow-up for thyroid dysfunction. Whether anti-TPO plays a protective role in LC remains to be elucidated.
Suggested Reviewers:	Eric Alexander Harvard Medical School ekalexander@bwh.harvard.edu
	Trevor Angell University of Southern California Trevor.angell@med.usc.edu
	George Kahaly JGU: Johannes Gutenberg Universitat Mainz george.kahaly@unimedizin-mainz.de
Opposed Reviewers:	
Additional Information:	
Question	**Response**

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Running Title: Thyroid and Long COVID

Authors: David Tak Wai Lui[1], MBBS; Chi Ho Lee[1], MBBS; Wing Sun Chow[1], MBBS; Alan Chun Hong Lee[1], MBBS; Anthony Raymond Tam[1], MBBS; Polly Pang[1], BNurs; Tip Yin Ho[1], MMedSc; Carol Ho Yi Fong[1], MStat; Chun Yiu Law[2], PhD; Eunice Ka Hong Leung[1], MBBS; Kelvin Kai Wang To[3], MD; Kathryn Choon Beng Tan[1], MD; Yu Cho Woo[1], MD; Ching Wan Lam[4], PhD; Ivan Fan Ngai Hung[1], MD; Karen Siu Ling Lam[1], MD

Affiliations: [1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China; [2]Division of Chemical Pathology, Queen Mary Hospital, Hong Kong, China; [3]Department of Microbiology, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China; [4]Department of Pathology, The University of Hong Kong, Hong Kong, China

Address Correspondence to

Professor Karen Siu Ling Lam, MD

Email address: kslam@hku.hk

Telephone number: +852 2255-4783

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Abstract

Objective: Long COVID (LC) is an emerging global health issue. Fatigue is a common feature. Whether thyroid function and autoimmunity play a role is uncertain. We aimed to evaluate the prevalence and predictors of LC and the potential role of thyroid function and autoimmunity in LC.

Methods: We included consecutive adults without known thyroid disorder, admitted to a major COVID-19 centre for confirmed COVID-19 from July to December 2020 who had thyroid function tests (TFTs) and anti-thyroid antibodies measured on admission and at follow-up. LC was defined by the presence or persistence of symptoms upon follow-up.

Results: In total, 204 patients (median age: 55.0 years; 46.6% men) were reassessed at a median of 89 days (IQR: 69–99) after acute COVID-19. Forty-one (20.1%) had LC. Female (adjusted odds ratio [aOR] 2.48, p=0.018) and SARS-CoV-2 PCR cycle threshold value <25 on admission (aOR 2.84, p=0.012) independently predicted the occurrence of LC. Upon follow-up, most abnormal TFTs in acute COVID-19 resolved, and incident thyroid dysfunction was rare. Nonetheless, we observed incident anti-TPO (anti-thyroid peroxidase) positivity. While baseline or follow-up TFTs were not associated with the occurrence of LC, among 172 patients symptomatic in acute COVID-19, symptom resolution was more likely in those with positive anti-TPO upon follow-up (p=0.043).

Conclusion: LC is common among COVID-19 survivors, with female and those with higher viral load in acute COVID-19 particularly vulnerable. The observation of incident anti-TPO positivity warrants further follow-up for thyroid dysfunction. Whether anti-TPO plays a protective role in LC remains to be elucidated.

Keywords: post-acute COVID-19 syndrome; COVID-19; SARS-CoV-2; thyroid function tests; autoimmunity; autoantibodies

Introduction

The coronavirus disease 2019 (COVID-19), due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in a global pandemic.[1] Many COVID-19 survivors continue to experience a range of symptoms after recovery from the acute COVID-19 illness,[2] variably described as 'long COVID', 'post-acute sequelae of SARS-CoV-2' and 'post-acute COVID-19 syndrome'.[3] This phenomenon is referred to as 'long COVID' in this paper. Typical presentations include fatigue and dyspnoea.[2] Long COVID can represent (i) residual symptoms that persist after recovery from acute infection; or (ii) new symptoms or syndromes that develop after initial asymptomatic or mild infection.[4] As the population of COVID-19 survivors is growing, long COVID could evolve into a 'pandemic of the pandemic'.[5] It is crucial to identify those prone to develop long COVID for the appropriate allocation of health care resources.

As acute COVID-19 is associated with multisystem involvement by SARS-CoV-2,[6] it is also increasingly recognized that sequelae may occur in multiple systems after acute COVID-19 illness.[2] The hypothalamic-pituitary-thyroid axis has attracted clinical interest in its relevance in acute COVID-19.[7] The volume of literature is growing regarding thyroid dysfunction in acute COVID-19, which mainly includes non-thyroidal illness (NTIS) and thyroiditis. However, relatively few studies addressed the thyroid status in the convalescent phase of COVID-19,

mainly reporting the resolution of thyroid dysfunction. Recently, the potential incident thyroid dysfunction and autoimmunity among 122 COVID-19 patients during convalescence has been described.[8] As manifestations of long COVID include fatigue, and immune dysregulation is one of the postulated mechanisms of long COVID,[9] it would be helpful to investigate whether thyroid function and autoimmunity play a role in long COVID.

Hence, we conducted this prospective study to evaluate the prevalence and predictors of long COVID, and the role of thyroid function and autoimmunity among COVID-19 survivors who suffered from long COVID.

Methods

This study included all COVID-19 survivors reassessed at around three months after acute COVID-19, with the inclusion and exclusion criteria detailed below.

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.[10] 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 21 December 2020 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.[10,11] 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. Each patient had baseline blood tests taken within 24 hours after admission before starting 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., 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 units. Basic haematology and biochemistry panel, glycated haemoglobin (HbA1c) and C-reactive protein (CRP) were measured. Abnormal laboratory parameters were

84 defined according to their respective reference ranges.[10] Elevated CRP was defined by >0.76
85 mg/dL. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney
86 Disease Epidemiology Collaboration (CKD-EPI) equation.[12] Anti-nuclear antibody (ANA) titres
87 were measured with Kallestad Human epithelial cell (HEp-2) immunofluorescence assays (Bio-
88 Rad, Hercules, CA, USA), with titres >1:80 considered positive.
89
90 Demographics and major comorbidities were recorded. Obesity was defined by the
91 International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code
92 278.0. Diabetes was defined by a known diagnosis of diabetes or HbA1c ≥6.5% on admission.
93 Charlson Comorbidity Index was calculated for each patient. COVID-19-related symptoms
94 were evaluated with a standard checklist. Respiratory rate, baseline oxygen saturation by
95 pulse oximetry, and oxygen requirement on admission were captured. Chest x-ray was
96 performed in each patient on admission, and abnormal chest x-ray images were graded as
97 mild (opacities in 1-2 lung zones), moderate (opacities in 3-4 lung zones) and severe (opacities
98 in >4 lung zones).[13] Cycle threshold (Ct) values were obtained from the qualitative LightMix
99 SarbecoV E-gene assay (TIB Molbiol, Berlin, Germany) performed on specimens from NPS or
100 DTS (whichever was lower) on admission. The Ct value represents the number of cycles
101 required for a gene target or a PCR product to be detected. While viral loads were not directly
102 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,[14,15] 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.[16] Patients' clinical outcomes were captured.

COVID-19 survivors were offered a follow-up visit at a dedicated COVID-19 clinic around three months from admission, in which they were systematically evaluated for symptoms with a standard checklist, and had reassessment chest x-ray, CRP, TFTs and anti-thyroid antibodies. IgG antibodies specific to the SARS-CoV-2 spike protein receptor-binding domain (anti-SARS-CoV-2 RBD IgG) were measured with live virus microneutralization assay, an in-house assay developed by the University of Hong Kong.[17] A titre of ≥1:20 was considered positive. Reassessment chest x-ray was classified according to the British Society of Thoracic Imaging guidance, in comparison with the chest x-ray during acute COVID-19: (i) normal/resolved; (ii) ≥50% improvement; (iii) <50% improvement; (iv) worsening.[18]

<u>Comparison of the current cohort with adult COVID-19 patients admitted to other centers</u>

Hospitalization records of adults admitted for COVID-19 in the matched period (21 July 2020 and 21 December 2020) in Hong Kong were retrieved from the territory-wide anonymized electronic health database of the Hospital Authority of Hong Kong (HA) – the Clinical Data

Analysis and Reporting System (CDARS). ICD-9-CM codes are used as the standard in Hong Kong. Data validation has demonstrated high coding accuracy in CDARS.[19] With the help of CDARS, high-quality large population-based studies had been published.[19–22] The HA is the only public-funded healthcare provider providing management to all COVID-19 cases in Hong Kong. Cases of COVID-19 were identified using a combination of ICD-9-CM codes 519.8, 079.89 and V75.9, together with a valid SARS-CoV-2 PCR Ct value. However, information on the clinical severity of COVID-19 could not be retrieved from CDARS. Nevertheless, SARS-CoV-2 PCR Ct value has been reported to correlate with COVID-19 severity.[23] Thus, age, sex, SARS-CoV-2 PCR Ct values were compared between our cohort and the patients admitted to other centers.

Definition of long COVID

Long COVID was defined in our study as the presence or persistence of symptoms after acute COVID-19. Long COVID can represent (i) residual symptoms that persist after recovery from acute infection, or (ii) new symptoms or syndromes that develop after initial asymptomatic or mild infection.[4] Hence, in this study, patients were also categorized into (i) those who were symptomatic in the acute COVID-19 (group A) and (ii) those who had asymptomatic mild acute COVID-19 (group B).

Statistical analyses

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. Multivariable logistic regression analysis was used to identify the variables independently associated with long COVID. All variables with statistical significance in the univariate analysis (p<0.05) were included in the multivariable regression analysis.

Results

Baseline characteristics

In total, 204 COVID-19 patients were included. Their baseline clinical characteristics are summarised in **Table 1**. The median age was 55.0 years (IQR: 44.3–63.0), and 46.6% were men. 172 (84.3%) were symptomatic in the acute COVID-19 illness, while 32 (15.7%) had asymptomatic mild acute COVID-19. The most common comorbidities were hypertension (23.0%), diabetes (13.7%) and obesity (7.4%). Most patients (77.0%) had a Charlson comorbidity index of 0. Regarding the severity of acute COVID-19, most (n=147, 72.1%) were mild, 49 (24.0%) were moderate, and 8 (3.9%) were severe. The median Ct value was 25.55

(IQR: 19.10–29.90). Most of them (n=147, 72.1%) received treatment during acute COVID-19, usually interferon beta-1b (n=126, 61.8%) and ribavirin (n=98, 48.0%). The median length of stay was 8 days (IQR: 6–12). Only 5 patients required intensive care unit admission.

In the matched inclusion period (21 July 2020 to 21 December 2020), we identified 5199 adult patients with COVID-19 admitted to other centers with valid Ct values. Comparison with our cohort (n=204) revealed comparable sex distribution (46.6% men in our cohort vs 47.5% men in other centers, p=0.788) and baseline Ct values (25.55 [IQR: 19.10–29.90] in our cohort vs 23.24 [IQR: 18.60 – 28.92] in other centers, p=0.245), although patients admitted to other centers were slightly younger (49.0 years [IQR: 35.0 – 62.0] in other centers vs 55.0 years [IQR: 44.3–63.0] in our cohort, p<0.001).

Results of reassessment

Upon reassessment at a median interval of 89 days (IQR: 69 – 99) after acute COVID-19, forty-one patients (20.1%) reported at least one symptom upon reassessment, i.e., having long COVID. Symptoms, in descending order of frequency, included dyspnoea (n=16), cough (n=16), anosmia (n=11), malaise/fatigue (n=7), loose stool (n=1), headache (n=1) and palpitation (n=1). Regarding symptom burden, 31 patients reported one symptom, 8 reported two and 2 reported three.

The evolution of the TFTs of all 204 patients is summarised in **Figure 1**. Forty-three of the 204 patients had abnormal TFTs on admission for acute COVID-19, 35 of them (81.4%) recovered spontaneously subsequently. Thirteen patients had subclinical thyrotoxicosis in acute COVID-19: 10 spontaneously resolved upon follow up, while 3 remained in subclinical thyrotoxicosis. Twenty-one patients had isolated low fT3 suggestive of NTIS, where 19 recovered. One patient had NTIS upon reassessment when he was admitted for fluid overload and was clinically ill. The other patient developed T3-toxicosis at three months, followed by spontaneous resolution another three months later, suggestive of painless thyroiditis. Six patients had isolated mildly abnormal fT4 or fT3 in acute COVID-19: all subsequently normalized upon follow-up. All three patients with subclinical hypothyroidism in acute COVID-19 had positive anti-TPO, likely representing pre-existing autoimmune thyroid disorder: they all had persistent subclinical hypothyroidism upon reassessment; one of them required thyroxine replacement because TSH was persistently >10 mIU/L. Incident TFT abnormalities detectable at 3 months were rare. Among 161 patients with normal TFTs in acute COVID-19, only three (1.9%) had abnormal TFTs upon follow-up: one had subclinical hypothyroidism (TSH 5.8 mIU/L, fT4 16 pmol/L, fT3 4.3 pmol/L), one had mildly elevated fT4 (TSH 1.1 mIU/L, fT4 25 pmol/L, fT3 4.7 pmol/L), and one had mildly elevated fT3 (TSH 1.8 mIU/L, fT4 21 pmol/L, fT3 6.9 pmol/L). Although the TFT changes in these three cases could be compatible with different phases of

198 thyroiditis, in the latter two cases assay variability could not be excluded entirely. None of
199 these three patients required treatment.
200
201 In total, 159 of all 204 patients (77.9%) had anti-TPO and anti-Tg assessed at baseline and
202 follow-up. The baseline characteristics were largely comparable between patients with and
203 without anti-TPO data – % male (p=0.988), clinical severity of COVID-19 (p=0.860), SARS-CoV-
204 2 PCR Ct value (p=0.849), Charlson comorbidity index (p=0.562) – except for younger age in
205 those with missing anti-TPO data (49.0 years [IQR: 36.0 – 61.5] vs 57.0 years [IQR: 46.0 – 64.0],
206 p=0.029). Among the 159 patients with paired anti-thyroid antibody data, 32 were positive for
207 anti-TPO and 18 were positive for anti-Tg in acute COVID-19. Interestingly, 7 of the 127
208 patients (5.5%) with negative anti-TPO in acute COVID-19 developed incident anti-TPO
209 positivity upon follow-up, whereas only one patient positive for anti-TPO in acute COVID-19
210 became negative upon follow up. On the other hand, regarding anti-Tg status, only one patient
211 converted from anti-Tg negative to positive, and another patient from anti-Tg positive to
212 negative.
213
214 <u>Predictors of long COVID</u>
215 We compared patients who did and did not have symptoms upon follow-up (**Table 2**). We
216 observed female preponderance, higher viral load (represented by Ct value <25) and a higher

likelihood of exposure to COVID-19 treatment among those who reported symptoms at follow-up. Of note, baseline clinical severity (p=0.508), symptom burden (p=0.293), laboratory parameters, elevated CRP (p=0.233), chest x-ray severity (p=0.822), requirement of prolonged stay (≥14 days) (p=0.471) and intensive care unit admission (p=0.056) in acute COVID-19 were not different between patients with and without long COVID. Elevated CRP (p=0.347), anti-SARS-CoV-2 RBD IgG positivity (p=0.613) and chest x-ray resolution (p=0.699) upon follow-up were also not different between patients who did and did not have long COVID. Besides, TFTs and anti-thyroid antibodies did not differ between the two groups. In the multivariable logistic regression analysis, both female (adjusted odds ratio [aOR] 2.48, 95% CI: 1.17–5.27, p=0.018) and Ct value <25 (aOR 2.84, 95% CI: 1.26–6.42, p=0.012) independently predicted the occurrence of long COVID, whereas COVID-19 treatment was no longer an independent predictor of long COVID (p=0.272).

According to the definition of long COVID,[4] our 204 patients were categorized into those who were symptomatic in the acute COVID-19 illness (group A; n=172) and those who had asymptomatic mild acute COVID-19 illness (group B; n=32).

In group A (**Table 3**), patients who experienced persistent symptoms upon follow-up were more likely to be female (p=0.017) and have a higher viral load in acute COVID-19 (p=0.004),

consistent with the findings in the whole cohort. Moreover, we observed a higher proportion of anti-TPO positivity at baseline and at follow-up among patients whose symptoms subsequently resolved, although complete anti-TPO data were only available in around 80% of the cohort. Regarding the clinical course of acute COVID-19, there was no difference in the baseline clinical severity (p=0.538), symptom burden (p=0.165), elevated CRP (p=0.320), requirement of prolonged stay (≥14 days) (p=0.351) and intensive care unit admission (p=0.053). Upon follow-up, there was no difference in the proportion of patients with elevated CRP (p=0.257), anti-SARS-CoV-2 RBD IgG positivity (p=0.977) and chest x-ray resolution (p=0.464). In the multivariable logistic regression model including female, Ct value <25 and exposure to COVID-19 treatment, the independent predictors of symptom persistence were female (aOR 2.88, 95% CI: 1.25–6.65, p=0.013) and Ct value <25 (aOR 3.13, 95% CI: 1.24–7.90, p=0.015), but not exposure to COVID-19 treatment (p=0.318). To explore the potential role of anti-TPO in symptom persistence, we analysed a subgroup of 138 patients who had complete anti-TPO status at baseline and reassessment (**Table 4**). Patients with symptom persistence had a higher Charlson comorbidity index (p=0.048) and higher viral load in acute COVID-19 (p=0.016). There was no difference in the baseline clinical severity (p=0.232), symptom burden (p=0.318) and CRP elevation (p=0.640), requirement of prolonged stay (≥14 days) (p=0.765) and intensive care unit admission (p=0.454) in acute COVID-19. Upon follow-up, there was no difference in the proportion of patients with elevated CRP (p=0.454), anti-SARS-CoV-2 RBD

IgG positivity (p=0.999) and chest x-ray resolution (p=0.635). On the other hand, more patients with symptom resolution had positive anti-TPO at baseline (p=0.046) and follow-up (p=0.027). As anti-TPO positivity at baseline and follow-up showed a significant and strong correlation (Kendall's tau-b 0.886, p<0.001), anti-TPO positivity at baseline and follow-up were separately entered into the multivariable logistic regression analysis models comprising Charlson comorbidity index and viral load. In the former model, only Ct value <25 (aOR 2.88, 95% CI 1.04–7.99, p=0.043) remained to be the significant factor associated with symptom persistence, but not baseline anti-TPO positivity (aOR 0.15, 95% CI 0.18–1.17, p=0.070) or Charlson comorbidity index (p=0.323). In the latter model, both Ct value <25 (aOR 2.83, 95% CI 1.02–7.86, p=0.046) and anti-TPO positivity at follow-up (aOR 0.12, 95% CI 0.01–0.94, p=0.043) were associated with symptom persistence, but not Charlson comorbidity index (p=0.229).

In group B, the clinical characteristics at baseline and follow-up were not different between patients who remained asymptomatic and those who developed new symptoms. (Data not shown)

Discussion

Our study added to the current growing literature of long COVID. We reported a 20% prevalence of long COVID, predicted by female and higher SARS-CoV-2 viral loads. Importantly, our study was the first to investigate the potential role of thyroid function and autoimmunity in long COVID. We demonstrated that, on follow-up, most thyroid dysfunction in acute COVID-19 had recovered spontaneously, and incident thyroid dysfunction was relatively rare. Nonetheless, we observed incident anti-TPO positivity upon follow-up. Interestingly, subgroup analysis revealed that symptom resolution was more likely among patients with positive anti-TPO at the time of reassessment, suggesting a potential protective role of anti-TPO in long COVID.

An accurate estimate of the prevalence of long COVID provides essential information to health care authorities for resource planning. The prevalence of long COVID may vary with the different populations studied, the interval from acute COVID-19 to reassessment, and the study instrument used.[24] Earlier studies in the United States, Europe and China have revealed prevalence varying from one-third to close to 90%,[2] with the higher prevalence usually reported among cohorts of patients with more severe acute COVID-19. Our reported prevalence of 20% was at the lower end of this range, which could be explained by the less severe disease spectrum and lower symptom burden in acute COVID-19 in our cohort. This prevalence likely applies to the general population for several reasons. Firstly, vigorous contact

293 tracing by the Centre of Health Protection and active surveillance with the Universal
294 Community Testing Programme, followed by early quarantine and isolation, likely allowed
295 identification of most COVID-19 patients in the territory. Secondly, most COVID-19 patients
296 belonged to the mild disease spectrum.[25] Thirdly, our cohort was largely similar to COVID-19
297 patients admitted to other centers in Hong Kong in terms of sex and initial Ct values, except
298 that patients in our cohort were slightly older. We were not able to compare the clinical
299 severity of COVID-19 between our cohort and patients admitted to other centers. Nonetheless,
300 admission to centers for COVID-19 management was arranged according to patients' area of
301 residence and the occupancy of the individual centers, but not according to clinical severity.
302 Furthermore, the initial Ct values were similar among patients admitted to our center and
303 those admitted to other centers. As Ct values have been reported to correlate with COVID-19
304 severity,[23] we do not expect a significant inter-center difference in the clinical severity of
305 COVID-19. Our list of reported symptoms of long COVID was consistent with the existing
306 studies, with dyspnoea, cough, anosmia/ageusia and fatigue/malaise more commonly
307 reported.[2]
308
309 While much has been focused on the description of long COVID, the predictors of long COVID
310 still require active research. A large contemporary symptom-based prospective observational
311 cohort study for long COVID in the UK revealed that older age, higher body mass index, female

312 and high symptom burden during acute COVID predicted long COVID.[26] We took a step further
313 to include a panel of laboratory parameters, viral loads, immune profiles and radiological
314 assessments in the study of predictors of long COVID, which may unveil mechanisms of long
315 COVID and improve risk stratification. Consistent with the UK study, female was predictive of
316 long COVID in our cohort. In addition, higher baseline SARS-CoV-2 viral load predicted long
317 COVID. Our finding echoed another single-centre longitudinal study in China which showed
318 that viral shedding time in acute COVID-19 was associated with specific symptoms upon
319 follow-up (physical decline/fatigue or post-activity polypnoea).[27] On the other hand, we did
320 not identify any association among the panel of haematological, biochemical, inflammatory
321 and radiological markers with the presence of long COVID. These findings may support the
322 hypothesis of a direct viral effect in the pathogenesis of long COVID, analogous to the
323 postulated potential of SARS-CoV-1 for direct neuro-invasion causing persistent
324 neuropsychiatric sequelae such as post-viral fatigue syndrome.[28,29] Moreover, our study
325 findings carry potential implications to encourage clinicians to be alerted to the Ct values
326 reported upon the diagnosis of COVID-19, and to triage female patients with higher initial
327 SARS-CoV-2 viral loads for more comprehensive assessment and post-acute COVID care.[30]
328
329 While thyroid dysfunction in acute COVID-19 has become better characterized with results
330 from various cohorts,[7] evidence on the longer-term impact of COVID-19 on the thyroid is still

eagerly awaited. The latest data from an Italian cohort of 51 COVID-19 patients presented in ENDO 2021 in March 2021 showed that both inflammatory markers and thyroid function normalized at three months, but one-third of them still had focal hypoechoic areas on thyroid ultrasonography suggestive of thyroiditis. This ultrasonographic finding has raised concern for the need for longer-term monitoring for potential incident thyroid dysfunction. Our study findings were in agreement with that follow-up study that most thyroid dysfunction in acute COVID-19 recovered and that incident thyroid dysfunction was rare. Furthermore, our novel observation of incident anti-TPO positivity post-acute COVID-19 suggested potential perturbation of thyroid autoimmunity after COVID-19, and an additional concern for potential incident thyroid dysfunction as the occurrence of anti-TPO can precede thyroid dysfunction.[31] Hence, our data further supported the need for follow-up TFTs.

Interestingly, anti-TPO positivity at three months was associated with a higher likelihood of symptom resolution among patients who were symptomatic in acute COVID-19. A trend towards statistical significance was observed for baseline anti-TPO positivity with symptom resolution. This phenomenon appeared to be confined to thyroid-specific antibodies as no statistically significant difference in ANA positivity was observed between patients with and without long COVID. No difference in anti-SARS-CoV-2 RBD IgG positivity was observed between the two groups either. A cross-sectional study of 641 community-dwelling older

women demonstrated a lower prevalence of frailty with positivity of thyroid-specific autoantibodies (anti-TPO and anti-Tg) but not with ANA positivity (more a marker of systemic autoimmunity), independent of thyroid function status.[32] Some symptoms of long COVID, such as malaise/fatigue, are in common with features of frailty, such as reduced physical strength and low energy level. Whether some form of beneficial autoimmunity may play a role in the link between anti-TPO positivity and long COVID remained to be determined. Chronic use of interferon-beta 1b was reported to be associated with altered thyroid function and autoimmunity,[33] but in fact numerically more patients in the long COVID group were treated with interferon than those with symptom resolution. Hence, the association between anti-TPO positivity and symptom resolution was less likely confounded by the effect of interferon beta-1b on anti-TPO positivity. Nonetheless, the possibility of other residual confounders not measured in this study cannot be excluded.

The strengths of our study included the following. Firstly, we described the prevalence and predictors of long COVID predominantly among patients with mild to moderate disease, generalizable to COVID-19 patients at large. Secondly, our study findings were based on structured face-to-face assessments, including blood tests for inflammatory markers and SARS-CoV-2 antibodies and chest x-ray, which allowed a systematic evaluation of residual objective abnormalities post-acute COVID-19, beyond symptoms perceived by patients.

Thirdly, our study was the first to evaluate the role of thyroid function and autoimmunity in long COVID, revealing interesting observation of incident thyroid autoimmunity post-acute COVID-19 and a potential protective role of anti-TPO in long COVID. Nevertheless, our study findings should be interpreted bearing certain limitations. Firstly, SARS-CoV-2 viral loads were represented by Ct values. Despite a good correlation,[14,15] direct quantitative measurements of viral loads would have been preferable if available. Secondly, obesity, which has been reported to be associated with long COVID,[26] 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. Thirdly, 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. Fourthly, a control group of non-COVID-19 pneumonia patients was not available for further characterization of long-term impact of COVID-19. Fifthly, we did not have data on the background rate of anti-TPO positivity in the community, but this has been reported to be 11-12% in Taiwan, a region also consisting of Han Chinese and situated near Hong Kong geographically.[34] Although the rate of positive anti-TPO in our cohort (20%) appeared numerically higher than the reported background rate of anti-TPO positivity in Taiwan, a direct comparison was not possible. Last but not least, our findings were based on a single-center study with a relatively small sample size and follow-up duration of around 3

months. Studies with a larger sample size and longer follow-up are necessary to provide more definitive conclusions.

Conclusion

Long COVID was not uncommon, with female and higher viral load in acute COVID-19 being the risk factors. Most thyroid dysfunction during acute COVID-19 recovered. Though incident thyroid dysfunction was rare, we observed incident anti-TPO positivity, suggesting the possibility of COVID-19 triggering autoimmunity. Patients with anti-TPO positivity at reassessment were more likely to have symptom resolution, the significance of which remained to be elucidated.

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Figure Legend

Figure 1. The evolution of the thyroid function of all 204 patients

Figure 1

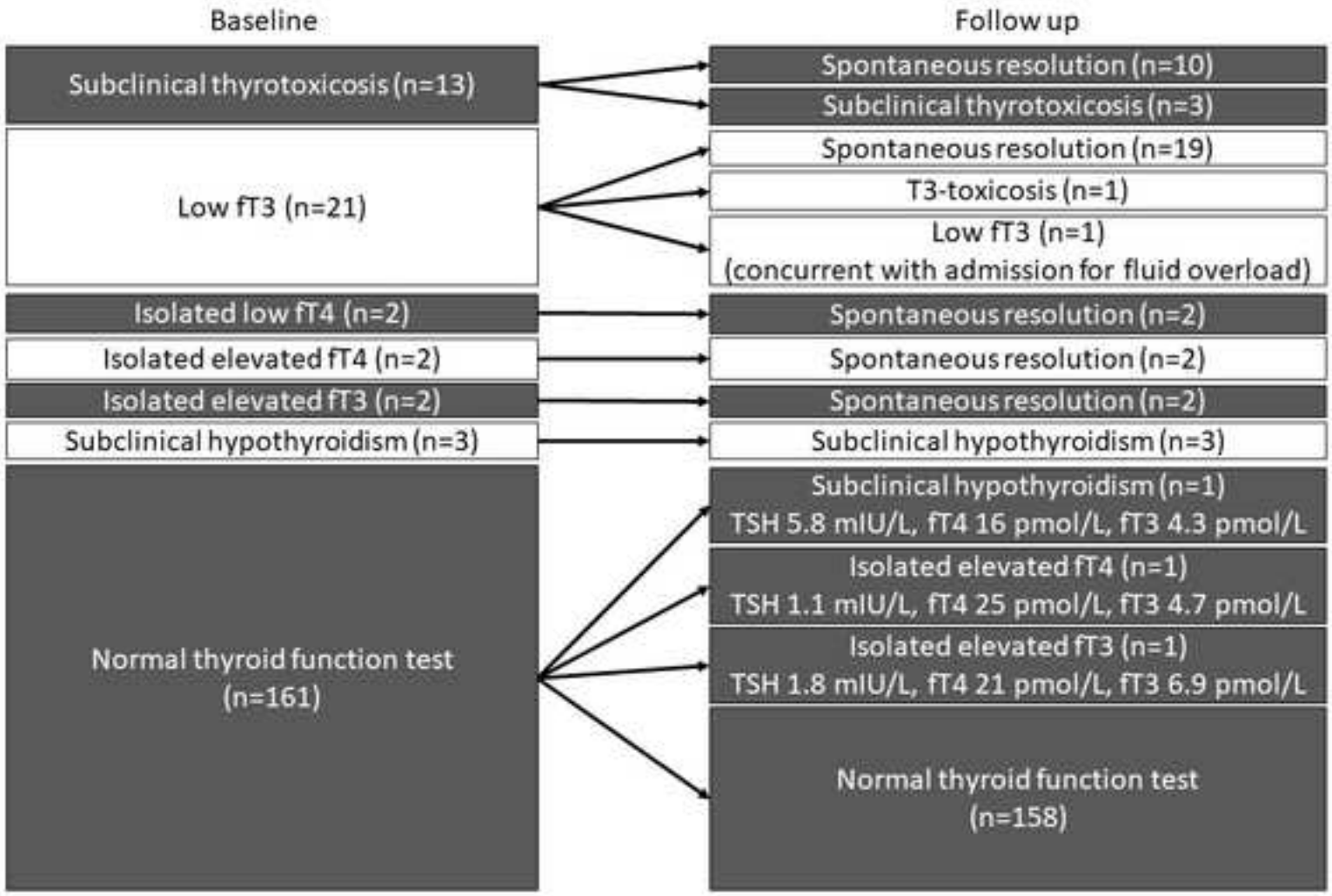

Table 1. Baseline characteristics of the cohort

	All	Symptomatic in acute COVID-19 illness (group A)	Asymptomatic in acute COVID-19 illness (group B)	P value[a]
Number	204	172	32	---
Age (years)	55.0 (44.3 – 63.0)	56.0 (45.0 – 63.0)	53.5 (32.0 – 64.5)	0.243
Female	109 (53.4%)	90 (52.3%)	19 (59.4%)	0.463
Charlson comorbidity index				0.333
0	157 (77.0%)	132 (76.7%)	25 (78.1%)	
1	27 (13.2%)	21 (12.2%)	6 (18.8%)	
≥2	20 (9.8%)	19 (11.0%)	1 (3.1%)	
ACUTE COVID-19 ILLNESS				
Baseline clinical severity				**<0.001**
Mild	147 (72.1%)	115 (66.9%)	32 (100%)	
Moderate	49 (24.0%)	49 (28.5%)	0 (0%)	
Severe	8 (3.9%)	8 (4.7%)	0 (0%)	
SARS-CoV-2 PCR Ct value	25.55 (19.10 – 29.90)	23.85 (19.00 – 28.51)	28.48 (17.97 – 33.05)	0.199
TSH (mIU/L)	1.10 (0.76 – 1.70)	1.10 (0.72 – 1.68)	1.30 (1.00 – 1.88)	0.080
fT4 (pmol/L)	17 (16 – 19)	17 (16 – 19)	18 (16 – 20)	0.470
fT3 (pmol/L)	4.1 (3.5 – 4.5)	4.0 (3.4 – 4.4)	4.3 (3.9 – 4.9)	**0.002**
Abnormal TFT	43 (21.1%)	38 (22.1%)	5 (15.6%)	0.410
Anti-TPO positivity	40/200 (20.0%)	32/171 (18.7%)	8/29 (27.6%)	0.269
Anti-Tg positivity	21/200 (10.5%)	18/171 (10.5%)	3/29 (10.3%)	0.999
ANA positivity	20/80 (25.0%)	16/69 (23.2%)	4/11 (36.4%)	0.454
COVID-19 treatment	147 (72.1%)	129 (75.0%)	18 (56.3%)	**0.030**
REASSESSMENT				
Interval from acute COVID-19 (days)	89 (69 – 99)	90 (71 – 101)	88 (33 – 97)	0.390
Long COVID	41 (20.1%)	34 (19.8%)	7 (21.9%)	0.785
TSH (mIU/L)	1.40 (0.95 – 1.98)	1.45 (0.96 – 2.00)	1.40 (0.88 – 1.78)	0.512
fT4 (pmol/L)	17 (16 – 19)	17 (15 – 19)	18 (16 – 19)	0.199
fT3 (pmol/L)	4.7 (4.4 – 5.1)	4.7 (4.3 – 5.0)	4.9 (4.4 – 5.2)	0.187
Abnormal TFT	12 (5.9%)	11 (6.4%)	1 (3.1%)	0.696
Anti-TPO positivity	38/162 (23.5%)	28/139 (20.1%)	10/23 (43.5%)	**0.014**
Anti-Tg positivity	18/162 (11.1%)	16/139 (11.5%)	2/23 (8.7%)	0.999

[a]Comparison between group A and group B

Abbreviations: TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free

triiodothyronine; TFT; thyroid function test; TPO; thyroid peroxidase; Tg, thyroglobulin; ANA; anti-nuclear antibody

Values reaching statistical significance are in bold

Table 2. Comparison of clinical characteristics between patients who did and did not have symptoms post-acute COVID-19 (n=204)

	Long COVID	Symptoms resolved	P value
Number	41	163	---
Age >50 years	21 (51.2%)	110 (67.5%)	0.052
Female	28 (68.3%)	81 (49.7%)	**0.033**
Charlson comorbidity index			0.135
0	28 (68.3%)	129 (79.1%)	
1	7 (17.1%)	20 (12.2%)	
≥2	6 (14.6%)	14 (8.6%)	
ACUTE COVID-19 ILLNESS			
Ct value <25	30 (73.2%)	78 (47.9%)	**0.005**
TSH (mIU/L)	1.10 (0.85 – 1.60)	1.10 (0.66 – 1.70)	0.952
fT4 (pmol/L)	17 (15 – 19)	17 (16 – 19)	0.999
fT3 (pmol/L)	4.0 (3.4 – 4.3)	4.0 (3.4 – 4.4)	0.643
Abnormal TFT	6 (14.6%)	37 (22.7%)	0.258
Anti-TPO positivity	4/39 (10.3%)	36/161 (22.4%)	0.118
Anti-Tg positivity	3/39 (7.7%)	18/161 (11.2%)	0.771
ANA positivity	2/17 (11.8%)	18/63 (28.6%)	0.214
COVID-19 treatment	35 (85.4%)	112 (68.7%)	**0.034**
Interferon	29 (70.7%)	97 (59.5%)	0.186
Ribavirin	22 (53.7%)	76 (46.6%)	0.420
Remdesivir	9 (22.0%)	30 (18.4%)	0.606
Dexamethasone	6 (14.6%)	22 (13.5%)	0.850
Clofazimine	1 (2.4%)	4 (2.5%)	0.999
REASSESSMENT			
Interval from acute COVID-19 (days)	89 (73 – 99)	89 (63 – 101)	0.823
TSH (mIU/L)	1.50 (0.91 – 2.05)	1.40 (0.99 – 1.90)	0.894
fT4 (pmol/L)	17 (16 – 18)	17 (15 – 19)	0.775
fT3 (pmol/L)	4.6 (4.2 – 4.9)	4.7 (4.4 – 5.2)	0.174
Abnormal TFT	2 (4.9%)	10 (6.1%)	0.999
Anti-TPO positivity	4/32 (12.5%)	34/130 (26.2%)	0.160
Anti-Tg positivity	2/32 (6.25%)	16/130 (12.3%)	0.530

Abbreviations: Ct, cycle threshold; TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; TFT; thyroid function test; TPO; thyroid peroxidase; Tg, thyroglobulin; ANA; anti-nuclear antibody

Values reaching statistical significance are in bold

Table 3. Comparison of clinical characteristics between patients who did and did not have persistent symptoms post-acute COVID-19 (n=172)

	Long COVID	Symptoms resolved	P value
Number	34	138	---
Age >50 years	18 (52.9%)	95 (68.8%)	0.080
Female	10 (29.4%)	72 (52.2%)	**0.017**
Charlson comorbidity index			0.070
0	22 (64.7%)	110 (79.7%)	
1	6 (17.6%)	15 (10.9%)	
≥2	6 (5.9%)	13 (9.4%)	
ACUTE COVID-19 ILLNESS			
Ct value <25	26 (76.5%)	68 (49.3%)	**0.004**
TSH (mIU/L)	1.10 (0.85 – 1.60)	1.10 (0.66 – 1.70)	0.952
fT4 (pmol/L)	17 (15 – 19)	17 (16 – 19)	0.999
fT3 (pmol/L)	4.0 (3.4 – 4.3)	4.0 (3.4 – 4.4)	0.643
Abnormal TFT	5 (14.7%)	33 (23.9%)	0.246
Anti-TPO positivity	2/33 (6.1%)	30/138 (21.7%)	**0.046**
Anti-Tg positivity	3/33 (9.1%)	15/138 (10.9%)	0.999
ANA positivity	1/14 (7.1%)	15/55 (27.3%)	0.162
COVID-19 treatment	30 (88.2%)	99 (71.7%)	**0.049**
Interferon	25 (73.5%)	86 (62.3%)	0.221
Ribavirin	18 (52.9%)	65 (47.1%)	0.542
Remdesivir	8 (23.5%)	28 (20.3%)	0.677
Dexamethasone	6 (17.6%)	22 (15.9%)	0.809
Clofazimine	1 (2.9%)	4 (2.9%)	0.999
REASSESSMENT			
Interval from acute COVID-19 (days)	90 (73 – 97)	90 (68 – 101)	0.844
TSH (mIU/L)	1.55 (0.95 – 2.10)	1.40 (0.98 – 2.00)	0.581
fT4 (pmol/L)	17 (16 – 18)	17 (15 – 19)	0.946
fT3 (pmol/L)	4.6 (4.2 – 4.9)	4.7 (4.4 – 5.1)	0.297
Abnormal TFT	1 (2.9%)	10 (7.2%)	0.695
Anti-TPO positivity	1/24 (4.2%)	26/108 (24.1%)	**0.027**
Anti-Tg positivity	2/24 (8.3%)	13/108 (12.0%)	0.999

Abbreviations: Ct, cycle threshold; TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; TFT; thyroid function test; TPO; thyroid peroxidase; Tg, thyroglobulin; ANA; anti-nuclear antibody

Values reaching statistical significance are in bold

Table 4. Subgroup analysis of patients in group A with complete anti-TPO information (n=138)

	Long COVID	Symptoms resolved	P value
Number	25	113	---
Age >50 years	15 (60.0%)	81 (71.7%)	0.251
Female	16 (64.0%)	56 (49.6%)	0.191
Charlson comorbidity index			**0.048**
0	16 (64.0%)	91 (80.5%)	
1	5 (20.0%)	13 (11.5%)	
≥2	4 (16.0%)	9 (8.0%)	
ACUTE COVID-19 ILLNESS			
Ct value <25	19 (76.0%)	56 (49.6%)	**0.016**
TSH (mIU/L)	1.20 (0.77 – 1.60)	1.05 (0.63 – 1.70)	0.446
fT4 (pmol/L)	18 (16 – 19)	17 (16 – 19)	0.748
fT3 (pmol/L)	3.8 (3.3 – 4.1)	4.0 (3.5 – 4.4)	0.398
Abnormal TFT	4 (16.0%)	23 (20.4%)	0.784
Anti-TPO positivity	1 (4.0%)	24 (21.2%)	**0.046**
Anti-Tg positivity	2 (8.0%)	14 (12.4%)	0.736
ANA positivity	1/11 (9.1%)	10/49 (20.4%)	0.670
COVID-19 treatment	22 (88.0%)	86 (76.1%)	0.284
Interferon	20 (80.0%)	78 (69.0%)	0.274
Ribavirin	17 (68.0%)	60 (53.1%)	0.175
Remdesivir	3 (12.0%)	21 (18.6%)	0.567
Dexamethasone	4 (16.0%)	17 (15.0%)	0.999
Clofazimine	1 (4.0%)	4 (3.5%)	0.999
REASSESSMENT			
Interval from acute COVID-19 (days)	91 (84 – 98)	92 (83 – 102)	0.682
TSH (mIU/L)	1.40 (0.92 – 2.08)	1.45 (0.93 – 2.00)	0.754
fT4 (pmol/L)	17 (16 – 18)	17 (15 – 19)	0.668
fT3 (pmol/L)	4.7 (4.3 – 4.9)	4.7 (4.4 – 5.2)	0.668
Abnormal TFT	1 (4.0%)	7 (6.2%)	0.999
Anti-TPO positivity	1 (4.0%)	27 (23.9%)	**0.027**
Anti-Tg positivity	2 (8.0%)	14 (12.4%)	0.736

Abbreviations: Ct, cycle threshold; TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; TFT; thyroid function test; TPO; thyroid peroxidase; Tg, thyroglobulin; ANA; anti-nuclear antibody

Values reaching statistical significance are in bold