‘OVIDIUS’ UNIVERSITY IN CONSTANŢA
DOCTORAL SCHOOL OF MEDICINE
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2023-2024 ACADEMIC YEAR

Ph.D. THESIS

Ph.D. supervisor: **Prof. Eduard Circo, Ph.D.**

Ph.D. Candidate: **Anca-Mihaela Belu**

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Implications of Thyroid Function in Patients with Liver Cirrhosis

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Note: *The datasets and scripts of all statistical analyses performed are available on the Open Science Framework platform and can be accessed at:*
https://osf.io/v4fdg/?view_only=e2347fc241c94523ac05078f26170fec

CONTENT

Key words - thyroid activity, liver cirrhosis, severity, mortality

INTRODUCTION

Liver cirrhosis is a complex condition with a guarded prognosis and multifactorial evolution, requiring complex monitoring. For this, the possibility of identifying connections between paraclinical aspects regarding thyroid activity and the evolution of liver cirrhosis was investigated, which could offer new opportunities for the development of more effective and customized therapeutic strategies.

This paper is intended at analysing thyroid function in patients with liver cirrhosis with the aim of demonstrating that this monitoring can significantly contribute to survival in liver cirrhosis, by choosing the right moment for liver transplantation, but also to prevent the evolution of the disease.

The aim of the thesis is to determine if there is a connection between thyroid changes and the progression of liver cirrhosis, but also the possibility that TSH, T3 and fT4 changes represent, along with MELD, a prognostic or survival assessment factor. The utility of thyroid function tests is considered in addition to the MELD score, to differentiate the unfavourable evolution of patients with liver cirrhosis, including their mortality.

The connection between thyroid function, disease progression, and mortality in liver cirrhosis remains a current topic and opens the door to studies designed to improve the management of this condition and increase survival rates.

I. Liver Cirrhosis – General Information

Liver cirrhosis is a global health problem, causing over a million deaths in 2020 (1), and is a significant cause of morbidity and mortality in people with chronic liver disease worldwide. In 2019, cirrhosis was associated with 24% of global deaths. However, with the rising prevalence of obesity and increased alcohol consumption on one hand, and improvements in the management of hepatitis B virus and hepatitis C virus infections on the other hand, the epidemiology and challenges of cirrhosis are changing (2).

A diagnosis of liver cirrhosis implies liver failure and portal hypertension. It can be said that the changes characteristic of the cirrhogenic process that appear in the advanced stages of the disease are, in fact, its so-called complications.

Some patients with cirrhosis are completely asymptomatic and have a normal life expectancy. The disease progresses from an asymptomatic phase (compensated cirrhosis) to a symptomatic phase (decompensated cirrhosis), whose complications often lead to hospitalization, impaired quality of life and high mortality. Progressive portal hypertension, systemic inflammation and liver failure lead to the disease, and management of liver cirrhosis is focused on treating the causes and complications, and liver transplantation may also be necessary. In general, patients have a multitude of the most severe symptoms of end-stage liver disease and a limited chance of survival (1), and in terms of complications, they appear in the decompensation phase of the disease (3).

The Child Pugh or Child-Turcotte-Pugh (CPS) classification is by far the most widely applied and reported system because it is easy to use as a bedside test (4). It contains five variables, including serum bilirubin and albumin levels, prothrombin time, ascites, and encephalopathy. The two clinical determinants are based on subjective assessment and can be modified by therapy, and the CPS divides patients into low (class A), intermediate (class B) and high (class C) risk, with 10 levels of difference between the patient least sick and most advanced. While the development of the CPS classification was based on empirical evaluation, subsequent studies have shown that the

CPS is predictive in assessing prognosis in patients with liver disease. These studies demonstrated that each of the five individual clinical variables as well as the overall CPS classification had prognostic significance (5), and the difficulties and interobserver variability for the subjective parameters in the CPS classification led to the development of the "final model" (6).

The Child-Pugh score system is extensively used to predict mortality in patients with cirrhosis, who are classified into three categories: A (good liver function), B (moderate liver function) and C (advanced liver dysfunction), the score helping and in predicting the risk of death and liver complications in patients with liver disease. The study of cirrhosis according to the Child-Pugh score, divided into grades A, B and C, and the correlation with serum thyroid hormone indices can provide valuable information, the results suggesting that as the Child-Pugh score is higher, the prognosis of patients with cirrhosis liver is less favorable (7).

The Child-Pugh scoring system was originally conceptualized by Child and Turcotte in 1964 to guide the selection of patients who would benefit from elective portal decompression surgery, dividing patients into three categories. The original scoring system used five clinical and laboratory criteria: serum bilirubin, serum albumin, ascites, neurological disorder, and clinical nutritional status (8), and was later modified by Pugh et al., replacing clinical nutritional status with time of prothrombin and entering variable points for each criterion based on increasing severity (1):

There were three main limitations to the use of the Child Pugh Score: 1. classification of ascites and encephalopathy requires subjective assessment, 2. the classification system does not take into account renal function, and 3. there are only ten different (point-based) scores available. This last limitation was a significant one, as patients could not be adequately differentiated according to the severity of liver disease (9). Basically, a patient with an INR of 6 and bilirubin of 14 could have the same Child-Pugh score as a patient with an INR of 2.3 and bilirubin of 4.0.

MELD score

The MELD score has a wider range of values, can be treated as a continuous variable, and was created to account for these differences. The initial calculation of the MELD score used the patient's bilirubin level, creatinine level, INR, and cause of liver disease (5). It has evolved over time to rule out the causes of the disease and takes into account the serum sodium level and whether or not the patient is on dialysis (10).

The model for end-stage liver disease (MELD) has been established as a reliable predictor of short-term survival in patients with end-stage liver disease. Recently, concerns have arisen regarding the reduced accuracy of mortality prediction by MELD (11), there being and a number of concerns, ranging from changes in the epidemiology of liver disease and the development of prognosis-modifying therapies, to changes in the distribution of MELD scores, increasing age and comorbidity in patients awaiting transplantation. In addition, there have been growing concerns that women are disadvantaged by the current system for several reasons, including serum creatinine overestimating renal function in women and thus underestimating their mortality risk (12).

II. Thyroid Hormones and Their Role in Liver Function

The thyroid gland produces two hormones: thyroxine (T4) and triiodothyronine (T3). These act through thyroid hormone receptors α and β, playing a critical role in cellular differentiation during development and maintaining thermogenic and metabolic homeostasis in adults (3). T4 is secreted by the thyroid gland at a rate about twenty times higher than T3, and both hormones are bound to plasma proteins, including thyroxine-binding globulin, transthyretin (thyroxine-binding prealbumin), and albumin (4).

The liver plays an important role in the metabolism of thyroid hormones, as it is the primary organ responsible for the peripheral conversion of thyroxine (T4) to T3 by type 1 deiodinase (5), (6). This enzyme, present in the liver at about 30%-40%, accounts for approximately 30%-40% of extrathyroidal production of T3 and can perform both 5'-deiodination and 5-deiodination of T4 to T3. Furthermore, the liver is involved in the conjugation and excretion of thyroid hormones, as well as in the synthesis of thyroxine-binding globulin (7). T4 and T3 regulate the basal metabolic rate of all cells, including hepatocytes, and consequently influence liver function (8). Thyroid diseases can disrupt liver function, while liver disease can modulate thyroid hormone metabolism. Clinical and laboratory associations exist between thyroid diseases and liver diseases (9). Patients with chronic liver disease may develop thyroiditis, hyperthyroidism, or hypothyroidism, and patients with subacute thyroiditis or hyperthyroidism may present with abnormal liver function tests that return to normal as the thyroid condition improves (10).

Studies to date have shown that the most frequent changes in plasma thyroid hormone levels include a decrease in total T3 and free T3 concentrations, which is associated with the severity of liver dysfunction. These changes in thyroid hormone levels are so well-established that some researchers have advocated for their use as a sensitive indicator of liver function (4,11).

Changes in thyroid hormone levels have been significant in predicting the degree of liver damage in patients with liver cirrhosis and are primarily related to the following reasons:

1. After the onset of liver cirrhosis, the thyroid gland may undergo atrophy or degenerative changes in the hormonal feedback mechanism, affecting the secretion and synthesis of thyroid hormones (12).
2. Liver cirrhosis is usually accompanied by complex changes in homeostasis. Under the influence of the body's feedback mechanism, the conversion of T4 is blocked, leading to a decrease in T3 levels (13).
3. Liver cirrhosis puts the body under stress, causing dysfunction of the hypothalamic-pituitary-thyroid axis and decreased regulation of thyroid hormones, which leads to alterations in serum thyroid hormone levels (14).
4. The body's energy metabolism is disrupted, hypersplenism induces anaemia, and Na+-K+-s
5. levels (15).

PERSONAL CONTRIBUTION

III. Evaluation of the Relationship between the Thyroid Function and the Severity of Liver Cirrhosis

III.1. Objectives

The general objective of the thesis is to evaluate the relationship between the thyroid function and the severity of liver cirrhosis, considering the assessment of thyroid hormone profiles in patients with liver cirrhosis and analysing the MELD score and thyroid hormones as evolution and prognosis factors. This study investigated the possibility of identifying connections between paraclinical aspects regarding thyroid activity and the progression of liver cirrhosis, which could

offer new opportunities for developing more effective and customized clinical and paraclinical monitoring strategies. Thyroid dysfunction was also studied in parallel with the progression of cirrhosis to improve the monitoring of these patients and to assess mortality risk.

Study 1 aimed at evaluating the relationship between the thyroid hormone profile and the progression of liver disease, assessing the severity of cirrhosis in patients without thyroid changes versus patients with cirrhosis and thyroid hormone profile changes.

The **objective of Study 2** was to examine mortality in cirrhotic patients in relation to changes in thyroid activity.

III.2. General Methodology

In the two studies, a cohort of 419 patients diagnosed with liver cirrhosis, who were hospitalized between March 2022 and March 2023 at the Constanța County Emergency Clinical Hospital, was analysed.

III.2.1. Inclusion Criteria

The cohort included patients aged at least 18 years, able to read and write, who gave their informed consent, diagnosed with liver cirrhosis following a clinical, paraclinical-biological examination and imaging investigations, classified into categories of severity and treated appropriately, present in the records of an internist or gastroenterologist, or recently diagnosed during hospitalization.

The diagnosis of liver cirrhosis was clinically confirmed by the presence of signs and symptoms of liver failure and portal hypertension, and paraclinically confirmed through biological tests and imaging methods. The MELD (Model for End-Stage Liver Disease) score was calculated so that each case could be placed into a category based on progression and severity.

III.2.2. Exclusion Criteria

Patients who refused to sign the informed consent were excluded from the study. Additionally, patients known to have thyroid pathology and those receiving medication that could influence thyroid hormone metabolism (e.g., iodine-based contrast agents and Amiodarone, which accentuates the conversion of T4 to triiodothyronine T3) were excluded, as were other drug classes such as glucocorticoids and Dopamine, which increase TSH secretion with subsequent decreases in T3 (16). The values of TSH, T3, and fT4 were monitored, along with the progression of cirrhosis, using laboratory tests, Child classification, days of hospitalization, and mortality. The *MELD (Model for End-Stage Liver Disease)* score, a reliable short-term survival indicator for patients with end-stage liver disease (17), conceived based on serum bilirubin, serum creatinine, and INR (international normalized ratio) was also studied.

STUDY I

EVALUATION OF THYROID ACTIVITY IN PATIENTS WITH LIVER CIRRHOSIS

IV. Evaluation of Thyroid Activity in Patients with Liver Cirrhosis

IV.1. Introduction

There is a bidirectional relationship between the liver and thyroid, in which the liver plays a major physiological role in the activation, transport, metabolism, and clearance of thyroid hormones (17), while thyroid hormones affect the activities of hepatocytes and hepatic metabolism (18).

V.2. Hypothesis/Objectives

The primary objective of the research is to investigate the relationship between thyroid hormone variability and the severity of liver cirrhosis.

IV.3. Materials and Methods

The study involved 419 patients diagnosed with liver cirrhosis, who were hospitalized between March 2022 and March 2023 at the Constanţa County Emergency Hospital. The inclusion and exclusion criteria presented in the General Methodology section were applied. Blood samples were collected, as described earlier, and patients were categorized according to severity classes based on the Child-Pugh classification. The MELD score was also calculated.

The experimental data were processed using *IBM SPSS Statistics 25* statistical processing software. The procedures used included: *Descriptive statistics* (for characterizing discrete and continuous variables defined at the database level), *Graphs*, *Parametric statistical tests* (t-test for

comparing the mean of two independent samples), *Non-parametric statistical tests* (Chi-square test of association between two categorical variables, determining the odds ratio (OR) and relative risk Rr where applicable, the z-test for comparing two proportions, the Mann-Whitney U test, and the median test) (18).

Patients were divided into two groups: **euthyroid** and **non-euthyroid**. The normal thyroid or Euthyroid group included patients with normal values of TSH, T3, and fT4 or who presented a change in one of these tests that did not persist dynamically. The Non-euthyroid group included patients who had at least two changes reflecting thyroid gland dysfunction, or where one test remained outside the normal range throughout hospitalization, confirmed by two determinations, or by two different thyroid function tests with abnormal values. For the classification into the two groups, the TSH, T3, and fT4 determinations upon admission were used as a reference and monitored in dynamics.

Table 1. Grouping of Patients

	Group	Relative frequency	Percentage frequency
Valid	Euthyroid	238	56.80%
	Non-euthyroid	181	43.20%
	Total	419	100.00%

Table 2. Descriptive Statistics of the Studied Groups

Group		N	Minimum	Maximum	Mean	Std deviation
Euthyroid	Age (years)	238	32.00	88.00	63.23	9.46
	MELD	238	5.00	30.00	12.61	3.48
	Days of hospitalization	238	.00	69.00	7.47	7.74
	TSH	238	.11	10.50	2.63	1.44
	T3	238	.80	8.20	1.95	.77
	fT4	238	10.10	23.00	14.33	2.48
	TOTAL PROTEINS (mg/dL)	238	4.60	7.60	6.47	.70

	ALBUMIN (mg/dL)	238	1.80	3.60	2.92	.42
	SODIUM (mEq/L)	238	118.00	144.00	131.00	4.15
	POTASSIUM (mEq/L)	238	1.80	6.40	3.54	.82
Non-euthyroid	Age (years)	181	32.00	91.00	63.56	10.32
	MELD	181	7.00	31.00	16.01	4.56
	Days of hospitalization	181	.00	37.00	7.03	6.46
	TSH (microUI/L)	181	.10	10.50	2.70	2.17
	T3 (nmol/L)	181	.30	6.20	1.85	.72
	fT4 (pmol/L)	181	8.80	22.90	13.87	2.77
	Total proteins (mg/dL)	181	4.10	7.80	6.24	.81
	Albumin (mg/dL)	181	1.60	3.40	2.79	.24
	Na (mEq/l)	181	116.00	144.00	130.78	4.64
	K (mEq/L)	181	2.00	6.40	3.78	.97

The descriptive statistical analysis of the two patient groups (Euthyroid and Non-euthyroid) is shown in the table above.

The alcoholic aetiology is most frequent in the Euthyroid group, followed by viral B aetiology, viral C aetiology, and mixed aetiology. The aetiology in the Non-euthyroid group is represented by alcohol, followed by viral infection C, viral infection B, and mixed aetiology (alcoholic and viral).

Table 3. z-Test Results for Comparing Two Proportions (Group vs. Aetiology of Cirrhosis)

Etiology of Cirrhosis	Euthyroid			Non-euthyroid					
	Total	N_1	p_1	Total	N_2	p_2	z	p	Decision
Alcoholic	238	121	0.5084	181	91	0.5028	0.114	0.909	NO
Viral B	238	53	0.2227	181	22	0.1215	2.675	0.007	YES
Viral C	238	42	0.1765	181	36	0.1989	-0.584	0.559	NO
Mixed	238	22	0.0924	181	32	0.1768	-2.553	0.011	YES

There are statistically significant differences between the proportion of patients with Viral B cirrhosis in the Euthyroid group (p_1 = 22.27%) and the proportion of patients with Viral B cirrhosis in the Non-euthyroid group (p_2 = 12.15%) (z = 2.675, p = 0.007). Similarly, there are significant differences between the proportion of patients with mixed cirrhosis in the Euthyroid group (p_1 = 9.24%) and the Non-euthyroid group (p_2 = 17.68%) (z = -2.553, p = 0.011).

Table 4. Contingency Table (Group vs. Child-Pugh Classification)

			CP			
			A	**B Score**	**C**	**Total**
Group	Euthyroid	Count	60	102	76	238
		% within Group	25.21%	42.86%	31.93%	100.00%
	Non-euthyroid	Count	18	104	59	181
		% within Group	9.94%	57.46%	32.60%	100.00%
Total		Count	78	206	135	419
		% within Group	18.62%	49.16%	32.22%	100.00%

There is a dependent relationship between the Group grouping variable (Euthyroid vs. Non-euthyroid) and the Child-Pugh class variable (A, B, C): χ^2_{calc} = 17.342, df = 2, p < 0.001 < α = 0.05 (Chi-square test for association between two categorical variables).

Table 5. z-Test Results for Comparing Two Proportions (Group vs. Child-Pugh Class)

CP Score	**Euthyroid**			**Non-euthyroid**					
	Total	**N_1**	**p_1**	**Total**	**N_2**	**p_2**	**z**	**P**	**Decision**
A	238	60	0.2521	181	18	0.0994	3.977	0.000	YES
B	238	102	0.4286	181	104	0.5746	-2.961	0.003	YES
C	238	76	0.3193	181	59	0.3260	-0.144	0.885	NO

There are statistically significant differences between the proportion of patients in Child-Pugh class A in the Euthyroid group p_1 = 0.2521 (25.21%) and the Non-euthyroid group p_2 = 0.0994 (9.94%) (z = 3.977, p < 0.001), respectively there are statistically significant differences between the proportion of patients in Child-Pugh class B in the Euthyroid group p_1 = 0.4286 (42.86%) and the proportion of patients in Child-Pugh class B in the Non-euthyroid group p_2 = 0.5746 (57.46%) (z = -2.961, p = 0.003).

Table 6. Contingency Table (Group vs. Encephalopathy Class (West Haven))

			Encephalopathy Class (West Haven)					
			Grade 0	Grade I	Grade II	Grade III	Grade IV	Total
Group	Euthyroid	Count	171	18	35	10	4	238
		% within Group	71.85%	7.56%	14.71%	4.20%	1.68%	100.00%
	Non-euthyroid	Count	113	10	39	11	8	181
		% within Group	62.43%	5.52%	21.55%	6.08%	4.42%	100.00%
Total		Count	284	28	74	21	12	419
		% within Group	67.78%	6.68%	17.66%	5.01%	2.86%	100.00%

There is no association between the Group grouping variable (Euthyroid vs. Non-euthyroid) and the Encephalopathy Class (West Haven) variable (Grades 0, I, II, III, IV): χ^2_{calc} = 8.124, df = 4, p = 0.087 > α = 0.05 (Chi-square test for association between two categorical variables).

Table 7. Contingency Table (Group vs. Mortality)

			Mortality		Total
			Yes	No	
Group	Euthyroid	Count	11	227	238
		% within Group	4.6%	95.4%	100.0%
	Non-euthyroid	Count	22	159	181
		% within Group	12.2%	87.8%	100.0%
Total		Count	33	386	419
		% within Group	7.9%	92.1%	100.0%

There is a dependent relationship between the Group grouping variable (Euthyroid vs. Non-euthyroid) and the Mortality variable (Yes vs. No): χ^2_{calc} = 8.041, df = 1, p = 0.005 < α = 0.05 (Chi-square test for association between two categorical variables). The risk of death in the Euthyroid group is 2.857 times lower than in the Non-euthyroid group: OR = 0.350, statistically significant value with a 95% confidence interval CI = (0.165, 0.743). The proportion of deaths in the

Euthyroid group (p = 0.046) is 2.631 times lower than in the Non-euthyroid group (p = 0.1220): Rr = 0.380, statistically significant value with a 95% confidence interval CI = (0.189, 0.764).

Table 8. Statistical Indicators Corresponding to the MELD Variable

Group	N	Mean	Median	SD	Minimum	Maximum	Percentiles	
							P25	P75
Euthyroid	238	12.58	13.00	3.35	5.00	22.00	9.00	15.00
Non-euthyroid	181	16.01	16.00	4.56	7.00	31.00	13.00	19.00

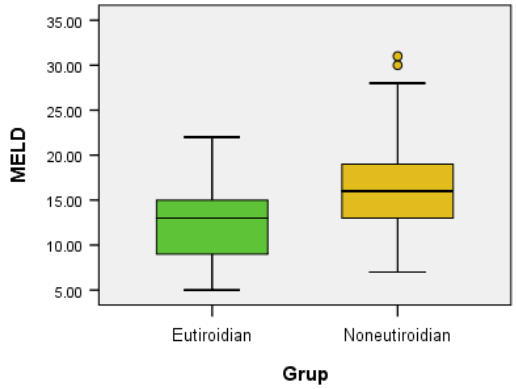

Figure 1. Box Plot Representation of MELD Score Distribution (Euthyroid/Non-euthyroid Groups)

The Kolmogorov-Smirnov test disproves the assumption of normal distribution for MELD values in both groups: KS = 0.109, df = 238, p < 0.001 for the Euthyroid group, and KS = 0.118, df = 181, p < 0.001 for the Non-euthyroid group. Thus, the Mann-Whitney U non-parametric test shows that there are statistically significant differences in the distribution of MELD values between the Euthyroid group (Median = 13, *IQR* = *6*, n = 238, Mean rank = 171.04) compared to the Non-euthyroid group (Median = 16, *IQR* = *6*, n = 181, Mean rank = 261.23): *U* = 12266.500, z = -7.579, p < 0.001 < α = 0.05). The Median test also confirms a statistically significant difference between the median MELD values of the two groups (p < 0.001 < α = 0.05). Patients in the group with thyroid changes have higher MELD scores and, therefore, a worse prognosis compared to cirrhotic patients without thyroid test changes.

Table 9. Contingency Table (Group vs. MELD Score Intervals)

			MELD				Total
			(...-10)	[10-20)	[20-30)	[30-40)	
Group	Euthyroid	Count	60	174	4	0	238
		% within Group	25.21%	73.11%	1.68%	0.00%	100.00%
	Non-euthyroid	Count	12	128	39	2	181
		% within Group	6.63%	70.72%	21.55%	1.10%	100.00%
Total		Count	72	302	43	2	419
		% within Group	17.18%	72.08%	10.26%	0.48%	100.00%

Table 10. Contingency Table (Group vs. TSH Intervals (microUI/L))

			TSH (microUI/L)			Total
			(...-0.27)	[0.27-4.20)	[4.20-...)	
Group	Euthyroid	Count	2	208	28	238
		% within Group	0.84%	87.39%	11.76%	100.00%
	Non-euthyroid	Count	6	91	84	181
		% within Group	3.31%	50.28%	46.41%	100.00%
Total		Count	8	299	112	419
		% within Group	1.91%	71.36%	26.73%	100.00%

It can be noticed that normal values are most frequently encountered in both groups, but in the Non-euthyroid group, values higher than the normal limit are present in 46.41% of cases, and values lower than normal in 3.31%.

Table 11. Contingency Table (Group vs. T3 Intervals (nmol/L))

			T3 (nmol/L)			Total
			(...-1.3)	[1.3-3.1)	[3.1-...)	
Group	Euthyroid	Count	19	216	3	238

		% within Group	7.98%	90.76%	1.26%	100.00%
	Non-euthyroid	Count	94	84	3	181
		% within Group	51.93%	46.41%	1.66%	100.00%
Total		Count	113	300	6	419
		% within Group	26.97%	71.60%	1.43%	100.00%

Patients in the Non-euthyroid group showed normal T3 values in 46.41% of cases, values above the normal limit in 1.66%, and values lower than normal in 51.93% of cases.

Table 12. Contingency Table (Group vs. fT4 Intervals (pmol/L))

			fT4 (pmol/L)			Total
			(...-12)	[12-22)	[22-...)	
Group	Euthyroid	Count	42	194	2	238
		% within Group	17.65%	81.51%	0.84%	100.00%
	Non-euthyroid	Count	62	118	1	181
		% within Group	34.25%	65.19%	0.55%	100.00%
Total		Count	104	312	3	419
		% within Group	24.82%	74.46%	0.72%	100.00%

The fT4 determinations showed that most values are within normal limits in both groups, but patients in the Non-euthyroid group had values above the normal limit in 0.55% of cases, and values below the normal limit in 34.25% of cases.

Table 13. Statistical Indicators Corresponding to Total Proteins Variable (mg/dL)

Group	N	Mean	Median	SD	Minimum	Maximum	Percentiles	
							P25	P75
Euthyroid	201	6.47	6.70	.70	4.60	7.60	5.90	7.00
Non-euthyroid	162	6.23	6.40	.80	4.10	7.40	5.80	6.90

The analysis of the contingency between groups in relation to the Child-Pugh classification showed that there is a connection between patients belonging to the two studied groups according to the Chi-square test for association between two categorical variables. This relationship is statistically significant in the case of patients with Child-Pugh class A and Child-Pugh class B cirrhosis. It is likely that patients with Child-Pugh class C cirrhosis do not present an association between the Euthyroid and Non-euthyroid groups due to the more advanced progression of the liver disease, which has repercussions on the entire organism (19).

The tendency for T3 to record low values more frequently than fT4 can be explained by two mechanisms. The first is the reduced conversion of fT4 to T3 in peripheral tissues, especially in the liver, due to impaired liver function (20). The second possible mechanism for the more frequent decrease in T3 levels compared to fT4 is the reduced serum protein levels that bind T3, while fT4 is a fraction that circulates freely (21). Serum protein levels ranged from 4.60 to 7.60 mg/dL in the Euthyroid group, with a mean of 6.47 and a median of 6.70 mg/dL. In the Non-euthyroid group, values ranged from 4.10 to 7.40 mg/dL, with a mean of 6.23 and a median of 6.40.

The TSH values are associated with the classification of patients according to the Child-Pugh score. As the disease progresses, statistically significant differences were observed, suggesting that TSH values increase with the severity of cirrhosis. Thus, there are statistically significant differences between TSH values in patients with Child-Pugh class C cirrhosis compared to those with Child-Pugh class B and A.

STUDY II

EVALUATION OF THE CORRELATION BETWEEN THYROID PROFILE AND MORTALITY IN PATIENTS WITH LIVER CIRRHOSIS

Evaluation of the Correlation between Thyroid Profile and Mortality in Patients with Liver Cirrhosis

Chronic liver disease and cirrhosis significantly contribute to global mortality, with limited improvements despite medical advances (21).

In the second study, the mortality of patients with liver cirrhosis was analysed in relation to changes in their thyroid profile.

V.1. Materials and Methods

Survival was evaluated by recording deaths after discharge in patients with liver cirrhosis.

The study involved 419 patients diagnosed with liver cirrhosis, who were hospitalized between March 2022 and March 2023 at the Constanta County Emergency Hospital. The inclusion and exclusion criteria presented in the General Methodology were applied.

V.2. Study Hypothesis

Thyroid hormone levels are statistically significantly different between the survivor group and the deceased group.

V.3 Results

V.3.1. Kaplan-Meier Analysis

The data analysis was performed using the Kaplan-Meier method, with survival curves plotted. To describe the 'mean' survival of patients in the study group, the median survival time was used, as the data was not normally distributed.

The log-rank test was used to evaluate differences between groups.

Kaplan-Meier analysis was performed only for data collected upon admission.

The data shows that for patients with low T3 values, the probability of survival was 89.0% (SE = 24.16%, CI 95% [84.3%, 93.9%]) at 9 days and 71.5% (SE = 6.46%, CI 95% [59.9%, 85.4%]) at 22 days, while for patients with normal T3 values, it was 92.3% (SE = 6.45%, CI 95% [80.5%, 100.0%]) at 12 days (see Figure 1). The log-rank test did not indicate any statistically significant difference (p = 0.10) between the median survival time of patients with abnormal T3 values and that of patients with normal T3 values.

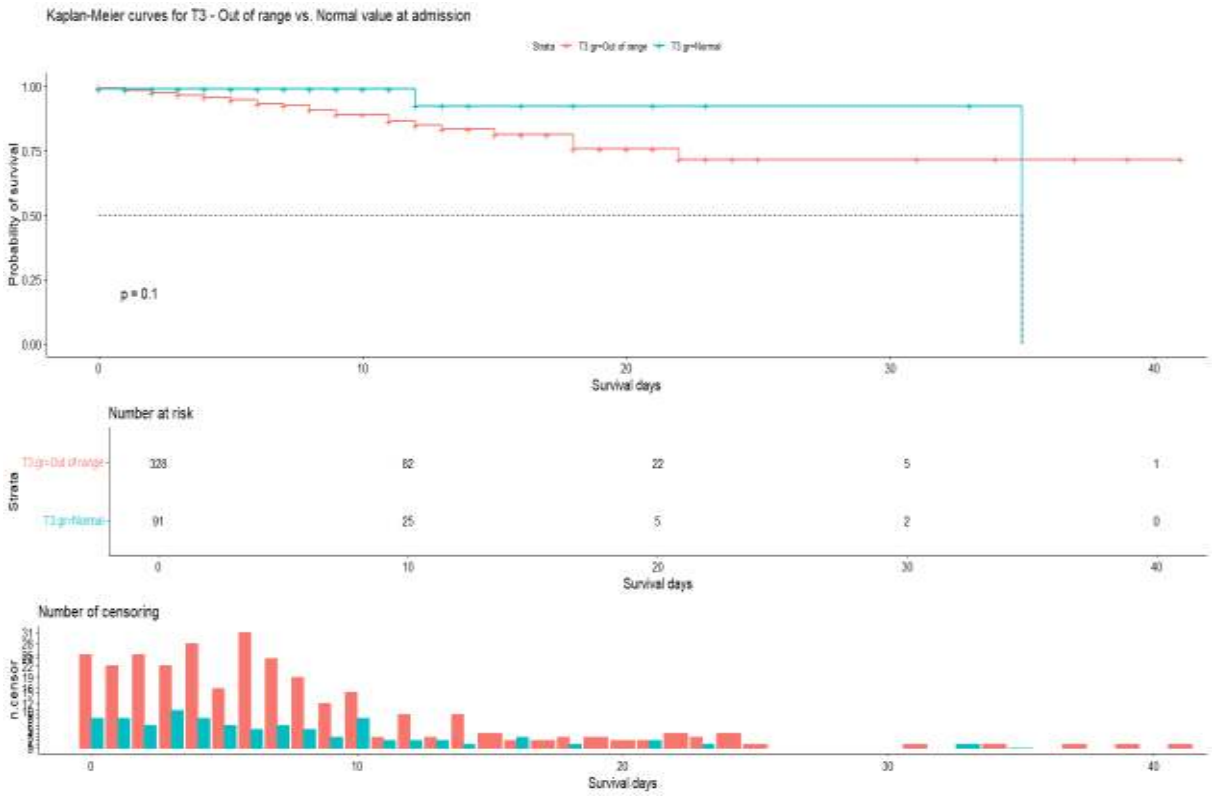

Figure 2. Kaplan-Meier Survival Curve for T3 upon Admission. Normal Values vs. Values Outside the Normal Range

For patients with abnormal fT4 values, the probability of survival was 90.40% (SE = 5.03%, CI 95% [81.0%, 100.0%]) at 12 days and 73.8% (SE = 11.84%, CI 95% [53.9%, 100.0%]) at 22 days, while for patients with normal fT4 values, the probability of survival was 85.3% (SE = 3.39%, CI 95% [79.0%, 92.3%]) at 12 days and 77.5% (SE = 5.37%, CI 95% [67.7%, 88.8%]) at 18 days (see Figure 2). The log-rank test did not indicate any significant difference (p = 0.93) between the median survival time of patients with abnormal fT4 values and those with normal fT4 values.

The data shows that all patients with normal TSH values survived, while for patients with abnormal TSH values, the probability of survival was 88.8% (SE = 2.39%, CI 95% [84.2%, 93.6%]) at 9 days and 46.6% (SE = 19.52%, CI 95% [20.5%, 100.0%]) at 35 days.

The log-rank test indicates a statistically significant difference (p = 0.003), with the mean survival time for patients in the group with abnormal TSH values being 35 days, significantly

shorter than the survival time for patients in the group with normal TSH values (41 days, see Figure 3).

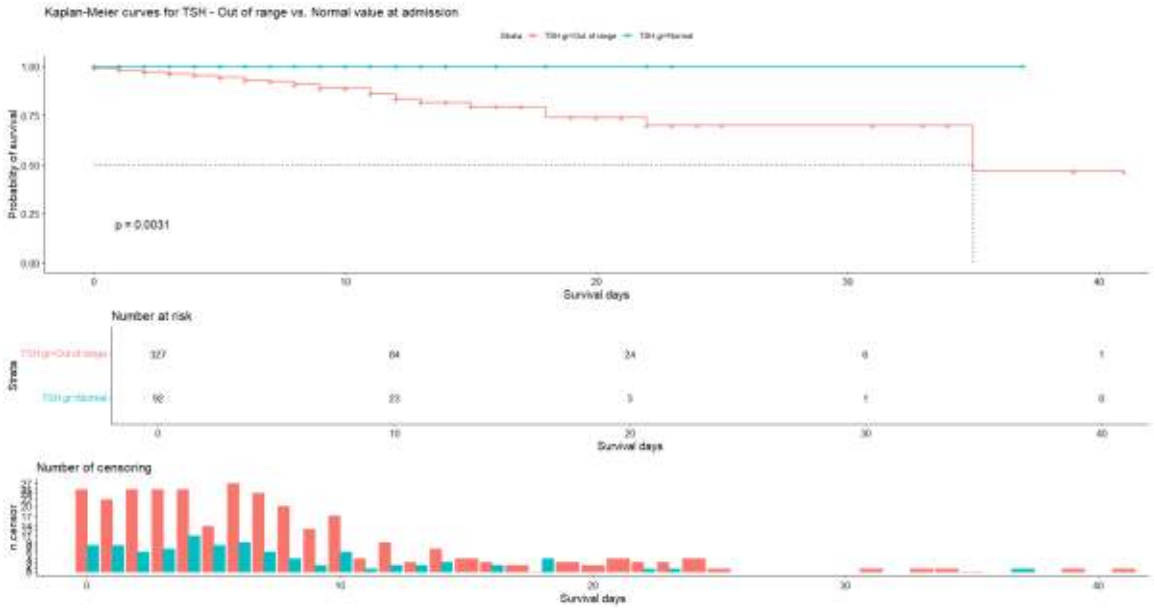

Figure 3. Kaplan-Meier Survival Curve for fT4 upon Admission. Normal Values vs. Values outside the Normal Range

V.3.2. ROC Curve for Survival Classification

The ROC analysis indicated an accuracy for classifying survival based on **TSH hormone** levels of 62.23% (CI 95% [52.44%, 70.52%]) upon **admission** , and 62.71% (CI 95% [53.19%, 70.88%]) upon **discharge**. Therefore, TSH can be considered an acceptable classifier for survival in patients with liver cirrhosis, even though the effects are small but statistically significant.

The classification of survival based on T3 hormone levels was acceptable in accuracy upon admission 64.63% (CI 95% [54.86%, 73.73%]) and marginal upon discharge, with a specificity of 60.87% (CI 95% [50.27%, 71.42%]) upon discharge (see Figure 8). Therefore, T3 can be considered an acceptable classifier for survival in patients with liver cirrhosis, especially based on admission values, but the discharge results had too many false-negative predictions.

The classification of survival based on fT4 hormone levels had very low accuracy both upon admission (52.12%, CI 95% [42.36%, 61.70%]) and upon discharge (52.07%, CI 95% [41.08%, 64.29%]), so fT4 cannot be considered an acceptable classifier for survival in patients with liver cirrhosis.

Kaplan-Meier analysis showed that all patients with normal TSH survived, while for those with abnormal TSH values, the probability of survival was 88.8% after 9 days of hospitalization and 46.6% after 35 days. The log-rank test did not show any statistically significant difference between T3 and fT4 values in survivors and non-survivors. A similar situation was noticed in the studies by Fei Ye (20).

TSH can be a prognostic factor related to mortality in patients with liver cirrhosis. Monitoring TSH not only improves understanding of disease progression but can significantly contribute to patient survival.

Conclusions

1. Patients with liver cirrhosis frequently present thyroid function disturbances. Among these changes, the most common are increased TSH levels, decreased T3 levels, and decreased fT4 levels.

2. There is a correlation between the severity of liver cirrhosis, as expressed by classes A, B, and C according to Child-Pugh Classification, and thyroid dysfunction. Thus, thyroid function changes are more commonly found in patients with Child-Pugh class B cirrhosis, while patients without thyroid changes are more frequently in Child-Pugh class A.

3. Increased TSH is most commonly found in patients with Child-Pugh class C cirrhosis, while normal TSH values are most frequently associated with Child-Pugh class A. Decreased T3 levels are more often found in patients in class B and less frequently in those in Child-Pugh class A. As for fT4, no correlation was found between this hormone and the Child-Pugh classification.

4. It was found that there is no statistically significant difference between the thyroid hormone profile and the degree of hepatic encephalopathy, which is diagnosed with similar frequency in both euthyroid and non-euthyroid patients.

5. Serum protein levels in patients with liver cirrhosis are correlated with thyroid function changes. Hypoproteinaemia occurs more frequently and is more severe in non-euthyroid patients compared to euthyroid patients.

6. There was no statistically significant difference in albumin levels between cirrhotic patients with normal thyroid profiles and those with thyroid changes.

7. Serum sodium levels in patients with liver cirrhosis show similar variations regardless of thyroid profile changes. The determination of serum potassium levels in cirrhotic patients shows that hyperkalaemia is more common in non-euthyroid patients, while low potassium levels are more frequent in euthyroid patients.

8. MELD scores above 20 are more frequently found in non-euthyroid patients, and MELD scores above 30 are exclusively found in patients with thyroid profile changes. MELD scores of 10 or lower are more often associated with normal thyroid profiles.

9. The results highlighted a higher risk of death in patients with thyroid function changes compared to those without thyroid changes.

10. The probability of survival for cirrhotic patients with abnormal T3 levels is lower than for those with normal T3 levels.

11. There is no statistically significant difference in survival probability between patients with abnormal fT4 values and those with normal fT4 values.

12. There is a connection between survival rate and TSH levels in cirrhotic patients. All patients who died had TSH levels higher than normal.

13. The association between thyroid changes and the severity of cirrhosis, as well as with the MELD score, represents an important factor in managing liver cirrhosis, optimally determining recommendations for liver transplantation, and assessing the severity of the disease to reduce mortality.

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