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PREDICTIVE BIOLOGICAL MARKERS IN ACUTE CHOLECYSTITIS

PhD SUMMARY

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Bucharest University Emergency Hospital is one of the largest hospitals in the southern part of the country, serving the capital city of Romania, with almost 2 million inhabitants [1].

In our study, we shall address acute cholecystitis lithiasis, which most often requires emergency intervention. The latter involves higher costs due to the risk of complications, a form that is most often associated with inflammation especially at the level of the Calot's triangle. Thus, the time needed for surgery may increase. The patient's comorbidities, especially neurological, cardiovascular, neoplasia, coagulopathies, anticoagulant treatments, also increase the hospitalization time. In some cases, costs may be even higher due to the patient's condition, which may require admission to the Resuscitation Department. Here, monitoring, multidisciplinary treatments, thorough care are needed.

As it can be seen, cholecystectomy in emergency increases resources used and costs, which is confirmed by the respective medical literature. Outpatient cholecystectomies are up to 90% cheaper than those performed by emergency service [2,3]. Cost differences between open and laparoscopic techniques are a widely debated topic in the literature. A study shows that although the logistics costs used for laparoscopic intervention are higher, in the end it is cheaper by 23% [4].

The importance of the chosen theme comes precisely from the desire to assist the surgeon from the first contact with the patient to easily anticipate the severity of the case, and thus be able to choose the most appropriate treatment method. Another important aspect is the possibility to explain when signing the consent for surgery, as clearly, succinctly and objectively as possible the degree of severity and possible complications that may occur. Thus, both the surgeon and the patient have an objective assessment of the situation.

Multiple scores have been developed in the literature with the aim of predicting mortality and morbidity of patients admitted to emergency [5,6]. In difficult cases where there is doubt, they can help make a decision. The aforementioned aspect is also confirmed in the literature that these scores help the doctor to assess the situation as clearly as possible and thus have an objective way of present it [7-10].

Several scores have been developed of which we shall mention but a few, given that this is not the object of our study.

Emergency surgery is the branch from which the development of scores started, probably out of the need to be able to predict complications and survival [7,11], in the case of polytraumatized patients. Starting from these scores, others have developed, among which we can mention: A.S.A., APACHE, II and III, Goldman Index [5], A.P.G.A.R. Score for surgery, SOFA, qSOFA, P.U.L.P. score [9].

For acute cholecystitis, several attempts have been made to develop scores that play a role in predictability of severity and difficulty of surgery. Among these it is worth mentioning the Tokyo criteria, which for the diagnosis of acute cholecystitis have proven that clinical and laboratory criteria are necessary [12,13].

Starting from the above, the purpose of this study is to determine biological variables that can predict both the severity of acute cholecystitis and the possibility of difficult surgery in emergency.

Laparoscopic cholecystectomy is one of the most performed surgeries, being the treatment of choice in complicated gallstones [14,15].

The complications of acute cholecystitis have implications for both surgeon and patient, being associated with malpractice for the former and morbidity and mortality for the latter [16].

Starting from those mentioned in the Bucharest University Emergency Hospital, acute cholecystitis is a common pathology to be dealt with. In fact, cholecystectomy is also frequently performed. Most of the time this is carried out in an emergency.

We consider it necessary to conduct a study aimed at determining biological markers that may have value in the management of patients with acute cholecystitis. In addition to this value, a predictive value is also required regarding the difficulty of the intervention and postoperative complications.

With the agreement of the research ethics committee, we conducted retrospective studies over a period of 5 years where we included all patients with an inpatient diagnosis of acute cholecystitis.

Using the InfoWorld program, we selected hospitalized patients with the diagnosis of acute cholecystitis, and then by studying electronic records and operating protocols we made a database.

Thus, we gathered a main group of 314 patients. We studied biological markers that can have a predictive role for the severity of acute cholecystitis, difficulty of intervention and postoperative complications. Most patients were included regardless of the surgery performed.

We noticed that for a better predictive value, multiple correlation of the variables used with the results of laboratory investigations is needed.

The results obtained were correlated with those in the specialized literature in the discussion chapter and in the conclusions chapter we listed the findings obtained.

Starting from this group of patients, we made a database and conducted the first study, which provided an overview of patients hospitalized with the diagnosis of acute cholecystitis.

Starting from this database, due to the need for greater specificity and sensitivity, which I wanted to offer to my doctoral thesis, I conducted two other studies.

The second study was intended to determine markers that may have predictive value in the severity and adverse effects of acute cholecystitis, being a retrospective study conducted over a period of 3 years.

The third study was aimed at determining the predictors of difficult laparoscopic cholecystectomy, being a retrospective study conducted over a period of 4 years.

For the compilation of the general part, we conducted a literature study involving surgical anatomy, pathophysiology, surgical technique and treatment of acute cholecystitis. The anatomical study focused on the idea that in the case of the bile tree anatomical variations are almost the rule [17]. The complications that can arise from its misinterpretation have major implications for both surgeon and patient.

In order to carry out the studies, an interdisciplinary collaboration was needed. In the case of patients undergoing surgery, we collaborated with the anaesthesiologist; this collaboration extended to patients with serious general condition admitted to the intensive care unit. The excised pieces were preserved and transferred to the department of pathological anatomy according to a clearly established protocol. Subsequently, the results were interpreted and transmitted by the pathologist.

The studies conducted were retrospective, did not interfere and did not influence therapeutic decisions. Patients included in the study agreed to participate in the studies.

The limitation of the studies stems from the fact that they are retrospective, unicentric and conducted from a limited number of patients.

Variants of further evolution are the realization of a large-scale study starting from the results obtained and having the experience of markers with predictive value, especially in the case of difficult laparoscopic cholecystectomy.

In the context of technological advances and availability of internet connection, we hope in the future to create a prognostic score that can be integrated in the form of an application and used on mobile phones to calculate the severity and risk of cholecystectomy burdened by complications.

Knowledge of surgical anatomy is important in performing any surgery, but especially in the case of bile duct surgery, where it is known that misinterpretation of anatomy and frequency of anatomical variations contribute to serious postoperative complications represented by bile duct damage. These injuries are a frequent cause of morbidity and mortality, generating litigation for the general surgeon. Due to the fact that laparoscopic cholecystectomy has now become the standard procedure in the treatment of gallstones, the number of bile duct lesions has increased [18,19].

Anatomical variations are frequently encountered during laparoscopic cholecystectomy, and their preoperative diagnosis through joint investigations is difficult to achieve. Lately, the rate of iatrogenic lesions has decreased considerably; yet, their incidence is still increased compared to laparoscopic cholecystectomy [20]. Iatrogenic injuries are between 0.3-0.5% [21]. Due to biliovascular lesions, the conversion rate to the open technique is between 0.1-1.9% [22].

Most bile duct lesions occur during dissection of the hepatocystic triangle and exposure of the cystic duct and cystic artery. Over time, several techniques have been developed to avoid bile duct damage. These include the concept of "Critical View of Safety", intraoperative use of fluoroscope with indocyanine [23,24], use of the laparoscope of 300, dissection as close as possible to the gallbladder and avoidance of electrocautery near the main bile duct [25,26]. According to a 2006 study, damage to the bile ducts during laparoscopy has been attributed to a lack of anatomical orientation.

The anatomical variations encountered in biliary surgery are confusing. These are more pronounced in traction manoeuvres performed in cholecystectomy [27,28]. Knowledge of this detail is very important, since basically there is a variation of anatomical landmarks. But sometimes the operator loses sight of these details, hence the risk of developing bile duct lesions.

According to the consensus of the American Society of Gastrointestinal and Endoscopic Surgeons ("SAGES") and as directed by Tokyo 2018 guidelines, achieving critical landscape ("C.V.S. = Critical View of Safety") this is a good method of reducing bile duct damage during laparoscopic cholecystectomy [29,30].

Obtaining CVS is one of the most important operative times in laparoscopic cholecystectomy, being practically the effective method to avoid bile duct damage. Basically, CVS is not an operative technique; it is the final image obtained after thorough dissection of the Calot's triangle.

- Addressing first the bottom of the gallbladder and then the Calot's triangle is a safe technique in case of increased risk of iatrogenicity due to inflammation or fibrosis. According to one study, this technique had a lower rate of biliary lesions compared to the classic onset of dissection [31];
- SAGES recommends obtaining CVS first for correct identification of the cystic duct and bile duct [32];
- Another step that can be used to perform a safe cholecystectomy is to identify the Rouviere's sulcus, it helps to recognize more easily the plane of the main bile duct [33];

- The use of intraoperative cholangiography is another strategy that can help in case of doubt [34];
- Intraoperative laparoscopic ultrasound is another safe method for visualizing the bile duct that does not necessarily increase operative time [35];
- We must also consider "bailout" procedures, which in translation means "abandoning a difficult situation". These are recommended by Tokyo criteria and can be performed laparoscopically [36,37];

Laparoscopic cholecystectomy in emergency is a common intervention in emergency hospital clinics. The major challenge that can occur in this surgery is represented by the intra-operative aspect. It can range from mild inflammation to inflammation and dangerous complex adhesion syndrome. In laparoscopic cholecystectomy an important step is to clearly explain before intervention the complications that may result. One must mention the risk of biliary lesions, conversion to classical cholecystectomy, drainage, risk of bleeding and, last but not least, comorbidities, morbidity and recovery in case of complications [38, 39].

Starting from the above, with the realization of these studies, we have the following objectives.

The study of patients with acute cholecystitis who came to the emergency room and their characteristics. Among the characteristics, we shall focus on:

- the time elapsed since the onset of symptoms,
- demographics, that is, the environment, gender, age:
- associated risk factors for lithiasis disease, comorbidities.
- features of the clinical examination, the presence of accompanying signs, fever, jaundice.
- changes on blood count, markers of inflammation, elements suggestive of liver disease, kidney damage
- features of imaging examinations suggestive of acute cholecystitis, type of lithiasis, dilation of bile ducts, features of calculi, the presence of double contour, liquid.

Subsequently, we intend to observe the weight of diagnostic elements according to the diagnostic guidelines in force and the classification in a degree of severity. The presence of complications of gallstones accompanying acute cholecystitis, cholelithiasis, angiocolitis, pancreatitis, endoscopic treatment are also of interest to us. Elements characteristic of surgery, classification in an A.S.A. risk class, type of surgery were also recorded.

Regarding surgery, we aim to determine macroscopic intra operative changes of the gallbladder. Another goal is to determine the factors and reasons for conversion in laparoscopic cholecystectomy.

After determining these elements, it is desired to form an overview of the pathophysiological elements in patients with acute cholecystitis.

The clinical and laboratory elements that can predict the intraoperative aspect and the risk of perioperative complications is again one of the important goals of the study.

The first study will be a general approach to all patients with acute cholecystitis. We want to have an overview, taking into account the associated problems, or recent surgical history. The idea of this first study is represented by the fact that in many cases the history of patients can greatly influence the therapeutic attitude and subsequent complications in patients with intra-abdominal infections. Especially a topic worth mentioning is the clinical picture, which in these patients can be atypical or even polymorphic.

Given that in this first study the selection criterion will be the diagnosis of acute cholecystitis, for greater accuracy we conducted two more studies. For both studies we applied exact selection criteria so that we selected the most suitable patients to be included in each study, so that they will have a higher specificity.

In the second study, we aimed at calculating systemic inflammatory biomarkers N.L.R., P.L.R., S.I.I. We integrated these values into the clinical context. The objective is to establish their role in anticipating a severe form of acute cholecystitis, the risk of conversion and the postoperative complications that may occur.

In the third study, we intended to determine the preoperative elements that will anticipate the difficulties encountered in laparoscopic cholecystectomy. We chose laparoscopic cholecystectomy because it is one of the most performed surgeries, being the gold standard in the treatment of gallstones.

Through analogies and statistical determinations, we intend to identify elements with predictive value in patients with other diseases in whom complicated gallstones may be secondary. On the other hand, we want to test the values of biological markers in patients with the exact diagnosis of acute cholecystitis, without other comorbidities that can influence the values of biological parameters. And, last but not least, we want to determine the suggestive elements for a laparoscopic surgery with a high degree of difficulty.

The idea of this approach started from the need to have an overview of gallbladder pathology, but especially from the need to accurately select patients who have a severe form of acute cholecystitis and those at high risk, complications caused by difficult surgery.

Through these elements, we want to bring more theoretical and practical knowledge, especially useful to those at the beginning of the road. Thus, at the end of these studies, we want us to have a clear look at severe cases, but especially to be able to anticipate the intraoperative difficulty from the first contacts with the patient.

We strongly believe that the presented elements are important for young specialists who will have the opportunity to request the presence in the operating room of an experienced surgeon from the clinical and paraclinical evaluation of the patient.

Microsoft Excel and Microsoft Word were used to collect data and write this doctoral thesis. The thesis was written in accordance with the provisions of the Doctoral School of the University of Medicine and Pharmacy "Carol Davila" Bucharest. For data analysis, the following were used: IBM SPSS Statistics 22, Microsoft Excel and Med Calc® Statistical Software

In the first study, the group of patients consists of 65% women and 35% men. The environment is predominantly urban with 40.4% women and 25.8% men. At the other extreme, patients from rural areas are present in a proportion of 33.8%.

We made a comparison between the type of surgery and the duration of the postoperative period and the days of hospitalization. If for C.L., the average days of hospitalization was 6.00, this increases to 6,254 C.L.C. and to 7,422 for C.C. This increase demonstrates that laparoscopic intervention is cheaper taking into account the period of hospitalization.

In the case of cholecystostomy, the hospitalization period is 2,828 days, which is explained by the high severity of cases in these patients. Given that, as mentioned, cholecystectomy is a life-saving intervention in critically ill patients who cannot undergo surgery.

As it can be seen, the shortest postoperative period is for C.L. 2.636 and the longest is for C.C. 6.860. As one can notice, from this point of view, C.L. involves lower costs.

In our study, we compared the severity according to Tokyo criteria with the hospitalization period, respectively the postoperative period. There were no significant differences between grades I and II. For both hospital and postoperative days, the periods were similar. Between the two and grade III severity, the differences were significant, with the latter showing the highest values.

Using the ANOVA test, we compared mean age with the TG13/TG18 severity criterion [30] and obtained statistically significant differences with $p<0.001$. In our study, the two variables are directly proportional, i.e. average age increases with severity. The result obtained is statistically significant, being confirmed by the linearity test with a $p<0.001$. In our study, the average age at grade III of severity was 66.79, extreme age

were between 23 and 91 years; practically we can say that in our study the average age that predicted a third degree of severity was about 67 years. This result can be attributed to the fact that most cases came in emergency before, during and after the pandemic. The result in the literature, during the pandemic the elective surgeries were postponed; this led to an increase in the severity of acute cholecystitis and a decrease in the average age of severity [40].

We can say that starting from the first contact with the patient in which we make the anamnesis, a first factor that draws our attention to a difficult cholecystectomy is age.

We made a comparison between the sex of patients and the degree of severity trying to find a correlation between them. For this we used Fisher's exact test to get a $p<0.001$. The conclusion is statistically significant, that is, the form of severity is higher for the male group.

In class II of severity, the percentage for male and female is approximately equal, around 40%. Things are different, for the other two classes: if in class I the female percentage is 43%, it decreases in class III of severity to 17%. For males, things are reversed: the percentage increases from 24.5% in class I to 34% in class III of severity.

Comparing with previous results, although acute cholecystitis is more common in women, its severity is greater in men, so we can consider the male a predictive factor for difficult cholecystectomy. Basically, we ignore the prevalence of acute cholecystitis higher in women and put its greater severity in men first.

Within the studied group, most interventions were performed laparoscopically in a percentage of 83.1%; on the second place, there were laparoscopies converted in a percentage of 8.9%, and on the last places there were interventions on open route and those with exploratory visa.

In grade I of severity, laparoscopic intervention predominates with a percentage of 42.9%.

In grade II, values are close between laparoscopic surgery and intervention converted to open surgery.

In the III degree of severity, classical intervention predominates, and on the second place are interventions converted to open surgery.

We have demonstrated that the percentage of C.L. has a minimum value for grade 3 severity. Another aspect highlighted is the increase in severity, which is directly proportional to the frequency of C.L.C. and C.C.

We used Fisher's exact test to analyse the relationship between the CT scan and the degree of severity. The combination of the two variables is statistically significant with a

p=0.001 for patients in severity grades II and III. In these patients, CT scans were performed more often.

We used Fisher's exact test and obtained a statistically significant result with a p=0.014 for the correlation between C.B.I.H dilation and severity degrees.

We used the ANOVA test and the linear dependency test; for these two we obtained that the diameter of the C.B.P. depends on the severity criterion.

The wall thickness is directly proportional to the degree of severity. For the assessment of statistical significance, we used the ANOVA test and the linear dependency test. The results were statistically significant with a $p<0.001$.

In our study, the double contour ultrasound sign was present in 35.4% of patients. In patients of category III severity, the sign was present in 60.3% of them.

In the study, we observed that the increased presence of sludge is correlated with the higher degree of severity, which is confirmed by a $p<0.001$.

This result brings to mind the idea that the presence of sludge increases the severity of the disease.

In our study, we made comparisons through linear tests dependence between the presence of fluid or abscess around the gallbladder and the degree of severity according to Tokyo criteria.

We obtained a $p<0.005$, having a 39.7% percentage of the presence of liquid in grade III of severity. This result is most likely attributable to associated organ dysfunction in these patients and/or acute cholecystitis.

We believe that this ultrasound aspect must be integrated into the clinical context and cannot be used for prediction.

The presence of Murphy's ultrasound sign was compared with the severity of acute cholecystitis. In our study, it was present in 21.9% of patients with grade III severity.

In our study, hepatic steatosis was found in 20.5% of patients classified in grade III severity and in 14.3% of the total number of patients. These results reflect for the first category the higher share of associated comorbidities in this category. The higher number of comorbidities causes a more severe response of the body to the inflammatory process and/or organ dysfunction, therefore this explains the higher presence of these patients in this category.

In contrast, white blood cell counts were statistically significant and had predictive value for severe inflammation, difficult dissection of the Calot’s triangle with a $p<0.001$.

An important result for surgical practice with statistical significance, p=0.006, was obtained when comparing uncomplicated adhesion syndrome with severe inflammation, difficult dissection of the triangle.

The result obtained has clinical significance, since most intra operative complications occur as a result of dissection of the Calot triangle.

Summing up, leukocytes can be used primarily to differentiate I and II degrees from acute cholecystitis. A large number of leukocytes suggests greater severity. With the number of leukocytes increases the risk of conversion and difficult cholecystectomy, in patients with inflammation, difficult dissection of the Calot's triangle [41].

The number of leukocytes is correlated with gangrenous cholecystitis, being statistically significant with a p=0.02. The white blood cell count correlated statistically significantly with a p=0.012, also for acute phlegmonous cholecystitis.

Neutrophils and lymphocytes play important roles in the host's immune response to infection, representing two types of immune systems, innate and adaptive. Neutrophil percentage and severity are directly proportional magnitudes, being highest in grade III. The percentage of lymphocytes and the degree of severity are inversely proportional in magnitudes, the percentage of lymphocytes being lowest in grade III of severity. Neutrophils and lymphocytes by their divergent values had predictive value for intraoperative difficulty. These results were statistically significant for the following shapes:

- Difficult Calot's triangle dissection and inflammation, $p<0.001$;
- Acute gangrenous cholecystitis, $p=0.002$;
- Phlegmonous acute cholecystitis, $p=0.021$;
- Around gallbladder abscess, $p=0.015$;
- The differentiation between, slight dissection, and difficult dissection shape of the triangle, was statistically significant $p=0.020$;

We compared the ratio of neutrophils and lymphocytes with the intraoperative aspect. Thus, we obtained the following statistically significant results:

- For difficult dissection of the Calot's triangle, $p=0.001$;
- For acute gangrenous cholecystitis, $p = 0.001$;
- For acute phlegmonous cholecystitis, $p=0.014$;
- For around gallbladder abscess, $p=0.043$.

The mean value of the ratio was 5,168, consistent with literature data. According to it, a ratio of more than 3.0 suggests the presence of a pathological process such as inflammation, infection.

We then compared the value of the ratio with the degree of severity of the acute cholecystitis form. The obtained results confirm the predictive value of the ratio for severity and prolongation of the hospitalization period in acute cholecystitis [42]. For statistically significant results with $p<0.05$, having predictive value for:

- adhesion syndrome with difficult dissection of the triangle Calot
- acute gangrenous cholecystitis;
- acute phlegmonous cholecystitis;
- around gallbladder abscess;

The increase in fibrinogen with the degree of severity, but also with the advancement of pathophysiological changes is a clear evidence of its value. For our study, the values of transaminases, I.N.R., platelets and creatinine were not statistically insignificant, but a slight increase is observed with the degree of severity.

Diabetic patients represent a special category due to the higher risk of developing cholecystitis, especially due to the evolution of complications of the disease.

Leukocyte values were higher in this category of patients in our group, with a $p=0.023$, neutrophils also had statistically significant higher values, $p=0.003$. Another statistically significant marker of inflammation was fibrinogen, with $p=0.001$, which correlated well in diabetic patients.

In the second study, we aimed to determine inflammatory factors with a role in the severity of acute cholecystitis and to anticipate complications.

We conducted a retrospective study over a period of 3 years, starting from the main batch; data were collected from electronic registers and operator protocols. Following the applied inclusion and exclusion criteria, we obtained a number of 235 patients. The presence of ischemic heart disease was statistically significant, with $p=0.01$; more common in also observed in the advanced disease group. Among the common risk factors for the two diseases, we can mention: sedentary lifestyle, diabetes, obesity, metabolic syndrome, dyslipidaemia [43]. In addition, a common risk factor for the two pathologies is cholesterol, present both in atheroma plaques and in calculi [43]. From the above, we have every reason to believe that the presence of gallstones may be associated with heart disease. Our results show a higher association in those with a more advanced degree of acute cholecystitis, so we can say that the common pathophysiological mechanism

between the two is confirmed by our results. The presence of heart failure or shock was correlated with the advanced acute cholecystitis group with a p=0.001.

In our study, an ascent of the ASA degree was observed within the advanced acute cholecystitis group, with p=0.008, for the chi-squared test, and p=0.0003, for the upward trend in the linear association test. In the context of acute cholecystitis, an increased ASA risk is associated with a difficult cholecystectomy, risk of postoperative complications, increased operating and hospitalization time.

Chronic liver disease was correlated with the advanced cholecystitis group, being statistically significant with a p=0.002. CBD lithiasis and/or angiocolitis was observed more frequently in the advanced acute cholecystitis group, p=0.013.

We observed a progressive rise in NLR, PLR and SII inflammation markers with increasing severity according to Tokyo criteria. For these determinations, we used the Scheffe and Chi-squared test. The alteration of these biomarkers represents the pathophysiological response of the body through inflammation-specific changes. This is confirmed by the differences found in the Scheffe test, which suggests that each biomarker characterizes specific changes in the inflammatory process.

Of these three, the difference between degrees of severity is best made by SII, an explanation in this regard being given by its calculation formula.

Local inflammation echoes at the systemic level by increasing PLR, in severe forms of acute cholecystitis. This phenomenon confirms the pathophysiological mechanism underlying the evolution of inflammation, when it begins to have systemic effects.

In contrast, regarding the mild and medium forms, we notice an increase in NLR between these two forms, being able to differentiate between these two.

We used R.O.C. curves to test the value of inflammatory markers as mentioned alongside leukocytes and TG13/18 criteria to predict advanced forms of acute cholecystitis. Thus, we wanted to test sensitivity and specificity. Of these, NLR had predictive value with AUC=0.824.

After the Clavien-Dindo classification [44], we centralized the postoperative complications encountered.

Of these, nosocomial infections and postoperative wound complications were statistically significant for the advanced acute cholecystitis group, p=0.043 and p=0.007, respectively. Patients in this group may have an evolution with a higher rate of open

interventions, conversions and a longer period of hospitalization. We noticed that in the group of advanced acute cholecystitis there were more frequent complications requiring admission to the intensive care unit, p=0.002, including sepsis, p=0.004, and malignant postoperative hypertension, p=0.043.

We then studied the existence of correlations between the type of surgery and inflammatory biomarkers. Thus, we observed that SII and PLR had higher average values for conversion rate, but were not statistically significant. In contrast, NLR and T.G.13/T.G.18 had significance and associated well with the type of intervention, p=0.001, and p<0.0001, respectively.

Postoperative complications were another area of interest for our study, so we studied the role of inflammatory parameters for correlation with the former. According to the ANOVA biomarker test studied, criteria T.G.13/T.G.18 correlated well with postoperative period (NLR p<0.001; PLR p<0.001; SII p<0.001; T.G.13/18 p=0.008) and hospitalization period at the time of admission (NLR p=0.002; PLR p<0.001; SII p<0.001; T.G.13/18 p=0.001).

In the third study, we aimed to determine the factors that predict a difficult laparoscopic cholecystectomy.

Gallstones have laparoscopic cholecystectomy as a surgical indication, the latter can be performed in selection or emergency [32,45,46]. Gallstone lesions are one of the complications of laparoscopic cholecystectomy and are becoming more frequent with the adoption of the latter worldwide [45-50]. Gallstone damage is a debilitating complication for the patient both physically and psychologically [51].

The term difficult laparoscopy is hard to define because the level of surgical aptitude differs from operator to operator. On the other hand, local conditions, the condition of the equipment, the skill of the cameraman, the helper who manipulates the gallbladder are other factors that influence the course of surgery.

In the group of 255 patients, they were between the age extremes, respectively between 19 years and 90 years.

In the group of laparoscopic cholecystectomy patients with a high degree of difficulty, the average age was higher, being 57.1, compared to the laparoscopic cholecystectomy group where it was 52.4. This difference is statistically significant with a confidence level represented by the value p=0.009.

In our study, males had a greater distribution within the difficult cholecystectomy group, with a p=0.0032. The male gender is among the risk factors in difficult cholecystectomy and among those in the conversion risk of cholecystectomy.

In the difficulty cholecystectomy group, diabetes mellitus was found in 22.8% of patients, compared to 11.3% in the laparoscopic cholecystectomy group., statistically significant difference being represented by p=0.02.

The onset of symptoms greater than 72 hours was associated with a difficult cholecystectomy being statistically significant with a p=0.03.

In the study, laboratory examination confirmed clinical suspicion by the following changes within the cholecystectomy group with high degree of difficulty:

- leukocyte counts were higher in the group earlier by a p<0.001.
- neutrophilia and increased NLR ratio were statistically significant with a p<0.001, thus confirming the more pronounced inflammatory character in the cholecystectomy group with increased degree of difficulty.
- in the latter group, the fibrinogen was statistically significant with a p<0.001.
- the presented elements suggest that the difficulty of laparoscopic cholecystectomy increases in proportion to the degree of inflammation in acute cholecystitis.
- increased fibrinogen levels have been associated with increased difficulty with laparoscopic cholecystectomy [52].

With a p<0.001, our results were statistically significant for the application of Tokyo criteria to the cholecystectomy group with increased degree of difficulty, and most patients are included in grade II.

The ultrasound examination revealed the following changes in the cholecystectomy group with an increased degree of difficulty:

- the thickness of the gallbladder wall was 6 mm, being statistically significant with a p<0.001; also the double ultrasound contour of the gallbladder was present in 48.5% of cases, being statistically significant with a p<0.001.
- gallbladder diameters were larger in this group and had statistical significance.

- Murphy's sign and fluid around the gallbladder were also statistically significant, with $p=0.002$ and $p<0.001$, respectively. It should be noted that the Murphy ultrasound sign is subjective, and its presence may be diminished by the administration of opioid analgesics.

Postoperative complications were higher in the cholecystectomy group with a high degree of difficulty, but, of these, postoperative wound infections were more frequent. This phenomenon can be explained on the one hand by the greater severity of acute cholecystitis in this group. This is confirmed by the higher number of leukocytes, neutrophils. Another explanation may be the higher rate of CLC, resulting in these patients being more prone to wound infections. Compared to CLS, the risk is higher given that wound size is smaller in patients with CLS.

In our study, postoperative complications were more common in the cholecystectomy group with a higher degree of difficulty, but those in grades 4 and 5 were missing, but those in grades 1, 2 and 3 were statistically significant, with $p=0.06$, $p=0.01$ and $p=0.03$. Treatment was conservative for grade 1 and 2 and interventional for grade 3 complications. Subsequently, there were no further complications.

Subsequently, the relationship between changes in certain preoperative parameters and cholecystectomy with difficulty was evaluated by applying a logistic regression analysis. After successive trials, the most predictive model was a combination of fibrinogen, wall thickness, and transverse diameter.

Of all the variables used, the best predictive value was the Tongyoo score [53], with a $p=0.857$, sensitivity of 67.6%, specificity of 88%, predictive values close to 79.7%, for positive, and 79.3%, for negative.

The logical regression model consisting of fibrinogen, transverse gallbladder diameter and gallbladder wall thickness was not statistically significant with $p=0.802$, sensitivity of 60.5%, specificity of 86.6%, the predictive values being very close to each other.

In our study, we came to the following conclusions. In the general, part we have conducted a literature review on surgical anatomy with practical utility in gallbladder interventions.

In the special part, we conducted 3 studies that focused on: clinical and laboratory characteristics in patients with acute cholecystitis, including those with associated

pathologies; the degree of severity of cholecystitis. Here, we focused on predictive factors of the difficulty of laparoscopic cholecystectomy and severity of acute cholecystitis.

Our conclusions are as follows:

- Laparoscopic cholecystectomy is one of the most performed surgeries worldwide. Hence the importance of its main complication, iatrogenic vasculobilious lesions. The latter did not increase significantly with the development of laparoscopy, due to the adoption of algorithms that increase the safety of the intervention. Lack of general access to educational resources or training as well as difficult laparoscopic cholecystectomy are to be addressed individually. Thus, each author proposes techniques adopted to the area population;
- Most injuries are caused by erroneous recognition of anatomy; therefore, methods must be developed to apply knowledge to the wound more easily;
- Anatomical landmarks were synthesized according to importance and surgical principles. We put special emphasis on anatomical variations and obtaining C.V.S.;
- The use of intraoperative methods of confirming anatomy having precise indications helps to avoid lesions;
- Among the inflammatory markers studied in acute cholecystitis have NLR, PLR, SII values;
- Of these, NLR is a useful marker, with a value higher than PLR and SII, and the total number of leukocytes;
- Compared to T.G.13/T.G.18, it has the advantage of being easy to determine and especially the fact that it is a continuous variable;
- The higher value of NLR than 4.19 suggests the presence of severe inflammation;
- Postoperative sepsis is suggested by an NLR value greater than 8.54;
- Instead, PLR and SII do not correlate well with the severity of acute cholecystitis and the risk of conversion;
- They represent well the body's inflammatory response and can suggest the severity of inflammation and its systemic resonance;

- Regardless of the degree of experience, always, for the surgeon, difficult cholecystectomy is a challenge, and this regardless of the method of approach;
- Following the study, cholecystectomy in this situation is associated with a higher degree of morbidity and a longer period of hospitalization;
- Factors that can predict a difficult cholecystectomy should be known to the surgeon and evaluated preoperatively. This is all the more important before informed consent is explained;
- Fibrinogen is an important marker of prediction of a difficult cholecystectomy along with ultrasound imaging results, namely changes in the gallbladder wall;
- In our study for evaluating cholecystectomy in emergency, we observed the usefulness of the Tongyoo score, the latter having a good specificity;
- Old age is a predictor of a severe case of acute cholecystitis and difficult surgery;
- Acute cholecystitis is more common in women;
- The degree of severity is higher in men;
- The possibility of converting laparoscopic cholecystectomy or classical approach *per primam* is directly proportional to the degree of severity;
- The need for the C.T. examination is higher since the degree of severity is higher;
- The double ultrasound contour sign has a predictive role in the difficulty of cholecystectomy;
- Paraclinical blood variables have a prognostic role in difficult cholecystectomy, as well as severity;
- The limitation of our studies comes from the following: unicentric studies, performed on fixed batches of patients, and being retrospective.

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