*Article*

# Interrelation Midst Low T3 Levels, Type 3 Deiodinase, Oxidative Stress And Mortality In Sepsis and Septic Shock: Defining Patient Outcome

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**Abstract:** Background: Low T3 syndrome occurs frequently in patients with sepsis. Type 3 deiodinase (Dio3) is present in immune cells but there is no description of its presence in this patients. Here we aimed to determine the prognostic impact of thyroid hormones levels (TH) measured on ICU admission on mortality and on evolution to chronic critical illness (CCI) and the presence of D3 in white cells. Methods: Prospective cohort study with a follow-up for 28 days or deceased. Results: Low T3 levels at admission were present in 86.5% of the patients. Dio3 was 55% induced in immune cells. The cut-off value of 60 pg/mL for T3 displayed a sensitivity and specificity of 81% and 64% for predicting death, odds ratio of 4.89. Lower T3 yielded an area under the receiver operating characteristic curve of 0.76 and 0.75 for mortality and evolution to CCI, respectively, thus displaying better performance than commonly used prognostic scores. Conclusions: We advance in the pathophysiology of low T3 showing induced D3 in immune cells during sepsis. Low T3 levels independently predicted a progression to CCI and mortality within 28 days in sepsis and septic shock.

**Keywords:** low T3 syndrome; oxidative stress; sepsis; septic shock; thyroid hormone; type 3 deiodinase

## 1. Introduction

Non-thyroidal illness syndrome (NTIS) refers to characteristic changes in thyroid hormone (TH) levels in response to systemic illnesses. Typical changes include low plasma concentrations of triiodothyronine (T3), low or normal thyroxine (T4), and elevated reverse T3 (rT3) (1, 2). Low serum T3 levels are associated with increased mortality (3-8), prolonged weaning from mechanical ventilation (9, 10), and reduced cardiac output (11); however, some studies have failed to demonstrate any prognostic implications (12-14).

Sepsis is one of the most common indications for intensive care unit (ICU) admission, and a major cause of morbidity and mortality worldwide (15). A considerable number of patients still endure prolonged complicated ICU stays, thus becoming chronically critically ill. The term "Chronic Critical Illness" (CCI) describes patients with ICU course lasting >7 days, requiring complex medical care, and becoming dependent of one or more life support techniques, causing functional dependence and poor long-term survival (16, 17). High levels of IL-6 is one of hallmarks of sepsis and augments the production of reactive oxygen species (ROS), compromising deiodinase function and altering the peripheral T3/T4 activation/inactivation process (18). The use of n-acetylcysteine (NAC), an antioxidant that replenishes glutathione (GSH)/thiols, conteracts the effects of IL-6 on deiodinase mediated T4 to T3 conversion, thereby indicating that IL-6 inhibits the function of deiodinases by increasing cellular ROS, which subsequently reduces the levels of GSH or a GSH-like endogenous cofactor (19). Among critically ill patients, those with sepsis present

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with pronounced increases in IL6 production, when compared with critically ill patients with other diagnoses of similar severity (20). Despite measuring the deiodinase function in deceased patients, the measurement in living is limited since it requires biopsy samples.

We have seen that despite the initial site of disease, oxidative stress occurs in multiple tissues and results in the systemic induction of D3, suggesting that normal circulating thyroid hormone levels are dependent on the maintenance of a reduced cellular redox environment throughout the whole body (21). Also, TH levels may predict mortality in hospitalized patients, in different clinical settings (22-25). Moreover, it is also known that D3 is present in neutrophils (26), and also in mice, on macrofages of injured muscle (27). Its role in humans under sepsis or septic shock has not been addressed yet. In this novel study, we aimed to measure D3 expression in blood obtained from patients with sepsis and septic shock and associate with the impact of low T3 on mortality and evolution to CCI. Therefore, we determined the relationship between T3 levels during sepsis stages and the levels of deiodinase expression in vivo.

## 2. Results

### *Study Population and TH Levels at ICU Admission*

Of the 260 patients, 66.1% presented with septic shock (Table 1). Pneumonia was the most common cause of sepsis (47%). The mean age was 58.3±14 years, and 56% of the patients were men. There were significant differences in the severity scores between survivors and non-survivors. Low T3 on admission was present in 225 (86.5%) patients. Of these, 100 patients (38.4%) manifested both T3 and T4 levels below the reference range. The T3 mean values were below the normal range in all patients, with a statistical difference between those with sepsis and septic shock ($p<0.001$). The TSH, T4, and FT4 mean values were within the normal range in all groups. The mortality was 14.8% and 47.1% in patients with sepsis and septic shock, respectively.

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**Table 1.** Baseline characteristics and blood concentration of biomarkers in all patients and a comparison by the diagnosis at admission to the ICU and outcomes.

| | All patients (n=260) | Sepsis (n=88) | | p-value | Septic Shock (n=172) | | p-value |
|---|---|---|---|---|---|---|---|
| | | Survivors (n=75) | Non -Survivors (n=13) | | Survivors (n=91) | Non -Survivors (n=81) | |
| Demographic characteristics | | | | | | | |
| Age, years | 58.3±14.7 | 56.8 ±14.2 | 56.9 ± 21.3 | 0.99 | 56 ±15.1 | 63.4 ± 11.8 | 0.001 |
| Male sex, % | 55.4 | 69.2 | 57.3 | 0.31 | 51.6 | 55.6 | 0.36 |
| Site of infection, % | | | | | | | |
| Pulmonary | 41.1 | 43.2 | 38.6 | 0.01 | 37.8 | 43.2 | 0.5 |
| Abdominal | 26.7 | 17.6 | 46.2 | | 28.9 | 29.6 | |
| Urinary tract | 10.9 | 13.5 | 0 | | 12.2 | 8.6 | |
| Others | 21.3 | 25.7 | 15.4 | | 21.1 | 18.5 | |
| Healthcare-associated infection (%) | 42.7 | 40.0 | 76.9 | 0.01 | 42.9 | 39.5 | 0.38 |
| Predictive scoring systems on admission | | | | | | | |
| SAPS | 71.8±17.7 | 62.7 ±14.2 | 68.1 ±14.2 | 0.22 | 73 ±16.8 | 81.8±18.8 | 0.003 |
| SOFA | 7.8±3.4 | 5.0 ±2.6 | 6.0±3.2 | 0.24 | 9.0±2.6 | 10.0±3.1 | 0.015 |
| CHARLSON | 4.5±2.2 | 3.9±2.3 | 5.1±1.9 | 0.11 | 4.3±2.2 | 5.6 ±1.9 | <0.001 |
| Routine laboratory findings | | | | | | | |
| Lactate (<2.2 mol/L) | 2.9±2.4 | 1.8±1.3 | 2.1±1.6 | 0.50 | 2.9±2.1 | 4.8±3.4 | <0.001 |
| C-reactive protein (mg/L) | 201.8±121 | 183.1±133 | 216.9±120 | 0.41 | 195±109 | 224±126 | 0.12 |
| White blood count (x10) | 13.6 (8-18.6) | 16.3 (11.5-19.7) | 13.1 (7.9-16.7) | 0.07 | 15.2 (8.9-23.1) | 11.4 (4.7-17.7) | 0.8 |
| Thyroid hormones | | | | | | | |
| NTIS (%) | 86.5 | 70.7 | 100 | 0.01 | 89.8 | 98.9 | 0.02 |
| TSH (0.27-4.2 UI/mL) | 2.26±2.34 | 2.1±2.1 | 2.3±1.8 | 0.74 | 2.4±2.4 | 2.1±2.4 | 0.44 |
| T4 (4.6-12 ng/dL) | 5.4±2.1 | 6.5±2.1 | 4.6±0.8 | 0.001 | 5.3±1.9 | 4.6±2.1 | 0.018 |
| fT4 (0.93-1.7 ng/dL) | 1.05±0.35 | 1.17±0.33 | 1.01±0.36 | 0.10 | 1.05±0.3 | 0.95±0.4 | 0.06 |
| T3 (75-200 ng/dL) | 56.2±16.9 | 68.1±17.3 | 49.7±8.8 | <0.001 | 57.2±16.4 | 46.6±12.5 | <0.001 |
| Outcomes | | | | | | | |
| LOS, days | 4 (3-10) | 4 (2-6) | 5 (3-12) | 0.98 | 6 (3-14) | 4 (2-9) | 0.002 |

Abbreviations: fT4: free T4; ICU: intensive care unit; LOS: length of stay; NTIS: non-thyroidal illness syndrome; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment score; T3: triiodothyronine; T4: thyroxine; TSH: thyrotropin. Categorical data are presented as frequencies and were analyzed using the $\chi 2$ test or Fisher's exact test. Quantitative data with normal distribution are presented as mean ± SD, and were analyzed using the Student's t test. Non-parametric variables are presented as median ± interquartile range and were analyzed using the Mann-Whitney's U test. P>0.05 is considered statistically significant.Correlation Between T3 and Oxidative Parameters.

The T3 levels were strongly correlated with sulfhydryl amount or the total circulating thiol groups (Figure 1). Levels of sulfhydryl are lower in septic shock patients, mirrowing the lowest levels of T3 ($R^2$=0.72; P<0.0001, Figure 1B).

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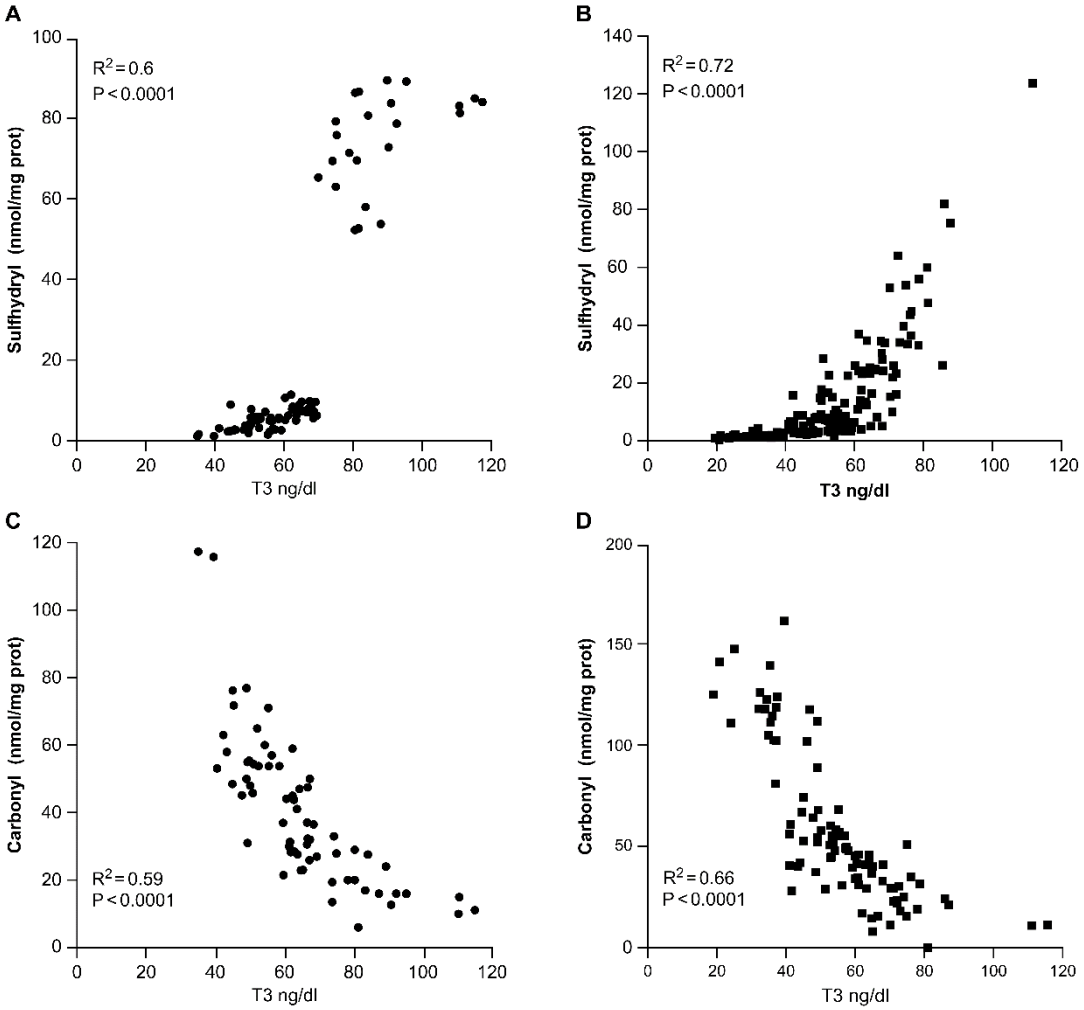


**Figure 1.** Sulfhydryl levels are correlated with serum T3 in patients with sepsis (A) and septic shock (B). Carbonyl levels are inversely correlated with T3 levels in patients with sepsis (C) and septic shock (D). No outliers have been omitted within this analysis. P-levels are denoted in the graphs. T3, triiodothyronine.

Interestingly, we observed an inverse correlation among the carbonyl levels (oxidized proteins) with T3 levels in septic patients ($R^2$=0.59, P<0.0001; Figure 1C) as well as in septic shock ($R^2$=0.66, P<0.0001; Figure 1D). These results thus confirm the strong dependence of circulating reduced groups that maintains normal levels of GSH and T3 levels maintenance in disease.

*Type 3 Deiodinase Is Substantially Induced in the immune Cells of Patients with Sepsis and Septic Shock and correlates with mortality*

Type 3 deiodinase expression was barely observed in the immune cells of the healthy control group. However, we detected a 55% difference of Dio3 expression in patients with sepsis (P=0.0002, Figure 3A). Of the patients with septic shock, Dio3 expression was not only higher then in sepsis but also 34% higher in the deceased ones (P=0.005, Figure 3B). Interestingly, when comparing survivors in both groups, we did not observed any increment in the D3 expression. However, we observed a 16% significant increment in the deceased. Taken together, D3 was substantially expressed in the immune cells, and could at least partially facilitate explaining the difference in the circulating T3 levels between both groups.

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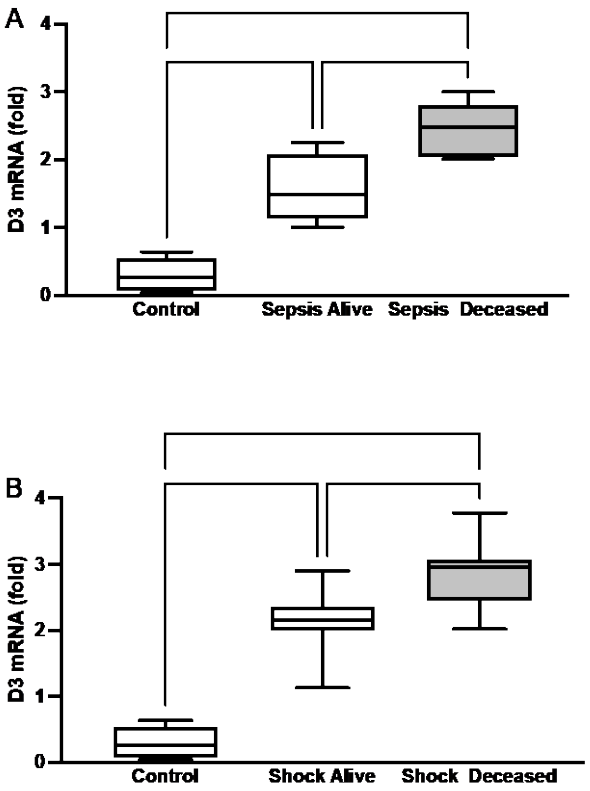


**Figure 2.** Dio3 expression in patients with sepsis (Figures A and B) and septic shock (Figures C and D). (A and B) mRNA levels of D3 measured by the Mean–SEM, *p<0.001 by the unpaired Student's t-test. (C and D) D3, Type 3 deiodinase.

*T3 Levels at ICU Admission were an Independent Predictor of 28-Day Mortality and Evolution to CCI*

Patients who presented with NTIS at ICU admission revealed higher mortality than those with normal TH levels (41.3% vs 2.9%, p<0.001). Patients with low T3 and low T4 levels had worse outcomes, compared with those with only low T3 levels (mortality of 55% vs 30%, p<0.001). There are significant differences between survivors and non-survivors in the univariate analysis for the following variables: age, serum lactate levels, c-reactive protein levels, T3, T4 and FT4 levels, SAPS III, SOFA score, and Charlson comorbidity index. Lower levels of T3 yielded an independent AUC of 0.76 (Figure 3A). The AUC for SAPS III and Charlson comorbidity index was 0.68 (95% CI, 0.60–0.76) and 0.67 (95% CI, 0.59–0.74), respectively. The cut-off value of 60 pg/mL for T3 levels displayed a sensitivity and specificity of 81% and 64% for predicting death, respectively, with an OR of 4.89 (95% CI, 2.48–9.66).

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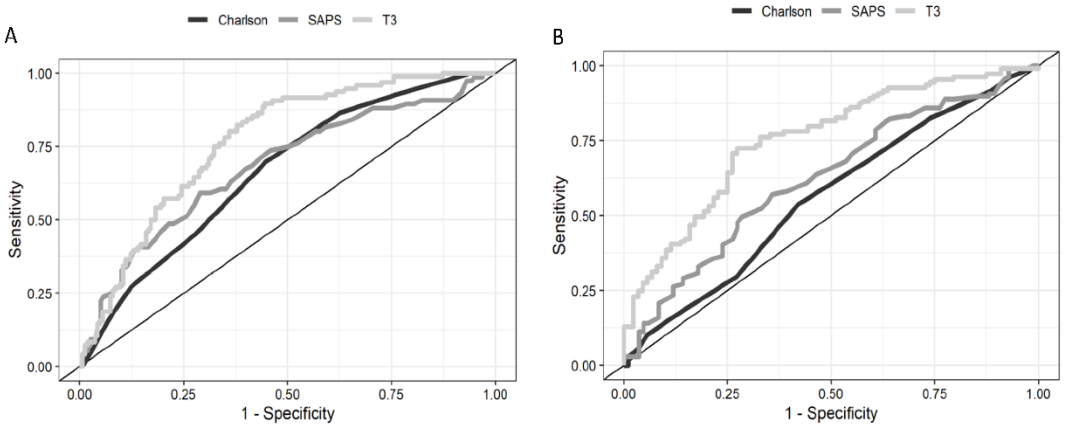


***Figure 3.*** Receiver-operator characteristic curves of T3 to predict the 28-day mortality (A). The AUC for lower levels of T3 is 0.76 (95% CI, 0.70–0.82). (B) ROC curves of T3 to predict the evolution to chronic critical illness. The AUC for lower levels of T3 is 0.75 (95% CI, 0.68–0.82). T3, triiodothyronine; ROC, receiver operating characteristic; and AUC, area under the ROC curve.

The evolution to CCI was more frequent in patients with NTIS than in those with normal TH levels (62% vs 23.5%, p<0.001). Only SAPS III and T3 levels independently predicted the evolution to CCI (Table 2). Lower levels of T3 yielded an AUC of 0.75 (Figure 3B). The cut-off value of 60 pg/mL displayed a sensitivity and specificity of 72.5% and 72% for predicting the evolution to CCI, respectively, with an OR of 2.20 (95% CI, 1.61–3.01).

**Table 2.** Predictors of death within 28 days as identified by the univariate analysis and multivariate logistic regression.

| Univariate analysis | | | | | |
|---|---|---|---|---|---|
| | Survivors (n=166) | | Non -Survivors (n=94) | | p-value |
| Age, years | 56.3 ±14.6 | | 62.5 ± 13.5 | | 0.001 |
| SAPS | 68.3 ±16.5 | | 79.3 ±18.7 | | <0.001 |
| SOFA | 7.2±3.3 | | 9.4±3.3 | | <0.001 |
| CHARLSON | 4.1±2.2 | | 5.4 ±1.9 | | <0.001 |
| Lactate (<2.2) | 2.4±1.9 | | 4.4±3.3 | | <0.001 |
| C-reactive protein | 190±120 | | 223±125 | | 0.04 |
| T4 (4.6-12 ng/dL) | 5.86±2.1 | | 4.55±2.01 | | <0.001 |
| FT4 (0.93-1.7 ng/dL) | 1.12±0.34 | | 0.96±0.37 | | 0.001 |
| T3 (75-200 ng/dL) | 62.09±17.64 | | 47.05±12.1 | | <0.001 |
| Multivariate analysis | | | | | |
| | Estimate | SE | Odds ratio | 95% CI | p-value |
| Intercept | -.849 | .4903 | .428 | .164 – 1.118 | .083 |
| T3 | -.038 | .0051 | .962 | .953 -.972 | .0001 |
| T3 <60 ng/dL | 1.58 | .34 | 4.89 | 2.48-9.66 | .0001 |
| SAPS III | .012 | .0052 | 1.012 | 1.002-1.023 | .017 |
| Charlson | .149 | .0411 | 1.161 | 1.071-1.256 | .0001 |

Abbreviations: FT4: free T4; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment score; T3: triiodothyronine; T4: thyroxine.

## 3. Discussion

This prospective translational study explored the independent association between low T3 levels, highly expressed D3 in immune cells and adverse outcomes in patients with

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sepsis and septic shock admitted to ICU. We observed high expression on D3 in the immune blood cells, which was related to the disease severity and outcomes. Patients with low T3 levels at ICU admission were at a higher risk of progression to CCI and mortality within 28 days. The T3 levels alone were independently associated with worse outcome, without covariance with other commonly used severity scores. T3 levels <60 ng/dL displayed the best sensitivity and specificity to predict mortality and the evolution to CCI.

The prevalence of low T3 syndrome in ICU settings varies substantially, ranging from 16% to 80% (6, 9, 30, 31, 35, 36). The prevalence of low T3 levels in 86.5% of the patients was partially explained by the cohort being exclusively composed of patients with sepsis. Sepsis is characterized by the simultaneous release of proinflammatory and anti-inflammatory mediators, with the intensity of responses depending on multiple factors of the host as well as the pathogen. Proinflammatory cytokines cause the activation and proliferation of leukocytes and elicit a complete immune response (37).

The interaction between the complex network of immunity and TH function plays a role in lowering T3 levels in sepsis, despite the difficulty to create a simplistic model (38-40). One of the possible paths involves derangements on deiodinase expression on the immune cells caused by oxidative stress. This has not been studied yet. In patients with sepsis, sulfhydryl levels paralleled to those of T3 in both groups, thereby suggesting the consumption of thiol products and the oxidation of protein-bound sulfhydryl groups altered thyroid hormone metabolism in these patients. Moreover, we observed an inverse correlation between protein oxidation (carbonyl levels) and T3 levels, which reinforced the role of oxidative stress in the system. The high levels of protein oxidation directly affect the thiol regenerating system, by modulating GSH and NADPH levels, in such patients (20). Herein, we elucidated the induction of D3 in immune cells, further decreasing the plasma T3 level and increasing the inactivation of T4 to rT3. Recently, we reported on a correlation between sulfhydryl and GSH levels with augmented D3 activity in several tissues under NTIS (21). The present study shows induced D3 on white cells, paralleled with augmented oxidative stress and diminished thiol levels. The sulphydryl correlating with Dio3 expression in blood favors the putative ideia that gluthatione regulates, or is one of the regulative agents of Dio3 expression. These findings add to the mechanism by which the administration of the antioxidant NAC, which sustains the thiol state of the cysteine protein residues, restores the redox equilibrium and prevents the derangement in TH concentrations in humans (41).

Some limitations of the study were as follows: a) the outcome data were limited to in-hospital events; b) we did not measure rT3 levels prevented the complete characterization of NTIS; and c) we did not measured Dio3 expression in separated neuthophils or macrophages, but on the whole pool, which could have underestimated Dio3 expression in one of these lineages.

Our findings have clinical and mechanistic relevance since low T3 concentrations were associated with poor outcomes at ICU admission, thereby independently predicting the evolution to CCI and death. Moreover, they added to the current knowledge that substantially induced Dio3 levels in defense cells play an important role in lowering T3 levels. Patients with lower T3 levels displayed a significantly higher risk of persistent organ dysfunction and mortality. Our observations unified the hypothesis of low T3 plus high Dio3 expression in immune cells as a major step toward unraveling this long-standing enigma, thus helping us identify a previously unrecognized combinatorial pathway. These results represent an integrative measure of multiple harmful pathological processes in patients with critical illness, such as inflammation and energetic imbalance, which are associated with adverse outcomes. This warrants investigating if antioxidants, such as NAC, could be beneficial as an adjuvant therapy with other therapeutic measures in critically ill patients.

## 4. Materials and Methods

*Patients*

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This prospective cohort study performed between October 2017 and April 2019 recruited 576 consecutive adults admitted to the ICU of a tertiary hospital in southern Brazil. Patients diagnosed with sepsis or septic shock within 24 h of evolution were eligible for the study. Sepsis was defined as organ dysfunction (an increase in the SOFA score by ≥2 points) caused by a dysregulated host response to infection. Septic shock was defined as a subset of sepsis characterized by profound circulatory, cellular, and metabolic abnormalities, clinically identified by vasopressor requirement to maintain a mean arterial pressure ≥65 mmHg and serum lactate level >2 mmol/L (>18 mg/dL) in the absence of hypovolemia (28). Low T3 referred to levels under the normal range, concomitant with normal/low-normal TSH serum levels (29). The exclusion criteria were as follows: 1) age <18 years or >80 years; 2) a history of primary thyroid disease; 3) pregnancy or immediate postpartum period; 4) imminent death or the directive of exclusive palliative care. Based on the prevalence of NTIS and local mortality for patients with septic shock (30, 31), we calculated the sample size to provide a statistical power of 90% for determining an absolute difference of 20% in the mortality between the two groups (assuming a two sided level.05) using Epitools Epidemiological Calculators (32). Accordingly, 260 patients were enrolled (Figure 1).

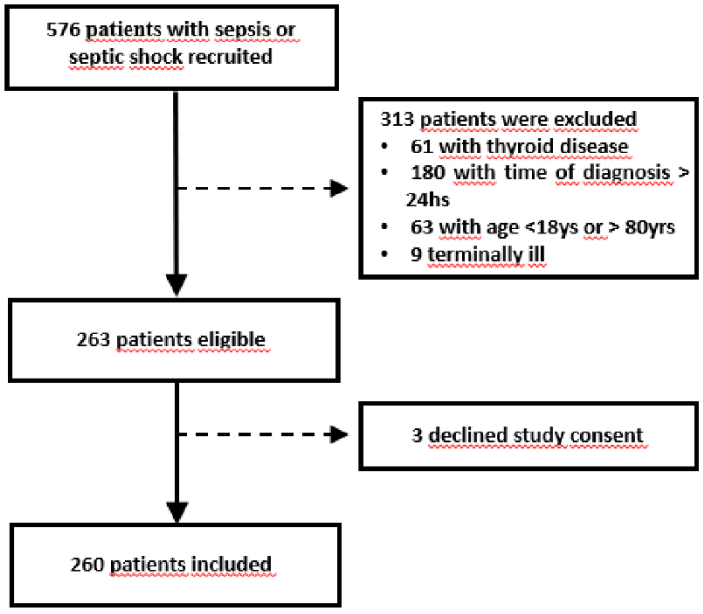


**Figure 1.** Flowchart showing patient selection.

*Blood Measurements*

Venous blood samples were obtained within the first 24 h. We collected data related to the severity score systems, namely the Charlson comorbidity index, SAPS III, and SOFA, on the first day following ICU admission. THs were measured by electrochemiluminescent immunoassay (ADVIA Centaur XP; Siemens, Munich, Germany). Its normal ranges were as follows: free T4 (FT4), 0.93–1.7 ng/dL; T4, 4.6–12 ng/dL; T3, 75–200 ng/dL; and TSH 0.27–4.2 mU/L. Sulfhydryl content was measured based on the reduction of 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) by thiols and measured at 412 nm. Carbonyls were measured from 10 mM 2.4-dinitrophenylhydrazine. The carbonyl content was determined at 370 nm and calculated using the millimolar absorption coefficient of hydrazine (e370 nm=21,000000 M-1 cm-1).

*Real-time* quantitative polymerase chain reaction (*PCR)*

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Reverse transcription of 250 ng of RNA into cDNA done using the SuperScript VILO Master Mix IV (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturers protocol. cDNA was then amplified by quantitative real-time PCR (qPCR). qPCR experiments were performed by monitoring in real-time the increase in fluorescence of the SYBR® Green dye (33). Primers for *Dio3 are* 5′-TCCAGAGCCAGCACATCCT-3′ and 5′-ACGTCGCGCTGGTACTTAGTG-3′ The reference genes beta-actin (*ACTB*) and beta2-microglobulin (*β2M*) were designed using published human gene sequences in the Primer Express 3.0 Software (Thermo Fisher Scientific, Waltham, MA, USA) and are PCR reactions were performed using 5 µL of 1X Fast SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL (1 ng/µL) of forward and reverse primers for the gene and 1 µL of cDNA (12.5 ng/µL for Dio3), in a total volume of 10 µL. Then, cDNA was amplified by qPCR in the ViiA7 Real Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). qPCR specificity was determined using melting curve analyses. Each sample was assayed in triplicate and a negative control was included in each experiment. Quantification was performed using the comparative ΔΔCq method. The ΔΔCq method calculates changes in gene expression as relative fold differences (n-fold changes) between an experimental and an external calibrator sample (34).

*Statistical Analysis*

Categorical data are presented as frequencies and were analyzed using the $\chi^2$ test or Fisher's exact test. Quantitative data with normal distribution are presented as mean ± SD and were analyzed using the Student's *t* test. Non-parametric variables are presented as median ± interquartile range, and were analyzed by the Mann-Whitney's *U* test. We determined the correlations between variables by the Pearson's correlation test. We performed a multivariate binary logistic regression by the independent mortality predictor's assessment. Moreover, we included associated factors ($p<0.05$ was the retention criterion for each factor) from the univariate analysis in the multivariable logistic regression analysis. The power of the association between risk factors and outcomes was expressed as the odds ratio (OR). We applied the receiver operating characteristic (ROC) curve to set the critical point for predicting the mortality for T3 levels. The results are presented as the area under the curve (AUC) with sensitivity and specificity. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 23 software (SPSS Inc., Chicago, IL, United States), with a significance of $p<0.05$.

## 5. Conclusions

The present study provided evidence for low T3 levels as an independent prognostic factor in patients with sepsis and septic shock, with reduced T3 levels at ICU admission being associated with a higher risk of evolution to CCI and death. Furthermore, the high expression of circulating D3 in white cells is a novel mechanism that further reduces the T3 available to tissues.

**Author Contributions:** Conceptualization: Josi Vidart, Simone Magagnin Wajner. Data curation: Josi Vidart, Rafael Aguiar Marschner, Andre Braun, Luiza Axelrud,. Formal analysis: Josi Vidart, Rafael Aguiar Marschner, Simone Magagnin Wajner. Funding acquisition: Simone Magagnin Wajner.

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**Institutional Review Board Statement:** The study was performed in accordance with the Declaration of Helsinki; the Institutional Ethics Committees of Hospital de Clinicas de Porto Alegre approved the study protocol (CAAE: 64743617.0.0000.5327). Written informed consent was obtained from all patients or their legally responsible next of kin.

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**Conflicts of Interest:** The authors declare no conflict of interest."

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