UNIVERSITY OF MEDICINE AND PHARMACY

"CAROL DAVILA", BUCURESTI

DOCTORAL SCHOOL

FACULTY OF MEDICINE

DOCTORAL THESIS

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Student-doctorand:

STANCU GEORGE

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DOCTORAL SCHOOL

FIELD OF INTERNAL MEDICINE

USEFULNESS OF SERUM PSEUDOCHOLINESTERASE

IN ASSESSMENT, STAGING AND PROGNOSIS

LIVER FAILURE

ABSTRACT OF DOCTORAL THESIS

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Coupons

Introduc tion

Chronic liver disease represents a frequent local and global pathology. It is estimated that 1,5 bilions people are suffering of a chronic liver disease (1) and for liver cirrhosis the age correlated incidence is 20.7/100,000, with an increase of 13% compared to year 2000.

It affects equally both sexes and all ages. Viral and toxic ethiologies are the most frequent followed by the fatty liver disease and autoimmune. (3).

In Romania, according to World Health Organisation the mortality secondary to cirrhosis is in top 10 on the 6th place for women and 4th place for men (5).

Identifying the aetiology of liver disease is a crucial moment in the patient's evolution that can completely change the therapeutic attitude. Sometimes it also involves the need to obtain an aetiological diagnosis under time pressure. The rapidly unfavourable evolution of the patient is what sometimes imposes a rapid pace on the efforts to identify the triggering agent concomitantly with the initiation of supportive treatment of liver function. (1).

The second plane is the assessment of the severity of liver disease and thus the prognosis. An attempt is made to assess liver function and structural integrity and to estimate life expectancy (7).

Currently, the staging of hepatopathy is done using scores (Child Pugh score, Meld Na score, Fibromax) or functional tests and the assessment of the degree of fibrosis by non-invasive methods using ultrasound or invasive techniques such as liver biopsy. (7). In daily practice assessment of the stage of liver disease helps in estimating the extent of drug therapy or the need for liver transplantation. Currently used scores identify in particular the presence of cirrhosis (end-stage liver disease) (8). For this reason, intermediate stages preceding the onset of cirrhosis are not easily identified and thus patients with chronic liver disease have the illusion of a stationary evolution until the onset of cirrhosis. (2).

Before a surgical or radiological procedure that would cause a fraction of the liver volume to be lost, it is necessary to assess the liver functional reserve in terms of the remaining post-procedural liver function (9).

Segmental or lobar liver resections cause a sudden loss of a fraction of the liver's structural volume. Surgically removed liver segments instantly cause a proportional decrease in liver function (10).

Interventional radiology procedures of chemoembolization of hepatic artery branches also cause a loss of liver mass both structurally and functionally. The loss of blood flow to the tributary segments of the embolized hepatic artery branch will cause ischemia of these segments and, subsequently, decreased hepatic functional reserve post procedure (11).

Both surgical resections and embolizations structurally affect liver functional reserve and require a thorough pre-procedural evaluation to avoid a situation where the remaining liver parenchyma does not provide sufficient liver functional reserve to sustain life and induce acute post-procedural liver failure. (12).

Scores, functional tests, volumetric imaging assessments and liver biopsies are used to avoid this.

Of the scores, the Child-Pugh score (CTP) is one of the most widely used globally and is a tool originally designed to predict mortality in cirrhotic patients. Originally designed by Child and Turcotte in 1964 to select patients who would benefit from surgery for portal decompression. This score divides patients into 3 categories : A - good liver reserve, B - moderately impaired liver function and C - severe liver dysfunction. This score uses 5 clinical and laboratory items to assess patients: serum bilirubin, serum albumin, ascites, neurological disorders and nutritional status. This score was later modified by Pugh et al. by replacing nutritional status with prothrombin time (13). In addition they introduced the point system correlated with severity. The Child-Pugh score is used successfully to date globally by clinicians on all continents but this score is, as stated above, designed for cirrhotic patients, using relatively arbitrarily chosen assessment items. For this reason, the CTP score may not reflect decreases in liver functional reserve that have occurred prior to reaching the cirrhosis stage of chronic liver disease. (14).

Serum pseudocholinesterase or butyrylcholinesterase (BCHE) could be the solution for evaluating patients with chronic liver disease in both precortical and cirrhotic stages (15). This enzyme is known to the scientific community but lacks the notoriety required for widespread use. Having a short hepatic synthesis half-life, plasma levels not easily influenced and an extremely low price, it may be a solution for the estimation of liver function and may be useful in the development of a new score for the staging of chronic

liver diseases with an increased sensitivity in the early stages of the disease. (15). These are the **reasons why serum pseudocholinesterase is the subject of this research topic**.

I. General part

1. Sericeous cholinesterases

Discovered in 1968 by Walo Leuzinger et al. who succeeded in purifying and crystallizing acetylcholinesterase from electric eels at Columbia University in New York. (16) serine cholinesterases are enzymes of the hydrolase class that hydrolyse choline esters (19). Some choline esters act as neurotransmitters, the best known substrate being acetylcholine. Acetylcholine is the neurotransmitter of the cholinergic synapse (20) and is hydrolysed by acetylcholinesterase (ACHE) to choline and acetic acid. By degrading acetylcholine the synapse returns to a resting state. Acetylcholinesterase is found in synapses and in the erythrocyte membrane.

There is another type of cholinesterase called butyrylcholinesterase (BCHE) or pseudocholinesterase or serum cholinesterase which is found in plasma and is synthesised by the liver. The difference between the 2 types of cholinesterase is the affinity of each for a particular substrate. Acetylcholinesterase hydrolyses acetylcholine more efficiently and pseudocholinesterase or butyrylcholinesterase (BCHE) is faster in hydrolysing butyrylcholine. (21), (22).

Butyrylcholinesterase (BCHE) is also referred to in the literature as serum cholinesterase or serum pseudocholinesterase but the accepted name today is butyrylcholinesterase (BCHE). (23).

Pseudocholinesterase deficiency may be useful in the choice of anaesthetic for surgery or dental procedures. This enzyme plays an important role in the metabolism of ester-type local anaesthetics. A deficiency of serum BCHE alters the safety limits and increases the risk of systemic effects with this type of anaesthetic. (19).

1.1 Serum pseudocholinesterase

Butyrylcholinesterase (HGNC symbol BCHE; EC 3.1.1.8), referred to alternatively ca șias BChE, BuChE, BuChase, pseudocholinesterase, plasma cholinesterase or serum cholinesterase, is a non-specific cholinesterase enzyme that hydrolyses choline-containing esters. In humans it is synthesised in the liver and found in plasma. It is encoded by the BCHE gene (19).

It is very similar to neuronal acetylcholinesterase. The term "serum cholinesterase" is generally used to describe a biochemical test that reflects the activity of these enzymes in the blood. Determination of BCHE activity in the blood can be used as a

test of liver function since both elevated and decreased values have pathological significance (34).

It has a half-life of 10-14 days. Serum BCHE levels are lower in patients with advanced liver disease. If the decrease in plasma level is greater than 75%, it will cause prolonged neuromuscular blockade when succinylcholine is administered (34), (24).

BCHE has no unique physiological function that cannot be compensated by other enzymes. Humans and laboratory animals lacking serum BCHE activity are healthy, fertile and live to old age (35), (36). The functions of pseudocholinesterase become apparent when it interacts with certain substances. In humans, cocaine is detoxified to a pharmacologically inactive product mainly by BCHE.

BCHE is one of the esterases that inactivate the appetite stimulating hormone octanoyl-ghrelin (38). That BCHE may have a role in lipid metabolism has been observed by the fact that BCHE-deficient mice become obese when fed a high-fat diet and wild-type mice do not become obese when fed the same diet.

BCHE is being studied as a therapeutic agent in the prevention of toxic combat gas poisoning (30). Protection is achieved by rapid inactivation of the toxic agent by BCHE.

1.2 Variants of serum pseudocholinesterase

The *BCHE* gene encodes pseudocholinesterase (BChE), which metabolizes succinylcholine. More than 75 genetic variants of BCHE have been identified. Variants of BCHE have been correlated with deficiency of BCHE enzyme activity leading to higher than expected levels of succinylcholine and therefore prolonged neuromuscular blockade. Patients with these variants are usually asymptomatic until they are exposed to succinylcholine as a surgery-related event. The best known variant of BCHE is the 'atypical' variant (variant A) (25).

1.3 Normal values of serum pseudocholinesterase

Serum pseudocholinesterase has a normal level reported by commercial clinical laboratories as shown in Table 1. (23).

Table 1 Normal serum pseudocholinesterase values at present

Age and gender	***Values (U/L)***

children, men, women >40 years	***5320-12920***
women <40 years old, who are not pregnant and do not take oral contraceptives	4260-11250
women <40 years old, pregnant or taking oral contraceptives[2]	3650-9120

Thus, a value between 3650 and 1290 U/L could be normal but these values are thought to be an indicator of possible insecticide poisoning. Preoperative screening of serum pseudocholinesterase is used to detect patients with atypical forms of this enzyme, who are at risk of prolonged apnoea after administration of muscle blocker anaesthetics. Low serum pseudocholinesterase values may occur in :

- cases of organophosphate insecticide poisoning,
- chronic hepatitis,
- cirrhosis,
- myocardial infarction,
- acute infections
- in the presence of atypical phenotypes of this enzyme (23).
- in case of exposure to anabolic steroids, carbamates, cimetidine, cyclophosphamide, estrogens, glucocorticoids, lithium, neostigmine, neuromuscular relaxants (e.g. pancuronium, succinylcholine), oral contraceptives, organophosphate insecticides, phenelzine, phenothiazines, physostigmine, radiocontrast agents (e.g. iopanoic acid), ranitidine, streptokinase, testosterone.

Elevated enzyme values may occur in the healing period of acute hepatitis, nephrotic syndrome (due to exaggeration of hepatic proteosynthetic functions) and in cholinesterase-secreting tumours.

2. Liver failure

Liver failure can be regarded as an acute condition occurring in a patient without pre-existing liver disease and in this situation the term acute liver failure is used.

A progressive, sustained or plateaued deterioration of liver function over many years is chronic liver failure.

Diagnosis of impaired liver function is made using "static" or "dynamic" laboratory tests such as the indocyanine green clearance test. Liver transplantation remains the treatment of choice for fulminant, acute or sub-acute liver failure with poor prognosis estimated by King's College or Clichy criteria ideally in the absence of pre-existing liver disease and before extrahepatic complications develop. (33).

Current preclinical research suggests that programmed cell death is an important mechanism in the pathogenesis of acute and acute-on-chronic liver failure and thus there is potential for anti-apoptosis drug treatments.

3. Methods for assessing liver failure

Determining the severity of liver disease is an important element in improving diagnostic and therapeutic possibilities. Several serum markers, liver indices and scoring systems have been proposed to address this issue and provide prognosis. The most widely used are the Child-Pugh classification (CTP) and the Model of End-Stage Liver Disease (MELD) score. In addition, mathematical scores combining serum markers such as the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), AST/thrombocyte ratio (APRI) and fibrosis-4 (FIB-4) score have been developed to identify the presence and severity of chronic liver disease. In addition, liver density measurements by transient elastography (TE; Fibroscan) - a technology based on the measurement of the velocity of a wave produced by a mechanical pulse - have attracted attention over the last few years. (43).

The occurrence of liver fibrosis is a turning point in the course of chronic liver disease and the presence and severity of fibrosis correlates with prognosis without being influenced by etiology (44).

Liver biopsy is still the gold standard for detailed assessment of the location and extent of fibrosis (45) Although it yields extensive information and remains a key investigation in hepatology, the size of the biopsy specimen must be long enough to be interpreted by experts and yield reliable information.

Non-invasive, repeatable and ideally cheaper techniques are an alternative in the assessment of fibrosis. It is important to note that diagnostic measures of fibrosis in

chronic liver disease should have low inter- and intra-operative variability to allow comparison of results over time and that fibrosis is a dynamic process and may regress. Non-invasive tests should also have prognostic capacity in addition to assessing fibrosis and its complications (2).

Tests that allow the assessment of chronic liver diseases can be classified into:

a) serological tests (serum markers of fibrosis; laboratory variables**);**

b) "dynamic" liver function tests (indocyanine green**);**

c) methods assessing the physical properties of liver **tissue** (e.g. liver stiffness; attenuation; viscosity);

d) imaging methods that assess the anatomy of the liver and other organs. These approaches can be considered complementary in various situations. It should be emphasised that non-invasive tests, liver biopsy and invasive methods and clinical elements must be integrated in order to obtain a correct diagnosis and staging in chronic liver diseases.

4. Limitations in the use of current scores

Assessment of liver damage is an important step in patients with chronic liver disease. Although liver biopsy is the standard investigation for establishing necroinflammation and fibrosis, it has limitations as any invasive procedure and the need for repetition for dynamic monitoring of liver disease has led to the development of noninvasive tests as an alternative to biopsy. Such non-invasive approaches include biological (serum biomarkers) or physical (imaging and liver density assessment) elements. However currently available tests have many limitations such as variability, lack of accuracy. Many of the current tests were originally developed for chronic hepatitis C and then refined for NAFLD and then refined for chronic liver disease prognosis. An important consideration despite their increasing use in everyday practice is that they are not designed to reflect the dynamics of the fibrosis process , differentiate between adjacent stages of disease, diagnose non-alcoholic steatohepatitis or track longitudinal changes in liver fibrosis due to natural disease progression or due to therapeutic interventions.

Understanding the strengths and limitations of these tests can be used judiciously in the clinical setting as they are complementary rather than a replacement for liver biopsy.

5. Usefulness of cholinesterase in the evaluation of liver failure

The relationship between cholinesterase and liver failure is being explored by a variety of researchers . Serum pseudocholinesterase is a marker of liver function in cirrhotic

patients (35) and not only because it is exclusively synthesized by the liver like albumin and coagulation factors and thus its level is expected to decrease in patients with cirrhosis.

Multiple other studies have examined the utility of serum pseudocholinesterase as a possible static liver function test but the conclusions have been equivocal (98) (29) (99) (100) (101). Most of these studies examined patients with liver tumours or obstructive jaundice, conditions irrelevant today due to technological advances in radiology. In addition, new techniques have emerged that have made the measurement of serum pseudocholinesterase levels much simpler to perform.

Cirrhotic patients have a much lower level of serum pseudocholinesterase compared to healthy patients. Also, patients with decompensated cirrhosis have a lower cholinesterase level compared to those with compensated cirrhosis. Even after stratifying patients by MELD score the differences remained significant. The presence of infection or variceal bleeding did not affect the results (102). Serum pseudocholinesterase levels showed excellent correlation with serum albumin and reasonable correlation with serum bilirubin and INR indicating that pseudocholinesterase is a good indicator of liver functional reserve.

The fact that serum pseudocholinesterase level correlates with liver functional reserve has been proven in various clinical situations (103) . For example in familial intrahepatic cholestasis, after external biliary drainage, increased serum pseudocholinesterase levels indicate successful surgery (104). Li Q et al showed that in patients with severe chronic hepatitis, serum pseudocholinesterase levels were very low and correlated with increased mortality. The authors suggested a logistic regression model using pseudocholinesterase with superior prognostic value MELD (36). Because serum pseudocholinesterase levels provide substantial information about liver functional reserve, it may be useful in decisions to initiate non-hepatic surgery, liver resection or to perform transjugular intrahepatic portosystemic shunting (TIPS).

Measurement of serum pseudocholinesterase levels is an extremely simple procedure compared to other liver tests. A new score using pseudocholinesterase, serum albumin, and bilirubin has been proposed for the prediction of postoperative liver dysfunction in cardiac patients with liver dysfunction (105). A prognostic model using serum albumin, variceal size, and serum pseudocholinesterase level was superior to the Child-Pugh score in predicting 1-, 3-, and 5-year mortality in a cohort of patients with alcoholic cirrhosis (106).

Although normal pseudocholinesterase values appear to vary quite widely, each individual appears to retain his or her normal serum pseudocholinesterase value throughout life under normal circumstances (107). Low serum pseudocholinesterase values in a cirrhotic patient require follow-up and, if they persist at a low level, may be considered a marker of poor prognosis of liver disease. Improvement in serum pseudocholinesterase levels may be interpreted as a response to treatment for cirrhosis such as initiation of antiviral therapy for hepatitis B, steroid therapy in autoimmune hepatitis or chelation therapy for Wilson's disease. Patient follow-up shows the potential role of pseudocholinesterase in identifying acute-on-chronic liver disease. Very important decisions such as the need for liver transplantation can be made following monitoring of this parameter.

Looking at the overall contribution of this test to the diagnosis, monitoring and prognosis of cirrhosis identifies several important elements. Serum pseudocholinesterase is a single biochemical test available very easily and cheaply. It can be repeated whenever needed. Serum pseudocholinesterase has a similar course regardless of the aetiology of liver disease (14). In anticoagulated patients such as those with Budd Chiari syndrome, prothrombin activity and therefore INR are unusable in the calculation of scores. Also in patients receiving human albumin treatment for refractory ascites or hepatorenal syndrome type 2 , the true synthetic function of the liver is masked but can be assessed by serum pseudocholinesterase determination. Thus it can play an important role in assessing liver function in situations where PT, INR and albumin cannot be used. Ogunkeye et al suggested that serum pseudocholinesterase is a means of differentiating between the presence of liver disease or other pathologies in which tests used to assess liver disease are affected (108). Values tend to decrease with decreasing liver functional reserve (109). In patients with coexisting renal failure, when the serum creatinine value is elevated, the MELD score cannot be used to estimate the severity of liver disease. However the potential role of serum pseudocholinesterase requires extensive studies before a conclusion can be drawn. Further studies are also needed to ensure the use of serum pseudocholinesterase in the organ allocation protocol for transplantation, especially in situations with low MELD score such as the presence of refractory ascites. Serial dosing in ACLF may provide additional information on the restoration of liver functional reserve. It should be remembered, however, that in addition to liver failure, low serum pseudocholinesterase levels may also occur in chronic anaemia, neoplasia, heart failure, burns and radiotherapy.

In conclusion, serum pseudocholinesterase is an excellent biomarker for cirrhosis with good sensitivity and specificity. It has a good correlation with serum albumin, PT, INR and MELD score.

Because they differentiate well between compensated and decompensated cirrhosis, low levels of serum pseudocholinesterase can be considered another prognostic marker in advanced liver disease. Further studies are needed to define its place in medical practice.

2 Personal contributions

6. Working hypothesis and general objectives

Assessment of the presence of liver failure is a necessity in both patients with chronic hepatopathies, patients with acute-on-chronic hepatitis and patients undergoing interventional surgical or radiological procedures that decrease liver volume.

Liver resections and hepatic arterial embolizations require very careful preprocedural evaluation so as not to reduce liver functional reserve to a level incompatible with post-procedural survival.

In advanced stages, the presence of liver failure is easily detected by any of the static laboratory tests or scores, dynamic tests or liver volume assessments. The presence of cirrhosis is a guarantee of concomitant liver failure, but in the early stages of liver failure, more accurate tests are needed because static tests are often not affected.

Current scores are associations of clinical, ultrasound, biochemical parameters, laboratory tests, physical estimates of liver density or invasive - liver biopsy. Chronic liver diseases disproportionately affect various liver functions.

A new, more sensitive chronic liver disease staging score is a tool that, by better estimating the stage of liver disease, can increase life expectancy and adjust treatment or potentially necessary procedures.

BCHE is commonly used in China and is included in the PRC Gastroenterology Society's guidelines for the evaluation of patients with liver disease (110). Although Asian countries use this parameter, in Europe and America it is not used to the same extent.

The serum BCHE value could estimate the degree of liver failure in terms of protein synthesis capacity and, if confirmed, together with the physical estimation of the degree of fibrosis of the liver parenchyma and other biochemical parameters could form the basis of a new score to assess the stage of liver disease.

Establishing the serum BCHE ranges corresponding to the 3 stages of liver cirrhosis established by the Child Pugh score for a sample of Caucasian patients and then comparing

these values with the ranges identified by the Chinese Gastroenterological Society to prove that they are similar is the **first research hypothesis.**

With the advent of new curative treatments for chronic hepatitis C virus, we are in a new situation: curing patients with chronic liver disease. This situation allowed us to identify the evolution of the BCHE level before and after the removal of the hepatic C virus and implicitly to evaluate the evolution of the hepatic protein synthesis capacity after cure. **The second research hypothesis** is the increase in BCHE levels after removal of liver virus C and the increase in liver functional reserve after healing.

The third research hypothesis is to prove an increase in BCHE levels after liver transplantation in a patient with liver cirrhosis. Theoretically the BCHE value after transplantation should normalize.

The fourth working hypothesis is to prove the predictive character of serum pseudocholinesterase levels on the ability to estimate survival of serum cholinesterase levels. The higher the serum pseudocholinesterase level, the higher the life expectancy

In this paper we have tried to resolve these working hypotheses, but the subject can have multiple research valences and can also be useful in evaluating the therapeutic efficacy of some drugs or supplements.

The scientific objective of this thesis is to assay serum pseudocholinesterase levels in as large a sample of patients with chronic liver disease as possible and then monitor its levels over the years to identify patterns of evolution, the possibility of influence by pharmacological methods and to assess the ability to estimate survival of serum BCHE levels.

We identified 463 Caucasian patients, 276 women (59.61%) and 187 men (40.3%) with various liver pathologies.

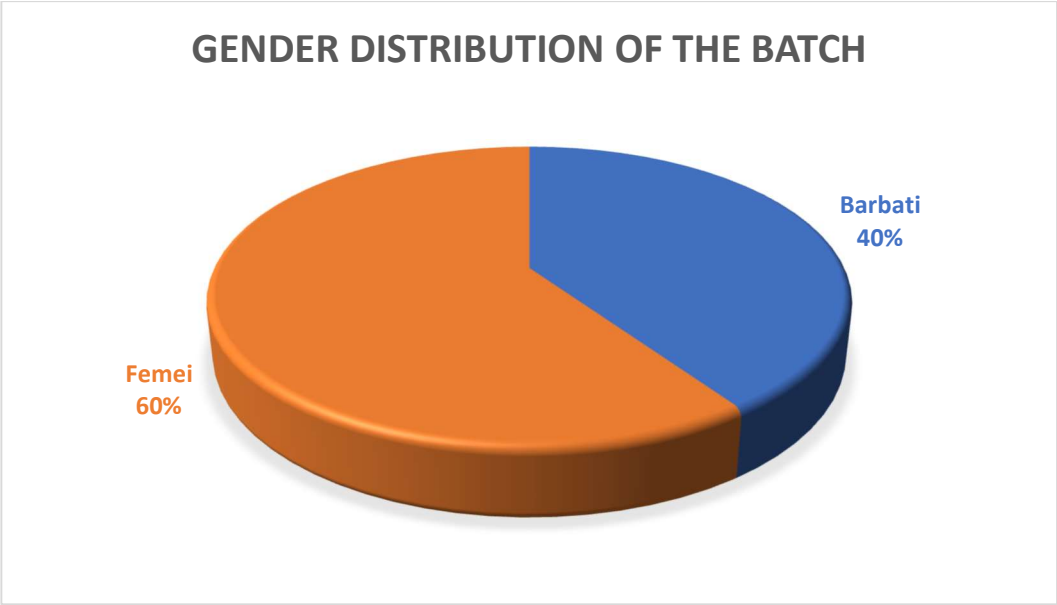

Figure 1 Gender distribution of the batch

The average age at study entry of patients included in the group is 58 years (minimum 21 years, maximum 87 years) and for women is 58.97 years SD 12, minimum 21 years, maximum 81 years and for men the average age is 55.56 years SD 13.99, minimum 24 years, maximum 87 years.

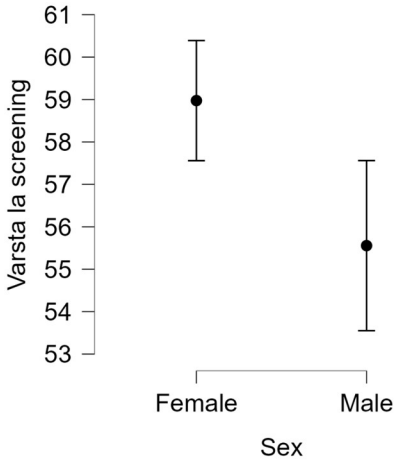

Figure 2 Interval plots / Age of screening patients compared by sex

In terms of chronic liver disease staging, the 463 patients fall into the Child-Pugh score (CTP) categories as follows: Child A 356 patients, Child B 63 patients and Child C 44 patients (Table 2, Figure 10).

Description of the lot according to the stage of the PTI

Child	Frequency	Percent	Valid Percent	Cumulative Percent
A	356	76.89	76.89	76.89
B	63	13.61	13.61	90.50

Description of the lot according to the stage of the PTI

Child	Frequency	Percent	Valid Percent	Cumulative Percent
C	44	9.50	9.50	100.00
Total	463	100.00		

Table 1 Batch distribution by TCO score

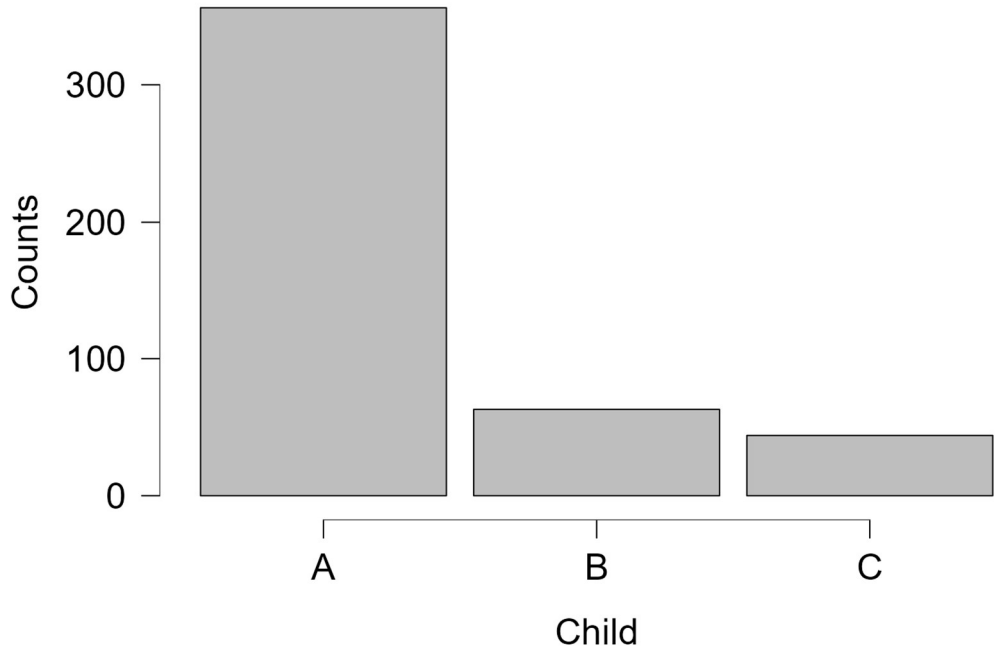

Figure 3 Lot distribution according to PTC stage

We started by assaying serum BCHE levels in patients with various liver pathologies and obtained a database of 463 patients with at least yearly BCHE levels in order to obtain time course curves.

For patients in each PTC class pseudocholinesterase dosages have a decreasing trend. Thus for Child A class mean BCHE value is 8748±2195, for Child B 4997±1481 and for Child C 2675±1372 (Table 3). This progressive decrease in BCHE value suggests that the BCHE level may correlate with the severity of liver disease and seems to be consistent with previous studies.

Descriptive statistics

	BCHE		
	A	B	C
Valid	356	63	44
Missing	0	0	0
Mean	8748.11	4997.00	2675.23
Std. Deviation	2195.84	1481.50	1372.16

Descriptive statistics

	BCHE		
	A	**B**	**C**
Minimum	3220.00	2054.00	980.00
Maximum	21197.00	9847.00	8416.00

Table 2 Pseudocholinesterase (BCHE) values for PTC stages

The first research hypothesis: to determine the serum BCHE ranges corresponding to the 3 stages of liver cirrhosis established by the Child Pugh score for a sample of Caucasian patients and then to compare these values with the ranges identified by the Chinese Gastroenterological Society showed that there is a concordance between the PTC stages and the serum BCHE level. In the light of these results I decided to follow the evolution of serum BCHE in patients with chronic HCV hepatitis receiving curative treatments for liver C virus infection.

Following BCHE dosing we observed that there is no increase in BCHE level after healing of liver C virus infection. Basically there is no increase in liver functional reserve after healing, this is the conclusion of the 2nd working hypothesis which is refuted.

To prove that the BCHE level reflects the functional liver reserve we performed BCHE measurements before and after liver transplantation in a patient with Child C nutritional toxic cirrhosis. Serum BCHE levels after liver transplantation increased to normal values, confirming that BCHE correlates with liver functional reserve and thus working hypothesis number 3 is true.

The next study followed the survival of patients with chronic liver disease over a period of 7 years. Serum BCHE levels were compared with survival. The results are promising but we could not obtain information on liver-related mortality. Thus the overall mortality, deeply influenced by COVID-19 mortality, and the rather small sample led to values that are difficult to interpret. A follow-up on a larger sample and over a longer time interval could lead to more valuable information. The results showed that serum BCHE levels correlate with survival, but the decrease in BCHE is not linear. At normal BCHE values decreases of 200 units are of less importance than decreases of 50 units from low basal values. Overall, however, BCHE correlates with survival, which led to the 4th working hypothesis: serum cholinesterase is predictive of survival. However, this last section requires a follow-up of a statistically significant group over decades.

Establishing the utility of BCHE in the assessment, staging and treatment of liver failure will benefit the approach to these patients in other specialties outside of Internal

Medicine and Gastroenterology. A patient with significant hepatic functional reserve will be able to undergo general surgery, liver resections or interventional radiology procedures that induce ischaemia of one or more liver segments or even liver lobes. Thus the usefulness of cholinesterase can be seen in patients in Surgery, Oncology, Cardiology and Transplant Medicine.

The evaluation of the 463 patients was not without limitations. The most important one was the COVID-19 pandemic which limited the mobility of patients and thus the number of visits during that period was lower, thus also the number of BCHE doses. The overall mortality of patients was also influenced by COVID-19 mortality. In addition, due to the small number of patients monitored we could not assess the average decrease in BCHE levels after resection or chemoembolization of one or more liver segments.

7. Study 1- Correlations between serum pseudocholinesterase value and Child-Pugh and MELD-Na scores

7.1 Introduction (working hypothesis and specific objectives)

We use liver function tests to determine the degree of liver failure in cirrhotic patients. These tests can also be influenced by other conditions. In contrast to these tests, serum pseudocholinesterase has low serum values in liver failure.

The purpose of this study is to determine whether there is a correlation between serum pseudocholinesterase levels and the level of liver failure.

The proteins synthesized by the liver are represented by ceruloplasmin, serum albumin, alpha1-antitrypsin, ferritin, lipoproteins and coagulation factors. These proteins are synthesised by the liver but serum levels may be influenced by other pathologies. Serum pseudocholinesterase is also synthesised by the liver and then released into the bloodstream (5).

7.2 Patients and Method

Patients. We selected 70 patients (28 males and 42 females) admitted to the Fundeni Clinical Institute , Bucharest , Romania, between 2017 and 2018 and were included in the study. Inclusion criteria were: diagnosis of liver cirrhosis (based on clinical examination or abdominal ultrasonography or elastography or histological data). Exclusion criteria were recent treatments with blood or plasma transfusions in the last month before enrollment,

clinical suspicion of recent variceal bleeding, known diagnosis of hepatocarcinoma or personal history of liver transplantation. I have obtained informed consent.

Measurement of serum markers. Blood samples were taken with minimal stasis. Serum was obtained by centrifugation within one hour of collection. Serum albumin, total bilirubin, serum creatinine, serum Na, ALT, INR and complete hemoleukogram were performed. Serum cholinesterase activity was determined by a chemiluminescence method with a Cobas e601 Roche analyzer within 2 hours of sample centrifugation..

Statistical analysis. Statistical analysis and database management was performed using JASP 0.16.3 Software for Windows11. Descriptive results were presented as mean ± standard deviation (SD) or number (percentage) of patients. We used multiple comparisons for ANOVA test and they were compared with the mean of the data. We applied The Pearson correction test to compare serum cholinesterase , serum albumin and prothrombin time (INR). The tests were two-tailed and $P<0.05$ was considered to indicate a statistically significant difference

7.3 Results

Patient characteristics. A total of 70 patients with a mean age of 66.47±10.41 years were selected. 28 patients were male with a mean age of 63.64±12.74 years and 42 patients were female with a mean age of 68.35±8.15 years.

In Child-Pugh class A there were 43 patients (61.42%), in class B 13 patients (18.57%) and in class C 14 patients (20%) (Table 9).

Serum pseudocholinesterase in Child-Pugh groups. The results showed that serum pseudocholinesterase levels tended to decrease significantly when moving from one grade to another: Child A (8055.465±1709.092 U/l), Child B (5415.769±1109.270 U/l) and Child C (2543.643±838.512 U/l) (Table 4). The difference between the mean serum pseudocholinesterase in Child A, B and C groups was statistically significant, as was the difference between the mean values for Child B and C groups (Table 9, Figure 17, Figure 18).

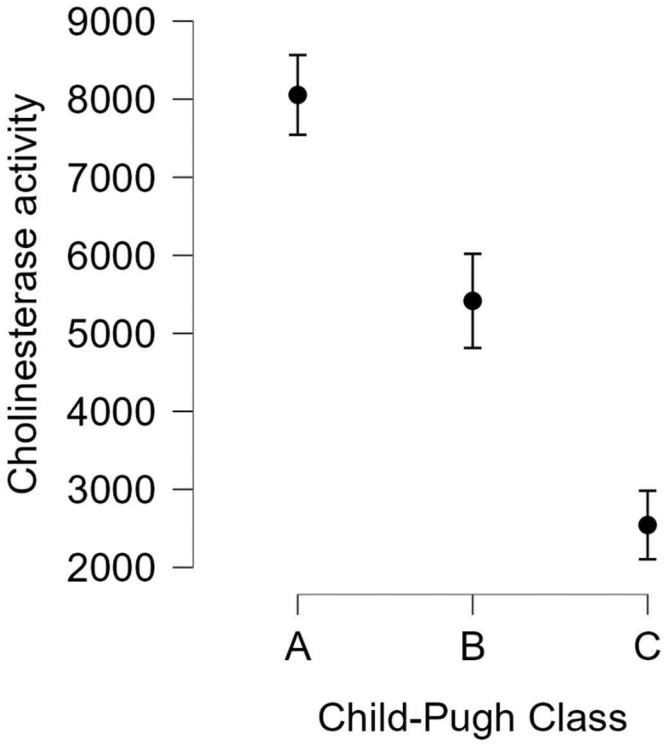

Figure 4 Pseudocholinesterase activity

Correlations between serum cholinesterase, albumin and INR. Serum cholinesterase correlated positively with albumin (r=0.633, P<0.001) and correlated negatively with INR (r=-0.404, P<0.001) in these patients. This confirms that these substances were synthesized by the liver and their decrease in serum in liver failure confirms decreased hepatic synthesis. Cholinesterase and INR (r=-0.404, p=0.001) have a strong negative correlation. Albumin and INR (r=0.782, p=0.001) have a strong positive correlation (Table 10, Table 11, Table 12).

Correlations between serum pseudocholinesterase, Child-Pugh score and MELD-Na score. Serum pseudocholinesterase and Child-Pugh score have a strong, negative correlation (r=-0.696, p=0.001). Pseudocholinesterase and MELD-Na score (r=-0.548, p=0.001) have the same strong, negative correlation. Between Child-Pugh score and MELD-Na score (r=-0.783, p=0.001) there is a strong positive correlation validating the data (Table 10, Table 11, Table 12, Table 13).

Serum pseudocholinesterase grouped according to Child-Pugh score for patients with liver C virus. We selected 52 patients with liver C virus infection and divided them into groups A, B and C according to Child-Pugh score. The results indicate that serum cholinesterase level has a significant decreasing trend in the 3 groups: Child A (8237.541±1572.758 U/l), Child B (5390.111±1167.291 U/l) and Child C

(2702.667±1011.552 U/l) (Table 14), similar to the results in the whole group. The difference between the mean serum cholinesterase activity in Child A, B and C groups was statistically significant, as was the difference between the mean values of Child B and C groups (Table 14, Figure 19). The values are similar to the values obtained for the whole group.

7.4 Discussion

In the Child A group, the serum cholinesterase value was 8055.46U/l with an SD of 1709.09U/l, in the Child B group the mean serum cholinesterase value was 5415.76U/l with an SD of 1109.27 and in the Child C group the mean serum cholinesterase value was 2543.64U/l with an SD of 838.51U/l. The results obtained are similar to the data published by Gu and Zhong (26). Their data showed that serum cholinesterase levels for each Child/Pugh group were: Child A (5978±535U/l), Child B (3957±454U/l) and Child C (2267±332U/l). The Child-Pugh score is calculated from five characteristic clinical parameters of liver disease. Two parameters, ascites and encephalopathy, are subjective in nature (118). Cirrhosis of the liver is stratified into 3 Child-Pugh classes A, B and C using the above score. Serum cholinesterase compared to the Child -Pugh score, has the advantage that it is easier to obtain (it is a single serological biochemical test), more objective in assessing liver functional reserve and has a very low cost.

Protein synthesis is carried out eminently by the liver. Serum cholinesterase, serum alumina and coagulation factors are synthesised by the liver and then released by the liver into the bloodstream. Thus, liver function tests including albumin, cholinesterase and prothrombin time (INR) have proven useful in estimating liver function reserve in a patient with liver cirrhosis. Serum cholinesterase correlates positively with albumin and negatively with INR, confirming that these proteins are produced by the liver and reduced in liver dysfunction secondary to decreased protein synthesis.

In patients with decompensated liver disease (Grade B and C of Child-Pugh score), albumin and blood transfusions are used as standard treatment, which may change the blood values of these parameters used to calculate the Child-Pugh score (46). If surgery is required, the risk calculated with the Child-Pugh score may be incorrect in patients treated with albumin or plasma transfusions or clotting factors. (119). In their study, Gu and Zhong (26) demonstrated that three cirrhotic patients (two with Child B and one with Child A) developed encephalopathy after porto-azygos disconnection surgery with cholinesterase levels below 2000U/l. (33) Thus, these authors suggested that cirrhotic patients with cholinesterase values <2000U/l may be at high risk of liver failure if they require surgery.

Thus, the combination of Child-Pugh score and serum cholinesterase level may be more objective and accurate in assessing the liver functional reserve of cirrhotic patients and may be useful for surgeons in deciding the timing of surgery or for gastroenterologists in adjusting the intensity of liver treatments accordingly.

7.5 Conclusions

Our study demonstrates that the severity of liver cell dysfunction correlates with serum cholinesterase value. Also serum cholinesterase can estimate liver functional reserve of cirrhotic patients. Compared to current scores (Child-Pugh score and MELD-Na score), serum cholinesterase is a less complex, cheaper, more readily available test, available in all emergency departments and not influenced by treatments for decompensated liver disease. The combination of cholinesterase and Child-Pugh score or MELD-Na score may be more objective and sensitive in assessing liver functional reserve in cirrhotic patients, but more studies are needed.

8. Study 2 - Variation in serum pseudocholinesterase levels during direct antiviral therapy for chronic hepatitis C virus

8.1 Introduction

The introduction of new treatments for chronic liver C virus infection brings us to a new situation where many variables are as yet unknown. In Romania, thousands of patients with chronic liver C virus infection are being treated with the new treatments.
On a group of 50 patients we determined the BCHE value before the initiation of AAD treatment and at the end of AAD treatment. All patients had undetectable viremia at the end of treatment.

8.2 Patients and methods

Patients. A total of 50 patients with chronic hepatitis C virus admitted to the Internal Medicine Department 2 of Fundeni Clinical Institute, Bucharest, Romania, between June 2017 and June 2018. Inclusion criteria were established by the National Health House for the chronic hepatitis C virus treatment program. Thus patients with positive viremia, Child-Pugh A score, Fibromax F3 or F4, no history of liver neoplasia, no HBV or HIV hepatitis were included. Exclusion criteria were decompensated liver disease and presence of neoplasia.

Measurement of serum markers. Blood samples were collected with minimal venostasis. Serum was obtained by centrifugation of the vaccutainers within 60min of collection. Serum butyrylcholinesterase, HCV-RNA and Fibromax were assayed in an external laboratory approved by CNAS - Regina Maria Bucharest.

Statistical analysis. Database management and statistical analysis were performed using SPSS ver.23, Chicago, USA. Descriptive results were expressed as mean ± standard deviation (SD) or as number or percentage.

8.3 Results

Patient characteristics.

The sample consists of 50 patients, 13 men and 37 women , mean age 62.5± 8.48 years known to have chronic hepatitis C virus. Demographic data are represented in Table 1.

After inclusion patients were treated with ombitasvir, paritaprevir, ritonavir and dasabuvir for 12 weeks. At the end of the 12 weeks they were reassessed clinically, sonographically and biologically. All patients had undetectable HCV-RNA viremia at the end of treatment and at 12 weeks after completion of treatment were classified as sustained viral response.

Dynamics of serum cholinesterase.

Serum cholinesterase had a mean value of 7304±2223.37 U/l before treatment and after 12 weeks of treatment it had a mean value of 6792±2063U/l.

Dynamics of liver function tests

Transaminases have a favourable evolution with a decrease in ASAT from 76 IU/L to 24.63 IU/L and ALAT from 62 IU/L to 24.6 IU/L, both values falling within physiological values after treatment. In the case of total bilirubin, serum creatinine, serum albumin, prothrombin time, and serum sodium the values are stationary.

HCV-RNA viremia decreased from an average of 1095696IU to undetectable.

8.4 Conclusions.

The results of the study showed a slight decrease in mean serum cholinesterase after treatment with direct-acting antivirals from 7304 U/l to 6792 U/l. Both values fall within Gu and Zhong's estimate for Child A stage of 5368.04±1657.32 U/l (YB, 2010).

Both the baseline serum pseudocholinesterase value and the mean value obtained after confirmation of sustained viral response show a maintenance of the Child-Pugh score

at stage A. We did not identify any increase in serum pseudocholinesterase levels or a significant decrease in serum pseudocholinesterase levels after confirmation of sustained viral response secondary to treatment of chronic liver C virus infection with direct-acting antivirals.

Since it has been previously shown that the level of serum pseudocholinesterase correlates with liver functional reserve, basically with the mass of functional hepatocytes, we can state that there is no variation in it after removal of liver virus C from hepatocytes.

This lack of change in serum pseudocholinesterase levels refutes the hypothesis of an improvement in liver functional reserve after removal of liver C virus infection. Long-term monitoring of these patients is necessary.

The other parameters monitored show a benefit only in the case of normalising transaminases, the rest of the parameters having a stationary evolution.

This shows that the process of hepatocytolysis is halted following removal of liver virus C.

9. Study 3 - Case presentation Serum Pseudocholinesterase variation before and after liver transplantation

9.1 Introduction

Liver transplantation is the last therapeutic option for patients with end-stage liver disease. In liver transplantation, an increase in serum cholinesterase activity may confirm the clinical value of this test.

We followed a patient admitted to the Fundeni Clinical Institute, Internal Medicine Department 2. This patient received a whole liver transplant from a deceased patient for Child C toxic-nutritional cirrhosis. We obtained informed consent and performed clinical examination, abdominal ultrasonography, laboratory tests and serum cholinesterase determination in our department. Liver transplantation was also performed in our hospital. We obtained blood samples for laboratory tests before and after liver transplantation. Postprocedurally we followed the patient for five years and repeated blood tests and serum cholinesterase.

9.2 Presentation of the case

We selected a male patient with end-stage liver disease of toxic-nutritional etiology who received a whole liver transplant from a deceased donor. The patient was 60 years old at the time of transplantation. Physical examination identified sclerotegumentary jaundice,

liver of cirrhotic consistency, splenomegaly and grade 3 encephalopathy. Blood tests identified moderate anemia and thrombocytopenia, prolonged clotting test times, low serum albumin, mild hepatocytolysis, and Child-Pugh and MELD-Na scores with values consistent with severe liver failure. All parameters indicated end-stage liver disease and urgent indication for liver transplantation. The pre-transplant serum cholinesterase value was very low , with a value of 2700 U/L (Table 1).

Parameter	Before liver transplant	After liver transplant
Age (years)	60,00	65,00
Cholinesterase BCHE (U/L)	2700,00	7634,00
Hb (g/dL)	10,10	14,50
Leu (x10^3/µL)	4,60	4,58
Plt (x10^3/µL)	51,00	90,00
PA (%)	36,00	69,00
INR	2,14	1,28
Fibrinogen (mg/dL)	194,00	351,00
Albumin (g/L)	2,40	4,49
ALAT (U/L)	63,00	47,00
ASAT (U/L)	105,00	40,00
Total Bilirubin (mg/dL)	10,10	0,90
Direct bilirubin (mg/dL)	9,30	0,30
GGT (U/L)	36,00	7,00
FA (U/L)	141,00	52,90
Creatinine (mg/dL)	1,27	1,25
Serum Na (mmol/L)	133,00	145,91
Encephalopathy grade	3,00	0,00
Ascites (ultrasound)	absent	absent
Child-Pugh score	12 pts (Child C)	5 pts (Child A)
Meld-Na Score	28 pts	11 pts

Table 3 Patient characteristics

The liver transplant was performed and the patient survived the surgery. We monitored the patient and after five years he was re-evaluated using the same procedures (Table 1).

9.3 Discussion

Liver transplantation had a positive effect on liver function and serum pseudocholinesterase levels. The Child-Pugh score decreased from 12 pct (Child C class) to 5 pct (Child A class) and the MELD-Na score decreased from 28 pct to 11 pct. These scores showed that liver function became normal after transplantation (Table 15).

Serum pseudocholinesterase increased from 2700 U/L to 7634 U/L and thus the serum cholinesterase (BCHE) value after liver transplantation is in the normal range.

This evolution of scores is consistent with the hypothesis that liver transplantation will increase serum cholinesterase. We also observed improvements in serum albumin, INR, prothrombin activity, haemoglobin, fibrinogen, transaminases, total bilirubin and serum Na levels.

Normalization of liver functional reserve resulted in improvement of all these parameters.

9.4 Conclusion

This study demonstrates the value of increased serum cholinesterase activity after liver transplantation. Improvements in Child-Pugh and Meld-Na scores confirm that liver functional reserve increased after liver transplantation.

The new liver is responsible for increasing the liver's functional reserve. Serum pseudocholinesterase can be a useful tool in assessing liver functional reserve before and after liver transplantation.

10. Study 4: Relationship between serum pseudocholinesterase and survival

10.1 Introduction

Serum pseudocholinesterase is an enzyme that is secreted, as mentioned above, by the hepatocyte. Over the lifetime of a cirrhotic patient the level of this serum enzyme has a downward trend. Levels are high in patients with normal liver function. Over time the cholinesterase value progressively decreases as the patient becomes cirrhotic in Child-Pugh A, then Child-Pugh B then Child Pugh C. As previously shown, if the serum cholinesterase value falls below 2000ui , the risk of perioperative death is extremely high. In addition patients with a value below 6000ui have a much higher risk of death than those with a value above 6000ui.

Contemplating these data the following question can be asked: is the value of pseudocholinesterase predictive of the life expectancy of a cirrhotic patient?

If so, then the serum cholinesterase value could be extremely useful as a prognostic element of the overall survival of a cirrhotic patient and not only for estimating perioperative mortality.

A serum pseudocholinesterase value could place the patient on an expected normal or abnormal curve. A faster or slower degradation of pseudocholinesterase values could provide additional information.

10.2 Patients and method

We selected 405 patients with a total of 1188 butyrylcholinesterase (BChE) doses for the study. Demographic and clinical characteristics of patients at enrollment included age, gender, etiology of liver disease, and degree of liver fibrosis. A summary of these characteristics is shown in Table 16.

Patient characteristics at enrolment	**N = 405[1]**
Age	60 (51, 67)
Sex (male)	156 (39%)
Etiology[2]	
TN	35 (8.6%)
VHB	90 (22%)
VHC	280 (69%)
Child-Pugh classification	
Class A	307 (76%)
Class B	57 (14%)
Class C	41 (10%)
Degree of fibrosis	
F0-1	105 (26%)
F2	100 (25%)
F3	142 (35%)
F4	58 (14%)

[1]Reported statistics: median (IQR); n (%)

2Abbreviations: NT, toxic-nutritional (alcoholic); HBV, chronic hepatitis B virus; HCV, chronic hepatitis C virus.

Table 4 Characteristics of patients at enrolment

On average, patients had 2 to 3 doses of serum cholinesterase, with a minimum and maximum of [1, 6] doses. The maximum duration of the follow-up period was 4 years (48 months), at the end of which 367 patients remained event-free (i.e. death) and 38 (9.4%) died. Overall survival was 74.41% (95% CI 84.77% - 65.31%) at the end of the study.

The Kaplan-Meier evolution of the overall survival probability is illustrated in Figure

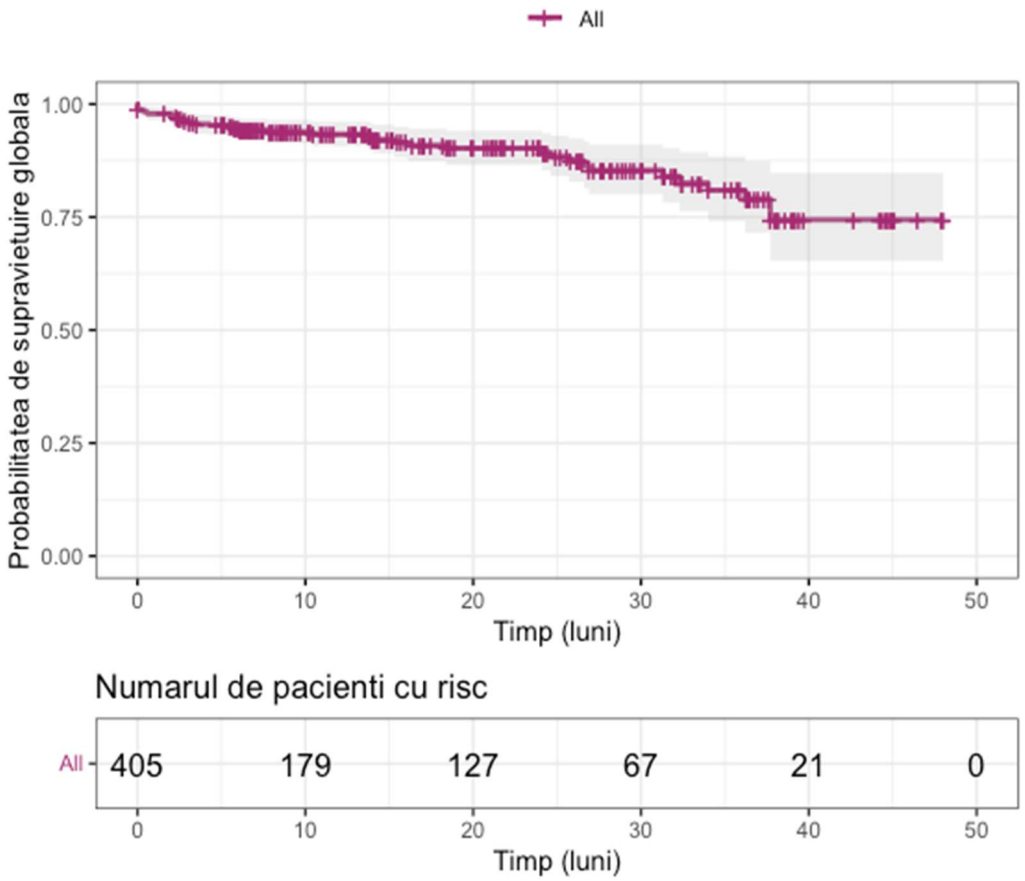

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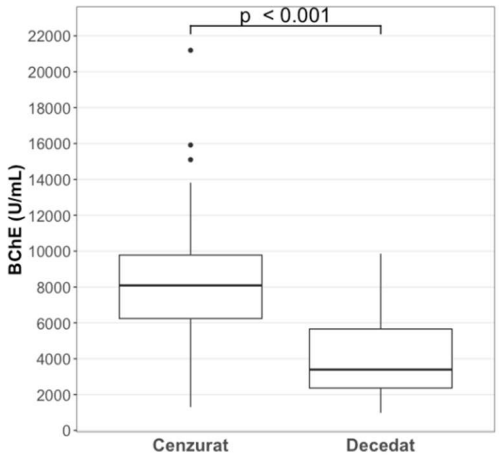
Figure 5 **Kaplan-Meier curve estimating overall survival together with 95% IÎ** .
Abbreviations: IÎ, Confidence Interval.

A first exploratory analysis of the data shows that, at the time of enrolment, BChE levels in the deceased patients group are significantly lower ($p < 0.001$) than the values of patients in the non-event group. Figure 21 shows the biomarker distributions in both groups together with the p-value of their statistical difference, calculated according to the Mann-Whitney *U* test.



21: BChE levels at enrollment for censored and deceased patients, respectively.

Similarly, survival probabilities for patients with a BChE level below 6000 U/mL at first dosing are significantly lower than those of patients with dosages above that level. In Figure 22, Kaplan-Meier analysis and log-rank tests show significant differences ($p < 0.0001$) between the 2 survival curves.

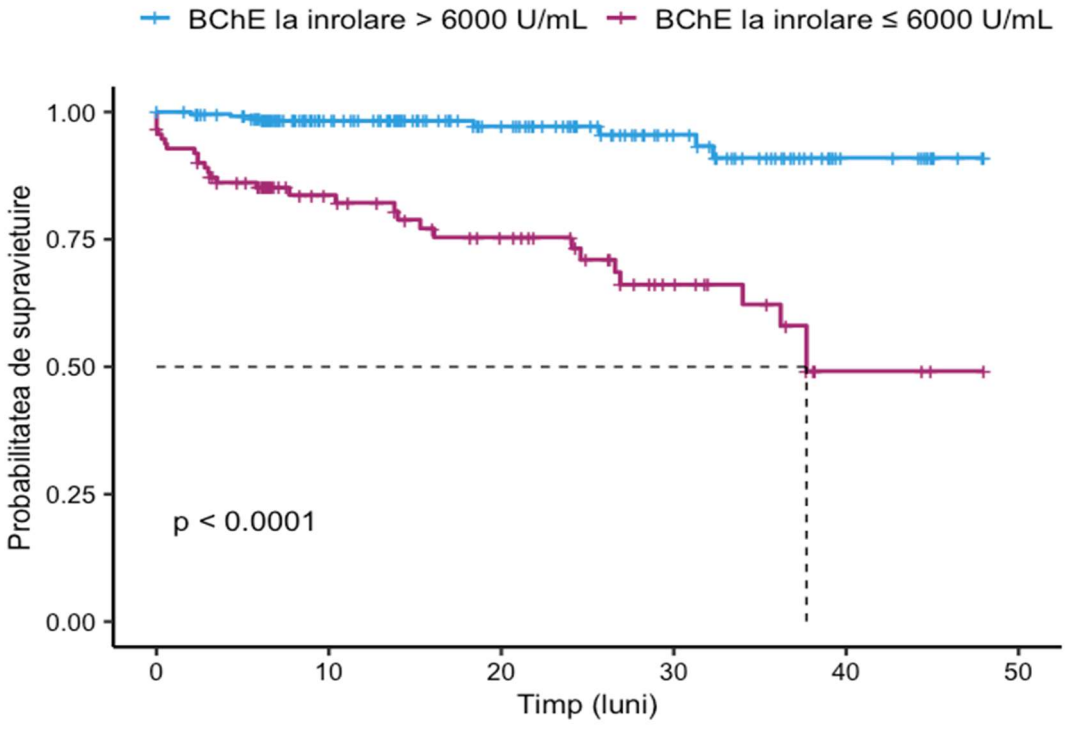


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Figure 6 Relationship between BChE level at enrolment and survival prognosis.

Etiology	BChE (U/mL)			
	Media	**Dev.Std.**	**Median**	**IQR**
TN	4622.11	2674.78	4517.0	3203.00
VHB	9112.04	2834.37	8919.0	2867.00
VHC	7429.50	2620.99	7613.5	3757.25

Table 5 Characteristics of BChE levels (U/mL) according to liver etiology.

Kaplan-Meier curves and log-rank tests demonstrate significant differences ($p < 0.0001$) between the survival probabilities of patients with different liver aetiologies.

The survival rate for patients in the TN group was 29.53% (95% CI, 14.2% - 61.2%), for those in the HCV group it was 78.49% (95% CI, 68.5% - 89.9%), and for HBV patients it was 100%.

The median survival time was 32.3 [7.7, -) months in the TN group. For the other groups, the survival rate never dropped below 50%, therefore, the mean survival time cannot be specified.

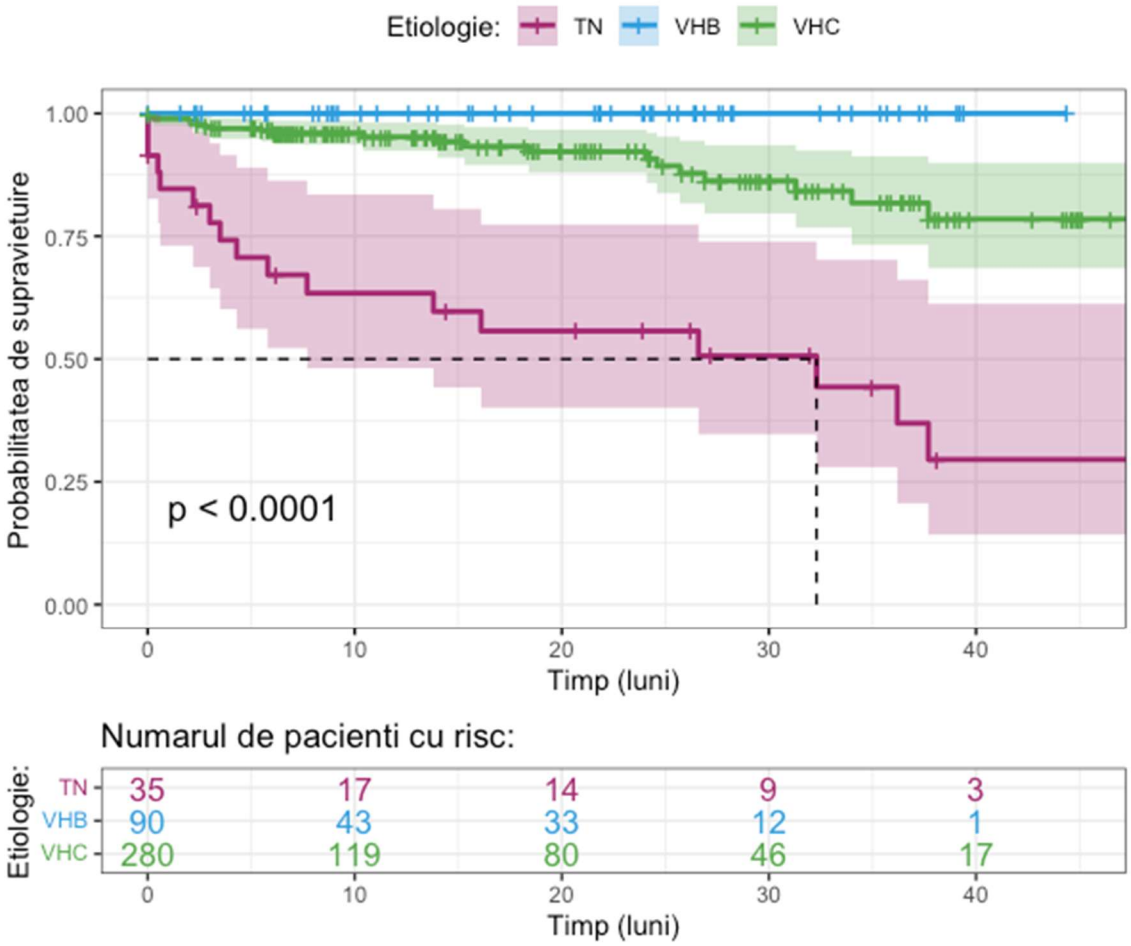


Figure 7 Kaplan-Meier survival curves and 95% IÎ by liver etiology .

The present work aims to describe the evolution over time of the BChE biomarker according to the aetiology of liver disease and to study its association with the risk of patient mortality.

In many studies, biomarker levels change during the follow-up period, and their evolution over time may be relevant in determining mortality risk, sometimes being more important than the actual biomarker level at enrollment or at last dosing. However, a difficulty related to such markers is that their levels are known only at medical visits. Therefore, in order to analyse the association between biomarker evolution over time and the probability of survival of an individual, an estimation of biomarker values between dosing has to be made.

10.3 Results

The hazard ratio is HR = 0.96 (95% CI: 0.93; 0.99), with all other risk factors held constant. If we want to interpret the result in terms of decreasing √BChE, then HR = 1.041. In this case, we would say that decreasing the √BChE level by one unit increases the risk of

death by 4%. Returning to the original terms of the BChE scale, we would say, for example, that we expect a 4% increase in the risk of death at a decrease of about 80 U/mL in the BChE level when it is around 1500 U/mL and at a decrease of about 250 U/mL when the BChE level is around 15 000 U/mL, respectively.

An analysis of mean serum pseudocholinesterase levels by age of patients in this study, divided by decades, shows a progressive decline. From decade to decade serum pseudocholinesterase decreases on average by 629 U/mL/decade. And the mean value of serum pseudocholinesterase in the 80-90 decade is 5591U/mL , close to the level from which increased mortality of 6000U/mL was observed.

The study group also includes 46 deceased patients consisting of 21 women with an average age of 66.43 years and 24 men with an average age of 66.83 years.

This group of patients had mean serum pseudocholinesterase values of 6687.93U/mL for Child-Pugh A, 3477.18U/mL for Child-Pugh B and 2634.47U/mL for Child-Pugh C (Table).

Table 6 - Mean serum pseudocholinesterase value of deceased patients grouped by Child-Pugh score

	BCHE value		
	A	B	C
Valid	15	11	19
Missing	0	0	0
Mean	6687.93	3477.18	2634.47
Std. Deviation	2472.47	979.69	1228.31
Minimum	2237.00	2054.00	980.00
Maximum	9856.00	5425.00	5398.00

Serum pseudocholinesterase values follow the trend of progressive decrease with Child-Pugh score from A to C, as does the overall group.

Grouping the subgroup of deceased patients according to the degree of liver fibrosis obtained by Fibroscan elastography shows that this is not an ideal correlation. For F0-1 liver fibrosis grade patients the mean serum pseudocholinesterase value was 5507.0 U/mL, for F2 the BCHE value was 7225.0U/mL, for F3 the BCHE value was 4720.71U/mL and for F4 high liver fibrosis grade patients the BCHE value was 3236.04 U/mL. There is a decreasing trend in mean BCHE from F2 to F4 but the mean BCHE value in those with F0-1 fibrosis is lower than those with F2 liver fibrosis. This may also be explained by the fact that there was

only one patient who died in the F1 liver fibrosis group or by the fact that in patients with early stages of fibrosis, liver functional reserve does not correlate with liver fibrosis.

Table 7 - Serum pseudocholinesterase value according to the degree of liver fibrosis of patients with deceased hepatopathies

	BCHE value			
	F1	F2	F3	F4
Valid	1	5	14	25
Missing	0	0	0	0
Mean	5507.00	7225.00	4720.71	3236.04
Std. Deviation	NaN	3899.35	2427.52	1521.86
Minimum	5507.00	980.00	1197.00	1127.00
Maximum	5507.00	9856.00	9560.00	7319.00

From the observations so far I believe that a classification of liver failure / liver functional reserve can be established, independent of liver fibrosis or other parameters, with 4 stages of liver failure table. Stage L0 represents the healthy patient with normal liver function and normal functional reserve. The serum pseudocholinesterase value in this stage is above 6000U/mL and is in line with current normal serum pseudocholinesterase values (see Table 2). In stage L1 patients have mild liver failure and the serum pseudocholinesterase value is between 4500 and 6000U/mL and corresponds to Child-Pugh stage A. In stage L2 - moderate liver failure , the serum pseudocholinesterase value is between 2500 and 4499U/mL and corresponds to Child-Pugh stage B. In stage L3 - severe liver failure - the pseudocholinesterase value is below 2500U/mL and corresponds to Child-Pugh stage C.

Table 8 - Proposed Stages of liver failure corresponding to serum pseudocholinesterase levels

Status	**Interpretation**	**BCHE value**
L0	Normal liver function reserve	Over 6000 U/mL
L1	Slightly decreased liver functional reserve	4500 - 6000 U/mL
L2	Moderately low liver functional reserve	2500 - 4499 U/mL
L3	Severely decreased liver function reserve	Below 2500 U/mL

Table 9 - BCHE value grouped according to proposed degrees of liver failure

	BCHE value			
	L0	L1	L2	L3
Valid	8	9	18	10
Missing	0	0	0	0
Mean	8488.38	5289.89	3137.39	1663.40
Std. Deviation	1470.83	529.44	536.67	551.56
Minimum	6201.00	4595.00	2509.00	980.00
Maximum	9856.00	5904.00	4049.00	2310.00

Table 10 Serum pseudocholinesterase values according to stages of liver failure L0-L3

The patients in the study group, divided into the 4 proposed liver failure groups, have a mean serum pseudocholinesterase value progressively decreasing from L0 to L3 confirming the proposed ranges in this sample (Table 24).

If we follow the mean BCHE values in patients with chronic liver disease grouped in 10-year age groups, we can see that by the age of 80 years all of them are in the L0 liver failure or normal liver functional reserve grade. In the decade 80-90 years the average value of BCHE is 5591 U/dL which falls in the L1 stage - Slightly decreased liver functional reserve (Figure 16).

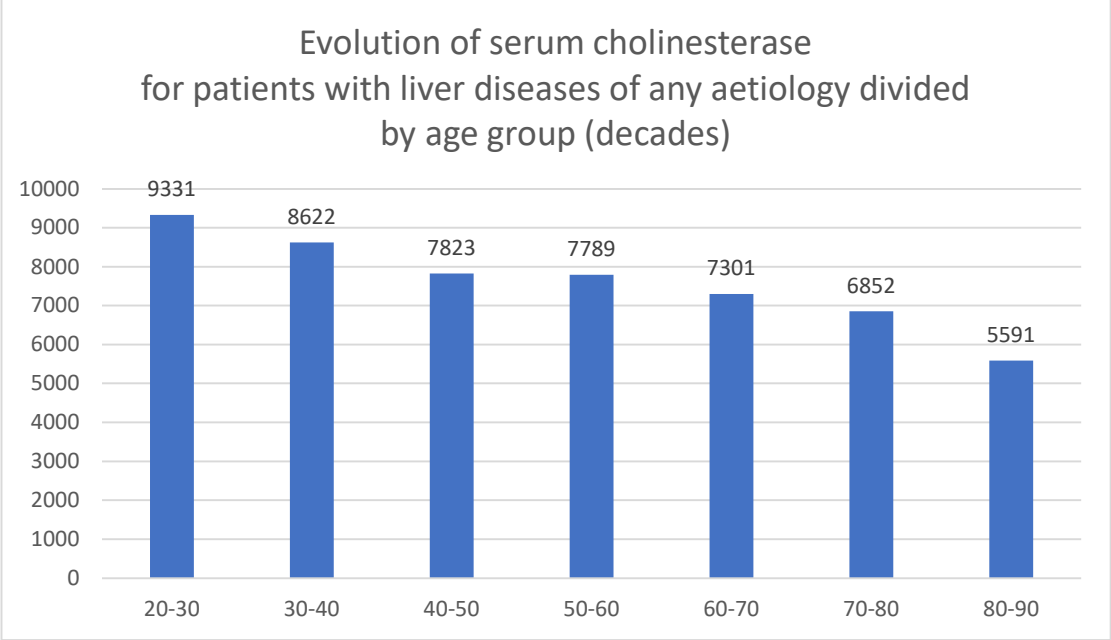


Figure 8 Mean serum pseudocholinesterase in patients with liver disease grouped by age (decades)

Analysing the BCHE value in Child-Pugh score groups along with days survived from BCHE dosing and age of death grouped in the same way it can be seen that BCHE has

progressively lower values towards Child C group. The mean survival in days does not have a typical distribution , the shortest being in Child B patients and the age at death of these patients is similar , around 68 years regardless of group.

Table 11 - BCHE descriptive statistics, Survival and age of death in the subgroup of deceased patients stratified by Child class

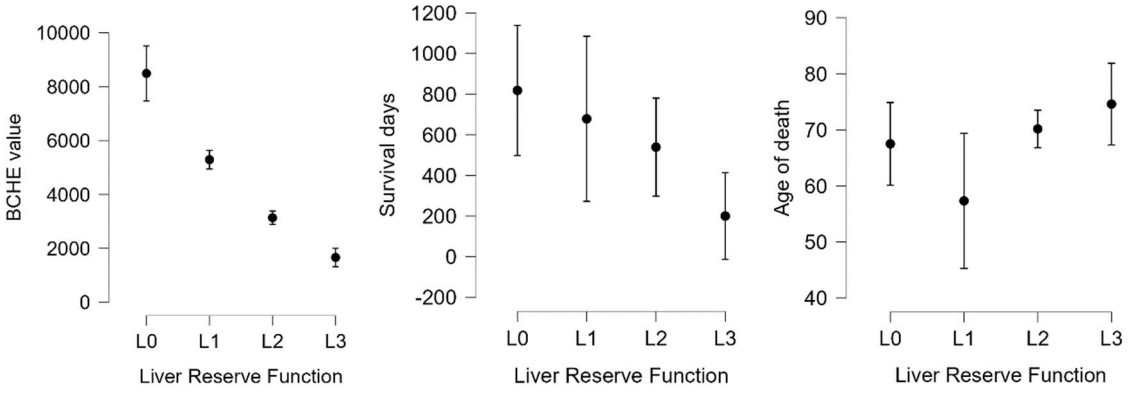
	BCHE value			Survival days			Age of death		
	A	B	C	A	B	C	A	B	C
Valid	15	11	19	15	11	19	15	11	19
Missing	0	0	0	0	0	0	0	0	0
Mean	6687.93	3477.18	2634.47	602.40	284.45	641.68	65.87	69.73	68.95
Std. Deviation	2472.47	979.69	1228.31	448.13	375.71	625.28	13.61	10.26	13.78
Minimum	2237.00	2054.00	980.00	61.00	0.00	0.00	28.00	55.00	29.00
Maximum	9856.00	5425.00	5398.00	1496.00	1146.00	1852.00	83.00	87.00	90.00

Table 6 - BCHE descriptive statistics, survival and age of death in the subgroup of deceased patients stratified by stage of liver failure

Descriptive Statistics

	BCHE value				Survival days				Age of death			
	L0	L1	L2	L3	L0	L1	L2	L3	L0	L1	L2	L3
Valid	8	9	18	10	8	9	18	10	8	9	18	10
Missing	0	0	0	0	0	0	0	0	0	0	0	0
Mean	8488.38	5289.89	3137.39	1663.40	818.25	678.44	539.11	200.10	67.50	57.33	70.17	74.60
Std. Deviation	1470.83	529.44	536.67	551.56	462.52	621.81	522.14	343.37	10.64	18.42	7.23	11.74
Minimum	6201.00	4595.00	2509.00	980.00	130.00	0.00	0.00	0.00	49.00	28.00	62.00	55.00
Maximum	9856.00	5904.00	4049.00	2310.00	1496.00	1852.00	1629.00	1102.00	83.00	78.00	87.00	90.00

Interval plots



Another risk factor statistically significantly associated d.p.d. with mortality was the etiology of liver disease ($p = 0.0252$). The degree of liver fibrosis was not found to be a significant risk factor for patient death in the JM analysis including BChE progression as one of the model predictors. However, the degree of liver fibrosis will remain relevant ($p < 0.0001$) in a survival model that does not include the biomarker as a risk factor. The results of the latter analysis appear in Table 25.

10.4 Conclusions

The degree of liver failure seems easy to estimate with current scores, but in everyday practice things are not very clear. We apply the Child-Pugh and Meld-Na scores at the initial assessment of patients with liver disease, at annual assessments or if we are in need of surgery or interventional radiology.

The application of these scores can provide relevant information on the patient's prognosis or survival under the conditions of surgery or chemoembolisation of one or more liver segments, TIPPS or even liver transplantation, but cannot be applied in every situation and to every patient.

Anticoagulated patients, those receiving transfusions or parenteral albumin cannot be correctly assessed with these scores. In addition, patients who do not have cirrhosis of the liver can be included in the Child-Pugh A stage because, as we have shown, total bilirubin, serum albumin, INR, presence of ascites and presence of encephalopathy are used to calculate the score. So a patient without significant liver disease will receive a Child-Pugh classification of A. This stage shows that the patient's prognosis is very good if they go through surgery but will also include patients who may not be at the stage of liver cirrhosis.

Serum cholinesterase activity can differentiate a patient with Child-Pugh A score with normal liver reserve (stage L0) from a patient with Child-Pugh A score with mild liver failure (stage L1).

11. Conclusions and personal contributions

Serum pseudocholinesterase is a plasma enzyme without a well-established role at this time. It is responsible for the rapid degradation of anaesthetics. It is a protein synthesised by the hepatocyte and then excreted into the plasma. The plasma level of this enzyme is not influenced by treatments and thus can be used in the assessment of hepatic protein synthesis function.

Liver protein synthesis function is useful in estimating liver functional reserve . Basically, liver functional reserve represents the capacity of the organ to tolerate transient insults to liver function and subsequently recover its previous capacity to function or a level of function that allows the patient to survive after the event.

A transient injury such as a viral liver infection or an episode of ALF is expected to be followed by a recovery of liver functional reserve but a procedure that surgically

removes or induces ischaemia of a fraction of the liver volume induces a permanent decrease in liver functional reserve.

Knowing the level of liver functional reserve helps us to estimate and predict a patient's outcome after surgery, radiological or drug treatment and life expectancy.

Currently this assessment can be done, as we have shown in previous chapters, by biochemical methods, functional tests, physical or volumetric assessments but pseudocholinesterase can be a new test in the battery of tests for assessing liver functional reserve.

As we have shown, serum pseudocholinesterase levels decrease as liver disease progresses and correlate with Child-Pugh and Meld-Na score stages. Basically it can be used in place of these tests to assess liver reserve.

The correlation of serum pseudocholinesterase levels with liver function is also demonstrated by an increase to normalization in the recipient of a liver transplant from a deceased patient. Normalization of serum pseudocholinesterase shows that the protein synthesis function of the liver of a patient initially classified as Child-Pugh class C becomes Child-Pugh class A after liver transplantation.

Because serum pseudocholinesterase cannot assess the degree of liver fibrosis, it may be beneficial to develop a score containing only 2 parameters: serum pseudocholinesterase and elastography assessment of fibrosis.

Because serum pseudocholinesterase can assess the protein synthesis function of the liver and thus the liver functional reserve, it can be used to evaluate this parameter over time. Thus we used it to investigate the evolution of liver functional reserve in patients infected with liver virus C before, during and after treatment with direct-acting antivirals. Serum pseudocholinesterase assays followed the hepatic functional reserve of these patients before and after sustained viral response. The conclusion of this study was that removal of liver virus C in patients with chronic viral hepatitis does not induce an increase in liver functional reserve.

Thus, the liver functional reserve of chronic hepatitis C patients was not temporarily inhibited by the presence of the virus. Hepatitis C virus decreases hepatic functional reserve over time through chronic hepatocytolysis and implicitly through a progressive decrease in hepatocyte mass. Elimination of HCV does not induce, at least in later years, an improvement in liver function.

All this information confirmed the hypothesis that serum pseudocholinesterase may have predictive properties on survival of patients with liver disease. We analysed the mean

survival of a group of patients with hepatopathies who died and tried to find out the mean survival time. First the serum cholinesterase value at which the risk of death can be said to be high was found to be 6000ui. The critical pseudocholinesterase value is 2500ui, from this value survival is only possible with sustained medical treatment or liver transplantation. These values can be extremely useful in medical and surgical decisions for patients with chronic liver disease.

By analysing mean annual pseudocholinesterase levels we obtained pseudocholinesterase decline curves over the lifetime of a patient with chronic liver disease. Analysis of these curves can provide information on the natural course of the disease, evaluating therapeutic interventions in terms of their beneficial or unfavourable influence on the evolution curves. These new research topics could confirm or refute the therapeutic effect of some drugs or supplements with therapeutic potential on liver diseases.

Serum pseudocholinesterase, in the light of these studies, may be useful in estimating liver function, but studies on larger samples of patients are needed to validate it.

12. List of published scientific papers

1 Stancu G, Iliescu EL. The Influence of Liver Transplant on Serum Cholinesterase Levels: A Case Report. Cureus. 2023 Jan 14;15(1):e33761. doi: 10.7759/cureus.33761. PMID: 36793850; PMCID: PMC9923630. (Found in Chapter 9)

2 Stancu G, Sorohan B, Gherlan G, Toma L, Iliescu LE. Correlations between the value of serum cholinesterase and Child-Pugh and Meld-Na scores in cirrhotic patients. Ro J Infect Dis. 2022;25(2):62-6. doi:10.37897/RJID.2022.2.5 (Found in Chapter 7)