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Clinical Research Article

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The Association of TSH and Thyroid Hormones With Lymphopenia in Bacterial Sepsis and COVID-19

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Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CV, coefficient of variation; FT4, free thyroxine; HPT, hypothalamic-pituitary-thyroid; ICU, intensive care unit; IL-6, interleukin 6; IQR, interquartile range; NTIS, nonthyroidal illness syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, Sequential Organ Failure Assessment; T3, 3,5,3′-triiodothyronine; T4, thyroxine; TH, thyroid hormone; TSH, thyroid stimulating hormone.

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Abstract

Context: Lymphopenia is a key feature of immune dysfunction in patients with bacterial sepsis and coronavirus disease 2019 (COVID-19) and is associated with poor clinical outcomes, but the cause is largely unknown. Severely ill patients may present with thyroid function abnormalities, so-called nonthyroidal illness syndrome, and several studies have linked thyrotropin (thyroid stimulating hormone, TSH) and the thyroid

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hormones thyroxine (T4) and 3,5,3′-triiodothyronine (T3) to homeostatic regulation and function of lymphocyte populations.

Objective: This work aimed to test the hypothesis that abnormal thyroid function correlates with lymphopenia in patients with severe infections.

Methods: A retrospective analysis of absolute lymphocyte counts, circulating TSH, T4, freeT4 (FT4), T3, albumin, and inflammatory biomarkers was performed in 2 independent hospitalized study populations: bacterial sepsis (n = 224) and COVID-19 patients (n = 161). A subgroup analysis was performed in patients with severe lymphopenia and normal lymphocyte counts.

Results: Only T3 significantly correlated ($\rho = 0.252$) with lymphocyte counts in patients with bacterial sepsis, and lower concentrations were found in severe lymphopenic compared to nonlymphopenic patients (n = 56 per group). Severe lymphopenic COVID-19 patients (n = 17) showed significantly lower plasma concentrations of TSH, T4, FT4, and T3 compared to patients without lymphopenia (n = 18), and demonstrated significantly increased values of the inflammatory markers interleukin-6, C-reactive protein, and ferritin. Remarkably, after 1 week of follow-up, the majority (12 of 15) of COVID-19 patients showed quantitative recovery of their lymphocyte numbers, whereas TSH and thyroid hormones remained mainly disturbed.

Conclusion: Abnormal thyroid function correlates with lymphopenia in patients with severe infections, like bacterial sepsis and COVID-19, but future studies need to establish whether a causal relationship is involved.

Key Words: thyroid, metabolism, inflammation, lymphocyte, sepsis, COVID-19

Lymphopenia is a commonly described clinical finding in patients with severe systemic infections (1). It is a key feature of immune dysfunction in bacterial sepsis, and particularly severe persistent lymphopenia is associated with poor clinical outcome in sepsis patients (2). Loss of effector T cells ($CD4^+$ and $CD8^+$), B cells, and dendritic cells through apoptosis is described as one of the main causes of lymphopenia in sepsis. Additionally, functional and phenotypical characteristics of T cell exhaustion have been reported, leading to a prolonged state of immunosuppression, or so-called sepsis-induced T cell immunoparalysis (3). T cell immunoparalysis is considered to be one of the key mechanisms that contributes to the clinically observed immunosuppressive state in critically ill sepsis patients, which is reflected by their failure to clear the primary infection and their increased susceptibility to secondary and opportunistic infections (4, 5).

In addition to bacterial and fungal infections (main causes of sepsis), systemic viral infections are also associated with lymphopenia (1). Prior studies reported the incidence of severe lymphopenia and its associations with poor clinical outcome in dengue and influenza virus infections in different cohorts (6, 7). These viruses may trigger severe immune dysregulation and organ failure in these patients, resulting in the clinical syndrome of sepsis (8). Currently, global research interest is dominated by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, the pathogen that has caused the coronavirus disease 2019 (COVID-19) pandemic with more than 100 million confirmed cases and more than 2 million deaths worldwide (9). A complex immune dysregulation has been proposed for COVID-19. Maladaptive patterns related to both innate and adaptive immunity have been reported (10, 11). Profound lymphopenia especially is a clinical hallmark of COVID-19, and multiple studies have demonstrated its association with severity and increased mortality in hospitalized COVID-19 patients (12). Although this prognostic link is clearly established, causative factors contributing to lymphopenia in COVID-19 are still largely unknown.

Many years of evidence support the bidirectional interaction of the hypothalamic-pituitary-thyroid (HPT) axis and immune function (13). Animal models showed that thyrotropin (thyroid stimulating hormone, TSH) as well as the thyroid hormones (THs) thyroxine (T4) and 3,5,3′-triiodothyronine (T3) play a crucial role in the homeostatic regulation and functional activity of lymphocyte populations (14, 15). Other studies have reported the important role of thyroid function in lymphopoiesis (16, 17). Moreover, TH balance is often abnormal in severely ill patients. Critically ill patients, particularly those in intensive care units (ICU), often present with strong decreased concentrations of T3 and may present with low to normal

plasma T4 concentrations, while TSH concentrations can be normal or even decreased, a condition referred to as nonthyroidal illness syndrome (NTIS) (18). This syndrome has been extensively described in patients with bacterial sepsis (19). Interestingly, decreased concentrations of TSH and T3 were also identified in COVID-19 patients (20).

To date, it is still unclear to what extent changes in TSH and TH concentrations can be linked to immune responses in patients with severe infections. Based on previous findings in the literature, we hypothesize that abnormal thyroid function correlates with the lymphopenia observed in these patients.

Therefore, in this study we comprehensively examined the relationship of thyroid function abnormalities and lymphopenia in 2 independent study populations of hospitalized patients with severe infections: a cohort of patients with bacterial sepsis and a series of patients with COVID-19.

Materials and Methods

Study Design and Setting

This retrospective study was performed in parallel in 2 independent study populations: a cohort of hospitalized patients with bacterial sepsis admitted in Greece (PROVIDE study, unpublished data, ClinicalTrials.gov identifier NCT 03332225, EudraCT No. 2017-002171-26) and a selected group of patients from a cohort of COVID-19 patients admitted to a tertiary university hospital (Radboudumc, Nijmegen) in the Netherlands (Fig. 1A and 1B).

Bacterial Sepsis Cohort

All patients (age ≥ 18 years) with bacterial sepsis admitted to one of the 14 recruiting study sites and hospitals in Greece were screened for eligibility. Informed consent was provided from all enrolled individuals by the patient or by a first-degree relative or spouse (approval by the National Ethics Committee of Greece 78/17). All patients were clinically diagnosed with sepsis or septic shock, according to the third definition of sepsis (21), due to community-acquired pneumonia, health care-associated pneumonia, ventilator-associated pneumonia, acute cholangitis, or primary bloodstream infection. Definitions and diagnostic criteria of eligible infections are provided in Table 1 (22-25). Exclusion criteria included a "do not resuscitate" decision, pregnancy or lactation, other causes of infections, any stage IV malignancy, active tuberculosis, HIV infection, primary immunodeficiency, oral or intravenously administered corticosteroids at a daily dose greater than or equal to 0.4 mg prednisone for the last 15 days or treatment with any anticytokine biological during the last month, medical history of systemic lupus erythematosus, or medical history of multiple sclerosis, or any other demyelinating disorder.

A stratification method was used to perform a subgroup analysis in the bacterial sepsis cohort (n = 224, median lymphocyte count 1.00 × 10^9/L (interquartile range [IQR] 0.64-1.58 × 10^9/L) comparing the extreme "severe lymphopenic" (0%-25%: lymphocyte counts ≤ 0.64 × 10^9/L) patients with the "nonlymphopenic" (75%-100%: lymphocyte counts ≥ 1.58 × 10^9/L) bacterial sepsis patients.

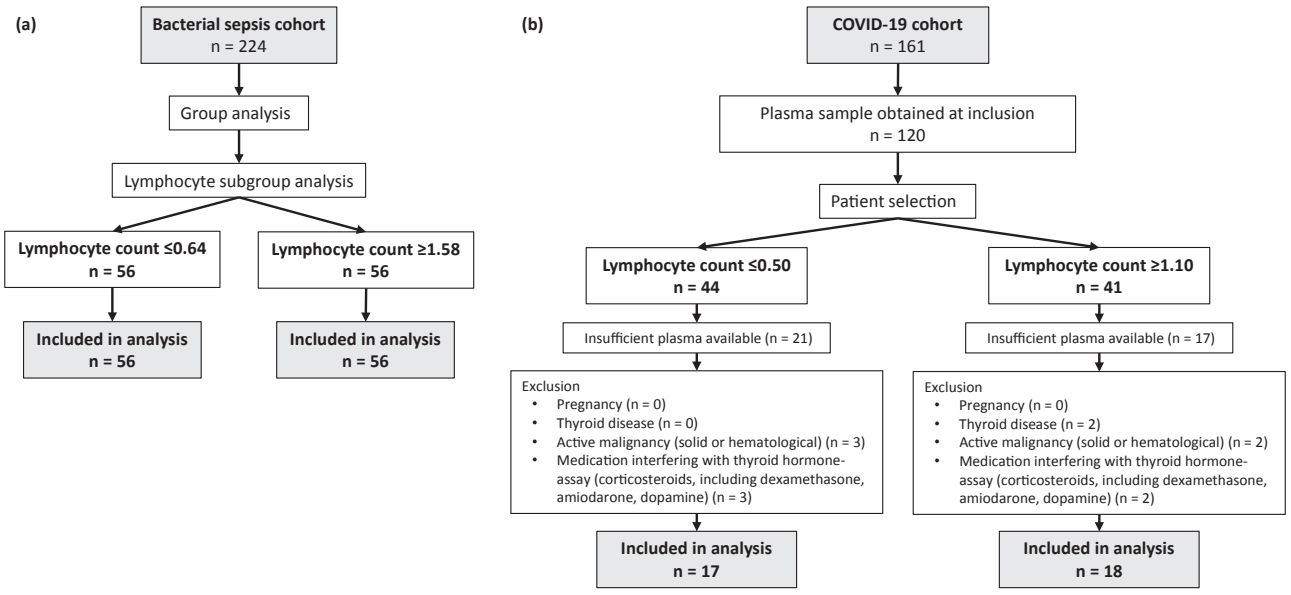

Figure 1. Flowchart of study design.

Coronavirus Disease 2019 Study Population

Patients (or their legal representatives) with a polymerase chain reaction–proven or clinically diagnosed SARS-CoV-2 infection admitted to a Dutch tertiary hospital during the first peak of the COVID-19 pandemic between March and April 2020 were asked to give informed consent to participate. The study protocol was approved by the local ethics committee (CMO 2020 6344 and CMO 2016 2963). Clinical diagnosis of COVID-19 infection was defined based on signs and symptoms, specific computed tomography findings according the Dutch COVID-19 Reporting and Data System (CO-RADS) classification, and final consensus of clinical experts (26).

Patient Selection and Subgroup Definitions

To test our hypothesis that lymphopenia correlates with thyroid function abnormalities in patients with severe infections, COVID-19 patients with very low and relatively high (normal range at the institutional laboratory, 1.00-3.50 × 10^9/L) counts were selected from an existing cohort (see Fig. 1B). This selection was performed based on the median and quartile distribution of absolute lymphocyte counts observed on admission in the entire COVID-19 population that was hospitalized at our institution. A median lymphocyte count of 0.74 × 10^9/L (IQR, 0.50-1.10 × 10^9/L) was observed on admission in 161 hospitalized COVID-19 patients, thereafter 0.50 or less and 1.10 or greater were used as predefined cutoff points for patient selection. Patients with pregnancy, preexisting thyroid disease, active hematological or solid malignancies, or use of any medication interfering with TH assay (eg, corticosteroids including dexamethasone, dopamine, and amiodaron) were excluded, or in case plasma volume for measurements of THs was insufficient. After the previously mentioned exclusion criteria were applied, 17 COVID-19 patients were included from the subgroup representing lymphocyte counts of less than

Table 1. Definitions of eligible infections in bacterial sepsis

Infection	All the following	At least 2 of the following	At least 1 of the following
AC (22)	• Pain at right upper quadrant • Fever (tympanic or oral temperature ≥ 38 °C, rectal ≥ 38.3 °C)	None	Consistent ultrasound or CT findings
BSI (22)	• At least 1 positive blood culture • Failure to identify a primary infection site despite thorough clinical and radiology investigation	None	None
CAP (23)	New or evolving infiltrate on chest x-ray	• New onset or worsening of cough • Dyspnea • Auscultatory findings consistent with pulmonary consolidation	• PCT ≥ 0.25 ng/mL • Hypoxemia pO_2 ≤ 60 mm Hg or oxygen saturation ≤ 90% in room air • Respiratory rate ≥ 20 breaths/min
HAP (or HCAP)[a] (24)	• Onset > 48 h from hospital admission • New or evolving infiltrate on chest x-ray	• New onset or worsening of cough or dyspnea • Purulent tracheobronchial secretions • Auscultatory findings consistent with pulmonary consolidation	• PCT ≥ 0.25 ng/mL • Hypoxemia pO_2 ≤ 60 mm Hg or oxygen saturation ≤ 90% in room air • Respiratory rate ≥ 20 breaths/min
VAP (24, 25)	• Onset > 48 h from start of mechanical ventilation • New or evolving infiltrate on chest x-ray	• Purulent tracheobronchial secretions • Auscultatory findings consistent with pulmonary consolidation	• PCT ≥ 0.25 ng/mL • CPIS ≥ 6

Abbreviations: AC, acute cholangitis; BSI, bloodstream infection; CAP, community-acquired pneumonia; CPIS, clinical pulmonary infection score; CT, computed tomography; HAP, hospital-acquired pneumonia; HCAP, health care–associated pneumonia; PCT, procalcitonin; pO_2, partial pressure of oxygen; VAP, ventilator-associated pneumonia.

[a]HCAP implies the same definition as *hospital acquired*, but here pneumonia was diagnosed in a patient who was a resident of an extended care facility or nursing home prior to hospital admission.

or equal to 0.50×10^9/L and thereafter defined as "severe lymphopenia." Another 18 COVID-19 patients were included with lymphocyte counts greater than or equal to 1.10×10^9/L, thereafter called "no lymphopenia."

Baseline Characteristics

Baseline patient characteristics, comorbidities, and clinical data were collected from medical files in both cohorts. The Acute Physiology and Chronic Health Evaluation (APACHE II) and Sequential Organ Failure Assessment (SOFA) score were calculated at first day of admission to assess disease severity in patients with bacterial sepsis.

Sampling Processing and Storage

Serum was obtained from patients with bacterial sepsis within 24 hours after diagnosis. EDTA plasma was collected from COVID-19 patients at 2 time points during their hospital stay: The first available sample was obtained in our hospital after diagnosis (referred to as *baseline*), and the second sample was obtained 1 week after the first sample. Serum was centrifuged at 3500*g* and EDTA plasma at 2954 relative centrifuge force, both at room temperature for 10 minutes. All samples were aliquoted and stored at –80 °C before further analysis.

Assays

Measurement of thyrotropin, thyroid hormones, and albumin

Measurements of TSH, free T4 (FT4), T3, and T4 were performed in serum or plasma using electrochemiluminescence immunoassay (ECLIA) on the Roche Cobas 8000 (E801). Albumin in serum or plasma was measured by a bromocresol purple colorimetric method, both on the Roche Cobas 8000 (C702). The reference ranges at our laboratory are 0.27 to 4.2 mU/L for TSH; 10.0 to 23.0 pmol/L for FT4; 1.3 to 3.1 nmol/L for T3; 66 to 181 nmol/L for T4, and 35 to 50 g/L for albumin. For TSH, the total coefficient of variation (CV) ranged between 3.5% at 0.17 mU/L and 2.1% at 2.3 mU/L (detection range, 0.005-100 mU/L). The total CV for FT4 ranged between 2.6% at 18.4 pmol/L and 2.2% at 49.7 pmol/L (detection range, 0.5-100 pmol/L). The total CV for T3 ranged between 1.9% at 1.7 nmol/L and 1.5% at 4.4 nmol/L (detection range, 0.3-10 nmol/L). The total CV for T4 ranged between 1.6% at 38 nmol/L and 3.0% at 123 nmol/L (detection range, 5.4-320 nmol/L). The total CV for albumin is 2.5% at 24.6 g/L and at 41.3 g/L (detection range, 2-100 g/L).

Lymphocyte Count Measurements

Lymphocytes were measured in whole blood by fluorescence flowcytometry on an XE-2100 (sepsis) and XE-5000 (COVID-19) hematology analyzer (Sysmex). The reference ranges are 1.0 to 3.5×10^9/L.

C-Reactive Protein Measurements

C-reactive protein (CRP) in plasma was measured for routine diagnostic assessment in sepsis patients using a nephelometric analyzer, Siemens BN II System, reference range less than 3 mg/L. For COVID-19 samples, plasma CRP was measured by immunoturbidimetry on the Roche Cobas 8000 (C702), reference range less than 10 mg/L.

Ferritin Measurements

For patients with bacterial sepsis, concentrations of ferritin were measured in serum by an enzyme-immunoassay (ORGENTEC Diagnostika GmbH) with a lower detection limit of 75 ng/mL. In COVID-19 patients, ferritin was measured in plasma using ECLIA on the Roche Cobas 8000 (E801).

Interleukin 6 Measurements

Concentrations of IL-6 were determined batchwise using enzyme-linked immunosorbent assays (ELISA, Quantikine, R & D Systems), with a lower detection limit of 16 pg/mL in both study populations.

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp) and GraphPad Prism, version 8.0 (GraphPad Software Inc). Continuous data are presented as the median with IQR following criteria for nonnormally distributed variables. Nominal data are presented as numbers (n) with percentages (%). Differences between groups (severe lymphopenia vs no lymphopenia) were assessed by Mann-Whitney *U* test or Kruskal-Wallis test for continuous variables, and Fisher exact/chi-square test for discrete variables. Correlations between lymphocyte counts, TSH, THs, and inflammatory markers were assessed by the Spearman rank correlation test. Two-tailed *P* values of less than .05 were considered statistically significant.

Results

Patient Characteristics of the Bacterial Sepsis Cohort

Plasma samples and absolute lymphocyte counts of 224 patients diagnosed with bacterial sepsis were available. To test our hypothesis that low lymphocyte counts may correlate with thyroid function abnormalities, a parallel subgroup analysis was performed. The median lymphocyte count in this study population was 1.00 × 10^9/L (IQR, 0.64-1.58 10^9/L). Based on the cutoffs defined using the IQRs, the total cohort was divided into groups of "severe lymphopenia" (≤ 0.64 × 10^9/L) and "no lymphopenia" (≥ 1.58 × 10^9/L) (see flowchart in Fig. 1A). Baseline and patient characteristics of the total cohort, severe lymphopenia and no lymphopenia subgroups are depicted in Table 2. Overall, no substantial differences in (baseline) patient characteristics were observed between these groups, except for a lower body mass index in the severe lymphopenic subgroup (24.2; IQR, 22.0-29.4) compared to the nonlymphopenic subgroup (28.1; IQR, 22.8-31.5).

Thyrotropin, Thyroid Hormones, and Lymphocyte Count Correlations in Bacterial Sepsis

Fig. 2 depicts the correlations between lymphocyte counts, inflammatory markers (IL-6, CRP, and ferritin) and TSH, T4, FT4, and T3 for the total bacterial sepsis cohort (n = 224). Only a weak positive correlation between lymphocyte counts and T3 was observed (ρ = 0.252, $P < .001$) in the entire cohort, whereas no correlations were found for other TH and lymphocyte counts. Of note, clear correlations between the T4, FT4, and T3 concentrations were present in these patients.

Next, the concentrations of TSH, T4, FT4, and T3 were analyzed for subgroups stratified based on lymphocyte counts as described earlier (n = 56 per group). Abnormal, mainly low, concentrations of TSH, T4, FT4, and T3 were measured in, respectively, 34.8% (39 of 112), 58.9% (66 of 112), 33.9% (38 of 112), and 95.5% (107 of 112) of these patients compared to a healthy population (based on the reference ranges of our institution [see Fig. 3A-3D]). In line with the correlation analysis, T3 was significantly lower in sepsis patients with severe lymphopenia, whereas no differences in TSH, T4, and FT4 concentrations were observed between patients with severe lymphopenia and without lymphopenia (Fig. 3A-3D; $P = .070$, .513, .549, and < .001 for TSH, T4, FT4, and T3, respectively). Furthermore, albumin concentrations were overall quite low in patients with bacterial sepsis (91.1% < 35 g/L; lower reference limit), but not different between patients with severe lymphopenia and patients without lymphopenia (Fig. 3E, $P = .625$).

Patient Characteristics of the Coronavirus Disease 19 Cohort

A recent meta-analysis showed that lower lymphocyte counts on admission are associated with severity and poor outcome in COVID-19 (12). Therefore, we further investigated our hypothesis by particularly selecting COVID-19 patients with or without profound lymphopenia. We applied the same method of stratification and analysis as performed in the bacterial sepsis study population, but patients were preselected before measurements. Absolute lymphocyte counts on hospital admission were available for 161 patients diagnosed with COVID-19 admitted to our university hospital. Among these patients, a median lymphocyte count of 0.74 × 10^9/L (IQR, 0.50-1.10 × 10^9/L) was observed; demographic and clinical characteristics are described in Table 3. Next, patients were selected based on plasma availability and exclusion criteria (see Fig. 1B). Finally, 17 COVID-19 patients could be selected from the group with the lowest lymphocyte counts (≤ 0.50 × 10^9/L), defined as "severe lymphopenia" and 18 COVID-19 patients were selected from the group of the highest lymphocyte counts (≥ 1.10 × 10^9/L), defined as "no lymphopenia." Baseline characteristics were different between the lymphopenia groups for CRP and IL-6, which were significantly higher in the severe lymphopenia group ($P = .023$ and $P = .001$, respectively). Moreover, ICU admissions and mortality rates were significantly higher in COVID-19 patients with severe lymphopenia (47% vs 6%, $P = .007$ and 41% vs 0%, $P = .003$, respectively; see Table 3).

Thyrotropin and Thyroid Hormones Are Associated With Severe Lymphopenia and Inflammatory Biomarkers in Coronavirus Disease-2019

Abnormal TSH, T4, FT4, and T3 concentrations were present in 11.4% (4 of 35), 17.1% (6 of 35), 17.1% (6 of 35), and 51.4% (18 of 35), respectively, of the selected patients with COVID-19 (Fig. 4A-4D). COVID-19 patients with severe lymphopenia showed significantly lower plasma concentrations of TSH, T4, FT4, and T3 compared to the group of patients without lymphopenia, with the most profound differences for T4 and T3 (Fig. 4A-4D, $P = .026$, < .001, .001, < .001 for TSH, T4, FT4, and T3 respectively). Importantly, plasma concentrations of albumin were again low (91.4% < 35g/L) in all patients, but not different between the groups, suggesting no substantial effects of plasma binding of the THs (Fig. 4E, $P = .258$).

The concentrations of the inflammatory biomarkers IL-6, CRP, and ferritin in patients with no lymphopenia and severe lymphopenia are presented in Fig. 5. Patients with severe lymphopenia demonstrate significantly higher

Table 2. Baseline characteristics of the bacterial sepsis cohort

	Total (n = 224)	Severe lymphopenia (n = 56)	No lymphopenia (n = 56)	*P*, severe vs no lymphopenia
Age, y	76 (65-83)	75 (65-83)	76 (67-84)	.347
Sex (n, %)				1.000
Male	129 (58)	30 (54)	30 (54)	
Female	95 (42)	26 (46)	26 (46)	
Body mass index, kg/m^2[a]	26.3 (22.5-30.9)	24.2 (22.0-29.4)	28.1 (22.8-31.5)	.045
Comorbidities (n, %)				
Diabetes mellitus	67 (30)	16 (24)	18 (27)	.681
Heart failure	61 (27)	11 (20)	13 (23)	.645
Coronary heart disease	51 (23)	9 (16)	11 (20)	.622
Chronic renal disease	24 (11)	4 (7)	3 (5)	.696
Chronic obstructive pulmonary disease	53 (24)	12 (21)	9 (16)	.468
History of hematological/solid malignancies	12 (5)	4 (7)	3 (5)	.401
Chronic intake of corticosteroids/biologicals (n, %)	10 (5)	4 (7)	0 (0)	.042
Source of infection (n, %)				
Acute cholangitis	13 (6)	3 (5)	4 (7)	.696
Primary bacteremia	38 (17)	14 (25)	10 (18)	.357
Community-acquired pneumonia	97 (43)	25 (45)	21 (38)	.442
Healthcare-associated pneumonia	51 (23)	12 (21)	11 (20)	.815
Ventilator-associated pneumonia	24 (11)	4 (7)	9 (16)	.140
Septic shock (n, %)	168 (75)	42 (19)	42 (19)	1.000
APACHE II[b]	24 (17-31)	24 (17-35)	28 (19-33)	.547
SOFA score	11 (9-14)	12 (9-15)	11 (8-14)	.163
CRP, mg/L	70 (21-159)	100 (19-204)	39 (19-88)	.051
Ferritin, µg/L	1370 (435-3326)	1407 (423-2878)	1117 (488-2460)	.805
IL-6, pg/mL	119 (26-217)	143 (52-314)	41 (18-109)	.109
Thyroid hormones				
TSH, mU/L	0.67 (0.24-1.94)	0.43 (0.19-1.28)	0.85 (0.28-0.85)	.070
T3, nmol/L	0.69 (0.55-0.88)	0.63 (0.53-0.79)	0.78 (0.64-1.00)	.001
T4, nmol/L	57.6 (40.0-81.1)	56.2 (35.1-74.5)	59.7 (34.4-92.3)	.513
FT4, pmol/L	12.9 (9.3-16.0)	12.5 (9.4-15.7)	13.8 (7.9-17.4)	.549
Albumin, g/L	22.5 (17.1-28.5)	21.4 (17.9-28.6)	20.7 (16.0-29.1)	.625
Mortality 28 days (n, %)	136 (61)	32 (57)	32 (57)	1.000

Data are presented as median (interquartile range) or n (%). Absolute lymphocyte counts equal to or below 0.64×10^9/L were classified as severe lymphopenia based on the lowest quartile (P0-25). Absolute lymphocyte counts equal to or above 1.58×10^9/L were classified as no lymphopenia based on the highest quartile (P75-100).

Abbreviations: APACHE II, The Acute Physiology and Chronic Health Evaluation; COVID-19, coronavirus diseases 2019; CRP, C-reactive protein; FT4, free thyroxine; IL-6, interleukin 6; SOFA, Sequential Organ Failure Assessment; T3, 3,5,3′-triiodothyronine; T4, thyroxine; TSH, thyrotropin.

[a]Fifty-five missing values.

[b]Eighteen missing values.

values of all inflammatory markers compared to patients with normal lymphocyte counts ($P < .001$, .023, and .008 for IL-6, CRP, and ferritin, respectively).

Follow-up Measurements of Lymphocytes and Thyroid Hormones and Thyrotropin in Coronavirus Disease-2019

To investigate the relationship between lymphopenia and TH in more depth, a second measurement of lymphocytes, THs, and TSH was performed 1 week after the first assessment in 15 patients of our COVID-19 study group (Fig. 6). Paired analysis revealed that THs did not significantly change over this time period, with T3 levels especially remaining relatively low in most patients after 1 week of follow-up. In contrast, almost all patients (12 of 15) showed increased lymphocyte counts approaching normal ranges, indicating a quantitative recovery of their adaptive immune response over this time period (see Fig. 6).

Discussion

In the present study, we investigated the relationship between lymphopenia and thyroid function abnormalities

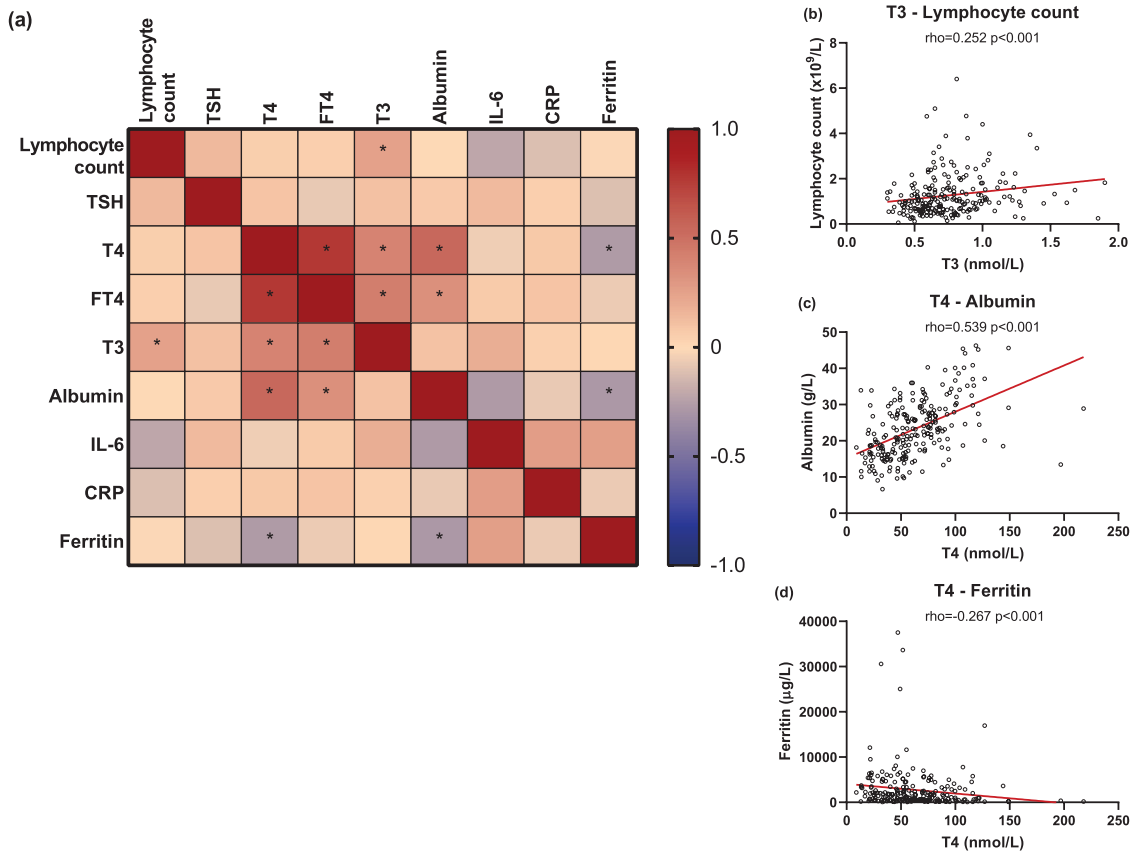

Figure 2. Correlations of lymphocytes, inflammatory markers, and thyroid hormones in patients with bacterial sepsis (n = 224). A, Heatmap of correlations between lymphocytes, inflammatory markers, and thyroid hormones. Scatterplots of correlation between B, 3,5,3′-triiodothyronine (T3) and lymphocyte count; C, thyroxine (T4) and albumin; and D, T4 and ferritin. Correlation coefficients and *P* values were calculated with Spearman rank correlation tests. After correction for multiple testing (Bonferroni), a *P* value of less than .002 was considered statistically significant. Interleukin 6 (IL-6) was available for only 30 patients. CRP, C-reactive protein. **P* less than .002.

in patients with severe infections, such as bacterial sepsis and COVID-19. In patients with bacterial sepsis, T3 weakly correlated with lymphocyte counts, but no difference in TSH and other THs between severe lymphopenic and nonlymphopenic subpopulations were observed. In contrast, in COVID-19 patients, lower concentrations of TSH and THs were detected in patients with profound lymphopenia. In parallel, inverse relationships of TSH and TH concentrations with common inflammatory biomarkers were observed in these patients. However, the majority of COVID-19 patients showed overall improvement of their lymphocyte counts after 1 week, whereas THs and TSH remained largely disturbed, and this may argue either against a direct causal relationship between lymphopenia and the thyroid function abnormalities, or for different kinetics of the recovery of lymphopenia and thyroid function.

Previous studies have investigated the extent and duration of lymphopenia in the early phase of bacterial sepsis and reported an association with increased mortality (2, 27). In this study, severe lymphopenia in sepsis (representing the lowest quartile, 0%-25%) was defined using a cutoff of less than or equal to 0.64×10^9/L, which is comparable to previous studies that used similar thresholds and reported a comparable prevalence (22%-26%) of severe lymphopenia among sepsis patients (2, 27). During the acute phase of critical illness, patients most typically present with a rapid decline of circulating T3, referred to as "low T3 syndrome," a synonym for NTIS (18). A previous study reported that NTIS affects 60% to 70% of critically ill patients in the ICU (28). Another study reported a similar incidence of 67% in sepsis patients (29). In our study, almost all the sepsis patients had relatively low T3 serum concentrations, particularly those with severe lymphopenia. This finding might be explained by the enrollment of mainly critically ill patients, illustrated by the overall high incidence of septic shock, high mortality rate, and high APACHE II and SOFA scores (see Table 1). Moreover, in this study sepsis was defined according to the Sepsis-3 definition, which is stricter and identifies more severe patients compared to the Sepsis-2 definition used by the earlier study (29). Overall, bacterial sepsis patients may present with severe lymphopenia and TH imbalances at the

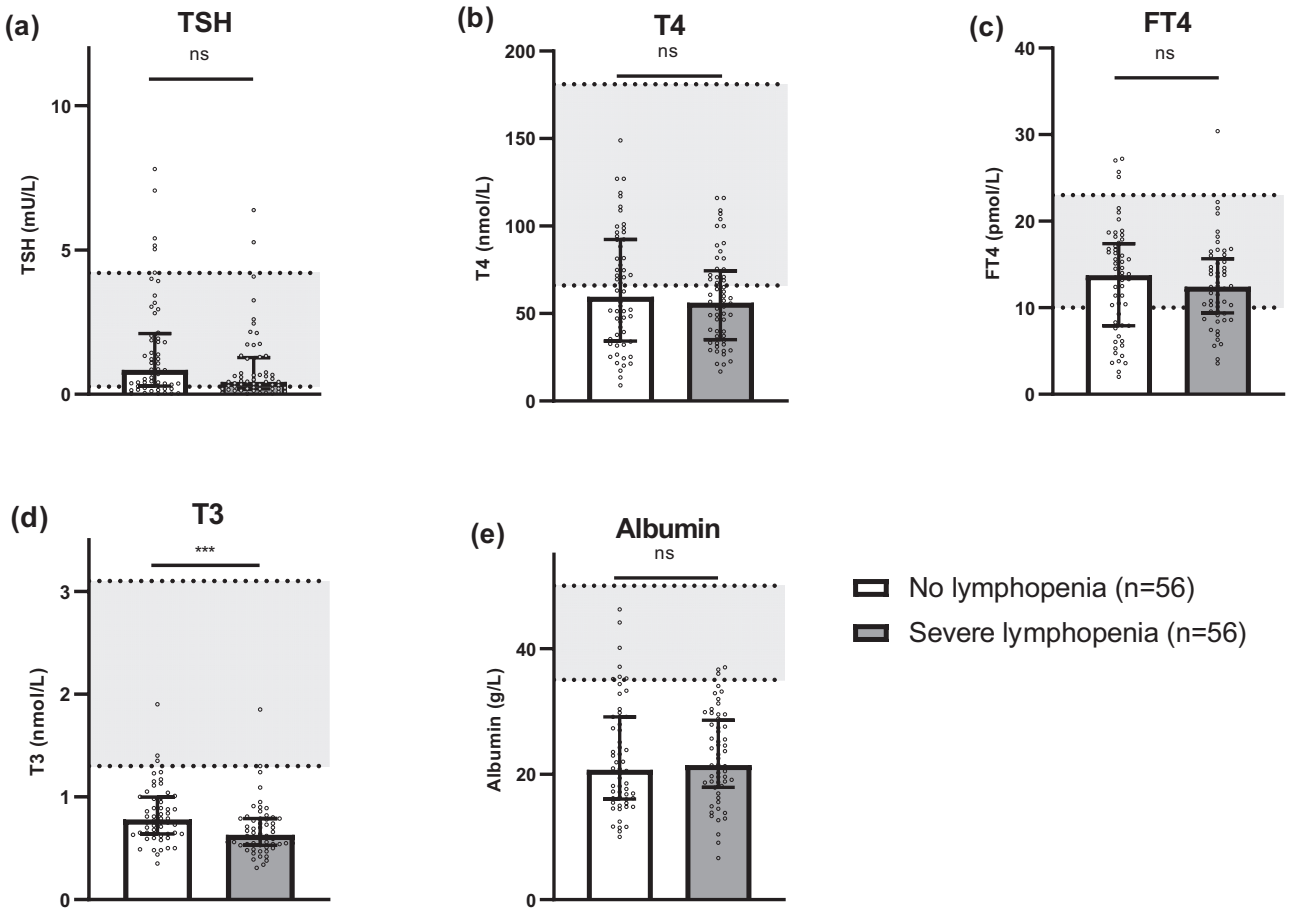

Figure 3. Thyroid hormones in bacterial sepsis patients with severe lymphopenia (n = 56) and no lymphopenia (n = 56). Concentrations of A, thyrotropin (TSH); B, thyroxine (T4); C, freeT4 (FT4); D, 3,5,3′-triiodothyronine (T3); and E, albumin in sepsis patients with severe lymphopenia and without lymphopenia. Absolute lymphocyte counts below 0.64 × 10^9/L were classified as severe lymphopenia based on the lowest quartile (P0-25). Absolute lymphocyte counts above 1.58 × 10^9/L were classified as no lymphopenia based on the highest quartile (P75-100). The gray area represents reference ranges used at our institution. Exact *P* values are A, .070; B, .513; C, .549; D, less than .001; and E, less than .625. *P* values were calculated with Mann-Whitney *U* tests. Data are presented as median with interquartile ranges. ns, not significant. ***P less than .001.

same time, while especially low T3 concentrations seemed to be linked to the extent of disease severity.

Recently, a small retrospective study showed that abnormal thyroid function was present in 64% of patients with COVID-19, and lower TSH and T3 concentrations were associated with severity of the disease (20). Accordingly, another study reported thyroid abnormalities in 52 of 84 (62%) COVID-19 patients, mostly found in severe and critical cases (30). In our study, thyroid abnormalities were particularly observed in severe lymphopenic COVID-19 patients; in addition, this subgroup showed an increased mortality rate. These findings suggest a possible relationship between abnormal thyroid function and unfavorable outcome in COVID-19, and highlights the need for further investigation to establish the prognostic value of TSH and THs in hospitalized COVID-19 patients.

The bidirectional interaction of the HPT axis and the proinflammatory immune response is complex and still poorly understood. Pathogens can trigger an acute-phase response that is accompanied by the production of cytokines, including IL-6 (31). Torpy et al showed that a single bolus infusion of IL-6 in healthy individuals introduced acute changes in THs, with declined concentrations of T3, the most typical characteristic of the acute phase of NTIS (32). The inverse correlation between serum IL-6 and serum T3 concentrations has been widely reported in hospitalized patients (33-36). Interestingly, a similar negative association was observed in this study, as the profound lymphopenic COVID-19 patients showed significantly higher IL-6 and lower T3 concentrations compared to patients with normal lymphocyte counts.

Currently, more insights have led to a better understanding of the relationship between NTIS and inflammation in critical illness. A biphasic pattern of NTIS can be recognized (37-39). The acute phase of NTIS is predominantly characterized by acute changes in peripheral TH concentrations and function, mainly mediated by altered

Table 3. Clinical characteristics of the coronavirus disease 2019 population and lymphocyte subgroups

	Total (n = 161)	Severe lymphopenia (n = 17)	No lymphopenia (n = 18)	*P*, severe vs no lymphopenia
Age, y	65 (54-73)	69 (60-77)	62 (43-73)	.096
Sex				1.000
Male (n, %)	105 (65)	12 (71)	12 (67)	
Female (n, %)	56 (35)	5 (29)	6 (33)	
Body mass index, kg/m^2	26.9 (24.0-29.6)	25.8 (24.4-29.0)	27.8 (23.6-30.5)	.463
Comorbidities (n, %)				
Diabetes mellitus	35 (22)	3 (18)	1 (6)	.338
Congestive heart failure	11 (7)	2 (12)	0 (0)	.229
Coronary heart disease	17 (11)	1 (6)	1 (6)	1.000
Chronic renal disease	10 (6)	1 (6)	0 (0)	.486
Chronic obstructive pulmonary disease	41 (25)	3 (18)	8 (44)	.146
History of hematological/solid malignancies	40 (25)	2 (12)	6 (33)	.088
Ward of admission at first sampling				
Hospital ward (n, %)	120 (75)	9 (53)	17 (94)	.007
ICU (n, %)	41 (25)	8 (47)	1 (6)	
Time between hospital admission and plasma sampling, d	NA	3 (2-5)	3 (2-5)	.807
CRP, mg/L	86 (46-151)[a]	92 (62-233)	53 (31-120)	.023
Ferritin, µg/L	800 (383-1490)[a]	1461 (916-2897)	705 (382-1174)	.008
IL-6, pg/mL	NA	110 (75-185)	22 (16-69)	< .001
Total white blood cell counts	6.9 (4.6-9.2)[a]	5.4 (3.7-7.0)	7.9 (5.6-10.3)	.017
White blood cell differentiation				
Absolute neutrophil counts	5.60 (3.36-7.56)[a]	4.31 (3.24-5.86)	4.63 (3.39-7.06)	.335
Absolute lymphocyte counts	0.74 (0.50-1.10)[a]	0.38 (0.33-0.45)	1.49 (1.23-1.76)	< .001
Absolute monocyte counts	0.40 (0.25-0.67)[a]	0.39 (0.16-0.63)	0.73 (0.44-1.11)	.011
Absolute eosinophil counts	0.00 (0.00-0.00)[a]	0.00 (0.00-0.03)	0.06 (0.00-0.19)	.019
Absolute basophil counts	0.00 (0.00-0.01)[a]	0.00 (0.00-0.01)	0.02 (0.00-0.03)	.027
Thyroid hormones				
TSH, mU/L	NA	0.87 (0.43-1.32)	1.41 (0.85-2.47)	.026
T3, nmol/L	NA	1.09 (0.89-1.22)	1.69 (1.31-1.88)	< .001
T4, nmol/L	NA	80.5 (64.9-95.6)	111 (103-137)	< .001
FT4, pmol/L	NA	13.9 (11.3-14.7)	17.1 (15.1-19.3)	.001
Albumin, g/L	NA	28.1 (19.9-31.8)	29.6 (28.7-31.2)	.258
Outcome				
Mortality (n,%)	20 (12)	7 (41)	0 (0)	.003
Duration of hospital stay, days	9 (5-21)	14 (8-28)	8 (6-11)	.005

Data are presented as median (interquartile range) or n (%). Selected patients with absolute lymphocyte counts equal to or below 0.50 × 10^9/L were classified as severe lymphopenia and patients selected with absolute lymphocyte counts equal to or above 1.10 × 10^9/L were classified as no lymphopenia.

Abbreviations: CRP, C-reactive protein; FT4, free thyroxine; ICU, intensive care unit; IL-6, interleukin 6; NA, not available; T3, 3,5,3′-triiodothyronine; T4, thyroxine; TSH, thyrotropin.

[a]Values on admission.

activity of iodothyronine deiodinases types 1 and 3 (18). The activity of liver deiodinase type 1, involved in the production of T3 out of T4 and in the clearance of reverse T3, decreases during acute illness resulting in a decline of serum T3 and an increase in serum reverse T3 (40, 41). It has been suggested that deiodinase alterations may represent a beneficial adaptation. This is supported by studies showing that changes in deiodinase activity may play a role in innate immune activation (neutrophils and macrophages) and support bacterial killing capacity of phagocytic cells (42-44). Particularly proinflammatory cytokines, such as interleukin 1β, IL-6, and hypoxia have been proposed as possible pathophysiological drivers for the altered deiodinase activity (45-47). However, a previous study showed that infusion of interleukin-1 receptor antagonist, the natural antagonist for interleukin 1β, could not restore NTIS induced by experimental endotoxemia in humans (48).

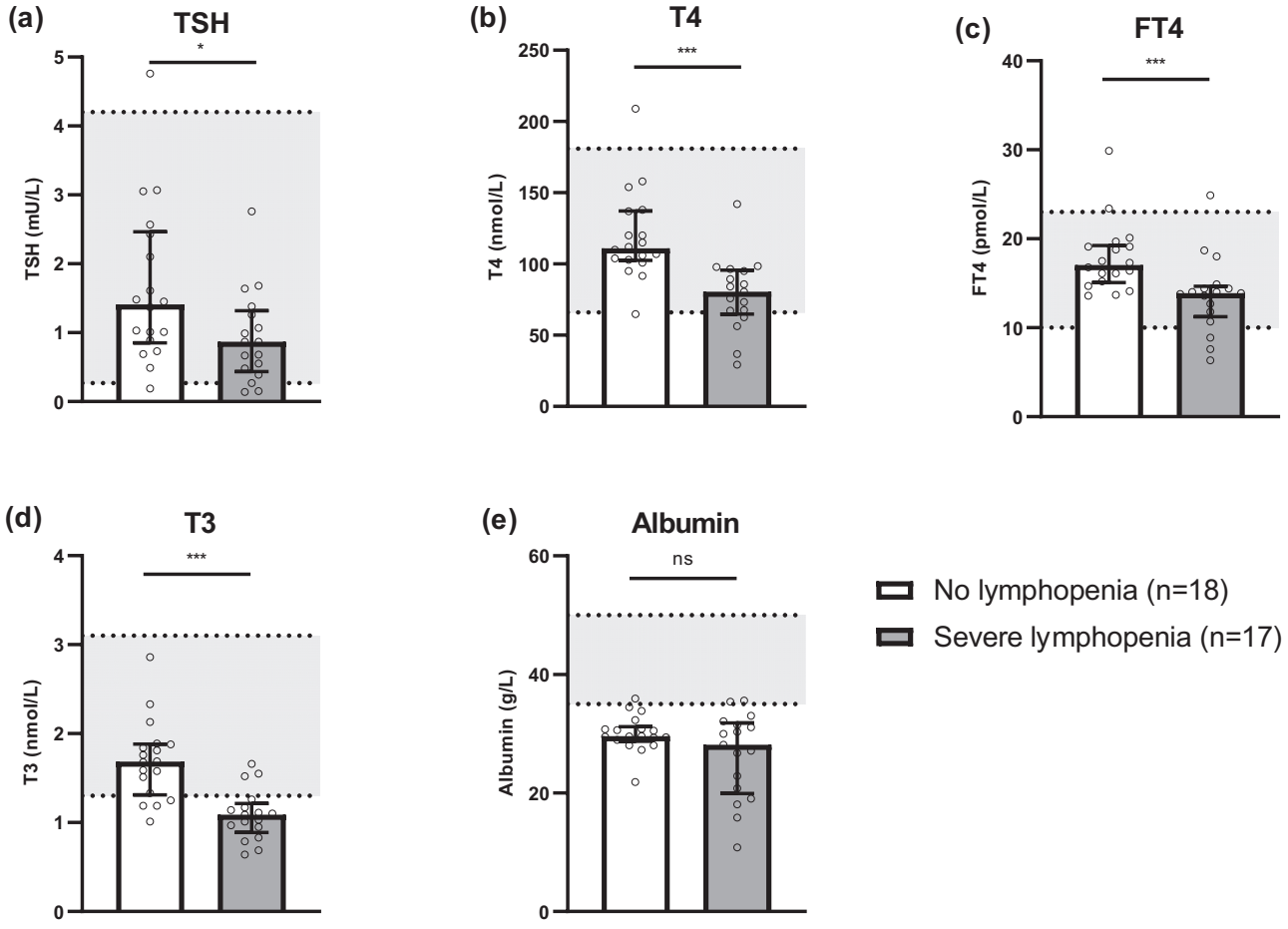

Figure 4. Thyroid hormones in coronavirus disease 2019 (COVID-19) patients with severe lymphopenia (n = 17) and no lymphopenia (n = 18). Concentrations of A, thyrotropin (TSH); B, thyroxine (T4); C, free T4 (FT4); D, 3,5,3′-triiodothyronine (T3); and E, albumin in COVID-19 patients with severe lymphopenia and without lymphopenia. Absolute lymphocyte counts below 0.50 × 10^9/L were classified as severe lymphopenia based on the lowest quartile (P0-25). Absolute lymphocyte counts above 1.10 × 10^9/L were classified as no lymphopenia based on the highest quartile (P75-100). Exact *P* values are A, .026; B, less than .001; C, .001; D, less than .001; and E, .258. *P* values were calculated with Mann-Whitney *U* tests. Data are presented as median with interquartile ranges. ns, not significant. **P* less than .05. ****P* less than .001.

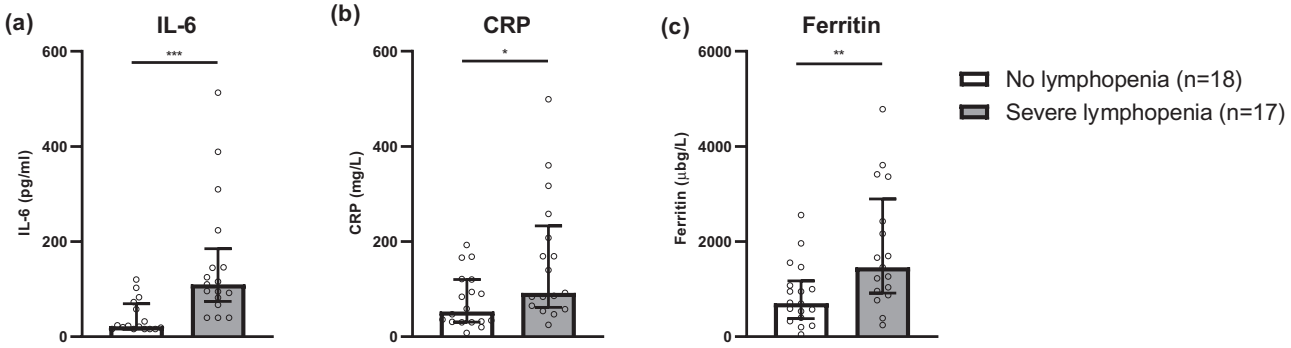

Figure 5. Inflammatory markers in coronavirus disease 2019 (COVID-19) patients with severe lymphopenia (n = 17) and no lymphopenia (n = 18). Concentrations of A, interleukin 6 (IL-6); B, C-reactive protein (CRP); and C, ferritin in COVID-19 patients with severe lymphopenia and without lymphopenia. Absolute lymphocyte counts below 0,50 × 10^9/L were classified as severe lymphopenia based on the lowest quartile (P0-25). Absolute lymphocyte counts above 1.10 × 10^9/L were classified as no lymphopenia based on the highest quartile (P75-100). Exact *P* values are A, less than .001; B, .023; and C, .008. *P* values were calculated with Mann-Whitney *U* tests. Data are presented as median with interquartile ranges. ns, not significant; **P* less than .05. ***P* less than .01.

Severely and prolonged critically ill patients may show central suppression of the HPT axis, hallmarked by markedly low T3/T4 concentrations and suppression of TSH concentrations, which further impairs TH homeostasis in addition to peripheral changes (18, 38). In our study, these alterations were observed in severely ill sepsis patients.

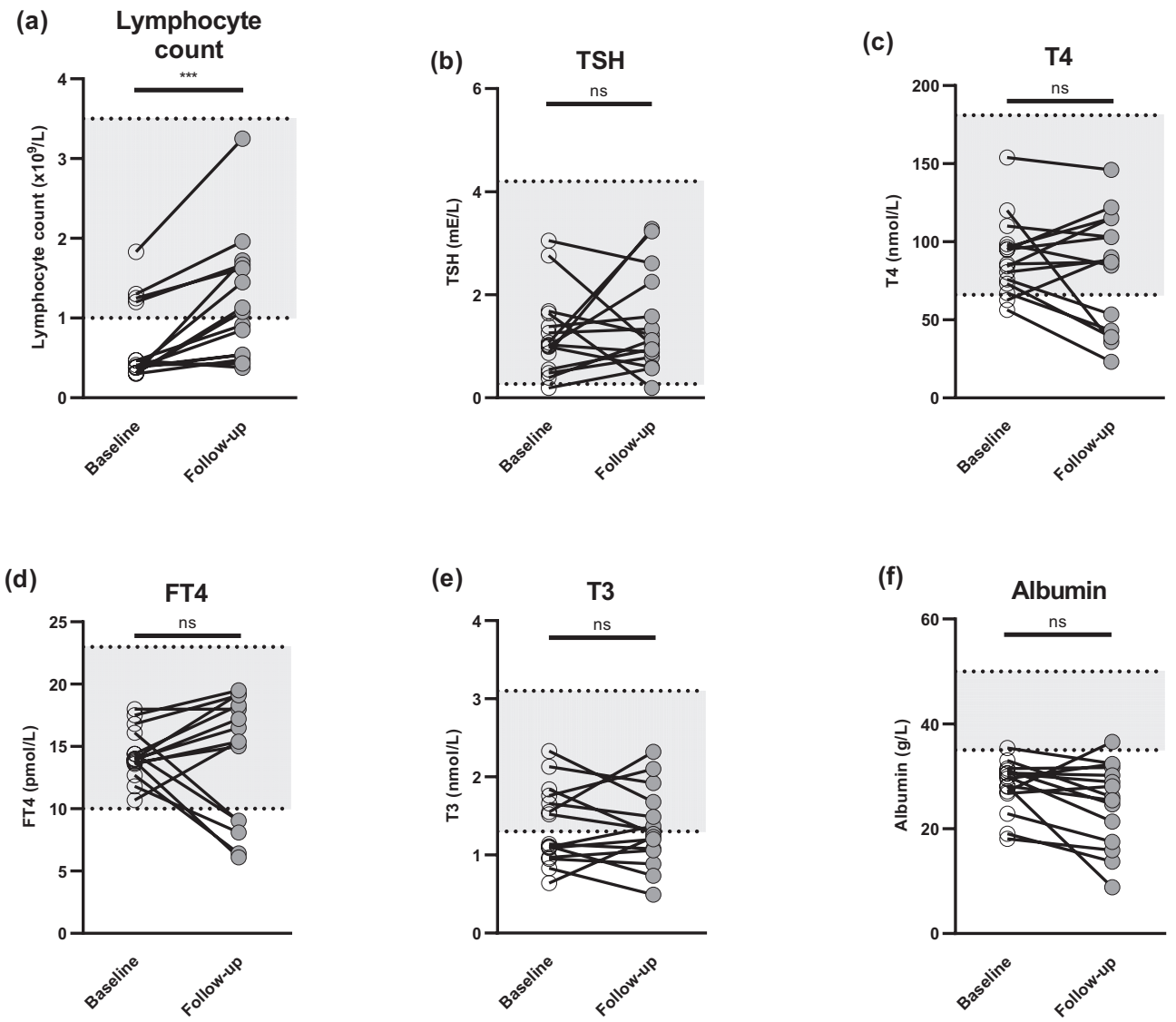

Figure 6. Individual values of follow-up of lymphocytes and thyroid hormones in the disease course of coronavirus disease 2019 (COVID-19) (n = 15). A, Individual lymphocyte counts and individual concentrations of B, thyrotropin (TSH); C, thyroxine (T4); D, free T4 (FT4); E, 3,5,3′-triiodothyronine (T3); and F, albumin in COVID-19 patients in the first week of admission (baseline) and 1 week after (follow-up). Exact *P* values are A, less than .001; B, .639; C, .534; D, .808; E, .720; and F, .050. *P* values were calculated with Wilcoxon signed rank test. Data are presented as the median with interquartile ranges. ns, not significant. ***P* less than .001.

Interestingly, a few COVID-19 patients showed similar altered patterns, which often remained present after 1 week of follow-up. It has been suggested that this phenotype could be maladaptive, as a prolonged catabolic state might indirectly contribute to muscle wasting, with potential negative clinical effects (49). Another study reported an increased risk of prolonged mechanical ventilation in patients with NTIS admitted to the ICU (50). In general, several randomized controlled clinical trials have investigated TH replacement therapies in hospitalized patients (51, 52), with inconclusive results regarding patient-centered outcomes.

Of note, although extensive research has been carried out on therapeutic intervention with T3, T4, TSH-releasing factor, or recombinant TSH in study populations with critical illness, none of these studies has reported information regarding circulating lymphocyte counts before and/or after treatment, or after cardiac surgery (53-62).

To reduce potentially interfering factors, we have excluded from our series patients who were treated with glucocorticoids. The rapidly changing treatment strategies in COVID-19 currently include adding dexamethasone therapy as standard care in hospitalized COVID-19 patients (63). This could also affect the HPT axis, leading to decreased TSH, T4 and T3 levels in these patients. Distinguishing the effect of dexamethasone treatment from

NTIS in COVID-19 patients can be difficult, particularly in severe and prolonged states of critical illness, as these patients may display low serum T4 and TSH levels (besides low T3 levels), which are indicative of a poor prognosis (64). Current guidelines recommend against the use of TH supplementation therapies in NTIS (65). The clinical dilemma of which patients need to be treated, or not, remains unsolved as causal mechanisms of the neuroendocrine immune system crosstalk and its relation to clinical outcomes in critically ill patients are still missing.

The detailed role of THs and their contribution in the regulation of the immune system has not yet been elucidated. Previous studies in mice have reported an important role for THs in regulating lymphocyte function (15, 16, 66-69). Klecha and colleagues showed that hypothyroid mice show lower T and B cell mitogen-induced proliferation compared with euthyroid animals, and reversion of the hypothyroid state by T3 administration was able to recover the proliferative responses (15). In mice subjected to chronic stress, TH levels and T cell reactivity was reduced, while T4 replacement therapy restored the hypothyroid state and reversed T cell proliferative responses in these mice (69). Interestingly, both research groups mentioned protein kinase C as being potentially involved in these processes (15, 69), with protein kinase C possibly acting as a mediator of TH metabolism, as well as a regulator of lymphocyte proliferation (70). Recently, a study published the results of a large cohort of healthy individuals supporting the role of TSH and T4 for the regulation of lymphoid cell compartments acquiring immune homeostasis (71). Among these individuals, TSH levels were strongly associated with T cell subpopulations, whereas T4 was associated with B cell populations. Further investigation linking genetic variance and immunological function suggested an important role of FT4 in the regulation of B cell function. Taken together, these observations in mice and humans do suggest a crosstalk between THs and lymphocyte homeostasis, which is in line with the associations reported in this study. How alterations of THs may be related to lymphopenia in severe infections is yet to be explored.

This study has some limitations. Our subgroup analysis of extreme (severe) lymphopenia and nonlymphopenia cases was primarily designed to test our hypothesis, which led to the exclusion of mild and moderate lymphopenic cases in both study populations and relatively small sample sizes, especially in our COVID-19 study selection. Both the prevalence and degree of lymphopenia, and the (variation of) disease severity within the study populations, might have influenced the strength of the correlations with THs observed in the bacterial sepsis and COVID-19 patients. Owing to methodological constraints, the results of the 2 populations cannot be directly compared. The pathogenesis and high prevalence of lymphopenia in COVID-19 patients (including those with less-severe disease) is not known; nevertheless, different mechanisms might be responsible as compared to the bacterial sepsis patients. In this regard, one should be cautious with the extrapolation of our results to all patients with severe infections (including those with bacterial sepsis or COVID-19) in the general population. The follow-up period of our COVID-19 cohort was relatively short; therefore, information about the long-term effects of TSH and TH changes in relation to the HPT axis feedback loop cannot be provided. Another limitation is that within our bacterial sepsis cohort, data were lacking on preexisting thyroid disease and potential drug confounders that could directly interfere with the HPT axis, or could have influenced analytical methods of TSH and TH measurements in this study.

In conclusion, this study confirms that lymphopenia and abnormal thyroid function tests are highly prevalent in patients with severe viral (COVID-19) and bacterial infections, yet a direct causal relationship between these 2 processes cannot be established. Therefore, future larger studies are needed to clarify their underlying pathophysiological mechanisms, including the identification of specific host- (eg, severity) and pathogen-derived factors contributing to the variety of clinical phenotypes. Moreover, we suggest that additional studies investigating the kinetic patterns and long-term effects of lymphocyte count and TH changes may further improve our understanding of this complex immunometabolic crosstalk. Such key studies could establish their independent roles as potential prognostic predictors in patients with severe infections, and this may potentially facilitate the implementation of personalized care strategies to improve the clinical outcomes in these patients.

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