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# The thyroid and COVID-19, volume II

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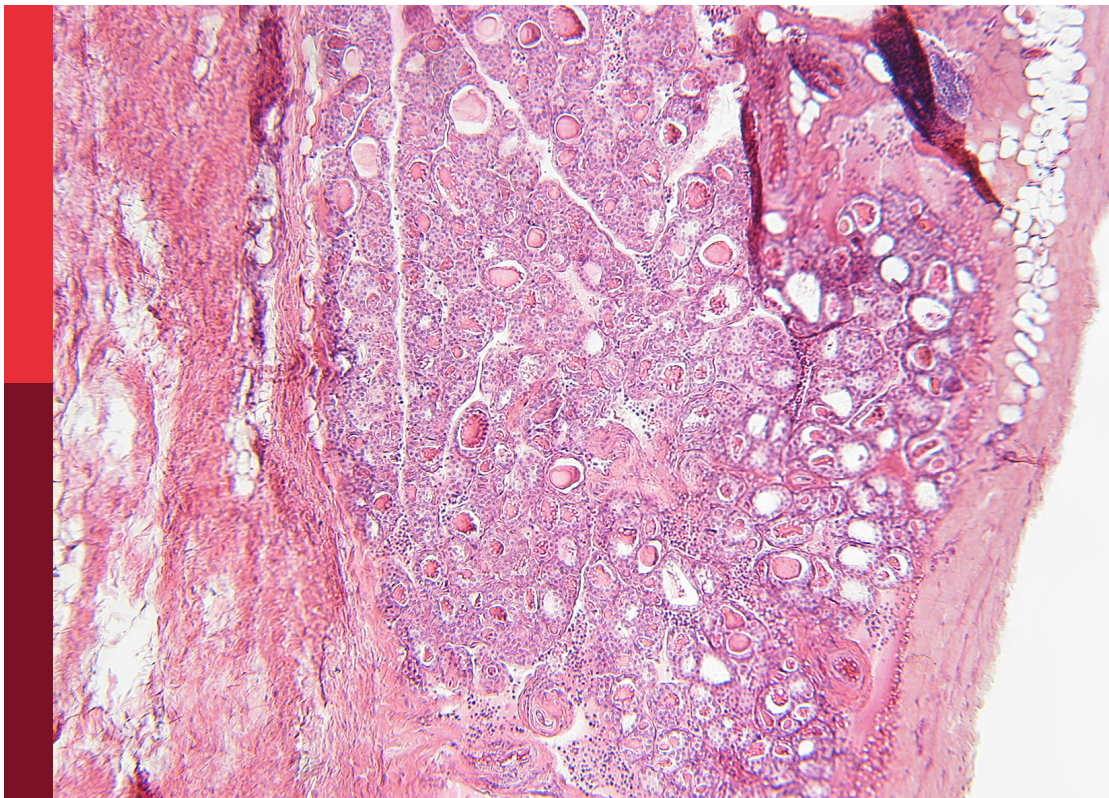

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# The thyroid and COVID-19, volume II

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# Editorial: The thyroid and Covid-19, volume II

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Editorial on the Research Topic
The thyroid and Covid-19, volume II

## More insights into thyroid and COVID-19

Coronaviruses are enveloped RNA viruses of wide distribution in humans associated with mild respiratory disease. By contrast, severe acute respiratory syndrome coronavirus (SARS-CoV) is one of those coronaviruses that can cause fatal illness. In late December 2019, an outburst of pneumonia of unknown cause in Wuhan, China, was identified as the early stage of the coronavirus disease (COVID-19) pandemic outbreak, and the SARS-CoV-2 was found responsible (1).

Two main proteins expressed by SARS-CoV-2 are essential for the manifestations of COVID-19. The first is the transmembrane protease serine 2 (TMPRSS2), which acts on the transcription and replication of the virus. The second is the Spike protein found on the surface of viral particles, which binds to angiotensin-converting enzyme 2 (ACE2) in tissue cells and is a determinant for transmitting infection. Therefore, SARS-CoV-2 infection depends on two steps: ACE2 receptor recognition via Spike protein and cell membrane fusion via transmembrane protease (2).

ACE2 is expressed in different tissues, and the thyroid is no exception (3). It has been shown that the thyroid gland has high expression levels of ACE2, which may explain the direct effects on the thyroid parenchyma, making it more susceptible to viral attack (3).

SARS-CoV-2 infection can lead to thyroid diseases by severely destroying parafollicular and follicular epithelial cells, leading to follicle rupture. As a result, SARS-CoV-2 virus infections are associated with inflammatory thyroid diseases such as subacute thyroiditis, Graves' disease, thyrotoxicosis, Hashimoto's thyroiditis, and euthyroid patient syndrome (4). As regards thyroid cancer, the COVID-19 pandemic has also affected its traditional

management, and the consequences of this strategic change are largely unknown. Furthermore, it is intriguing that if the virus attacks the thyroid gland, it can also modulate thyroid cancer behavior (5).

In this second volume of COVID-19 and the Thyroid of Frontiers in Endocrinology, nine articles have been included that describe the association between the thyroid gland and the SARS-CoV-2 infection. The topics covered referred to thyroid hormone dysregulation in COVID-19-infected patients and after COVID-19 vaccination, as well as the strategic management of thyroid nodular disease during the pandemic.

In this regard, Silveira et al. described the adapted management of a whole country by retrospectively analyzing the data of thyroid cancer-related procedures in the Brazilian public health system from 2019 to 2021. According to medical guidelines recommendations, there was a considerable reduction in the number of FNABs (29%), oncologic thyroidectomies (17%), and RAI therapies (28%) in 2020. Due to the lack of information about patients' clinical and oncological features, the long-term consequences of this adopted strategy on thyroid cancer care could not be evaluated. However, since the proportion of thyroidectomies decreased during the pandemic but not significantly, it may be speculated that surgery for high-risk thyroid cancer was prioritized. Therefore, this study may exemplify active surveillance for low-risk thyroid tumors in a large-scale model.

In other terms, the challenging situation of managing respiratory distress due to giant goiter and concomitant severe COVID-19 disease was reported in four patients by Wang et al. In three cases where emergency thyroid surgery was implemented, the respiratory tract obstruction was relieved, and dyspnea improved post-surgically. However, the Authors reckon that anesthesia stimulation may have aggravated the inflammatory response attributable to the viral infection. Given the small size of this study, solid conclusions are difficult to obtain. However, as thyroid surgery was generally avoided during the COVID-19 pandemic, this small case-series study reveals critical details of the pros and cons of emergency thyroid surgery in this exceptional context.

As regards thyroid dysfunction during COVID-19, a systematic review and meta-analysis of the English and Chinese population thyroxine levels carried out by Li et al. found that compared to healthy patients, COVID-19 infection showed decreased TSH and FT3 levels, whereas FT4 was increased. Moreover, these findings also indicate that the severity of the infection was positively correlated with the observed changes in thyroxine levels. These results support the notion that FT3, in particular, could constitute an essential clinical index to understand the impact of COVID-19 infection on thyroid function.

It is particularly interesting if post-COVID-19 patients are more predisposed to develop thyroid autoimmunity. Rossini et al. addressed this question by assessing thyroid function and antibodies in a prospective cohort of 599 COVID-19 survivors. The results were compared to a historical control group from the same institution without thyroid disease. TPOab prevalence was almost double (15.7% vs 7.7%) in the COVID-19 group, raising awareness of the immunogenic potential of this disease upon the thyroid, a gland with a high propensity to autoimmunity.

Among the many thyroid function markers found to be impaired by COVID-19 is thyroglobulin. Świątkowska-Stodulska et al. assessed thyroglobulin (TG) levels as a marker of possible thyroid destruction in 174 patients hospitalized for COVID-19 and after glucocorticoid treatment. TG levels decreased over time in the whole group and did not differ between the patients with normal and abnormal thyroid function tests. However, it is interesting that the decrease was primarily observed in the subgroup of individuals under glucocorticoid therapy. Although this therapeutical approach is widely used in subacute thyroiditis, its potential use to protect the thyroid gland in the setting of SARS-CoV-2 is entirely original.

Using a cohort of Japanese health workers immunized against COVID-19 with the SARS-CoV-2 BNT162b2 mRNA vaccine, Morita et al. found that this vaccine can disrupt thyroid autoimmunity, leading to Graves´ disease. The 12-month follow-up study found that after two doses of the SARS-CoV-2 BNT162b2 mRNA vaccine, several serum markers of Graves´ disease, most notably anti-TSH receptor antibody, increased in female patients. Moreover, after the third dose, there was also an increase in anti-thyroglobulin antibodies. Although larger cohort epidemiologic studies are required, this evidence argues that clinical surveillance on thyroid status should be considered after immunization against COVID-19 to prevent relapse of new onset of Graves' disease and allow early identification and clinical management of this condition.

To investigate mood changes and thyroid disorders after COVID-19 vaccination in susceptible populations, Ma et al. performed a retrospective multi-center study in Hashimoto's thyroiditis patients. The thyroid function tests, thyroid antibodies, CRP levels, and Beck Depression Inventory scores of 2765 patients before and 24 weeks after two doses of inactivated CoronaVac (BBIBP-CorV) vaccines were compared to propensity score matched 1288 non-vaccinated controls. Increased levels of TSH were observed in the vaccinated patients, followed by mood changes. Increased baseline levels of TSH, CRP, and TPOab were predictors of a higher incidence of mood changes. These findings might serve to support the long-debated argument on the relationship between depression and thyroid autoimmunity.

Finally, Rossetti et al. elaborated a comprehensive narrative review of several studies reporting the consequences of COVID-19 on the thyroid, focusing on the mechanistic pathways whereby COVID-19-induced cytokine storm might lead to NTIS and thyrotoxicosis. This review is complemented by the study by Zhang et al. also included in this second volume of the Research Topic on the Thyroid and COVID-19. Data obtained from the ThyroidOmics Consortium served for thyroid function analysis, while data on COVID-19 susceptibility and severity were extracted from the COVID-19 Host Genetics Initiative. It was shown that COVID-19 susceptibility might be a risk factor for hypothyroidism,

opening the possibility of adding COVID-19 infection to the list of case-finding for hypothyroidism.

## Author contributions

GB: Writing – original draft, Writing – review & editing. MC: Writing – review & editing. CN: Writing – review & editing. JS: Writing – review & editing.

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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.

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# Causal associations between thyroid dysfunction and COVID-19 susceptibility and severity: A bidirectional Mendelian randomization study

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**Background:** Observational studies have reported an association between coronavirus disease 2019 (COVID-19) risk and thyroid dysfunction, but without a clear causal relationship. We attempted to evaluate the association between thyroid function and COVID-19 risk using a bidirectional two-sample Mendelian randomization (MR) analysis.

**Methods:** Summary statistics on the characteristics of thyroid dysfunction (hypothyroidism and hyperthyroidism) were obtained from the ThyroidOmics Consortium. Genome-wide association study statistics for COVID-19 susceptibility and its severity were obtained from the COVID-19 Host Genetics Initiative, and severity phenotypes included hospitalization and very severe disease in COVID-19 participants. The inverse variance-weighted (IVW) method was used as the primary analysis method, supplemented by the weighted-median (WM), MR-Egger, and MR-PRESSO methods. Results were adjusted for Bonferroni correction thresholds.

**Results:** The forward MR estimates show no effect of thyroid dysfunction on COVID-19 susceptibility and severity. The reverse MR found that COVID-19 susceptibility was the suggestive risk factor for hypothyroidism (IVW: OR = 1.577, 95% CI = 1.065–2.333, $P$ = 0.022; WM: OR = 1.527, 95% CI = 1.042–2.240, $P$ = 0.029), and there was lightly association between COVID-19 hospitalized and hypothyroidism (IVW: OR = 1.151, 95% CI = 1.004–1.319, $P$ = 0.042; WM: OR = 1.197, 95% CI = 1.023-1.401, $P$ = 0.023). There was no evidence supporting the association between any phenotype of COVID-19 and hyperthyroidism.

**Conclusion:** Our results identified that COVID-19 might be the potential risk factor for hypothyroidism. Therefore, patients infected with SARS-CoV-2 should strengthen the monitoring of thyroid function.

KEYWORDS

**COVID-19, hypothyroidism, hyperthyroidism, Mendelian randomization, thyroid dysfunction**

# Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has rapidly become a global epidemic and a serious health threat (1). Currently, several studies have shown that some susceptible populations include type 2 diabetes, obesity, hypertension, and other metabolic diseases (2–5). However, thyroid disease is not covered in the report.

SARS-CoV-2 virus infection and thyroid-promoted inflammatory responses are related and interact in a complex way. Several small observational case studies now support that thyroid dysfunction is a predisposing factor for COVID-19 (6, 7) and that hospital mortality is higher in patients with COVID-19 hypothyroidism than in patients with COVID-19 normal thyroid function (8). However, some studies have taken the opposite view (9–11), suggesting that the real cause of COVID-19 progression is antithyroid drug-induced neutropenia rather than thyroid disease status. In addition, SARS-CoV-2 may impair the hypothalamic–pituitary–thyroid axis or damage thyroid problems through a viral inflammatory process, which may increase the risk of future partial thyroid dysfunction (12–14). However, these findings remain vulnerable to confounding factors and reverse causality that cannot be fully excluded in observational studies. Further investigation is needed to determine the causal relationship between thyroid dysfunction and COVID-19 infection.

Mendelian randomization (MR) is a new epidemiological approach that uses genetics as a tool to study correlations between exposure and outcome (15). MR is biologically based on the random distribution of gametes in meiosis and has the advantage of overcoming the limitations of confusion and reverse causality often encountered in observational studies (16). In this study, we performed bidirectional MR to explore the causal association between thyroid dysfunction and COVID-19 risk.

# Methods

## Study design

We conducted a bidirectional two-sample MR study to assess the causal relationship between thyroid dysfunction (hypothyroidism and hyperthyroidism) and COVID-19 susceptibility and its severity. The instrumental variables must satisfy three basic principles: (1) Genetic variation is strongly correlated with exposure; (2) genetic variation is not strongly related to potential confounders; and (3) genetic variation does not directly affect the outcome (17). We performed bidirectional MR in this study, and forward MR: thyroid dysfunction genome-wide association study (GWAS) data were used to explore the effect of thyroid dysfunction on COVID-19. Reverse MR: COVID-19 GWAS was used as an exposure to explore the effect of COVID-19 on thyroid dysfunction (Supplementary Figure 1).

## Data sources

Data from genome-wide association studies of COVID-19 cases were obtained from the COVID19 Host Genetics Initiative GWAS meta-analysis, round 5 (18). In our study, the COVID-19 susceptibility phenotype comprised 38,984 European patients with COVID-19 defined as individuals with laboratory confirmation of SARS-CoV-2 infection or electronic health records [using the International Classification of Diseases (ICD) or physician annotations] or self-reported, with 1,644,784 control individuals. We then used two cohorts to assess the COVID-19 severity phenotype. The first cohort was compared to 9,986 hospitalized patients versus 1,877,672 control individuals. The second cohort compared 5,101 very severe patients, defined as patients who died or required respiratory support (including continuous positive airway pressure (CPAP), bilevel positive airway pressure (BiPAP), intubation, or high-flow nasal cannula). A total of 1,383,241 control individuals were not included as cases (https://www.COVID19hg.org/blog/2021-03-02-freeze-5-results/). Details of the phenotypes are shown in Table 1.

Summary statistics of thyroid dysfunctions were from The ThyroidOmics Consortium (https://transfer.sysepi.medizin.uni-greifswald.de/thyroidomics/datasets/). The GWAS summary comprised 19 independent cohorts (19). The hypothyroidism cohort included 3,340 cases with thyroid-stimulating hormone (TSH) levels above the reference range, and the hyperthyroidism cohort included 1,840 cases with TSH levels below the cohort-specific reference range. In addition, 49,983 control individuals with TSH levels within the reference range were included in the cohort. Patients receiving medication for thyroiditis were excluded. Genotype data were estimated from the 1,000 genomes, Project Phase 1, Version 3 ALL Population Reference Panel; and all analyses were adjusted for age, age squared, and sex. Details of the 19 cohorts can be found in the original article (19).

The ethical approval and consent information for the above summary statistics were taken from the original publication.

## Selection of genetic instruments

Single nucleotide polymorphisms (SNPs) that met the $p < 5 \times 10^{-8}$ threshold and minor allele frequency >1% were included to avoid potential statistical bias from the original GWAS. We then retained only those SNPs with linkage

TABLE 1 Sources of data for the analysis.

| Phenotype | Source of Genetic Variants | |
|---|---|---|
| | Consortium | Participants |
| **Hypothyroidism** | – | **Cases:** 3,340 cases with TSH levels above the reference range.<br>**Controls:** 49,983 individuals with TSH levels in the reference range. |
| **Hyperthyroidism** | – | **Cases:** 1,840 cases with TSH levels below the reference range.<br>**Controls:** 49,983 individuals with TSH levels in the reference range. |
| **COVID-19 susceptibility** | **Susceptibility** | **Cases:** 3,8984 individuals with COVID-19 by laboratory confirmation of SARS-CoV-2 infection, or by electrical health records (using ICD or physician notes), or self-reporting.<br>**Controls:** 1,644,784 individuals enrolled in the cohorts and not included as cases. |
| **COVID-19 severity** | **Hospitalized** | **Cases:** 9,986 hospitalized individuals with COVID-19.<br>**Controls:** 1,877,672 individuals enrolled in the cohorts and not included as cases |
| | **Very severe disease** | **Cases**: 5,101 very severe patients defined as patients who died or required respiratory support (including CPAP, BiPAP, intubation, or high-flow nasal cannula).<br>**Controls:** 1,383,241 individuals enrolled in the cohorts and not included as cases. |

disequilibrium (R2 < 0.01) clustered in genomic regions 5,000 kbp apart. We used the PhenoScanner database (http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner) to examine the selected instruments variables associated with other phenotypes that may at risk of affecting outcome. rs597808, selected SNP for exposure of hypothyroidism, was correlated significantly with hematological phenotypes (e.g., platelet count, total eosinophil basophil count, percentage of neutrophils in granulocytes, and lymphocyte count). Some studies have shown that changes in blood counts are a marker of SARS-CoV-2 infection and severity, with 25% of patients with COVID19 showing various forms of leucopenia (WBC < 4.00E+09 cells/liter) and the majority (63%) showing lymphocytopenia (20). One other study found that reduced platelet counts can lead to severe COVID-19 (21). Therefore, we conducted MR analysis before and after removed rs597808. We did not detect any SNP association with body mass index, smoking, or drinking, which may be at risk of affecting hyperthyroidism or hypothyroidism when the outcome of MR was thyroid dysfunction (22, 23).

$R^2$ represents the ability of genetic variables to explain the exposures. In our study, the explained variances ranged from 7.1% to 17.6%. In addition, we used F statistics to avoid any weak instrumental variables (F > 10). The detailed information for the selected SNP is shown in the Supplementary File.

## Mendelian randomization analysis

We performed a Wald ratio to assess the effect of exposure on an outcome for each genetic instrument. Then, we used the inverse variance-weighted (IVW) method by combining each effect size as the main analysis to estimate the causal effect of exposure on outcome in the fixed-effects model (24). Moreover, the MR-Egger and weighted-median (WM) methods were applied as supplements. The MR-Egger method was based on the Instrument Strength Independent of Direct Effect assumption. The MR-Egger method often takes inaccurate and statistically low results, especially in the case of the small size of SNPs (e.g., <10) (25). In addition, the value of the MR-Egger intercept term is far from zero, indicating horizontal pleiotropy ($P$ < 0.05). Therefore, the MR-Egger method was mainly conducted to detect pleiotropy in our MR study. The WM analysis was more reliable if more than one-half of SNPs are invalid genetic instruments (e.g., due to pleiotropy) (26). We applied Cochrane's Q-value to examine the heterogeneity, and we adopted the IVW random-effects method as the main effect size if heterogeneity existed. In addition, MR-PRESSO (27) was performed to detect any outlier which may lead to the heterogeneity. If we detected outliers, then they would be removed, and we reassessed the MR effect. We adjusted the multiple testing by a Bonferroni-corrected threshold of $p$ < 0.0083 (p< 0.05/3/2). The $p$-values between 0.0083 and 0.05 were considered suggestive associations. A flow chart about how the MR analysis was performed step by step is shown in Figure 1.

We applied the "TwoSampleMR" and "MR-PRESSO" packages to our MR study; all statistical analyses were performed on the basis of the R software 4.1.1, and the data visualization was conducted in R software and STATA 12.0.

# Results

## Causal association of thyroid dysfunction with COVID-19 *via* forward MR

There was no evidence supporting hypothyroidism as a risk or protective factor for COVID-19 susceptibility, hospitalization, and very severe disease phenotype (IVW: OR = 0.971, 95% CI = 0.826–1.083, $P$ = 0.193; OR = 0.983, 95% CI = 0.862–1.121, $P$ = 0.798; and OR = 0.911, 95% CI = 0.746–1.112, $P$ = 0.359; respectively). In addition, the association between

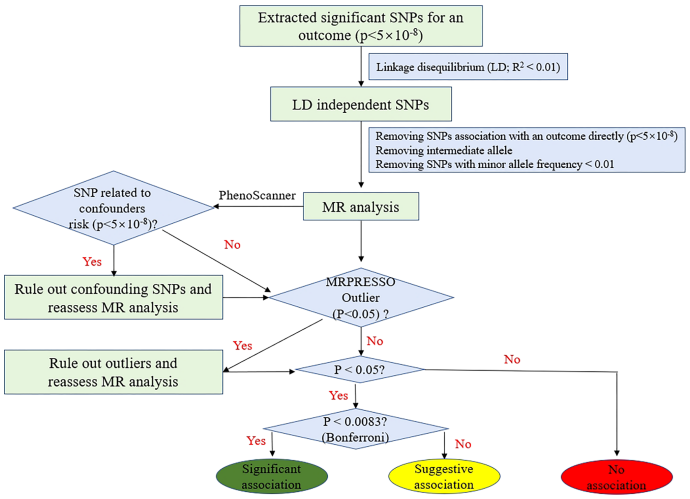


FIGURE 1
Flow chart about the analytical methods and how the MR analysis was performed step by step.

hyperthyroidism and COVID-19 susceptibility, hospitalization, and very severe disease phenotype risk was not observed (IVW: OR = 0.983, 95% CI = 0.948–1.019, *P* = 0.705; OR= 0.938, 95% CI= 0.873-1.007, p=0.077; and OR = 0.921, 95% CI = 0.826–1.026, *P* = 0.136; respectively).

We detected a significant correlation between the genetic instrument rs597808 and hematological features of hyperthyroidism, which may be a confounding factor for the COVID-19 phenotype. We then removed rs597808 and reassessed the MR analysis but reached the same conclusions as the former. On the basis of Q-tests, MR-Egger intercepts, and MR-PRESSO to detect associations between thyroid dysfunction and COVID-19 risk, there was no significant heterogeneity, horizontal pleiotropy, or outliers. Detailed results of the different MR analyses are shown in Table 2.

## Causal association of COVID-19 with thyroid dysfunction *via* reverse MR

The IVW estimate suggested that the susceptibility of COVID-19 and its severity may increase the risk of hypothyroidism. In addition, we deem that the association was suggestive because the p-values were between 0.0083 and 0.05 (Bonferroni-corrected threshold P = 0.0083). The MR results are shown in Table 3 and Figures 2, 3.

COVID-19 susceptibility (IVW: OR = 1.577, 95% CI = 1.065–2.333, *P* = 0.022; WM: OR = 1.527, 95% CI = 1.042–2.240, *P* = 0.029) and hospitalization (IVW: OR = 1.151, 95% CI = 1.004–1.319, *P* = 0.042; WM: OR = 1.197, 95% CI = 1.023-1.401, *P* = 0.023) have a suggestive association with hypothyroidism. There was no association between the severe disease phenotype of COVID-19 and hypothyroidism. The light heterogeneity was excited when the susceptibility and the severe disease phenotype of COVID-19 were used as the exposure, so we performed the IVW random-effects method as the main analysis. The directional pleiotropy was not detected by the MR-Egger intercept. An outlier was detected by the MR-PRESSO test when the severe disease phenotype was used as the exposure, and we reassessed the MR after removing the outlier (Table 3).

Any type of COVID-19 phenotypes was not the risk or protective factor for hyperthyroidism (P > 0.05) in our study. The light heterogeneity was excited when the susceptibility phenotype of COVID-19 was used as the exposure, so we used the IVW random-effects method for our MR analysis. The directional pleiotropy and outliers were not detected by the MR-Egger intercept and MR-PRESSO, respectively.

The detailed MR results and sensitivity analysis for the previous results are presented in Table 3.

# Discussions

Our bidirectional two-sample MR study included two independent consortiums, and only European ancestry was retained. All genetic instruments were rigorously screened by conducting the PhenoScanner. Finally, our MR studies inferred suggestive causal effects of COVID-19 susceptibility and its severity on a higher risk of hypothyroidism.

Some previous observational studies have provided evidence of COVID-19–related primary hypothyroidism (8, 28, 29). Several studies have suggested that hypothyroidism may be caused by direct damage caused by SARS-CoV-2. Angiotensin-converting enzyme 2 (ACE2) and transmembrane protease

TABLE 2 MR estimates for the causal effect of thyroid dysfunction on COVID-19.

| | Outcome | NSNP | IVW | | Weighted Median | | MR-Egger | | $P(I^2)$ | $P_{(pleiotropy)}$ | $P_{(Global)}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | *P* | OR (95% CI) | *P* | OR (95% CI) | *P* | | | |
| **Hyper** | COVID-19 susceptibility | 8 | 0.983 (0.948, 1.019) | 0.705 | 0.972 (0.930, 1.016) | 0.202 | 0.970 (0.835, 1.127) | 0.705 | 0.847 | 0.864 | 0.835 |
| | COVID-19 hospitalization | 8 | 0.938 (0.873, 1.007) | 0.077 | 0.936 (0.853, 1.028) | 0.165 | 0.918 (0.684, 1.231) | 0.752 | 0.619 | 0.587 | 0.619 |
| | COVID-19 very severe | 8 | 0.921 (0.826, 1.026) | 0.136 | 0.947 (0.820, 1.094) | 0.458 | 0.935 (0.587, 1.488) | 0.786 | 0.448 | 0.950 | 0.473 |
| **Hypo** | COVID-19 susceptibility | 7 | 0.971 (0.826, 1.083) | 0.193 | 0.959 (0.907, 1.015) | 0.153 | 0.946 (0.826, 1.083) | 0.458 | 0.711 | 0.701 | 0.730 |
| | COVID-19 hospitalization | 7 | 0.983 (0.862, 1.121) | 0.798 | 0.903 (0.792, 1.028) | 0.141 | 1.195 (0.791, 1.807) | 0.436 | 0.037 | 0.373 | 0.053 |
| | COVID-19 s very severe | 7 | 0.911 (0.746, 1.112) | 0.359 | 0.852 (0.689, 1.053) | 0.138 | 0.943 (0.466, 1.908) | 0.877 | 0.057 | 0.923 | 0.060 |
| **Hypo-excluded*** | COVID-19 susceptibility | 6 | 0.973 (0.928, 1.021) | 0.267 | 0.956 (0.899, 1.016) | 0.149 | 0.944 (0.825, 1.082) | 0.456 | 0.593 | 0.667 | 0.646 |
| | COVID-19 hospitalization | 6 | 1.001 (0.871, 1.167) | 0.914 | 0.995 (0.866, 1.143) | 0.939 | 1.181 (0.759, 1.837) | 0.503 | 0.043 | 0.497 | 0.063 |
| | COVID-19 very severe | 6 | 0.948 (0.765, 1.174) | 0.627 | 0.971 (0.796, 1.185) | 0.776 | 0.918 (0.446, 1.887) | 0.827 | 0.061 | 0.930 | 0.116 |

Hypo-excluded*: MR analysis (exposure: hypothyroidism; outcome: COVID-19) after excluded rs597808 (PMID: 27863252), which significantly associated with hematological traits by performing PhenoScanner datasets; hematological parameters are markers of COVID-19 infection and severity; NSNP, number of single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance weighting; OR, odds ratio. The $I^2$ statistic was used to present the heterogeneity among estimates for each SNP in one analysis. $P_{(Global)}$: The p-value for the global test in the MR-PRESSO. $P_{(pleiotropy)}$: The p-value for the intercept in the MR-Egger regression was used present the pleiotropy ($p < 0.05$). Hyper, hyperthyroidism; Hypo, hypothyroidism.

serine 2 (TMPRSS2) binding is a key complex in SARS-CoV-2–infected hosts (30, 31). Notably, ACE2 and TMPRSS2 are expressed at high levels in the thyroid gland, even higher than in the lung tissue (32). Tee et al. (29) reported a case of overt primary hypothyroidism induced by autoimmune thyroiditis a week after mild COVID-19 symptoms were resolved. A case of hypothyroidism due to Hashimoto's thyroiditis in a 45-year-old man was described (29). A study described that about 7% of

TABLE 3 MR estimates for the causal effect of COVID-19 on thyroid dysfunction.

| | Outcome | NSNP | IVW | | Weighted Median | | MR-Egger | | $P(I^2)$ | $P_{(pleiotropy)}$ | $P_{(Global)}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | *P* | OR (95% CI) | *P* | OR (95% CI) | *P* | | | |
| **COVID-19 susceptibility** | Hyper | 7 | 0.963 (0.589, 1.575) | 0.881 | 1.072 (0.667, 1.725) | 0.773 | 2.745 (0.825, 9.132) | 0.161 | 0.027 | 0.128 | NA |
| | Hypo | 7 | 1.577 (1.065, 2.333) | **0.022** | 1.527 (1.042, 2.240) | **0.029** | 2.358 (0.682, 8.032) | 0.228 | 0.037 | 0.525 | 0.062 |
| **COVID-19 hospitalization** | Hyper | 5 | 1.131 (0.936, 1.367) | 0.203 | 1.200 (0.980, 1.469) | 0.077 | 1.221 (0.889, 1.670) | 0.214 | 0.284 | 0.185 | 0.324 |
| | Hypo | 5 | 1.151 (1.004, 1.319) | **0.042** | 1.197 (1.023, 1.401) | **0.023** | 1.342 (1.017, 1.771) | **0.038** | 0.599 | 0.302 | 0.574 |
| **COVID-19 very severe** | Hyper | 8 | 1.069 (0.959, 1.193) | 0.228 | 1.094 (0.948, 1.263) | 0.220 | 1.238 (0.955, 1.607) | 0.158 | 0.612 | 0.269 | 0.532 |
| | Hypo | 8 | 1.103 (0.963, 1.263) | 0.158 | 1.133 (1.006, 1.277) | **0.039** | 1.334 (0.979, 1.818) | 0.118 | 0.019 | 0.234 | 0.049 |
| **COVID-19 very severe outlier#** | Hypo | 7 | 1.060 (0.954, 1.177) | 0.276 | 1.126 (1.002, 1.262) | **0.043** | 1.182 (0.920, 1.528) | 0.191 | 0.245 | 0.106 | 0.251 |

Severity outlier#: MR analysis was reassessed (exposure: very severe COVID-19; outcome: hypothyroidism) after removed the MRPRESSO outlier (rs111837807; outlier test, p = 0.008); NSNP, number of single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance weighting; OR, odds ratio. The $I^2$ statistic was used to present the heterogeneity among estimates for each SNP in one analysis. $P_{(Global)}$: The p-value for the global test in the MR-PRESSO. $P_{(pleiotropy)}$: The p-value for the intercept in the MR-Egger regression was used present the pleiotropy ($p < 0.05$). Hyper, hyperthyroidism; Hypo, hypothyroidism. Bold values indicate $p<0.05$.

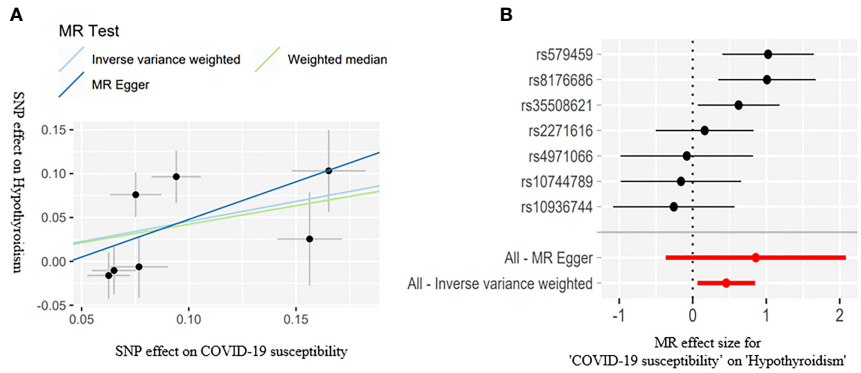


FIGURE 2
Scatter plots **(A)** for estimating causal effects of genetically predicted susceptibility on risk of hypothyroidism. Each black point representing the effect sizes of each SNP on the exposure (horizontal axis) and on the outcome (vertical axis) is plotted with error bars corresponding to each standard error (SE). The slope of each line corresponds to the combined estimate using each method of the IVW (light blue line), the MR-Egger regression (blue line), and the weighted median (light green line). Forest plots **(B)** of susceptibility on the risk of hypothyroidism; the red points showed the combined causal estimate using all SNPs together in a single instrument, using two different methods (MR-Egger and IVW).

patients who survived from severe COVID-19 might have a risk of persistent hypothyroidism, mostly due to Hashimoto's thyroiditis (33). Another study reported that an 18-year-old woman who was hospitalized with severe viral thyroiditis had a positive oropharyngeal swab for SARS-CoV-2 15 days earlier and had mild symptoms of COVID-19 (34). In addition, some other studies reported that subacute viral thyroiditis appears 16–36 days after symptoms of COVID-19 disappear (35, 36). It is worth noting that the original GWAS defined hyperthyroidism and hypothyroidism based on TSH levels, and some patients with subclinical hypothyroidism might be included in the GWAS. There are some observational studies regarding subclinical hypothyroidism after COVID-19 infection. A clinical study by Burekovic et al. (37) showed that, at an average of 2 months after COVID-19 infection, some patients developed subclinical hypothyroidism, and another study (38) showed that seven of the 71 patients infected with COVID-19 included developed subclinical hypothyroidism.

Hypothyroidism is a broad spectrum of disorders that may be related to autoimmune disease or other processes such as viral thyroiditis. COVID-19 is associated with some autoimmune diseases other than thyroid disorder, which have been

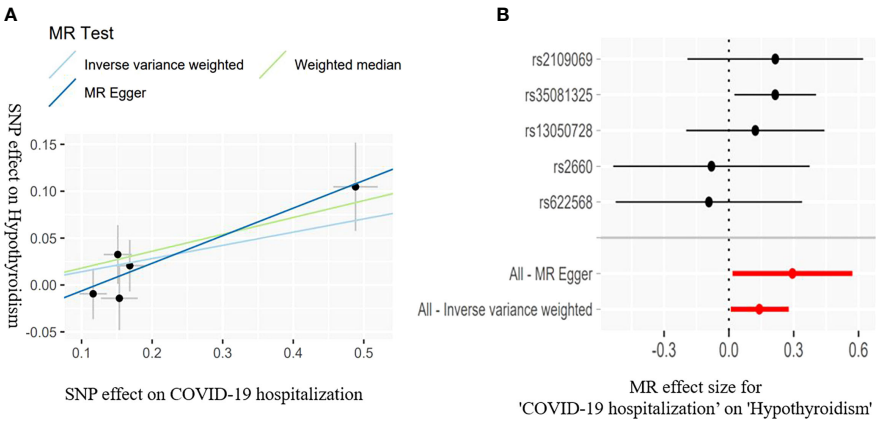


FIGURE 3
Scatter plots **(A)** for estimating causal effects of genetically predicted susceptibility on risk of hypothyroidism. Each black point representing the effect sizes of each SNP on the exposure (horizontal axis) and on the outcome (vertical axis) is plotted with error bars corresponding to each standard error (SE). The slope of each line corresponds to the combined estimate using each method of the IVW (light blue line), the MR-Egger regression (blue line), and the weighted median (light green line). Forest plots **(B)** of susceptibility on the risk of hypothyroidism; the red points showed the combined causal estimate using all SNPs together in a single instrument, using two different methods (MR-Egger and IVW).

described in many studies, such as Guillain–Barre syndrome, autoimmune hemolytic anemia, and systemic lupus erythematosus (39). Antibodies against SARS-CoV-2 can react with various organs, including the thyroid gland, which can cause damage (40). ACE-2, the receptor through which SARS-COV-2 infects the host cells, is expressed by various tissues, including the thyroid. Therefore, the SARS-COV-2 virus will be present not only in the lung tissue but also in the thyroid cells. Hence, the patients may develop subacute thyroiditis following SARS-CoV-2 infection (41). Our MR study was limited by the original GWAS datasets, which were unable to distinguish between these different types of thyroid dysfunction, which may be the main source of heterogeneity. However, combining the results of this study with previous observational studies, we deem that COVID-19 susceptibility and its severity on the higher risk of any type of hypothyroidism.

At present, WHO has already made elderly, pregnant women, and people with heart failure, uncontrolled diabetes, and cancer-related complications as susceptible people of COVID-19. However, such general guidelines do not provide information about the COVID-19 risk of patients with thyroid problems. Most (42–46) epidemiological studies deem that thyroid dysfunction did not have higher rates of COVID-19 susceptibility and its severity, which is consistent with our conclusion. In an epidemiological study in Europe, patients with hyperthyroidism and hypothyroidism did not have an increased risk of COVID-19 infection (45). Two retrospective studies in the US (10) and Iran (46) show that patients with COVID-19 with hypothyroidism will not increase the rates of hospitalization or mortality. There may be multiple impacts of thyroid dysfunction on the risk of COVID-19 susceptibility and its severity due to mixed confounding factors such as an antithyroid drug. Boelaert et al. (47) reported that antithyroid drug-induced neutropenia, but not thyroid disorder status, may promote the progression of COVID-19 and its symptoms (47). We think that whether thyroid dysfunction increases the risk of COVID-19 requires further study, which can provide guidance on the management of thyroid disorder during the COVID-19 pandemic. We note that a recent MR study on COVID-19 and thyroid function showed that patients with COVID-19 susceptibility but not severe were more likely to develop hypothyroidism (48). We point out that the reason our study also found a potential correlation between patients with severe COVID-19 and hypothyroidism is that we included a larger number of patients with COVID-19 GWAS compared to study by Li et al. In addition, our inclusion criteria for genetic instruments were more stringent, as we excluded any genetic instruments that could be associated with confounding factors, whereas the study by Li et al. did not, thus giving our study more credibility.

Our study used the MR method by performing genetic instruments to examine the causal relationship between the characteristics of thyroid dysfunction and COVID-19 risk, and MR analysis can eliminate confounding factors as much as possible. In addition, a large size of independent datasets was used to obtain genetic instruments, which can mitigate the risk of bias in our results due to a few cases. However, there are some limitations to our study. This study included heterogenous in datasets, and the original GWAS datasets are unable to distinguish between these different types of thyroid dysfunction. However, combined with previous epidemiological studies, we point out that COVID-19 is associated with various types of hypothyroidism; thus, further research is required. As a two-sample MR analysis requires that both samples come from the same population, our study includes only participants of European ancestry; therefore, the generalization of these results to other populations requires further study.

## Conclusion

Our results identified that COVID-19 susceptibility and its severity might increase the risk of hypothyroidism. Therefore, patients infected with SARS-CoV-2 should strengthen the monitoring of thyroid function. However, the effect of thyroid dysfunction on COVID-19 susceptibility and its severity was not found in this MR study.

## 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

This study only used published or publicly available data. Ethical approval for each study included in the investigation can be found in the original publications (including informed consent from each participant).

## Author contributions

ZZ and TF designed the study. ZZ and TF conducted data analysis. ZZ conceived the project and wrote the manuscript. YL revised and approved the paper. 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



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.961717/full#supplementary-material

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# Effect of COVID-19 pandemic on diagnosis and treatment of thyroid cancer in Brazil

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**Introduction:** The COVID-19 pandemic delayed the diagnosis, treatment, and follow-up visits of patients with thyroid cancer. However, the magnitude with which these restrictions affected the Brazilian health care is still unknown.

**Methods:** Retrospective analysis of thyroid cancer-related procedures performed in the Brazilian public health system from 2019 to 2021. Data were retrieved from the Department of Informatics of the Unified Health System (DATASUS). The following procedures were evaluated: fine-needle aspiration biopsies (FNABs), oncologic thyroidectomies, and radioiodine (RAI) therapies for thyroid cancer. The year of 2019 served as baseline control.

**Results:** Compared with 2019, FNABs, oncologic thyroidectomies, and RAI therapies performed in 2020 decreased by 29%, 17% and 28%, respectively. In 2021, compared with 2019, FNABs increased by 2%, and oncologic thyroidectomies and RAI therapies decreased by 5% and 25%, respectively. Most pronounced reductions were observed in the first months of the pandemic. In April 2020, FNABs decreased by 67%, oncologic thyroidectomies by 45%, and RAI therapies by 75%. In 2021, RAI therapies were the only procedure with a statistically significant decrease.

**Conclusion:** The restrictions to public health care during the COVID-19 pandemic resulted in a significant reduction in diagnostic and treatment procedures for thyroid cancer in Brazil. The effects of these transitory gaps in thyroid cancer care, due to COVID-19, are still unclear.

KEYWORDS

**thyroid carcinoma, COVID-19, fine-needle aspiration (FNA) biopsy, thyroidectomies, radioiodine (131I) treatment**

# Introduction

In late March 2020, community transmission of COVID-19 was identified within Brazil, and actions were taken to reduce exposure (1). Healthcare providers temporarily postponed cancer screenings, in-person consultations were shifted to telemedicine, and surgeries and other in-office procedures were delayed. In addition to restrictions and sparing of resources to fight an unprecedented health crisis, oncologic patients were oriented to keep distance from hospitals due to a greater risk of SARS-CoV-2 infection complications and death (2).

Most thyroid cancers are considered low risk and have an excellent prognosis (3). Hence, during the COVID-19 pandemic, medical societies released statements suggesting that fine-needle aspiration biopsies (FNABs), thyroid cancer surgeries, and radioiodine (RAI) therapies could be safely postponed without changes to individual prognosis for most cases (4, 5). Restrictions for these services were observed worldwide (6, 7). In Italy, data from 28 surgical units showed that the number of oncologic thyroidectomies decreased by 27.1% during the first wave of the COVID-19 pandemic (from March 2020 to August 2020) (8).

The Brazilian health system, a mix of public-private services, has three parts: the public (SUS), the private, and the private health insurance subsectors. Most of the Brazilian health services are provided by the public subsector, in which services are financed by the government (9). Indeed, data from 2019 show that more than 70% of Brazilians (approximately 150 million people) rely exclusively on the public subsector (10).

Since the peak of COVID-19, cases and hospitalizations have decreased, and it is time to look back and evaluate the effect those restrictions had on the diagnosis and treatment of thyroid cancer. We performed a retrospective analysis of thyroid cancer-related procedures, pre- and post-pandemic. The procedures analyzed were: FNABs, oncologic thyroidectomies, and RAI therapies.

# Methods

A retrospective analysis of thyroid cancer-related procedures was performed in the Brazilian public health system from 2019 to 2021. Data were retrieved from the Department of Informatics of the Unified Health System (DATASUS). The FNABs, oncologic thyroidectomies, and RAI therapies for thyroid cancer were analyzed. Data were collected following standardized procedure codes used in DATASUS (Supplemental Table 1). April 2020 was considered as the onset of the COVID-19 pandemic in Brazilian healthcare, and 2019 as the baseline control year. All comparisons included the period of January to December of each year.

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) version 18 (IBM Corp., Armonk, NY). Data for continuous variables were expressed as mean ± standard deviation of the mean. Welch's ANOVA was used with Games-Howell *post-hoc* test. Results were considered statistically significant if P-value < 0.05.

# Results

## Fine-needle aspiration biopsies

In 2019, 59,411 FNABs were performed in the Brazilian public health system. The number of FNABs decreased to 42,087 (29% decrease) in 2020 and increased to 60,583 (2% increase) in 2021 (Figure 1). Comparing FNABs performed in 2020 and 2021 with the monthly average of FNABs performed in 2019, April 2020 represented the most pronounced decline (67% decrease). FNABs persisted below the monthly average of 2019 from April 2020 to May 2021 (Figure 2A). Table 1 shows a summary of the data and statistical analysis. Figure 3A shows the *post-hoc* pairwise comparisons.

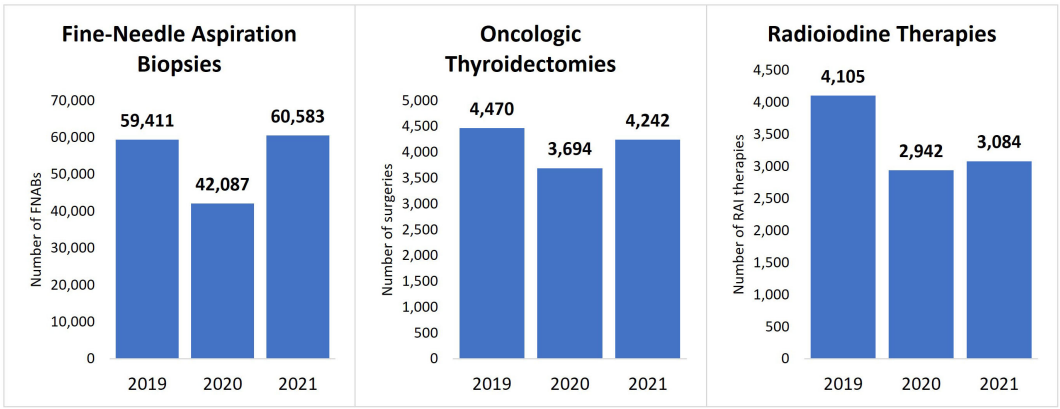


FIGURE 1
Number of procedures performed by year in Brazil's public health system. FNABs, fine-needle aspiration biopsies; RAI, radioiodine therapy.

## Oncologic thyroidectomies

In 2019, 4,470 oncologic thyroidectomies were performed in the Brazilian public health system. The number of oncologic thyroidectomies decreased to 3,694 (17% decrease) in 2020 and to 4,242 (5% decrease) in 2021 (Figure 1). The greatest reductions occurred from April to July 2020, with new significant drops in December 2020 and April 2021 (Figure 2B). Table 1 shows a summary of the data and statistical analysis. Figure 3B shows the *post-hoc* pairwise comparisons.

## Radioiodine therapy

In 2019, 4,105 RAI therapies were performed in the Brazilian public health system. The number of RAI therapies decreased to

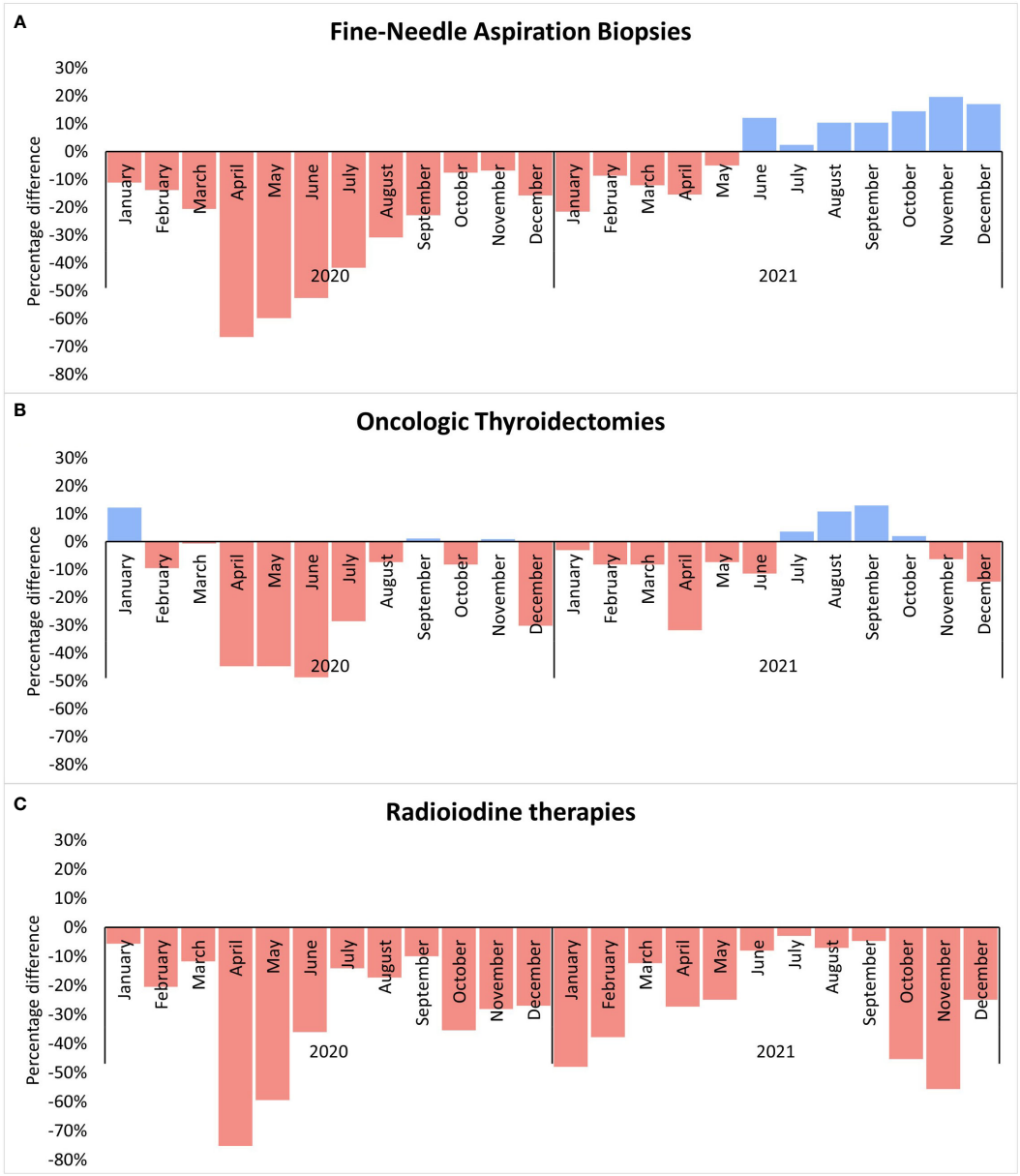


FIGURE 2
The percentage difference for each month of 2020 and 2021 in comparison with the 2019 monthly average of each procedure. **(A)** Fine-Needle Aspiration Biopsies; **(B)** Oncologic Thyroidetomies; **(C)** Radioiodine therapies.

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TABLE 1 Number of fine-needle aspiration biopsies, oncologic thyroidectomies, and radioiodine therapies in 2019, 2020, and 2021.

| | 2019 | 2020 | 2021 |
|---|---|---|---|
| **FNABs (n)** | 59,411 | 42,087 | 60,583 |
| **Mean FNABs per month*** | 4,951 ± 520 | 3,507 ± 1,040 | 5,049 ± 694 |
| **Oncologic thyroidectomies (n)** | 4,470 | 3,694 | 4,242 |
| **Mean thyroidectomies per month*** | 373 ± 39 | 308 ± 78 | 354 ± 45 |
| **RAI (n)** | 4,105 | 2,942 | 3,084 |
| **Mean RAI per month*** | 342 ± 46 | 245 ± 71 | 257 ± 63 |

Comparison between the monthly average of procedures of 2019, 2020, and 2021. Welch's ANOVA test was used. *Statistically significant. FNABs: p<0.001; Oncologic Thyroidectomies: p = 0.025; RAI: p = 0.001.
FNABs, fine-needle aspiration biopsies; RAI, radioiodine therapy.

2,942 (28% decrease) in 2020 and to 3,084 (25% decrease) in 2021 (Figure 1). Since January 2020, all months had fewer RAI therapies than the monthly average of 2019. April 2020 presented the greatest reduction (Figure 2C). Table 1 shows a summary of the data and statistical analysis. Figure 3C shows the *post-hoc* pairwise comparisons.

## Discussion

Our study aimed to evaluate the effect of COVID-19 on thyroid cancer care in Brazil. The number of FNABs, oncologic thyroidectomies, and RAI therapies largely decreased over the first few months of the pandemic. Despite following an

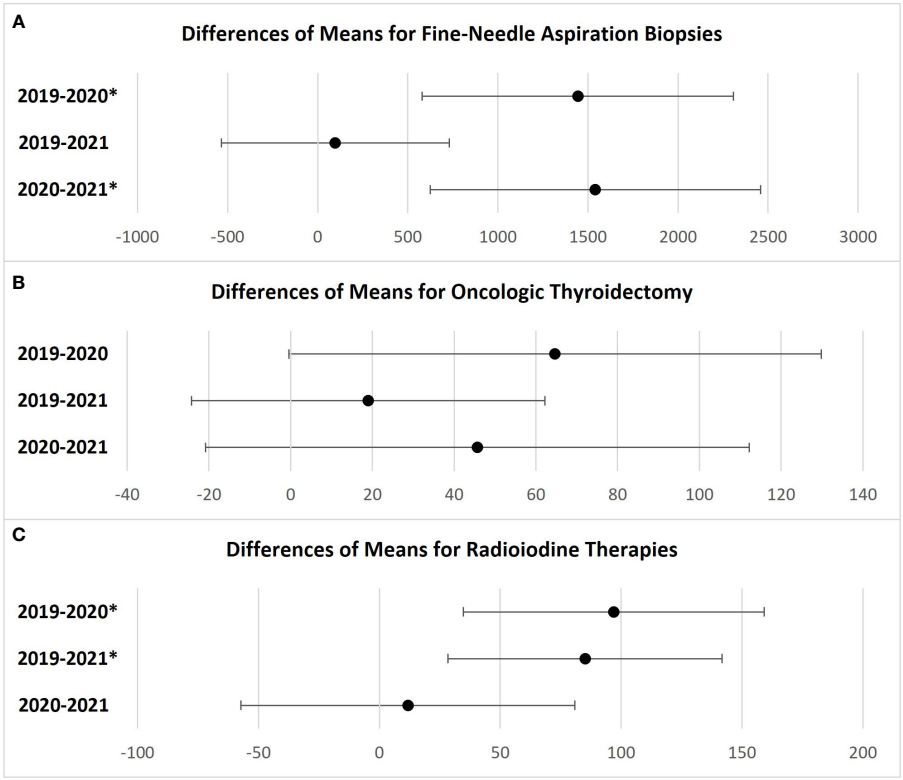


FIGURE 3
Games-Howell post-hoc test. Simultaneous 95% confidence intervals of differences between means of each procedure. **(A)** Fine-Needle Aspiration Biopsies; **(B)** Oncologic Thyroidetomies; **(C)** Radioiodine therapies. *Statistically significant. If an interval does not contain zero, the corresponding means are significantly different. Differences are expressed as absolute values.

increasing trend thereafter, oncologic thyroidectomies and RAI procedures have not returned to pre-pandemic levels.

In the context of diagnosis, experts in the area recommended that FNABs be deferred for most asymptomatic thyroid nodules (5). In Brazil, 17,324 (−29%) fewer FNABs were performed in 2020 in comparison to 2019. Considering that 6% of all FNABs are classified as Bethesda V or VI (11), the number may imply that more than 1,000 malignant or suspicious for malignancy lesions were not diagnosed.

In 2021, the number of FNABs was similar to pre-pandemic levels, despite still having new reductions from January to May 2021, as seen in Figure 2A. However, a consistent increase in FNABs procedures from June to December 2021 is observed, and it compensated for the prior reductions. Possibly, FNABs had a faster recovery because they are not dependent on hospital beds.

On the other hand, we should consider that, to some extent, nodules with relevant clinical and ultrasound features must have been prioritized during periods of service restrictions. In Brazil, the number of oncologic thyroidectomies decreased by 17% and 5% in 2020 and 2021, respectively, when compared to 2019. These results did not achieve statistical significance when we compared the monthly average of oncologic thyroidectomies (Figure 3B). The smaller reductions observed in oncologic thyroidectomies, compared with FNABs and RAI therapies, could be attributed to efforts to prioritize patients in more need of care.

Brazilian data shows that almost one-third of RAI therapies for thyroid cancer were postponed in 2020. The greatest reductions were in the first two months of service restrictions: a 75% reduction in April and a 59% reduction in May 2020. Different from FNABs and oncologic thyroidectomies, the number of RAI therapies had a significant reduction in 2021 as well. There was a 25% drop for RAI therapy in 2021 when compared with 2019, a percentage close to what was observed in 2020. We hypothesize that this discrepancy is due to disruptions observed in the national RAI supply chain in 2021 (12). Moreover, previous studies showed that there is a great disparity in the availability of RAI therapies between Brazilian states (13). Therefore, it is possible that the COVID-19 outbreak, and supply chain disruptions decreased the availability of this resource in states which were already underserved.

Efforts to reestablish thyroid cancer care to pre-pandemic levels should not result in low-value care. Thyroid cancer overdiagnosis due to the widespread use of diagnostic procedures has also been present in developing countries, including Brazil (14). In the context of current thyroid cancer epidemiology being composed mostly of low-risk tumors, some authors suggest that the effect the COVID-19 pandemic had on thyroid cancer management may serve as an opportunity to implement more conservative treatment options on a large scale, such as active surveillance (15). Our study quantified numbers of thyroid cancer-related procedures showing that COVID-19 disrupted thyroid cancer management patterns in a high magnitude. Nevertheless, little is known about the consequences due to the unprecedented nature of this pandemic. Thus, in addition to strategies to manage a growing amount of patients, discouragement of thyroid exams/procedures related to low-value care is timely (16, 17).

Additionally, thyroid cancer patients have suffered from more pronounced emotional and psychological distress when compared to the general population since the COVID-19 outbreak, as demonstrated in surveys conducted in China (18) and Italy (19). In this matter, appropriate psychological support is also necessary.

This study has limitations. The DATASUS database is composed of aggregated ecological data. Thus, since DATASUS was not designed as a cancer registry, it is prone to bias and does not include information about patients' clinical and oncological features. Notwithstanding, considering that the DATASUS system is based on billing information, it is audited by competent authorities, which contributes to a minimal curation of the data. Additionally, our study encompasses data retrieved from the public health system, with no records from the private subsector or the private health insurance subsector. The SUS, however, is the world's largest public health system, providing health care coverage for a population of 210 million people. This study aimed to measure the effect of the COVID-19 pandemic on thyroid cancer procedures in a developing country at a national level.

This study shows that the restrictions during the COVID-19 pandemic resulted in a significant reduction in diagnostic and treatment procedures for thyroid cancer within Brazil's public health system. The effects of these transitory gaps in thyroid cancer care due to COVID-19 are still unclear.

## 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

VS, WS, GH, RS, and JD contributed to the study conception and design, data analysis and interpretation, and manuscript preparation. AZ and AM contributed to data analysis and interpretation, and manuscript preparation. 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.

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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.2022.995329/full#supplementary-material

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# COVID-19 and thyroid function: What do we know so far?

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Coronavirus disease 2019 (COVID-19) was characterized as a pandemic in March, 2020 by the World Health Organization. COVID-19 is a respiratory syndrome that can progress to acute respiratory distress syndrome, multiorgan dysfunction, and eventually death. Despite being considered a respiratory disease, it is known that other organs and systems can be affected in COVID-19, including the thyroid gland. Thyroid gland, as well as hypothalamus and pituitary, which regulate the functioning of most endocrine glands, express angiotensin-converting enzyme 2 (ACE2), the main protein that functions as a receptor to which SARS-CoV-2 binds to enter host cells. In addition, thyroid gland is extremely sensitive to changes in body homeostasis and metabolism. Immune system cells are targets for thyroid hormones and T3 and T4 modulate specific immune responses, including cell-mediated immunity, natural killer cell activity, the antiviral action of interferon (IFN) and proliferation of T- and B-lymphocytes. However, studies show that patients with controlled hypothyroidism and hyperthyroidism do not have a higher prevalence of COVID-19, nor do they have a worse prognosis when infected with the virus. On the other hand, retrospective observational studies, prospective studies, and case reports published in the last two years reported abnormal thyroid function related to acute SARS-CoV-2 infection or even several weeks after its resolution. Indeed, a variety of thyroid disorders have been documented in COVID-19 patients, including non-thyroidal illness syndrome (NTIS), subacute thyroiditis and thyrotoxicosis. In addition, thyroid disease has already been reported as a consequence of the administration of vaccines against SARS-CoV-2. Overall, the data revealed that abnormal thyroid function may occur during and in the convalescence post-COVID condition phase. Although the cellular and molecular mechanisms are not completely understood, the evidence suggests that the "cytokine storm" is an important mediator in this context. Thus, future studies are needed to better investigate the pathophysiology of thyroid dysfunction induced by COVID-19 at both molecular and clinical levels.

KEYWORDS

**COVID-19, SARS-CoV-2, subacute thyroiditis, non-thyroidal illness syndrome, NTIS, hypothyroidism, hyperthyroidism**

# 1 Introduction

In December, 2019, a pneumonia of unknown origin emerged in Wuhan, China. On January, the virus responsible for the pneumonia was identified as a new coronavirus, later named severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, and the disease was named coronavirus disease 2019 (COVID-19) (1). The genetic material of the virus was rapidly sequenced (2), and the transmission could not be prevented. The viruses rapidly spread across China and then all over the world, so on March 11$^{th}$ 2020, World Health Organization (WHO) declared that COVID-19 reached pandemic levels. Since then, life on earth was totally affected by the pandemic, with changes in the way humans interact with each other, millions of deaths, ills and a great economic impact. On August 20$^{th}$, 2022, according to WHO, the number of confirmed cases was higher than 595 million and confirmed deaths surpassed 6.4 million.

Most coronavirus strains that infect humans cause a mild respiratory disease; however, SARS-CoV-2 causes a serious illness, which can lead to severe respiratory syndrome and eventually to death. The most frequent symptoms include fever, dyspnea, sore throat, anosmia, dysgeusia, fatigue, and can progress to pneumonia, acute respiratory distress syndrome, and multiorgan dysfunction. SARS-CoV-2 genetic material is a positive-sense single-stranded RNA and the virus consists of a spherical particle, enveloped, with a diameter of approximately 120 nm (3). The origin of SARS-CoV-2 is controversial and the Huanan seafood wholesale market in Wuhan was suggested to be the place where the virus jumped to humans, with bat (*Rhinolophus affinis*) and pangolin (*Manis javanica*) being most probably the natural and intermediate hosts, respectively (4).

**Abbreviations:** ACE2, angiotensin-converting enzyme 2; ACTH, adrenocorticotrophic hormone; AUC, area under the curve; COVID-19, Coronavirus disease 19; CRP, C-reactive protein; D1, iodothyronine deiodinase 1; D2, iodothyronine deiodinase 2; D3, iodothyronine deiodinase 3; ESR, erythrocyte sedimentation rate; GH, growth hormone; HPT, hypothalamus-pituitary-thyroid axis; ICU, Intensive Care Unit; IFN, Interferon; IL, Interleukin; IG, immunoglobulins; NO, nitric oxide; NOS, nitric oxide synthase; NTIS, non-thyroidal illness syndrome; PVN, paraventricular nucleus; OR, Odds ratio; RAS, renin-angiotensin system; ROC, receiver operating characteristic; ROS, reactive oxygen species; RT-PCR, real-time reverse-transcriptase-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SAT, subacute thyroiditis; T3, triiodothyronine; T4, thyroxine; TBG, thyroxine-binding protein; TG, thyroglobulin; THs, thyroid hormones; TNF-α, tumor necrosis factor-alpha; TPO, thyroperoxidase; TRH, thyroid-releasing hormone; TSH, thyroid-stimulating hormone or thyrotropin; WHO, World Health Organization.

# 2 COVID-19 and Thyroid gland: General concepts

Despite being initially described as a respiratory disease, in the course of time it was observed that other organs and systems could be affected by COVID-19, such as cardiovascular system (5), central nervous system (6), kidney (7), liver (8), among others. Endocrine system is also affected by COVID-19, including pancreas, adrenal, testicle, reproductive tract, parathyroid gland and the thyroid gland (9–14) (Figure 1). Moreover, endocrine-metabolic disturbances, such as diabetes mellitus and obesity, are highly associated to severe illness (15, 16).

It is not surprising that the endocrine system can be affected by SARS-CoV-2 since both hypothalamus and pituitary, which regulate the functioning of most endocrine glands, express angiotensin-converting enzyme 2 (ACE2), the main protein to which SARS-CoV-2 binds to enter host cells (17, 18). ACE2 is a transmembrane protein with carboxypeptidase activity, which cleaves angiotensin I to angiotensin 1-9 and angiotensin II to angiotensin 1-7. The viral envelope contains a spike glycoprotein, which interacts with ACE2 with high specificity and affinity, which contributes to the high transmissibility and infectivity of SARS-CoV-2. Then, virus particle enters the cell by endocytosis or by fusion of the viral envelope with the cell membrane. The spike protein is not cleaved, thus the enzymatic activity of ACE2 is not relevant for the virus entry in host cell (19).

Besides hypothalamus and pituitary, thyroid gland also express ACE2 and may be directly affected by COVID-19 (20, 21). Thyroid gland is responsible for the production of thyroid hormones: the prohormone tetraiodothyronine ($T_4$) and the active hormone triiodothyronine ($T_3$). In fact, around 90% of the circulating $T_3$ in humans is produced by the peripheral conversion of $T_4$ to $T_3$, by enzymes called deiodinases. $T_3$ and some of its metabolites are the main regulators of basal metabolic rate, with effects on central nervous system, cardiovascular system, respiratory system, skeletal muscles, among others.

Thyroid hormones regulate to some extent the immune system (22). It has already been shown that one of the targets of thyroid hormones are immune system cells and that THs modulate specific immune responses, including cell-mediated immunity, natural killer cell activity, the antiviral action of interferon (IFN) and proliferation of T- and B-lymphocytes (22–24). In healthy subjects, there is a positive correlation between serum thyroid hormone levels and inflammatory markers, monocyte-activated IL-6 expression, the percentage of memory T cells, the quantity of natural killer T cells and the quantities of $CD3^+/CD4^+/CD45RO^+$ memory T helper cells. On the other hand, serum thyroid hormones are negatively correlated with lymphocyte death and to the ratio of naïve:

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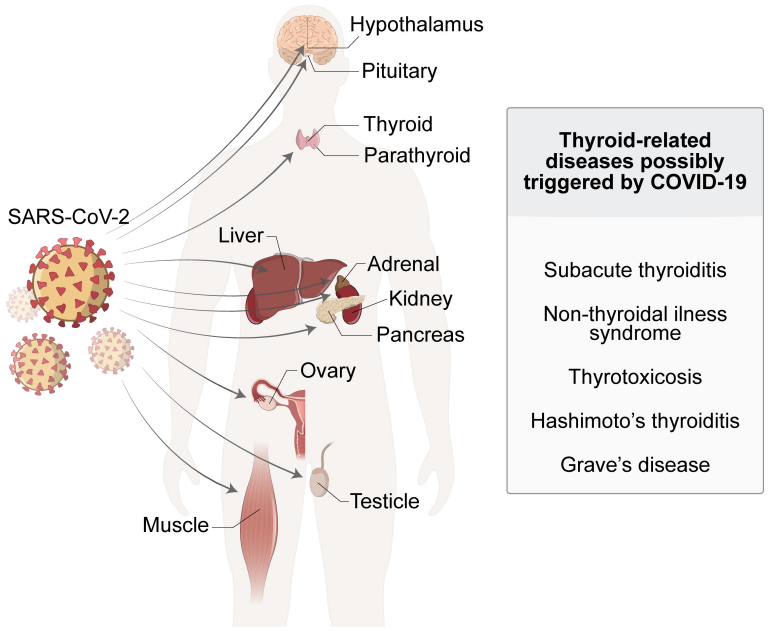


FIGURE 1
SARS-Cov-2 infection affects and damages a wide-ranging of human organs and systems. The manifestation of endocrine disorders, including thyroid-related pathologies, have been diagnosed in COVID-19 patients, suggesting a possible causal relationship between these conditions.

cytotoxic $CD3^+/CD8^+/CD45RO^+$ memory T cells (25). These data suggest that THs stimulate the immune system to strongly react to infection.

Even though it has already been shown that hypothyroidism and hyperthyroidism have opposite effects on some parameters of the immune response, there is no evidence that patients with poorly controlled thyroid disorders are more susceptible to contract viral infections. However, considering the role of thyroid hormones on the immune system, it is plausible that patients with uncontrolled thyroid dysfunction may be at higher risk of complications due to these infections (26). Contrasting results have been reported for other immune functions, and so it is difficult to establish a clear correlation between immune function and hyper- or hypothyroid conditions (22). Overall, hypothyroidism tends to impair the activation of the immune system while hyperthyroidism results in the activation of the immune response (27).

Therefore, thyroid function could have an impact in the prognosis of COVID-19 and conversely COVID-19 could have an impact on thyroid function. In fact, abnormal thyroid function has been reported during SARS-CoV-2 infection or even several weeks after its resolution. Indeed, a variety of thyroid disorders have been documented in COVID-19 patients including non-thyroidal illness syndrome (NTIS), subacute thyroiditis (SAT), thyrotoxicosis and hypothyroidism in retrospective observational studies and case-reports (Figure 1). Although a cause-effect association between the infection and the onset of thyroid dysfunction has not yet been demonstrated from a mechanistic point-of-view, these reports raise the concern about whether thyroid function should or not receive a special attention in COVID-19 patients. Herein, we review important aspects of the relationship between COVID-19 and thyroid, including the interaction of thyroid hormones with immune system, the effect of infectious agents on the incidence of thyroid disorders and recent data regarding the relationship between COVID-19 and thyroid.

# 3 Pre-existing thyroid dysfunction and COVID-19

## 3.1 Hypothyroidism

Hypothyroidism is the insufficient production of thyroid hormones. This disease can be congenital, due to mutations in proteins that are essential for thyroid hormones synthesis pathway and defects in thyroid gland formation, or even the absence of the gland. Hypothyroidism can also be acquired, due to iodide deficiency, tumors or infections in the thyroid gland or in the pituitary, and autoimmunity. Hashimoto's thyroiditis is an idiopathic thyroid atrophy due to a chronic autoimmune inflammatory reaction, being the most common form of hypothyroidism in humans (28). Hashimoto's autoimmune

thyroid disease is characterized by the production of autoantibodies against thyroglobulin (Tg-Ab) and thyroperoxidase (Tpo-Ab) that are essential for hormonal synthesis. Hypothyroid patients have lower metabolic rate, decreased thermogenesis, bradycardia, lethargy and drowsiness (29).

Animal models have shown that the experimental induction of hypothyroidism leads to an involution of the spleen and lymph nodes as well as a decrease in the humoral and cell-mediated immune response (30, 31). Hypothyroidism induced by chronic restraint stress seems to be related to the reduction of T-cell lymphoproliferative response, since T4 replacement reversed it. Besides, in these chronic stress mice bearing tumors, T4 reversed the alteration of lymphoma growth, interleukin-2 production and specific cytotoxic response against tumor cells (32). Furthermore, B-lymphocytes can also be regulated by thyroid hormones. In mouse strains deficient in the production of anterior pituitary-derived hormones, and consequently secondary hypothyroid, the frequency and absolute number of pro-B- and B-lymphocytes are lower, showing that THs can regulate the proliferative potential of T- and B-lymphocytes (33–35). Clinically, patients with severe hypothyroidism due to autoimmune thyroiditis experience a dramatic decrease in lymphocyte function, which is restored when T4 is normalized by exogenous hormone administration (36).

Considering that hypothyroidism leads to immune system dysfunctions, and that ACE2 is expressed in thyroid gland, one could speculate that hypothyroidism might impact the outcomes in COVID-19 patients. A retrospective study conducted in the New York City health system evaluated a cohort of 3703 COVID-19 patients, of which 251 patients (6.8%) had pre-existing hypothyroidism. The authors found that hypothyroidism was not associated with increased risk of hospitalization or an increased risk of mechanical ventilation or death (37). Other studies have also shown that the prevalence of hypothyroidism appears similar in COVID-19 patients compared to the general population, which indicates that hypothyroidism does not increase the chance of COVID-19 infection, and also that hypothyroidism is not associated with a greater COVID-19 death risk (38, 39). Despite this, previous studies show that, although well-managed hypothyroidism is not associated with increased infection risk, poorly controlled hypothyroidism may increase the susceptibility to infections (36, 40). Therefore, it is important that patients with thyroid disorders maintain their treatment during the COVID-19 pandemic.

## 3.2 Hyperthyroidism

Hyperthyroidism is characterized by higher levels of circulating thyroid hormone. It is mostly an acquired condition, which is most frequently caused by Graves' disease, toxic multinodular goiter or toxic adenoma (41). Graves' disease is an autoimmune disorder, in which thyroid-stimulating antibodies activate the thyroid-stimulating hormone (TSH) receptors, triggering increased thyroid hormone synthesis. The clinical condition observed in Graves' disease is thyrotoxicosis, resulting from excessive amounts of thyroid hormones in the tissues and blood. In those patients, whole-body metabolism is activated leading to body weight loss, sweating, heat intolerance, increased heart rate, overactive bowel movement, tremor, nervousness, and exophthalmos (42).

Hyperthyroidism is associated with unbalanced immune responses, including abnormal antibody production (either increased or decreased) (43), increased migration of polymorphonuclear leukocytes (44), increased lymphocyte proliferation (45) and increased macrophages reactive oxygen species (ROS) production (27, 46). Compared to healthy controls, hyperthyroid patients present higher levels of serum immunoglobulins M and G (IgM, IgG) and higher levels of p65 and p-IκBα in B-lymphocytes, which are indicators of NF-κB activation. In addition, these patients have higher serum oxidative stress levels (47). These results suggest that hyperthyroidism increases ROS production activating the NF-κB pathway that, in turn, enhances the production of Ig's by B-lymphocyte.

Due to this hyper-responsiveness of the immune system during hyperthyroidism, it is plausible that uncontrolled hyperthyroid patients, especially with thyrotoxicosis, may be at higher risk of complications from any infection (42). On the other hand, angiotensin converting enzyme activity and the counter-regulatory components of the RAS (renin-angiotensin system) is increased in patients with hyperthyroidism (48, 49). A study conducted in China shows that COVID-19 patients with thyroid disease had a significantly higher fatality rate (20% vs 0%) and they were more likely to stay in the hospital for more than 28 days than were those without thyroid disease (80% vs 56.52%) (50). However, the authors defined thyroid disease as an abnormal thyroid function test result, and included patients with overt thyrotoxicosis, overt hypothyroidism, subclinical hypothyroidism, subclinical hyperthyroidism, and euthyroid sick syndrome. Therefore, it is not possible to estimate the contribution of each thyroid disease to a worse prognosis of COVID-19.

In general, previously published data show that patients with controlled hyperthyroidism are not considered to be at higher risk of contracting COVID-19 (26, 51, 52), but there are two exceptions. It is known that patients taking antithyroid drugs present higher risk of developing neutropenia or agranulocytosis, which occurs in 0.2-0.5% of patients taking these medications (42, 53). Neutropenia is associated with increased risk of infections. Therefore, patients with neutropenia caused by the administration of antithyroid drugs may be more prone to complications during COVID-19 infection due to reduced immune response. Likewise, patients with Graves' ophthalmopathy who are undergoing immunosuppressive agents, like glucocorticoids, may also be considered to be more vulnerable to COVID-19 infection (26,

51). Additionally, since thyroid hormones regulate vascular tonus and multi-organ dysfunction is associated to hypoxia, T3 treatment has been suggested to be potentially useful in the treatment of severe COVID-19 (54).

# 4 Thyroid dysfunction during COVID-19

## 4.1 Non-thyroidal illness syndrome (NTIS) and COVID-19

The first reports of abnormal serum thyroid hormone concentrations after severe illness or starvation in patients with no history of thyroid disease has been made nearly 60 years ago (55, 56). In mild-to-moderate illness, the most typical laboratory finding is a reduction in serum $T_3$ and, remarkably, no concomitant increase in TSH. Accordingly, this condition has been named "low $T_3$ syndrome", "euthyroid sick syndrome" or "non-thyroidal illness syndrome". Elevated reverse $T_3$ ($rT_3$) has long been considered another hallmark of NTIS, but now some authors argue that its levels may be normal or even reduced in some patients with NTIS (57). Reduced levels of rT3 have already been observed in AIDS patients that present NTIS, and this phenomenon has also been observed in COVID-19 and NTIS patients (58–60). Additionally, a decline in $T_4$ and TSH may also be observed in critically ill patients. Thus, NTIS is a complex condition with no unique phenotype, which greatly depends on disease severity. Pro-inflammatory cytokines, like interleukin-6 and IL-1β, are recognized as major players in the pathogenesis of NTIS since the 90's, when an inverse correlation between serum $T_3$ and IL-6 in hospitalized patients was first observed (61). Moreover, the chronic treatment of rodents with these cytokines recapitulated several hallmarks of human NTIS, including low $T_3$, $T_4$ and hypothalamic TRH mRNA expression (62, 63).

The entire hypothalamus-pituitary-thyroid (HPT) axis is profoundly affected by severe illness (Figure 2). At the hypothalamic level, TRH decrease is largely mediated by a $T_3$-induced feedback mechanism in illness. The TH transporters MCT10 and OATP1C1 are upregulated in the hypothalamus of prolonged ill rabbits, which may further contribute to the

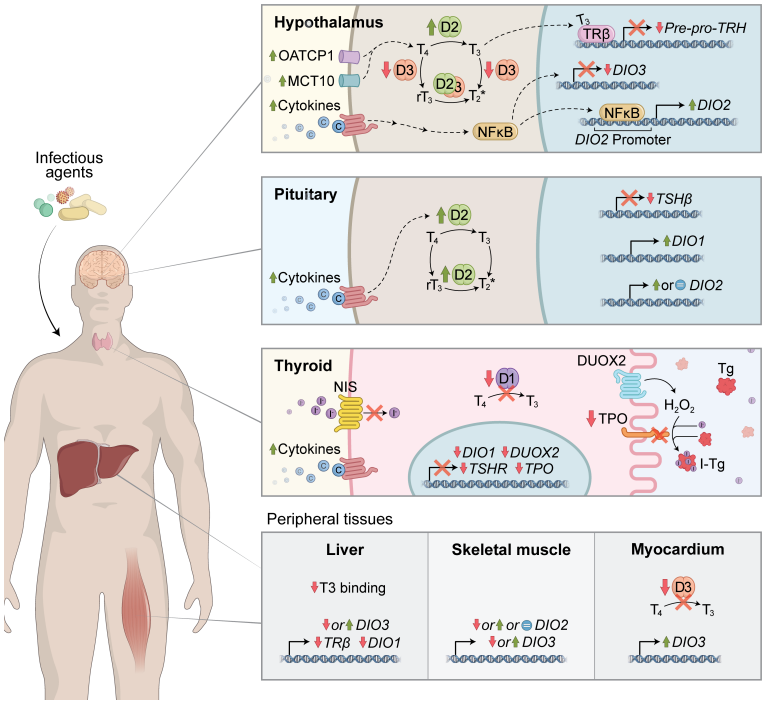


FIGURE 2
Mechanistic insights into COVID-19-induced Non-thyroidal illness syndrome (NTIS). Multiple mechanisms might be involved in the pathogenesis of NTIS. During severe illness, the hypothalamus-pituitary-thyroid (HPT) axis is profoundly affected, which is primarily mediated by pro-inflammatory cytokines. In the hypothalamus, illness promotes TRH downregulation due to abnormal T3-induced negative feedback. Potential mechanisms involved include increased local T3 production by D2 and increased expression in TH transporters (MCT10 and OATP1C1). At the pituitary level, illness impairs TSH response to low levels of circulating $T_3$ and $T_4$, which can be secondary to reduced TRH levels or the direct effect of pro-inflammatory cytokines. Modulation of deiodinase activity might be additionally involved. Cytokines also inhibit multiple steps of TH biosynthesis and D1 activity in the thyroid gland, decreasing hormonal synthesis and secretion. In other peripheral tissues, suppressed D1 and increased D3 expression/activity could contribute to low $T_3$ and high $rT_3$ levels observed in sick individuals.

negative feedback (64). Several subsequent animal studies using different illnesses models revealed a consistent upregulation of iodothyronine deiodinase 2 (D2) both in the PVN and in tanycytes, suggesting an increase in local $T_3$ production, which helped explain the abnormal negative feedback resulting in TRH downregulation besides low levels of serum $T_3$ (65, 66). Deiodinase 3 (D3) is negatively regulated by LPS in the PVN of mice and in cultured neuroblastoma cells, leading to increased intracellular $T_3$ levels in these cells (67, 68). Although logical, the increase in intra-hypothalamic levels of $T_3$ still lacks direct experimental evidence *in vivo*. At the pituitary level, illness leads to an impaired TSH response to low levels of circulating $T_3$ and $T_4$ (64) and lack of pulsatile secretion (69). This disturbed TSH response can be partially attributed to diminished TRH stimulus and to a direct effect of cytokines like IL-1β and TNF-α (70, 71). The contribution of pituitary's D2 and/or D1 activity to these responses is still disputable.

The thyroid gland is also directly influenced by illness (Figure 2). Data from a vast collection of *in vivo* and *in vitro* models report that cytokines (e.g., IL-1α, IL-1β, TNF-α, IFNγ) can inhibit several steps of the TH synthesis machinery, including TSH receptor expression (72), iodide incorporation by NIS (73–75), iodide organification by TPO (76–78), DUOX expression (77), thyroglobulin production (79) and deiodinase 1 expression and activity (80–82). Collectively, these effects lead to a decrease in the synthesis and secretion of TH by the thyroid gland.Yet, most of the circulating $T_3$ is not produced by the gland itself but through deiodination of $T_4$ in peripheral tissues, such as liver, kidney and muscle (83, 84). During illness, alterations in the expression and function of D1 (85, 86), D2 (85, 87–89) and D3 (85, 86) might also contribute to serum TH abnormal levels (Figure 2).

There is still limited data on the literature outlining NTIS during viral infectious diseases. Although the few reports involve different populations and different viruses, they congregate in terms of clinical manifestations. In a cohort of $HIV^+$ and AIDS patients, $fT_3$ levels were lower than in healthy controls while calculated TBG capacity was increased (59). A subsequent study from Nigeria showed that, among 108 HIV-$1^+$ individuals, 52% had abnormalities in thyroid function. Of these, 8.5% had subclinical hypothyroidism and 45.5% had NTIS. The HIV–$1^+$ individuals had significantly lower TSH, $T_3$ and $T_4$ when compared with HIV-$1^-$ controls (90).

Viruses that attack the respiratory tract have been also linked to the development of NTIS. The influenza A virus subtype H7N9 (A/H7N9) is a bird flu strain of the Influenza virus A (avian influenza) that infected humans in China in 2013. Of patients infected with H7N9, 70.6% presented abnormally low total $T_3$ levels, 58.8% had low free $T_3$ and TSH levels and 29.4% had abnormally low total and free $T_4$ levels (below the lower limit of the reference ranges for each hormone) (91).

At the time of this publication, thyroid function tests of more than 2,000 COVID-19 patients have been reported in the literature. Collectively, they make clear that low serum $fT_3$ and NTIS at admission strongly predict poor outcomes in these patients, although the utility of measuring $fT_3$ at admission is still disputable in terms of cost-effectiveness since other biochemical indicators provide similar predictive value. Here we present some of the relevant clinical data regarding NTIS in COVID-19 patients.

Only three months after the beginning of the outbreak the first report of altered thyroid hormones in COVID-19 patients was presented in a retrospective analysis of 274 COVID-19 cases in the region of Wuhan. The authors revealed that serum levels of $fT_3$ and TSH were significantly diminished in deceased patients compared to recovered patients, even though the decrease in TSH was still within the normal range (92). Similar observations were consistently reported in several other papers, although the incidence of decreased levels of $fT_3$ and NTIS varied greatly, probably as a result of the huge discrepancies in the disease severity of the cohort included in each study.

In a study with 50 patients confirmed with moderate to critical COVID-19 with no history of thyroid disease, Chen et al. reported altered thyroid function in more than 60% of patients (93). Low TSH with or without lower-than-normal levels of $tT_3$ were the most frequent alterations found in these patients. Interestingly, TSH and $tT_3$ levels were lower in patients with COVID-19 when compared with healthy patients and non-COVID-19 pneumonia patients, suggesting that these clinical observations could be characteristic of SARS-CoV-2 infection. However, it is important to highlight that in this study thyroid function was evaluated while most of COVID-19 patients were under glucocorticoid treatment, which may affect thyroid function. In another cohort, Zhang and collaborators identified thyroid disorders in 28% of 71 COVID-19 patients, which included mainly NTIS (48%) and subclinical hypothyroidism (28%) (50). In agreement, NTIS was also found in more than 25% of COVID-19 in another Chinese cohort and its occurrence was associated with inflammation and disease severity (94).

A retrospective study published in October of 2020 conducted in Changsha (China) analyzed clinical and laboratory data of 149 patients with mild COVID-19 infection within the first 3 months of the pandemic and detected that 28% had NTIS, characterized by $fT_3$<2.3 pg/mL and low or normal TSH. Compared to non-NTIS patients, NTIS-patients had lower $tT_3$ (0.66 *vs.* 0.96 ng/mL, p<0.0001), $tT_4$ (8.3 *vs.* 9.5 μg/dL, p<0.0001), and non-statistically significant lower TSH (1.36 *vs.* 1.74 μIU/mL, p = 0.06). Also, NTIS patients had a higher ESR, CRP and lymphopenia. NTIS was identified as an independent risk factor for disease severity by Cox-regression model (HR = 2.5 [95%CI 1.05-6.02]) and receiver operating characteristic (ROC) analysis (AUC = 0.81) (94). Another study searched for predictors of mortality in 121 ICU-admitted severe COVID-19 patients and detected that $fT_3$ was the second-best predictor of death (AUC from ROC = 0.86), only after Sequential Organ

Failure Assessment (SOFA) (AUC from ROC = 0.96) (95). Similar results were obtained in a cohort of moderately severe COVID-19 patients in which only 31.5% were admitted to the ICU (AUC from ROC = 0.84) (96).

Likewise, Gao et al. followed 100 COVID-19 patients (66% of then were severely or critically ill) and showed that $fT_3$, TSH and $fT_3/fT_4$ ratio decreased with clinical deterioration and were lower in non-survivors. Moreover, the reduction in $fT_3$ levels was independently associated with all-cause mortality (97). The prospective analysis of 115 COVID-19 patients in Italy, in which 18% had NTIS also observed that low $fT_3$ was associated with mortality and inflammation. The following of these patients thought the hospitalization revealed that, not unexpectedly, the number of patients with decreased levels of $fT_3$ and TSH increased during hospitalization. However, most of them were under corticosteroids therapy, emphasizing the need for cautious interpretation of TSH and TH after ICU admission (98).

A recent prospective analysis of 245 hospitalized patients with moderate or critical (ICU-admitted) COVID-19 patients in Brazil corroborated previous findings that $fT_3$ is negatively associated with survival. Similar to previous findings, only 6.5% of patients had NTIS (characterized as $fT_3$<2.0 pg/mL and low or normal TSH) but this diagnosis significantly increased the risk of death (OR = 7.05). This was the first study to measure $rT_3$ levels in COVID-19 patients and it revealed that they were elevated (>0.35 ng/mL) in 63% of the patients and were significantly higher in critical patients compared to non-critical patients. Interestingly, although $rT_3$ is frequently considered a hallmark of NTIS and NTIS increases the risk of death by COVID-19, serum $rT_3$ levels were not associated to death risk, but the opposite. Patients with simultaneously $fT_3$<2.6 pg/mL and $rT_3$<0.38 ng/mL had a significantly worse clinical outcome (36% mortality rate) than patients with only $fT_3$<2.6 pg/mL (17%), only $rT_3$<0.38 ng/mL (20%), or none of these alterations (5%). Moreover, the authors showed that the value obtained from the mathematical product of $T_3 \times rT_3$ had the strongest predictive value for mortality amongst all analyzed parameters (AUC from ROC = 0.7). Remarkably, the OR for death in patients with $T_3 \times rT_3$<1.29 was 8.08 (60).

These results shed light on a possible underappreciated protective role of $rT_3$ in critically ill patients. Rastogi et al. (2018) evaluated the efficacy of intravenous rT3 administration as a neuroprotective agent in rat model of middle cerebral artery occlusion induced cerebral ischemia-reperfusion and in an *in vitro* model of oxygen glucose deprivation/reoxygenation. The authors demonstrated that the administration of rT3 significantly reduced markers of neuronal injury, oxidative stress [levels of malondialdehyde, glutathione and reactive oxygen species (ROS)], infarct size and neurological deficit after ischemic insult (99). The authors' explanation would be the inhibition of rT3-induced DIO2 synthesis, with a decrease in T3 action at the brain level, reducing O2 consumption and oxidative stress. Interestingly, this finding contrasts with the prognostic value of $rT_3$ in other conditions such as acute myocardial infarction, in which $rT_3$ > 0.27 ng/mL increases the risk of 1-year mortality (HR = 3.0) (100). Of note, a rapid decrease in $rT_3$ has also been observed in animal models of acute illness by turpentine-induced sterile abscess (67) and in critically ill $HIV^+$ patients with or without secondary infections (59).

Consistent data has been obtained about the prognostic value of thyroid hormones abnormalities during COVID-19 illness and their impact on patient outcome. However, no advances have been obtained yet regarding the mechanisms accountable for $fT_3$ reduction. More specifically, the contribution of thyroid hormone production and peripheral tissue TH metabolism (both through deiodination and minor metabolization routes) and also the role of TH metabolites like $rT_3$. Increased $rT_3$ is often observed in critically ill patients and is mostly attributed to decreased liver D1 activity and, with less certainty, to increased liver D3 (85). However, the finding by Beltrão et al. (2021) that decreased levels of $rT_3$ leads to less favorable outcomes in COVID-19 raises the question of whether and how disturbances in TH metabolism in peripheral tissues may impact the course of the disease (60).

Recently, Beltrão et al. (2022) showed a protective role of the polymorphic variant DIO2 (Thr92Ala) heterozygous state in COVID-19 mortality. In his study, heterozygous in-hospital patients were protected by 47-62% of risk mortality. The protective role of Thr92Ala's heterozygous advantage was supported in a meta-analysis of 21 studies on thousands of cases with different diseases, such as ischemic stroke, myocardial infarction, and left ventricular hypertrophy. This protection could be explained by the gene Thr92Ala-DIO2 expression association with endoplasmic reticulum stress, inflammation, oxidative stress, apoptosis, and mitochondrial dysfunction that are mechanisms also related to the pathophysiology of COVID-19 (101).

It must also be highlighted that even mild decreases in $fT_3$ that remain within the normal range can indicate a poor clinical outcome. Schwarz et al. (2021) divided a small cohort of COVID-19 patients into $fT_3$ tertiles and observed that the bottom tertile (that included patients below the normal range and in the lower part of the normal range) had a mortality rate 6-times higher than the two greater tertiles (96). Other studies also proposed $fT_3$ cut-off values above the bottom limit of the normal reference range that efficiently indicated increased risk of death in COVID-19 (60, 95).

Several studies were published about COVID-19 patients and poor outcomes related to thyroid dysfunction during hospitalization. However, it is still unclear if the relation is accurate. To answer this question, we did a systematic review. We only selected studies with a sample of adults and greater than 50 subjects, excluding studies with children and pregnant women. After applying the eligibility criteria, we found 27 studies on the subject, counting 4554 patients. Our research

focused on two main types of studies: (i) retrospective studies (Table 1) and prospective studies (Table 2). Most studies were retrospective (18 studies). The number of patients evaluated in the studies ranged from 50 to 506. Most patients were critical and had several comorbidities (mainly hypertension and DM), and the mortality rate ranged from 0 to 29%. In thyroid function assessment in-hospital, most studies evaluated the levels of TSH, fT4, and fT3 in their patients, while only two studies evaluated rT3 and thyroglobulin. Some patients were diagnosed with NTIS and thyrotoxicosis during hospitalization, and the prevalence ranged from 1.7-66.3% and 0-28%, respectively (Tables 1, 2).

The abnormalities in TH and TSH in COVID-19 seem to be, as for other critical illnesses, transient. Khoo et al. (124) compared the levels of $T_4$ and TSH at admission and after COVID-19 recovery with the patient-matched baseline level assayed in 2019 (i.e., before the pandemic) and confirmed that after recovery serum hormone levels returned to baseline. The lack of knowledge about the precise role of thyroid hormone fluctuations during illness makes it challenging to estimate the potential of a TH replacement therapy in COVID-19. An ongoing randomized placebo-controlled clinical trial (NCT04348513) aims to investigate whether the administration of $T_3$ (liothyronine, 0.8g/kg i.v.) to ICU-admitted COVID-19 patients alleviates their need for cardiorespiratory support (54).

Importantly, most of the above-mentioned studies presented clinical data of patients admitted throughout the first 6 months of 2020, when SARS-CoV-2 variants of concern (VOC) were still not widespread. Whether the clinical course of patients infected by VOC will differ from the course of the disease caused by the original strain is still to be elucidated.

## 4.2 Subacute thyroiditis

Subacute Thyroiditis (SAT), also known as De Quervein's thyroiditis or subacute granulomatous thyroiditis, is a self-limited inflammatory disorder of the thyroid gland that usually disappears in a few months. Patients usually show neck pain and enlarged thyroid and tenderness upon palpation. Symptoms as low fever, fatigue, malaise and myalgia are common (125). The development of subacute thyroiditis has been linked to viral infection or post inflammatory reaction to several different viruses, including mumps virus, measles virus, rubella virus, adenovirus, cytomegalovirus, enterovirus, Coxsackie virus, HIV, influenza virus and dengue fever virus (126). The inflammatory process causes damage and rupture of follicular cells, which leads to the release of $T_3$ and $T_4$ in circulation, inducing thyrotoxicosis symptoms in the first weeks of disease (Figure 3).

A study published in 1967 demonstrated that 45% of patients with subacute thyroiditis presented an increase of at least four times in viral antibodies during their thyroid disease (127). Curiously, clusters of subacute thyroiditis have been reported during outbreaks of viral infection (126) and a higher prevalence of this disease has been reported during the summer, which is the season of the highest incidence of enteroviruses and enterobacteria (128). It is well known that infections can cause direct effects as tissue damage, and also non-infectious consequences, such as malignancies, immunodeficiency syndrome, peptic ulcer and autoimmune diseases. It has been demonstrated that several infectious agents are involved in the development of autoimmune diseases, such as rheumatic fever, lupus erythematosus, insulin-dependent diabetes, among others (129). In this context, thyroid diseases thought to be of infectious etiology *(e.g.* subacute thyroiditis) have been shown to be associated with thyroid autoimmune phenomena. When follicular destruction is extensive, subacute thyroiditis can progress and cause hypothyroidism (Hashimoto's thyroiditis) (Figure 3). In fact, thyroid autoantibodies (anti-thyroglobulin and anti-thyroid peroxidase) have been found in 40-60% of patients with subacute thyroiditis (127, 130). Around 30% of those patients will experience subsequent hypothyroidism before returning to euthyroid state (125). There are also reports of the sequential occurrence of Graves' disease and subacute thyroiditis (131, 132).

Several mechanisms have been proposed for induction of thyroid autoimmunity by viral agents including: (1) viral induction of changes in self antigen expression, or exposure of cryptic epitopes; (2) induction of local inflammation (e.g. by cytokine release), resulting in activation of autoreactive T-cells (bystander mechanism); (3) molecular mimicry between viral antigens and thyroidal antigens; (4) induction of heat shock proteins in the thyroid; and (5) induction of aberrant expression of MHC class II molecules on thyroid cells (129). However, these autoimmune phenomena seem to represent a non-specific and transient response to the inflammatory release of thyroid antigens and classical autoimmune thyroid disease is only rarely triggered by viral infections (128).

Viruses that attack the respiratory tract have been linked to the development of thyroiditis. Cases of De Quervain thyroiditis, with low TSH levels and high levels of free $T_3$ and $T_4$, were described in the course of H1N1 influenza infection (133, 134). The H1N1 virus has also been linked to the occurrence of thyroid storm, which is a potentially fatal intensification of thyrotoxicosis, and is characterized by hyperthermia, tachycardia, severe agitation and altered mental status (135, 136). In 2009, a 31-year-old female diagnosed with community-acquired bronchopneumonia with possible influenza A (H1N1) viral pneumonia, presented tachycardia and $T_3$ levels 16 times higher than the threshold. The diagnosis of thyroid storm was made, but despite treatment with propylthiouracil (PTU), the patient progressed from multiorgan failure to brain death (135). A recent case report also presented the occurrence of a thyroid storm associated with Influenza A infection in a 10-year old girl (137).

A prospective study followed 61 survivors of the 2002 SARS epidemy (with no pre-existing endocrine diseases) 3 months

TABLE 1 Retrospective studies that analyzed thyroid function in COVID-19 patients during admission.

| Author | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings | Thyroid function and clinical outcomes |
|---|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations | |
| Wang et al | 84 | Not mentioned | Moderate 25% Critical 75% | TSH, TT3, TT4 | Overt thyrotoxicosis 4% Subclinical thyrotoxicosis 4% | TT3 and TSH levels were significantly lower in COVID-19 patients ($p < 0.001$). Thyroid dysfunction was more commonly found in critical than in mild/moderate cases (74.6 vs 23.8%, $p < 0.001$). The group with thyroid dysfunction also had an increased level of leukocytes ($p < 0.001$), neutrophils ($p < 0.001$), CRP ($p = 0.002$), and PCT ($p = 0.054$); and a decreased level of lymphocytes ($p < 0.001$). | Thyroid dysfunction tended to be associated with longer viral nucleic acid cleaning time (14.13 ± 9.39 vs. 10.56 ± 8.29 days, $p = 0.088$). |
| China Feb/2021 (102) | 53 (63.1%) 57.3 ± 14.5 | Not mentioned | 0% | Procalcitonin, PCR, IL-6, IL-10, TNF-α, interferon-γ | Not mentioned | Small sample size. Free T3, free T4, and reverse T3 were not measured. | |
| Gao et al | 100 | Not mentioned | Moderate 34% Critical 66% | TSH, FT4, FT3 | Overt thyrotoxicosis 17% | FT3 levels are lower in severe ill patients (4.40 ± 0.88 vs 3.41 ± 0.90, $p < 0.001$). TSH levels are lower in severe ill patients (2.03 (1.24, 3.31) vs 1.20 (0.45, 2.05), $p = 0.002$). | The lower (versus upper) two-thirds of FT3 were associated with all-cause mortality HR (95% CI) of 9.23 (2.01, 42.28). |
| China Nov/2020 (97) | 52 (52%) 66.1 ± 16 | 14 days ± 6 | 22% | PCR, D-dimer, IL-6, TNF-alfa, NT-proBNP | 28% | Small sample size. Sample composed mainly of patients with severe COVID-19. | |
| Sun et al | 336 | Hypertension 35.3% Diabetes 15.3% CVD 9.3% | Mild/ Moderate 92.3% Critical 7.7% | FT3, FT4, TT3, TT4 | Not mentioned | TT3, FT3, TT4 and FT4 were significantly lower in moderate/critical patients; TT3 AUROC 0.96. | Thirty-six of the clinical and laboratory features analyzed were found to be statistically associated with severe/critical symptoms of COVID-19. |
| China Jul/2020 (103) | 117 (34.8%) 50 | Not mentioned | 0% | CD3, CD4, CD19, CRP | Not mentioned | TSH and reverse T3 were not measured. | |

*(Continued)*





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TABLE 1 Continued

| Author | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings | Thyroid function and clinical outcomes |
|---|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations | |
| Lania et al | 287 | Hypertension 49.5% Diabetes 24.4% CVD 14.3% COPD 12.2% | Critical (100% ICU) | TSH, FT3, FT4 | Overt thyrotoxicosis 10.8% Subclinical thyrotoxicosis 19.9% | In the multivariate analysis, thyrotoxicosis was associated with higher IL-6 levels (odds ratio: 3.25, 95% CI: 1.97-5.36; P < 0.001). 16% of patients with overt thyrotoxicosis developed thromboembolic events. | The in-hospital mortality rate was higher in patients with either thyrotoxicosis or hypothyroidism. In discharged patients, the duration of hospitalization resulted to be significantly longer in cases with thyrotoxicosis as compared to those with either normal TSH or hypothyroidism. |
| Italy Oct/2020 (104) | 193 (67.2%) 66 (27-92) | Not mentioned | 21.4% | IL-6 | Not mentioned | In several patients, thyroid function was assessed in the course of treatment with low-molecular-weight heparin. | |
| Sen et al | 60 | Not mentioned | Mild 43.3% Moderate 26.7% Critical 30% | TSH, FT3, FT4, TT3, TT4, TPOAb | Not mentioned | 35% of the patients showed one or more abnormality in thyroid function. The commonest abnormality was low TSH, found in 11 patients (18.33%). | FT4 is associated with the severity of the disease ($P$ = 0.009). |
| India Jan/2021 (105) | Not mentioned | Not mentioned | 0% | Ferritin, D-dimer | Not mentioned | Small sample size. | |
| Chen et al | 50 | Not mentioned | Mild 30% Moderate 46% Critical 24% | TSH, FT3, FT4 | 56% | 64% of the patients had abnormal thyroid function parameters. | The more severe the COVID-19, the lower the levels of TSH and TT3 (p <0.001). |
| China Jan/2021 (106) | 33 (66%) 48,4 ± 13,7 | Not mentioned | 0% | Albumin | Not mentioned | Small sample size. | |
| Guo et al | 121 | Not mentioned | Critical 100% | FT4, FT3 | Not mentioned | In the ROC curve, the FT3 variable was the best laboratory variable in predicting hospital mortality (AUC 0.863), 3.25 pmol/L cut-off. | Not mentioned. |
| China Jan/2021 (95) | 57.0% 66 (56–72) | Not mentioned | 28.9% | IL-2R, IL-6, IL-8, IL-10, TNF-α, NT-proBNP, | Not mentioned | Small sample size. TSH and reverse T3 were not measured. | |

*(Continued)*





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TABLE 1 Continued

| Author | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings | Thyroid function and clinical outcomes |
|---|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations | |
| | | | | troponin, and hs-CRP | | | |
| Schwarz et al | 54 | Hypertension 38.8% Diabetes 33.3% CVD 29.6% | Moderated 68.5% Critical 31.4% | TSH, FT4, FT3 | Not mentioned | Patients in the lowest FT3 tertile had significantly lower mean room air oxygen saturation on presentation (81%, 92.7%, and 93.7%, respectively; p = 0.006). | Patients in the lowest FT3 tertile had a significantly higher mortality rate (40%, 5.9%, and 5.9% in the first, second, and third tertiles, respectively; P = 0.008), more mechanical ventilation (45%, 29.4%, and 0.0%, respectively; P = 0.007), and ICU hospitalization (55%, 29.4%, and 5.9%, respectively; P = 0.006). |
| Israel Feb/2021 (96) | 37 (68.5%) 58.7 ± 17.5 | Not mentioned | 10% | CRP, D-dimer, ferritin, troponin, LDH | Not mentioned | Small sample size. Reverse T3 was not dosed. | |
| Vassiliadi et al. | 87 | Not mentioned | Moderate 47.1% Critical 52.9% | TSH, FT4, TT3, TG | Overt thyrotoxicosis 6.9% Subclinical thyrotoxicosis 6.8% | T3 and TSH levels were lower in the ICU patients (70.5 ± 31.9 vs 89.7 ± 42.0, $P = 0.001$ and 0.95 ± 0.93 vs 1.66 ± 1.46, $P \leq 0.001$, respectively). | The prevalence of thyroid hormone abnormalities increased with increasing disease severity. |
| Greece Jun/2021 (107) | 69 (66.3%) 59.3 ± 18.3 | Not mentioned | 14.9% | IL-6 | 47.1% | Small sample size. Free T3 and reverse T3 were not measured. | |
| Yazan et al | 205 | Hypertension 42.6% Diabetes 26.3% CVD 15.2% COPD 12.3% Neoplasia 5.8% | Moderate 85% Critical 15% | TSH, FT3, FT4, TGAb, TPOAb | Overt thyrotoxicosis 3.9% Subclinical thyrotoxicosis 4.3% | Thyroid dysfunction rate was 65.8% in this study. FT3 (rho = -0.34, $p < 0.001$), and TSH (rho = -0.21, $p = 0.002$) had weak negative correlations with WHO illness severity scores. | Length of hospitalization, rate of oxygen demand, ICU admission and mortality were lower in euthyroid patients. FT3 and TSH levels were significantly lower in patients admitted to ICU (p < 0.001 and p = 0.005, respectively). |
| Turkey Aug/2021 (108) | 113 (55.1%) | Not mentioned | 4.3% | CRP, D-dimer, ferritin, DHL | 52.6% | Absence of a control group. | |
| Ahn J et al. | 119 | Hypertension 52.1% Diabetes 30.3% CVD 18.4% COPD 5.9% | Moderate 26.9% Critical 73.1% | TSH, FT3, FT4 | Overt thyrotoxicosis 0% Subclinical thyrotoxicosis 14.3% | Patients with severe to critical COVID-19 disease had lower TSH (median: 0.90 mIU/L vs 1.67 mIU/L, $p = 0.006$) and T3 (median: 0.82 ng/mL vs 1.11 ng/mL, $p < 0.001$) levels compared with those with non-severe disease. T3 was negatively correlated with hs- | COVID-19 patients in the lower third of T3 levels (compare to middle and upper third of T3 levels) had poor outcomes: ICU admission (61.5% vs 32.5% vs. 30%, p = 0.005), mechanical ventilation (46.2% vs 27.5% vs 12.5%, p = 0.001), and death (48.7% vs 32.5% vs 5%, p < 0.001). The Kaplan-Meier curves for survival showed increased mortality of the lowest third T3 (log-rank P=0.014). |

*(Continued)*





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TABLE 1 Continued

| Author | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings | Thyroid function and clinical outcomes |
|---|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations | |
| | | | | | | CRP ($r$=–0.373, $p < 0.001$) and WBC count ($r$=–0.463, $p < 0.001$). | |
| Korea Aug/2021 (109) | 62 (52.1) 64.3 ± 16.8 | Not mentioned | 28.6% | CRP | 18.5% | Sample composed mainly of patients with severe COVID-19. Reverse T3 was not measured. | |
| Clausen et al. | 116 | Hypertension 46% Diabetes 33% Asthma 10% COPD 8% | Moderate 83% Critical 17% | TSH, FT4 | Overt thyrotoxicosis 1.7% Subclinical thyrotoxicosis 9.5% | 18.1% patients had biochemically thyroid dysfunction. IL-8 (r = –0.248, P = 0.008), IL-10 (r = –0.253, P = 0.007), IL-15 (r = –0.213, P =0.02), IP-10 (r = –0.334, P = 0.0003) and GM-CSF (r =–0.254, P =0.007) were inversely correlated with TSH. IL-8 levels, IP-10, and GM-CSF were higher in patients with serum TSH < 0.4 mIU/L. | Neither TSH in the whole cohort nor in the group with TSH levels <0.4 mIU/L was associated with 30- and 90-day mortality in crude and adjusted logistic regression models (adjusted for age, sex, and IL-6). |
| Denmark Set/2021 (110) | 44 (38%) | Not mentioned | 24% | 35 cytokines | 1.7% | Small sample size. Free T3 and reverse T3 were not measured. | |
| Okwor et al | 90 (45 control) | Not mentioned | Not mentioned | TSH, FT3, FT4 | Overt thyrotoxicosis 2.2% | Plasma levels of FT3 (4.19 ± 1.32 vs 2.42 ± 0.83) and TSH (2.60 ± 1.04 vs 1.68 ± 0.67) were significantly higher in COVID-19 patients compared to healthy controls (p< 0.001). | Amongst COVID-19 patients 7 (15.6%) presented euthyroid sick syndrome whereas no cases were found in the control group. |
| Nigeria Set/2021 (111) | 34 (75.6%) 35.3 ± 12.4 | Not mentioned | 0% | CPR | 15.6% | Small sample size. Young population and mostly men. Severity criteria were not used. | |
| Dutta et al | 236 | Hypertension 43.2% Diabetes 50.4% Hypothyroidism 18% CVD 8% | Moderate 94.1% Critical 5.9% | TSH, FT3, FT4 | Subclinical thyrotoxicosis 3,8% | Low FT3, high TSH and low TSH were seen in 56 (23.7%), 15 (6.4%) and 9 (3.8%) patients, respectively. | Cox regression analysis showed that low FT3 was associated with severe COVID-19 (P =0.032, HR 0.302; CI 0.101–0.904). The duration of hospital stay correlated negatively with both FT3 and TSH. |

*(Continued)*





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TABLE 1 Continued

| Author | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings | Thyroid function and clinical outcomes |
|---|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations | |
| India Nov/2021 (112) | 159 (6%) 54 (15-91) | Eight days (1-44) | 4.7% | CPR, D-dimer, IL-6, ferritin, DHL. | 23,7% | Most patients with moderate disease. | |
| Lang et al | 127 | Hypertension 41.7% Diabetes 21,3% CVD 10.2% COPD 10.2% | Mild 44.1% Moderate 42.5% Critical 13.4% | TSH, FT4, FT3 | Not mentioned | The serum levels of TSH [0.8 (0.5–1.7) *vs.* 1.9 (1.0–3.1) μIU/mL, *P* = .031] and FT3 [2.9 (2.8–3.1) *vs.* 4.2 (3.5–4.7) pmol/L, *P* <.001] were lower in non-survivors than in survivors. | Patients with low FT3 (<3.1 pmol) had a higher risk of death (adjusted OR 13.2, 95% CI 3.87–55, p < 0.001). |
| China Nov/2021 (113) | 62 (48.8%) 66 (53-71) | Not mentioned | 8.6% | CRP, D-dimer, IL-6. | 16,5% | Small sample size. Corticosteroids in the treatment of COVID-19. | |
| Zheng et al. | 235 | Hypertension 35.3% Diabetes 15.3% DCV 9.3% COPD 5.9% | Moderate 20.8% Critical 79.2% | TSH, FT3, FT4 | Not mentioned | The proportion of subclinical hypothyroidism was 7.23% in COVID-19 patients. Patients with NTIS had higher CRP (17.6 (2.6) vs 67.4 (7.4), p<0.001), WBC count (6.26 (0.2) vs 7.59 (0.6), p=0.001) and ESR (43.9 (2.7) vs 81.5 (8.5), p<0.001). | Patients with NTIS had higher incidences of COVID-related complications, including ARDS (9.1–13.0% vs 0.0–1.1%), acute cardiac injury (54.5–70.0% vs 15.3–23.5%), acute kidney injury (21.7–27.3% vs 0.0–2.7%), shock (36.4 47.8% vs 0.0–1.6%), hypoalbuminemia (45.5–52.2% vs 18.6– 23.5%), and coagulopathy (27.3–30.0% vs 0.0–10.9%), as well as higher severe types of COVID-19 (100% vs 75.5–76.5%) compared to patients with normal thyroid function. . |
| China Nov/2021 (114) | 112 (47.6%) 61 (51-69) | Not mentioned | 6.8% | PCR, D-dimer, IL-6, BNP | 14.47% | Most critically ill patients. Reverse T3 was not measured. | |
| Sethi et al | 57 | Not mentioned | Mild 33.3% Moderate 33.3% Critical 33.3% | TSH, FT3, FT4 | Overt thyrotoxicosis 28% Subclinical thyrotoxicosis 9% | 28% of the patients had raised T4 and around 9% had decreased TSH. A negative correlation was found between TSH and CRP (r=-0.541). | T3 (H = 11.98, p =0.02) and T4 (H = 6.71, p = 0.035) were lower in higher disease severity (p <0.05). |
| India May/2022 (115) | 39/57 (68%) 47.1 | Not mentioned | Not mentioned | CPR | Not mentioned | Small sample size. | |
| Okoye et al | 95 | Not mentioned | Mild 55.4% Moderate 19.3% | TSH, FT3, FT4 | Not mentioned | There is no difference in the incidence of NTIS between patients with COVID-19 (66.3%) and patients with | Among COVID-19 patients, a slightly lower mortality of NTIS patients was observed (23.8% vs 31.2% respectively, *p*=0.43), while |

*(Continued)*



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TABLE 1 Continued

| Author | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings | Thyroid function and clinical outcomes |
|---|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations | |
| | | | Critical 25.3% | | | non-COVID-19 pneumonia (67,9%) ($p$ = 0.82). | non-COVID-19 patients with NTIS showed a three times higher mortality than non-NTIS (14.5% vs 3.8% respectively, $p$=0.09). |
| Italy May/2022 (116) | 52.6% 81.9 ± 7.8 | Not mentioned | 26.3% | CPR | 66.3% | Small sample size. Only hospitalized elderly. | |

after recovery and observed that 6.7% of them became biochemically hypothyroid (with one case of primary and three of central hypothyroidism) and 39% had hypocortisolism (two of hypocortisolic patients had also transient subclinical thyrotoxicosis). The authors speculated that these effects might be due to SARS-induced reversible hypophysitis or a direct effect of the virus on the hypothalamus (138). In addition, in subjects who died of SARS, follicular epithelial damage in the thyroid gland were found during the autopsy, with large numbers of cells exfoliated into the follicle and undergoing apoptosis (139). A subsequent study also observed in autopsies that the adenohypophysis of SARS patients had profound alterations including lower positive-cell count and staining intensity for TSH, GH and ACTH (140).

In May of 2020, an Italian case-report provided the first case of subacute thyroiditis potentially associated with a prior mild COVID-19 infection (141). An 18-year-old female patient reported neck pain radiated to the jaw, fever and palpitation 15 days after a positive RT-PCR for SARS-CoV-2. The patient showed painful and enlarged thyroid to palpation and laboratory findings typical of acute phase of destructive thyroiditis, including elevated $fT_3$ and $fT_4$, undetectable TSH, detectable thyroglobulin (Tg) and anti-Tg antibodies. Antibodies against TPO and TSH receptor were absent, and the inflammatory markers CRP, ESR and white blood count were elevated. Neck ultrasound revealed diffuse hypoechoic areas. The patient was diagnosed with SAT and treated with prednisone. Forty days after diagnosis thyroid function and inflammatory markers were normalized. Subsequent studies also reported additional isolated cases of painful symptomatic SAT developed 16 to 42 days after COVID-19 infection (142, 143) and also cases during active COVID-19 disease (144–147), reinforcing a possible association between SARS-CoV-2 infection and SAT (Table 1).

The THYRCOV study retrospectively evaluated the thyroid function in a cohort of 287 patients hospitalized for COVID-19 in non-intensive care units and found that thyrotoxicosis was the thyroid disorder with higher prevalence (104). In this cohort, around 55 patients (20.2%) showed low TSH (≤0.10 mU/L), from whom 31 patients presented overt thyrotoxicosis. From those 31 patients, 10 had atrial fibrillation. Fifteen patients (5.2%) were diagnosed with high TSH (>4.80 mU/L), and overt hypothyroidism was present in two of those patients (104). Thyrotoxicosis was significantly associated with higher serum IL-6 levels. Thyroid function was spontaneously improved during the follow-up, which raised the hypothesis that the thyrotoxic state could be related to destructive thyroiditis, but it was not properly investigated in this cohort.

Corroborating this findings Muller and colleagues found a thyrotoxicosis' (TSH <0.28 mU/L and/or $fT_4$>1.7 ng/dL) prevalence of 15% (13 in 85 patients) in patients with COVID-19 (HICU-20) admitted in high intensity of care units (HICUs), compared to 2% (2 in 41 patients) in those admitted in low

TABLE 2 Prospective studies that analyzed thyroid function in COVID-19 patients during admission.

| Author, | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings |
|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations |
| Muller et al. | 145 (COVID-19), 93 (ICU), 52 (non-ICU); 101 (non-COVID-19) | Not mentioned | Moderate 35.9% Critical 64.1% | TSH, FT4, FT3 | Overt thyrotoxicosis 11.8% Subclinical thyrotoxicosis 17.7% | FT4 were higher in the COVID-19 ICU group (18.7 ± 5.4) than in the COVID-19 NICU (13.5 ± 4.6) group (p=0·016) but not in non-COVID-19 group (16.2 ± 2.4) (p=0·38). |
| Italy Set/2020 (117) | 89 (61.4%) COVID-19 ICU 65.3 ± 12.9 years COVID-19 non-ICU 70.3 ± 18.1 years | COVID-19 ICU (23.8 ± 15.8 days) COVID-19 NICU (22.3 ± 15.5 days) | COVID-19 ICU (18.7%) COVID-19 NICU (7.8%) | PCR, D-dimer, ferritin, DHL. | Not mentioned | Small sample size. Reverse T3 and other biomarkers were not measured. |
| Khoo et al. | 334 | Hypertension 48,5% Diabetes 39,5% CVD 23,7% COPD 17,4% CKD 13,2% | Moderate 89,3% Critical 10,7% | TSH, FT4 | Overt thyrotoxicosis 0% Subclinical thyrotoxicosis 5.4% | Patients with COVID-19 had lower TSH (1.03 mU/L) and FT4 (12.60 pmol/L) than patients without COVID-19: TSH (1.48 mU/L, P = 0.01) and T4L (13.11 pmol/L, P = 0.01). |
| United Kingdom Jan/2021 (118) | 203 (60,8%) 66,1 ± 16 years | 8 days (IQR 6-11). | 26% | CPR, cortisol, albumin | Not mentioned | Free T3 was not measured; therefore, patients with NTIS were not analyzed. |
| Guven et al. | 250 | Not mentioned | Moderate 50% Critical 50% | TSH, FT4 | Overt thyrotoxicosis 4% Subclinical thyrotoxicosis 5.2% | The FT3 level showed a negative correlation with length of hospital stay and CRP (r = −0.216, p=0.001; r = −0.383, P < 0.0001). |
| Turquia Mar/2021 (119) | 157 (63%) 68 (54-78) years | 9 days (IQR 5-15). | 15,2% | PCR, D-dimer, ferritin | 13% | Small sample size. Diabetic and nephropathy patients were excluded. |
| Lui et al | 367 | Hypertension 24,3% Diabetes 16,3% CVD 5,4% CVA 2,7% COPD 3,5% | Mild 75,2% Moderate 21% Critical 3,8% | TSH, FT3, FT4 | Subclinical thyrotoxicosis 8,2% | Patients with NTIS had a higher risk of death (adjusted OR 3.18, 95% CI 1.23–8.25, p = 0.017), |
| China Apr/2021 (120) | 172 (46,9%) 54 ± 15 years | 8 days (IQR 6-13). | 1% | CPR, CPK, TGP, DHL | 7,4% | Most mild COVID-19 patients. Reverse T3 not measured. |
| Campi et al | 115 | Hypertension 64% Diabetes 17,5% Cardiopathy 6,3% CVA 4,2% Pneumopathy 3,1% | Critical 100% (ICU) | TSH, FT3, FT4, Tg, anti-Tg | Subclinical thyrotoxicosis: during admission (10,4%), during hospitalization (23,5%) | Low TSH levels were found either at admission or during hospitalization in 39% of patients, associated with low FT3 in half of the cases. In the univariate analysis, the predictors of mortality were low FT3 (P <0.0001) and low FT4 (P = 0.01). |

*(Continued)*

TABLE 2 Continued

| Author, | Number of patients | Comorbidity | Severity | Thyroid function markers | Hyperthyroidism (%) | Main statistical findings |
|---|---|---|---|---|---|---|
| Local Month/ year | Men's n (%) – Age | Length of stay | Mortality | Inflammatory markers | NTIS (%) | Limitations |
| Italy Mai/2021 (98) | 97 (67%) 68.1 ± 14 years | 21 ± 19 days | 31.3% | PCR, Cortisol | 9% | Only severe COVID-19 patients. |
| Beltrao et al | 245 | Hypertension 66.5% Diabetes 44.6% DCV 13.8% Pneumopathy 4.4% | Non-critical 73.9% Critical 26.1% | TSH, FT3, FT4, TT3, rT3, Tg anti-Tg | Subclinical thyrotoxicosis 27.3% | fT3 levels were lower in critically ill compared with non-critical patients [fT3: 2.82 (2.46–3.29) pg/mL vs. 3.09 (2.67–3.63) pg/mL, p = 0.007]. Serum reverse triiodothyronine (rT3) was mostly elevated but less so in critically ill compared with non-critical patients [rT3: 0.36 (0.28–0.56) ng/mL vs. 0.51 (0.31–0.67) ng/mL, p = 0.001]. There is correlation between in-hospital mortality and serum fT3 levels (odds ratio [OR]: 0.47; 95% confidence interval [CI 0.29–0.74]; p = 0.0019), rT3 levels (OR: 0.09; [CI 0.01–0.49]; p = 0.006) and the product fT3 · rT3 (OR: 0.47; [CI 0.28–0.74]; p = 0.0026). |
| Brazil Nov/2021 (60) | 145 (59.1%) 62 (49-75) years | 6 (4–10) days | 16.7% | PCR, D-dimer, fIL-6, DHL, albumin | 6.5% | It is unclear whether a decrease in caloric intake, a weight loss, or a combination of these factors are the cause of decreased fT3 levels in COVID-19 critically ill patients. |
| Vizoso et al | 78 | Hypertension 55.1% Diabetes 25.6% CVD 15.4% COPD 12.8% Cancer 11.5% | Critical 100% | TSH, FT3, FT4, T3, rT3 | Not mentioned | FT3 levels were lower in non-survivors (1.6 ± 0.2) vs survivors (1.8 ± 0.5) p = 0.02. |
| Spain Nov/2021 (121) | 55/78 (70.5%) Survivors 59 ± 12 Non-survivors 68 ± 12 | Survivors 37 (22–83) days Non-survivors 18 (7–39) days | 29.5% | Not evaluated | 46.2% | Small sample size. Critical patients only. |
| Ilera et al. | 55 | Not mentioned | Mild 22% Moderate 27.1% Critical 50.8% | TSH, FT3, TT3, FT4, TT4, anti-TPO | 0% | The T3/T4 ratio was significantly lower in patients with severe disease compared with those with mild/moderate infection [7.5 (4.5–15.5) vs. 9.2 (5.8–18.1); p =0.04] and lower in patients who died than in patients who were discharged [5.0 (4.53–5.6) vs. 8.1 (4.7–18.1); p = 0.03] |
| Argentina Dez/2021 (122) | 28 (50.9%) 56 (21-89) years | Not mentioned | 7.4% | CPR, D-dimer, ferritin, DHL, VHS, fibrinogenin | 54.5% | Small sample size. |
| Sparano et al | 506 | Hypertension 51.3% Diabetes 17% CVD 26.9% COPD 7.1% Cancer 18.4% | Mild/ Moderate 73.7% Critical 26.3% | TSH, FT3, FT4 | Overt thyrotoxicosis 12.4% | In Kaplan–Meier and Cox regression analyses, fT3 was independently associated with poor outcome and death ($p$ = 0.005 and $p$ = 0.037, respectively). A critical fT3 threshold for levels < 2.7 pmol/l (sensitivity 69%, specificity 61%) was associated with a 3.5-fold increased risk of negative outcome (95%CI 2.34–5.34). |
| Italy 2022 (123) | 62.3% 68.8 ± 1.6 years | 12.5 ± 9.1 days | 19% | IL-6, NT-ProBNP, PCR, procalcitonin, D-dímer, DHL, | 57% | Monocentric study, without a control group. Most mild patients. Reverse T3 levels were not evaluated. |

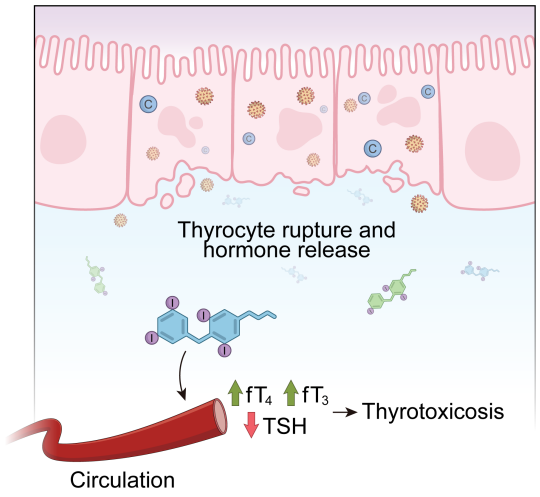


FIGURE 3
The course of Subacute Thyroiditis (SAT). Subacute Thyroiditis is a self-limited inflammatory disorder of the thyroid gland that often follows a viral infection. The damage and rupture of follicular cells, caused by the inflammatory process, results in the release of $T_3$ and $T_4$ in circulation, inducing thyrotoxicosis symptoms in the first weeks of disease. When extensive follicular destruction occurs, subacute thyroiditis can progress to hypothyroidism before returning to the euthyroid state.

intensity of care units (LICU-20) and only 1% (1 of 78 patients) in patients admitted in HICUs in 2019 for non-COVID-19 related reasons (HICU-19). Free $T_3$ equally low in all groups. TSH levels were lower in HICU-20 than other groups, while $fT_4$ was higher in HICU-20 only when compared to LICU-20 (117). However, it is important to mention that $fT_4$ and $fT_3$ were only measured in patients whose TSH levels were less than 0.45 mIU/L. Since a greater proportion of HICU-20 group (24,7%) showed low TSH levels (TSH <0.45 mIU/L) when compared to HICU-19 (17,7%) and LICU-20 (9,8% groups), TH levels were more frequently measured in HICU-20, which might be a bias. CRP levels were higher in COVID-19 patients than the non-COVID-19 group. From the 6 patients with thyrotoxicosis that were followed post-discharge, all had normal thyroid function 1.5-2 months later and were negative to thyroid autoantibodies and 3 of them had ultrasound and CT scans suggestive of subacute thyroiditis. However, since no patient reported neck pain, TSH levels were not extremely low, $fT_4$ was not very high and lymphopenia and not leukocytosis were present, it was probably not a typical case of subacute thyroiditis. The authors suggested a routine evaluation of thyroid function in patients in ICU due to increased risk of thyrotoxicosis.

A Chinese cohort of 367 patients with predominantly mild to moderate COVID-19 detected abnormal thyroid function in 62 patients (16,9%) (120). Twenty-seven patients (7,4%) had non-thyroidal illness syndrome (NTIS) and 30 patients (8,2%) had biochemical alterations that were suggestive of distinct phases of thyroiditis such as: isolated low TSH and high-normal fT4, isolated slightly elevated $fT_3$, high-normal Ft4 or isolated low fT4. None had overt thyrotoxicosis. Of these 30 patients with subnormal TSH, 5 presented anti-TPO or anti-TSHR autoantibodies, suggesting an autoimmune component in these cases. Pre-existing autoimmune thyroid disorder was present in 5 patients.

In contrast with the previous studies, Khoo and collaborators, did not found any case of overt thyrotoxicosis in a cohort 334 patients admitted with COVID-19 in intensive therapy unit, either during the disease or follow-up (124). Most COVID-19 patients (86.6%) were euthyroid but 5.7% present subclinical hyperthyroidism and a small proportion present overt hypothyroidism (0,6%), which did not differ from non-COVID patients. A small significant reduction in TSH and $fT_4$ was observed in patients with COVID-19 when compared with non-COVID-19 patients which might be compatible with a nonthyroidal illness syndrome and did not justify any treatment. A negative correlation between TSH and Cortisol or CRP was observed.

Of note, since the beginning of COVID-19 vaccination campaigns, some studies show the development of thyroid disease, especially thyroiditis, after the vaccines administration. Currently, more than 30 articles reporting data of SAT onset after COVID-19 vaccine. This relation seems more prevalent in women and the main symptoms are neck pain, palpitations, fatigue, fever and weight loss (148, 149). The patients almost always presented with thyrotoxicosis and elevated serum inflammatory markers (148, 150). Two cases of vaccine-induced Graves' disease have been reported in two female health-care workers of 28 and 40 years of age. The patients presented typical symptoms of thyrotoxicosis 2-3 days after receiving the first dose of the Pfizer-BioNTech vaccine (151). A sistematic review concluded that the thyroid disease onset occurred an average of 11 days after the administration of the vaccine (150). Cases of SAT had already been observed after the administration of the H1N1 vaccine (152, 153). The first mechanism suggested to explain the relation between COVID-19 vaccination and thyroid disease is the autoimmune/inflammatory syndrome induced by adjuvants (ASIA) (148, 151). But it is also already shown that antibodies against SARS-CoV-2 proteins could cross-react with tissue antigens, including thyroid peroxidase (TPO) (154). Despite that, considering that billions of vaccines against COVID-19 have already been administered around the world, the development of thyroid disorders following SARS-CoV-2 vaccination is a very rare side effect.

## 4.3 Thyroid features in COVID-19 patients

In 2002, the Severe Acute Respiratory Syndrome (SARS) caused by SARS-CoV, a member of the Coronaviridae family,

became an epidemic and rapidly spread to 26 countries (155). Extensive follicular damage, with large numbers of cells exfoliated in the follicle, was observed in the thyroid glands obtained from five SARS patients. The follicular architecture was prominently affected, showing follicular distortion and collapse (139).

In the first report of subacute thyroiditis (SAT) associated with COVID-19 infection, diffuse hypoechoic areas in the thyroid ultrasound were reported, in addition to the alterations in FT3, FT4, TSH, and the presence of TgAb (142). Subsequently, other studies found alterations in the thyroid ultrasonography of COVID-19 patients that developed SAT, including bilateral hypoechoic areas (145–147, 156), heterogeneity in the parenchyma (144), a relative diffuse decrease of vascularity (144, 146, 147) and increased vascularity (145) and inflammation (104). Thoracic computed tomography also showed that COVID-19 patients present altered thyroid tissue density during their infective states compared to prior infection. In these patients, the iodine content in thyroid tissue decreased, suggesting thyroiditis (157). Likewise, a case report of SARS-CoV-2-associated thyroid storm also detected ultrasound changes in the thyroid. The patient, a 25-year-old woman, presented exophthalmos, tachycardia, diffusely enlarged goiter with a bruit, and fine tremor. Laboratory results demonstrated very low TSH levels (TSH<0.01 mIU/L) and high levels of FT4 (5.34 ng/dL) and TT3 (654 ng/dL). Thyroid ultrasound revealed heterogeneous echotexture with increased vascularity (158).

As discussed before, viral infections are considered a major factor in the pathogenesis of autoimmune thyroid diseases, and a link between SARS-CoV-2 infection and Hashimoto thyroiditis and Graves' disease has already been reported. In a patient who started to present hyperthyroidism symptoms (fatigue, shortness of breath, palpitations, and weight loss) nearly 3 weeks after a mild SARS-CoV-2 infection, the ultrasound showed a diffusely heterogeneous and irregular thyroid and a nodular image below the sternal notch. Thyroid scintigraphy confirmed Graves' disease pattern (159). Not many studies have evaluated the *post-mortem* thyroid of patients who died of COVID-19. Of these, some found no significant damage of follicular thyroid cells (102), while others reported chronic inflammation of the thyroid, follicular epithelial cell disruption, or interstitial lymphocytic infiltration (160, 161).

Importantly, thyroid morphological changes persist even after COVID-19 resolution. A study conducted in China evaluated the thyroid of 79 COVID-19 survivors approximately one month after acute COVID-19 infection. At this time, all patients presented normal T4, T3 and TSH levels. Interestingly, higher SARS-CoV-2 viral load on presentation was associated with smaller thyroid volumes among the male survivors. The authors also observed that 13.9% of the COVID-19 survivors had ultrasonographic features suggestive of thyroiditis (5 had heterogeneous echogenicity, 6 had abnormal vascularity and 3 had micro-nodulation) (162). A similar result was also found in patients whose thyroid was evaluated 6 months after COVID-19 infection. In the Turkish cohort, the mean thyroid gland volume was significantly lower in COVID-19 survivors (10.3 ± 3.4 mL) than in non-COVID patients (14 ± 5.3 mL). There were no differences in thyroid gland volume between males and females (163). These findings encourage longitudinal follow-up to clarify a possible direct viral effect of thyroid atrophy.

## 5 COVID-19 and thyroid: State-of-art

Herein, we present an overview of the current knowledge regarding the relationship between thyroid dysfunction and SARS-CoV-2 infection. Overall, these data revealed that abnormal thyroid function may occur during and in the convalescence post-COVID condition phase. Although the cellular and molecular mechanisms are not completely understood, the evidence suggests that the "cytokine storm" is an important mediator in this context. It is very likely that indirect mechanisms (e.g., increased serum cytokines and immune cells) are responsible for most of the effects observed in the whole HPT axis. On the other hand, some authors have also proposed that the thyroid cells could be directly infected by SARS-CoV-2. It has been consistently demonstrated in multiple datasets that ACE2 mRNA is expressed in both human thyroid tissues and primary cultured cells, suggesting that the thyroid could be vulnerable to direct viral infection and its cytopathic effects (21, 164). However, stringent immunohistochemistry analysis from the The Human Protein Atlas performed with 7 different antibodies against human ACE2 reveals that thyrocytes do not have ACE2 protein. Indeed, ACE2 protein has been detected in endothelial cells within the thyroid gland, which may explain the detection of ACE2 mRNA in whole tissue extracts. Hence, thyroid function alteration during COVID-19 is more likely a result of pro-inflammatory signals and impaired central control than a direct infection of follicular cells by SARS-CoV-2.

However, it is important to highlight that the studies found in the literature have limitations. First of all, they were retrospective and, in most of them, thyroid function tests were performed only at admission and/or days after resolution, which did not allow the observation of dynamic alterations in thyroid function during disease progression. Some studies did not assess thyroid function on all cohort, while others measured only TSH or limited $fT_3$ or $fT_4$ measurements only to patients with abnormal TSH. Moreover, only one study measured $rT_3$ levels. Thus, future studies are needed to better investigate the pathophysiology of thyroid dysfunction induced by COVID 19 at both molecular and clinical levels. Furthermore, future prospective studies are crucial to clarify the prevalence of thyroid function alterations in COVID-19 patients, as well as to provide more clinical data to elucidate how it could impact the disease outcome.

## Author contributions

The attributions the authors had in the production of the manuscript were: Literature review and article writing: CR, JC and FH; Text review and interpretation of data for the work: AF, RF, DC and HR; Figure creation: FH; Data collection: FB, Text review: FB, DC and HR and research coordinator and text review: DC. 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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# Effect of SARS-CoV-2 BNT162b2 mRNA vaccine on thyroid autoimmunity: A twelve-month follow-up study

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**Objectives:** Graves' disease (GD) has been highlighted as a possible adverse effect of the respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccine. However, it is unknown if the SARS-CoV-2 vaccine disrupts thyroid autoimmunity. We aimed to present long-term follow-up of thyroid autoimmunity after the SARS-CoV-2 BNT162b2 mRNA vaccine.

**Methods:** Serum samples collected from seventy Japanese healthcare workers at baseline, 32 weeks after the second dose (pre-third dose), and 4 weeks after the third dose of the vaccine were analyzed. The time courses of anti-SARS-CoV-2 spike immunoglobulin G (IgG) antibody, thyroid-stimulating hormone receptor antibody (TRAb), and thyroid function were evaluated. Anti-thyroglobulin antibodies (TgAb) and anti-thyroid peroxidase antibodies (TPOAb) were additionally evaluated in thirty-three participants.

**Results:** The median age was 50 (IQR, 38-54) years and 69% were female. The median anti-spike IgG antibody titer was 17627 (IQR, 10898-24175) U/mL 4 weeks after the third dose. The mean TRAb was significantly increased from 0.81 (SD, 0.05) IU/L at baseline to 0.97 (SD, 0.30) IU/L 4 weeks after the third dose without functional changes. An increase in TRAb was positively associated with female sex ($\beta$ = 0.32, $P$ = 0.008) and low basal FT4 ($\beta$ = -0.29, $P$ = 0.02) and FT3 ($\beta$ = -0.33, $P$ = 0.004). TgAb was increased by the third dose. Increase in TgAb was associated with history of the thyroid diseases ($\beta$ = 0.55, $P$ <0.001).

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**Conclusions:** SARS-CoV-2 BNT162b2 mRNA vaccine can disrupt thyroid autoimmunity. Clinicians should consider the possibility that the SARS-CoV-2 vaccine may disrupt thyroid autoimmunity.

KEYWORDS

SARS-CoV-2, COVID-19, thyroid autoimmunity, BNT162b2 vaccine, thyroid-stimulating hormone receptor antibody, Graves' disease

# Introduction

For the COVID-19 pandemic, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines have been authorized and have provided durable benefits in reducing the risk of the severe outcomes of hospitalization and death (1, 2). To date (May 2022), three doses of SARS-CoV-2 mRNA vaccination (the initial two doses and the third 'booster' dose) have been introduced in the many countries, including Japan. As the fourth doses of the SARS-CoV-2 mRNA vaccines were only authorized for those older than 50 years in the US, the vaccination program is speculated to be continued specifically in the population who are at greatest risk and who might gain most benefit from vaccination. This includes immunocompromised individuals and people older than 50 years, given the prevalence of comorbidities that increase the risk of severe disease and death in such individuals (1, 2).

Despite the robust beneficial data on the SARS-CoV-2 mRNA vaccine, adverse effects of the vaccines related to endocrine disorders have also recently been highlighted (3–8). Among the endocrine organs possibly targeted as adverse effects of the vaccine, the thyroid gland is the most common one (4, 7). Notably, an increasing number of cases of new-onset or relapse of Graves' disease (GD) has been recently reported to occur following the SARS-CoV-2 mRNA vaccines (3, 4, 7, 9). Such case reports and series have raised speculation of the development of thyroid autoimmunity due to the mRNA vaccine. However, there is limited epidemiological evidence or basic findings to clarify if the SARS-CoV-2 mRNA vaccine induces thyroid autoimmunity. Furthermore, if the mRNA vaccine disrupts thyroid autoimmunity, further clinically important questions arise: if the disruption has the potential to induce functionally overt situations, how long does the vaccine affect thyroid autoimmunity, and what factors predict its disruption?

In this study, we present a twelve-month clinical follow-up of thyroid-stimulating hormone (TSH) receptor antibody (TRAb) as a marker of thyroid autoimmunity disease, GD, and thyroid function from the baseline to after the third dose of the SARS-CoV-2 BNT162b2 (Pfizer/BioNTech) mRNA vaccine in Japanese healthcare workers. In addition, if induction of TRAb could be observed after the vaccine, we aimed to investigate factors that could be used to predict it.

**Abbreviations:** ASIA, Autoimmune/inflammatory syndrome induced by adjuvants; FT3, Free T3; FT4, Free T4; GD, Graves' disease; IQR, Interquartile range; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; TgAb, Antithyroglobulin antibody; TPOAb, Antithyroid peroxidase antibody; TRAb, Thyroid-stimulating hormone receptor antibody; TSH, Thyroid-stimulating hormone.

# Materials and methods

## Study design and timeline

In this study, 99 healthcare workers, all employees of Wakayama City Medical Association, were enrolled. The initial interviews and the first serum sampling for the baseline were conducted in March and April 2021. Negativity for SARS-CoV-2 antibodies was confirmed at baseline. Participants received first and second doses of the BNT162b2 mRNA vaccine 3 weeks apart in April and May 2021, and a third dose in January 2022. Serum samples were prospectively collected at 4, 24, and 32 weeks after the second dose, and 4 weeks after the third dose in March 2022 as shown on the time schedule in Figure 1. Anti-SARS-CoV-2 antibody titers were measured at each time point. The remaining serum samples were preserved at -80°C until the assessment of thyroid autoimmunity and function. Participants who had consistently elevated titers were evaluated for infection *via* anti-nucleocapsid antibodies.

Exclusion criteria were as follows: i) pregnant and breastfeeding females, ii) thyroid cancer, iii) individuals with serious medical diseases including liver or kidney dysfunction, iv) individuals with breakthrough infections of COVID-19, and v) non-completion of the time schedule. Regarding criterion iii), all the participants were assessed by both medical interview and laboratory test at baseline, especially for liver and renal function, including blood cell counts, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, γ-glutamyl transpeptidase, creatinine, and creatinine-based estimated glomerular filtration rate.

Among the 99 participants enrolled, several participants were excluded from analysis: three contracted breakthrough infections, five did not receive a third dose of the vaccine, and one was treated for GD with positive TRAb (3.7 IU/L) at baseline were excluded from the analysis. Among the 90 remaining participants, samples from 70 participants whose serum samples at all the time points were sufficient for measurement of thyroid autoimmune antibodies and functions were retrospectively analyzed (Figure S1). None of these 70 participants met the exclusion criteria. In a limited number of the participants (n = 33), anti-thyroglobulin antibodies (TgAb) and anti-thyroid peroxidase antibodies (TPOAb) were also analyzed at 32 weeks after the second dose (pre-third dose) and 4 weeks after the third dose (post-third dose) to assess the response of these antibodies to the third dose.

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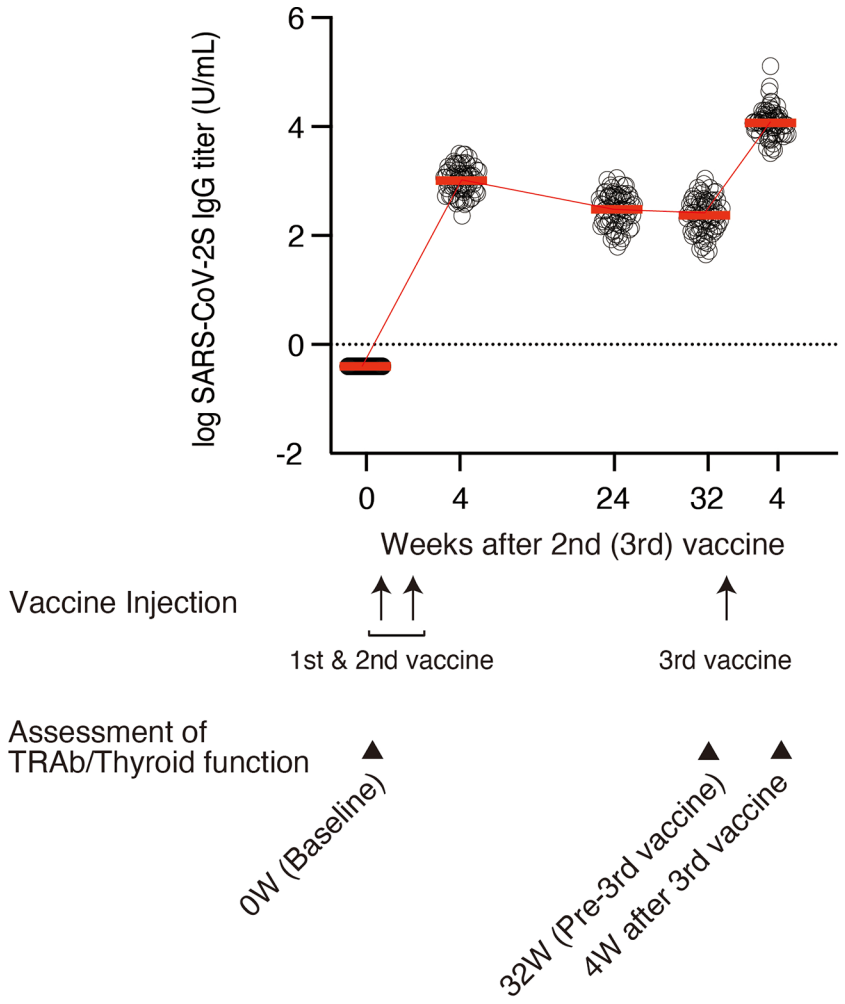


FIGURE 1
Timelines and trends of logarithmically transformed anti-SARS-CoV-2 S IgG titers at 0 (baseline), 4, 24, and 32 weeks after the second dose of vaccine and 4 weeks after the third dose of vaccine (n = 70). Each circle denotes individual log IgG titers. Mean values of anti-SARS-CoV-2 S IgG titer are indicated by red bars. Mean values are also presented in Table 1. TRAb and thyroid function were analyzed at 0 (baseline), 32 weeks after the second dose, and 4 weeks after the third dose of vaccine. TgAb and TPOAb were also assessed at 32 weeks after the second dose and 4 weeks after the third dose of vaccine in 33 participants.

This study was approved by the ethics committee of Wakayama City Medical Association Seijinbyo Center Ethics Committee (No. 202103-1). All participants provided written informed consent.

## Measurement of Anti-SARS-CoV-2 S antibodies

Elecsys Anti-SARS-CoV-2 S immunoassay (Roche Diagnostics, Basel, Switzerland) was used to measure anti-spike immunoglobulin G (IgG) SARS-CoV-2 antibody titers on a Roche Cobas e411 analyzer (Roche Diagnostics) according to the manufacturer's instructions. Samples with a titer >250 U/mL were serially diluted until the titer became ≤250 U/mL, according to the manufacturer's protocol. Anti-nucleocapsid antibodies (Elecsys Anti-SARS-CoV-2, Roche Diagnostics) were measured according to the manufacturer's protocol.

## Measurement of thyroid autoimmune antibodies and functions

TRAb (Elecsys Anti-TRAb v2, Roche Diagnostics; reference range, <2.0 IU/L), TgAb (Elecsys Anti-Tg, Roche Diagnostics; reference range, <28.0 IU/mL), and TPOAb (Elecsys Anti-TPO, Roche Diagnostics; reference range, <16.0 IU/mL) were measured on a Roche Cobas e801 analyzer (Roche Diagnostics) according to the manufacturer's instructions. TSH (Elecsys TSH v2, Roche Diagnostics; reference range, 0.500-5.000 μIU/mL), free T4 (FT4, Elecsys FT4III, Roche Diagnostics; reference range, 0.90-1.7 ng/dL), and free T3 (FT3, Elecsys FT3III, Roche Diagnostics; reference range, 2.30-4.00 pg/mL) were measured on a Roche Cobas e801 analyzer (Roche Diagnostics) according to the manufacturer's instructions. Increases of TRAb, TSH, FT4, and FT3 from baseline to 4 weeks after the third dose were defined as ΔTRAb, ΔTSH, ΔFT4, and ΔFT3, respectively, which were calculated as:

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(value at 4 weeks after the third dose) - (value at baseline).

Increases of TgAb and TPOAb from 32 weeks after the second dose (pre-third dose) to 4 weeks after the third dose (post-third dose) were also defined as ΔTgAb and ΔTPOAb, respectively, and calculated as:

(vales at 4 weeks after the third dose) - (values at 32 weeks after the second dose).

Responders to increase in TRAb were defined as the subjects who exhibited increase in TRAb in a time dependent manner and had TRAb >1.2 IU/L at 4 weeks after the third dose.

## Statistical analysis

The differences in TRAb, TSH, FT4, and FT3 of each group at each pair of time-points were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons test. The differences in TgAb and TPOAb between 32 weeks after the second dose (pre-third dose) and 4 weeks after the third dose (post-third dose) were analyzed by Wilcoxon matched-pairs signed-rank test. To identify the predictive factors of increase TRAb and TgAb, the associations between ΔTRAb or ΔTgAb and baseline characteristics were analyzed using univariate linear regression. Similarly, the associations between TRAb or TgAb at 4 weeks after the third vaccine and baseline characteristics were also analyzed using univariate linear regression. Differences between responders and non-responders were analyzed using Mann-Whitney U test. A two-sided $P < 0.05$ was considered significant. Statistical analysis was performed using GraphPad Prism version 9.1.0 (GraphPad Software, San Diego, CA, USA) and JMP Pro16 (SAS Inc., Cary, NC, USA).

# Results

## Clinical characteristics and time course of anti-SARS-CoV-2 S antibodies

Clinical characteristics of the 70 participants are shown in Table 1. The median age was 50 (interquartile range [IQR], 38-54) years and 69% were female. The time course of anti-SARS-CoV-2 S

TABLE 1 Baseline cohort characteristics and time course of Anti-SARS-CoV-2 S IgG antibody, TRAb, TSH, FT4, and FT3 (n = 70).

| Characteristics | Value |
|---|---|
| Age (y), median (IQR) | 50 (38 – 54) |
| Sex (Female), n (%) | 48 (69) |
| Body mass index (kg/m$^2$), median (IQR) | 22 (20 – 24) |
| Smoking, n (%) | 14 (20) |
| Alcohol (g/week), median (IQR) | 0 (0 – 31) |
| Fever, n (%) | 23 (33) |
| History of thyroid disease, n (%) | 4 (6) |
| Family history of thyroid disease, n (%) | 3 (4) |
| Comorbidities | |
| Asthma, n (%) | 3 (4) |
| Hypertension, n (%) | 9 (13) |
| Dyslipidemia, n (%) | 8 (11) |
| Malignancy, n (%) | 2 (3) |
| Diabetes mellitus, n (%) | 0 (0) |
| Autoimmune disease, n (%) | 2 (3) |
| Cerebral infarction, n (%) | 1 (1) |
| Current medication | |
| Allergy, n (%) | 9 (13) |
| Hypertension, n (%) | 8 (11) |
| Dyslipidemia, n (%) | 8 (11) |
| Diabetes mellitus, n (%) | 0 (0) |
| Immunosuppressant, n (%) | 2 (3) |
| Anti-SARS-CoV-2 S IgG antibody | |
| Baseline IgG titer (U/mL), median (IQR) | <0.4 (<0.4-<0.4) |
| 4 weeks after vaccination | |
| Days after 2nd dose, median (IQR) | 28 (28-28) |
| IgG titer (U/mL), median (IQR) | 1158 (717-1611) |
| 24 weeks after vaccination | |
| Days after 2nd dose, median (IQR) | 168 (168-168) |
| IgG titer (U/mL), median (IQR) | 417 (223-712) |
| 32 weeks after vaccination | |
| Days after 2nd dose, median (IQR) | 224 (224-224) |
| IgG titer (U/mL), median (IQR) | 325 (169-546) |
| 4 weeks after 3rd vaccination | |
| Days after 3rd dose, median (IQR) | 28 (28-28) |
| IgG titer (U/mL), median (IQR) | 17627 (10898-24175) |
| TRAb (IU/L) | |
| Baseline, mean (SD) | 0.81 (0.05) |
| 32w, mean (SD) | 0.91 (0.15) |
| 4 weeks after the 3rd dose, mean (SD) | 0.97 (0.30) |
| TSH (μIU/mL) | |
| Baseline, mean (SD) | 2.06 (1.89) |
| 32w, mean (SD) | 1.95 (1.36) |
| 4 weeks after the 3rd dose, mean (SD) | 1.72 (1.13) |
| FT4 (ng/mL) | |
| Baseline, mean (SD) | 1.21 (0.15) |
| 32w, mean (SD) | 1.22 (0.16) |

(Continued)

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TABLE 1 Continued

| Characteristics | Value |
|---|---|
| 4 weeks after the 3rd dose, mean (SD) | 1.19 (0.17) |
| FT3 (pg/mL) | |
| Baseline, mean (SD) | 2.98 (0.41) |
| 32w, mean (SD) | 3.08 (0.36) |
| 4 weeks after the 3rd dose, mean (SD) | 2.93 (0.41) |

IQR, interquartile range.
Sex was recorded as 0: male and 1: female.

antibody titer is shown in Table 1 and Figure 1. During the period between 32 weeks after the administration of the second dose and 4 weeks after the third dose, the median antibody titer increased from 325 (IQR, 169-546) U/mL to 17627 (IQR, 10898-24175) U/mL (Table 1 and Figure 1). The period from 32 weeks after the administration of the second dose to 4 weeks after the third dose was 91 (IQR, 91-93) days. All participants had an increase in the antibody titer (Figure 1). No major adverse events were reported.

## Time course of TRAb and thyroid function

The mean TRAb increased from 0.81 (SD, 0.05) IU/L at baseline to 0.91 (SD, 0.15) IU/L 32 weeks after the second dose ($P$ <0.0001) and 0.97 (SD, 0.30) IU/L 4 weeks after the third dose ($P$ <0.0001) (Figures 2A, 3, Table 1). On the other hand, thyroid functions assessed by TSH, FT4, and FT3 were not significantly changed between each time points, although TSH showed a trend of time-dependently decrease following the mRNA vaccine (Figures 2B–D, Table 1). No participants showed definite symptoms of hyperthyroidism at any time point.

## Predictive factors for the increase of TRAb

To investigate the factors which could contribute to the increase of TRAb, we first calculated ΔTRAb as described in the Materials and Methods section. Univariate analysis for ΔTRAb was then performed to identify the predictive variants at baseline. ΔTRAb was positively associated with female sex (β = 0.315, $P$ = 0.008), low basal FT4 (β = -0.285, $P$ = 0.02), and low basal FT3 (β = -0.333, $P$ = 0.004) (Table 2). As confirmation, similar findings were also observed by univariate analysis for TRAb 4 weeks after the third dose (Table 2).

## Response of TgAb and TPOAb by the third dose of SARS-CoV-2 BNT162b2 mRNA vaccine

Clinical characteristics of the 33 participants, whose serum samples were analyzed for TgAb and TPOAb, are shown in Table 3. The mean TgAb was increased from 140 (SD, 459) IU/mL 32 weeks after the second dose (pre-third dose) to 174 (SD, 626 IU/mL) 4 weeks after the third dose (post-third dose) ($P$ = 0.003). The mean TPOAb was not significantly changed between those two time points [mean (SD); 48 (128) vs. 51 (133) (IU/mL), $P$ = 0.08]. To investigate the predictive factors for the increase of TgAb, ΔTgAb was calculated as described in the Materials and Methods section,

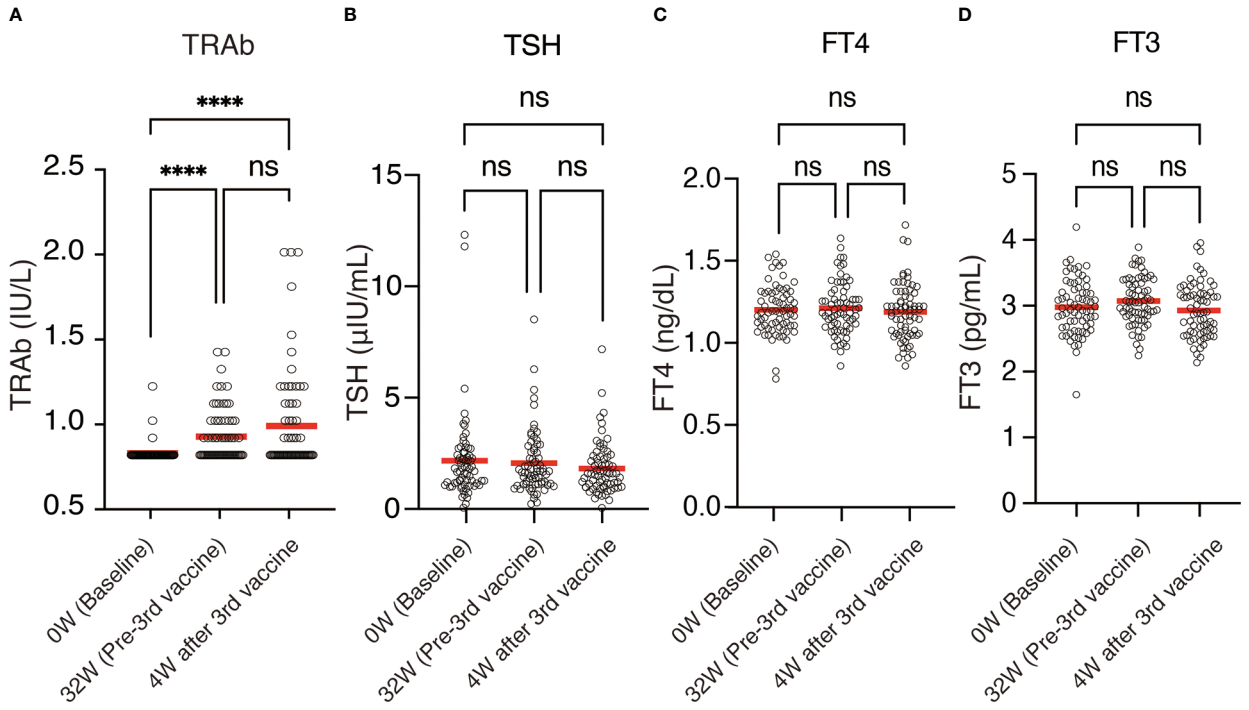


FIGURE 2
Time course of **(A)** TRAb, **(B)** TSH, **(C)** FT4, and **(D)** FT3 (n = 70). Each circle denotes individual values. Mean values were indicated by red bars. Mean values are also presented in Table 1. ns, not significant; ****$P$ <0.0001.

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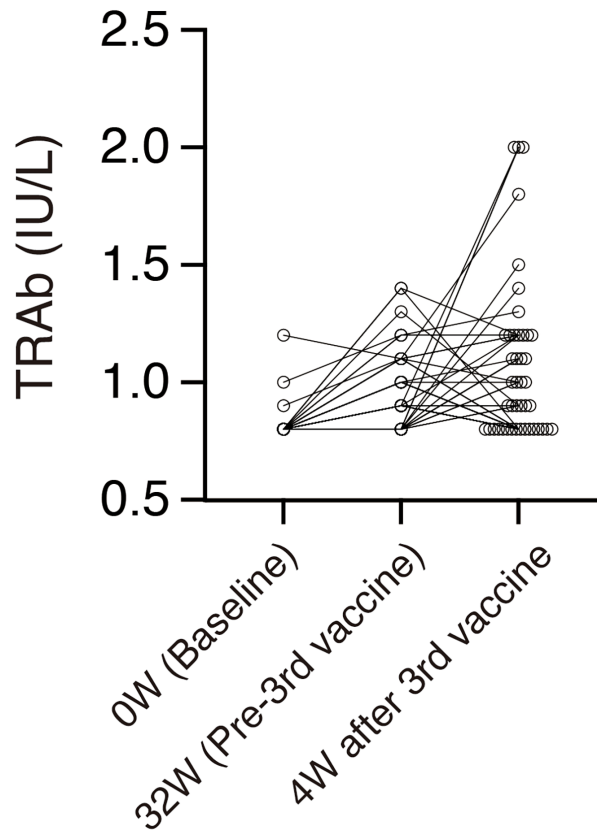


FIGURE 3
Time courses of TRAb in individuals whose TRAb is more than the lower limit of the range (0.8 IU/L) at any time point. Each circle denotes an individual value. n = 44.

similarly to TRAb. By univariate analysis, ΔTgAb was positively correlated with the history of thyroid disease (β = 0.553, $P$ = 0.0008) and TRAb (β = 0.384, $P$ = 0.03), TgAb (β = 0.975, $P$ <0.0001) and TPOAb (β = 0.763, $P$ <0.0001) 32 weeks after the second dose (pre-third dose) (Table 4). TgAb 4 weeks after the third dose was also associated with history of thyroid disease (β = 0.605, $P$ = 0.0002) (Table 4).

## Discussion

In this twelve-month follow-up study in a cohort of Japanese healthcare workers, we showed the first evidence of the SARS-CoV-2 BNT162b2 vaccine increasing TRAb, as a maker of thyroid autoimmunity. In addition, we presented candidate predictive factors to increase TRAb by the vaccine: female sex, baseline low FT4 and FT3. Furthermore, as confirmation of the disruption of the thyroid autoimmunity by the vaccine, we demonstrated that the third dose of mRNA vaccine increased TgAb in 4 weeks.

One of the main findings of this study is that TRAb was significantly increased by the first two doses of the SARS-CoV-2 BNT162b2 vaccine and that the effect was still evident at 32 weeks. Furthermore, although without statistical significance, TRAb tended to be additionally increased by the third dose of the vaccine within 4 weeks. Since most of the participants (67/70) had baseline TRAb under the lower limit of the reference range (<0.8 IU/L), it is assumed that the SARS-CoV-2 BNT162b2 vaccine not only disrupted the steady state of GD, but also newly induced the disruption of thyroid autoimmunity. These findings support previous cases and series that showed the relapse or even new onset of GD potentially associated with the SARS-CoV-2 BNT162b2 mRNA vaccine (3, 7, 10). Similar to TRAb, TgAb was also increased after the third dose, additionally supporting the evidence about the disruption of thyroid autoimmune owning to the vaccine. Although all the participants received the BNT162b2 mRNA vaccine in this study, since GD was also newly induced or enhanced by other SARS-CoV-2 mRNA vaccines in previous case reports, the effects may be considered as a class effect of the drug (3, 7, 10, 11). Supportively, a recent study in patients with GD also showed an increasing trend of TRAb from one month after to three months after the administration of inactivated SARS-CoV-2 vaccines (12). Together, our data with the results of previous case reports provide evidence that the SARS-CoV-2 mRNA vaccine disrupts thyroid autoimmunity.

In this study, no participants had new onset of GD with clinically overt hyperthyroidism. The criteria for GD was immunologically fulfilled in 4.3% of the participants (3/70) with the positivity of TRAb (TRAb>2), but none had overt hyperthyroidism. Conversely, although not significantly, TSH was decreased in a time-dependent

TABLE 2 Univariate analysis for increases of TRAb (ΔTRAb) and TRAb 4 weeks after the third dose vaccine (n = 70).

| | ΔTRAb | | TRAb (4 weeks after the third dose) | |
|---|---|---|---|---|
| Variable | β (95% CI) | *P value* | β (95% CI) | *P value* |
| Age (y) | -0.19 (-0.41 to 0.05) | .11 | -0.14 (-0.36 to 0.10) | .24 |
| Sex | 0.32 (0.09 to 0.51) | .01 | 0.31 (0.08 to 0.51) | .01 |
| Body mass index (kg/m$^2$) | -0.04 (-0.27 to 0.20) | .75 | -0.05 (-0.28 to 0.19) | .70 |
| Smoking | -0.08 (-0.31 to 0.16) | .52 | -0.08 (-0.31 to 0.16) | .51 |
| Alcohol (g/week) | -0.04 (-0.27 to 0.20) | .74 | -0.01 (-0.25 to 0.22) | .92 |
| Fever | 0.21 (-0.03 to 0.42) | .08 | 0.18 (-0.06 to 0.40) | .14 |
| History of thyroid disease | 0.15 (-0.09 to 0.37) | .23 | 0.13 (-0.11 to 0.35) | .28 |
| Family history of thyroid disease | 0.03 (-0.21 to 0.26) | .80 | 0.02 (-0.21 to 0.26) | .85 |

*(Continued)*

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TABLE 2 Continued

| | ΔTRAb | | TRAb (4 weeks after the third dose) | |
|---|---|---|---|---|
| Variable | β (95% CI) | *P value* | β (95% CI) | *P value* |
| **Comorbidities** | | | | |
| Asthma | -0.02 (-0.25 to 0.22) | .88 | -0.03 (-0.26 to 0.21) | .84 |
| Hypertension | -0.08 (-0.31 to 0.16) | .51 | -0.03 (-0.26 to 0.21) | .80 |
| Dyslipidemia | -0.03 (-0.26 to 0.21) | .83 | 0.02 (-0.21 to 0.26) | .85 |
| Thyroid disease | 0.15 (-0.09 to 0.37) | .23 | 0.13 (-0.11 to 0.35) | .28 |
| Malignancy | 0.15 (-0.09 to 0.37) | .23 | 0.13 (-0.10 to 0.36) | .27 |
| Autoimmune disease | 0.09 (-0.15 to 0.31) | .48 | 0.08 (-0.16 to 0.31) | .53 |
| Cerebral infarction | -0.07 (-0.30 to 0.17) | .58 | -0.07 (-0.30 to 0.17) | .57 |
| Current medication | | | | |
| Allergy | -0.11 (-0.34 to 0.13) | .36 | -0.12 (-0.34 to 0.12) | .33 |
| Hypertension | -0.06 (-0.29 to 0.18) | .63 | -0.01 (-0.24 to 0.23) | .95 |
| Dyslipidemia | -0.03 (-0.26 to 0.21) | .83 | 0.02 (-0.21 to 0.26) | .85 |
| Thyroid disease | 0.08 (-0.16 to 0.31) | .50 | 0.07 (-0.17 to 0.30) | .56 |
| Immunosuppressant | 0.09 (-0.15 to 0.31) | .48 | 0.08 (-0.16 to 0.31) | .53 |
| **Anti-SARS-CoV-2 S Ab IgG titer (U/mL)** | | | | |
| Pre-vaccination (Baseline) | N/A | | N/A | |
| 4 weeks after the 2nd dose | -0.05 (-0.28 to 0.19) | .68 | -0.07 (-0.30 to 0.17) | .55 |
| 24 weeks after the 2nd dose | 0.10 (-0.14 to 0.32) | .43 | 0.06 (-0.18 to 0.29) | .62 |
| 32 weeks after the 2nd dose | 0.04 (-0.20 to 0.27) | .76 | 0.01 (-0.23 to 0.24) | .95 |
| 4 weeks after the 3rd dose | -0.09 (-0.31 to 0.15) | .48 | -0.09 (-0.32 to 0.15) | .45 |
| **TRAb (IU/L)** | | | | |
| Baseline | 0.17 (-0.07 to 0.39) | .16 | 0.34 (0.12 to 0.53) | .00 |
| 32w | 0.09 (-0.14 to 0.32) | .44 | 0.14 (-0.10 to 0.36) | .25 |
| 4 weeks after the 3rd dose | 0.98 (0.97 to 0.99) | .00 | N/A | |
| ΔTRAb | N/A | | 0.98 (0.97 to 0.99) | .00 |
| TSH (μIU/mL) | | | | |
| Baseline | -0.06 (-0.29 to 0.18) | .64 | -0.04 (-0.27 to 0.19) | .73 |
| 32w | -0.14 (-0.36 to 0.10) | .26 | -0.09 (-0.32 to 0.15) | .45 |
| 4 weeks after the 3rd dose | -0.19 (-0.40 to 0.05) | .12 | -0.15 (-0.38 to 0.08) | .20 |
| ΔTSH | -0.07 (-0.30 to 0.17) | .56 | -0.07 (-0.30 to 0.17) | .59 |
| FT4 (ng/dL) | | | | |
| Baseline | -0.29 (-0.49 to -0.05) | .02 | -0.29 (-0.49 to -0.06) | .02 |
| 32w | -0.16 (-0.38 to 0.08) | .18 | -0.16 (-0.38 to 0.07) | .17 |
| 4 weeks after the 3rd dose | -0.09 (-0.32 to 0.15) | .46 | -0.11 (-0.34 to 0.13) | .37 |
| ΔFT4 | 0.16 (-0.07 to 0.38) | .17 | 0.15 (-0.09 to 0.37) | .22 |
| **FT3 (pg/mL)** | | | | |
| Baseline | -0.33 (-0.53 to -0.11) | .00 | -0.34 (-0.54 to -0.12) | .00 |

*(Continued)*

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TABLE 2 Continued

| | ΔTRAb | | TRAb (4 weeks after the third dose) | |
|---|---|---|---|---|
| Variable | β (95% CI) | *P value* | β (95% CI) | *P value* |
| 32w | -0.20 (-0.41 to 0.04) | .10 | -0.21 (-0.43 to 0.03) | .08 |
| 4 weeks after the 3rd dose | -0.26 (-0.47 to -0.03) | .03 | -0.29 (-0.49 to -0.05) | .02 |
| ΔFT3 | 0.10 (-0.13 to 0.33) | .39 | 0.09 (-0.15 to 0.31) | .48 |

Sex was recorded as 0: male and 1: female. The other dichotomous variables were recorded as 0: no/absent and 1: yes/present. N/A, Not applicable.

TABLE 3 Baseline cohort characteristics for TgAb and TPOAb analysis (n = 33).

| Characteristics | Value |
|---|---|
| Age (y), median (IQR) | 49 (41-51) |
| Sex (Female), n (%) | 20 (61) |
| Body mass index (kg/m$^2$), median (IQR) | 21 (20-23) |
| Smoking, n (%) | 8 (24) |
| Alcohol (g/week), median (IQR) | 0 (0-32) |
| Fever, n (%) | 10 (30) |
| History of thyroid disease, n (%) | 3 (9) |
| Family history of thyroid disease, n (%) | 1 (3) |
| Comorbidities | |
| Asthma, n (%) | 2 (6) |
| Hypertension, n (%) | 5 (15) |
| Dyslipidemia, n (%) | 2 (6) |
| Malignancy, n (%) | 2 (6) |
| Diabetes mellitus, n (%) | 0 (0) |
| Autoimmune disease, n (%) | 1 (3) |
| Cerebral infarction, n (%) | 0 (0) |
| Current medication | |
| Allergy, n (%) | 4 (12) |
| Hypertension, n (%) | 4 (12) |
| Dyslipidemia, n (%) | 2 (6) |
| Diabetes mellitus, n (%) | 0 (0) |
| Immunosuppressant, n (%) | 1 (3) |
| Anti-SARS-CoV-2 S IgG antibody | |
| Baseline IgG titer (U/mL), median (IQR) | <0.4 (<0.4-<0.4) |
| 4 weeks after vaccination | |
| Days after 2nd dose, median (IQR) | 28 (28-28) |
| IgG titer (U/mL), median (IQR) | 923 (642-1377) |
| 24 weeks after vaccination | |
| Days after 2nd dose, median (IQR) | 168 (168-168) |
| IgG titer (U/mL), median (IQR) | 399 (233-682) |
| 32 weeks after vaccination | |
| Days after 2nd dose, median (IQR) | 224 (224-224) |
| IgG titer (U/mL), median (IQR) | 316 (176-538) |
| 4 weeks after 3rd vaccination | |
| Days after 3rd dose, median (IQR) | 28 (28-28) |
| IgG titer (U/mL), median (IQR) | 16885 (10566-21842) |
| TRAb (IU/L) | |
| Baseline, mean (SD) | 0.82 (0.07) |
| 32w, mean (SD) | 0.91 (0.14) |
| 4 weeks after the 3rd dose, mean (SD) | 1.07 (0.34) |
| TSH (μIU/mL) | |
| Baseline, mean (SD) | 2.02 (1.94) |
| 32w, mean (SD) | 1.91 (1.52) |
| 4 weeks after the 3rd dose, mean (SD) | 1.71 (1.32) |
| FT4 (ng/mL) | |
| Baseline, mean (SD) | 1.20 (0.12) |
| 32w, mean (SD) | 1.20 (0.15) |
| 4 weeks after the 3rd dose, mean (SD) | 1.18 (0.16) |
| FT3 (pg/mL) | |
| Baseline, mean (SD) | 2.94 (0.39) |
| 32w, mean (SD) | 3.02 (0.35) |
| 4 weeks after the 3rd dose, mean (SD) | 2.87 (0.39) |
| TgAb (IU/mL) | |

*(Continued)*

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TABLE 3 Continued

| Characteristics | Value |
|---|---|
| 32w, mean (SD) | 140 (459) |
| 4 weeks after the 3rd dose, mean (SD) | 174 (626) |
| TPOAb (IU/mL) | |
| 32w, mean (SD) | 48 (128) |
| 4 weeks after the 3rd dose, mean (SD) | 51 (133) |

IQR, interquartile range.
Sex was recorded as 0: male and 1: female.

manner, suggesting the possibility of progression in subclinical hyperthyroidism after the vaccine. Supportively, the responders to increase in TRAb showed less increase of TSH and more increase of FT4 than non-responders (Table S1). Conversely, FT3, and to a lesser extent FT4, was tended to be decreased after the third vaccine in the overall subjects. This might be because of non-thyroidal illness or disturbance of FT4 to FT3 conversion following the mRNA vaccination in non-responders. Further clinical evidence from studies with larger samples is required to clarify if the mRNA vaccine could have the potential to induce GD.

TABLE 4 Univariate analysis for increases of TgAb (ΔTgAb) and TgAb 4 weeks after the third dose vaccine (n = 33).

| | ΔTgAb | | TgAb (4 weeks after the third dose) | |
|---|---|---|---|---|
| Variable | β (95% CI) | *P value* | β (95% CI) | *P value* |
| Age (y) | 0.08 (-0.27 to 0.41) | .67 | 0.11 (-0.25 to 0.43) | .56 |
| Sex | 0.16 (-0.19 to 0.48) | .38 | 0.20 (-0.16 to 0.51) | .27 |
| Body mass index (kg/m$^2$) | 0.12 (-0.24 to 0.44) | .52 | 0.11 (-0.24 to 0.43) | .55 |
| Smoking | -0.11 (-0.44 to 0.24) | .55 | -0.10 (-0.43 to 0.25) | .58 |
| Alcohol (g/week) | -0.11 (-0.44 to 0.24) | .55 | -0.14 (-0.46 to 0.22) | .45 |
| Fever | -0.10 (-0.43 to 0.25) | .57 | -0.08 (-0.42 to 0.27) | .64 |
| History of thyroid disease | 0.55 (0.26 to 0.75) | .00 | 0.61 (0.33 to 0.79) | .00 |
| Family history of thyroid disease | -0.03 (-0.37 to 0.31) | .85 | -0.04 (-0.38 to 0.30) | .80 |
| Anti-SARS-CoV-2 S Ab IgG titer (U/mL) | | | | |
| Pre-vaccination (Baseline) | N/A | | N/A | |
| 4 weeks after the 2nd dose | -0.05 (-0.39 to 0.30) | .78 | 0.03 (-0.32 to 0.37) | .87 |
| 24 weeks after the 2nd dose | -0.02 (-0.36 to 0.32) | .90 | 0.04 (-0.31 to 0.38) | .84 |
| 32 weeks after the 2nd dose | 0.07 (-0.28 to 0.40) | .69 | 0.15 (-0.21 to 0.47) | .42 |
| 4 weeks after the 3rd dose | 0.02 (-0.32 to 0.36) | .90 | 0.08 (-0.28 to 0.41) | .68 |
| TRAb (IU/L) | | | | |
| Baseline | -0.04 (-0.38 to 0.31) | .82 | -0.06 (-0.39 to 0.29) | .76 |
| 32w | 0.38 (0.05 to 0.64) | .03 | 0.38 (0.04 to 0.64) | .03 |
| 4 weeks after the 3rd dose | 0.12 (-0.23 to 0.45) | .49 | 0.11 (-0.25 to 0.43) | .55 |
| ΔTRAb | 0.14 (-0.21 to 0.46) | .44 | 0.13 (-0.23 to 0.45) | .48 |
| TSH (μIU/mL) | | | | |
| Baseline | 0.00 (-0.35 to 0.34) | .99 | -0.01 (-0.35 to 0.34) | .97 |
| 32w | -0.06 (-0.39 to 0.29) | .76 | -0.06 (-0.39 to 0.29) | .75 |
| 4 weeks after the 3rd dose | -0.09 (-0.42 to 0.26) | .62 | -0.09 (-0.42 to 0.26) | .63 |
| ΔTSH | -0.09 (-0.42 to 0.26) | .63 | -0.08 (-0.41 to 0.27) | .66 |
| FT4 (ng/dL) | | | | |
| Baseline | -0.23 (-0.53 to 0.13) | .21 | -0.22 (-0.52 to 0.14) | .22 |
| 32w | -0.30 (-0.59 to 0.05) | .09 | -0.29 (-0.58 to 0.06) | .10 |
| 4 weeks after the 3rd dose | -0.27 (-0.56 to 0.08) | .12 | -0.27 (-0.56 to 0.09) | .14 |
| ΔFT4 | -0.14 (-0.46 to 0.21) | .43 | -0.14 (-0.46 to 0.21) | .43 |

*(Continued)*

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TABLE 4 Continued

| | ΔTgAb | | TgAb (4 weeks after the third dose) | |
|---|---|---|---|---|
| Variable | β (95% CI) | *P value* | β (95% CI) | *P value* |
| FT3 (pg/mL) | | | | |
| Baseline | -0.12 (-0.44 to 0.23) | .51 | -0.17 (-0.49 to 0.18) | .34 |
| 32w | -0.12 (-0.44 to 0.24) | .52 | -0.14 (-0.46 to 0.22) | .45 |
| 4 weeks after the 3rd dose | -0.19 (-0.50 to 0.17) | .30 | -0.23 (-0.53 to 0.13) | .21 |
| ΔFT3 | -0.11 (-0.43 to 0.25) | .56 | -0.08 (-0.42 to 0.27) | .64 |
| TgAb (IU/mL) | | | | |
| 32w | 0.98 (0.95 to 0.99) | .00 | 1.00 (1.00 to 1.00) | .00 |
| 4 weeks after the 3rd dose | 0.99 (0.97 to 0.99) | .00 | N/A | |
| TPOAb (IU/mL) | | | | |
| 32w | 0.76 (0.57 to 0.88) | .00 | 0.76 (0.56 to 0.87) | .00 |
| 4 weeks after the 3rd dose | 0.73 (0.52 to 0.86) | .00 | 0.73 (0.51 to 0.86) | .00 |

Sex was recorded as 0: male and 1: female. The other dichotomous variables were recorded as 0: no/absent and 1: yes/present. N/A, Not applicable.

The mechanisms of how the SARS-CoV-2 mRNA vaccine developed thyroid autoimmunity is still debatable. Besides GD and subacute thyroiditis, silent thyroiditis, concurrent GD and SAT, and painless thyroiditis have also been reported as COVID-19 vaccination-related thyroid diseases (4, 13). Two possible mechanisms that have been mainly discussed to date are the induction of the autoimmune/inflammatory syndrome induced by adjuvants (ASIA), and molecular mimicry of the SARS-CoV-2 mRNA vaccines encoding proteins that may cross-react with thyroid antigens (3, 14–18). ASIA is induced by adjuvants and develops through disruption of the host's immunological balance in genetically-susceptible subjects, involving post-vaccination autoimmune phenomena. Adjuvants are included in vaccines to enhance immune responses specifically against pathogens. However, some adjuvants can trigger adverse immune reactions, as evidenced in animal models (15, 16). The RNA-based vaccines are delivered to the host cells *via* adjuvants including lipid nanoparticles (19). Exposure to the adjuvants can potentially induce an exaggerated immune response (20) and precipitate the development of thyroid autoimmunity (3, 14, 17, 21). A review article of 83 reported cases of patients that received various types of COVID-19 vaccines, including mRNA-based, viral vector-based, and inactivated vaccines, revealed that the most cases of thyroid abnormalities following the vaccinations were observed after mRNA-based vaccines (4). The mechanisms are still unknown, but one of the reasons may be because mRNA itself acted as an adjuvant due to its intrinsic immunostimulatory properties (22). Furthermore, since the intrinsic adjuvant activity of the vaccines can trigger the innate sensors, the study to evaluate the associations between production by innate sensors, such as cytokines and thyroid function or thyroid autoimmune antibodies, would be expected to further support the effect of ASIA on thyroid autoimmunity (22).

As for the molecular mimicry of the mRNA vaccines, SARS-CoV-2 spike protein is known to cross-react with thyroid peroxidase (18). This may explain the discrepancy of the induction between TgAb and TPOAb following the mRNA vaccine, suggesting the predominant possibility of ASIA rather than molecular mimicry. As for TRAb, molecular similarity of SARS-CoV-2 spike protein and TSH receptor remains unclear, but the expression of TSH receptor has been reported to be increased during SARS-CoV-2 infection (23). We therefore speculate that both TSH receptor and SARS-CoV-2 spike protein epitope could be bound and presented by antigen-presentation cells in disease-susceptible hosts, as was reported in patients with GD (24–26). In addition, the cross-reactivity of SARS-CoV-2 with thyroid target proteins may also facilitate the triggering of ASIA syndrome. Conversely, considering incidences of the other autoimmune antibody-related diseases possibly due to the SARS-CoV-2 mRNA vaccine, such as type 1 diabetes or isolated adrenocorticotropic hormone deficiency, there may be production of new autoimmune antibodies against target endocrine organs (8, 21, 27, 28). Further studies to elucidate how the mRNA vaccine disrupts the thyroid autoimmunity are anticipated.

Another feature of this study is that we presented the factors associated with the increase of TRAb (ΔTRAb) and TgAb (ΔTgAb) after the vaccine, which could be possible predictive factors for future development of thyroid autoimmunity. Female sex and low FT4 and FT3 at baseline were candidate predictive factors for the increase of TRAb by the vaccine. The factor of female sex may suggest that those with the clinical background of autoimmune thyroid disease are prone to be affected by the vaccine. Similarly, low FT4 and FT3 at baseline associated with ΔTRAb may suggest pre-existing thyroid autoimmunity to induce hypothyroidism, such as Hashimoto's thyroiditis undetected at the initial interview. On the other hand, past history of thyroid disease and TRAb, TgAb and TPOAb before the third vaccine were candidates for the increase of TgAb after the third vaccine. These findings further suggest that thyroid autoimmune disease-prone clinical backgrounds may play an important role in disruption of thyroid autoimmunity triggered by the vaccine. Clinically, since some of these factors (sex and history of thyroid diseases) were ascertained by simple interview, this knowledge might be useful for the endocrinologists and primary care physicians in prediction of future progression or new onset of autoimmune thyroid disease at the

timing of the additional booster shots.

Regarding the interpretation and the limitations of this study, it should be noted that the majority of the participants were female, and the sample size was comparatively small. Overt autoimmune thyroid disease was not discovered in this vaccination study with the limited sample size. Nonetheless, an increase in TRAb, which was in the absence of disease manifestations, may be due to a bystander activating effect of certain B-cell clones which potentially contributes to the development of GD. On the other hand, the evaluation of thyroid autoimmunity by TgAb and TPOAb was performed in limited subjects and over a relatively short time periods, and this could be a cause of discrepancies in the significant increase in between TRAb and TgAb/TPOAb. Careful interpretation is required if the effect of current study is specific to SARS-CoV-2 mRNA vaccine. Setting a control group of participants who had received another vaccine such as seasonal flu shot would be helpful in future studies to clarify the specific effect of SARS-CoV-2 mRNA vaccine.

Other limitations of this study include the lack of measurement of cellular immunity and neutralizing antibody titers against SARS-CoV-2 and morphological assessment of thyroid with ultrasonography. In this study, there was no association between SARS-CoV-2 IgG antibody and TRAb. Despite evidence of a correlation between IgG response and protection against COVID-19, cellular immunity or neutralizing antibody titers might provide a more accurate association with thyroid autoimmunity (29, 30).

In conclusion, this is the first study to demonstrate the induction of TRAb after the SARS-CoV-2 BNT162b2 mRNA vaccine. Furthermore, this study presented several candidate factors to predict the increase of TRAb after the vaccine. In addition, this study also presented the increase of TgAb after the third dose. These findings support the increasing number of previous case reports regarding disruption of thyroid autoimmunity. There is abundant beneficial evidence of the mRNA vaccine against the severe outcomes of hospitalization and death. However, early identification of relapse or even new-onset of GD should also be considered to facilitate prompt diagnosis and initiation of treatment. Further epidemiological evidence with a larger cohort within mechanistic studies is required to confirm the associations between the SARS-CoV-2 mRNA vaccines and the development of thyroid autoimmunity.

## 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 Wakayama City Medical Association Seijinbyo Center. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

SM and TT had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. SM and TT contributed equally to this work. Concept and design: SM and TT. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: all authors. Critical revision of the manuscript for important intellectual content: SM and TT. Statistical analysis: SM and TT. Obtained funding: not applicable. Administrative, technical, or material support: SM and TT. Supervision: SM and TT. Competing interests: All the 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



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1058007/full#supplementary-material

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# Thyroxine changes in COVID-19 pandemic: A systematic review and meta-analysis

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**Objective:** COVID-19 infection may affect thyroid function. However, changes in thyroid function in COVID-19 patients have not been well described. This systematic review and meta-analysis assess thyroxine levels in COVID-19 patients, compared with non-COVID-19 pneumonia and healthy cohorts during the COVID-19 epidemic.

**Methods:** A search was performed in English and Chinese databases from inception to August 1, 2022. The primary analysis assessed thyroid function in COVID-19 patients, comparing non-COVID-19 pneumonia and healthy cohorts. Secondary outcomes included different severity and prognoses of COVID-19 patients.

**Results:** A total of 5873 patients were enrolled in the study. The pooled estimates of TSH and FT3 were significantly lower in patients with COVID-19 and non-COVID-19 pneumonia than in the healthy cohort (P < 0.001), whereas FT4 were significantly higher (P < 0.001). Patients with the non-severe COVID-19 showed significant higher in TSH levels than the severe ($I^2$ = 89.9%, *P* = 0.002) and FT3 ($I^2$ = 91.9%, *P* < 0.001). Standard mean differences (SMD) of TSH, FT3, and FT4 levels of survivors and non-survivors were 0.29 (*P*= 0.006), 1.11 (*P* < 0.001), and 0.22 (*P* < 0.001). For ICU patients, the survivors had significantly higher FT4 (SMD=0.47, *P*=0.003) and FT3 (SMD=0.51, P=0.001) than non-survivors.

**Conclusions:** Compared with the healthy cohort, COVID-19 patients showed decreased TSH and FT3 and increased FT4, similar to non-COVID-19 pneumonia. Thyroid function changes were related to the severity of COVID-19. Thyroxine levels have clinical significance for prognosis evaluation, especially FT3.

KEYWORDS

**free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), COVID-19, severity (S)**

# Introduction

COVID-19 pneumonia is caused by SARS-CoV-2 and broke out in 2019, causing unprecedented issues worldwide. SARS-CoV-2 infection can trigger systemic inflammatory symptoms involving systemic multi-organ multisystem dysfunction (1, 2). Since the pandemic outbreak, several studies have demonstrated varying degrees of impaired thyroid function in patients with COVID-19 (3, 4). Viral infections may affect thyroid function through hormones and immunoregulatory signaling molecules. However, changes in thyroid function in patients with COVID-19 have not been well described, and the particular mechanism is still controversial. Some mechanisms proposed are the virus's direct or indirect invasion of the thyroid gland, effects of systemic inflammatory immune responses, and nonspecific adaptive mechanisms (5, 6). Previous studies have examined whether thyroid diseases increase the risk of adverse outcomes in patients with COVID-19. Some studies concluded that thyroid diseases do not affect the progression of COVID-19, whereas some reported poor outcomes in patients with COVID-19 and thyroid diseases (7, 8). Several narrative and systematic reviews have revealed conflicting results about the relationship between thyroid and COVID-19, arguing that thyroid diseases are unrelated to SARS-CoV-2 infection and deterioration (9–11). Conclusions may be controversial due to the lack of large-scale clinical studies. This study aims to evaluate available evidences systematically, assess the level of thyroid function in patients with COVID-19 through meta-analysis, and analytically compare differences in thyroid function among different populations during the epidemic. Non-COVID-19 pneumonia patients and healthy people during the epidemic were included in the study as controls.

# Methods

## Protocols and registration

Our methods were based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (MOOSE) guidelines (eMethods 1 in the Supplement)and were registered in the International Prospective Register of Systematic Reviews (PROSPERO) database. The protocol number is CRD42022346568.

## Search strategy

We systematically searched PubMed/MEDLINE, Cochrane Reviews, Cochrane Central Register of Controlled Trials (Central), Web of Science and Embase databases in August 2022 without language restrictions. All published articles related to thyroid-related hormones and COVID-19 were searched. The literature search strategy was based on the following keywords: ([T3 OR FT3 OR triiodothyronine] or [T4 OR FT4 OR thyroxine] or [TSH or thyrotropin]) and (COVID-19 OR SARS-CoV-2 OR 2019 novel coronavirus). We then performed a manual search of studies meeting our inclusion criteria to identify articles apart from those found in the electronic databases. Two independent reviewers (ZL and PH) performed the first step of title/abstract screening and the second step of full-text assessment in the search process, and any disagreement that arose during this process was discussed until an agreement was reached.

## Study selection

We included observational studies in China and English language, to evaluate the correlation between COVID-19 disease and thyroxine levels. The complete list of articles obtained through the systematic search was screened to remove duplicates and exclude ineligible articles, including reviews, case reports, and studies with less than 20 patients. According to the inclusion or exclusion criteria, the full texts of all potentially qualified studies were independently reviewed by two reviewers (ZL) and (PH). Disagreements were addressed through discussion. A third reviewer (SM) resolved disagreements when a consensus could not be reached.

## Main outcomes and measures

The primary analysis assessed thyroid function in COVID-19 patients, comparing non-COVID-19 pneumonia and healthy cohorts. Secondary outcomes included different severity and prognoses of COVID-19 patients. Thyroxine levels of follow-up were also included.

## Data extraction

Basic Information, including author, country, type of study, sample size, mean or median age, sex ratio, and primary outcomes, such as death, severity, and survival, was extracted from the selected studies. The levels of thyroid hormones (FT3, FT4, and TSH) were extracted from patients in acute admission, survivors during follow-up, and deceased patients, in addition to those of healthy people and non-COVID-19 pneumonia patients during the pandemic. All extracted data were tabulated, and indexes measured by each research center were converted and unified.

We extracted data using standardized data abstraction forms. In case of missing data needed to conduct our meta-analyses, we contacted the authors, with a reminder 2 weeks later. Non-published data obtained from authors by communication are mentioned in the results section below, as applicable, with permission, and authors who responded are listed in Acknowledgments section.

## Quality assessment

Two reviewers independently assessed the risk of biases, including selection, performance, detection, attrition, and reporting biases, rated as low, high, or unclear risk. The quality of the included studies was evaluated using the Newcastle–Ottawa scale (eTables 1, 2 in the Supplement). The scale has a score of nine, and a seven or

higher indicates high quality. Two reviewers performed data extraction and quality assessment independently, and a third reviewer checked the results. Disagreements were resolved by discussion.

## Statistical analysis

Continuous data (thyroxine levels: TSH, FT3, and FT4) were synthesized using mean difference (MD) with standard deviation from each study for the calculation of the average MD with a 95% confidence interval (CI). We applied Wan's formula to estimate the relative means and standard deviations when continuous data were presented as medians and ranges (12). Adjusted SMD based on corrected data and other potential confounders were also presented. Meta-analyses were performed using the inverse variance method with the random-effects model when heterogeneity was statistically significant. The DerSimonian-Laird method with the fixed-effect model was used when heterogeneity was not statistically significant. Heterogeneity was assessed using Cochrane Q-test and $I^2$ statistic, and a p-value of <0.05 indicated statistically significant heterogeneity. According to the Cochrane Handbook for Systematic Reviews of Interventions, the ranges of interpretation for $I^2$ are as follows: 0%–40%, unimportant; 30%–60%, moderate heterogeneity; 50%–90%, substantial heterogeneity; and 75%–100%, considerable heterogeneity. All analyses were conducted using RevMan (version 5.4.1).

# Results

The search time was up to August 2022, 635 relevant articles were screened, and 523 articles were excluded according to the titles and abstracts (Figure 1). The full texts of 112 articles were reviewed. Finally, 41 articles from 38 studies were included in the final analysis, totaling 5873 COVID-19 patients (13–38) (Figure 2). The control group included 269 patients with non-COVID-19 pneumonia and 1052 healthy people during the epidemic. The Characteristics of the included studies are presented in Table 1.

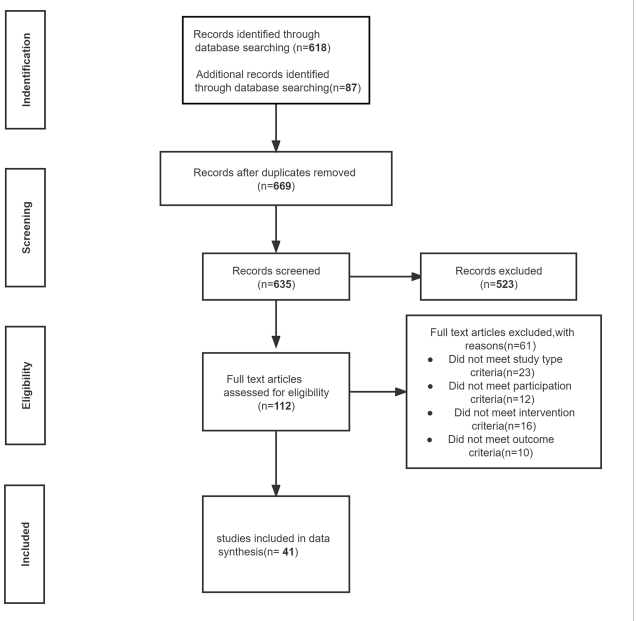


FIGURE 1
Flow diagram of the study selection process.

## Thyroxine levels at admission

A total of 36 studies recorded thyroxine levels at admission, including 5752 cases (13–24, 26, 38, 39). eFigure 1 in the Supplement depicts the pooled estimates of TSH (SMD = 1.46, 95% CI, [1.29, 1.62]), FT3 (SMD = 3.86, 95% CI, [3.59, 4.13]), and FT4 levels (SMD = 15.97, 95% CI, [15.24, 16.70]) by a mixed-effected model, including thirteen East Asian studies, six South Asian studies, three Asian studies, twelve European studies, one American study, and one African study. Subgroup analyses of Asian and European studies were performed (eFigure 2 in the Supplement). The pooled results suggested that high heterogeneity in TSH level ($I^2$ = 92.9%, $p$ < 0.001) and FT3 level ($I^2$ = 80.3%, $p$ = 0.02) but not in FT4 levels ($I^2$ = 0.0%, $p$= 0.81).

## Thyroxine levels in non-COVID-19 pneumonia and healthy cohort in the COVID-19 pandemic

Six studies recorded the thyroxine levels of healthy cohorts as control groups (n = 1052). The pooled estimates of TSH, FT3, and FT4 levels were 1.78 (95% CI, [1.56, 2.01]), 4.76 (95% CI, [4.44, 5.08]), and 14.03 (95% CI, [12.93, 15.13]), respectively (eFigure 3 in the Supplement) (14, 18, 36, 40–42). Five studies included the thyroxine levels of non-COVID-19 pneumonia patients (n = 269) (14, 18, 42–44). The pooled estimates of TSH, FT3, and FT4 levels were 1.22 (95% CI, [1.05, 1.40[), 3.66 (95% CI, [3.48, 3.83]), and 18.19 (95% CI, [15.61, 20.78]), respectively. TSH and FT3 levels were significantly lower in patients with COVID-19 and non-COVID-19 pneumonia than in the healthy cohort ($p$< 0.001), whereas FT4 levels were significantly higher than in the healthy cohort ($p$< 0.001).

## Thyroxine levels in patients with different levels of COVID-19 severity

According to the results of single-arm meta-analysis (eFigure 4 in the Supplement), patients with non-severe COVID-19 (n = 1697) and severe COVID-19 (n = 1022) showed significant heterogeneity in TSH level ($I^2$ = 89.9%, $P$ = 0.002) and FT3 level ($I^2$ = 91.9%, $P$ < 0.001). Patients with non-severe COVID-19 had higher TSH and FT3 levels, and no heterogeneity in FT4 levels was found between the groups ($I^2$ = 0.0%, $P$ = 0.72). Ten articles compared patients with severe and non-severe COVID-19 in terms of thyroid function. Differences in the three indicators were statistically significant, and the pooled estimates of TSH, FT3, and FT4 levels were 0.40 ($P$ = 0.010), 0.79 ($P$ < 0.001), and −0.18 ($P$ = 0.03), respectively. Three studies of the WHO classified COVID-19 severity compared patients with non-severe and severe COVID-19 (7, 28, 45). The results

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| Firts author, Year of publication | | NO. of study | NO. of patients | SMD 95% CI |
|---|---|---|---|---|
| **TSH (μIU/mL)** | | | | |
| Healthy Cohort (I²=89%) | | 6 | 1052 | 1.78 [1.56, 2.01] |
| Non-COVID-19 pneumonia (I²=33%) | | 5 | 269 | 1.22 [1.05, 1.40] |
| Covid-19 pheumonia (I²=97%) | | 36 | 5739 | 1.46 [1.29, 1.62] |
| Severity (P=0.002) | Non-severe (I²=96%) | 13 | 1697 | 1.77 [1.42, 2.13] |
| | Severe (I²=95%) | 19 | 1022 | 1.09 [0.86, 1.32] |
| Prognosis (P=0.002) | Suvivor (I²=95%) | 13 | 1311 | 1.21 [0.98, 1.45] |
| | Non-survivor (I²=0%) | 9 | 275 | 0.83 [0.73, 0.92] |
| Follow_up (I²=79%) | | 7 | 543 | 1.53 [1.36, 1.70] |
| **FT3 (pmol/L)** | | | | |
| Healthy Cohort (I²=93%) | | 5 | 245 | 4.76 [4.44, 5.08] |
| Non-COVID-19 pneumonia (I²=0%) | | 2 | 100 | 3.66 [3.48, 3.83] |
| Covid-19 pheumonia (I²=99%) | | 25 | 4385 | 3.86 [3.59, 4.13] |
| Severity (P<0.001) | Non-severe (I²=99%) | 10 | 1648 | 4.10 [3.67, 4.53] |
| | Severe (I²=95%) | 13 | 734 | 3.19 [2.92, 3.46] |
| Prognosis (P=0.008) | Suvivor (I²=99%) | 8 | 987 | 3.57 [3.01, 4.14] |
| | Non-survivor (I²=87%) | 7 | 225 | 2.77 [2.59, 2.95] |
| Follow_up (I²=98%) | | 4 | 388 | 4.74 [4.36, 5.11] |
| **FT4 (pmol/L)** | | | | |
| Healthy Cohort (I²=95%) | | 4 | 188 | 14.03 [12.93, 15.13] |
| Non-COVID-19 pneumonia (I²=85%) | | 2 | 100 | 18.19 [15.61, 20.78] |
| Covid-19 pheumonia (I²=98%) | | 28 | 5119 | 15.97 [15.24, 16.70] |
| Severity (P=0.72) | Non-severe (I²=97%) | 9 | 1141 | 16.25 [14.96, 17.55] |
| | Severe (I²=95%) | 13 | 1027 | 15.98 [15.16, 16.79] |
| Prognosis (P=0.80) | Suvivor (I²=99%) | 9 | 1028 | 15.41 [13.73, 17.08] |
| | Non-survivor (I²=97%) | 10 | 370 | 15.11 [13.45, 16.77] |
| Follow–up (I²=99%) | | 5 | 438 | 14.07 [11.90, 16.25] |

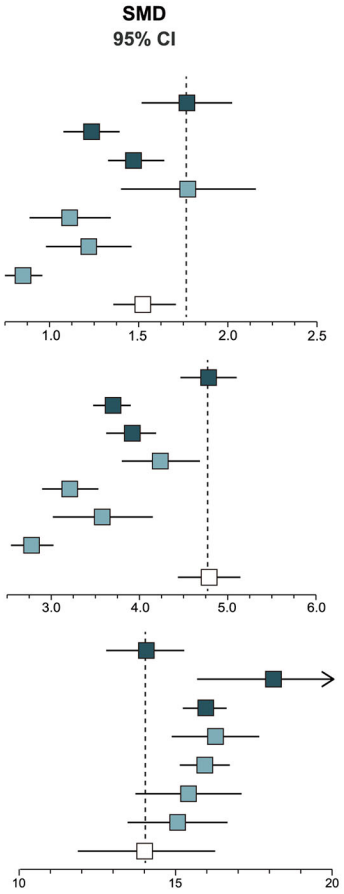


FIGURE 2
Forest plot for all studies.

suggested no significant differences in TSH level ($P$ = 0.54) and FT3 level ($P$ = 0.06) between non-severe and severe patients, and only FT4 levels ($P$ = 0.006) were significantly different (eFigure 5 in the Supplement), which were higher in patients with severe COVID-19. Significant heterogeneity in differences in TSH level ($I^2$ = 91.0%, $P$ < 0.001) and FT3 level ($I^2$ = 91.0%, $P$ < 0.001) was found in studies that used the WHO's classification, whereas no significant heterogeneity was found in difference in FT4 levels ($I^2$ = 0.0%, $P$ = 0.39). Non-WHO analysis used an SpO2 of 93% or 94% as the cutoff between severe and non-severe COVID-19. Seven studies that used non-WHO criteria compared the thyroid functions of patients with severe and non-severe COVID-19 (13, 14, 18, 29, 43, 46, 47). The pooled estimates suggested that TSH levels were significantly lower in patients with severe COVID-19 ($P$ < 0.001), and moderate heterogeneity in differences in TSH levels was found ($I^2$ = 52.0%, $P$ = 0.05). FT3 levels were lower in patients with severe COVID-19 ($P$ < 0.001), and no significant heterogeneity in differences in FT3 levels was found ($I^2$ = 0.0%, $P$ =0.47). However, no difference in FT4 level was observed ($P$ = 0.61). Only differences in FT4 levels were heterogeneous in the subgroup heterogeneity analysis ($I^2$ = 64.9, 6%, $P$ = 0.09). No significant differences in TSH level ($I^2$ = 0.0%, $P$ = 0.61) and FT3 level ($I^2$ = 0.0%, $P$ = 0.92) were found between the WHO and non-WHO clinical classifications.

## Thyroxine levels in different prognoses of patients with COVID-19

The single-arm meta-analysis (eFigure 6 in the supplement) showed no significant heterogeneity in FT4 levels ($I^2$ = 0.0%, $P$=0.80) and significant heterogeneity in TSH level ($I^2$ = 89.4%, $P$=0.002) and FT3 level ($I^2$ = 85.8%, $P$=0.008) in survivors (n = 1311) and non-survivors (n = 275). Nine articles compared the thyroxine levels of survivors and non-survivors(eFigure 7 in the Supplement). Differences in TSH, FT3, and FT4 levels were statistically significant, with a SMD values of 0.29 ($P$ = 0.006), 1.11 ($P$ < 0.001), and 0.22 ($P$ < 0.001), respectively. According to the reference ranges, differences in TSH and FT4 levels were small. In the analysis of ICU patients, three studies compared survivors and non-survivors (17, 24, 32). No significant difference in TSH ($P$ = 0.74) levels was found among ICU patients, but significant difference in FT4 ($P$ = 0.003) and FT3 ($P$ = 0.001) levels were found. The survivors had significantly higher FT4 and FT3 levels than non-survivors. The pooled mean differences in FT3 and FT4 levels were 0.51 (95% CI, [0.21, 0.82]) and 0.47 (95% CI, [0.16, 0.77]). Six studies included COVID-19 patients in all wards (13, 19, 24, 29, 35, 48). Significant differences in TSH ($P$ < 0.001), FT3 ($P$ < 0.001) and FT4 ($P$ = 0.02) levels were found, with mean differences of 0.46 (95% CI,[0.25,0.67]),

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TABLE 1 Description of eligible studies reporting the association between thyroid-related hormones and COVID-19.

| Author | Country | No. patients | Sex | Average age | Severity | Outcome | Control group | Thyroxine |
|---|---|---|---|---|---|---|---|---|
| Ahn (2021) | Korea | 119 | Male: 62 | 64 | Non-severe: 32 | Survivor: 85 | | TSH FT4 |
| | | | Female: 57 | | Severe: 87 | Non-survivor: 34 | | FT4 |
| Ardes (2021) | Italy | 118 | Male: 64 | 73 | Non-severe: 75 | Survivor: 92 | | TSH |
| | | | Female: 54 | | Severe: 43 | Non-survivor: 26 | | |
| Assimakopouls (2021) | Greece | 22 | Male: 11 | 62 | Non-severe: 13 | | Healthy cohort: 19 | TSH |
| | | | Female: 11 | | Severe: 9 | | Non-COVID-19 pneumonia: 19 | FT3 |
| | | | | | | | | FT4 |
| Baldelli (2021) | Italy | 46 | Male: 32 | 60 | Non-ICU: 23 | | | TSH |
| | | | Female: 14 | | ICU: 23 | | | FT3 |
| Beltrao (2021) | Brazil | 245 | Male: 145 | | Non-critical: 181 | Survivor: 204 | | TSH |
| | | | Female: 100 | | Critical: 64 | Non-survivor: 41 | | FT3 |
| | | | | | | | | FT4 |
| Campi (2021) | Italy | 73 | | | ICU: 73 | Survivor(ICU): 57 | | TSH |
| | | | | | | Non-survivor(ICU): 16 | | |
| Chen (2020) | China | 274 | Male: 171 | 59 | | Recover: 161 | | TSH |
| | | | Female: 103 | | | Death: 113 | | FT3 |
| Chen (2021) | China | 50 | Male: 33 | 48 | Non-severe: 15 | Survivor: 50 | Healthy cohort: 54 | TSH |
| | | | Female: 21 | | Severe: 35 | | Non-COVID-19 pneumonia: 50 | |
| Clarke (2021) | UK | 70 | Male: 47 | 56 | Non-severe: 42 | Survivor: 70 | | TSH |
| | | | Female: 23 | | Severe: 28 | | | FT3 |
| | | | | | | | | FT4 |
| Clausen (2021) | Denmark | 116 | Male: 44 | 71 | | Survivor: 82 | | TSH |
| | | | Female: 72 | | | Non-survivor: 34 | | FT4 |
| Dabas (2021) | India | 164 | Male: 107 | 41 | Non-severe: 100 | | | TSH |
| | | | Female: 57 | | Severe: 64 | | | FT3 |
| | | | | | | | | FT4 |
| Das (2021) | India | 84 | Male: 42 | 41 | Non-severe : 499 | | | TSH |

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| Author | Country | No. patients | Sex | Average age | Severity | Outcome | Control group | Thyroxine |
|---|---|---|---|---|---|---|---|---|
| | | | Female: 42 | | Severe: 35 | | | FT3 |
| | | | | | | | | FT4 |
| Dutta (2021) | India | 236 | Male: 159 | 54 | Non-severe: 200 | Survivor: 225 | | TSH |
| | | | Female: 77 | | Severe: 36 | Non-survivor: 11 | | FT3 |
| | | | | | | | | FT4 |
| Gao (2021) | China | 100 | Male: 52 | 63 | Non-sever: 34 | Survivor: 44 | | TSH |
| | | | Female: 48 | | Severe: 66 | Non-survivor: 22 | | FT3 |
| | | | | | | | | FT4 |
| Gong (2021) | China | 150 | Male: 81 | 70 | Non-sever: 25 | Survivor: 118 | | TSH |
| | | | Female: 69 | | Severe: 125 | Non-survivor: 42 | | FT3 |
| | | | | | | | | FT4 |
| Grondman (2021) | Netherlands | 161 | Male: 56 | 65 | Non-ICU: 120 | Survivor: 141 | | TSH |
| | | | Female: 105 | | ICU: 41 | Non-survivor: 20 | | FT4 |
| Gliven (2021) | Turkey | 250 | Male: 157 | 67 | Non-ICU: 125 | Survivor(ICU): 88 | | TSH |
| | | | Female: 93 | | ICU: 125 | Non-survivor(ICU): 37 | | FT3 |
| | | | | | | | | FT4 |
| Khoo (2020) | UK | 334 | Male: 203 | 66 | | Survivor: 239 | | TSH |
| | | | Female: 131 | | | Non-survivor: 95 | | FT4 |
| Kumar (2021) | India | 235 | Male: 147 | 49 | Non-severe: 202 | Survivor: 222 | | TSH |
| | | | Female: 88 | | Severe: 33 | Non-survivor: 13 | | FT3 |
| | | | | | | | | FT4 |
| Lang (2021) | China | 127 | Male: 62 | 64 | Non-severe: 56 | Survivor: 116 | | TSH |
| | | | Female: 65 | | Severe: 71 | Non-survivor: 11 | | FT3 |
| | | | | | | | | FT4 |
| Li (2020) | China | 40 | | 44 | Non-severe: 40 | | Healthy cohort: 57 | TSH |
| | | | | | | | | FT3 |
| | | | | | | | | FT4 |
| Lui (2020-2021) | China | 541 | Male: 245 | 50 | Non-severe: 499 | Survivor: 283 | | TSH |
| | | | Female: 196 | | Severe: 42 | | | FT3 |

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| Author | Country | No. patients | Sex | Average age | Severity | Outcome | Control group | Thyroxine |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | FT4 |
| Malik (2021) | Pakistan | 48 | Male: 31 | 51 | Non-severe: 22 | Survivor: 48 | Non-COVID-19 pneumonia: 28 | TSH |
| | | | Female: 17 | | Severe: 26 | | | |
| Nakamura (2021) | Japan | 147 | Male: 95 | 70 | Non-severe: 63 | | | TSH |
| | | | Female: 52 | | Severe: 84 | | | FT3 |
| | | | | | | | | FT4 |
| Okoye (2022) | Italy | 95 | Male: 50 | 82 | | Survivor: 70 | Non-COVID-19 pneumonia: 81 | TSH |
| | | | Female: 45 | | | Non-survivor: 25 | | FT3 |
| | | | | | | | | FT4 |
| Okwor (2021) | Nigeria | 45 | Male: 95 | 35 | | | Healthy cohort: 45 | TSH |
| | | | Female: 52 | | | | | FT3 |
| | | | | | | | | FT4 |
| Schwarz (2021) | Israel | 54 | Male: 37 | 59 | Non-ICU: 37 | Survivor: 44 | | TSH |
| | | | Female: 17 | | ICU: 17 | Non-survivor: 10 | | FT3 |
| | | | | | | | | FT4 |
| Sciacchitano (2021) | Italy | 62 | Male: 29 | 67 | | | | TSH |
| | | | Female: 33 | | | | | FT3 |
| | | | | | | | | FT4 |
| Sen (2021) | India | 60 | | | Non-severe: 42 | | | TSH |
| | | | | | Severe: 18 | | | FT3 |
| | | | | | | | | FT4 |
| Sparano (2022) | Italy | 506 | Male: 315 | 69 | Non-severe: 506 | | | TSH |
| | | | Female: 191 | | | | | FT4 |
| Urhan(2022) | Turkey | 64 | Male: 32 | 39 | Non-severe: 38 | Survivor: 64 | Healthy cohort: 70 | FT3 |
| | | | Female: 32 | | Severe: 26 | | | |
| Vassiliadi (2021) | Greece | 102 | Male: 76 | 55 | Non-ICU: 61 | Survivor: 88 | | TSH |
| | | | Female: 26 | | ICU: 41 | Non-survivor: 14 | | FT4 |
| Vizoso (2021) | Spain | 78 | Male: 55 | 62 | ICU: 78 | Survivor(ICU): 55 | | TSH |
| | | | Female: 23 | | | Non-survivor(ICU): 23 | | FT3 |
| | | | | | | | | FT4 |

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| Author | Country | No. patients | Sex | Average age | Severity | Outcome | Control group | Thyroxine |
|---|---|---|---|---|---|---|---|---|
| Wang (2021) | China | 84 | Male: 53 | 57 | Non-severe: 21 | Survivor: 84 | Healthy cohort: 807 | TSH |
| | | | Female: 31 | | Severe: 63 | | Non-COVID-19 pneumonia: 91 | |
| Yazan (2021) | Turkey | 205 | Male: 113 | 58 | Non-ICU: 174 | Survivor: 196 | | TSH |
| | | | Female: 92 | | ICU: 31 | Non-survivor: 95 | | FT3 |
| | | | | | | | | FT4 |
| Zhao (2022) | China | 384 | Male: 197 | 64 | Non-severe: 161 | Survivor: 219 | | TSH |
| | | | Female: 87 | | Severe: 212 | Non-survivor: 16 | | FT3 |
| | | | | | | | | FT4 |
| Zheng (2021) | China | 235 | Male: 112 | 60 | | Survivor: 219 | | TSH |
| | | | Female: 123 | | | Non-survivor: 16 | | FT3 |
| | | | | | | | | FT4 |
| Zou (2020) | China | 149 | Male: 71 | 49 | Non-severe: 123 | Survivor: 146 | | TSH |
| | | | Female: 78 | | Severe: 26 | Non-survivor: 1 | | FT4 |

1.39 (95% CI,[0.86,1.92]), and 0.17 (95% (49) CI,[0.03.0.31]), respectively. All three indexes were higher in survivors.

## Thyroxine levels during follow-up

Seven studies reassessed thyroid function after acute COVID-19 (eFigure 8 in the Supplement) (17, 18, 27, 34, 41, 43, 50). The pooled estimates of TSH, FT3, and FT4 levels were 1.53 (95% CI, [1.36, 1.70]), 4.74 (95% CI, [4.36, 5.11]), and 14.07 (95% CI, [11.90, 16.25]), respectively, without significant heterogeneity across studies. TSH, FT3, and FT4 levels were recovered during follow-up compared with the acute period. TSH and FT3 levels increased, whereas FT4 levels decreased compared with the acute phase.

# Discussion

SARS-CoV-2 can damage multiple organs, including the lungs, liver, heart, brain, and kidneys, leading to systemic symptoms. The thyroid gland highly expresses the ACE2 receptor. Thus, the hypothalamic–pituitary–thyroid axis may be susceptible to the disturbance in patients with COVID19 (22, 34, 41, 51, 52). SARS-CoV, a coronavirus related to SARS-CoV-2, injures thyroid parafollicular and follicular cells (53). Lui et al. found that high SARS-CoV-2 viral loads were associated with small thyroid volumes (37). This association suggested a direct viral effect on the thyroid gland. Lania et al. revealed that COVID-19 might be associated with the high risk of thyrotoxicosis (n = 31, 10.8%) in a retrospective study that enrolled 287 patients (54). However, the autopsy results suggested the absence of the virus was in thyroid tissues (55–57). The number of reported thyrotoxicosis cases in literature did not exponentially increase, including critically ill patients (13, 15, 28, 32, 34). Thyrotoxicosis may be a rare complication of COVID-19 (51).

Many studies revealed that thyroid function significantly changes during COVID-19 infection. The trends of thyroxine level fluctuations in patients with COVID-19 and non-COVID-19 pneumonia were similar (decreased TSH and FT3 levels and increased FT4 levels). This similarity suggests that COVID-19 and non-COVID-19 pneumonia affect the thyroid gland through similar mechanisms. In addition, thyroxine levels during follow-up indicated progressive improvement and transient hormone changes (17, 34). Patients suffering from COVID-19 and thyroxine fluctuation potentially encountered non-thyroidal illness syndrome (NTIS) induced by systemic inflammation (58, 59). NTIS is an adaptive response to stress, critical illness, and malnutrition, manifested by a decrease in FT3 levels or decreases in TSH, FT3, and FT4 levels in severe disease (58, 60). Since systemic inflammation potentially impacts the de-iodinase activity, it inhibits T4–T3 conversion decreases FT3 levels and increases FT4 levels (17, 61, 62). Elevated FT4 level upon admission tends to be mistaken for thyrotoxicosis. Most studies have investigated thyroxine changes in COVID-19 at admission. NTIS in patients with long-term critical illness shows symptoms similar to hypothyroidism (59, 63, 64). Unlike thyrotoxicosis, treatment with thyroid hormone is not recommended without clinical signs of hypothyroidism. Even mild hypothyroidism can be considered a physiologically favorable

condition that can suppress energy expenditure and eventually restrict catabolism by decreasing thyroid hormone activity (63). Thyroid function can recover in patients without intervention (18, 58, 65, 66).

Immunoassays for thyroxine can be affected by alterations in serum binding protein that occurs in various physiological states (20). Decreased TSH and increased FT4 levels were reported in the healthy cohort during the COVID-19 outbreak (67). During the pandemic, relationships were found between thyroid diseases and psychiatric factors, such as anxiety and depression (68, 69). The pandemic socio-psychological sequelae can constitute stressors for the population, potentially affecting the thyroid gland. Collectively, patients with COVID-19 are at risk of thyroid diseases and require attention.

Thyroid function parameters have clinical significance in determining disease severity and prognosis of COVID-19. Whether the severity of COVID-19 is associated with thyroid function remains unclear. Some studies reported no significant relationship between COVID-19 severity and thyroid function, whereas other studies have suggested that only some statistically significant indicators compare disease severity (7, 13, 14, 28, 45, 47). It may be related to multiple factors, including different criteria adopted in different regions. The classifications of COVID-19 severity vary. The WHO uses SpO2 of less than 90% as a cutoff between severe and non-severe COVID-19. By contrast, the United States, China, and Japan use 93% or 94% as a division basis in diagnosis and treatment guidelines (14, 19, 47, 70). In the present study, the single-arm meta-analysis results revealed that only TSH and FT3 levels were significantly different compared to patients with severe-critical and non-severe COVID-19. The case-control study showed that although the FT4 levels differed significantly, the difference was only 0.18, with minor practicality. In the acute phase, TSH and FT3 levels at admission in COVID-19 patients can be used in assessing patient severity.

Thyroid function is not a routine test indicator in patients infected with COVID-19. Some studies have suggested that thyroid function cannot be used in evaluating the prognosis of patients, or only some indicators can be used in the prognostic analysis (13, 32, 48, 71). In the present study, TSH, FT3, and FT4 levels presented significant differences between survivors and deceased patients at admission. The results of the single-arm meta-analysis suggested that only TSH and FT3 levels were heterogeneous between survivors and non-survivors. The controlled studies showed statistically significant differences in the three thyroid function parameters. However, we found that differences in TSH (SMD = 0.29) and FT4 (SMD = 0.22) levels were limited for assessing prognosis. Compared with TSH and FT4, FT3 levels may be more effective (SMD = 0.79). A low FT3 level is an outcome predictor, especially in severe patients (5). The excessive production of proinflammatory cytokines during SARS-CoV-2 infection aggravates ARDS and tissue damage resulting in multi-organ failure and death (72, 73). In patients with COVID-19, FT3 levels decrease with the increasing levels of inflammatory cytokines. Some studies have suggested that improving thyroid function can improve patient outcomes. However, a reduction in FT3 levels occurs before clinical symptoms worsen. This reduction can be used in assessing changes in patient condition and prognosis (74).

Survivor bias may have been present when prognostic thyroid function was being determined. Thyroid function is an unconventional test indicator for patients with COVID-19. Medical centers tend to assess the thyroid function in patients with underlying thyroid diseases or other severe illnesses. Moreover, the pharmacological doses of steroids in severe COVID-19 can affect thyroid function. Therefore, comprehensive extensive clinical studies are needed to evaluate the significance of thyroid function assessment for patients with COVID-19.

## Limitations

This study has several limitations. First, thyroid function is not a routine test indicator for COVID-19. Many studies have a selection bias for patients tested for thyroid function levels and have an incomplete recording of test results, especially TT3 and TT4 results. Second, heterogeneity was found among the included studies. Third, thyroid function often showed non-normal distribution. The median percentage transformation was used in the meta-analysis. Fourth, the cohort studies' sample size with a detailed thyroxine record was limited after reasonable sorting.

# Conclusion

Thyroxine levels (TSH, FT3, and FT4) fluctuated in patients with COVID-19. Compared with the healthy cohort, patients with COVID-19 showed decreased TSH and FT3 levels and increased FT4 levels. There were differences in thyroxine levels between severe and non-severe patients. No significant difference in TSH levels between severe and non-severe patients according to the WHO classification criteria, whereas FT4 levels were not significantly different in the studies using non-WHO classification criteria. However, FT3 levels were significantly lower in severe patients than in non-severe patients in the included study. In addition, different thyroid function parameters were assessed differently with regard to patient outcomes. TSH and FT4 levels have limitations in the prognostic evaluation of ICU patients and are ineffective in assessing patient outcomes. In general wards, TSH and FT4 are still effective, but the clinical application value is limited due to slight differences. FT3 levels can be adapted as an outcome assessment indicator for patients in ICU or not, with a reliable scope of application. Following the resolution of COVID-19 pneumonia, thyroid function gradually recovers in survivors during follow-up.

# 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

This is a meta-analysis based on observational studies. Informed consent from patients and ethical approval for this type of study are not required.

## Author contributions

ZL and PH had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. ZL and PH contributed equally. Concept and design: ZL, PH, HM, SM, MF, SW, YF. Acquisition, analysis, or interpretation of data: ZL, PH, SM, MF, HW, WZ, YC, SW, YF. Drafting of the manuscript: ZL, PH, SW, YF. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: ZL, SW, YF. Administrative, technical, or material support: SM, MF, HW, WZ. Supervision: HM, SM, MF, HW, WZ, YC, PH, SW, YF. Other - protocol review: ZL. 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.

The reviewer CL declared a shared affiliation with the authors PH and SM to the handling editor at the time of review.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1089190/full#supplementary-material

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# Increased prevalence of autoimmune thyroid disease after COVID-19: A single-center, prospective study

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**Introduction:** Thyroid dysfunctions associated with SARS-CoV-2 acute infection have been extensively described since the beginning of COVID-19 pandemics. Conversely, few data are available on the occurrence of thyroid autoimmunity after COVID-19 resolution. We assessed the prevalence of autoimmune thyroid disease (ATD) and thyroid dysfunctions in COVID-19 survivors three months after hospital admission.

**Design and methods:** Single-center, prospective, observational, cohort study performed at ASST Papa Giovanni XXIII Hospital, Bergamo, Italy. 599 COVID-19 survivors were prospectively evaluated for thyroid function and autoimmunity thyroperoxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb). When a positive antibody concentration was detected, thyroid ultrasound was performed. Multiple logistic regression model was used to estimate the association between autoimmunity and demographic characteristics, respiratory support, and comorbidities. Autoimmunity results were compared to a cohort of 498 controls referred to our Institution for non-thyroid diseases before the pandemic onset. A sensitivity analysis comparing 330 COVID-19 patients with 330 age and sex-matched controls was performed.

**Results:** Univariate and multivariate analysis found that female sex was positively associated (OR 2.01, SE 0.48, p = 0.003), and type 2 diabetes (T2DM) was negatively associated (OR 0.36, SE 0.16, p = 0.025) with thyroid autoimmunity; hospitalization, ICU admission, respiratory support, or COVID-19 treatment were not associated with thyroid autoimmunity (p > 0.05). TPOAb prevalence was greater in COVID-19 survivors than in controls: 15.7% vs 7.7%, p = 0.002. Ultrasonographic features of thyroiditis were present in 94.9% of the evaluated patients with positive antibodies. TSH was within the normal range in 95% of patients.

**Conclusions:** Autoimmune thyroid disease prevalence in COVID-19 survivors was doubled as compared to age and sex-matched controls, suggesting a role of SARS-CoV-2 in eliciting thyroid autoimmunity.

KEYWORDS

SARS-CoV-2, COVID-19, thyroid peroxidase (TPO) antibodies, thyroiditis, autoimmune diseases, thyroid dysfunctions, thyroid autoimmunity, autoimmune thyroid disease

# 1 Introduction

Viral infections may trigger autoimmune diseases (1). Reports of autoimmune conditions occurring after SARS-CoV-2 infection have been described (2), including anecdotal cases of Graves' disease (3–5) and Hashimoto's thyroiditis (6, 7). However, only few studies systematically evaluated the impact of COVID-19 in the development of autoimmune thyroid disease (ATD). Anaya et al. (8) found an increased prevalence of thyroperoxidase antibodies (TPOAb) in 120 patients hospitalized for COVID-19 as compared to healthy, pre-pandemic controls, suggesting an activation of thyroid autoimmunity by SARS-CoV-2. Consistently, Lui et al. (9) reported an increase in TPOAb concentration in COVID-19 survivors three months after hospital admission. However, most patients in this cohort were treated with interferon beta (IFN-beta) that has been associated per se with the induction of thyroid autoimmunity; the reassessment of a larger cohort of patients not exposed to IFN-beta was thus advocated by the Authors to provide a conclusive answer.

Alterations of thyroid function tests (TFTs) during the acute phase of COVID-19 have been more extensively characterized since the beginning of the pandemics (10, 11). Low TSH levels, attributed either to a destructive thyroiditis associated with thyrotoxicosis or to a non-thyroidal illness (NTI), were reported in several studies (12–18). According to most studies (12–14, 16, 19), TFTs usually normalize after COVID-19 recovery, but this finding has not been established in a large population.

Aim of our study was to assess the prevalence of ATD and thyroid dysfunction in a large cohort of COVID-19 survivors at a medium-term (three months) follow-up after hospitalization.

# 2 Materials and methods

## 2.1 Study cohort

COVID-19 survivors participating to our outpatient service program were eligible for the study. The enrollment protocol has been described in a previous paper (20). Briefly, a list of all patients with COVID-19 discharged from the emergency department or admitted to the hospital wards of our Institution (ASST Papa Giovanni XXIII, Bergamo, Italy) was obtained from the hospital electronic health records database. Asymptomatic positive patients admitted for planned procedures were excluded. Other exclusion criteria were: age less than 18 years, pregnancy, history of thyroid disease or previous thyroid surgery, concomitant medications known to interfere with thyroid function (lithium, amiodarone, interferon-α and antiretroviral drugs), severe kidney insufficiency (eGFR < 30 ml/min), and severe liver failure. Patients' enrollment took place between 2 May and 31 July 2020, before availability of SARS-CoV-2 vaccines, to avoid potential biases due to occurrence of post-vaccination thyroid disorders (21, 22).

To compare thyroid autoimmunity data, a control group was retrieved from the hospital electronic health records database. Controls were included if i) had one assessment of TPOAb and/or thyroglobulin antibodies (TgAb) from January 2016 to January 2020, ii) their medical history was negative for thyroid disease, and iii) they referred to our Institution for reasons other than a suspected thyroid disease.

## 2.2 Assays

Thyroid stimulating hormone (TSH), TPOAb, and TgAb were measured in all patients; free thyroxine (fT4) and free triiodothyronine (fT3) were measured in patients with abnormal TSH levels. A chemiluminescent immunoassay (Atellica Solution, Siemens) was employed. Normal range for TSH, fT4 and fT3 were 0.5-5.0 mIU/L, 0.7-1.8 ng/dL, and 2.3-4.5 pg/mL, respectively. For TPOAb, measuring interval was 28-1300 U/mL and range of normality was below 60 U/mL. For TgAb, measuring interval was 15-500 U/mL and range of normality was below 60 U/mL.

## 2.3 Ultrasound assessment

When positive antibodies were detected, ultrasonography of the thyroid was prescribed. Thyroid volume was calculated with the ellipsoid formula (23): width (mm) x length x thickness x 0.52 = volume (mL) for each lobe. Ultrasonographic diagnosis of thyroiditis was made if one or more of the following features were present: hypoechogenity of gland parenchyma, non-homogeneous parenchymal texture, and increased vascularity. All thyroid ultrasound examinations were performed by two operators (AR and SC) with the same instrument (My Lab Seven, Esaote, Italy), using a 3- to 13-MHz linear transducer.

## 2.4 Statistical analysis

Descriptive statistics was used to summarize clinical characteristics of COVID-19 patients during the acute phase of the disease and at the subsequent clinical evaluation. Continuous variables were expressed as medians and interquartile ranges (IQRs) and categorical variables were presented as frequencies and percentages. The study population was then stratified based on the presence of thyroid autoimmunity (yes/no), and differences between groups were tested using the Mann-Whitney test for continuous variables and the chi-square test (or Fisher's exact test when appropriate) for categorical variables. To evaluate the association of thyroid autoimmunity and COVID-19, we conducted a sensitivity analysis comparing 330 COVID-19 patients with 330 age and sex-matched subjects retrieved from control group.

A multiple logistic regression model was used to estimate odds ratios (ORs) of autoimmunity and their corresponding 95% confidence intervals (CIs) for the following variables: age (at entrance), sex, respiratory support (no support/low need/high need), and diabetes mellitus (yes/no). In the multivariable analysis were included demographic characteristics, respiratory support (as proxy of disease severity) and covariates that resulted significantly different between groups in the univariate analysis. For all tested hypotheses, two-sided p-values of 0.05 or less were considered significant. Statistical analysis was performed using STATA Software, release 16.1 (StataCorp LP, College Station TX, USA) and was carried out at the biostatistical laboratory of the Foundation for Research (FROM) at Papa Giovanni XXIII Hospital in Bergamo.

# 3 Results

The search in hospital electronic health records database identified 2965 patients eligible for the study (946 discharged from emergency department and 2019 admitted to Hospital), of which 646 died before the enrollment and 405 declined to participate. Of the remaining 1914, 767 were screened by 31 July 2020. In total, 168 patients met the exclusion criteria for this study. The final population therefore consisted of 599 patients (180 females). Figure 1 shows the flow-chart describing screened, included, and excluded subjects. Median time at evaluation was 102.5 days after hospital admission.

TPOAb were above the normal range in 85 patients (14.2%), TgAb in 43 (7.2%) and both antibodies in 23 (3.8%) patients. At least one antibody was positive in 105 patients (48 females), with an overall prevalence of thyroid autoimmunity of 17.5%.

Median TPOAb was 102 U/mL (IQR 68.5 – 611) in patients with positive TPOAb and 36 U/mL (IQR 27-44) in patients with negative TPOAb; median TgAb was 174 U/mL (IQR 89.5 – 285.7) in patients with positive TgAb and 18 U/mL (IQR 14-23) in patients with negative TgAb.

Median TSH was 1.55 mIU/L (IQR 1.09 - 2.15); thirty patients (5.0%) showed abnormal TSH values, of which 19 (3.2%) had values below 0.5 mIU/L and 11 (1.8%) above 5.0 mIU/L. All patients with TSH levels < 0.5 mIU/L had normal fT3 and fT4 levels. Nine out of the eleven patients with TSH levels > 5.0 mIU/L had normal fT4 levels, exhibiting a condition of subclinical hypothyroidism. The other two patients displayed overt hypothyroidism. Median TSH of patients with thyroid autoimmunity was 1.77 mIU/L (1.25 - 2.55). Ten patients (9.6%) in this subgroup showed abnormal TSH values, of which six (5.7%) had values below 0.5 mIU/L and 4 (3.9%) had values above 5.0 mIU/L.

Ultrasonography was prescribed to every patient with positive thyroid antibodies; however, only 59 patients (26 females) accepted to undergo the examination, which was performed at a median time of 23 days after the blood tests. Mean thyroid volume was 11.5 mL in males and 9.5 mL in females. Ultrasonographic features of thyroiditis were present in 56 patients (94.9%).

Univariate analysis found that thyroid autoimmunity was positively associated with female sex ($p < 0.001$) and negatively associated with type 2 diabetes (T2DM) ($p = 0.009$), but not with hospitalization, ICU admission, respiratory support, or COVID-19 treatment (Table 1). Multivariable analysis confirmed the association between thyroid autoimmunity and both female sex and T2DM (see Table 2).

The control group included 498 patients (320 females, median age 52.7 years). TPOAb were available in 444 patients, TgAb in 373 and both autoantibodies in 325. TPOAb were above the normal range in 37/444 patients (8.3%), TgAb in 33/373 (8.8%) and both antibodies in 14/325 (4.3%) patients.

The sensitivity analysis included 660 subjects (330 patients and 330 controls) matched for age and sex, with a female prevalence of 49.7% for both groups and a median age of 60 (IQR 51-70) in patients and of 59 (47–68) in controls. Positive TPOAb prevalence was higher in patients than in controls (52/330, 15.7% vs. 23/297, 7.7%; $p = 0.002$),

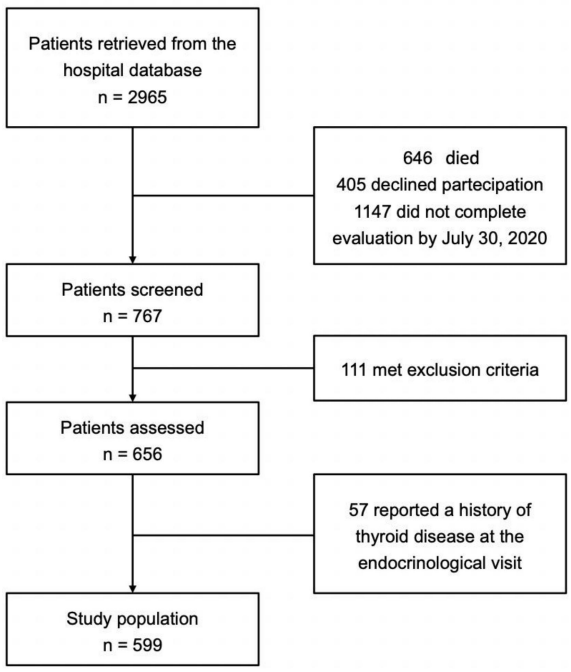


FIGURE 1
Flow chart of screened, included, and excluded patients.

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TABLE 1 Clinical characteristics of COVID-19 patients according to thyroid autoimmunity status.

| | Thyroid autoimmunity | | p-value |
|---|---|---|---|
| | No (N=494) | Yes (N=105) | |
| Age, median (IQR) | 65.0 (55.0-73.0) | 61.0 (54.0-72.0) | 0.30 |
| Gender, n (%) | | | |
| Females | 132 (26.7) | 48 (45.7) | **<0.001** |
| Males | 362 (73.3) | 57 (54.3) | |
| BMI ($Kg/m^2$) | 26.4 (24.5-29.7) | 26.7 (24.6-29.3) | 0.89 |
| Hospitalization, n (%) | 434 (87.9) | 93 (88.6) | 0.84 |
| ICU admission, n (%) | 50 (10.1) | 7 (6.7) | 0.27 |
| Respiratory support, n (%) | | | |
| None | 91 (18.8) | 22 (21.4) | 0.54 |
| Nasal cannula | 115 (23.7) | 27 (26.2) | 0.59 |
| Venturi mask | 54 (11.1) | 7 (6.8) | 0.19 |
| Reservoir bag | 85 (17.5) | 16 (15.5) | 0.63 |
| CPAP | 92 (19.0) | 23 (22.3) | 0.43 |
| Orotracheal intubation | 47 (9.7) | 8 (7.8) | 0.54 |
| ECMO | 1 (0.2) | 0 (0.0) | 0.64 |
| Comorbidity, n (%) | | | |
| Diabetes | 76 (15.4) | 6 (5.7) | **0.009** |
| Atrial fibrillation | 24 (4.9) | 2 (1.9) | 0.18 |
| Hypertension | 184 (37.2) | 32 (30.5) | 0.19 |
| IHD | 58 (11.7) | 9 (8.6) | 0.35 |
| Chronic heart failure | 22 (4.5) | 3 (2.9) | 0.46 |
| CKD | 46 (9.3) | 11 (10.5) | 0.71 |
| COPD | 20 (4.0) | 5 (4.8) | 0.74 |
| Autoimmune disease | 14 (2.8) | 1 (1.0) | 0.26 |
| Solid cancer | 10 (2.0) | 2 (1.9) | 0.94 |
| Hematological cancer | 9 (1.8) | 2 (1.9) | 0.95 |
| Liver failure | 4 (0.8) | 0 (0.0) | 0.35 |
| Cerebrovascular disease | 12 (2.4) | 4 (3.8) | 0.43 |
| COVID-19 treatment n (%) | | | |
| Lopinavir/ritonavir | 215 (43.5) | 51 (48.6) | 0.34 |
| Hydroxychloroquine | 299 (60.5) | 62 (59.0) | 0.78 |
| Glucocorticoids | 169 (34.2) | 26 (24.8) | 0.061 |
| Antibiotics | 312 (63.2) | 65 (61.9) | 0.81 |
| Tocilizumab | 27 (5.5) | 2 (1.9) | 0.12 |
| Siltuximab | 5 (1.0) | 3 (2.9) | 0.13 |
| Remdesivir | 10 (2.0) | 2 (1.9) | 0.94 |

*(Continued)*

TABLE 1 Continued

| | Thyroid autoimmunity | | p-value |
|---|---|---|---|
| | No (N=494) | Yes (N=105) | |
| Chronic treatment n (%) | | | |
| ACE inhibitors | 80 (16.2) | 16 (15.2) | 0.81 |
| ARBs | 74 (15.0) | 13 (12.4) | 0.49 |
| Other antihypertensive drugs | 154 (31.2) | 23 (21.9) | 0.059 |
| Glucocorticoids | 19 (3.8) | 2 (1.9) | 0.33 |
| Oral antidiabetic drugs | 56 (11.3) | 4 (3.8) | **0.020** |
| Insulin | 14 (2.8) | 1 (1.0) | 0.26 |
| Oral anticoagulants | 43 (8.7) | 4 (3.8) | 0.090 |
| Antiplatelet drugs | 88 (17.8) | 21 (20.0) | 0.60 |
| PPIs | 112 (22.7) | 18 (17.1) | 0.21 |

BMI, body mass index; ICU, intensive care unit; CPAP, continuous airway positive pressure; ECMO, extra-corporeal membrane oxygenation; IHD, ischemic heart disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; PPIs, proton pump inhibitors.
Bold values denote statistical significance at the $p < 0.05$ level.

while no difference was observed in positive TgAb prevalence (22/330, 6.7% vs. 20/250, 8%; $p = 0.539$). Median TPOAb (40 (IQR 31-51) vs. 31 (IQR (27-40)) and TgAb (18 (IQR 14-25) vs. 14 (IQR 14-20)) were within the normal range but significantly higher in COVID-19 patients as compared to controls (both $p < 0.001$) (Table 3).

# 4 Discussion

Studies evaluating the impact of SARS-CoV-2 infection on thyroid mainly focused on the alterations of TFTs during the acute phase of the disease, with less evidence about possible long-term effects on thyroid autoimmunity. Our aim was indeed to characterize thyroid autoimmunity and function in the largest cohort of COVID-19 survivors to date.

Most patients had normal TSH levels three months after hospital admission, as already reported in previous studies with smaller cohorts (12–14, 16, 19). Accordingly, the rate of newly diagnosed thyroid dysfunction was comparable to general population (24, 25). This finding seems to rule out a permanent direct damage to the thyroid gland induced by SARS-CoV-2. In this view, the alterations of TFTs in the acute phase of COVID-19 could more probably be secondary to a NTI (16) or a transient, self-limiting, thyroiditis.

The overall prevalence of thyroid autoimmunity in our cohort was 17.5%. Interestingly, the prevalence of positive TPOAb in COVID-19 patients was doubled as compared to controls

TABLE 2 Multivariable analysis of factors correlated with thyroid autoimmunity in the cohort of COVID-19 survivors.

| Outcome: Thyroid Autoimmunity | Odds Ratio | Std. Err. | z | P>\|z\| | [95% Conf. Interval] | |
|---|---|---|---|---|---|---|
| **Age (admission)** | 0.9969255 | 0.0083459 | -0.37 | 0.713 | 0.9807 | 1.01342 |
| Sex | | | | | | |
| Male | 1 (Ref) | | | | | |
| **Female** | **2.01324** | **0.47481** | **2.97** | **0.003** | **1.26808** | **3.19629** |
| Respiratory support | | | | | | |
| No respiratory support | 1 (Ref) | | | | | |
| Low need* | 0.9432334 | 0.3005707 | -0.18 | 0.854 | 0.5051 | 1.76142 |
| High need* | 1.079216 | 0.3266112 | 0.25 | 0.801 | 0.59635 | 1.95305 |
| Diabetes mellitus | | | | | | |
| No | 1 (Ref) | | | | | |
| **Yes** | **0.360492** | **0.1640879** | **-2.24** | **0.025** | **0.14772** | **0.87972** |

*Low need: Nasal cannula, Venturi mask; High need: reservoir bag, CPAP, orotracheal intubation ECMO.
Bold values denote statistical significance at the $p < 0.05$ level.

TABLE 3 Sensitivity analysis comparing 330 COVID-19 patients with 330 pre-pandemic controls.

| | COVID-19 Patients | Controls | *p*-value |
|---|---|---|---|
| Female Sex* (n, %) | 164/330 (49.7%) | 164/330 (49.7%) | |
| Age* (median, IQR) | 60 (51-70) | 59 (47-68) | |
| Positive TPOAb (n, %) | 52/330 (15.7%) | 23/297 (7.7%) | **0.002** |
| Positive TgAb (n, %) | 22/330 (6.7%) | 20/250 (8%) | 0.539 |
| TPOAb (U/mL) (median, IQR) | 40 (31-51) | 31 (27-40) | < **0.001** |
| TgAb (U/mL) (median, IQR) | 18 (14-25) | 14 (14-20) | < **0.001** |

TPOAb, anti-thyroperoxidase antibodies; TgAb, anti-thyroglobulin antibodies.
*Age and sex were matched in cases/controls for the sensitivity analysis.
Bold values denote statistical significance at the $p < 0.05$ level.

matched for sex and age (15.7% vs 7.7%). Few authors evaluated thyroid autoimmunity in COVID-19 patients, mostly during the acute phase. Anaya et al. (8) reported an increased prevalence of TPOAb in 120 patients hospitalized for COVID-19 as compared to healthy, pre-pandemic controls (36.7% vs. 20%). Lui et al. (9) identified TPOAb in 20.5% of patients hospitalized for COVID-19; the Authors reevaluated thyroid autoimmunity three months after the admission, reporting a significant increase in TPOAb with 4 out of 82 patients becoming TPOAb positive and an overall prevalence of TPOAb positivity of 25%. The same group confirmed these results in a subsequent study including also asymptomatic COVID-19 patients (19). Our finding of an increased concentration and prevalence of TPOAb in COVID-19 survivors strengthens the hypothesis that SARS-CoV-2 could be able to trigger thyroid autoimmunity; similarly, we found a slightly increased TgAb concentration in patients as compared to controls, though positive TgAb prevalence did not differ between the two groups; TgAb, however, are less useful than TPOAb in predicting thyroid dysfunction (26). Besides, the presence of an actual autoimmune process was consistently confirmed by the evidence of ultrasonographic features of thyroiditis in almost all patients with positive thyroid antibodies.

As already described for other viruses, SARS-CoV-2 may elicit autoimmune conditions through an hyperactivation of both the innate and adaptive immune response (27). Specifically, SARS-CoV-2 may directly trigger thyroid autoimmunity infecting thyroid follicular cells, where ACE-2 receptor is abundantly expressed (28); viral presence has indeed been retrieved in thyroid specimens (29, 30) and reactivity between TPO antigen and SARS-CoV-2 has been demonstrated *in vitro*, favoring the hypothesis of molecular mimicry (31). Alternatively, the hyperinflammatory status caused by severe COVID-19 may induce thyroid damage through the systemic increase of cytokines, unleashing thyroid autoimmunity in genetically predisposed individuals (32). Our findings may suggest that the latter mechanism plays a minor role in triggering thyroid autoimmunity, since the presence of ATD was unrelated with clinical parameters of COVID-19 severity.

In our cohort, thyroid autoimmunity directly correlated with female sex, as expected, and inversely correlated with type 2 diabetes mellitus (T2DM). Diabetes is generally characterized by an impairment of the immune system (33) and diabetic patients with non-severe COVID-19 have a reduced antibodies response to SARS-CoV-2 (34). It is therefore conceivable that diabetic survivors were also characterized by a decrease of autoantibodies against thyroid. Moreover, since during hospitalization in our hospital most diabetic patients were treated with sitagliptin, the immunomodulatory role exerted by the drug (35) may have limited the onset of thyroid immunity.

The strength of our study relies on i) being a large monocentric study, with all patients treated at the same institution and subsequently evaluated by two endocrinologists, and ii) the inclusion of a sensitivity analysis that allowed a direct comparison with age and sex matched controls evaluated before the pandemics.

The main limitations are i) the lack of baseline data about thyroidal status (function and antibodies) of the patients; and ii) not having assessed TSH-receptor antibodies in patients with low TSH. However, it has to be taken into account that routine assessment of thyroid function and autoimmunity is not recommended in the clinical care of acute COVID-19 patients (36).

In conclusion, our study showed that a relatively high proportion of COVID-19 survivors develop both serological and ultrasonographic features of thyroiditis, with only a minority displaying TFTs abnormalities. It is thus possible that the activation of immune response occurring during the acute phase of COVID-19 may induce or precipitate the onset of ATD in some patients. Since the development of thyroid autoimmunity usually precedes the onset of thyroid dysfunction, further longitudinal studies are needed to evaluate thyroid function in a long-term follow-up. Accordingly, the assessment of TPOAb and TFTs could be considered in patients evaluated for long COVID (19), as symptoms of this condition may overlap with those associated with ATD.

## Data availability statement

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

## Author contributions

AR, SC, MR, SB, SV, and RT designed the study. AR and SC evaluated the patients. AR, SI, GC, AG, and TB designed and

performed the analyses. AR, FP, and SI drafted the manuscript and prepared figure and tables. All authors contributed to the article and approved the submitted version.

## 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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# Thyroglobulin levels in COVID-19-positive patients: Correlations with thyroid function tests, inflammatory markers, and glucocorticoid use

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COVID-19 often results in generalized inflammation and affects various organs and systems. Endocrine research focused on the possible sequelae of COVID-19, with special interest given to the thyroid gland. Clinical problems such as thyroid function in non-thyroidal illness (NTI), autoimmune thyroiditis, and COVID-19-related subacute thyroiditis (SAT) quickly gained wide coverage. Thyrotoxicosis of various origins leads to the release of peripheral thyroid hormones and thyroglobulin (TG), the main glycoprotein contained within the thyroid follicular lumen. In our study, we evaluated TG levels in COVID-19-positive patients and investigated the possible relationships between TG, thyroid function tests (TFTs), and inflammatory markers. Our approach included separate subanalyses of patients who received and those who did not receive glucocorticoids (GCs). In the entire population studied, the concentration of TG tended to decrease with time ($p<0.001$; $p1,2 = 0.025$, $p1,3 = 0.001$, $p2,3 = 0.003$), and this pattern was especially clear among patients treated with GCs ($p<0.001$; $p1,2=<0.001$; $p1,3=<0.001$; $p2,3=<0.001$). The concentration of TG differed significantly between patients treated and those not treated with GC at the second and third time points of observation ($p=0.033$ and $p=0.001$, consecutively). TG concentration did not differ between the patients with normal and abnormal TFTs. The correlations between TG, TFTs, and inflammatory markers were very limited. 19 patients had elevated TG levels, but a TFT pattern suggestive of thyrotoxicosis was not common in this group. There were no statistically significant differences between patients who met and those who did not meet the predefined combined primary endpoint.

KEYWORDS

COVID-19, SARS-CoV-2, thyroid, thyroglobulin, TSH, glucocorticoids, IL-6, CRP

## Introduction

After nearly three years of the pandemic, COVID-19 remains an important and widespread clinical problem. Multiple aspects of the disease were extensively studied, with the thyroid function in infected patients being one of the most interesting and complex topics in endocrinology. Clinical problems such as thyroid function in non-thyroidal illness (NTI), autoimmune thyroiditis, and subacute thyroiditis (SAT) related to COVID-19 gained wide coverage (1). The majority of thyroid function abnormalities appear mild and transient, often fading once the patients recover from COVID-19 (2, 3). The novel onset of thyroid pathologies was hypothesized to be due to the direct and indirect effects of SARS-CoV-2 on the gland; the direct effect could be tied to viral infiltration of thyroid tissue, while the indirect effect was due to the generalized and uninhibited release of cytokines (1, 4, 5).

Trending topics related to thyroid and COVID-19 included, among others, NTI, thyrotoxicosis, and SAT. It seems that especially SAT associated with COVID-19, after being reported for the first time in 2020 by Brancatella et al., quickly gained special interest (6). SAT is known to follow viral infections of various origins, therefore, its connection with COVID-19 caused by SARS-CoV-2 coronavirus is highly plausible (7, 8). However, original studies with larger numbers of participants remain scarce and most of the available literature consists of case reports, case series, or systematic reviews (9–12). At the same time, a growing body of data supports the idea of thyrotoxicosis triggered by COVID-19, most of which seems to be caused by uncontrolled immune activation (13, 14). In our article, the objective was to evaluate thyroglobulin (TG) levels in patients hospitalized with COVID-19, perceiving TG as a biomarker of thyroid damage. We correlated TG concentrations measured at different time points of follow-up with the results of thyroid function tests (TFTs) and inflammatory markers. If TG levels increased, we provided an in-depth analysis for individual cases. Finally, we compared TG concentrations between patients who met the predefined endpoints and those who did not. Whenever possible, the analyzes were conducted separately for the patients who received and those who did not receive GCs.

## Materials and methods

In our observational study, we analyzed 174 patients hospitalized for COVID-19. All recruited patients were adults (≥18 years) and COVID-19 infection was confirmed by a PCR test in all patients. Our project recruited new patients between 14 February 2021 and 1 December 2021 at the 7 Navy Hospital in Gdańsk, Poland.

The study was approved by the Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk, Gdańsk, Poland (permissions NKBBN/373/2020, NKBBN/373-96/2021, NKBBN/373-184/2021), and was conducted according to the Declaration of Helsinki. Patients provided their informed consent to participation; if patients were unable to consent in writing due to a poor general condition resulting from COVID-19 and/or other diseases, the recruiters made a clear remark on the consent card. The only exclusion criterion we implemented was the patient's dissent to participate. The study was registered at ClinicalTrials.gov (NCT05070091).

Blood was collected for laboratory tests in the morning (6 a.m. - 8 a.m.) by qualified medical personnel. The collection took place at three preset hospitalization time points: on days 1, 4, and 10, later referred to as the first, second, and third time points, respectively. Biochemical analyzes were performed in contracted commercial laboratories, and detailed data on laboratory norms and methods are available as a Supplementary Table 1. The evaluated parameters included TFTs such as thyrotropin (TSH), total thyroxine (T4), free thyroxine (fT4), free triiodothyronine (fT3), reverse triiodothyronine (rT3), and TG, anti-thyroglobulin antibodies (anti-TG abs), C-reactive protein (CRP), interleukin-6 (IL-6), total blood count including leukocytes (LEU), neutrocytes (NEU), and lymphocytes (LYMPH). Demographic and anthropometric data were collected. Every day the blood was drawn, the patients' basic vital parameters (heart rate, blood pressure, blood oxygen saturation) and supplementary oxygen demand were recorded. Data on concomitant diseases and medication use were collected (Table 1). 7 patients (4.0%) used GCs to treat their chronic conditions, and in these cases GCs were administered continuously after admission. Additional patients required GC treatment for COVID-19 – in total, 93 patients (53,4%) received GCs at least once during their hospital stay. Due to the use of GCs by some patients, the studied group was divided into two subgroups for statistical analysis: those treated with steroids (the glucocorticoid group – GCG) and those not treated with steroids (the no-glucocorticoid group - NGCG). GCG consisted of patients who received GCs at least once during their hospitalization and the medication was administered at least one day before blood collection.

### Statistical analysis

Continuous data are presented as mean values and standard deviation (SD). Categorical data are presented as percentages. The normal distribution was verified by the Kolmogorov-Smirnov test. Continuous data from two groups were compared using the Student's t test or the Mann Whitney U test, depending on the distribution. More than two groups were compared using the Cochrane Q test for reliable variables. Categorical data were compared using the Chi-square test and Fisher's exact test. Correlations, depending on the distribution of variables, were evaluated using the Pearson correlation test or the Spearman

TABLE 1 General characteristics at admission.

| Parameter | Value |
|---|---|
| Mean age (years) ±SD | 66.8 ± 15.1 |
| Mean BMI (kg/m$^2$) ±SD | 27.6 ± 5.3 |
| Female sex (number of cases/percentage* [%]) | 84/48.3 |
| Supplemental oxygen use (number of cases/percentage* [%]) | 100/57.8 |
| Death (number of cases/percentage* [%]) | 30/17.2 |
| NIV/CPAP/HFNO (number of cases/percentage* [%]) | 12/6.9 |
| Mechanical ventilation (number of cases/percentage* [%]) | 3/1.7 |
| Catecholamine infusion (number of cases/percentage* [%]) | 6/3.5 |
| Hospital stay for at least 10 days (number of cases/percentage* [%]) | 87/50 |
| Steroid use at any point of observation (number of cases/percentage* [%]) | 93/53.4 |
| Chronic steroid use before hospital stay (number of cases/percentage* [%]) | 7/4.0 |
| Abnormal TFTs at any point of observation (number of cases/percentage* [%]) | 140/80.5 |
| History of hypothyroidism (number of cases/percentage* [%]) | 18/10.3 |
| Levothyroxine supplementation prior to hospital stay (number of cases/percentage* [%]) | 15/8.6 |
| History of hyperthyroidism (number of cases/percentage* [%]) | 2/1.1 |
| Thyrostatic use prior to hospital stay (number of cases/percentage* [%]) | 2/1.1 |
| History of arterial hypertension (number of cases/percentage* [%]) | 102/58.6 |
| History of diabetes (number of cases/percentage* [%]) | 53/30.5 |
| History of asthma and/or chronic obstructive pulmonary disease (number of cases/percentage* [%]) | 18/10.3 |
| History of chronic kidney disease (number of cases/percentage* [%]) | 26/14.9 |
| History of hemodialysis (number of cases/percentage* [%]) | 17/9.8 |
| History of heart failure (number of cases/percentage* [%]) | 17/9.8 |
| History of atrial fibrillation(number of cases/percentage* [%]) | 23/13.2 |
| History of stroke (number of cases/percentage* [%]) | 11/6.3 |
| History of pulmonary embolism (number of cases/percentage* [%]) | 3/1.7 |
| History of active neoplasia (number of cases/percentage* [%]) | 14/8.1 |

All numbers were mathematically rounded to the first decimal place. *Related to patients with available information.

correlation test. A P value less than 0.05 was considered statistically significant. Data were analyzed using the SPSS software v.21 (IBM, USA).

## Results

The final analysis included 174 COVID-19 positive individuals, of whom 160 had TG assessed at the baseline, 147 at the second time point of observation, and 102 at the third time point of observation. The baseline characteristics of the studied population are shown in Table 1. 12 of 174 (6,9%) patients had positive anti-TG abs. According to our analysis of the entire studied population, TG concentration decreased over time ($p<0.001$; $p1,2 = 0.025$, $p1,3 = 0.001$, $p2,3 = 0.003$), and a similar pattern was observed in GCG ($p<0.001$; $p1,2=<0.001$; $p1,3=<0.001$; $p2,3=<0.001$), but not in NGCG ($p=0.806$) (Table 2). The number and percentage of cases with an increased TG concentration (>77 ng/ml) is displayed in Table 2. Upon subanalysis of GCG and NGCG, we found that TG concentration was significantly lower in GCG at the second and third time points ($p=0.033$ and $p=0.001$, consecutively). There were no statistically significant differences for the first time point ($p=0.787$) (Table 3).

At the same time, TG concentration did not differ between the patients with normal and abnormal TFTs at any of the

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TABLE 2 TG concentration and the number of cases that exceeded the upper normal limit of TG concentration at three observation points in the general population and those treated versus those not treated with glucocorticoids.

| | Time point of observation | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Group | General n=174 | | | p value |
| TG (ng/ml), mean±SD | 38.3 ± 55.0 | 32.2 ± 45.2 | 26.6 ± 41.3 | <0.001* p1,2=0.025 p1,3=0.001 p2,3=0.003 |
| TG>N, number of cases (%) | 15 (8.6) | 13 (7.5) | 5 (3.0) | 0.174* |
| Subgroup | GCG n=53 | | | X |
| TG (ng/ml), mean±SD | 31.3 ± 45.2 | 26.9 ± 46.3 | 21.7 ± 46.2 | <0.001* p1,2<0.001 p1,3<0.001 p2,3<0.001 |
| TG>N, number of cases (%) | 7 (13.2) | 7 (13.2) | 2 (3.8) | 0.379* |
| Subgroup | NGCG n=33 | | | X |
| TG (ng/ml), mean±SD | 44.3 ± 65.6 | 40.3 ± 54.7 | 34.2 ± 38.0 | 0.806* |
| TG>N, number of cases (%) | 8 (24.2) | 6 (18.2) | 3 (9.1) | 0.804* |

GCG, patients treated with glucocorticoids; NGCG, patients not treated with glucocorticoids; N, upper limit of the norm. The General group: the mean TG concentration and the number of cases exceeding N were calculated for the entire population. The GCG and NGCG subgroups: the mean TG concentration and the number of cases exceeding N were calculated for patients with relevant data available for all three observation points.
*The marked p values were calculated for patients with relevant data available for all three observation points (General: n=86; GCG: n=53; NGCG: n=33).
Other p values were calculated for patients with relevant data available, with time points compared as indicated by numbers.

TABLE 3 TG concentration and cases with TG exceeding the upper limit of the norm: A. in individuals with abnormal versus normal results of thyroid function tests; B. in individuals treated versus not treated with glucocorticoids.

| | | Time point of observation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | | 2 | | | 3 | | |
| A | Parameter | Abnormal TFTs n=98 | Normal TFTs n=62 | p | Abnormal TFTs n=98 | Normal TFTs n=48 | p | Abnormal TFTs n=79 | Normal TFTs n=23 | p |
| | TG (ng/ml), mean ±SD | 42.6 ± 66.7 | 31.5 ± 26.9 | 0.365 | 32.3 ± 44.9 | 32.5 ± 46.4 | 0.305 | 26.2 ± 45.4 | 27.9 ± 23.2 | 0.864 |
| | TG>N, number of cases (%) | 11 (11.2) | 4 (6.5) | 0.313 | 10 (10.2) | 3 (6.1) | 0.545 | 4 (5.1) | 1 (4.3) | 1.000 |
| | | | | | | | | | | |
| B | | GCG n=53 | NGCG n=107 | p | GCG n=68 | NGCG n=77 | p | GCG n=59 | NGCG n=39 | p |
| | TG (ng/ml), mean ±SD | 38.1 ± 55.6 | 38.4 ± 55.0 | 0.787 | 25.2 ± 32.3 | 38.2 ± 53.8 | 0.033 | 21.3 ± 44.6 | 34.9 ± 37.2 | 0.001 |
| | TG>N, number of cases (%) | 5 (9.4) | 10 (9.3) | 1.000 | 5 (7.2) | 8 (10.4) | 0.506 | 2 (3.4) | 3 (7.7) | 0.384 |

TFTs, thyroid function tests; GCG, patients treated with glucocorticoids; NGCG, patients not treated with glucocorticoids; N, upper limit of the norm.
Values were calculated for patients with relevant data available.

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predesigned follow-up time points (Table 3). Abnormal TFTs were defined as laboratory results that deviated from the standardized norms provided by the manufacturer, and the parameters evaluated included TSH, T4, fT4, fT3, and rT3.

Next, we wanted to verify the possible correlations between the concentration of TG, the parameters of thyroid function, and the inflammatory parameters. We did not find statistically significant correlations within the GCG and NGCG (Table 4). Statistical significance was found only after the analysis of the entire population studied at the third time point and included a positive correlation between TG and fT3 (r=0.215, p=0.030), TG and T4 (r=0.196, p=0.048), and TG and CRP (r=0.225, p=0.027).

To provide further insight, we analyzed the 19 cases of patients who had elevated TG levels at various points in the observation period (Table 5). Interestingly, only one patient had a TFT pattern suggestive of thyrotoxicosis with undetectably low TSH and increased fT4 (case #7), and one additional patient showed only a slight decrease in TSH with elevated fT4 (case #15). Two additional patients presented with a decrease in TSH without an increase in fT3 or fT4 (cases #6 and #16), and one of them had a known history of hyperthyroidism. The rest of the patients who showed elevated TG concentrations had TSH, fT3, and fT4 not indicative of thyrotoxicosis.

TABLE 4 Relationship between TG concentration and various inflammatory and hormonal parameters.

| Group | General n=174 | | | | | |
|---|---|---|---|---|---|---|
| | Time point | | | | | |
| | 1 | | 2 | | 3 | |
| Parameters | r | p | r | p | r | p |
| TG-TSH | -0.149 | 0.061 | 0.097 | 0.245 | 0.082 | 0.413 |
| TG-fT3 | -0.002 | 0.982 | 0.022 | 0.792 | 0.215 | 0.030 |
| TG-fT4 | 0.076 | 0.336 | -0.068 | 0.412 | 0.029 | 0.771 |
| TG-T4 | 0.010 | 0.903 | -0.050 | 0.551 | 0.196 | 0.048 |
| TG-LEU | -0.065 | 0.411 | -0.054 | 0.515 | -0.175 | 0.078 |
| TG-NEU | -0.068 | 0.393 | -0.094 | 0.263 | -0.172 | 0.083 |
| TG-LYMPH | -0.099 | 0.214 | 0.094 | 0.261 | -0.009 | 0.930 |
| TG-CRP | 0.054 | 0.500 | 0.053 | 0.524 | 0.225 | 0.027 |
| TG-IL-6 | 0.019 | 0.817 | 0.082 | 0.341 | 0.101 | 0.334 |
| Subgroup | GCG | | | | | |
| | Time point | | | | | |
| | 1 | | 2 | | 3 | |
| Parameters | r | p | r | p | r | p |
| TG-TSH | -0.146 | 0.296 | 0.116 | 0.346 | 0.061 | 0.644 |
| TG-fT3 | 0.250 | 0.071 | 0.123 | 0.319 | 0.076 | 0.568 |
| TG-fT4 | 0.158 | 0.258 | 0.005 | 0.965 | 0.026 | 0.843 |
| TG-T4 | 0.191 | 0.176 | -0.060 | 0.625 | 0.093 | 0.482 |
| TG-LEU | -0.270 | 0.050 | -0.049 | 0.694 | 0.040 | 0.765 |
| TG-NEU | -0.270 | 0.050 | -0.100 | 0.421 | 0.048 | 0.719 |
| TG-LYMPH | 0.148 | 0.289 | 0.113 | 0.362 | -0.010 | 0.940 |
| TG-CRP | -0.002 | 0.987 | 0.114 | 0.357 | 0.250 | 0.061 |
| TG-IL-6 | -0.120 | 0.397 | 0.138 | 0.281 | 0.094 | 0.512 |

*(Continued)*

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TABLE 4 Continued

| Subgroup | NGCG | | | | | |
|---|---|---|---|---|---|---|
| | Time point | | | | | |
| | 1 | | 2 | | 3 | |
| Parameters | r | p | r | p | r | p |
| TG-TSH | -0.128 | 0.193 | 0.007 | 0.955 | -0.104 | 0.533 |
| TG-fT3 | -0.092 | 0.348 | -0.200 | 0.083 | 0.120 | 0.468 |
| TG-fT4 | 0.039 | 0.687 | -0.140 | 0.226 | 0.169 | 0.304 |
| TG-T4 | -0.070 | 0.472 | -0.052 | 0.654 | 0.135 | 0.413 |
| TG-LEU | 0.038 | 0.694 | -0.001 | 0.991 | -0.270 | 0.097 |
| TG-NEU | 0.021 | 0.831 | -0.012 | 0.918 | -0.205 | 0.210 |
| TG-LYMPH | -0.176 | 0.070 | -0.044 | 0.705 | -0.131 | 0.428 |
| TG-CRP | 0.063 | 0.516 | 0.008 | 0.944 | -0.070 | 0.684 |
| TG-IL-6 | 0.091 | 0.358 | 0.022 | 0.856 | -0.121 | 0.469 |

Values were calculated for patients with relevant data available. GCG, patients treated with glucocorticoids; NGCG, patients not treated with glucocorticoids.

Finally, we wanted to verify whether the TG concentrations differed between patients who met and those who did not meet the predefined endpoints. The combined primary endpoint consisted of death, mechanical ventilation, noninvasive ventilation/high flow nasal oxygenation, use of vasopressors, hospitalization for at least 10 days; the secondary endpoints were any of the listed events. First, we analyzed the entire study population: there were no statistically significant differences between patients who met and those who did not meet the combined primary endpoint, both in terms of TG concentration and the incidence of high TG concentration. No differences between groups were found at any of the follow-up points for secondary endpoints, such as prolonged hospital stay, mechanical ventilation, and vasopressor infusion. Statistically significant results were found in relation to death (the first time point [p=0.031] and the second time point [p=0.005], but not the third time point [p=0.091]), and noninvasive ventilation (only the second time point with p=0.043). GCG and NGCG were not evaluated separately.

## Discussion

The relationship between COVID-19 and thyroid function quickly gained widespread coverage from medical researchers. The range of topics evaluated was broad and included NTI, SAT, autoimmune thyroiditis, and a possible link between TFTs and survival in COVID-19 (1, 3, 15–18). The involvement of the gland appeared to be multifactorial, resulting both from the direct effects of viral infiltration and the local response it provoked, as well as systemic inflammation, often exaggerated up to the phase of the cytokine storm (1, 4, 19). The cytokine storm, with its uncontrolled and robust release of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α), is the peak of the detrimental immune hyperactivation seen in COVID-19 (20–22). SARS-CoV-2 penetrates cells using ACE2 receptors, which are widespread throughout the human system, and viral entry is co-facilitated by the serine protease TMPRSS2 (23–26). Therefore, the virus can infiltrate numerous tissues and organs, including thyroid (19, 24).

A systematic review of 1,237 cases of COVID-19 from 7 selected studies carried out by Giovanella et al. proved that abnormal thyroid function in COVID-19 is common and up to 64% of patients display abnormal TFTs (3). Our recent study on thyroid function in patients hospitalized for COVID-19 supported these findings: up to 80% of recruited individuals showed abnormal TFTs at least once during the observation period (27). In particular, NTI appeared to be common in patients with COVID-19 (27, 28). NTI features tended to fade over time in patients recovering from COVID-19, but remained prominent in patients who succumbed to their illness (29). A positive correlation was observed between abnormal TFTs and high inflammatory markers, and abnormal TFTs could herald an unfavorable clinical course of the disease and a prolonged hospital stay (27, 30).

The results of the THYRCOV study by Lania et al. proved that thyrotoxicosis might not be rare during COVID-19, as it was found in more than 20% of the studied patients. At the same time, thyrotoxicosis was positively correlated with elevated circulating IL-6 (14). On the other hand, a study by Campi et al. showed a considerable prevalence of transiently decreased TSH, often accompanied by low fT3. In this group, TG levels remained normal despite the decrease in TSH at baseline or after follow-up (29). The authors concluded that the pattern of

TABLE 5 Summary of patients who showed elevated TG.

| Case number | Age (years) | Sex | History of hypothyroidism | History of hyperthyroidism | Positive anti-TG abs | Time point of observation | Steroid use prior to blood collection | TSH (μIU/ml; N: 0.27-4.2) | fT3 (pmol/l; N: 3.1-6.8) | fT4 (pmol/l; N: 12-22) | TG (ng/ml; N: 3.5-77) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 71 | F | No | No | No | #2 | Yes | 2.82 | 1.78 | 15.17 | 88.5 |
| 2. | 94 | M | No | No | No | #1 | No | 2.15 | 2.23 | 11.1 | 117 |
| | | | | | | #2 | | 1.78 | 2.1 | 11.56 | 88 |
| 3. | 83 | F | No | No | No | #1 | No | 0.493 | 2.92 | 14.46 | 205 |
| | | | | | | #2 | | 0.635 | 3.11 | 12.97 | 138 |
| | | | | | | #3 | | 1.37 | 3.32 | 14.4 | 103 |
| 4. | 83 | M | Yes | No | No | #1 | No | 8.55 | 2.5 | 14.65 | 296 |
| | | | | | | #2 | | 17.42 | 2.73 | 15.78 | 311 |
| | | | | | | #3 | Yes | 6.24 | 1.96 | 14.37 | 332 |
| 5. | 57 | F | Yes | No | No | #1 | No | 1.03 | 4.2 | 16.54 | 97.5 |
| | | | | | | #2 | | 1.79 | 3.96 | 14.84 | 80 |
| 6. | 65 | F | No | Yes | No | #1 | Yes | 0.29 | 3.15 | 13.62 | 158 |
| | | | | | | #2 | | 0.215 | 3.32 | 15.08 | 103 |
| 7. | 73 | M | No | No | No | #1 | No | <0.005 | 3.18 | 23.66 | 133 |
| | | | | | | #2 | | <0.005 | 2.63 | 15.76 | 165 |
| 8. | 71 | F | Yes | No | No | #1 | Yes | 0.827 | 1.58 | 15.73 | 194 |
| | | | | | | #2 | | 0.897 | 2.25 | 17.17 | 165 |
| 9. | 70 | F | No | No | No | #2 | No | 4.62 | 3.78 | 15.48 | 88.3 |
| | | | | | | #3 | | 4.92 | 4.14 | 17.99 | 91.2 |
| 10. | 80 | M | No | No | No | #1 | No | 0.719 | 3.17 | 15.61 | 94.4 |
| 11. | 73 | M | No | No | No | #1 | Yes | 3.37 | 3.76 | 15.12 | 81.3 |
| | | | | | | #2 | | 1.18 | 2.56 | 13.57 | 139 |
| 12. | 69 | F | No | No | No | #1 | No | 0.787 | 1.8 | 15.33 | 87.5 |
| 13. | 59 | M | No | No | No | #1 | No | 1.66 | 6.1 | 16.03 | 92.4 |
| 14. | 61 | F | No | No | No | #1 | No | 0.851 | 2.4 | 10.72 | 271 |

*(Continued)*





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TABLE 5 Continued

| Case number | Age (years) | Sex | History of hypothyroidism | History of hyperthyroidism | Positive anti-TG abs | Time point of observation | Steroid use prior to blood collection | TSH (µIU/ml; N: 0.27-4.2) | fT3 (pmol/l; N: 3.1-6.8) | fT4 (pmol/l; N: 12-22) | TG (ng/ml; N: 3.5-77) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 15. | 85 | F | No | No | No data | #3 | Yes | 0.24 | 3.14 | 25.38 | 77.9 |
| 16. | 72 | M | No | No | No | #1 | Yes | 0.145 | 1.85 | 12.05 | 333 |
| 17. | 82 | F | No | No | No | #2 | No | 0.793 | 3.08 | 11.44 | 120 |
| 18. | 86 | F | No | No | No | #1 | Yes | 1.19 | 3.06 | 12.25 | 131 |
| | | | | | | #2 | | 1.31 | 2.61 | 16.35 | 132 |
| 19. | 90 | F | No | No | No | #1 | No | 1.28 | 3.04 | 16.96 | 346 |
| | | | | | | #2 | | 2.29 | 3.15 | 18.04 | 304 |
| | | | | | | #3 | | 2.52 | 3.05 | 19.12 | 207 |

F, female; M, male; N, normal value.

observed TFT abnormalities was most likely not related to destructive thyroiditis, and rare instances of elevated TG could be associated with a known preexisting condition (nodular goiter) (29). In another study, Vassiliadi et al. reported that TG levels were comparable between COVID-19 patients with normal TFTs, NTI, or a thyrotoxic pattern. The thyrotoxic pattern was observed in 8.8% of all patients who suffered from COVID-19, and it was seen in 14.6% of severely ill individuals who needed intensive care unit treatment (28).

An observational study by Mondal et al. showed that the prevalence of SAT in COVID-19 convalescents was 6.8% (n=11) within 3 months of follow-up - most of the patients were young (mean age 44 years) and female, the mean delay between COVID-19 and the diagnosis of SAT was 23,8 days, and the clinical course of the disease was more severe in atypical SAT, which presented earlier (10,6 days since the recovery from COVID-19), with an exaggerated inflammatory response and more pronounced thyrotoxicosis (12). SAT became an interest in endocrine research after Brancatella et al. presented the first known case report of COVID-19-related SAT in a young woman in mid-2020 (6). There is evidence that certain populations are more at risk of developing SAT due to their specific human leukocyte antigen (HLA) system, COVID-19-related SAT included (7, 8, 31, 32). The first stage of thyroiditis, thyrocyte breakdown resulting in hyperthyroidism, is marked not only by an uninhibited leak of peripheral thyroid hormones from the damaged gland, but also by a simultaneous release of TG, a glycoprotein stored within the thyroid follicular lumen, crucial for hormonogenesis and considered a biomarker of destructive thyroiditis (29, 33). In our project, we wanted to assess markers of possible thyroid destruction during COVID-19. Increased TG concentration in the bloodstream is not limited to SAT; it can reflect the amount of differentiated thyroid tissue, the degree of stimulation of the gland related to thyrotropin, and the history of recent thyroid trauma (33, 34). Therefore, TG concentration can increase in various clinical settings, including SAT, differentiated thyroid cancer, nodular goiter, or hyperthyroidism. As mentioned above, a pattern of TFTs suggesting thyrotoxicosis in patients who showed elevated TG concentration was not a common finding in our study. In general, we did not find clear, repeatable, and unequivocal relationships between TG levels, TFTs, and inflammatory parameters, which may suggest an overall limited destructive effect of COVID-19 on thyroid tissue. Interestingly, TG levels dropped after the introduction of GCs, a phenomenon previously described by researchers who focused on the management of SAT, which could be explained, among others, by impaired intrathyroidal hydrolysis of colloid due to the use of GC or a decline in systemic inflammation (35–37).

The main limitations of this study include the lack of post-discharge follow-up, especially since the available data highlight the frequent delay between COVID-19 and SAT; no in-hospital assessment specific for clinical signs and symptoms of SAT; the absence of radiological evaluation of the thyroid in terms of SAT or other pathologies that cause increased TG release, such as goiter. A

combination of the aforementioned factors and additional laboratory work could give better insight into the characteristics necessary to adequately document the occurrence of SAT. However, the assessment we designed was not meant to unequivocally determine the number of SAT cases in the studied population, but to investigate possible generalized thyroid damage based on TG levels, TFTs, and their correlation with inflammatory markers. Listing the limitations of the study, we must mention that a small percentage of the recruited patients (12 out of 174 – 6,9%) had positive anti-TG abs, which can sometimes interfere with the evaluation of TG. In addition, some of the presented statistical analyses, especially related to the entire studied population, might be biased due to inconsistent study samples (e.g. exogenous steroid use in some patients – GCG) and, sometimes, a wide disproportion between the number of studied cases.

The strengths of our study are the prolonged period of in-hospital observation with three separate assessment points, the subanalyses based on the use of GCs or its lack, the consistent set of performed assays, the in-depth analysis of detected cases with increased TG, and the assessment of TG levels in relation to predefined endpoints. We believe that, despite its limitations, our study still sheds new light on the complicated matter of TFTs and thyroid function abnormalities in COVID-19.

## Summary

The thyroid function in COVID-19 is complex and sometimes confusing. COVID-19-related NTI, SAT, and thyrotoxicosis quickly gained the attention of researchers. TFTs undergo distinct and very often transient changes in COVID-19-positive patients. In our material, the concentration of TG did not differ between the patients with normal and abnormal TFTs; however, it differed significantly depending on the use of GCs and tended to decrease over time. We found that 19 patients had elevated TG levels at least once during observation, but a TFT pattern suggestive of thyrotoxicosis was not common in this group. There were no statistically significant differences between patients who met and those who did not meet the combined primary endpoint.

# Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

# Ethics statement

The studies involving human participants were reviewed and approved by the Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk. 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 provide it due to the 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. EP-R – patient recruitment, data collection. 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



# Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1031188/full#supplementary-material

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# Thyroid function and associated mood changes after COVID-19 vaccines in patients with Hashimoto thyroiditis

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**Context:** Severe acute respiratory syndrome-coronavirus 2 (COVID-19) vaccines may incur changes in thyroid functions followed by mood changes, and patients with Hashimoto thyroiditis (HT) were suggested to bear a higher risk.

**Objectives:** We primarily aim to find whether COVID-19 vaccination could induce potential subsequent thyroid function and mood changes. The secondary aim was to find inflammatory biomarkers associated with risk.

**Methods:** The retrospective, multi-center study recruited patients with HT receiving COVID-19–inactivated vaccines. C-reactive proteins (CRPs), thyroid-stimulating hormones (TSHs), and mood changes were studied before and after vaccination during a follow-up of a 6-month period. Independent association was investigated between incidence of mood state, thyroid functions, and

inflammatory markers. Propensity score–matched comparisons between the vaccine and control groups were carried out to investigate the difference.

**Results:** Final analysis included 2,765 patients with HT in the vaccine group and 1,288 patients in the control group. In the matched analysis, TSH increase and mood change incidence were both significantly higher in the vaccine group (11.9% versus 6.1% for TSH increase and 12.7% versus 8.4% for mood change incidence). An increase in CRP was associated with mood change ($p< 0.01$ by the Kaplan–Meier method) and severity ($r = 0.75$) after vaccination. Baseline CRP, TSH, and antibodies of thyroid peroxidase (anti-TPO) were found to predict incidence of mood changes.

**Conclusion:** COVID-19 vaccination seemed to induce increased levels and incidence of TSH surge followed by mood changes in patients with HT. Higher levels of pre-vaccine serum TSH, CRP, and anti-TPO values were associated with higher incidence in the early post-vaccine phase.

KEYWORDS

COVID-19 vaccines, Hashimoto thyroiditis, thyroid function, inflammation, mood change

# Introduction

Hashimoto thyroiditis (HT) is an autoimmune condition featured by lymphocytic destruction and chronic inflammation of thyroid gland (1, 2), and pathological process involves the interaction between the genetic and environmental factors (2). Antibodies of thyroid peroxidase (anti-TPO) and/or thyroglobulin (anti-TG) are increased, which serve as the main criteria for diagnosis if combined with symptoms of hypothyroidism or low echogenicity in ultrasound. HT is usually underdiagnosed because of symptomatic dormancy. Foreign antigen–associated immune alterations, followed by internal inflammation, were hypothesized as one of the probable reasons for symptomatic conversion from long-term dormancy (3–5).

In the era of COVID-19 posing the greatest threat to public health, the introduction of various types of vaccines successfully decreased the rate of COVID-19 infection for both healthy populations and those with underlying diseases (6, 7). Immune response and safety in patients with HT have been concretely evidenced, and all patients are recommended to receive vaccines (8, 9). To date, sparse cases of thyroiditis in the general population have been reported after vaccination, and thyroid function changes are reported in healthy subjects recently (10–13). However, inflammatory and corresponding phenotypical changes have not been investigated after the introduction of a foreign antigen into immune system in the current stage when all patients are suggested to receive COVID-19 vaccines.

Next, whether vaccine-associated inflammatory marker changes are inducible to thyroid dysfunction and symptom changes in patients with HT has not been studied. Thyroid functions are critically linked to mood states, and the rate of mood swings is significantly higher in the HT population, with reports in the large samples of three times of incidence in 1 year as compared to general population (14, 15). Considering the association with antigen presentation and subsequent immune upregulation, changes in disease status are possible from subclinical to apparent symptoms. Incidence of mood state disorders in the era of COVID-19 pandemic requires further investigation due to the high levels of stress-afflicted HT people, which represents a psychiatrically susceptible group of patients with potential immune and thyroid dysregulation (11, 16). Such investigation may offer an added value to clinicians and patients who demand data on long-term influence of vaccines on subclinical HT. Thus, in the current study, we aim to find the vaccination-associated thyroid function and mood changes and to investigate how related inflammatory markers influence the outcomes.

# Methods

## Patients and study design

The retrospective, multi-center study recruited patients with clinical or subclinical HT to receive standard two-dose inactivated CoronaVac (BBIBP-CorV) vaccines during May 2021 to January 2022. Because there have not been guidelines for vaccine safety in China for patients with HT during recruitment period, vaccination was primarily decided voluntarily by patients. Thus, patients not receiving vaccines during study period were included as the control group, and corresponding matching by propensity scores was done to reduce bias. Diagnosis of HT was the presence of clinical symptoms of hypothyroidism combined with the increased levels

of anti-TPO and/or anti-TG antibodies. Ethical approval was obtained at the Second Affiliated Hospital of Shantou University Medical College as a waiver option for the retrospective study protocol. This study was performed according to the principles of Declaration of Helsinki, Good Clinical Practice, and all patients provided informed consent before participation.

The primary outcome was to evaluate the mood and thyroid function changes after COVID-19 vaccination, and an exploratory analysis was carried out to identify potential associations between inflammation markers and primary outcome. To illustrate role of vaccines and to control for the potential psychiatric-related conditions during COVID-19 pandemic, a control cohort with HT was recruited in the same hospitals that did not receive vaccines as geographical match during study period. The smallest sample size was calculated by PASS (V 15.0) before recruitment to reach the effect size of the thyroid-stimulating hormone (TSH) levels of 5% difference with pre-specified alpha of 0.05 and beta of 0.20, and the upper limit was dependent upon the actual size of recruited during study period (consecutive recruitment). Key exclusion criteria of the study are as follows: 1) critically ill conditions with a survival prognosis of less than 1 year; 2) diagnosis of Grave's disease; 3) history of anaphylaxis to contents of vaccine products; and 4) acutely ill patients or patients with active cancers, who may show increased inflammation markers.

## Baseline and follow-up assessment

The demographic data were extracted from Case Record Forms of the five tertiary referral hospitals, and the electronic data capture systems were applied to save and monitor de-identified profiles, in which each patient was coded for follow-up track. Demographic information included gender and age. Patients were followed up online to test their mood state on a weekly basis, and serum tests were performed in clinical laboratories of enrollment centers. Follow-up started from the date of vaccination to the 24th week after vaccination (end of follow-up).

The serum levels of markers were tested one to three times during a 3-month period prior to vaccination and at the end of follow-up. Mean values were applied in final calculation to represent baseline inflammation profiles. Markers included C-reactive protein (CRP, μg/dl; 1 mg/L = 100 μg/dl), interleukin-6 (IL-6), TSHs, and anti-TPO and anti-TG antibodies. At the full length of follow-up, Beck Depression Inventory (BDI) was administered online at each weekend to assess the mood change levels of patients dating back 2 weeks. Definition of mood changes was a BDI score of more than or equal to 13 (17, 18). Diagnosis of mood changes was further validated by symptoms during past 2 weeks in clinical settings at community or referral psychiatry clinics if the patient scored more than 13. Patients with suicidal or aggressive symptoms, if any, were medically managed and properly recorded.

Clinic coordinators with supervision gather data in community clinics when patients were not reachable in the tertiary hospitals (either as inpatients or outpatients). All data were extracted from Case Record Form of the follow-up sites, and the electronic data capture systems were applied to save and monitor de-identified profiles.

## Multivariate regression, survival analysis, and nomogram development

Incidence rate of mood change during follow-up was determined with the Kaplan–Meier method. In both vaccine and control groups, the incidence curves were drawn, and the log-rank test was applied to compare difference between different groups. Markers significant in univariate survival analysis were subject to multivariate Cox proportional hazards models to identify independent pre-vaccination markers that predict outcomes, with the corresponding hazard ratio and 95% confidence intervals. Two nomograms were formulated by using package of rms in R version 4.0.5 (http://www.r-project.org/). First, nomogram was constructed to predict incidence of TSH increase and, second, was built to predict disease-free survival (DFS) time during follow-up (19). The performance of nomograms was measured by concordance index (C-index) and by comparing the nomogram-predicted versus observed rates of events (i.e., DFS and TSH increase). Bootstraps with 1,000 resampling were used.

## Propensity score–based outcome analysis between the vaccine and control groups

To further decrease confounding bias across chronologically and geographically different enrollment and to alleviate pandemic-related psychiatric confounders, propensity score–based matching was performed between the vaccine and control groups. Propensity scores were calculated with the logistic conditional regression models. Variables included were those significantly associated with incidence of mood change and/or the increase of TSH levels in multivariate regression and survival analysis (20). Nearest neighbor head-to-head (1:1) method was adopted to match each participant in the vaccine and control groups, respectively, with a caliper width of 0.2 without replacement (20). Standardized mean difference (SMD) was calculated and compared between unmatched and matched data to evaluate matching performance as imbalance test. An SMD over $(\sqrt{((n1 + n2)/(n1 \star n2))}) \star 1.96$ was regarded as an imbalanced test result (20). Statistical tests of difference in matched samples included McNemar tests for categorical variables and Wilcoxon signed-rank tests for continuous variables (20).

## Statistic calculation

The categorical factors were represented as numbers and percentages, and the continuous factors were represented as means ± standard deviations and median (25th to 75th quartile). Each statistical test was based on a pre-determined statistical hypothesis with a type I error of 0.05. The paired Student's t-test was applied to compare continuous variables of the same cohort in chronological settings for parametric tests, including changes in

CRP, BDI scores, and TSH values. Statistics used in comparison study were carried out in SPSS V.24.0 software.

# Results

## Patient characteristics

The study enrolled 4,556 patients with HT, and 503 patients did not consent to follow-up and thus were excluded. Thus, final analysis included 2,765 patients in the vaccine group and 1,288 patients in the control group (3,198 female patients and 855 male patients; mean age, 42.34 ± 14.99 years). Before vaccination, mean CRP levels of the vaccine group were 581.12 ± 789.94 μg/dl, and mean TSH values were 293.29 ± 111.70 μIU/dl. Anti-TG antibody levels were 92.17 ± 375.92 IU/ml, and BDI scores were 8.52 ± 5.17. Anti-TPO antibody levels were 38.36 ± 100.55 IU/ml. Among all participants, 499 patients had a prior history of psychiatric diseases, and 104 patients had other autoimmune diseases (AIDs). Baseline demographics and serum markers of both groups are shown in Table 1.

## Thyroid function and mood changes after COVID-19 vaccination

As there could be episodes of incident mood and thyroid function changes during course of HT that were potentially unrelated to vaccination, baseline and follow-up data of the control group were compared with those of the vaccine group after propensity score matching to find whether incident mood changes were associated with vaccination. Matching yielded a total of 1,039 pairs, with the imbalance test results shown in Supplementary Table 1. Bias-corrected total incidence of mood changes during follow-up was 12.7% in the vaccine group and 8.4% in the control group. Kaplan–Meier survival analysis between the two groups showed that there was

TABLE 1 Baseline variables of patients with Hashimoto thyroiditis.

| Variables | | Before matching | | | After matching (N = 1,039 pairs) | | |
|---|---|---|---|---|---|---|---|
| | | Vaccine group N = 2,765 | Control N = 1,288 | $P^1$ | Vaccine group | Control | $P^2$ |
| Age (years) | Mean (SD) | 42.34 (13.99) | 51.84 (14.91) | <0.01 | 47.39 (12.78) | 48.69 (14.01) | 0.03 |
| | Median (quartile) | 41 (31–54) | 52 (42–63) | | 48 (37–56) | 50 (39–58) | |
| Gender | Male | 415 (15.0) | 440 (34.2) | <0.01 | 286 (27.5) | 261 (25.1) | 0.21 |
| | Female | 2350 (85.0) | 848 (65.8) | | 753 (72.4) | 778 (74.9) | |
| Time since diagnosis (months) | Mean (SD) | 9.60 (10.95) | 10.14 (6.65) | 0.05 | 9.40 (8.87) | 9.74 (6.64) | 0.33 |
| | Median (quartile) | 5 (3–10) | 9 (5–14) | | 6 (4–10) | 8 (4–14) | |
| TSH (μIU/dl) | Mean (SD) | 293.29 (111.70) | 292.60 (119.35) | 0.86 | 291.13 (108.37) | 292.14 (119.30) | 0.84 |
| | Median (quartile) | 291 (201–384) | 292 (189–393) | | 290 (204–378) | 293 (189–394) | |
| CRP (μg/dl) | Mean (SD) | 581.12 (789.94) | 435.02 (476.71) | <0.01 | 487.52 (684.91) | 447.42 (489.24) | 0.13 |
| | Median (quartile) | 190 (80–740) | 240 (130–650) | | 180 (70–620) | 240 (140–180) | |
| IL-6 (pg/ml) | Mean (SD) | 146.97 (103.33) | – | – | 148.52 (105.02) | – | – |
| | Median (quartile) | 117 (64–204) | – | | 118 (64–207) | – | |
| Anti-TG (IU/ml) | Mean (SD) | 92.17 (375.92) | – | – | 117.62 (448.82) | – | – |
| | Median (quartile) | 15.81 (13.91–33.83) | – | | 16.14 (13.97–14.03) | – | |
| Anti-TPO (IU/ml) | Mean (SD) | 38.36 (100.55) | 56.82 (121.81) | <0.01 | 48.30 (121.10) | 50.92 (112.68) | 0.61 |
| | Median (quartile) | 11.37 (8.67–17.31) | 14 (8–19) | | 11.48 (8.73–18.94) | 14 (8–19) | |
| Comorbidity with other AIIDs | Yes | 2661 (96.2) | 1168 (90.7) | <0.01 | 85 (8.1) | 68 (6.5) | 0.15 |
| | No | 104 (3.8) | 120 (9.3) | | 954 (91.9) | 971 (93.5) | |
| BDI values | Mean (SD) | 8.52 (5.17) | 8.29 (4.40) | 0.08 | 8.91 (5.35) | 8.26 (4.41) | 0.03 |
| | Median (quartile) | 8 (4–12) | 8 (5–12) | | 9 (4–12) | 8 (5–12) | |
| Psychiatric disease history | No | 2266 (82.0) | 984 (76.4) | <0.01 | 806 (77.6) | 806 (77.6) | 1.00 |
| | Yes | 499 (18.0) | 304 (23.6) | | 233 (22.4) | 233 (22.4) | |

SD, standard deviation; AIIDs, autoimmune inflammatory diseases; BDI-13, Beck Depression Inventory; TSH, thyroid stimulating hormones; CRP, C-reactive proteins.
[1]Independent t-test (continuous variable) and chi-square test (categorical variable).
[2]Wilcoxon signed-rank test (continuous variable) and McNemar test (categorical variable).

a significant difference between the two groups in incidence of mood changes (Supplementary Figure 1).

Using the McNemar test, the percentage rate of TSH increase was significantly different between the two groups (11.9% versus 6.1% for TSH increase in the vaccine and control groups, respectively). Further comparison of mean TSH values also showed significance (316 ± 195 μIU/dl versus 259 ± 90 μIU/dl, p< 0.01 by the Wilcoxon signed-rank test, Figure 1A). Another significant finding was the difference in the CRP values in the vaccine and control groups (583 ± 732 μg/dl versus 414 ± 428 μg/dl, p< 0.01 by Wilcoxon signed-rank test, Figure 1B). Changes in TSH and CRP values were also seen in the original, unmatched vaccine group before and after vaccination, respectively (p< 0.01, Figures 1C, D). To test the relationship between mood change severity and the level of CRP in the vaccine group, BDI scores were found to be correlated with the CRP levels during follow-up in the subgroup with mood changes (r = 0.75), and correlation was not found in the subgroup without mood changes (Figures 1E–H).

## Serum markers may independently predict incident mood change

To test the predicting values of serum markers before and after vaccination and to evaluate the relationship between demographics and rates of mood changes, univariate and multivariate survival analyses were carried out to find the independent markers associated with incidence of mood changes (Table 2). Initial Kaplan–Meier methods found that the baseline TSH values, CRP levels, IL-16 levels, and anti-TG antibody levels were associated with incidence of mood changes during follow-up. All variables but IL-6 levels were significant in multivariate survival analysis (p< 0.01). The Kaplan–Meier survival curves of CRP, TSH, and anti-TPO levels are shown in Figure 2. Incidence rate of mood changes was 17.3% in patients with increased CRP and 10.9% in patients without an increase. CRP increase after vaccination was associated with the increased mood change incidence (Figure 3).

## Nomogram predicting thyroid function changes and early-onset mood change incidence

Final analysis was done to see whether there was an association between mood change–predicting serum markers with change in thyroid function after vaccination. Baseline demographic and serum markers were subject to univariate and multivariate regression analyses of TSH increases in the vaccine group, and results are shown in Supplementary Table 2. It was demonstrated that the same markers were associated with TSH changes after vaccination. Thus, nomograms were developed in the vaccine group to quantify the risk of mood changes and the risk of TSH increases by means of anti-TPO antibodies, TSH, and CRP values before vaccination. In nomogram to predict TSH increases (Supplementary Figure 2), the value of C-index was 0.69, and the calibrating curve demonstrated a relatively good contingency between the predicted and actual rates of TSH increases.

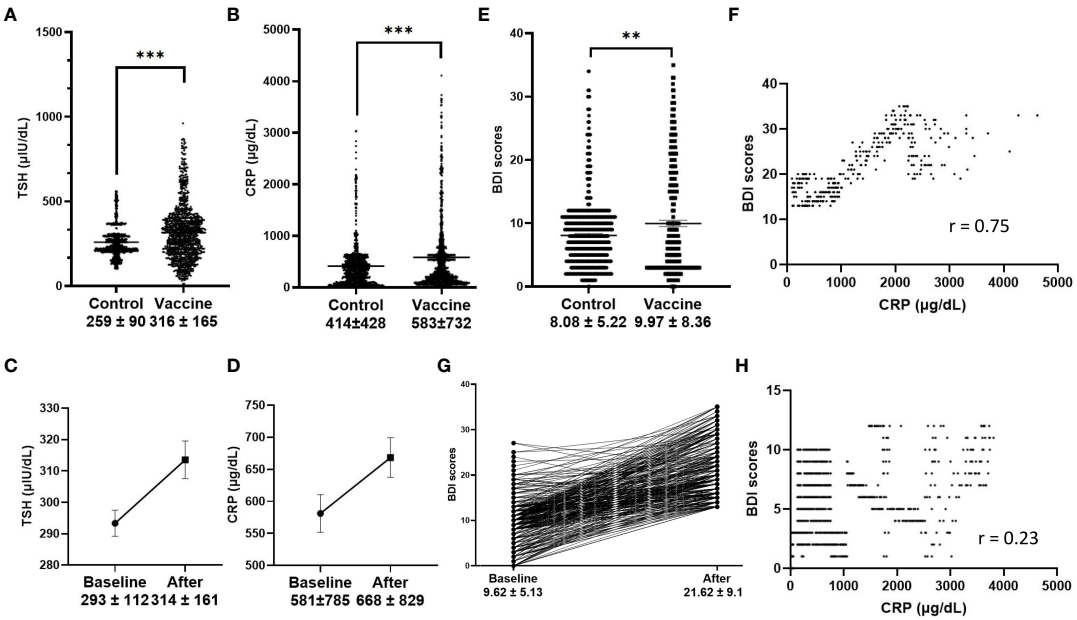


FIGURE 1
Serum markers and Beck Depression Index (BDI) scores. **(A, B)**, TSH and CRP values in the vaccine group and control group (p < 0.001 in matched analysis); **(C, D)**, TSH and CRP values before and after vaccination (p < 0.001); **(E)**, BDI scores in the vaccine group and control group, (p < 0.001 in matched analysis); **(F)**, CRP values were correlated with BDI scores after vaccination in the subgroup of patients with greater mood changes (defined as BDI score > 13, r = 0.75), and no correlation was found in subgroup without greater mood changes **(H)**; Changes of BDI scores before and after vaccination in the subgroup with greater mood changes **(G)**.

TABLE 2 Survival analysis of incident mood changes after vaccination.

| Variables | | *P* (univariate) | *P* (multivariate) | HR (95% CI) |
|---|---|---|---|---|
| **Gender** | | 0.56 | – | – |
| **Age** | | 0.27 | – | – |
| **Time since diagnosis** | | 0.96 | – | – |
| **TSH (μIU/dl)** | **<201** | <0.01 | <0.01 | 0.27 (0.18–0.40) |
| | **201–291** | <0.01 | <0.01 | 0.50 (0.38–0.67) |
| | **291–384** | <0.01 | <0.01 | 0.71 (0.54–0.93) |
| | **>384** | Reference | Reference | Reference |
| **CRP (μg/dl)** | **<80** | <0.01 | <0.01 | 0.36 (0.27–0.48) |
| | **80–190** | <0.01 | <0.01 | 0.10 (0.06–0.17) |
| | **190–740** | <0.01 | <0.01 | 0.56 (0.43–0.74) |
| | **>740** | Reference | Reference | Reference |
| **IL-6 (pg/ml)** | **<64** | <0.01 | 0.85 | 0.97 (0.69–1.36) |
| | **64–117** | <0.01 | 0.84 | 1.03 (0.75–1.42) |
| | **117–204** | <0.01 | 0.32 | 1.17 (0.86–1.58) |
| | **>204** | Reference | Reference | Reference |
| **Anti-TG antibody (IU/ml)** | | 0.67 | – | – |
| **Anti-TPO antibody (IU/ml)** | **<8.67** | <0.01 | <0.01 | 0.09 (0.06–0.15) |
| | **8.67–11.37** | <0.01 | <0.01 | 0.08 (0.05–0.13) |
| | **11.37–17.31** | <0.01 | <0.01 | 0.28 (0.21–0.37) |
| | **>17.31** | Reference | Reference | Reference |
| **Comorbidity with other AIIDs** | | 0.22 | – | – |
| **BDI values** | | 0.55 | – | – |
| **Psychiatric disease history** | | 0.31 | – | – |

AIIDs, autoimmune inflammatory diseases, BDI-13, Beck Depression Inventory, TSH, thyroid stimulating hormones; CRP, C-reactive proteins; HR, hazard ratio; CI, confidence interval.

# Discussion

As COVID-19 vaccines became widely covered in general population, thyroid function changes became evident in a small range of samples characterized by subclinical to clinical thyroiditis (12, 21). Thyroid function fluctuations in HT, autoimmune inflammatory thyroiditis (AIT), and mood state changes that ensued have seldom been reported. This study offered first observatory, real-world evidence that COVID-19 vaccination could incite changes in mood changes levels and thyroid function in HT by means of matched analysis. Specifically, associations between the increased CRP and incidence and the severity of mood changes were investigated in a relatively large sample.

Mood changes, a mood disorder that affects 6% to 7% of the general population, is quite commonly seen in patients with hypothyroid states due to the deranged levels of thyroid hormones and persistent inflammation (15, 22). Autoimmune thyroiditis was reported to be the most prevalent pathology type in such a population, and, by meta-analysis, 1-year prevalence of mood changes could be as high as 17% (14). The inflammatory state, represented by CRP levels and being a major trigger of mood swings in AIT, could be subject to fluctuations by means of an external stimulus such as COVID-19 vaccine antigens (3). Antigen-related CRP level increases were seen after vaccination (from 581 to 688 μg/dl), and the result was validated by comparison with the control group. Similar results have been reported in patients with psychiatric conditions, and an earlier study on influenza vaccination indicated changes in the inflammation levels (23, 24). Consistent with prior research, higher baseline CRP levels predict a higher probability of mood changes after immune stimulus, and, combined with hypothyroid states, an even higher risk could be predicted in current nomogram.

Safety of COVID-19 vaccines has been widely evidenced in recent studies of autoimmune conditions, and most effort has been intensified on immunogenicity and vaccine-related adverse events following vaccination (11). Reports on immune-related events after COVID-19 vaccination in autoimmune patients mainly focused on the immediate phenotypes in the small samples, and few studies gave real-world evidence on changes of inflammatory or hormone changes of AID, which were shown to predict future outcomes in a

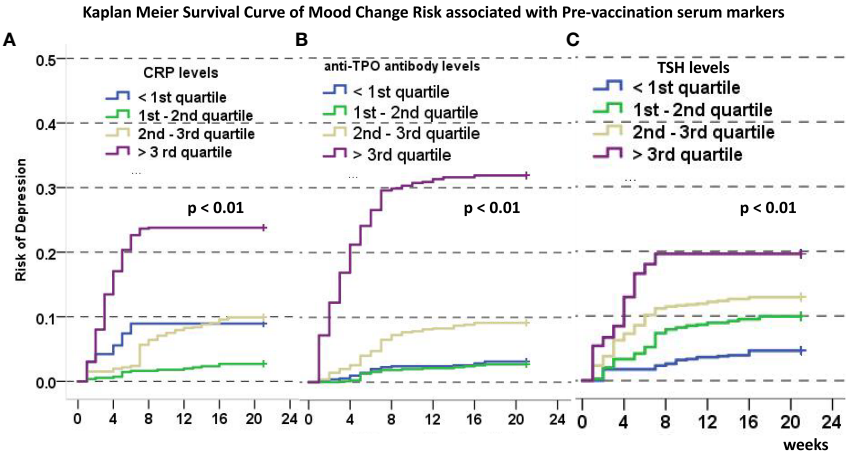


FIGURE 2
Kaplan-Meier Curve of disease-free survival (defined as time to mood changes) associated with CRP **(A)**, anti-TPO **(B)**, and TSH **(C)** levels categorized by the 25th, 50th, and 75th quartile. In all images, a higher ladder of marker categories predict higher incidence of disease-free survival.

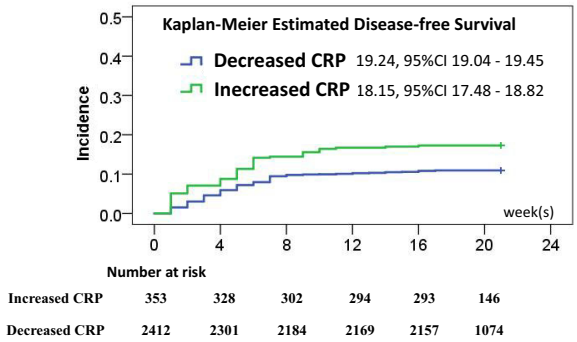


FIGURE 3
Disease-free survival rate estimates after vaccination associated with CRP changes, and the risk of mood changes was significantly different ($p < 0.01$).

number of chronic conditions (25, 26). This work demonstrated that TSH values were increased after vaccination, and independent analysis found associations between CRP values and incidence of TSH increase after vaccination. Moreover, the same serum markers were shown associated with incidence and severity of mood changes. Considering prior research, clinicians are suggested to monitor potential changes in disease progression after COVID-19 vaccination. Future research is encouraged to find molecular mechanisms of vaccine-induced hormone and mood changes.

Our work bears several limitations. Follow-up time was relatively short such that long-term disease-related outcomes cannot be assessed. In addition, third-dose vaccines took place 6 months after the second dose, and we did not assess outcomes after booster vaccination, which may provide more data on serum marker changes and their associations with mood change outcomes. In addition, we did not report immune response to vaccines, although response has been widely evidenced to be robust. Trajectory of these values could be evaluated in long-term follow-up and may be a source of validation for current study. Although nomogram was developed over a multi-center research protocol, a validation by an independent cohort is required to find predicting values.

## Conclusions

Patients with HT seemed to have increased incidence of mood changes after COVID-19 vaccines. This outcome seems related to changes in the CRP and TSH levels. Baseline serum markers—CRP, TSH, and anti-TPO antibodies—could predict outcome of mood changes and TSH increase after vaccination.

## 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 Second Affiliated Hospital of Shantou University Medical College. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

Prof XW and Dr AZ conceptualized and designed the study, and reviewed and revised the manuscript. Drs YM, NL designed the data collection instruments, carried out the initial analyses, and wrote the first draft of the manuscript. Drs JZ, YJ, YX, YOW, GZ, GJ, YAW, ZZ, GF, SH and CL coordinated and supervised data collection and critically reviewed the manuscript for important intellectual content. Drs YL, SC, XW, PZ, XL, YN, ML and SL collected data and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. The work reported in the paper has been performed by the authors, unless clearly specified in the text. 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.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1129746/full#supplementary-material

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# Case report: Preliminary study on the diagnosis and treatment of respiratory distress in patients with giant nodular goiter complicated with severe COVID-19

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**Background:** To investigate the practicality of emergency surgical and conservative medical treatments in patients with giant nodular goiter complicated by severe coronavirus disease 2019 (COVID-19)-related respiratory distress, evaluate the prognosis based on the two interventions, and explore the diagnosis and treatment plan of COVID-19-related respiratory distress in patients with giant nodular goiter.

**Methods:** Four cases were retrospectively collected. Among them, two cases underwent emergency surgery, one case was treated with conservative treatment, whereas the fourth case underwent emergency surgery after failure of conservative therapy.

**Results:** Dyspnea was significantly improved postoperatively, and the endotracheal tube was successfully removed 10.5 h after the operation, but inflammatory markers were greatly enhanced as compared to the preoperative values, patients with different degrees of fever, cough, and other discomforts postoperatively. Case 1 showed complete remission of all symptoms after 3 weeks, while case 2 displayed fever, cough, drowsiness, and other symptoms after the discharge and was eventually readmitted. In case 3, the conservative COVID-19 treatment marginally improved the pulmonary infection, fever, and other symptoms, but cough and other discomforts were persistent, along with delirium in later stages. Moreover, case 4 reported extubation failure after undergoing treatment with the standard new coronary pneumonia regimen in the tracheal intubation state; however, the patient was successfully weaned and extubated 9 days after emergency surgery to relieve the obstruction.

**Conclusion:** Our preliminary exploration suggested that patients with giant nodular goiter and respiratory tract obstruction post-acute COVID-19 infection can undergo early surgery after surgical tolerance evaluation for a better prognosis.

KEYWORDS

**giant nodular goiter, COVID-19, respiratory distress, diagnosis and treatment plan, surgery**

# Introduction

Since the outbreak of novel coronavirus disease 2019 (COVID-19), which is caused by SARS-CoV-2, several COVID-19-related thyroid diseases have emerged, including subacute thyroiditis, autoimmune thyroid disorders, non-thyroidal illness syndrome, and thyroid dysfunction due to unknown causes (1–3). Although a few respiratory distress cases have been recorded in which patients presented with tracheal compression due to giant nodular goiter superimposed with novel COVID-19 infection, only one case has been reported by Amin et al. (4). Moreover, it is still unknown whether novel COVID-19 infection can aggravate the tracheal compression symptoms and whether tracheal compression in giant nodular goiter cases can lead to prolonged COVID-19 pneumonia, which is difficult to treat. Furthermore, the efficacy of emergency tracheal intubation, ventilator-assisted support, or emergency thyroidectomy for relieving tracheal obstruction or a conservative treatment after controlling pulmonary infection in patients with dyspnea is still ambiguous. Does an emergency surgery increase the severity of lung infection and leads to enhanced inflammatory markers? Does the conservative treatment delay the optimal timing of surgery? How does an extended tracheal intubation time result in tracheomalacia, extubation failure, etc.? A precise evaluation of the need for surgical or conservative treatment is a key factor governing our current clinical decisions. Combined with the experience of treating four giant nodular goiter cases having tracheal compression coupled with severe coronavirus pneumonia post-COVID-19 pandemic in *The Second Affiliated Hospital Zhejiang University School of Medicine*, our study has discussed the development of an individualized treatment plan for emergency surgery adapted to the general population as mentioned below.

# Case introduction

## Inclusion criteria

Patients with giant nodular goiter with tracheal compression and superimposed severe COVID-19 infection with accompanying symptoms.

## Clinical data

This study was approved by the Human Research Ethics Committee of the Second Affiliated Hospital of Zhejiang University, and obtained the patient's consent.

Case 1: A 24-year-old female patient, after reporting dyspnea for >3 h and fever, with the highest body temperature of 37.9°C, without any cough or expectoration, was admitted on December 16, 2022. Physical examination showed deep breathing, respiratory rate 40 breaths/min, cyanotic lips and nail bed, clear breath sounds in both lungs, and no obvious rales. She had a previous history of hearing loss, while the remaining findings were unremarkable. After admission, the COVID-19 antigen test was positive, whereas the oxygen saturation was 86% (reference value 95–100%), white blood cell (WBC) $9.7 \times 10^9$/L (reference value 4–$10 \times 10^9$/L), neutrophil percentage 76.4% (reference value 50.0–70.0%), c-reactive protein (CRP) 1.7 mg/L (reference value<10.0 mg/L), and interleukin (IL)-6133.50 pg/mL (reference value<7.0 pg/mL). An emergency computed tomography (CT) revealed marked bilateral thyroid enlargement with uneven density, compression, and tracheal as well as esophageal narrowing, with no significant positive chest CT signs. Thyroid hormone level: TT3 1.11 nmol/L (reference value 0.98–2.33), TT4 70.5 nmol/L (reference value 62.7–150.8), FT3 3.27 pmol/L (reference value 2.43–6.01), FT4 11.45 pmol/L (reference value 9.01–19.05), TSH 0.29 mIU/L (reference value 0.35–4.49), TPOAB 3.38 IU/mL (reference value<5.61), ATGAB 1.20 IU/mL (reference value < 4.11), TG >500 μg/L (reference value 3.50–77.00), Diagnostic considerations: novel coronavirus pneumonia (severe). After a multidisciplinary consultation, and excluding dyspnea caused by cardiopulmonary function, "bilateral total thyroidectomy (retrosternal thyroidectomy)" was performed under general anesthesia with endotracheal intubation at 00: 10 AM on December 17, 2022. The operative time was 1 h and 35 min. Subsequently, the patient was shifted to ICU postoperatively and was provided assisted ventilation. The patient was successfully extubated 10 h after the operation and transferred to the isolation ward after becoming stable. One day after the operation, the patient developed a fever, with the highest temperature of 39.1°C. WBC count was $7.8 \times 10^9$/L (reference value 4–$10 \times 10^9$/L), neutrophil percentage was 79.6%L (reference value 95–100%), CRP was 30.8 pg/mL, procalcitonin (PCT) was 1.52 ng/mL (reference value<0.5 ng/mL), and IL-6 was 30.8 pg/mL. The patient got relief after symptomatic cooling treatment. As there was no cough, expectoration, muscle soreness, or any other discomfort during hospitalization, the patient was discharged 3 days postoperatively. A postoperative pathological examination showed thyroid nodules in the bilateral lobes of the thyroid gland. Postoperative follow-up was: The patient reported cough and expectoration after discharge, which was completely relieved after 3 weeks, without dyspnea, fever, fatigue, or other discomforts. Currently, the patient is in good physical condition.

Case 2: A 74-year-old female patient was admitted due to dyspnea for >1 h on December 19, 2022, without any fever, cough, expectoration, etc. Physical examination: dysphoria, extreme dyspnea, cyanotic lips and nail bed, respiratory rate 40 breaths/min, clear breath sounds in both lungs, no obvious rales heard. Being a deaf-mute patient, she had a history of hypertension and right partial thyroidectomy >20 years ago, a COVID-19 positive antigen test, oxygen saturation of 67%, and emergency tracheal intubation. The auxiliary examination showed WBC as $5.8 \times 10^9$/L, neutrophil percentage 74.6%, CRP 11.0 mg/mL, PCT 0.44 ng/mL, and IL-6 20.40 pg/mL. An emergency CT showed bilateral thyroid enlargement with multiple nodules and necrotic areas, right airway stenosis, throat edema, pleural effusion, and thoracic cavity effusion. Thyroid hormone level: TT3 1.33 nmol/L (reference value 0.98–2.33), TT4 74.7 nmol/L (reference value 62.7–150.8), FT3 3.87 pmol/L (reference value 2.43–6.01), FT4 9.47 pmol/L (reference value 9.01–19.05), TSH 1.56 mIU/L (reference value 0.35–4.49). Diagnostic considerations: novel coronavirus pneumonia (critical type). After a multidisciplinary consultation, cardiopulmonary function-related dyspnea was excluded. On December 19, 2022, the patient underwent "left total thyroidectomy + right partial thyroidectomy" under general anesthesia with endotracheal intubation. The operative time was 1 h and 49 min. The patient was admitted to ICU postoperatively and given assisted ventilation. Furthermore, 11 h later, the patient was successfully extubated and transferred to the general ward. The patient was given anti-infection drugs, fluid infusion, and supportive

treatment. One day after the operation, the patient developed a fever, with a body temperature of 38.4°C, shortness of breath with a rate of about 40 breaths/min, and oxygen saturation of 93%. The patient was immediately given nasal catheter oxygen inhalation of 3–5 L/min. Laboratory investigations revealed the WBC count as $12.6 \times 10^9$/L, neutrophil percentage 90.4%, CRP 97.1 mg/mL, and PCT 0.65 ng/mL. Based on this, the patient underwent dexamethasone intravenous bolus, anti-infection, atomization inhalation, phlegm reduction, and other symptomatic treatment. The vital signs were stable 3 days after the operation, without any recurrence of shortness of breath, fever, and oxygen saturation reduction. Although the patient was discharged 3 days postoperatively, the postoperative pathological examination revealed a nodular goiter of the left thyroid gland and isthmus with hemorrhagic cystic degeneration and focal follicular epithelial dysplasia. For right thyroid nodular goiter, 2 days after discharge, the patient displayed repeated fever (maximum body temperature: 38.5°C), fatigue, drowsiness, cough, and expectoration, without dyspnea. Subsequently, the patient was hospitalized in a local hospital and discharged after an improvement.

Case 3: An 89-year-old female patient was admitted due to a cough for >3 days on December 28, 2022. On admission day, the patient was diagnosed with a new COVID-19 infection, significant cough, and expectoration, respiratory rate 40 breaths/min, cyanotic lips and nail bed, without fever, and moist rales could be heard in both lungs. The patient also had a history of previous thyroid surgery, hypertension, and Parkinson's disease. After the patient visited our emergency department, the investigations revealed oxygen saturation of 92%, WBC $3.0 \times 10^9$/L, neutrophil percentage of 78.1%, CRP 5.3 mg/mL, PCT 0.04 ng/mL, and IL-6 27.3 pg/mL. A CT scan suggested bilateral thyroid enlargement, right deviation of tracheal compression, and scattered cord shadows, as well as exudations in both lungs, pleural effusion. Thyroid hormone level: TT3 1.34 nmol/L (reference value 0.98–2.33), TT4 88.9 nmol/L (reference value 62.7–150.8), FT3 4.07 pmol/L (reference value 2.43–6.01), FT4 12.00 pmol/L (reference value 9.01–19.05), TSH 0.32 mIU/L (reference value 0.35–4.49), TPOAB 4.22 IU/mL (reference value<5.61), ATGAB 9.60 IU/mL (reference value < 4.11), TG >500 μg/L (reference value 3.50–77.00). Diagnostic considerations: novel coronavirus pneumonia (severe). After admission, the patient was given oxygen inhalation, nutritional support, oral antiviral therapy with nelmativir+ritonavir, anti-infection drugs, atomization inhalation, phlegm reduction, anticoagulation drugs, and other symptomatic treatment. By the end of the study, after >50 days of hospitalization, the patient's lung infection, fever, and other symptoms were marginally improved, but cough and other discomforts, as well as delirium and other psychiatric symptoms at the later stages, were still observed. Furthermore, the patient was still hospitalized by the time our study's deadline ended.

Case 4: An 84-year-old female patient with airway foreign body obstruction for >2 weeks was admitted to our hospital on January 11, 2023. Having a history of left partial thyroidectomy, she reported chest tightness, shortness of breath, and cyanotic lips after eating the "pill" >2 weeks ago and visited a local hospital for an oxygen saturation level of 50%. She underwent emergency endotracheal intubation, airway clearance, and foreign body aspiration, while her oxygen saturation was maintained at >95%. An emergency CT revealed possible local compression of retrosternal goiter, multiple patchy high-density shadows in both lungs, and bilateral pleural effusion with adjacent lung tissue atelectasis. After admission, her COVID-19 antigen test was positive; however, her general condition improved after the administration of anti-infection, hormonal, and anti-inflammatory drugs with other treatments. Three days later, the removal of tracheal intubation further decreased the patient's oxygen saturation level to 70%. The tracheal intubation was performed again. Seven days post-admission, the patient maintained oxygen saturation > 93% under endotracheal intubation and spontaneous breathing. After the removal of the endotracheal tube, the oxygen saturation decreased to about 70%, 1.5 h after observation. The patient came to our hospital for emergency treatment after second endotracheal intubation. Physical examination showed sedation, endotracheal intubation, ventilator-assisted breathing, and crackles heard in both lungs. Emergency CT revealed thyromegaly with less uniform density, tracheal compression, two pulmonary inflammatory exudates, partial right upper lung consolidation, and bilateral pleural effusion with adjacent lung tissue atelectasis. Her investigations revealed WBC count as $17.8 \times 10^9$/L, neutrophil percentage 93.6%, CRP 37.8 mg/mL, PCT 0.35 ng/mL, IL-6 19.7 pg/mL, and albumin 22.5 g/L. Thyroid hormone level: TT3 1.77 nmol/L (reference value 0.98–2.33), TT4 65.6 nmol/L (reference value 62.7–150.8), FT3 2.98 pmol/L (reference value 2.43–6.01), FT4 15.85 pmol/L (reference value 9.01–19.05), TSH 0.03 mIU/L (reference value 0.35–4.49), TPOAB <0.5 IU/mL (reference value < 5.61), ATGAB 6.67 IU/mL (reference value<4.11), TG 99.81 μg/L (reference value 3.50–77.00). Diagnostic considerations: novel coronavirus pneumonia (critical type). After admission to ICU, the patient was given assisted ventilation, nutritional support, anti-infection drug, atomization inhalation, expectorant, anticoagulant, correction of hypoproteinemia, and other symptomatic treatment. On January 18, 2023, the patient underwent "retrosternal right thyroidectomy and isthmectomy+left thyroidectomy" under emergency general anesthesia with an operation time of 2 h and 10 min. The patient was admitted to the ICU postoperatively and given assisted ventilation, an anti-infection drug, fluid infusion, and symptomatic treatment. After trying to remove the endotracheal tube 6 days after the operation, the patient developed three concave signs (refers to significant depression of the suprasternal fossa, supraclavicular fossa, and intercostal space during inspiration when obstructive ventilatory dysfunction occurs), with profuse sweating, a decrease in oxygen saturation, and other symptoms. Moreover, the patient underwent endotracheal intubation and assisted ventilation again. Fiberoptic bronchoscopy revealed poor vocal cord mobility and marked swelling. Corticosteroids were administered to improve airway edema along with anti-infection drugs, nutritional support, and other treatments. The patient was successfully extubated 9 days postoperatively. After extubation, the patient displayed no obvious hoarseness, dyspnea, or other symptoms. The oxygen saturation level was 98% at rest. The patient was transferred to a local hospital 10 days after the operation to continue rehabilitation. A postoperative pathological examination showed bilateral thyroid and isthmic nodular goiter with calcification, collagenization, and hemorrhagic cystic degeneration. Ten days after the discharge, postoperative follow-up revealed that the patient experienced smooth breathing, oxygen saturation of 97–98% at rest, well-controlled pulmonary infection, intermittent delirium, and other psychiatric symptoms (Tables 1–4; Figures 1, 2).

## Discussion

COVID-19 infection is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is mainly

TABLE 1 Inflammatory markers of 4 cases.

| | WBC | Neutrophil percentage | CRP | IL-6 | PCT |
|---|---|---|---|---|---|
| Case 1 | | | | | |
| Preoperative | 9.70×10^9/L | 76.4% | 1.7 pg/mL | 133.50 pg/mL | (−) |
| Postoperative | 7.80×10^9/L | 79.6% | 30.8 pg/mL | 30.8 pg/mL | 1.52 ng/mL |
| Case 2 | | | | | |
| Preoperative | 5.81×10^9/L | 74.6% | 11.0 pg/mL | 20.40 pg/mL | 0.44 ng/mL |
| Postoperative | 12.60×10^9/L | 90.4% | 97.1 pg/mL | (−) | 0.65 ng/mL |
| Case 3 | 3.00×10^9/L | 78.1% | 5.3 pg/mL | 27.3 pg/mL | 0.04 ng/mL |
| Case 4 | 17.80×10^9/L | 93.6% | 37.8 pg/mL | 19.7 pg/mL | 0.35 ng/mL |
| Reference value | 4–10×10^$^9$/L | 50.0–70.0% | <10.0 mg/L | <7.0 pg/mL | 0.5 ng/mL |

WBC, white blood cell; CRP, C reactive protein; IL-6, Interleukin- 6; PCT, procalcitonin.

TABLE 2 Clinical presentation and treatment summary of 4 cases.

| | Thyroid size | Tracheal diameter at narrowest point | Chest computed tomography | Surgery or not | Thyroid hormone levels | Oxygen saturation | Symptoms and signs | Inflammatory markers |
|---|---|---|---|---|---|---|---|---|
| Case 1 | Left 44 × 46 mm Right 44 × 46 mm | 3 mm | No positive findings | Yes | Table 4 | Table 3 | | Table 1 |
| Case 2 | Left 61 × 60 mm Right 41 × 24 mm | – | Pleural effusion, and thoracic cavity effusion | Yes | | | | |
| Case 3 | Left 79 × 65 mm, Right 64 × 41 mm | 4 mm | Scattered streak shadows, scattered exudation, pleural effusion | | | | | |
| Case 4 | Left thyroidectomy, Right 87 × 38 mm | – | Patchy high-density shadows, bilateral pleural effusion with multiple adjacent lung tissues atelectasis | Yes | | | | |

TABLE 3 Thyroid hormone levels.

| | FT3 | FT4 | TSH | TG | TPOAB | ATGAB | TT3 | TT4 |
|---|---|---|---|---|---|---|---|---|
| Case 1 | 3.27 | 11.45 | 0.29 | >500 | 3.38 | 1.20 | 1.11 | 70.5 |
| Case 2 | 3.87 | 9.47 | 1.56 | – | – | – | 1.33 | 74.7 |
| Case 3 | 4.07 | 12.00 | 0.32 | >500 | 4.22 | 9.60 | 1.34 | 88.9 |
| Case 4 | 2.98 | 15.85 | 0.03 | 99.81 | <0.5 | 6.67 | 0.77 | 65.6 |
| Reference value | 2.43–6.01 (pmol/L) | 9.01–19.05 (pmol/L) | 0.35–4.49 mIU/L | 3.50–77.00 Ug/L | <5.61 IU/mL | <4.11 IU/mL | 0.98–2.33 (nmol/L) | 62.7–150.8 (nmol/L) |

transmitted by the spread of respiratory droplets, with an incubation period of generally 3–7 days, usually ≤14 days. The main clinical manifestations are fever, fatigue, dry cough, respiratory failure, septic shock, and/or multiple organ dysfunction, and failure in severe cases, thus, endangering the patient's life (5). On April 10, 2020, the World Health Organization (WHO) characterized COVID-19 as a global epidemic, as the infection involved >210 countries/regions (6). According to a WHO estimate, >670 million people worldwide have been infected, with >6.7 million deaths, however, 14–17% of infected patients may develop

TABLE 4 Symptoms, signs, severity grade of COVID-19.

| | Symptoms | Signs | Oxygen saturation | Novel coronavirus pneumonia grade |
|---|---|---|---|---|
| Case 1 | Deep breathing, respiratory rate 40 times/min, lips and nail bed cyanosis | T 37.9°C Clear breath sounds in bilateral lung, without obvious rales | 86% | Severe |
| Case 2 | Dysphoria, extreme dyspnea, cyanotic lips and nail bed respiratory rate 40 breaths/min | No fever, clear breath sounds in bilateral lung, without obvious rales | 67% | Critical type |
| Case 3 | Cough, expectoration, shortness of breath 30 beats/min, cyanotic lips and nail bed | No fever, moist rales palpable in bilateral lung | 92% | Severe |
| Case 4 | Ventilator-assisted breathing status at admission to our hospital | Crackles heard in bilateral lung | 50% | Critical type |

| Grading | Clinical presentation |
|---|---|
| Mild | Mild clinical symptoms, no imaging findings of pneumonia |
| Common type | With fever, respiratory tract and other symptoms, imaging showed pneumonia |
| Severe | Meet any of the following:<br>1. Respiratory distress, RR ≥ 30 beats/;<br>2. Oxygen saturation ≤ 93% at rest;<br>3. Arterial partial pressure of oxygen (PaO2)/oxygen concentration (FiO2) ≤ 300 mmHg;<br>4. Progressive aggravation of clinical symptoms, pulmonary imaging showed that the lesion significantly progressed >50% within 24–48 h |
| Critical type | Patients who meet one of the following conditions:<br>1. Respiratory failure, and need mechanical ventilation;<br>2. Shock;<br>3. Combined with other organ failure need ICU monitoring treatment. |

The grading criteria for COVID-19 in China.

COVID-19-related acute respiratory distress syndrome (7), and 2.3% of them require endotracheal intubation intervention (8). However, Piazza et al. reported that long-term endotracheal intubation in COVID19 patients, might lead to tracheomalacia, stenosis, post-intubation granuloma, and tracheoesophageal leakage. Additionally, a tracheotomy performed 7–14 days after endotracheal intubation significantly enhances successful weaning rates and reduces associated complications as well as mortality as compared to long-term maintenance endotracheal intubation (9).

Giant nodular goiter is mostly caused by nodular goiter or thyroid adenoma, which can compress, shift and narrow the cervical trachea, thus, leading to ventilatory dysfunction. The incidence of tracheal compression and dyspnea caused by giant nodular goiter is 10–50%, while the incidence of dyspnea caused by retrosternal goiter is ≥75% (10). In the initial stage, the patient has no obvious discomfort and can experience dyspnea, along with other associated symptoms, whenever there is an increased oxygen demand in the case of long-distance exercise or weather changes. Furthermore, the probability of dyspnea significantly increases if such patients are suffering from COVID-19. So the question in these cases is whether COVID-19 infection or thyroid enlargement compressing the trachea is the main cause of respiratory distress. We believe that the respiratory distress observed in our cases was the result of the interplay between obstructive ventilatory dysfunction caused by compression from the giant nodular goiter and diffuse ventilatory dysfunction caused by COVID-19. We hypothesize that if other severe viral pneumonias were substituted, similar outcomes would be observed, given the persistence of obstructive ventilatory dysfunction caused by the goiter compression and diffuse ventilatory dysfunction caused by pneumonia. According to the clinical manifestations, examination, and imaging data of four patients, case 1 presented with low inflammatory indicators and no infection findings on pulmonary imaging. Since cervical CT showed that the diameter of the narrowest part of the trachea was 3 mm, dyspnea may be caused by an upper respiratory tract obstruction. Case 2 examination after endotracheal intubation revealed exudative bilateral pleural effusions, along with increased CRP and IL-6 values and unknown airway stenosis. In case 3 lung exudation, IL-6 increased significantly, while the diameter of the narrowest part of the trachea was 4 mm. Moreover, in case 4, examination after tracheal intubation revealed two pneumonic exudates, elevated inflammatory markers, and airway stenosis of unknown etiology. In COVID-19, the human cells are primarily attacked through angiotensin-converting enzyme 2 (ACE2) to induce an inflammatory response. Although ACE2 is expressed in the nose, oral mucosa, throat, and tongue, edema manifestations in the corresponding organs might occur (11–13). Another study by Tasnuva disclosed that dyspnea in giant nodular goiter with new COVID-19 pneumonia may be related to airway edema, thus, aggravating tracheal stenosis (4), and our findings in case 1 confirmed this view. However, we did not compare the relevant imaging data before the disease onset and did not perform a bronchoscopy to identify whether airway edema led to aggravated airway stenosis.

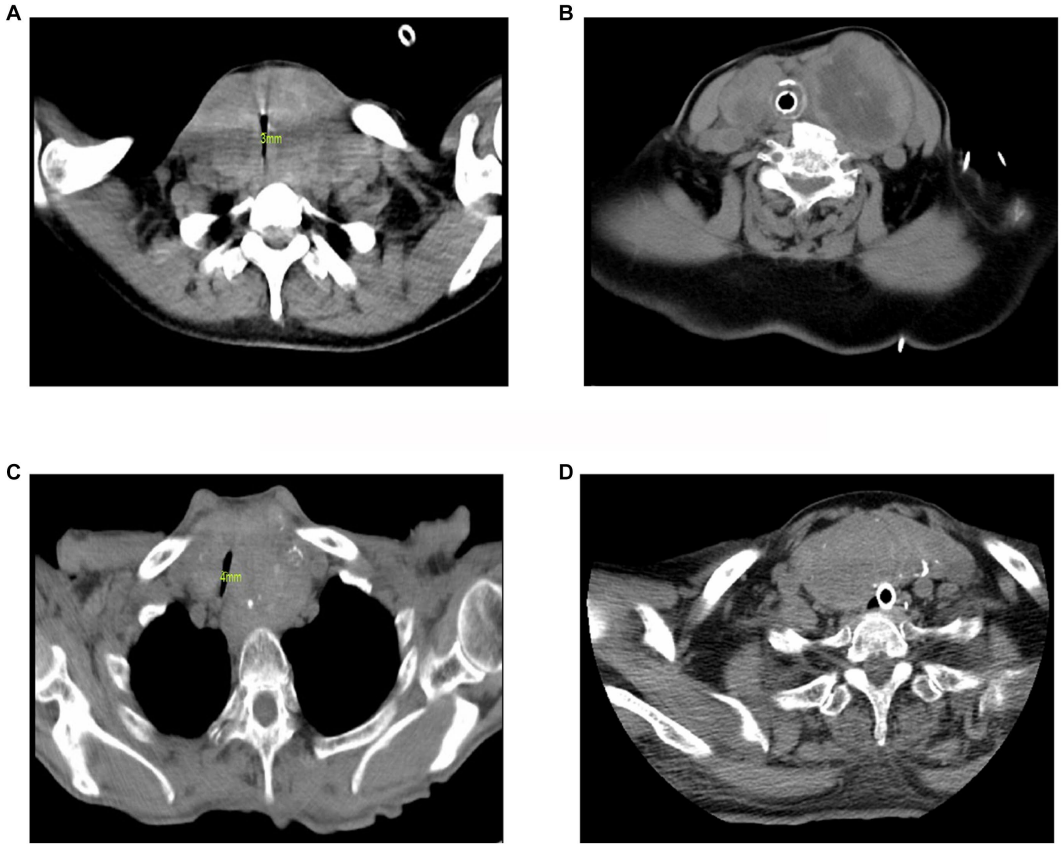


FIGURE 1
Preoperative cervical CT images of 4 cases. **(A)** Case 1. The size of the left thyroid gland was 44 × 46 mm, and the size of the right thyroid gland was 40 × 28 mm. Protruding behind the sternum, the trachea was significantly compressed, and the diameter of the narrowest part was 3 mm. **(B)** Case 2. Under tracheal intubation, the trachea moved to the right, the size of the left thyroid gland was about 61 × 60 mm, and the size of the right thyroid gland was about 41 × 24 mm. **(C)** Case 3. The size of the left thyroid gland was about 79 × 65 mm, the size of the right thyroid gland was about 64 × 41 mm, the trachea was shifted to the right, and the diameter of the narrowest part was 4 mm. **(D)** Case 4. Under tracheal intubation, after the left partial thyroidectomy, the size of the right thyroid gland was about 87 × 38 mm, protruding behind the sternum.

The Conservative management of the four cases is not the same, because we encountered numerous challenges during that period. In December 2022, China underwent a shift in the management and control of COVID-19. Consequently, a sudden surge in infections occurred, surpassing the preparedness of medical resources. There was a shortage of specific drugs, medical devices, and various supplies, making it difficult to ensure uniform treatment management for each patient during that time. Recent literature suggests that the time from COVID-19 onset to tracheal intubation intervention is usually 8–9 days (14, 15), while the mortality rate of critically ill patients is 16.7% ~ 61.5% (16, 17), of which the mortality rate at 24 h after tracheal intubation is 10.4%, and the 28-day mortality rate is ~61% (18). If dyspnea occurs in such patients, it requires tracheal intubation. Quick relief from respiratory tract obstruction and removal of tracheal intubation without delay is the key to improving the survival rate. While treating the four patients with giant nodular goiter complicated with severe COVID-19, we found that the dyspnea of patients undergoing emergency surgery significantly improved postoperatively, and the tracheal intubation was successfully removed 10.5 h after the operation on average. However, various inflammatory indicators postoperatively were significantly increased to different extents when compared with those before the operation, accompanied by different degrees of fever, cough, and other discomforts postoperatively. Since case 1 was younger, symptoms such as cough and sputum resolved completely after 3 weeks and recovered quickly after discharge, while case 2 was an elderly patient who was readmitted due to fever, cough, drowsiness, and other symptoms after discharge, however, emergency surgery in this patient resulted in aggravated inflammation. Although case 3 underwent the standard COVID-19 regime for long time after hospitalization, the patient's lung infection, fever, and other symptoms were marginally improved, but cough and other discomforts, along with delirium and other psychiatric symptoms in later stages, were still observed. Case 4 failed multiple extubation attempts after the standard COVID-19 treatment regimen, which might have been caused by aggravated tracheal stenosis due to throat edema as a result of prolonged intubation time, patient lung inflammation causing lung dysfunction. The patient was successfully extubated 9 days after emergency surgery to relieve the obstruction. Ten days after discharge, the patient follow-up revealed smooth breathing and good control of pulmonary infection, but psychiatric symptoms such as delirium occurred in later stages. Additionally, psychiatric symptoms like delirium occurred in later stages in both the patients who underwent prolonged conservative treatment, which was either purely coincidental or might be related to other factors, but due to the small number of cases in our study, any relevant conclusions could not be made.

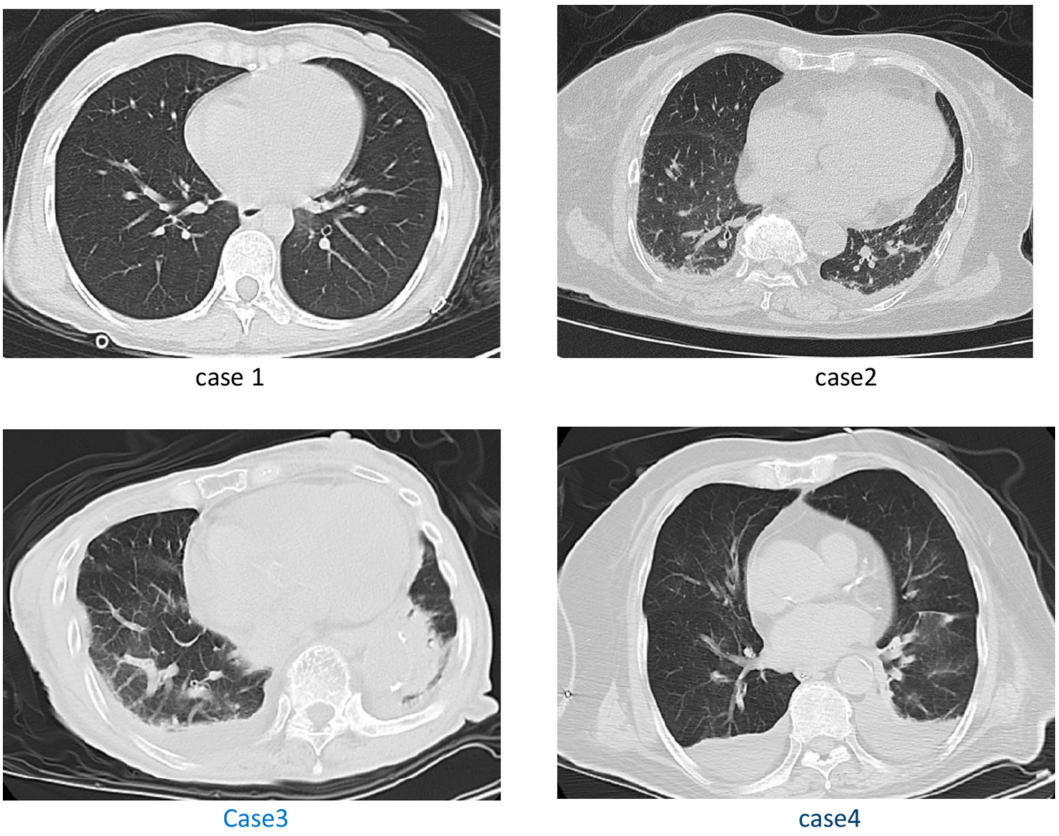


FIGURE 2
Case 1: with no significant positive chest CT signs. Case 2: pleural effusion, and thoracic cavity effusion. Case 3: scattered streak shadows in bilateral lung, scattered exudation in bilateral lung, pleural effusion. Case 4: multiple patchy high-density shadows in both lungs, and bilateral pleural effusion with adjacent lung tissue atelectasis.

In patients requiring emergency surgery at our center, we perform preoperative emergency chest and neck CT as well as evaluation of various inflammatory indicators to assess tracheal compression, pulmonary inflammation, presence of non-recurrent laryngeal nerve, and immediate endotracheal intubation for patients showing significant reduction in oxygen saturation. During the operation, the operation time should be shortened to reduce the spread of inflammation along with precise ligation of the blood vessels to avoid second surgical trauma and protect the recurrent laryngeal nerve to avoid aggravated dyspnea caused by vocal cord injury and difficult extubation at a later stage. In order to shorten the operative time, such patients can undergo thyroidectomy with a large cervical incision, subtotal thyroidectomy, or near-total thyroidectomy.

After emergency tracheal intubation for patients with giant nodular goiter showing tracheal compression combined with an acute attack of severe COVID-19 with low oxygen saturation, the selection of the extubation and operation time is a crucial parameter. After 3–4 days of an acute attack, the tracheal intubation can be removed by reassessing the levels of inflammatory indicators and the patient's general condition. If the extubation is smooth, symptomatic treatment can be continued or deferred to elective thyroidectomy later on. If such patients fail in multiple extubation attempts, like in our case number 4, and for patients with severe thyroid-related tracheal compression, emergency surgery can be performed simultaneously as anti-COVID-19 treatment after the general condition is corrected.

After a comparative analysis, we suggest the following advantages of emergency surgery: it can quickly relieve upper respiratory tract obstruction, and postoperative patients recover faster. However, this technique has a disadvantage, namely, surgery may aggravate the inflammatory response. Furthermore, using a conservative treatment can avoid surgical stimulation that might aggravate the patient's inflammatory response. Although it has a few disadvantages: it may lead to extubation difficulties, as long-term intubation may lead to aggravation of pulmonary infection, tracheomalacia, and other complications, and even delirium and other psychiatric symptoms at the later stages. In contrast, emergency surgery in such patients can provide a better prognosis. For choosing the timing of surgery, we suggest that before the COVID-19 progresses to critical illness, early surgery should be performed when the function of other organs is not significantly impaired, the earlier the operation, the greater the benefit to patients. Amin et al. (4) also recommended that in nodular goiter patients with respiratory obstruction, a timely intervention to relieve airway obstruction is the best choice. By analogy, patients with other diseases that may lead to acute exacerbation of pulmonary inflammation (such as chronic obstructive pulmonary disease) superimposed with giant nodular goiter and tracheal compression may also give priority to surgical treatment.

## Conclusion

It is difficult to choose an accurate treatment plan for a giant nodular goiter with COVID-19-related respiratory tract obstruction. Our preliminary exploration suggests that an early surgery to relieve respiratory tract obstruction in such patients after evaluation of surgical tolerance can provide a better prognosis. However, due to fewer study cases in our center, this specific situation requires more samples for precise analysis to derive better conclusions.

## 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 humans were approved by Human Research Ethics Committee of the Second Affiliated Hospital of Second Affiliated Hospital of Zhejiang University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The 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. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

## Author contributions

FW, XY, ZR, and YW performed the material preparation, data collection, and analysis. FW wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript, contributed to the study conception and design, read, and approved the final manuscript.

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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.

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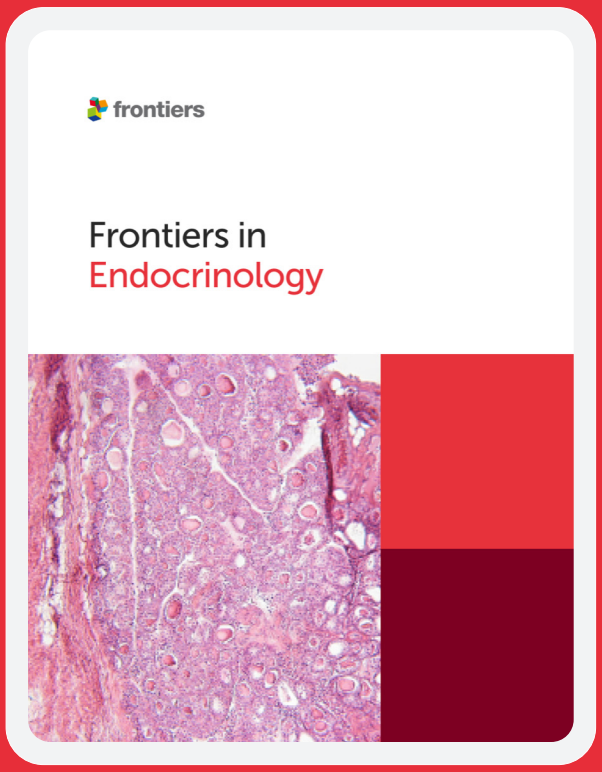


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