The association of TSH and thyroid hormones with lymphopenia in bacterial sepsis and COVID-19

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Abstract

Context

Lymphopenia is a key feature of immune dysfunction in patients with bacterial sepsis and COVID-19 and associated with poor clinical outcomes, but the cause is largely unknown. Severely ill patients may present with thyroid function abnormalities, so-called non-thyroidal illness syndrome (NTIS), and several studies have linked TSH and the thyroid hormones thyroxine (T4) and triiodothyronine (T3) to homeostatic regulation and function of lymphocyte populations.

Purpose

To test the hypothesis that abnormal thyroid function correlates with lymphopenia in patients with severe infections.

Methods

Retrospective analysis of absolute lymphocyte counts, circulating TSH, T4, free T4 (FT4), T3, albumin and inflammatory biomarkers was performed in two 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 non-lympopenic 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 one week follow-up, the majority (12/15) of COVID-19 patients showed quantitative recovery of their lymphocyte numbers, while 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.

Keywords: Thyroid, Metabolism, Inflammation, Lymphocyte, Sepsis, COVID-19

Introduction

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 (the main cause of sepsis), also systemic viral infections are 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 causes the coronavirus disease 2019 (COVID-19) pandemic with above 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 in several different studies 10, 11. Especially, profound lymphopenia 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 supports the bidirectional interaction of the hypothalamic-pituitary thyroid (HPT) axis and immune function 13. Animal models showed that TSH as well as the thyroid hormones thyroxine (T4) and triiodothyronine (T3) play a crucial role in the homeostatic regulation and functional acitivity of lymphocyte populations 14, 15. Other studies have reported the important role of thyroid function in lymphopoeisis 16, 17. Moreover, thyroid hormone balance is often abnormal in severely ill patients. Critically ill patients, particularly those in the 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 non-thyroidal 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 thyroid hormone concentrations can be linked to immune responses in patients with severe infections. Based on previous findings in literature, we hypothesize that abnormal thyroid function correlates with lymphopenia observed in these patients.

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

Methods

Study design and setting

This retrospective study was performed in parallel in two independent study populations; a cohort of hospitalized patients with bacterial sepsis admitted in Greece (PROVIDE study, unpublished data, ClinicalTrials.gov identifier NCT 03332225, EudraCT number 2017-002171-26) and selected group of patients from a cohort with COVID-19 patients admitted to a tertiary university hospital (Radboudumc, Nijmegen) in the Netherlands (Figure 1a and b).

Bacterial sepsis cohort

All patients (≥ 18 years) with bacterial sepsis admitted to one of the 14 recruiting study sites and hospitals in Greece were screened for eligibility. From all subjects enrolled informed consent was provided by the patient or by one's first-degree relative/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, healthcare-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: 'do no resuscitate' decision, pregnancy or lactation, other causes of infections, any stage IV malignancy, active tuberculosis, human immunodeficiency virus infection, primary immunodeficiency, oral or intravenously administered corticosteroids at a daily dose ≥0.4 mg prednisone for the last 15 days, or treatment with any anti-cytokine biological during the last one-month, medical history of systemic or 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 x109/L (interquartile range [IQR] 0.64-1.58 x109/L) comparing the extreme "severe lymphopenic" (0-25%: lymphocyte counts ≤0.64 x109/L) patients with the "non-lymphopenic" (75-100%: lymphocyte counts ≥1.58 x109/L) bacterial sepsis patients.

COVID-19 study population

Patients (or their legal representatives) with a PCR-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 (CT) 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 between 1.00 – 3.50 x109/l) counts were selected from an existing cohort (Figure 1b). This selection was performed based on the median and quartile distribution (interquartile range) of absolute lymphocyte counts observed upon admission in the entire COVID-19 population that was hospitalized at our institution. A median lymphocyte count of 0.74 x109/L (IQR 0.50-1.10 x109/L) was observerd upon admission in (n=161) hospitalized COVID-19 patients, thereafter ≤0.50 and ≥1.10 were used as pre-defined cutt-off points for patient selection. Patients with pregnancy, pre-existing thyroid disease, active hematological or solid malignancies, or use of any medication interfering with thyroid hormone-assay (e.g. corticosteroids, including dexamethasone, dopamine, amiodaron) were excluded, or in case plasma volume for measurements of thyroid hormones was insufficient. After the abovementioned exclusion criteria were applied, 17 COVID-19 patients were included from the subgroup representing lymphocyte counts ≤0.50x109/L and thereafter defined as “severe lymphopenia”. Another 18 COVID-19 patients were included with lymphocyte counts ≥1.1x109/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. Ethylenediaminetetraacetic acid (EDTA) plasma was collected from COVID-19 patients at two timepoints during hospital stay; the first available sample obtained in our hospital after diagnosis (referred as baseline), the second sample was obtained one week after the first sample. Serum was centrifuged at 3500 and EDTA plasma at 2954 Relative Centrifuge Force (RCF), both at room temperature for ten minutes. All samples were aliquoted and stored at -80 °C before further analysis.

Assays

Measurement of TSH, 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 Cobas 8000 (E801) (Roche, Almere, the Netherlands). Albumin in serum or plasma was measured by a bromocresol purple colorimetric method, both on the Cobas 8000 (C702) (Roche, Almere, The Netherlands). The reference ranges at our laboratory are 0.27-4.2 mU/L for TSH; 10.0-23.0 pmol/L for FT4; 1.3-3.1 nmol/L for T3; 66-181

nmol/L for T4 and 35-50 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 XE 2100 (sepsis) and XE-5000 (COVID-19) hematology analyzer (Sysmex, The Netherlands). The reference ranges are 1.0 – 3.5x109/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 (Munich, Germany), reference range <3 mg/L. For COVID-19 samples plasma CRP was measured by immunoturbidimetry on the Cobas 8000 (C702) (Roche, Almere, The Netherlands), reference range <10 mg/L.

Ferritin measurements

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

IL-6 measurements

Concentrations of IL-6 were determined batchwise using enzyme-linked immunosorbent assays (ELISA, Quantikine, R&D systems, Minneapolis, USA), 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., Armonk, NY, USA) and GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA, USA). Continuous data are presented as the median with interquartile range (IQR) following criteria for non-normally 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, thyroid hormones and inflammatory markers were assessed by Spearman's rank correlation test. Two tailed P-values < 0.05 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 studypopulation was 1.00 x109/L (IQR 0.64-1.58). Based on the cutt-offs defined using the interquartile ranges the total cohort was divided into groups of "severe lymphopenia" (≤0.64 x109/L) and "no lymphopenia" (≥1.58 x109/L) (flowchart Figure 1a). Baseline and patient charcteristics 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, exept for a lower Body Mass Index (BMI) in the severe lymphopenic sugroup (24.2, IQR 22.0-29.4) compared to the non-lymphopenic subgroup (28.1, IQR 22.8-31.5).

TSH, thyroid hormones and lymphocyte counts correlations in bacterial sepsis

Figure 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 (Rho=0.252, p-value: <0.001) 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 above (n=56 per group). Abnormal, mainly low, concentrations of TSH , T4 , FT4 and T3 were measured in, respectively, 34.8 % (39/112), 58.9% (66/112), 33.9 % (38/112) and 95.5% (107/112) of these patients compaired to a healthy population (based on the reference ranges of our instution [see Figure 3a-d). 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 could be observerd between patients with severe lymphopenia and without lymphopenia (Figure 3a-d, p-values: 0.070, 0.513, 0.549, <0.001 for TSH, T4, FT4 and T3 respectively). Furthermore, albumin concentrations were overall quite low in patients with bacterial sepsis (91.1% <35 g/L; the lower reference limit), but not different between patients with severe lymphopenia and patients without lymphopenia (Figure 3e, p-value: 0.625).

Patient characteristics of COVID-19 cohort

A recent meta-analysis showed that lower lymphocyte counts upon 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 profound lymphopenia or without. We applied the

same method of stratificiation and analysis as performed in the bacterial sepsis studypopulation, but patients were pre-selected before measurements. Absolute lymphocyte counts upon hospital admission were available in 161 patients diagnosed with COVID-19 admitted to our university hospital. Among those patients, a median lymphocyte count of 0.74 x109/L (IQR 0.50-1.10 x109/L) was observerd and demographic and clinical characteristics are described in Table 3. Next, patients were selected based on plasma availability and exlcusion critera (Figure 1b). Finally, 17 COVID-19 patients could be selected from the group with the lowest lymphocyte counts (≤0.50 x109/L) defined as "severe lymphopenia" and 18 COVID-19 patients were selected from the group of the highest lymphocyte counts (≥1.10 X109/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-value: 0.023 and p-value: <0.001 respectively). Moreover, ICU admissions and the mortality rates were significantly higher in COVID-19 patients with severe lymphopenia (47% versus 6%, p:-value:

0.007 and 41% versus 0%, p-value: 0.003, respectively; Table 3).

TSH and thyroid hormones are associated with severe lymphopenia and inflammatory biomarkers in COVID-19

Abnormal TSH, T4, FT4 and T3 concentrations were present in 11.4% (4/35), 17.1% (6/35), 17.1% (6/35) and 51.4% (18/35), respectively, of the selected patients with COVID-19 (Figure 4a-d). 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 (Figure 4a-d, p-values: 0.026, <0.001, 0.001, <0.001 for TSH, T4, FT4 and T3 respectively). Importantly, plasma concentrations of albumin were again low (91.4 % <35 g/L) in all patients, but not different between groups, suggesting no substantial effects of plasma binding of the thyroid hormones (Figure 4e, p-value: 0.258).

The concentrations of inflammatory biomarkers IL-6, CRP and ferritin in patients with no lymphopenia and severe lymphopenia are presented in Figure 5. Patients with severe lymphopenia demonstrate significantly higher values of all inflammatory markers compaired to patients with normal lymphocyte counts (p-value: <0.001, 0.023 and 0.008 for IL-6, CRP and ferritin respectively).

Follow-up measurements of lymphocytes and thyroid hormones and TSH in COVID-19

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

Discussion

In the present study we investigated the relationship between lymphopenia and thyroid function abnormalities 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 thyroid hormones between severe lymphopenic and non-lymphopenic subpopulations were obsevered. In contrast, in COVID-19 patients, lower concentrations of TSH and thyroid hormones were detected in patients with profound lymphopenia. In parallel, inverse relationships of TSH and thyroid hormone concentrations with common inflammatory biomakers were observed in these patients. However, the majority of COVID-19 patients showed overall improvement of their lymphocyte counts after one week, whereas thyroid hormones 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 assocation with increased mortality 2, 27. In this study, severe lymphopenia in sepsis (represented the lowest quartile; 0-25%) was defined using a cut-off of ≤0.64 x 109/L, which is comparable to previous studies that used similar tresholds and reported comparable prevalances (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 the ”low T3-syndrome”, synonym for NTIS 18. A previous study reported that NTIS affects 60-70% of critically ill patients in ICU 28. Another study reported a similar incidence of 67% in sepsis patients 29. In our study, almost all 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 en SOFA scores (Table 1). Moreover, in this study sepsis was defined according to the Sepsis-3 definition, which is more strict and identifies more severe patients compared to the Sepsis-2 definition used by the earlier study 29. Overall, it can be stated that bacterial sepsis patients may present with severe lymphopenia and thyroid hormones imbalances at the 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 the patients with COVID-19, and lower TSH and T3 concentrations were associated with severity of the disease 20. Accordingly, another preprint study reported thyroid abnormalities in 52/84 (62%) COVID-19 patients, mostly found in severe and critical cases 30. In our study, thyroid abnormalities were particularly observerd 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 unfavourable outcome in COVID-19, and highlights the need for further investigation to establish the prognostic value of TSH and thyroid hormones in hospitalized COVID-19 patients.

The bidirectional interaction of the hypothalamic-pituitary-thyroid axis and the proinflammatory immune response is complex and still poorly understood. Pathogens can trigger a acute phase response which is accompanied by the production of cytokines, including IL-6 31. Torpy at al. showed that a single bolus infusion of IL-6 in healthy humans introduced acute changes in thyroid hormones, 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 observerd in this study, as the profound lymphopenic COVID-19 patients showed signicantly 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 thyroid hormones concentrations and function, mainly mediated by altered activity of iodothyronine deiodinases type 1 (D1) and 3 (D3) 18. The activity of liver D1, involved in the production of T3 out of T4 and in the clearance of reverse T3 (rT3), decreases during acute illness resulting in a decline of serum T3 and an increase in serum rT3 40, 41. It has been suggested that deiodinases alterations may represent a beneficial adaptation. This is supported by studies showing that changes in deiodinases 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-1β) , IL-6 and hypoxia have been proposed as possible pathofysiological drivers for the altered deiodinases activity 45-47. However, a previous study showed that infusion of interleukin-1 receptor antagonist (IL-1RA), the natural antagonist for IL-1β, could not restore NTIS induced by experimental endotoxemia in humans 48.

Severely and prolonged critically ill patients may show central suppression of the HPT axis, hallmarked by markely low T3/T4 concentrations and supression of TSH concentrations, which further impairs thyroid hormone homestasis additional to peripheral changes 18, 38. In our study, these alterations could be obseverd in severly ill sepsis patients. Interestingly, a few COVID-19 patients showed similar altered patterns, which often remained present after one 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 in patients admitted to the ICU 50. In general, several randomized controlled clinical trials have investigated thyroid hormone 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, TRH or recombinant TSH in study populations with critical illness, none of these studies have reported information regarding circulating lymphocyte counts before and/or after treatment, or after cardiac surgery53-62.

In order to reduce potentially interfering factors, we have excluded in our series the patients that were treated with glucocorticoids. The rapidly changing treatment strategies in COVID-19 currently include adding dexamethasone therapy as standard care in hospitalized COVID-19 patients63. This could also affect the hypothalamic-pituitary-thyroid axis leading to decreased TSH and T4 and T3 levels in these patients. Distinguishing the effect of dexamethasone treatment from the 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), suggested indicative for a poor prognosis64. Current guidelines recommend against the use of thyroid hormone supplementation therapies in NTIS 65. The clinical dilemma which patients need to be treated, or not, remains unsolved as causal mechanisms of the neuro-endocrine immune system crosstalk and its relation to clinical outcomes in critically ill patients are still missing.

The detailed role of thyoid hormones and their contibution in regulation of the immune system has not yet been elucidated. Previous studies in mice have reported an important role for thyroid hormones in regulating lymphocyte function 15, 16, 66-69. Klecha et al. showed that hypothyroid mice show lower T- and B-cell mitogen-induced proliferation compared with euthyroid animals, and reversion of hypothyroid state by T3 administration was able to recover the proliferative responses15. In mice subjected to chronic stress, thyroid hormone 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 authors mentioned Protein Kinase C (PKC) to be potentially involved in these processes 15, 69, whereas PKC may act as a mediator of thyroid hormones metabolism, as well as a regulator of lymphocyte proliferation 70. Recently, a study published the results of a large cohort of healthy indiviuals 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 thyroid hormones and lymphocyte homestasis, which is in line with the associations reported in this study. How alterations of thyroid hormones 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 non-lymphopenia cases was primarly designed to test our hypothesis, which have led to the exclusion of mild and moderate lymphopenic cases in both study populations and a 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 thyroid hormones observed in bacterial sepsis and COVID-19 patients. Due to the methodological constraints the results of the two populations cannot be directly compared. The pathogenesis and the high prevalence of lymfopenia in COVID-19 patients (including those with less severe disease) is not known, nonetheless 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 of the longterm effects of TSH and thyroid hormone changes in relation to the HPT-axis feedback loop cannot be provided. Another limitation is that within our bacterial sepsis cohort data was lacking on pre-existing thyroid disease and potential drug confounders that could directly interfere with the HPT axis or have influenced analytical methods of TSH and thyroid hormone 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 two processes cannot be established. Therefore, future larger studies are needed to clarify their underlying pathophysiological mechanisms, including the identification of specific host-(e.g. 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 counts and thyroid hormones changes may further improve our understanding of this complex immunometabolic crosstalk. Such studies are the key to 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 clinical outcomes in these patients.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions:

R.T.N.M., E.J.G.B, M.G.N., L.A.B., F.L.v.d.V., P.P. and M.K. conceptualized and designed the study.

R.T.N.M., E.J.G.B. and M.G.N. supervised the project. I.G, A.H.d.N., N.A., N.A.F.J., M.M., K.L., collected blood samples, and collected clinical data. I.G., A.H.d.N., N.A.F.J., M.J., A.E.v.H. processed samples and performed laboratory measurements. I.G., A.H.d.N., M.J, performed the data analysis and drafted the manuscript, I.G. took the lead in the writing process. All co-authors reviewed the manuscript and gave approval of the final version to be submitted.

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Figure legends:

Figure 1. Flowchart of study design

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 T3 and lymphocyte count (b), T4 and albumin (c) and T4 and ferritin (d) Correlation coefficients and p-values were calculated with Spearman's rank correlation tests. After correction for multiple testing (Bonferroni) we considered p-value of <0.002 statistically significant. IL-6 was only available for 30 patients. Abbreviations: IL-6, interleukin 6; CRP, C-reactive protein. *: p-value <0.002.

Figure 3. Thyroid hormones in bacterial sepsis patients with severe lymphopenia (n=56) and no lymphopenia (n=56). Concentrations of TSH (a), T4 (b), FT4 (c), T3 (d) and Albumin (e) in sepsis patients with severe lymphopenia and without lymphopenia. Absolute lymphocyte counts below 0.64 x109/L were indicated as severe lymphopenia based on the lowest quartile (P0-25). Absolute lymphocyte counts above 1.58 x109/L were indicated as no lymphopenia based on the highest quartile (P75-100). Grey area represents reference ranges used at our institution. Exact p-values are 0.070 (a), 0.513 (b), 0.549 (c), <0.001 (d), 0.625 (e). P-values were calculated with Mann-Whitney U tests. Data is presented as median with interquartile ranges. Abbreviations: ns, not significant; ***: p-value <0.001.

Figure 4. Thyroid hormones in COVID-19 patients with severe lymphopenia (n=17) and no lymphopenia (n=18). Concentrations of TSH (a), T4 (b), FT4 (c), T3 (d) and Albumin (e) in COVID-19 patients with severe lymphopenia and without lymphopenia. Absolute lymphocyte counts below 0.50 x109/L were indicated as severe lymphopenia based on the lowest quartile. Absolute lymphocyte counts above 1.10 x109/L were indicated as no lymphopenia based on the highest quartile. Exact p-values are 0.026 (a), <0.001 (b), 0,001 (c), <0.001 (d), 0.258 (e). P-values were calculated with Mann-Whitney U tests. Data is presented as median with interquartile ranges. Abbreviations: COVID-19, coronavirus disease 2019; ns, not significant; *: p-value <0.05, ***: p-value <0.001.

Figure 5. Inflammatory markers in COVID-19 patients with severe lymphopenia (n=17) and no lymphopenia (n=18). Concentrations of IL-6 (a), CRP (b), Ferritin (c) in COVID-19 patients with severe lymphopenia and without lymphopenia. Absolute lymphocyte counts below 0,50 x109/L were indicated as severe lymphopenia based on the lowest quartile. Absolute lymphocyte counts above 1.10 x109/L were indicated as no lymphopenia based on the highest quartile. Exact p-values are <0.001 (Aa), 0.023 (b), 0.008 (c). P-values were calculated with Mann-Whitney U tests. Data is presented as median with interquartile ranges. Abbreviations: COVID-19, coronavirus disease 2019; IL-6, interleukin 6; CRP, C-reactive protein; ns, not significant; *: p-value <0.05, **: p-value <0.01.

Figure 6. Individual values of follow-up of lymphocytes and thyroid hormones in the disease course of COVID-19 (n=15)

Individual lymphocyte counts (a) and individual concentrations of TSH (b), T4 (c), FT4 (d), T3 (e) and Albumin (f) in COVID-19 patients in the first week of admission (Baseline) and 1 week after (Follow-up). Exact P-values are <0.001 (a), 0.639 (b), 0.534 (c), 0.808 (d), 0.720 (e), 0.050 (f). P-values were calculated with Wilcoxon signed rank test. Data is presented as the median with interquartile ranges. Abbreviations: COVID-19, coronavirus disease 2019; ns, not significant; ***: p-value <0.001.

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 the right upper quadrant • Fever (tympanic or oral temperature ≥38^{0}C, rectal ≥38.3^{0}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 pO2≤60mmHg or oxygen saturation ≤90% in room air • Respiratory rate ≥20 breaths/min
HAP (or HCAP)*[24]	• Onset >48 hours 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 pO2≤60mmHg or oxygen saturation ≤90% in room air • Respiratory rate ≥20 breaths/min
VAP[24,25]	• Onset >48 hours 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 • Clinical pulmonary infection score (CPIS)[25] ≥6

* Healthcare-associated pneumonia (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.

Abbreviations: AC, acute cholangitis; BSI, Blood stream infection; CT, computer tomography; CAP, community-acquired pneumonia; CPIS, the clinical pulmonary infection score; HAP, hospital-acquired pneumonia; HCAP, healthcare-associated pneumonia; PCT, procalcitonin; VAP, ventilator-associated pneumonia

Table 2. Baseline characteristics bacterial sepsis cohort

	Total (n=224)	Severe Lymphopenia (n=56)	No lymphopenia (n=56)	P-value (severe vs. no lymphopenia)
Age (years)	76 (65-83)	75 (65-83)	76 (67-84)	0.347
Gender (n,%)				
Male	129 (58)	30 (54)	30 (54)	1.000
Female	95 (42)	26 (46)	26 (46)	
Body Mass Index (kg/m^2)*	26.3 (22.5-30.9)	24.2 (22.0-29.4)	28.1 (22.8-31.5)	0.045
Comorbidities (n,%)				
Diabetes mellitus	67 (30)	16 (24)	18 (27)	0.681
Heart failure	61 (27)	11 (20)	13 (23)	0.645
Coronary heart disease	51 (23)	9 (16)	11 (20)	0.622
Chronic renal disease	24 (11)	4 (7)	3 (5)	0.696
Chronic obstructive pulmonary	53 (24)	12 (21)	9 (16)	0.468
disease	12 (5)	4 (7)	3 (5)	0.401
History of hematological/solid				
malignancies				
Chronic intake of corticosteroids/biologicals (n,%)	10 (5)	4 (7)	0 (0)	0.042
Source of infection (n,%)				
Acute cholangitis	13 (6)	3 (5)	4 (7)	0.696
Primary bacteremia	38 (17)	14 (25)	10 (18)	0.357
Community-acquired pneumonia	97 (43)	25 (45)	21 (38)	0.442
Healthcare-associated pneumonia	51 (23)	12 (21)	11 (20)	0.815
Ventilator-associated pneumonia	24 (11)	4 (7)	9 (16)	0.140
Septic shock (n,%)	168 (75)	42 (19)	42 (19)	1.000
APACHE II#	24 (17-31)	24 (17-35)	28 (19-33)	0.547

SOFA score	11 (9-14)	12 (9-15)	11 (8-14)	0.163
CRP (mg/L)	70 (21-159)	100 (19-204)	39 (19-88)	0.051
Ferritin (µg/L)	1370 (435-3326)	1407 (423-2878)	1117 (488-2460)	0.805
IL-6 (pg/mL)	119 (26-217)	143 (52-314)	41 (18-109)	0.109
Thyroid hormones				
TSH (mU/L)	0.67 (0.24-1.94)	0.43 (0.19-1.28)	0.85 (0.28-0.85)	0.070
T3 (nmol/L)	0.69 (0.55-0.88)	0.63 (0.53-0.79)	0.78 (0.64-1.00)	0.001
T4 (nmol/L)	57.6 (40.0-81.1)	56.2 (35.1-74.5)	59.7 (34.4-92.3)	0.513
FT4 (pmol/L)	12.9 (9.3-16.0)	12.5 (9.4-15.7)	13.8 (7.9-17.4)	0.549
Albumin (g/L)	22.5 (17.1-28.5)	21.4 (17.9-28.6)	20.7 (16.0-29.1)	0.625
Mortality 28 days (n,%)	136 (61)	32 (57)	32 (57)	1.000

Data are presented as median (IQR) or n (%). Absolute lymphocyte counts equal or below 0.64 $x10^9$/L were indicated as severe lymphopenia based on the lowest quartile (P0-25). Absolute lymphocyte counts equal or above 1.58 $x10^9$/L were indicated as no lymphopenia based on the highest quartile (P75-100). Abbreviations: APACHE, The Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; COVID-19, coronavirus diseases 2019; CRP, C-reactive protein; IL-6, interleukin 6. * 55 missing; # 18 missing values

Table 3. Clinical characteristics COVID-19 population and lymphocyte subgroups

	Total (n=161)	Severe lymphopenia (n=17)	No lymphopenia (n=18)	P-value (severe vs. no lymphopenia)
Age (years)	65 (54-73)	69 (60-77)	62 (43-73)	0.096
Gender				
Male (n,%)	105 (65)	12 (71)	12 (67)	1.000
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)	0.463
Comorbidities (n,%)				
Diabetes mellitus	35 (22)	3 (18)	1 (6)	0.338
Congestive heart failure	11 (7)	2 (12)	0 (0)	0.229
Coronary heart disease	17 (11)	1 (6)	1 (6)	1.000
Chronic renal disease	10 (6)	1 (6)	0 (0)	0.486
Chronic obstructive pulmonary disease	41 (25)	3 (18)	8 (44)	0.146
History of hematological/solid malignancies	40 (25)	2 (12)	6 (33)	0.088
Ward of admission at first sampling				
Hospital ward (n,%)	120 (75)	9 (53)	17 (94)	0.007
ICU (n,%)	41 (25)	8 (47)	1 (6)	
Time between hospital admission and plasma sampling (days)	NA	3 (2-5)	3 (2-5)	0.807
CRP (mg/L)	86 (46-151)*	92 (62-233)	53 (31-120)	0.023
Ferritin (µg/L)	800 (383-1490)*	1461 (916-2897)	705 (382-1174)	0.008
IL-6 (pg/mL)	NA	110 (75-185)	22 (16-69)	<0.001
Total white blood cell counts	6.9 (4.6-9.2)*	5.4 (3.7-7.0)	7.9 (5.6-10.3)	0.017
White blood cell differentiation				
Absolute neutrophil counts	5.60 (3.36-7.56)*	4.31 (3.24-5.86)	4.63 (3.39-7.06)	0.335

Absolute lymphocyte counts	0.74 (0.50-1.10)*	0.38 (0.33-0.45)	1.49 (1.23-1.76)	<0.001
Absolute monocyte counts	0.40 (0.25-0.67)*	0.39 (0.16-0.63)	0.73 (0.44-1.11)	0.011
Absolute eosinophil counts	0.00 (0.00-0.00)*	0.00 (0.00-0.03)	0.06 (0.00-0.19)	0.019
Absolute basophil counts	0.00 (0.00-0.01)*	0.00 (0.00-0.01)	0.02 (0.00-0.03)	0.027
Thyroid hormones				
TSH (mU/L)	NA	0.87 (0.43-1.32)	1.41 (0.85-2.47)	0.026
T3 (nmol/L)	NA	1.09 (0.89-1.22)	1.69 (1.31-1.88)	<0.001
T4 (nmol/L)	NA	80.5 (64.9-95.6)	111 (103-137)	<0.001
FT4 (pmol/L)	NA	13.9 (11.3-14.7)	17.1 (15.1-19.3)	0.001
Albumin (g/L)	NA	28.1 (19.9-31.8)	29.6 (28.7-31.2)	0.258
Outcome				
Mortality (n,%)	20 (12)	7 (41)	0 (0)	0.003
Duration of hospital stay, days	9 (5-21)	14 (8-28)	8 (6-11)	0.005

Data are presented as median (IQR) or n (%). Selected patient with absolute lymphocyte counts equal or below 0.50 $x10^9$/L were classified as “severe lymphopenia” and patients selected with absolute lymphocyte counts equal or above 1.10 $x10^9$/L were classified as “no lymphopenia”. *Values upon admission. Abbreviations: COVID-19, coronavirus diseases 2019; ICU, Intensive care unit; CRP, C-reactive protein; IL-6, interleukin 6.

Figure 1

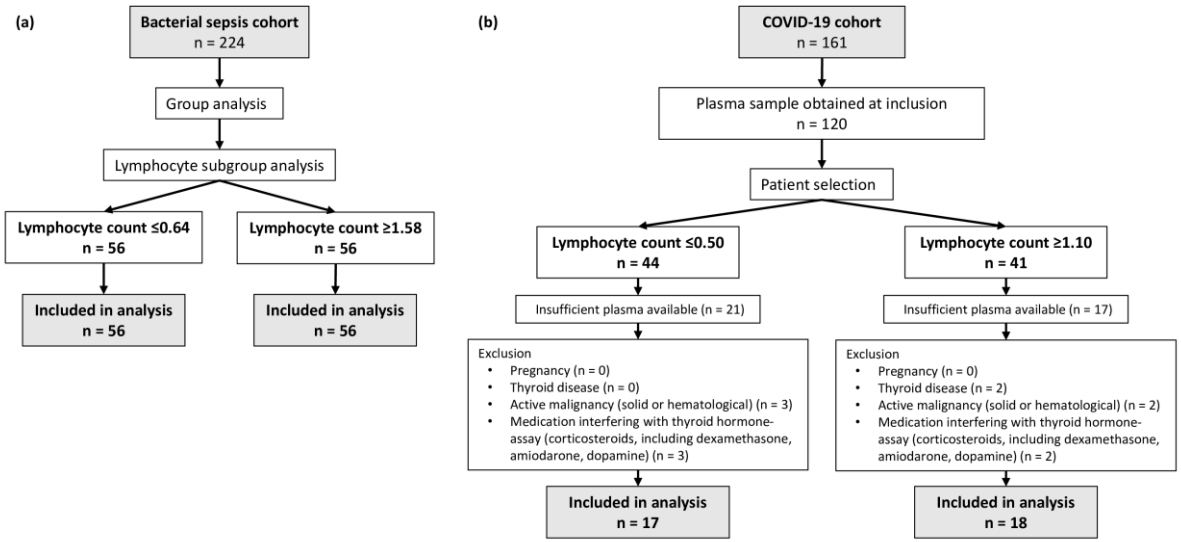

Figure 2

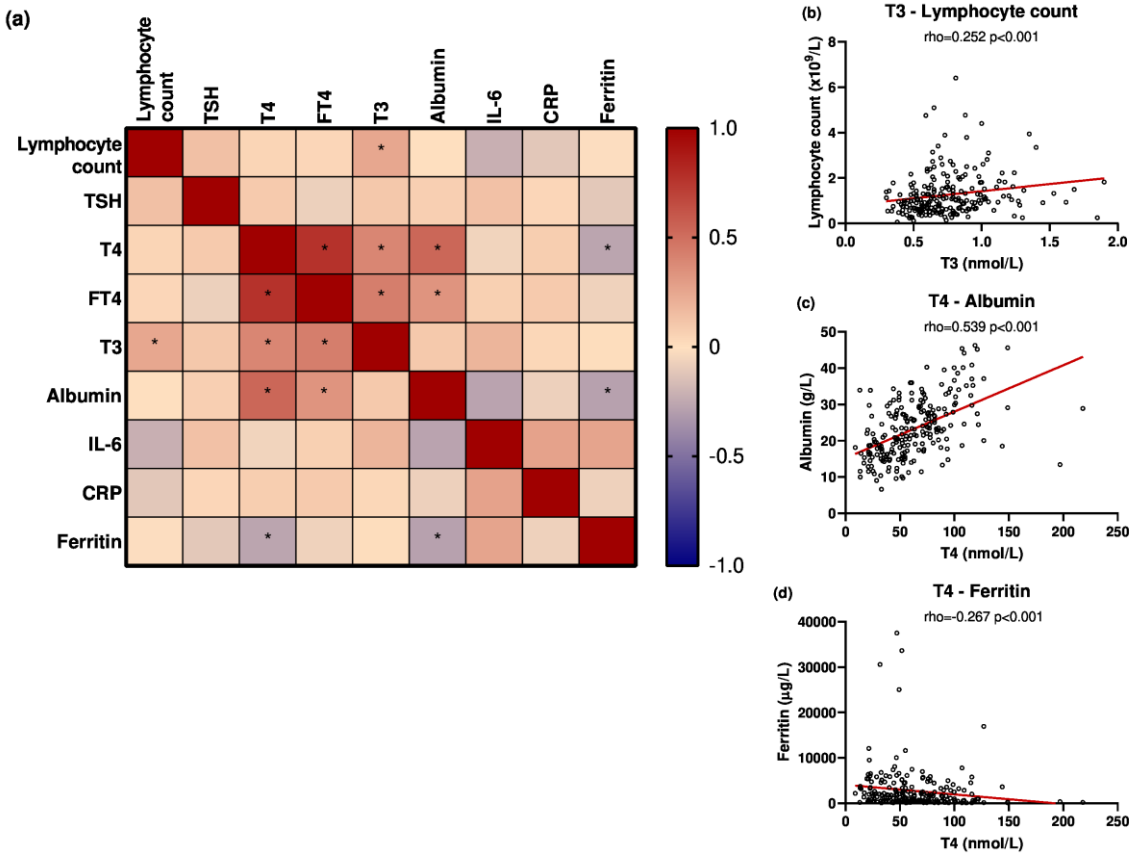
(a)
Lymphocyte count
TSH
T4
FT4
T3
Albumin
IL-6
CRP
Ferritin
1.0
0.5
0
-0.5
-1.0
(b)
T3 - Lymphocyte count
rho=0.252 p<0.001
Lymphocyte count (x10^9/L)
T3 (nmol/L)
(c)
T4 - Albumin
rho=0.539 p<0.001
Albumin (g/L)
T4 (nmol/L)
(d)
T4 - Ferritin
rho=-0.267 p<0.001
Ferritin (µg/L)
T4 (nmol/L)

Figure 3

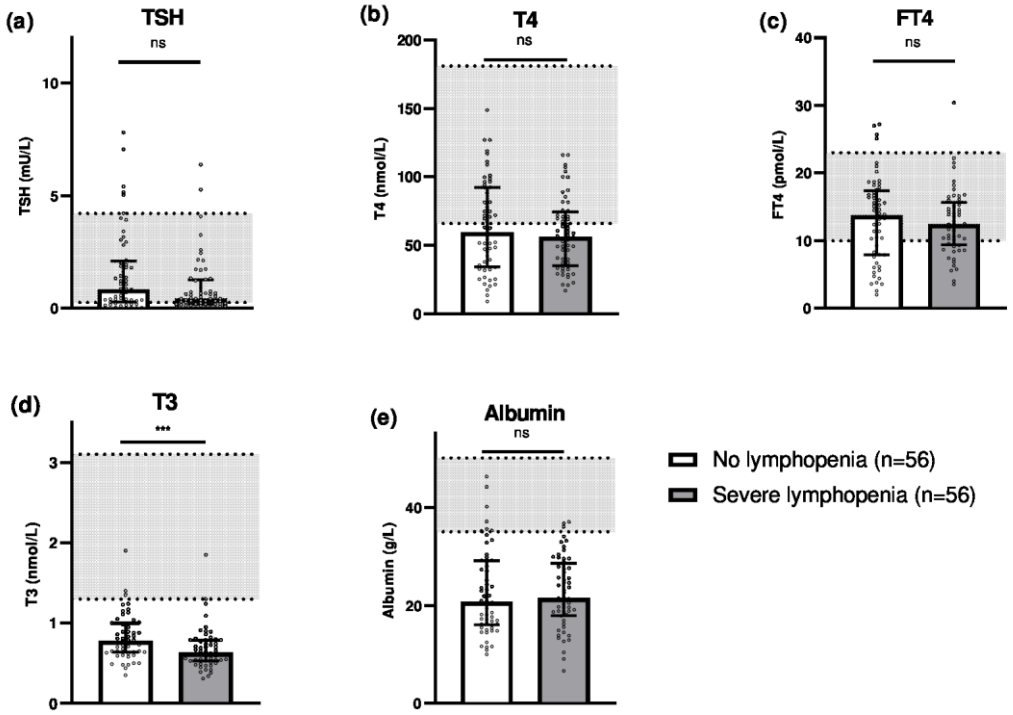

Figure 4

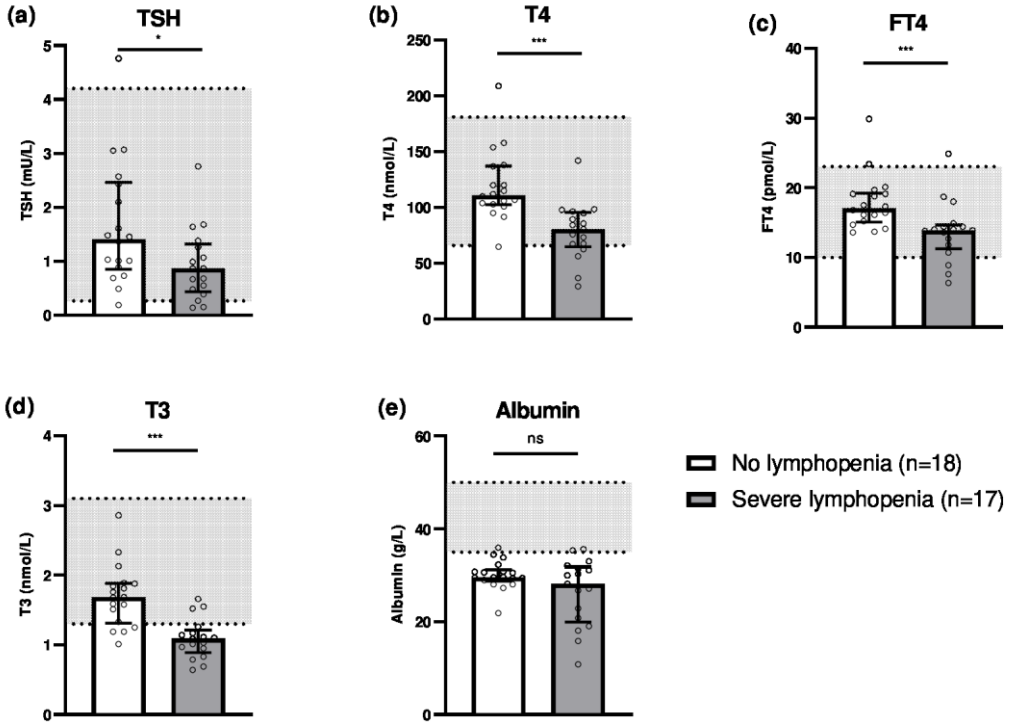

Figure 5

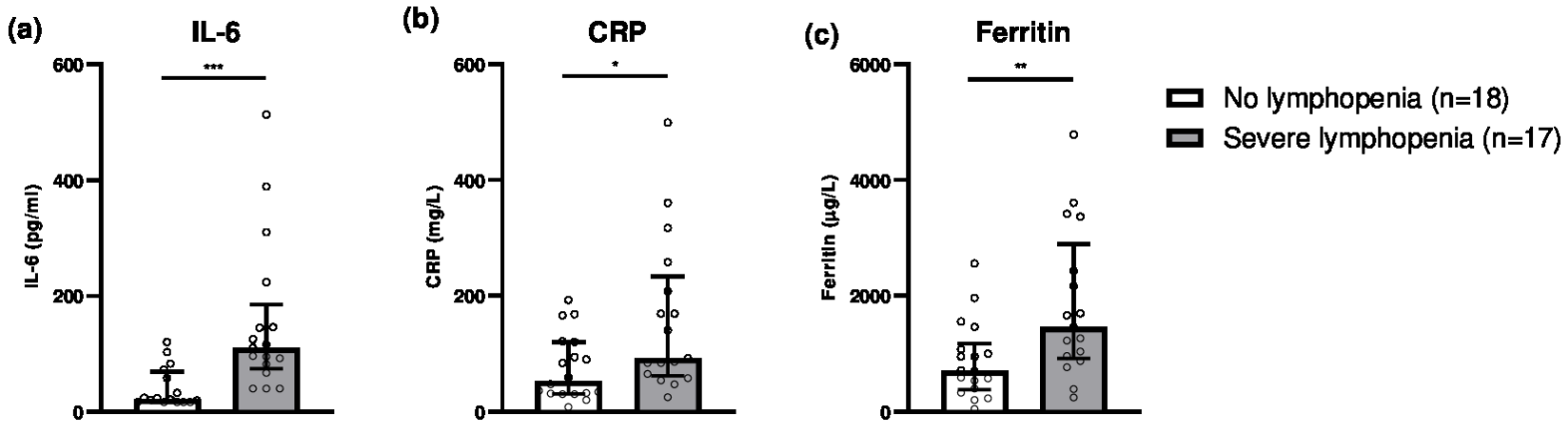

Figure 6