ORIGINAL ARTICLE

The impact of covid-19 on thyroid function tests in pregnancy

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Abstract

Purpose To investigate the effects of laboratory-confirmed SARS-CoV-2 infection on thyroid function tests (TFTs) in pregnant women and to evaluate whether TFT changes are related to the severity and prognosis.

Methods Consecutive pregnant women tested for SARS-CoV-2 by RT-PCR at Ankara City Hospital were recruited between January 2021 and September 2021. Thyroid-stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3), thyroid peroxidase antibody (anti-TPO), and thyroglobulin antibody (anti-TG) were measured on admission.

Results Among 747 eligible pregnant women with the same baseline characteristics, 369 RT-PCR-positive women in the patient group and 378 RT-PCR-negative women in the control group were included in the analyses. Pregnant women in the patient group had significantly lower TSH, FT4, FT3, Anti TPO, and Anti TG concentrations compared with those in the control group ($p < 0.001$). The proportion of patient groups with mild, moderate, severe, and critical diseases were 297 (80.4%), 40 (10.8%), 17 (4.6%), and 15 (4.2%), respectively, among which with the moderate, severe, and critical disease had significantly lower FT3 values (2.5 vs 2.19 pg/mL, $p < 0.001$) and higher nonthyroidal illness syndrome (NTIS) (29.2 vs. 8.4%, $p < 0.001$) than those with mild disease. Lower FT3 values increased the risk of ICU admission, NICU admission, and severe disease ($p < 0.001$). FT3 and TSH correlated positively with lymphocytes ($p < 0.001$) and negatively correlated with C-reactive protein (CRP) ($p < 0.001$, $p = 0.005$).

Conclusion The SARS-CoV-2 infection seems to have an impact on the TFTs of pregnant women, and particularly FT3 level seems to be correlated with disease severity.

Keywords COVID-19 · Pregnancy · SARS-CoV-2 · RT-PCR · Thyroid function tests

Introduction

The coronavirus disease 2019 (COVID-19), was declared a global pandemic by the World Health Organization (WHO) in March 2020 [1]. By July 18, 2022, more than 565 million confirmed cases and more than 6 million deaths have been reported worldwide [2].

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which uses angiotensin-converting enzyme 2 (ACE2) as a host cell receptor to infect humans [3, 4]. ACE2 is not only an enzyme as it is known but also a receptor on tissue cells through which SARS-CoV-2 enters the host cells. According to recent studies, it is highly expressed in many organs such as the lung, heart, kidney, liver, and endocrine organs (e.g., thyroid and pituitary), making the hypothalamic-pituitary-thyroid (HPT) axis a susceptible target of SARS-CoV-2 for both direct (due to ACE2 expression) and indirect damage (due to abnormal immune-inflammatory responses to the virus) [5–9]. Based on this pathophysiology of the virus, previous studies have shown that COVID-19 could induce reversible thyroid dysfunction and could be associated with the severity of the disease [10, 11].

Pregnancy has a considerable impact on the thyroid gland and its function. Depending on the need for maternal thyroid hormone production, the importance of thyroid hormones increases significantly during

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pregnancy. Two sources supply thyroid hormones to fetal tissues: the developing fetal thyroid gland and the maternal thyroid gland. Although the fetal thyroid gland begins to develop by the 5th or 6th week of gestation and begins to produce thyroid hormones by about the 12th week of gestation, it is not functionally mature until the 18th to 20th week of gestation, and nearly 30% of the free thyroxine (FT4) in the umbilical cord blood serum at delivery is of maternal origin. This means that maternal FT4 is transferred to the fetus throughout pregnancy and is critical for normal fetal brain development, especially in the first half of pregnancy [12–15]. In addition, thyroid disorders in pregnancy have been associated with serious maternal, fetal, and neonatal complications in numerous previous studies: spontaneous abortions, preterm delivery, preeclampsia, gestational diabetes mellitus, admission to the neonatal intensive care unit (NICU), placental abruption, and breech presentation [16–19]. Thus, timely detection and treatment of pregnant women with thyroid dysfunction are clinically important because even mild thyroid dysfunction can lead to adverse pregnancy outcomes. Almost all data in the literature on pregnant women are limited by the absence of FT3 measurement as the fetus majorly relies on transplacental delivery of maternal T4.

There are many studies investigating the association between thyroid functions and COVID -19, but similar studies on pregnant women are limited and restricted to comparing the pandemic period with the pre-pandemic period [20, 21]. To our knowledge, there has been no study comparing SARS-CoV-2 positive pregnant women with negative pregnant women during the pandemic period with respect to the analysis of thyroid function.

Therefore, the objectives of the present study were to investigate the effects of laboratory-confirmed SARS-CoV-2 infection on thyroid function tests (TFT) in pregnant women and to determine whether it leads to thyroid dysfunction. We also evaluated whether HPT axis and autoantibody changes are related to the severity and prognosis of COVID-19 in pregnancy and whether there is a correlation between TFT and inflammatory disease markers in patient pregnant women.

Materials and methods

This single-center case-control study was performed at Ankara City Hospital, a tertiary pandemic referral hospital with approximately 20,000 deliveries per year, accounting for the vast majority of SARS-CoV-2 infected pregnant women antenatal examinations and deliveries in Ankara, Turkey. Ethics approval was obtained from the institutional review board (No. E2-21-269). All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki [22].

Pregnant women from all trimesters were enrolled, who underwent thyroid function analysis at our hospital between January 01, 2021, and September 30, 2021, and were simultaneously tested for SARS-COV2 by reverse transcriptase-polymerase chain reaction (RT-PCR) and divided into 2 groups. RT-PCR-positive pregnant women considered as the study group was compared to an RT-PCR-negative gestational age-matched control group of pregnant women at the same period. According to the national guideline for COVID-19 management, the study group was divided into subgroups; mild, moderate, severe, and critical [23]. In all pregnant women, SARS-Cov-2 infection was confirmed by quantitative RT-PCR from nasal-oropharyngeal swab and blood samples were drawn from participants from antecubital vein between 07:00 a.m. and 09:00 a.m. along with RT-PCR test upon their first admission to the hospital or first overnight fasting after RT-PCR testing before initiation of any treatment and medication.

The following information, including sociodemographic and clinical data, was recorded by the researchers in the electronic medical record: Maternal age, gravidity, parity, previous miscarriage, living child, pre-pregnancy body mass index (BMI), the gestational week at the time of testing, gestational trimester at the time of diagnosis, the severity of COVID-19, maternal mortality, ICU admission rate, neonatal ICU admission rate, thyroid function tests (TFT); thyrotropin (TSH), free thyroxine (FT4), free triiodothyronine (FT3), antithyroid peroxidase antibody (anti-TPO), and antithyroglobulin antibody (anti-TG) concentrations.

Pregnant women were excluded from the analysis if they were obese (BMI before pregnancy >40 kg/m^2), underwent in vitro fertilization (IVF), had multiple pregnancies, had a history of thyroid disease or thyroid autoantibody positivity, were taking thyroid medication, or were using steroids and heparin for any reason. We did not include the cases if there were any missing values of TSH, FT3, FT4, and thyroid autoantibodies. We also wanted to exclude vaccinated pregnant women, based on a systematic review that analyzed data from 51 patients who developed thyroid problems after the COVID-19 vaccination [24]. Indeed, one study reported low acceptance of COVID -19 vaccination among pregnant women in Turkey [25], none of the participants were vaccinated in the present study. in the present study, none of the participants were vaccinated. A total of 747 pregnant women-study groups ($n = 369$), and control group ($n = 378$)-were included in the final analysis (Fig. 1).

The lower limits of detection of thyroid function tests in our laboratory were 28 and 0.1 IU/ml for Anti TPO and Anti TG, respectively; 0.01 mU/L for TSH; 1.36 ng/L for FT3; 0.63 ng/dl for FT4. Anti TPO≥60 IU/ml was considered positive. Anti TG ≥1.3 IU/ml was considered positive. The trimester-specific
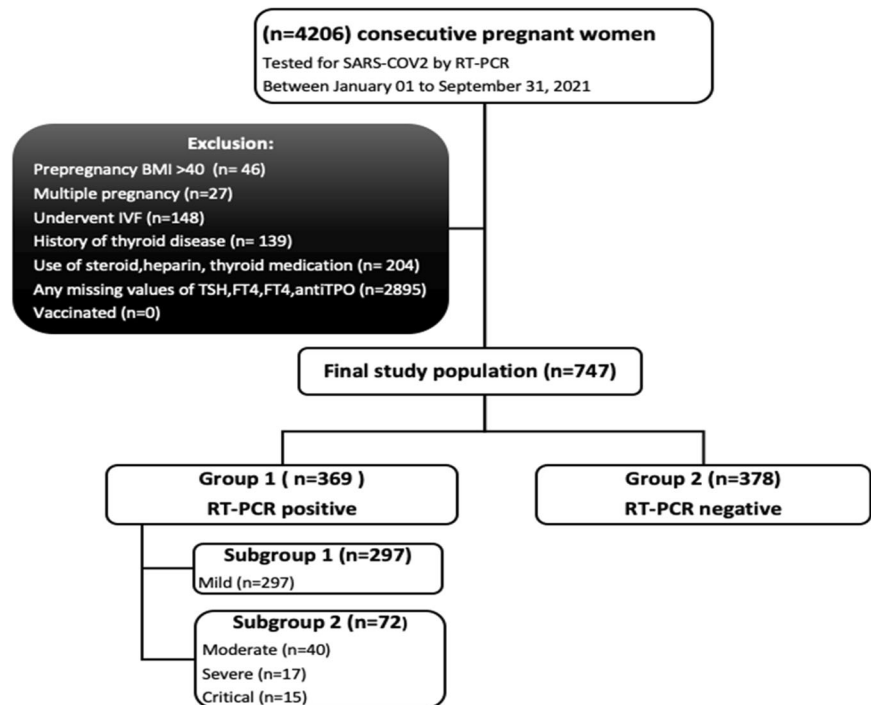
reference intervals in our hospital were set according to the 2017 guidelines of the American Thyroid Association and the Turkey Endocrinology and Metabolism Society [26, 27]. For the first trimester, the reference intervals were 0.1–2.5 mU/L for TSH and 0.8–1.53 ng/dL for FT4, and 2.46–3.89 ng/L for FT3. For the second trimester, they were 0.2–3 mU/L for TSH and 0.7–1.2 ng/dl for FT4, and 2.09–3.55 ng/L for FT3, and in the third trimester, they were 0.3–3 mU/L for TSH and 0.7–1.2 ng/dl for FT4 and 2.01–3.27 ng/L for FT3. TSH, FT4, FT3, anti-TPO, and anti-TG were measured by electro-chemiluminescence immunoassay (ECLIA) on a Roche Cobas 6000 e 601 analyzer (Roche Diagnostics).

The study group was divided into four categories of thyroid dysfunction (subclinical hypothyroidism, subclinical hyperthyroidism, isolated hypothyroxinemia, and nonthyroidal illness syndrome). We defined subclinical hypothyroidism as high TSH with normal FT4 and FT3; subclinical hyperthyroidism as low TSH with normal FT4 and FT3; isolated hypothyroxinemia as low FT4 with normal TSH and FT3; nonthyroidal illness syndrome (NTIS) as low FT3 with normal TSH and FT4. Finally, correlation analysis was performed in the study group to evaluate basic inflammatory biomarkers such as C-reactive protein (CRP), interleukin-6 (IL -6), and lymphocyte levels with TFT analysis. IL Interleukin-6 levels were measured using commercially available ELISA kits according to the kit data sheet (eBioscience, Thermo Fisher Scientific USA).

Statistical analyzes were performed using the SPSS package program (IBM SPSS Statistics 24). Frequency tables and descriptive statistics were used to interpret the results. Parametric methods were used for the measured values suitable for normal distribution. In accordance with the parametric methods, the "independent sample t"-test (t-table value) was used to compare the measured values of two independent groups. Nonparametric methods were used for the measured values that did not conform to the normal distribution. "Mann–Whitney U"-test (Z-table value) to compare the measured values of two independent groups by nonparametric methods; "Kruskal–Wallis H" method (χ^2-table value) to compare the measured values of three or more independent groups. "Spearman" correlation coefficient was used to study the relationships between two quantitative variables that do not have a normal distribution. "Pearson-χ^2" cross-tabulations were used to analyze the relationships between two qualitative variables. Binary logistic regression: the backward model LR was used to determine the factors affecting the risk situations.

Results

Baseline characteristics and TFT analysis between groups

There was no statistically significant difference between groups ($p > 0.05$) in baseline characteristics. Pregnant women in the study group had significantly lower TSH, FT4, FT3, Anti TPO, and Anti TG concentrations than those in the control group ($p < 0.001$) (Table 1).

Table 1 Comparison of the baseline characteristics and TFT analysis between groups

Variables	Study group (n = 369)	Control group (n = 378)	P value
Maternal age (years) (mean ± SD)	28.8 ± 6.08	29.45 ± 4.03	0.153[a]
Gravidity (median) (min–max)	2 (1–12)	2 (1–7)	0.618[a]
Parity (median) (min–max)	1 (0–9)	1.0 (0–8)	0.544[a]
Previous miscarriage (median) (min–max)	2 (1–3)	2 (1–3)	0.388[a]
Living child (median) (min–max)	1 (0–7)	1 (0–8)	0.756[a]
Pre-pregnancy BMI (kg/m^2) (mean ± SD)	26.11 ± 4.77	26.23 ± 4.83	0.844[a]
Gestational week at diagnosis (median) (min–max)	27 (5–41)	27 (7–40)	0.201[a]
COVID-19 severity			
Mild (n, %)	297 (80.4%)		
Moderate (n, %)	40 (10.8%)		
Severe (n, %)	17 (4.6%)		
Critical (n, %)	15 (4.2%)		
ICU admission (n, %)	38 (10.2%)		
Maternal mortality (n, %)	9 (2%)		
NICU admission (n, %)	25 (6.8%)		
TSH, μIU/mL (mean ± SD)	1.32 ± 1.10	1.67 ± 1.11	**<0.001**[b]
(median) (min–max)	1.1 (0.1–9.1)	1.4 (0.1–8.8)	
FT4, ng/mL (mean ± SD)	1.04 ± 0.52	1.05 ± 0.14	**<0.001**[b]
(median) (min–max)	1 (0.6–10.1)	1.1 (0.7–1.6)	
FT3, pg/mL (mean ± SD)	2.43 ± 0.45	2.87 ± 0.42	**<0.001**[b]
(median) (min–max)	2.4 (1.4–6.3)	2.8 (1.4–4.3)	
Anti TPO, IU/mL (mean ± SD)	58.07 ± 205.43	173.29 ± 515.45	**<0.001**[b]
(median) (min–max)	28 (28–3501)	37 (28–6636)	
Anti TG, IU/mL (mean ± SD)	2.33 ± 34.22	4.67 ± 11.73	**<0.001**[b]
(median) (min–max)	0.4 (0.1–621)	0.8 (0.1–183.8)	

[a]Statistical analysis was performed by independent sample *t*-test in data with normal distribution

[b]Mann–Whitney *U*-test was performed in the data without normal distribution

Bold values indicates statistically significant *P* values less than 0.05

TFT analysis between groups according to gestational-specific reference intervals

Then, we classified the TFT level as low/normal/high and thyroid autoantibodies as positive/negative according to trimester-specific references (Table 2). We found that the patient group was significantly more likely to have low TSH and low FT3 levels, while the control group had a predominance of high levels ($p = 0.034$ and $p < 0.001$). We also found that Anti TPO positivity was significantly higher in the control group ($p < 0.001$), while Anti TG positivity was significantly higher in the patient group ($p < 0.001$).

TFT analysis between groups according to Covid-19 severity

The study population was divided into two groups based on the severity of COVID-19: a mild group and a moderate/severe/critical group. Table 3 compares the thyroid function tests of each group. Patients with moderate/severe/critical disease had significantly lower FT3 values than patients with mild disease ($p < 0.001$), while there was no significant difference in FT4, TSH values, and thyroid autoantibody positivity ($p > 0.05$). To investigate the risk of developing a non-mild disease, logistic regression was performed with the parameters that were significant in the univariate analysis. FT3 level was found to be an important parameter inversely affecting disease severity ($p < 0.001$) (OR = 0.913; 1- OR = 0.087) (95% CI: 0.857–0.944).

FT3 levels according to COVID-19 severity (including the control group) were (2.87 ± 0.42 ng/dl) for control; (2.50 ± 0.46 ng/dl) for mild; (2.24 ± 0.37 ng/dl) for moderate; (2.17 ± 0.35 ng/dl) for severe; (2.11 ± 0.35 ng/dl) for critical patients and those levels show that the degree of FT3 reduction was related to confirmed disease and disease severity.

Table 2 Comparison of thyroid function analysis between groups according to gestational-specific reference intervals

Variables	Patient group (n = 369)		Control group (n = 378)		P value*
	n	%	n	%	
TSH					
low	8	2.2	3	0.8	**0.034**
normal	338	91.6	335	88.6	
high	23	6.2	40	10.6	
FT4					
low	14	3.8	12	3.2	0.307
normal	349	94.6	364	96.3	
high	6	1.6	2	0.5	
FT3					
low	49	13.3	8	2.1	**<0.001**
normal	308	83.4	314	83.1	
high	12	3.3	56	14.8	
Anti TPO					
negative	346	93.8	284	75.1	**<0.001**
positive	23	6.2	94	24.9	
Anti TG					
negative	306	82.9	353	93.4	**<0.001**
positive	63	17.1	25	6.6	

*Pearson-χ^2 cross tables were used to analyze the relationships between two qualitative variables

Bold values indicates statistically significant P values less than 0.05

Table 3 Comparison of thyroid function analysis between groups according to Covid-19 severity

Variables	Mild (n = 297)	Moderate/ Severe/Critical (n = 72)	P value
TSH (mean ± SD) (median) (min–max)	1.34 ± 1.08 1.1 (0.1–9.1)	1.21 ± 1.18 1.0 (0.1–8.7)	0.194[a]
FT4 (mean ± SD) (median) (min–max)	1.05 ± 0.58 1 (0.6–10.1)	1.00 ± 0.17 1 (0.8–1.8)	0.157[a]
FT3 (mean ± SD) (median) (min–max)	2.50 ± 0.46 2.4 (1.4–6.3)	2.19 ± 0.35 2.2 (1.4–3.4)	**<0.001**[a]
Anti TPO n (%)			0.791[b]
Negative	278 (93.6%)	68 (94.4%)	
Positive	19 (6.4%)	4 (5.6%)	
Anti TG n (%)			0.065[b]
Negative	241 (81.1%)	65 (90.3%)	
Positive	56 (18.9%)	7 (9.7%)	

[a]The "Mann–Whitney U"-test (Z-table value) statistics were used to compare the measurement values of two independent groups in the data that did not have a normal distribution

[b]Pearson-χ^2 cross tables were used to analyze the relationships between two qualitative variables

Data were presented as mean ± SD, median (IQR: interquartile range), and number (%) as appropriate

Bold values indicates statistically significant P values less than 0.05

Table 4 Comparison of thyroid diseases according to COVID-19 severity

Variables	Mild (n = 297)		Moderate/ Severe/ Critical (n = 72)		Total (N = 369)		P value*
	n	%	n	%	N	%	
Subclinical hypothyroidism							
Yes	11	3.7	4	5.6	15	4.1	0.475
No	286	96.3	68	94.4	354	95.9	
Subclinical hyperthyroidism							
Yes	5	1.7	—	—	5	1.4	0.268
No	292	98.3	72	100	364	98.6	
Isolated Hypothyroxinemia							
Yes	1	0.3	—	—	1	0.3	0.622
No	296	99.7	72	100	368	99.7	
Nonthyroidal Illness Syndrome							
Yes	25	8.4	21	29.2	46	12.5	**<0.001**
No	272	91.6	51	70.8	323	87.5	

*"Pearson-χ^2" crosstabs were used to analyze the relationships of two qualitative variables

Bold values indicates statistically significant P values less than 0.05

Thyroid disease and the severity of COVID-19

In Table 4, we analyzed the severity of COVID-19 and thyroid disease. While no statistically significant association was found between disease severity and subclinical hypothyroidism, subclinical hyperthyroidism, and isolated hypothyroxinemia ($p > 0.05$), the NTIS rate was significantly higher (29.2–8.4%) in the moderate/severe/critical group ($p < 0.001$).

Comparison of TFT analysis with COVID-19 according to ICU and NICU admissions and risk analysis

The study population was divided into two groups based on maternal ICU admission. As shown in Table 5, FT3 levels of ICU-admitted patients were significantly lower than those of non-ICU-admitted patients ($p < 0.001$), while there was no statistically significant difference between groups for TSH, FT4, anti-TPO, and anti-TG ($p > 0.05$). Logistic regression analysis was performed with the parameters that were significant in the univariate analysis to investigate the association between FT3 and ICU admission. It was found that FT3 level was a significant parameter inversely affecting ICU admission ($p < 0.001$, OR = 0.912; 1- OR = 0.088) (95% CI: 0.811–0.969).

Subsequently, the study population was divided into two groups based on fetal NICU admission. As shown in Table 6,

Table 5 Comparison of TFT parameters between ICU-admitted and non-ICU-admitted patients

ICU admission Variables	No ($n = 331$)	Yes ($n = 38$)	P value
FT4 (mean ± SD)	1.04 ± 0.55	0.99 ± 0.17	0.282[a]
(median)(min–max)	1.0 (0.6–10.1)	1.0 (0.8–1.7)	
FT3 (mean ± SD)	2.47 ± 0.46	2.17 ± 0.34	**<0.001**[a]
(median)(min–max)	2.4 (1.4–6.3)	2.2 (1.4—2.8)	
TSH (mean ± SD)	1.35 ± 1.13	1.08 ± 0.81	0.151[a]
(median)(min–max)	1.1 (0.1–9.1)	1.0 (0.3–4.3)	
Anti TPO *n* (%)			
Negative	302 (91.3%)	36 (94.7%)	0.200b
Positive	29 (8.7%)	2 (5.3%)	
Anti TG *n* (%)			
Negative	285 (86.2 %)	35 (92.2%)	0.190b
Positive	46 (13.8%)	3 (7.8%)	

[a]The "Mann–Whitney U"-test (Z-table value) statistics were used to compare the measurement values of two independent groups

[b]Pearson-χ^2 cross tables were used to analyze the relationships between two qualitative variables

Bold values indicates statistically significant P values less than 0.05

Table 6 Comparison of thyroid function analysis in pregnant women with COVID-19 according to NICU admission of neonates

NICU admission Variables	No ($n = 344$)	Yes ($n = 25$)	P value
FT4 (mean ± SD)	1.04 ± 0.54	0.94 ± 0.13	**=0.013**[a]
(median)(min–max)	1.0 (0.6–10.1)	0.9 (0.8–1.3)	
FT3 (mean ± SD)	2,46 ± 0.45	2.12 ± 0.35	**<0.000**[a]
(median)(min–max)	2.4 (1.4–6.3)	2.0 (1.4–2.9)	
TSH (mean ± SD)	1.31 ± 1.10	1.36 ± 1.17	=0.763[a]
(median)(min–max)	1.1 (0.1–9.1)	1.0 (0.3–4.3)	
Anti TPO n (%)			
Negative	321 (93.3%)	25 (100.0%)	=0.182[b]
Positive	23 (6.7%)	—	
Anti TG n (%)			
Negative	282 (82 %)	24 (96.0%)	=0.072[b]
Positive	62(18.0%)	1(4.0%)	

[a]The "Mann–Whitney *U*"-test (Z-table value) statistics were used to compare the measurement values of two independent groups in the data that did not have a normal distribution

[b]Pearson-χ^2 cross tables were used to analyze the relationships between two qualitative variables

Data were presented as mean ± SD, median (IQR), and number (%) as appropriate

Bold values indicates statistically significant P values less than 0.05

not only were the FT3 scores of the NICU-admitted infants significantly lower than those of the non-NICU-admitted infants ($p < 0.001$), but the FT4 scores were also significantly lower in the NICU-admitted group ($p = 0.013$). Logistic regression analysis with the parameters that were significant in univariate analysis was used to investigate the relationship between FT3, FT4, and NICU admission. It was found that the FT3 value was an important parameter that inversely affected NICU admission ($p < 0.001$, OR = 0.909; 1- OR = 0.091) (95% CI: 0.824–0.961).

Correlation analysis of laboratory biomarkers and TFTs

Finally, we examined the correlation of lymphocyte count, IL-6, CRP level, and TFT analysis in pregnant women with COVID -19, as shown in Table 7. We observed a significant positive correlation between lymphocytes with FT3 and TSH levels ($p < 0.001$) and a significant negative relationship between CRP with FT3 and TSH levels ($p < 0.001$, $p = 0.005$).

Discussion

Data on the relationship between COVID-19 and pregnancy have been published since March 2020 [28–30]. However, to the best of our knowledge, the current study is the first on the analysis of thyroid function during the COVID-19 outbreak, comparing confirmed SARS-CoV-2 positive pregnant women

with confirmed negative pregnant women and establishing an association with disease severity in pregnancy.

As mentioned earlier, most data in the literature on pregnant women are limited by the absence of FT3 measurement and thyroid autoantibody status, so further studies with these measurements were needed to determine the full effects of COVID -19 on the HPT axis. Therefore, one of the greatest strengths of our study was the inclusion of all TFTs. Here, we report the effect of COVID -19 on the HPT axis in pregnancy.

In the first part of the present study, we performed an analysis of positive and negative pregnant cases. The comprehensive analysis of our study showed that pregnant women infected with COVID -19 had significantly lower levels of FT3, FT4, TSH, anti-TPO, and anti-TG compared to the confirmed negative pregnant cases with the same baseline characteristics. Although all TFTs were suppressed in the study group, there was no difference in low FT4, while low FT3 and low TSH were significantly higher in the patient group. Wang et al. reported that TT3 and TSH levels were lower in COVID -19 patients than in the healthy group [31], which is consistent with our results, while no significant difference was found in TT4, in contrast to our results, which showed significantly lower FT4 levels. The contrast in FT4 may be due to the difference between the control groups in both studies because healthy individuals who underwent a routine annual physical examination before the onset of

Table 7 Correlation of Lymphocyte count, IL-6, and CRP levels with TFT analysis in pregnant women with COVID-19 infection

Correlation[a]		Anti TPO	Anti TG	FT4	FT3	TSH
Lymphocyte	r	0.004	0.007	−0.004	0.182	0.271
	p	0.937	0.897	0.942	**0.000**	**0.000**
CRP	r	−0.041	−0.113	−0.020	−0.278	−0.148
	p	0.429	0.131	0.750	**0.000**	**0.005**
IL-6	r	−0.263	−0.227	0.005	−0.234	−0.203
	p	0.153	0.219	0.979	0.206	0.274

CRP C-reactive protein, *IL* interleukin

[a]"Spearman" correlation coefficient was used to examine the relationships between two quantitative variables that do not have a normal distribution

Bold values indicates statistically significant *p* values less than 0.05

COVID-19 served as controls in their study. Indeed, Lin et. al. found that exposure to the COVID-19 outbreak was independently associated with lower FT4 levels in pregnant women [20]. Based on this study, it can be interpreted that anxiety triggered by COVID-19 during pregnancy [32] significantly affects maternal thyroid function. Of note, the overall decrease in all TSH, FT4, and FT3 levels in SARS-CoV-2 infected pregnant women are indicative of an indirect nonthyroidal clinical picture rather than a direct thyroid effect.

Anti TPO and Anti TG positivity are known to be present in 15.2 and 8.9% of unselected pregnant women, respectively [33], and a recent meta-analysis found isolated positivity of 4.1 and 4.8%, respectively [34]. Data suggest that thyroid autoantibody positivity is associated with poor fetal outcomes [35], and, in particular, with an increased risk of preterm birth [36, 37]. Previous studies in non-pregnant women have shown an increase in antithyroid antibody levels 3 months after COVID-19 [22] and following COVID-19 vaccination [38]. However, data on the impact of SARS-CoV-2 infection on thyroid autoantibodies during acute disease are still limited. In our analysis of unvaccinated pregnant women, we found that anti-TPO positivity was significantly higher in the control group (24.9–6.2%) and, paradoxically, anti-TG positivity was significantly higher in the patient group (17.1–6.6%), although both had significantly lower concentrations in the patient group. However, we found that none of the auto-antibodies were related to disease severity, ICU, and NICU admission, both in terms of concentration and positivity.

In the second part of our study, we performed a subgroup analysis in the positive pregnant cases according to disease severity, thyroid dysfunction, ICU admission, and NICU admission. We found that TSH and FT4 levels were suppressed with disease severity, but this was not statistically significant. On the other hand, moderately severe, severe, and critical cases were found to have significantly lower FT3 levels and a higher rate of NTIS than mild cases, which is confirmed by the literature in recent studies on the non-pregnant population [39–41]. Consequently, thyroid

function testing in pregnant patients seems reasonable, although WHO does not recommend systematic thyroid function testing in hospitalized COVID-19 patients [42].

In addition, FT4 and FT3 levels of positive pregnant women with NICU admission were significantly lower than those without NICU admission, but in logistic regression analysis, we found an increased risk for NICU admission only with decreased FT3 levels. Although maternal FT4 level is very important for the intrauterine life of fetuses, it does not increase the risk for NICU admission in newborns, even if it was significantly suppressed in the mother.

According to recent guidelines from ATA [26], subclinical hyperthyroidism during pregnancy is not associated with adverse outcomes and only needs to be monitored, whereas subclinical hypothyroidism is associated with adverse obstetric and fetal outcomes, and treatment with thyroxine is recommended in selected cases. Data regarding the association between isolated hypothyroxinemia and adverse maternal and fetal outcomes are controversial. Some studies suggest that isolated maternal hypothyroxinemia (IMH) is associated with an increased risk of preterm birth and hence admission to the neonatal intensive care unit [43, 44], whereas others have found no association [45, 46].

Because the severity of COVID-19 is known to increase the risk of perinatal complications, we found no association between disease severity and subclinical hyperthyroidism and IMH in the present study. On the other hand, COVID-19 severity was significantly associated with NTIS and insignificantly associated with subclinical hypothyroidism in our study, as described in previous studies [47, 48]. In view of analyzes using the logistic regression model to evaluate the predictive potential of low FT3 for the risk of non-mild COVID-19, ICU admission, and NICU admission, it has ensured that non-mild cases of COVID-19 may lead to NTIS in pregnant women as well.

Finally, we investigated the correlation between TFTs and some laboratory immune parameters at admission, which are commonly used in COVID-19 diagnosis and follow-up, because the thyroid gland is a neuroendocrine

organ effective in immune regulation. We found that FT3 and TSH had a significant inverse correlation with CRP and a direct correlation with lymphocytes, whereas there was no correlation with IL-6. However, most data have shown that IL -6 is also inversely related to FT3 levels, in contrast to our results [49]. In the absence of a study on pregnant women, similar studies in nonpregnant patients are consistent with our results in CRP and lymphocytes [50–52]. Of note, FT4 levels showed no correlation with these parameters, which may be due to the fact that FT3 is the active form of FT4. Consequently, all these results support the potential interactions between the HPT axis and the immune system in COVID-19.

The main strengths of the present study were a large number of cases despite the monocentric design, the inclusion of all TFTs in all groups, and the fact that all pregnant women were not vaccinated and were not taking any medication that could affect TFT levels. Another strength is the presence of a control group and a group of patients with different clinical scenarios classified as mild, moderate, severe, and critical. On the other hand, we have some limitations. We matched the study groups based on a molecular test (RT-PCR), which is highly specific and the probability of false positive results is low, but false negative results are possible [53]. In addition, thyroid imaging was not performed in the patient group to assess the potential effects of COVID -19 on the thyroid beyond TFTs and antithyroid antibodies, particularly with regard to subacute thyroiditis (also called de Quervain's thyroiditis or viral thyroiditis), which is an inflammatory disease of the thyroid gland that usually follows or coexists with a viral infection, especially in women, and in a recent review of case reports and case series linking subacute thyroiditis to a viral complication of COVID-19 [54].

In conclusion, SARS-CoV-2 infection appears to affect thyroid function analysis in pregnant women, and TFT levels, particularly FT3 levels, appear to be correlated with disease severity. It is well established in the literature that NTIS may be an early marker of poor outcomes of COVID-19, and the current study shows that a suppressed FT3 level has prognostic significance not only for disease severity and ICU admission but also for neonatal ICU admission. Considering the results of the present study as inferential, our study justifies the inclusion of FT3 in routine investigations alongside TSH and FT4 in pregnancies complicated with COVID-19. Given the ongoing COVID-19 pandemic, further studies in pregnancy are certainly needed to determine the full impacts of COVID -19 on the HPT axis, whereas similar data in pregnancy are still limited.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval Approval was granted by the Ethics Committee of Ankara City Hospital (Approval, March 24, 2021 /Protocol No. E2-21-269).

Consent to participate All participants signed informed written consent before being enrolled in the study. This study was performed in line with the principles of the Declaration of Helsinki.

References

1. Organization W.H. WHO Director-General's opening remarks at the media briefing on COVID-19 March 2020, https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020
2. World Health Organization WHO Coronavirus (COVID-19) dashboard July 2022, https://covid19.who.int
3. M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell **181**(2), 271–80.e8 (2020). https://doi.org/10.1016/j.cell.2020.02.052
4. C.G.K. Ziegler, S.J. Allon, S.K. Nyquist, I.M. Mbano, V.N. Miao, C.N. Tzouanas et al. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. Cell **181**(5), 1016–1035.e19 (2020). https://doi.org/10.1016/j.cell.2020.04.035
5. M.Y. Li, L. Li, Y. Zhang, X.S. Wang, Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect. Dis. Poverty **9**(1), 45 (2020). https://doi.org/10.1186/s40249-020-00662-x
6. L. Scappaticcio, F. Pitoia, K. Esposito, A. Piccardo, P. Trimboli, Impact of COVID-19 on the thyroid gland: an update. Rev. Endocr. Metab. Disord. **22**(4), 803–815 (2021). https://doi.org/10.1007/s11154-020-09615-z
7. W. Ni, X. Yang, D. Yang, J. Bao, R. Li, Y. Xiao, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care. **24**, 422 (2020). https://doi.org/10.1186/s13054-020-03120-0
8. A. Java, A.J. Apicelli, M.K. Liszewski, A. Coler-Reilly, J.P. Atkinson, A.H. Kim et al. The complement system in COVID-19: friend and foe? JCI Insight. **5**(15), e140711 (2020) . https://doi.org/10.1172/jci.insight.140711
9. S. Goncu Ayhan, D. Oluklu, S. Sinaci, A. Atalay, S.A. Erol, E. Ozden Tokalioglu et al. Fetal thymus size in pregnant women with COVID-19 infection. Gynecol. Obstet. Reprod. Med. **27**(2), 84–88 (2021). https://doi.org/10.21613/GORM.2021.1222
10. G. Lisco, A. De Tullio, E. Jirillo, V.A. Giagulli, G. De Pergola, E. Guastamacchia et al. Thyroid and COVID-19: a review on

pathophysiological, clinical and organizational aspects. J. Endocrinol. Invest **44**(9), 1801–1814 (2021). https://doi.org/10.1007/s40618-021-01554-z

11. J. Zheng, Z. Cui, N. Shi, S. Tian, T. Chen, X. Zhong et al. Suppression of the hypothalamic-pituitary-thyroid axis is associated with the severity of prognosis in hospitalized patients with COVID-19. BMC Endocr. Disord. **21**, 228 (2021). https://doi.org/10.1186/s12902-021-00896-2

12. J. Bernal, Thyroid hormone receptors in brain development and function. Nat. Clin. Pr. Endocrinol. Metab. **3**(3), 249–259 (2007). https://doi.org/10.1038/ncpendmet0424

13. R.M. Calvo, E. Jauniaux, B. Gulbis, M. Asunción, C. Gervy, B. Contempré et al. Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. J. Clin. Endocrinol. Metab. **87**(4), 1768–1777 (2002). https://doi.org/10.1210/jcem.87.4.8434

14. T.I. Korevaar, R. Muetzel, M. Medici, L. Chaker, V.W. Jaddoe, Y.B. de Rijke et al. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. Lancet Diabetes Endocrinol. **4**(1), 35–43 (2016). https://doi.org/10.1016/s2213-8587(15)00327-7

15. Thyroid disease in pregnancy: ACOG practice bulletin, number 223. Obstet Gynecol. **135**(6), e261–e274 (2020). https://doi.org/10.1097/aog.0000000000003893

16. T. Männistö, P. Mendola, J. Grewal, Y. Xie, Z. Chen, S.K. Laughon, Thyroid diseases and adverse pregnancy outcomes in a contemporary US cohort. J. Clin. Endocrinol. Metab. **98**(7), 2725–2733 (2013). https://doi.org/10.1210/jc.2012-4233

17. W.C. Allan, J.E. Haddow, G.E. Palomaki, J.R. Williams, M.L. Mitchell, R.J. Hermos et al. Maternal thyroid deficiency and pregnancy complications: implications for population screening. J. Med Screen **7**(3), 127–130 (2000). https://doi.org/10.1136/jms.7.3.127

18. M. Abalovich, S. Gutierrez, G. Alcaraz, G. Maccallini, A. Garcia, O. Levalle, Overt and subclinical hypothyroidism complicating pregnancy. Thyroid **12**(1), 63–68 (2002). https://doi.org/10.1089/105072502753451986

19. S. Singh, V.G. Nair, V.V. Singh, S. Tiwari, D. Arora, M. Dey et al. Pregnancy-specific concerns and psychological impact of COVID-19 on antenatal women. Gynecol. Obstet. Reprod. Med **28**(2), 129–134 (2022). https://doi.org/10.21613/GORM.2021.1172

20. T.T. Lin, C. Zhang, H.Q. Zhang, Y. Wang, L. Chen, C.L. Dennis et al. Thyroid hormone changes in early pregnancy along with the COVID-19 pandemic. Front. Endocrinol. **11**, 606723 (2020). https://doi.org/10.3389/fendo.2020.606723

21. J. Hua, J. Shen, J. Zhang, Y. Zhou, W. Du, G.J. Williams, The association between COVID-19 pandemic and maternal isolated hypothyroxinemia in first and second trimesters. Psychoneuroendocrinology **128**, 105210 (2021). https://doi.org/10.1016/j.psyneuen.2021.105210

22. World Medical Association, World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. **310**(20), 2191–2194 (2013). https://doi.org/10.1001/jama.2013.281053

23. Turkish Ministry of Health. General Directorate of Public Health, COVİD-19 (SARS-CoV-2 infection) Guideline, Scientific Committee Report, https://covid19.saglik.gov.tr/. Accessed May, 2020

24. S. Ippolito, D. Gallo, A. Rossini, B. Patera, N. Lanzo, G.F.M. Fazzino et al. SARS-CoV-2 vaccine-associated subacute thyroiditis: insights from a systematic review. J. Endocrinol. Invest **45**, 1189–1200 (2022). https://doi.org/10.1007/s40618-022-01747-0

25. S. Goncu Ayhan, D. Oluklu, A. Atalay, D. Menekse Beser, A. Tanacan, O. Moraloglu Tekin et al. COVID-19 vaccine acceptance in pregnant women. Int J. Gynaecol. Obstet. **154**(2), 291–296 (2021). https://doi.org/10.1002/ijgo.13713

26. E.K. Alexander, E.N. Pearce, G.A. Brent, R.S. Brown, H. Chen, C. Dosiou et al. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. Thyroid **27**(3), 315–389 (2017). https://doi.org/10.1089/thy.2016.0457

27. Türkiye Endokrinoloji ve Metabolizma Derneği (TEMD) Tiroid Hastalıkları Tanı ve Tedavi Kılavuzu 2020 [Available from: https://file.temd.org.tr/Uploads/publications/guides/documents/20200929134733-2020tbl_kilavuzf527c34496.pdf?a=1.

28. P. Dubey, S.Y. Reddy, S. Manuel, A.K. Dwivedi, Maternal and neonatal characteristics and outcomes among COVID-19 infected women: an updated systematic review and meta-analysis. Eur. J. Obstet. Gynecol. Reprod. Biol. **252**, 490–501 (2020). https://doi.org/10.1016/j.ejogrb.2020.07.034

29. M.J. Delahoy, M. Whitaker, A. O'Halloran, S.J. Chai, P.D. Kirley, N. Alden et al. Characteristics and maternal and birth outcomes of hospitalized pregnant women with laboratory-confirmed COVID-19 - COVID-NET, 13 States, March 1-August 22, 2020. MMWR Morb. Mortal. Wkly Rep. **69**(38), 1347–1354 (2020). https://doi.org/10.15585/mmwr.mm6938e1

30. D. Sahin, A. Tanacan, A.T. Anuk, S. Sinaci, B. Besimoglu, D. Oluklu, et al. Comparison of clinical features and perinatal outcomes between pre-variant and post-variant periods in pregnant women with SARS-CoV-2: analysis of 1935 cases. Arch Gynecol Obstet. **306**(6), 1939–1948 (2022). https://doi.org/10.1007/s00404-022-06493-5

31. W. Wang, X. Su, Y. Ding, W. Fan, W. Zhou, J. Su et al. Thyroid function abnormalities in COVID-19 patients. Front Endocrinol. **11**, 623792 (2020). https://doi.org/10.3389/fendo.2020.623792

32. S. Sinaci, E. Ozden Tokalioglu, D. Ocal, A. Atalay, G. Yilmaz, H.L. Keskin et al. Does having a high-risk pregnancy influence anxiety level during the COVID-19 pandemic. Eur. J. Obstet. Gynecol. Reprod. Biol. **255**, 190–196 (2020). https://doi.org/10.1016/j.ejogrb.2020.10.055

33. A. McELDUFF, J. Morris, Thyroid function tests and thyroid autoantibodies in an unselected population of women undergoing first trimester screening for aneuploidy. Aust. N.Z. J. Obstet. Gynaecol. **48**(5), 478–480 (2008)

34. S. Bliddal, A. Derakhshan, Y. Xiao, L.M. Chen, T. Männistö, G. Ashoor et al. Association of thyroid peroxidase antibodies and thyroglobulin antibodies with thyroid function in pregnancy: an individual participant data meta-analysis. Thyroid **32**(7), 828–840 (2022). https://doi.org/10.1089/thy.2022.0083

35. S. Athar, S.F. Beer, Z. Martis, M.I. Alloub, The prevalence of thyroid autoimmunity in pregnancy and adverse neonatal outcomes at a secondary care hospital in the Middle East. Cureus **14**(5), e24814 (2022). https://doi.org/10.7759/cureus.24814

36. S. Thangaratinam, A. Tan, E. Knox, M.D. Kilby, J. Franklyn, A. Coomarasamy, Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. Bmj **342**, d2616 (2011). https://doi.org/10.1136/bmj.d2616

37. R. Negro, Thyroid autoimmunity and pre-term delivery: brief review and meta-analysis. J. Endocrinol. Invest **34**(2), 155–158 (2011). https://doi.org/10.1007/bf03347047

38. D.T.W. Lui, C.H. Lee, C.Y.Y. Cheung, J.H. Cheung Mak, C.H.Y. Fong, B.W.C. Lui, et al. Impact of COVID-19 vaccines on thyroid function and autoimmunity and impact of thyroid autoimmunity on antibody response. J. Clin. Endocrinol. Metab. **107**(9), e3781–e3789 (2022). https://doi.org/10.1210/clinem/dgac355

39. R. Zou, C. Wu, S. Zhang, G. Wang, Q. Zhang, B. Yu et al. Euthyroid sick syndrome in patients with COVID-19. Front. Endocrinol. **11**, 566439 (2020). https://doi.org/10.3389/fendo.2020.566439

40. D.T.W. Lui, C.H. Lee, W.S. Chow, A.C.H. Lee, A.R. Tam, C.H.Y. Fong et al. Thyroid dysfunction in relation to immune profile, disease status, and outcome in 191 patients with COVID-

19. J. Clin. Endocrinol. Metab. **106**(2), e926–e935 (2021). https://doi.org/10.1210/clinem/dgaa813

41. F.E.L. Beltrão, D.C.A. Beltrão, G. Carvalhal, F.E.L. Beltrão, A.D.S. Brito, K. Capistrano et al. Thyroid hormone levels during hospital admission inform disease severity and mortality in COVID-19 patients. Thyroid **31**(11), 1639–1649 (2021). https://doi.org/10.1089/thy.2021.0225

42. World Health O. COVID-19 clinical management: living guidance, 25 January 2021. Geneva: World Health Organization; 2021 2021. Contract No.: WHO/2019-nCoV/clinical/2021.1.

43. X. Yang, Y. Yu, C. Zhang, Y. Zhang, Z. Chen, L. Dubois et al. The association between isolated maternal hypothyroxinemia in early pregnancy and preterm birth. Thyroid **30**(12), 1724–1731 (2020). https://doi.org/10.1089/thy.2019.0818

44. T.I. Korevaar, S. Schalekamp-Timmermans, Y.B. de Rijke, W.E. Visser, W. Visser, S.M. de Muinck Keizer-Schrama et al. Hypothyroxinemia and TPO-antibody positivity are risk factors for premature delivery: the generation R study. J. Clin. Endocrinol. Metab. **98**(11), 4382–4390 (2013). https://doi.org/10.1210/jc.2013-2855

45. G. León, M. Murcia, M. Rebagliato, M. Álvarez-Pedrerol, A.M. Castilla, M. Basterrechea et al. Maternal thyroid dysfunction during gestation, preterm delivery, and birthweight. the Infancia y Medio Ambiente Cohort, Spain. Paediatr. Perinat. Epidemiol. **29**(2), 113–122 (2015). https://doi.org/10.1111/ppe.12172

46. M.P. Hamm, N.M. Cherry, J.W. Martin, F. Bamforth, I. Burstyn, The impact of isolated maternal hypothyroxinemia on perinatal morbidity. J. Obstet. Gynaecol. Can. **31**(11), 1015–1021 (2009). https://doi.org/10.1016/s1701-2163(16)34345-6

47. M. Świstek, M. Broncel, P. Gorzelak-Pabiś, P. Morawski, M. Fabiś, E. Woźniak, Euthyroid sick syndrome as a prognostic indicator of COVID-19 pulmonary involvement, associated with poorer disease prognosis and increased mortality. Endocr. Pract. **28**(5), 494–501 (2022). https://doi.org/10.1016/j.eprac.2022.02.006

48. M. Chen, W. Zhou, W. Xu, Thyroid function analysis in 50 patients with COVID-19: a retrospective study. Thyroid **31**(1), 8–11 (2021). https://doi.org/10.1089/thy.2020.0363

49. C. Sparano, E. Zago, A. Morettini, C. Nozzoli, D. Yannas, V. Adornato et al. Euthyroid sick syndrome as an early surrogate marker of poor outcome in mild SARS-CoV-2 disease. J. Endocrinol. Invest **45**, 837–847 (2022). https://doi.org/10.1007/s40618-021-01714-1

50. D.T.W. Lui, C.H. Lee, W.S. Chow, A.C.H. Lee, A.R. Tam, P. Pang et al. The independent association of TSH and Free triiodothyronine levels with lymphocyte counts among COVID-19 patients. Front Endocrinol. **12**, 774346 (2021). https://doi.org/10.3389/fendo.2021.774346

51. V. Ilera, L.C. Delfino, A. Zunino, P. Glikman, M. Drnovsek, A. Reyes et al. Correlation between inflammatory parameters and pituitary-thyroid axis in patients with COVID-19. Endocrine **74**, 455–460 (2021). https://doi.org/10.1007/s12020-021-02863-2

52. J. Deng, S. Zhang, F. Peng, Q. Zhang, Y. Li, Y. Zhong, The association between FT3 with the outcome and inflammation/coagulopathy/fibrinolysis of COVID-19. Front Endocrinol. **13**, 877010 (2022). https://doi.org/10.3389/fendo.2022.877010

53. T. Ai, Z. Yang, H. Hou, C. Zhan, C. Chen, W. Lv et al. Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology **296**(2), E32–e40 (2020). https://doi.org/10.1148/radiol.2020200642

54. M. Aemaz Ur Rehman, H. Farooq, M.M. Ali, M. Ebaad Ur Rehman, Q.A. Dar, A. Hussain, The association of subacute thyroiditis with COVID-19: a systematic review. SN Compr. Clin. Med. **3**, 1515–1527 (2021). https://doi.org/10.1007/s42399-021-00912-5