MINISTRY OF HEALTH OF UKRAINE
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY
FACULTY OF PHARMACY
DEPARTMENT OF CLINICAL PHARMACOLOGY
AND CLINICAL PHARMACY

Theme of master's work:

CLINICAL-PHARMACOLOGICAL ANALYSIS OF HEPATOTOXICITY OF NONSTEROIDAL ANTI-INFLAMMATORY AND CARDIOTROPIC DRUGS

Department of Clinical Pharmacology and Clinical Pharmacy

(Head – Professor, Doctor of Medical Sciences *Khaitovich.MV*)

Scientific Supervisor: Doctor of medical sciences,

Professor *Pinsky.L.L*

creator:

Hachami Othmane

Kyiv 2022

CONTENT

LIST OF ABBREVIATIONS

ADRs - Adverse drug reactions

ALT - Alanine Transaminase

AST- Aspartate transferase

AUC - Area under the curve

CLD - Cholestatic liver disease

COX-2 - Cyclooxygenase-2

DILI - Drug-induced liver injury

FAS - Fatigue scale analysis

GGTP - Gamma-glutamyl transpeptidase

HCV - Hepatitis C virus

MFI - Multivariate fatigue inventory

NAFLD -NAFLD disease

NSAIDs - NSAIDs

PBC - Primary biliary cirrhosis

RA - Rheumatoid arthritis

RM - Reactive metabolite

INTRODUCTION

Relevance of the topic. Significant prevalence of hepatotoxic reactions in the appointment of cardiotropic drugs (MPs) and NSAIDs is increasing every year.

The aim of the study was to evaluate screening methods for early verification of drug-induced liver damage, the effect of NSAIDs on the state of hepatocytes, and to determine the pharmacokinetic interaction of the drugs - substrates and inhibitors of CYP2D6 in the occurrence of side effects.

Research objectives:

1. Analysis of the prevalence of toxic drug-induced hepatitis in patients who take NSAIDs for a long time.
2. The study of biochemical markers of cytolytic and cholestatic syndromes in toxic liver damage in the appointment of diclofenac.
3. Analysis of fatigue scale (FAS) scores in patients with toxic liver injury to assess this parameter as an early predictor of drug-induced hepatitis.
4. Analysis of the possibility of using Celecoxib, which has low hepatotoxicity, in the group of cardiac patients, taking into account its inhibitory properties on CYP2D6 and P-glycoprotein.

The study materials included 89 extracts from the medical history and outpatient cards of patients who were prescribed NSAIDs, FAS fatigue level survey cards.

Object of study: NSAIDs, diclofenac, Celecoxib, FAS scale of patient fatigue.

Subject of study: the use of NSAIDs by cardiological patients, the assessment of the severity of FAS fatigue as an early screening marker of toxic drug-induced liver damage, the severity of cytolytic and cholestatic syndromes when using NSAIDs.

Research methods. Bibliosemantic, statistical and graphical methods were used in the work.

Practical significance of the obtained results. Practical recommendations have been developed for physicians and pharmacists for early screening verification of toxic drug-induced liver injury using the FAS fatigue scale, the most significant biochemical tests for the diagnosis of toxic hepatitis when using diclofenac have

been identified, and to prevent complications, it has been proposed to exclude dangerous combinations of Celecoxib with cardiotropic drugs from clinical use.

Approbation of the results of master's work. The main results of the master's work are presented at: The Sixth Universiade in Clinical Pharmacology "Clinical-pharmacological analysis of hepatotoxicity of nonsteroidal anti-inflammatory and cardiotropic drugs" (April 12, 2022, Kyiv); April student scientific session of the Bogomolets National Medical University "Clinical-pharmacological analysis of hepatotoxicity of nonsteroidal anti-inflammatory and cardiotropic drugs" (April 19, 2022, Kyiv); 26th International medical congress of students and young scientists (13-15th of April, 2022, Ternopil, Ukraine) "Clinical-pharmacological analysis of hepatotoxicity of nonsteroidal anti-inflammatory and cardiotropic drugs".

Scientific novelty of the obtained results.

1. For the first time, discriminant intergroup properties of biochemical markers of cytolysis and cholestasis in toxic liver damage with diclofenac were established: ALT activity (F coefficient = 68.2; $p<0.05$), GGTP activity (F coefficient = 52.3; $p<0.05$), AST activity (F coefficient = 48.9; $p<0.05$).
2. For the first time, when conducting a non-parametric correlation analysis of Spearmen between the indices of cytolysis and cholestasis and the intensity of hepatogenic fatigue (according to the FAS scale), a significant correlation was established: between the activity of ALT and FAS - $R=+0.68$ ($p<0.05$), between the activity of GGTP and FAS – $R=+0.61$ ($p<0.05$), between ALT and FAS activity – $R=+0.58$ ($p<0.05$).
3. For the first time, the potentially most dangerous pharmacokinetic interactions of Celecoxib, as a potent inhibitor of CYP2D6, with substrates of this isoenzyme: the beta-blocker carvedilol and the calcium channel blocker Diltiazem have been established for the first time. A high probability was established when prescribing these combinations: negative inotropic, chronotropic effects, a sharp decrease in blood pressure, bradycardia and collapse.
4. For the first time, it has been established that the simultaneous administration of Celecoxib (a CYP2D6 inhibitor) with the indirect anticoagulant Warfarin can lead

to an increase in the concentration of Warfarin and the development of symptoms of hypocoagulation. Co-administration of Celecoxib with amiodarone (a CYP2D6 substrate) may increase the concentration of this antiarrhythmic drug and induce sinus bradycardia, ventricular arrhythmia, and liver toxicity. The potentially dangerous interactions of Celecoxib with cardiotropic drugs found are more significant than its low hepatotoxicity, which does not allow it to be recommended to therapeutic patients with heart pathology.

SECTION 1. LITERATURE REVIEW.

1.1. Modern problems of drug toxic damage to the liver with non-steroid anti-inflammatory drugs

There are limited data on the proportion of drug-induced liver injury among outpatients seen in hepatology clinics. Therefore, it is important to assess the proportion of cases of drug-induced liver damage and identify the most important hepatotoxic agents, the nature of liver damage. When analyzing a computerized database of diagnoses at the outpatient hepatology clinic at the Swedish University Hospital, a causal relationship of cases with suspected drug-induced liver injury was established. In total, 1164 cases of the disease were detected for the first time during this period. Drug-induced liver injury was found in 77 cases (6.6%), 38 (3.3%) of which were sent for examination to the clinic, while 3% underwent dynamic follow-up after hospitalization of patients with a diagnosis of drug-induced liver injury. The median age of the examined patients was 58 years, of which 43 (56%) were women, cytolytic damage was observed in 37 cases (48%), cholestatic - in 31 (40%) and mixed - in 12%. Antibiotics were the most common agents that induced drug-induced liver injury, followed by NSAIDs, with diclofenac being the most common cause of drug-induced liver injury. Thus, cases of drug-induced liver injury account for 6% of all outpatients and 3% of visits and are more common in women. Antibiotics and diclofenac were the most common causes of drug-induced liver injury in outpatients [1].

When analyzing the hepatotoxicity of various groups of drugs, it was found that TB drugs, dietary supplements and antibacterial drugs were the biggest problem in Asia, while antibiotics, cardiotropic drugs and NSAIDs also caused the greatest damage in Europe [2, 3].

Reye's syndrome occurs in children under the age of 16 after taking aspirin or tetracycline (especially with influenza and chicken pox). Glycogen disappears in hepatocytes, microvesicular steatosis progresses, severe damage to mitochondria occurs, hypoglycemia, lactic acidosis, hyperammonemia and cerebral edema develop. The dramatic rapid development of multiple organ pathology is accompanied by a

rapid increase in the activity of cytolytic enzymes in almost anicteric hepatic encephalopathy [4, 5].

The ibuprofen, naproxen, and diclofenac, ibuprofen had minimal association with gastrointestinal complications in patients of all ages. Co-administration of ibuprofen, aspirin, naproxen, and ketoprofen with acetaminophen results in the gastrointestinal complications, and this possibility is greatly increased in alcoholics [6].

In the analysis of hepatotoxicity, it was found that the effect of diclofenac on the liver is greater than that of ibuprofen. Diclofenac is cardiotoxic and adversely affects hepatic parameters [7].

According to the degree of liver damage and the induction of drug-induced hepatitis caused by NSAIDs, diclofenac ranks second after nimesulide.

However, diclofenac-induced liver damage has been shown to reverse in most cases. The recommended time for analysis of the level of cytolytic and cholestatic liver enzymes is four to eight weeks after the start of treatment with diclofenac [8].

Indomethacin, unlike ibuprofen, may aggravate the symptoms of Parkinson's disease. Indomethacin has a cardiotoxic effect, can provoke cardiac arrest. To assess the adverse effects of the use of diclofenac, it is advisable to dynamically study biochemical markers of kidney and liver pathology, coagulogram and macronutrient levels [9].

Naproxen is the safest non-steroidal drug for patients with heart disease [10]. The half-life of the drug is very high (about 50 hours). There are complications from the liver, kidneys and gastrointestinal tract. The multiorgan nature of complications in the application of this product has led to the minimization of its use in medical practice. Piroxicam should be avoided in patients with any degree of hepatic or renal problems, as well as in elderly patients (especially women during menopause) [11].

An increased risk of cardiovascular complications is a serious problem with this drug. In addition, Celecoxib also accelerates the progression of renal failure and is therefore contraindicated in patients with any degree of renal failure [12].

Many NSAIDs cause gastrointestinal, liver, and bone marrow toxicity in some patients, resulting in gastrointestinal bleeding, ulceration, fulminant liver failure,

hepatitis, agranulocytosis, and aplastic anemia. Drugs of the NSAID group form prooxidant radicals when metabolized by peroxidases. GSH, NADH and/or ascorbate are oxidized with catalytic amounts of NSAIDs, hydrogen peroxide in the presence of peroxidase. When GSH and NADH are co-oxidized, oxygen is taken up and activated. The prooxidant catalytic efficacy rating of the NSAIDs fenamate and aryl acetic acid was as follows: mefenamic acid > tolfenamic acid > flufenamic acid, meclofenamic acid, or diclofenac. Diphenylamine, a common moiety for all of these NSAIDs, was a more active prooxidant for the co-oxidation of NADH and ascorbate than these NSAIDs, suggesting that the oxidation of the NSAID's diphenylamine moiety to the cation and/or nitroxy radical was responsible for the NSAID's prooxidant activity. Although indomethacin had little prooxidant activity, its major in vivo metabolite, N-deschlorobenzoyl indomethacin, had significant prooxidant activity. Amino antipyrine, the major metabolite of aminopyrine or analgin in vivo, was also more prooxidant than the parent drugs. It is hypothesized that NSAID radicals and/or the resulting oxidative stress initiate cytotoxic processes leading to idiosyncratic toxicity [13].

The relationship between exposure to NSAIDs (NSAIDs) and liver injury was analyzed using the French pharmacovigilance database. A case/non-case methodology was used, where "cases" were reports of reactions of interest (liver lesions recorded in the database according to the WHO classification, including cytolytic and cholestatic hepatitis, acute hepatitis, elevated liver enzymes). "Non-cases" were all reports of reactions other than those under study. Amineptine and acetaminophen were used as positive controls. Of the 42,913 adverse drug reactions reported in the database, 5,708 (13 percent) were liver injury. Compared to other drugs in the database, liver injury was inversely associated with NSAID exposure, regardless of drug class. In contrast, liver injury was significantly associated with acetaminophen (OR 2.1 [1.9–2.3]) and amineptine (OR 14.0 [10.5–18.7]). Naproxen and diclofenac were associated with a higher incidence of liver injury, 15.7% and 11.5%, respectively. The risk associated with NSAIDs alone was significantly reduced when the analysis

was performed after the exclusion of NSAID-related hepatotoxic drugs (with the exception of naproxen) [14].

Diclofenac-induced acute hepatitis without any symptoms also occurs in clinical practice [15, 16].

A clinical example of the use of diclofenac demonstrates a patient's history of osteoarthritis and the development of severe hepatitis. This clinical example highlights the importance of being vigilant for the hepatic side effects of diclofenac [17].

Hepatotoxicity of diclofenac varies from an asymptomatic increase in transaminase activity to significant liver damage. The literature in English, French and Spanish has already reported 31 cases of diclofenac-induced hepatitis with five deaths. The paper describes the case of a 64-year-old patient who was admitted to the hospital with a sudden onset of icteric hepatitis. The only drug taken prior to admission was diclofenac at a daily dose of 150-200 mg for spondylodiscitis. Examination of the patient included laparoscopy and liver biopsy and excluded other causes of cholestatic hepatitis. Cancellation of diclofenac led to the normalization of transaminase activity and bilirubin concentration within four months. The frequent use of diclofenac and the possibility of fatal liver damage emphasize the need to take into account the toxicity of diclofenac in the differential diagnosis of acute cholestatic hepatitis [18, 19].

Some studies have found that Diclofenac does not have significant hepatotoxicity when administered. In this regard, the fact of diclofenac hepatotoxicity requires a more study and consideration of genetic risk factors [20].

The purpose of the analyzed study was to compare the hepatotoxicity of NSAIDs with highly active metabolites and NSAIDs with weak metabolites in the VigiBase™ database. The pharmacological properties of the drugs were compared with recorded adverse reactions. Thus, although spontaneous reporting has many limitations, the results are consistent with previous studies on the concept of reactive metabolites [21].

Many drugs can cause drug-induced liver injury (DILI); however, the underlying mechanisms of liver injury are significantly different. The concept of different

pathways of adverse outcome has become a method for assessing the risk of hepatotoxic effects of different types of drugs. Analyzing specific for immune-mediated drug-induced allergic hepatitis, taking into account the genomic, histological and clinical data of the pathology of experimental animals, the consequences of taking diclofenac were studied [22].

1.2. The study of fatigue in diagnostic and prognostic algorithms in patients with liver pathology

Experimental studies on experimental animals and male athletes revealed the involvement of the serotonin neurotransmitter system in fatigue of central origin [23, 24, 25, 26].

Fatigue is a common and often debilitating symptom for people living with chronic hepatitis C virus infection. Numerous published reports over the past decade have attempted to address the nature and etiology of fatigue in chronic hepatitis C; however, there is a lack of clarity in this area about how hepatitis C virus (HCV)-related fatigue occurs and when an infected person experiences it. Consequently, both patients and clinicians lack a clear understanding of how to mediate or prevent the negative effects of HCV-related fatigue. Research related to fatigue in chronic diseases aims to suggest future directions for research and interventions in the field of HCV-related fatigue [27, 28, 29].

Fatigue is an important symptom and a determinant of quality of life in patients with cholestatic liver disease. The pathogenesis of fatigue is unclear, although changes in peripheral muscle dysfunction have been blamed. There is currently no effective treatment [30].

Fatigue is the symptom with the greatest impact on quality of life, especially when it is associated with social dysfunction. The pathogenesis of fatigue in cholestatic liver disease is complex, poorly understood, and likely has central and peripheral components. Fatigue management in cholestatic liver disease presents a challenge for clinicians given the complexity and multiple associations [31].

The autoimmune liver disease, primary biliary cirrhosis (PBC), is associated with debilitating fatigue in a significant proportion of patients. The pathogenesis of fatigue in PBC is unclear, but preliminary studies suggest that it has central mechanisms and may have peripheral manifestations. Research is beginning to elucidate the biological causes of fatigue in PBC, especially sleep disturbances and autonomic dysfunction. Comprehensive studies investigating the pathogenesis of fatigue in PBC are urgently needed, as are large-scale prospective outcome studies [32, 33, 34, 35].

1.3. Technologies for studying the severity of fatigue in clinical practice

The Fatigue Impact Scale (FIS) elements reflect the intended impact on cognitive, physical and psychosocial functioning. Internal agreement for FIS was > 0.87 for all analyses. The effect of fatigue was highest in the ChF group, although reported fatigue in the MS group also exceeded fatigue in the HT group [36, 37].

The Functional Assessment System for Chronic Disease Therapy (FACIT) is a set of health, fatigue, quality of life (HRQOL) questionnaires designed for the management of chronic diseases [38].

The Visual Analogue Fatigue Severity Scale (VAS-F) was developed and tested on a sample of 75 healthy individuals and a sample of 57 patients undergoing a medical examination for sleep disorders. The VAS-F compares favorably with the Stanford Sleepiness Scale and the Mood State Profile, and the reliability of its internal consistency is high. Healthy subjects showed significant differences between their evening and morning VAS-F scores, while patients with sleep disorders had no such differences [39, 40].

A self-rating scale was also developed to measure the severity of fatigue. Two hundred and seventy-four new GP enrollments completed the 14-point fatigue scale. In addition, 100 consecutive GP attendees completed the Clinical Inquiry Schedule Revised Fatigue Scale and Fatigue Item (CIS-R). They were compared using relative operating characteristic (ROC) analysis. Both datasets were tested for internal consistency and principal component analysis. The validation coefficients for the fatigue

scale using an arbitrary threshold score of 3/4 and a CIS-R item were: sensitivity 75.5 and specificity 74.5 [41, 42, 43].

A fatigue scale was proposed when analyzing the psychometric qualities of a new fatigue score. In this Fatigue Rating Scale (FAS) study, a longitudinal study was performed [44, 45].

1.4. Possible hepatotoxicity of the specific COX-2 inhibitor CELECOXIB.

When conducting epidemiological studies associated with hospitalization due to hepatotoxicity of traditional NSAIDs (NSAIDs), it was found that the number of reports of hepatotoxicity of nimesulide, Celecoxib and rofecoxib is constantly increasing. We analyzed the relationship between the use of hepatotoxic NSAIDs and an increase in the number of hospitalizations associated with acute hepatitis. We used two types of models. for analysis with unidirectional and bidirectional cross-sectional models over 28-day exposure periods and running conditional logistic regression models. There were 4519 hospitalizations for acute hepatitis, and the odds ratios for Celecoxib, nimesulide, diclofenac, ibuprofen, and other hepatotoxic NSAIDs were significantly higher. Compared with the adjusted odds ratio of other hepatotoxic NSAIDs (OR = 2.13, 95% CI = 2.00, 2.28), Celecoxib caused acute drug-induced toxic hepatitis in fewer cases (OR = 1.92, 95% CI = 1.38, 2.69) [46].

Cyclooxygenase-2 inhibitors such as Celecoxib have been shown to be associated with fewer adverse drug reactions compared to other NSAIDs (NSAIDs). However, associations of Celecoxib with cholestasis and hepatitis have been rarely reported. Only isolated cases of toxic liver injury with Celecoxib have been reported in the literature. One of these examples demonstrates a clinical situation in which a young Hispanic woman developed biochemical signs of hepatic cholestasis after 3 weeks of Celecoxib. Liver chemistry returned to normal after medication was discontinued. This case is rather a rare exception to the rule, since Celecoxib generally has low hepatotoxicity [47, 48, 49].

Selective COX-2 inhibitors, coxibs, are NSAIDs (NSAIDs) that have a much better gastrointestinal safety profile compared to non-selective NSAIDs. The epidemiological features of coxib-induced hepatotoxicity and the clinical consequences of coxib-associated liver damage were analyzed. Data on hepatotoxicity caused by Celecoxib and etoricoxib indicate that this group of drugs has a negligible toxic effect on the liver. Liver damage caused by coxibs is an uncommon event. Improvements in this group of drugs and increased COX-2 selectivity in drugs such as rofecoxib, valdecoxib, parecoxib, and lumiracoxib have been associated with higher cardiovascular risk as well as dermatological and more severe hepatic reactions [50, 51].

SECTION 2. MATERIALS AND RESEARCH METHODS

2.1. Justification of the expediency of choosing objects and methods of research

After studying and working out theoretical sources, statistical data on the prevalence of toxic liver damage by NSAIDs, in particular diclofenac, it became relevant to study the prevalence of toxic hepatopathy in the group of patients with somatic pathology. Important is the early screening verification of liver pathology through the detection of hepatogenic fatigue. Identification of a complex of clinical signs of fatigue will determine the need for an in-depth biochemical examination of patients who take NSAIDs for a long time. A possible alternative to diclofenac, which is widely used in clinical practice, but has a pronounced hepatotoxic effect, could be replaced by Celecoxib. This specific COX-2 non-steroidal anti-inflammatory drug has a minimal hepatotoxic effect. To analyze its safety and possible use in cardiac patients, it is necessary to study its interaction with cardiotropic drugs to prevent adverse complications.

Given the relevance of the problem under consideration, the results of literary sources, as well as previous studies on this topic, the following areas of research were chosen:

- analysis of the prevalence of toxic drug-induced hepatitis in patients who take NSAIDs for a long time;
- analysis of biochemical markers of cytolytic and cholestatic syndromes in toxic liver damage when diclofenac is prescribed;
- analysis of fatigue scale (FAS) indicators in patients with toxic liver damage to assess this parameter as an early prognostic factor of drug-induced hepatitis, and indications for in-depth laboratory biochemical examination of the risk group;
- analysis of the possibility of using Celecoxib, which has low hepatotoxicity in the group of cardiac patients, given its inhibitory properties to CYP2D6 and P-glycoprotein.

Conducting research allowed to fulfill the main tasks that were formed at the beginning of writing the master's work.

2.2. Methodology and research methods

This section of the work describes the main methods and characteristics of research materials. To achieve the goals and objectives, the corresponding algorithm for conducting a master's study was used (Table 1).

Table 1

Algorithm for conducting a master's study

Research stages	The content of research areas
1. The study of the prevalence of toxic drug-induced hepatitis in patients who take NSAIDs for a long time	Analysis of extracts from case histories of cardiac patients with toxic drug-induced hepatitis who have been taking NSAIDs for a long time
2. The study of biochemical markers of cytolytic and cholestatic syndromes in toxic liver damage when prescribing diclofenac	Analysis of 89 extracts from the case histories of patients with toxic drug-induced liver damage with the analysis of variance and discriminant analysis of laboratory tests.
3. The study of indicators of the fatigue scale (FAS) in patients with toxic liver damage to predict drug-induced hepatitis.	Analysis of the results of a study of the level of fatigue using the FAS scale in a group of patients and the establishment of boundary values at which an in-depth set of biochemical tests should be carried out.
4. Studying the possibility of prescribing a specific COX-2 inhibitor Celecoxib in cardiac patients	Analysis of the effect of Celecoxib, as a powerful inhibitor of CYP2D6 and P-glycoprotein, on the concentration of cardiotropic drugs in somatic patients.

The research was carried out using such methods as bibliosemantic, statistics and graphic.

The bibliosemantic method was used to study Internet resources and scientific literature on the results of epidemiological studies of the prevalence of toxic drug-induced liver damage, modern methods for verifying drug-induced hepatitis when using NSAIDs, screening methods for verifying the severity of fatigue in somatic patients.

An analytical method to establish possible correlations between FAS fatigue indices and biochemical markers of cytolysis and cholestasis in patients with toxic drug-induced liver injury.

The statistical method was used to assess the prevalence of toxic liver damage, search for the FAS boundary value at which the likelihood of developing hepatitis increases, and conduct a discriminant analysis of laboratory parameters in cardiac patients.

Graphical methods were used to display the material and systematize the research results.

We analyzed 89 extracts from the medical history of patients with cardiac pathology (53 men and 36 women) aged 42 to 68 years, who were observed in the cardiology department of the Kiev Clinical Emergency Hospital. The structure of the pathology of these patients included the following diagnoses: hypertension, coronary heart disease (rest angina, exertional angina), cardiosclerosis, chronic gastritis with low and high acidity, ulcerative deformity of the duodenal bulb, chronic osteoarthritis, chronic osteoarthritis. Of the 89 patients, 21 (23.6%) were diagnosed with drug-induced toxic hepatitis induced by long-term use of the non-steroidal anti-inflammatory drug diclofenac, which was prescribed for the treatment of chronic joint pathology. Drug-induced toxic hepatitis was confirmed by a complex of clinical and laboratory studies, which included markers of cytolysis, cholestasis, bilirubin metabolism and thymol test, as well as the results of a physical examination and ultrasound examination of the abdominal organs. At the same time, the activity levels of enzymes were determined - alanine aminotransferase (according to the international

classification of enzymes 2.6.1.2.), aspartate aminotransferase (EC 2.6.1.1.), alkaline phosphatase (EC 2.3.2.4), gamma-glutamyl transpeptidase (EC 2.3.2.2), the concentration of total bilirubin, thymol test level.

The results of biochemical analysis were statistically processed using EXCEL 2016 spreadsheets and the STATISTICA 8.0 software package in the Microsoft Windows 10 Pro operating system. In each of the examined groups. In case of non-compliance with the normality criteria in each group, we calculated the values of the median (Me), its errors (m Me), the lower (25%) and upper (75%) quartile (Q25 - Q75) [52].

SECTION 3. RESULTS OF OUR RESEARCH

3.1. Analysis of the prevalence and biochemical parameters of toxic liver damage in the appointment of diclofenac

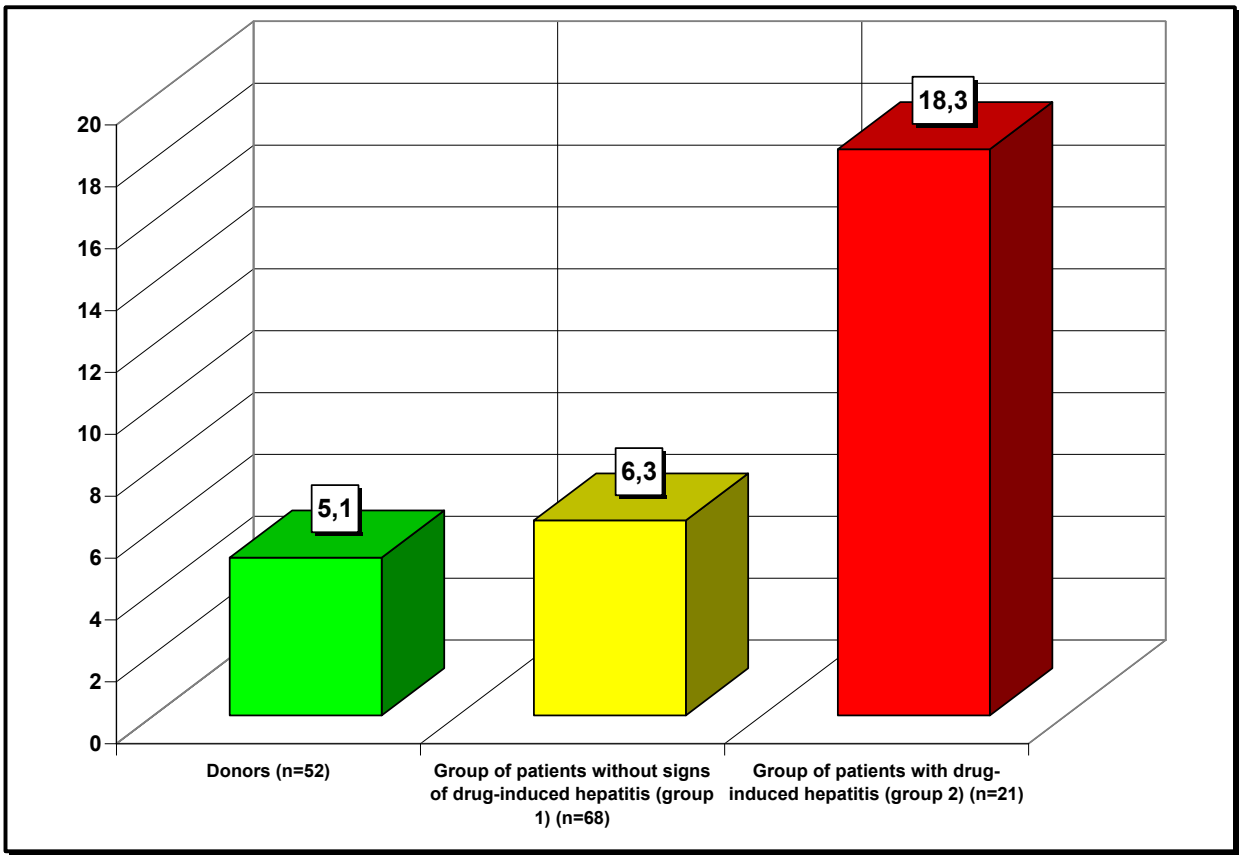
To identify intergroup differences in biochemical parameters of blood serum between groups of patients with the presence (n=21) and absence (n=68) of clinical and laboratory signs of drug-induced toxic hepatitis, we used the nonparametric Mann-Whitney method. As a result of our studies, we found that toxic liver damage was accompanied by a significant increase in the concentration of total bilirubin up to 18.3±0.2 μmol/l, which is 2.9 times higher than in the group without toxic liver damage (6.3±0.1 μmol/l; p<0.05 according to Mann-Whitney) and 3.6 times more than the level of bilirubin in the donor group (5.1±0.05 μmol/l; p<0.05 according to Mann-Whitney) (Table 2, picture 1).

Table 2

Biochemical parameters of blood serum of patients with or without drug-induced toxic hepatitis

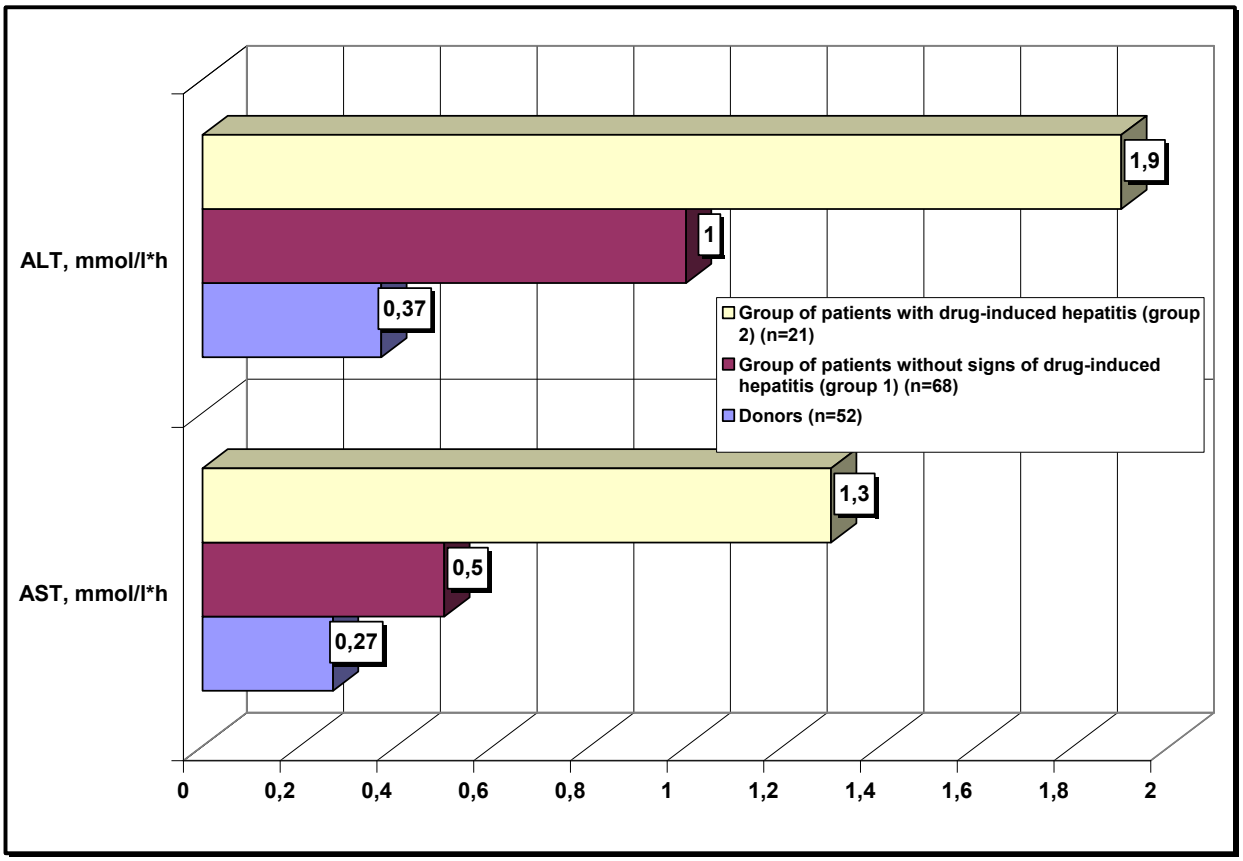
Groups Biochemical indicators	Donors (n=52) Me±m Me (Q25 - Q75)	Group of patients without signs of drug-induced hepatitis (group 1) Me±mMe (Q25 - Q75) (n=68)	Group of patients with drug-induced hepatitis (group 2) Me±mMe (Q25-Q75) (n=21)	Mann-Whitney score between groups of patients with and without drug-induced hepatitis
Total bilirubin, μmol/l	5.1±0.05 (2.91-5.82)	6.3±0.1* (4.2-8.5)	18.3±0.2* (14.5-21.6)	<0.05
ASAT, mmol/lg	0.27±0.01 (0.11-0.39)	0.5±0.07 (0.45-0.72)	1.3±0.09* (0.98-1.42)	<0.05
ALT, mmol/lg	0.37±0.01 (0.17-0.51)	1.0±0.04* (0.85-1.22)	1.9±0.07* (1.41-2.1)	<0.05
Alkaline phosphatase, mmol/lg	1.28±0.01 (1.11-1.38)	1.6±0.05* (1.44-1.9)	2.5±0.08* (1.7-2.61)	<0.05
GGTP, mmol/lg	531.26±1.89 (470-610)	701.0±18.6* (625-722)	1321.8±44.7* (782-945)	<0.05
Thymol test, units	1.68±0.02 (0.84-2.16)	2.9±0.03* (1.51-3.34)	4.8±0.11* (2.22-6.45)	<0.05

Note:
In the table, biochemical parameters are presented by the median (Me) and its error (mMe) (Me±mMe).
In brackets in the second line are indicated - 25% and 75% quartile (Q25 -Q75).
* - with a significant difference P<0.05 in the analysis of Mann-Whitney in comparison with the indicators of donors.



Pict. 1. The level of total bilirubin (µmol/l) in groups with and without drug-induced hepatitis

Analyzing the severity of the cytolytic syndrome, we found that the activity of AST in the group of patients without toxic liver damage practically did not differ from the indicators of donors (0.5 ± 0.07 mmol / l * h; 0.27 ± 0.01 mmol / l * h, respectively ; $p>0.1$ according to Mann-Whitney), and in the 2nd group, the activity of AST exceeded the level of group 1 by 2.6 times (1.3±0.09 mmol/l*h; $p<0.05$ according to Mann -Whitney), and donor indicators by 4.8 times (0.27±0.01 mmol/l*h; $p<0.05$ according to Mann-Whitney) (Table 2, (Fig. 2).

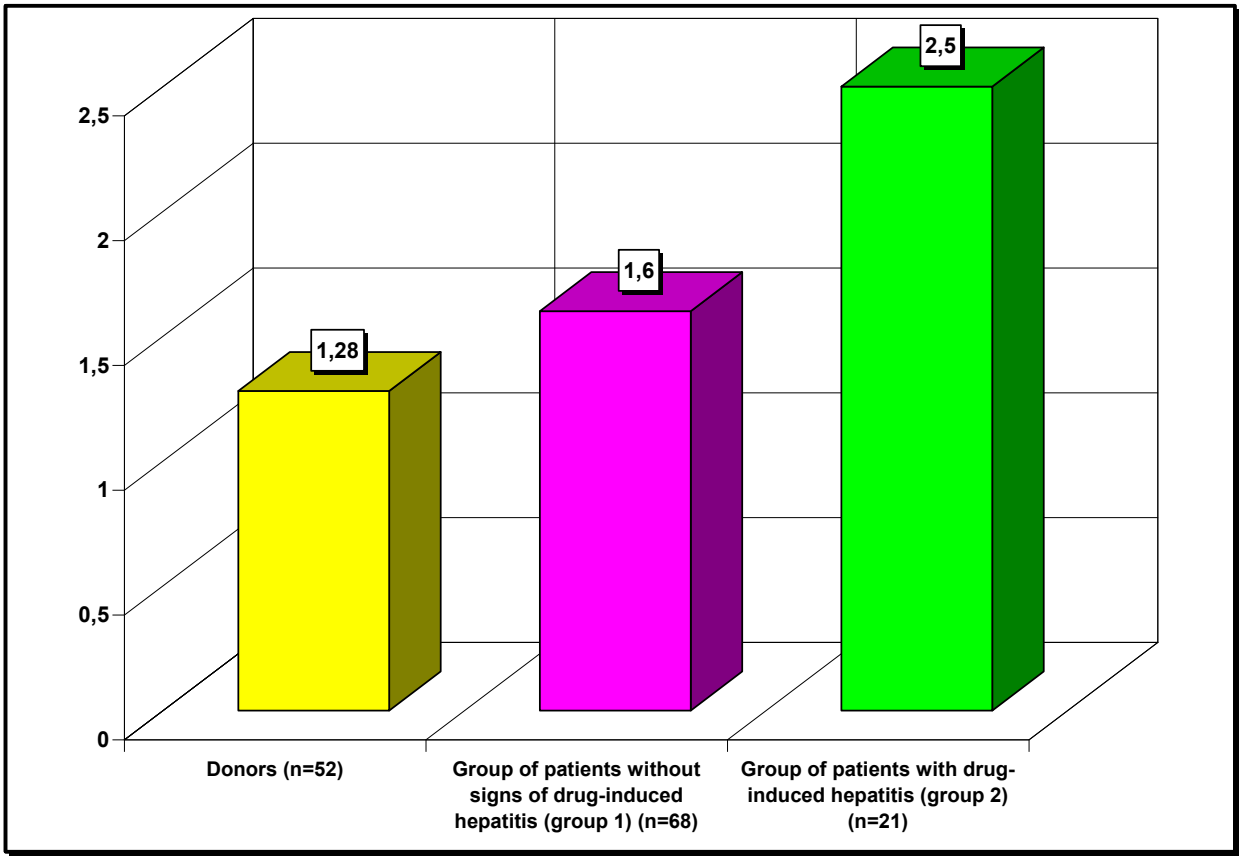


Pict. 2. The level of ALT and AST activity (mmol/l*h) in groups with and without drug-induced hepatitis

AlAT activity was higher than in donors in both groups 1 and 2. The indicator of group 1 was 2.7 times higher than the ALT level of donors (1.0±0.04 mmol/l*h; 0.37±0.01 mmol/l*h, respectively; $p<0.05$ according to Mann-Whitney). In toxic liver damage (Group 2), ALT activity (1.9±0.07 mmol/L*h) was significantly higher than in Group 1 (1.9 times; $p<0.05$ according to Mann-Whitney) and exceeded the level donors by 5.1 times ($p<0.05$ according to Mann-Whitney) (table, (Fig. 2).

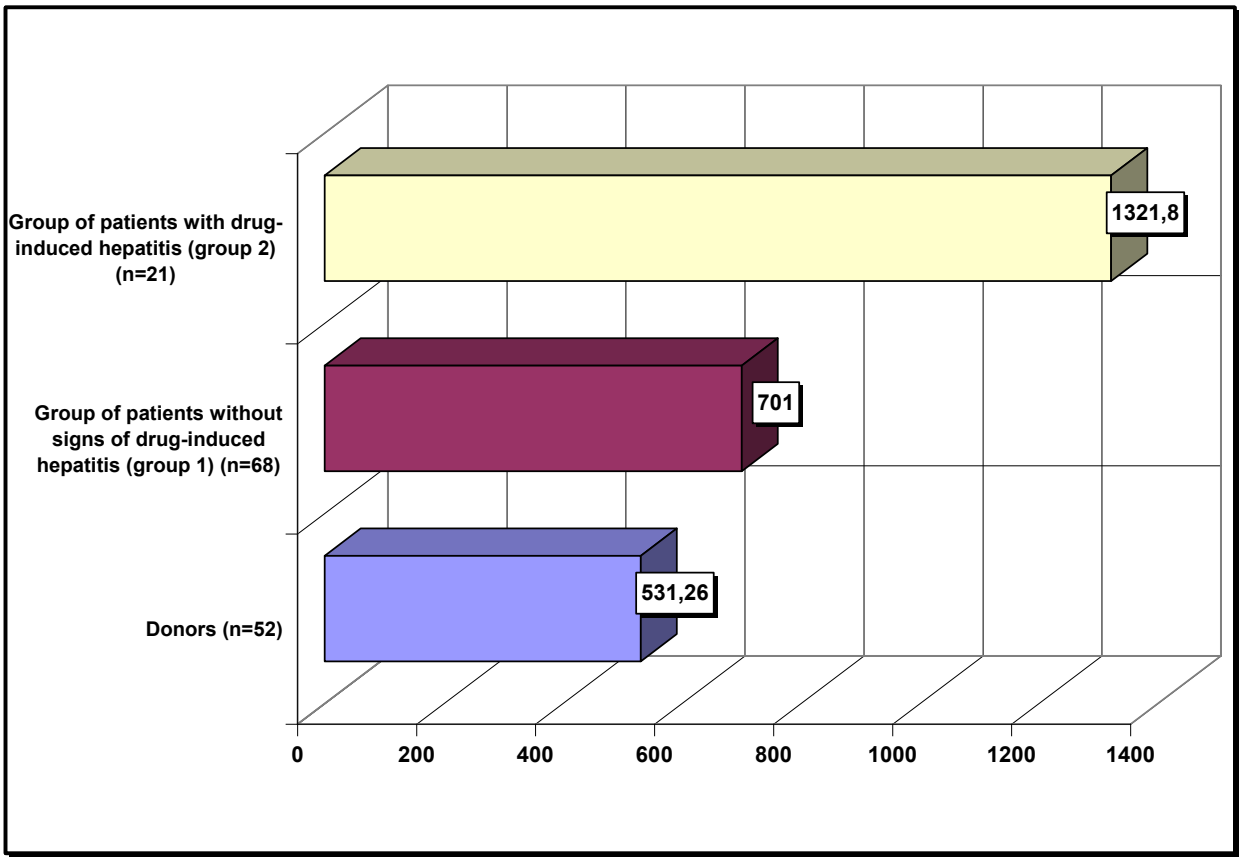
When studying the parameters of the cholestatic syndrome, we found that the activity of alkaline phosphatase in the group without toxic liver damage (group 1) exceeds the level of the donor group by 1.3 times (1.6±0.05 mmol/l*h; 1.28±0 .01 mmol/l*h, respectively). In the presence of drug-induced hepatitis, the level of alkaline phosphatase becomes even higher, and exceeds the indicators of group 1 by 1.6 times (2.5 ± 0.08 mmol / l * h; 1.6 ± 0.05 mmol / l * h, respectively; $p <0.05$ Mann-Whitney). The level of alkaline phosphatase of the 2nd group is 2.0 times higher

than the level of activity of this enzyme than in donors ($p<0.05$ according to Mann-Whitney) (Table 2, Fig. 3).



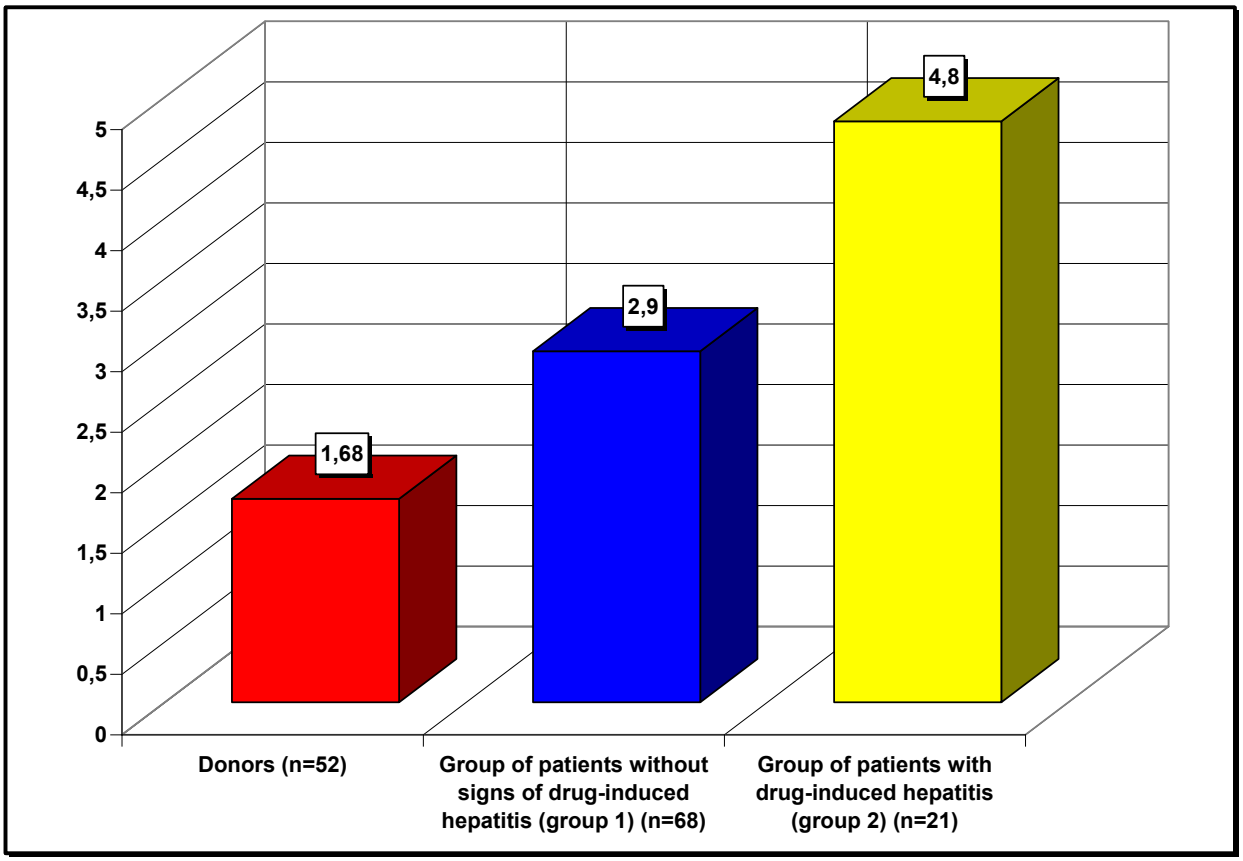
Pict. 3. The level of activity of alkaline phosphatase (mmol/l*h) in groups with and without drug-induced hepatitis

Analyzing the activity of group 2 gamma-glutamyl-transferase with the presence of toxic hepatitis, we found an increase in its level in relation to donors by 2.5 times (1321.8±44.7 mmol/l*h; 531.26±1.89 mmol/l *h, respectively; $p<0.05$ according to Mann-Whitney) and 1.9 times in relation to the indicators of group 1 (701.0±18.6 mmol/l*h; $p<0.05$ according to Mann-Whitney) (Fig. 4).



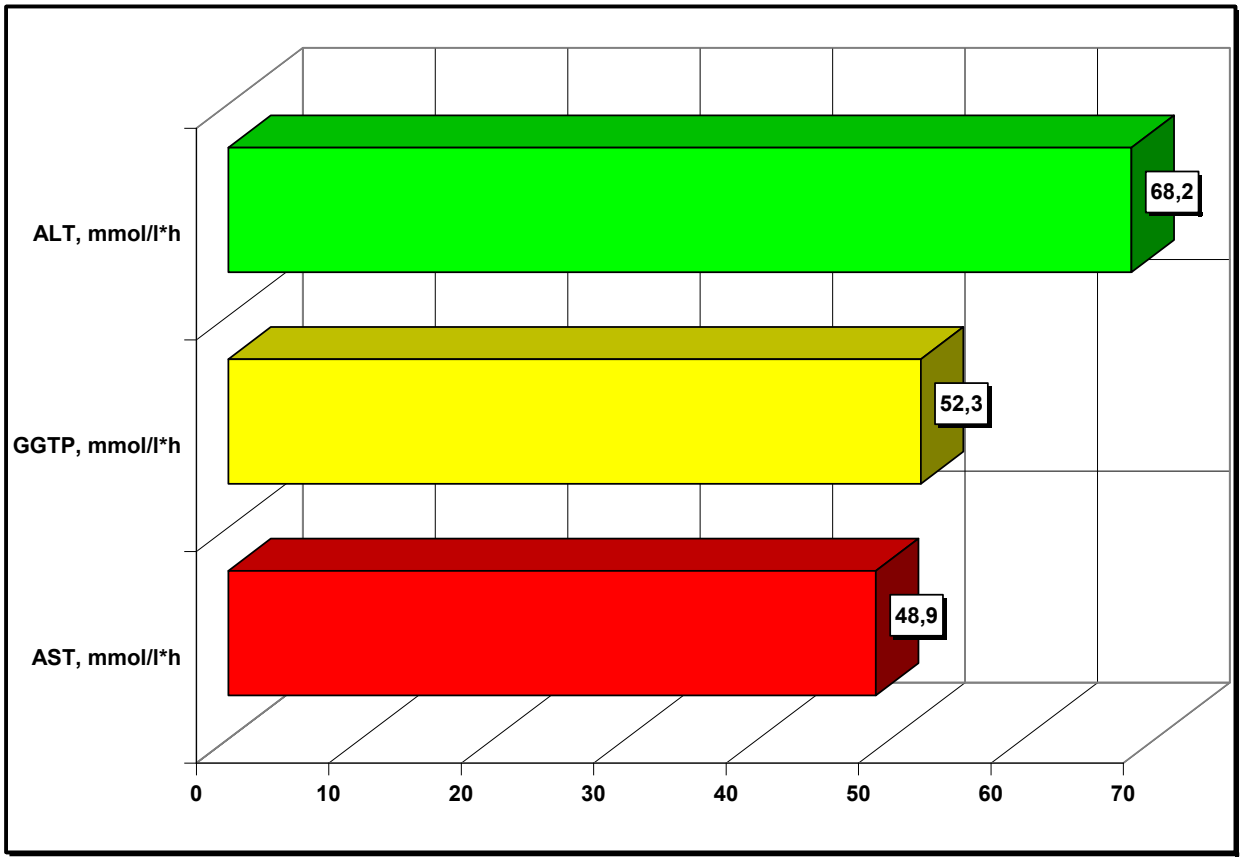
Pict. 4. Level of gamma-glutamyl transferase activity (mmol/l*h) in groups with and without drug-induced hepatitis

The level of thymol test was significantly higher than that of donors in both the 1st and 2nd groups. Thus, the level of this indicator in group 1 was 2.9±0.03 U, which exceeded the normal value by 1.7 times (1.68±0.02 U; $p<0.05$), and in the second group there was an increase of this parameter to 4.8±0.11 U, which is significantly more than in the 1st observation group by 1.7 times ($p<0.05$ according to Mann-Whitney) (Fig. 5).



Pict. 5. Thymol test level (mmol/L*h) in groups with and without drug-induced hepatitis

Next, we carried out a discriminant analysis, which allowed us to calculate the laboratory parameters of a group of patients with drug-induced toxic hepatitis, which most distinguish the 1st and 2nd groups. It was established that the following have the greatest discriminant properties: AlAT activity (F coefficient = 68.2; $p<0.05$), GGTP activity (F coefficient = 52.3; $p<0.05$), AST activity (F coefficient = 48, 9; $p<0.05$) (Figure 6).



Pict. 6. The value of the intergroup discriminant coefficient F of biochemical parameters

3.2. Parameters of the FAS fatigue scale in toxic drug-induced hepatitis and its correlations with biochemical parameters of cytolysis and cholestasis.

We analyzed the results of testing on the Fatigue Assessment Scale (FAS). The total score ranges from 10 to 50. A total FAS score < 22 indicates no fatigue, a score greater than 22 indicates fatigue.

When analyzing the FAS values in patients of group 1, we found that out of 68 patients, 29 patients (42.6%) had a value from 0-10 points, 26 people (38.2%) had a value from 11 to 20 points, from 21 to 30 points - 13 people (19.2%), more than 30 points - there were no patients (0%) (figure).

When using the limit of 22 points to verify hepatogenic fatigue in group 1, there were 13 such patients (19.1%).

In the second group with toxic drug-induced hepatitis, the distribution of scores was as follows: there were no patients from 0-10 points (0%), from 11 to 20 points

- there were 2 patients (9.5%), from 21 to 30 points - 11 people (52.4%), more than 30 points - 8 (38.1%) patients (figure).

When using the limit of 22 points to verify hepatogenic fatigue in group 2, there were 17 such patients (81.0%) (Figure 7).

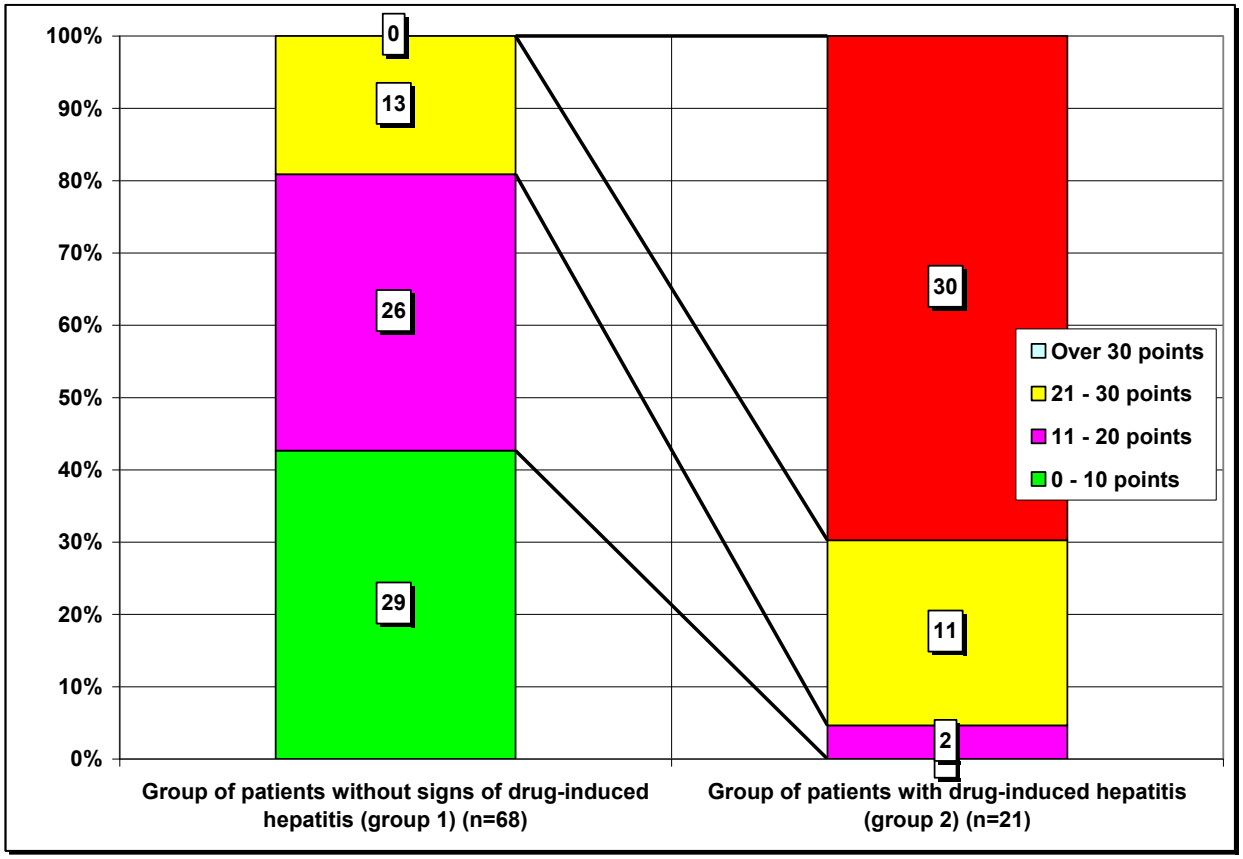


Fig. 7. Fatigue Assessment Scale (FAS) scores in both groups of patients

When conducting a non-parametric correlation analysis of Spearmen between the indices of cytolysis and cholestasis and the intensity of hepatogenic fatigue (according to the FAS scale), we found a significant correlation: between ALT and FAS - R = +0.68 ($p<0.05$), between GGTP and FAS - R=+0.61 ($p<0.05$), between ALT and FAS – R=+0.58 ($p<0.05$). This suggests the possibility of using the FAS scale for screening verification of hepatogenic fatigue (with a FAS value of more than 22 points) and early laboratory verification of drug toxic liver damage.

3.3. The possibility of using Celecoxib in combination with cardiotropic drugs.

It could potentially be possible to prescribe Celecoxib (a drug that has a minimal hepatotoxic effect) as an alternative to Diclofenac. But we have established the potentially most dangerous pharmacokinetic interactions of Celecoxib, as a potent inhibitor of CYP2D6, with substrates of this isoenzyme. The simultaneous use of Celecoxib with the beta-blocker Carvedilol and the calcium channel blocker Diltiazem can lead to a negative inotropic, chronotropic effect, a sharp decrease in blood pressure, bradycardia and collapse.

Drug-drug interactions and analysis between CYP2D6 inhibitors and substrates:

According to drug bank, the drug interactions are as follows with their severity state (major, moderate, minor):

Celecoxib/Diltiazem: (moderate state):

When Diltiazem is taken with Celecoxib, its metabolism might be slowed. The impacted medicine is metabolized and the relevant drug is a moderate inhibitor. Concurrent administration may reduce the affected drug's metabolism, resulting in higher blood concentrations as well as an increased risk and severity of side effects.

Celecoxib/amiodarone: (major state):

When Celecoxib is taken with Amiodarone, its metabolism can be slowed. The impacted medicine is metabolized by CYP3A4, and the relevant drug is a potent CYP3A4 inhibitor. Concurrent dosing reduces the affected drug's metabolism, raising blood concentrations as well as the risk and severity of side effects.

Celecoxib/propafenone: (moderate state):

Propafenone's antihypertensive activity may be reduced by Celecoxib. NSAIDs can cause vasoconstriction, which raises blood pressure. This can raise the risk of hypertension in people on beta-blocker anti-hypertensive medications.

Quinidine/propafenone: (major state):

Propafenone is a proarrhythmic drug that can produce new arrhythmias or aggravate pre-existing ones. Because quinidine has the ability to extend the QTc interval, co-administration of propafenone with quinidine may enhance the degree or severity of proarrhythmic effects. Furthermore, quinidine is a strong inhibitor of CYP2D6, which is essential for propafenone 5-hydroxylation. Co-administration of quinidine with propafenone may make all patients receiving medication slow metabolizers of

propafenone, resulting in reduced clearance and higher steady-state propafenone concentrations. Increased systemic exposure to the medicine may affect the therapeutic impact of the treatment and increase the likelihood of drug-related side effects.

Drug-drug interactions and analysis between P-glycoprotein inhibitors and substrates:

According to drug bank, the drug interactions are as follows with their severity state (major, moderate, minor):

Nifedipine/losartan: (moderate state):

In certain situations, the use of more than one antiarrhythmic medicine is justified, but it is critical to be aware of the potential risks associated with this combination. Depending on the substances employed, various pharmacokinetic or pharmacodynamic pathways may result in an antiarrhythmic medication interaction, offering a considerable risk of life-threatening cardiac problems. Concurrent use of two antiarrhythmic drugs may result in additive arrhythmogenic effects, such as excessive expansion of the cardiac QRS complex and/or prolonging of the QT interval. Furthermore, several antiarrhythmics may displace other drugs from their binding sites, interfering with their mode of action or changing serum concentrations. Furthermore, Losartan may increase the arrhythmogenic activities of nifedipine.

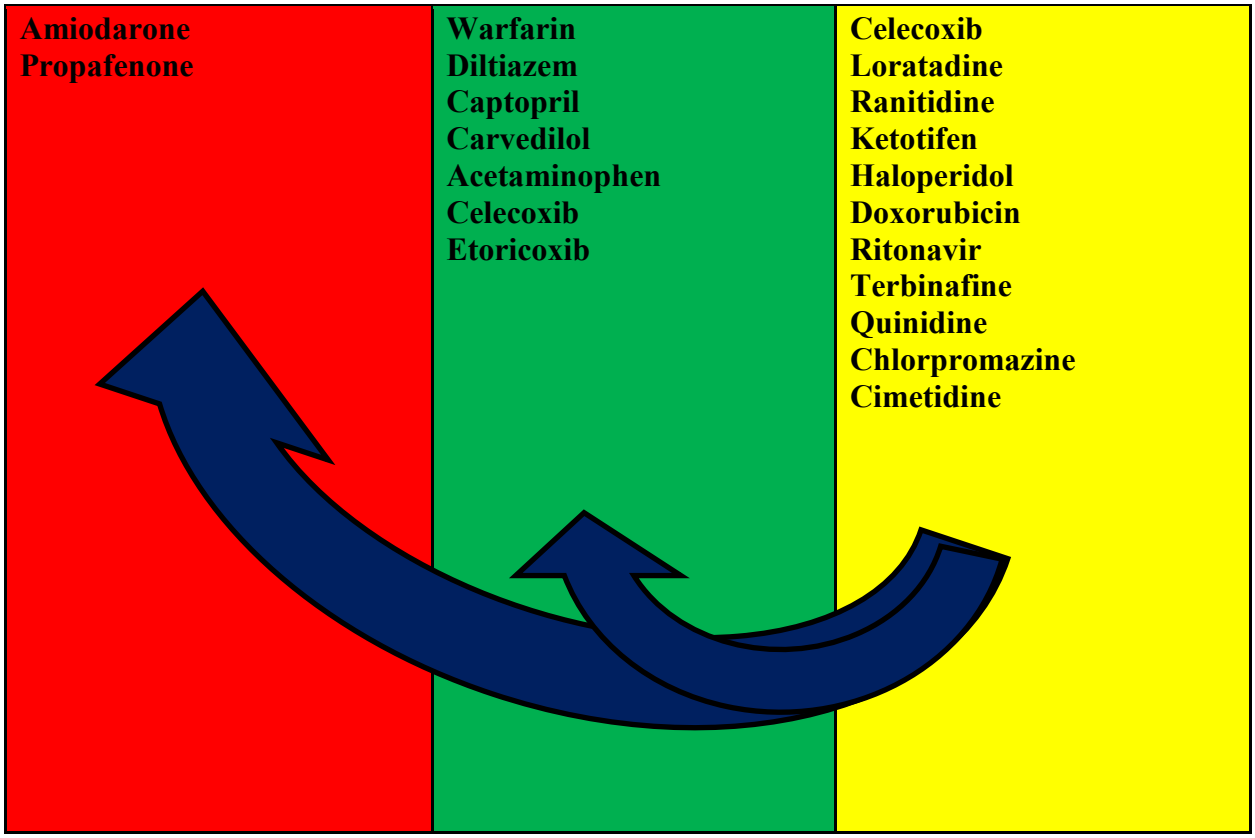
Spironolactone/Amiodarone: (major state):

When combined with Spironolactone, Amiodarone's metabolism can be slowed. Serum concentrations have noticeably increased. If the substrate has a narrow therapeutic index, there is only a small serum concentration range within which its administration is considered safe, and any potential increases or changes in serum concentration would result in an increased risk, incidence, and/or severity of adverse effects and toxicity associated with exposure to the given substrate.

Nicardipine/lovastatin: (moderate state):

When combined with Nicardipine, the metabolism of lovastatin can be reduced. Concurrent usage reduces lovastatin metabolism, raising blood concentrations as well as the risk and severity of side effects such as myopathy and rhabdomyolysis.

Co-administration of Celecoxib (a CYP2D6 inhibitor) with the indirect anticoagulant Warfarin may lead to an increase in Warfarin concentration and the development of symptoms of hypocoagulability. Early symptoms of a decrease in hemocoagulation are hematuria, then hemorrhagic rashes appear on the mucous membranes (enanthems) and on the skin (exanthems). If the early symptoms of high concentrations are not recognized by the doctor, inhibition of CYP2D6 by Celecoxib while taking Warfarin may develop internal bleeding with the development of anemia, tachycardia, melena (Figure 8).



Pict. 8. Interaction of drugs - substrates and inhibitors of CYP2D6

Co-administration of Celecoxib with amiodarone (a CYP2D6 substrate) may increase the concentration of this antiarrhythmic drug and induce sinus bradycardia, ventricular arrhythmia, and liver toxicity. The induction of a negative inotropic effect can lead to a sharp decrease in blood pressure, collapse and poor outcome (Figure 8).

The potentially dangerous interactions of Celecoxib with cardiotropic drugs found are more significant than its low hepatotoxicity, which makes it possible not

to recommend its appointment in clinical practice in therapeutic patients with heart pathology.

FINDINGS

1. In the analysis of 89 extracts from the medical history of patients with cardiac pathology (53 men and 36 women) aged 42 to 68 years, it was found that the most common cause of the development of drug toxic liver damage from NSAID preparations was Diclofenac (in 21 patients; 23.6 %).
2. Toxic liver damage was accompanied by a significant increase in the concentration of total bilirubin up to 18.3±0.2 μmol/l, which was 2.9 times higher than in the group without toxic liver damage (6.3±0.1 μmol/l; p<0, 05 according to Mann-Whitney), an increase in AST activity by 2.6 times (1.3±0.09 mmol/l*h; 0.5±0.07 mmol/l*h, respectively; p<0.05 according to Mann - Whitney), an increase in ALT activity by 1.9 times (1.9±0.07 mmol/l*h; p<0.05 according to Mann-Whitney).
3. In the event of toxic damage to the liver, cholestasis indicators also increased - alkaline phosphatase activity increased by 1.6 times (2.5±0.08 mmol/l*h; 1.6±0.05 mmol/l*h, respectively; p<0, 05 according to Mann-Whitney) and GGTP activity by 1.9 times (1321.8±44.7 mmol/l*h; 701.0±18.6 mmol/l*h; p<0.05 according to Mann-Whitney). Also significantly, by 1.7 times, the level of thymol test increased to 4.8±0.11 U (p<0.05).
4. The highest discriminant intergroup properties in toxic liver damage are: AlAT activity (F coefficient = 68.2; p<0.05), GGTP activity (F coefficient = 52.3; p<0.05), AST activity (F coefficient = 48.9, p<0.05).
5. When conducting a non-parametric Spearman correlation analysis between the indices of cytolysis and cholestasis and the intensity of hepatogenic fatigue (according to the FAS scale), we found a significant correlation: between ALT and FAS - R=+0.68 (p<0.05), between GGTP and FAS - R=+0.61 (p<0.05),

between ALT and FAS – R=+0.58 ($p<0.05$). Thus, for early screening verification of drug-induced toxic hepatitis, it is advisable to use the Fatigue Scale (FAS). If the total FAS coefficient exceeds more than 22 points, patients need in-depth clinical and biochemical examination and hepatotropic treatment.

6. Potentially, it could be possible to prescribe Celecoxib (a drug with minimal hepatotoxic effect) as an alternative to Diclofenac. But we have established the potentially most dangerous pharmacokinetic interactions of Celecoxib, as a potent inhibitor of CYP2D6, with substrates of this isoenzyme. The simultaneous use of Celecoxib with the beta-blocker Carvedilol and the calcium channel blocker Diltiazem can lead to a negative inotropic, chronotropic effect, a sharp decrease in blood pressure, bradycardia and collapse.
7. Co-administration of Celecoxib (a CYP2D6 inhibitor) with the indirect anticoagulant Warfarin may lead to an increase in Warfarin concentration and the development of symptoms of hypocoagulability. Co-administration of Celecoxib with amiodarone (a CYP2D6 substrate) may increase the concentration of this antiarrhythmic drug and induce sinus bradycardia, ventricular arrhythmia, and liver toxicity. Found potentially dangerous interactions of Celecoxib with cardiotropic drugs are more significant than its low hepatotoxicity, which does not allow it to be prescribed in therapeutic patients with heart pathology.

BIBLIOGRAPHY

1. De Valle MB, Klinteberg V. Av., Alem N. et al. / Drug-induced liver injury in a Swedish University hospital out-patient hepatology clinic // Aliment Pharmacol Ther.- 2006.- Oct 15;24(8).- p. 1187-95.

2. En Xian Sarah Low, Qishi Zheng, Edwin Chan, Seng Gee Lim / Drug induced liver injury: East versus West – a systematic review and meta-analysis // Clin. Mol. Hepatol.- 2020. - Apr;26(2). - P.142-154.

3. Katzung BG, Masters SB, Trevor AJ / Basic and clinical pharmacology. 12-ed. // New York: Mc Graw Hill Lange.- 2012.

4. Kleiner DE, Chalasani NP, Lee WM et al./ Hepatic histological findings in suspected drug-induced liver injury: systematic evaluation and clinical associations // Hepatology.- 2014.-59. – p.661–670.

5. Durkin E., Moran AP, Hanson PJ / Apoptosis induction in gastric mucous cells in vitro: lesser potency of Helicobacter pylori than Escherichia coli lipopoly-saccharide, but positive interaction with ibuprofen // J. Endotoxin. Res.- 2006; 12(1). - p.47-56

6. Perez-Gutthann S., Garcia-Rodriguez LA, Duque-Oliart A. et al. / Low-dose diclofenac, naproxen, and ibuprofen cohort study // Pharmacotherapy.- 1999.-19 (7) .- p.854-859

7. Naidoo V., Swan GE / Diclofenac toxicity in Gyps vulture is associated with decreased uric acid excretion and not renal portal vasoconstriction // Comp. Bio-chem. physiol. C. Toxicol. Pharmacol. // 2009.- 149 (3). - p.269-274.

8. Bessone F. / NSAIDs: What is the actual risk of liver damage? // World J. Gastroenterol.- 2010.- 16 (45) .- p.5651 - 5661.

9. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Principles of Internal Medicine. In: Peter K, editor. 18 ed. New York: McGraw-Hill; 2012

10. Katzung BG, Masters SB, Trevor AJ / Basic and clinical pharmacology. 12-ed. New York: Mc Graw Hill Lange; 2012

11. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Principles of Internal Medicine. In: Peter K, editor. 18 ed. New York: McGraw-Hill; 2012

12. Derry S., Moore RA / Single dose oral Celecoxibum for acute postoperative pain in adults // Cochrane. database. Syst. Rev. - 2013.- (10). -CD004233

13. Giuseppe Galati, Shahrzad Tafazoli, Omid Sabzevari et al. / Idiosyncratic NSAID drug induced oxidative stress // Chem. Biol. Interact. – 2002.- Nov 10;142(1-2). – p.25-41.

14. Bareille MP, Montastruc JL, Lapeyre-Mestre M. / Liver damage and non-steroidal anti-inflammatory drugs: case non-case study in the French Pharmacovigilance Database // Case Reports Therapie.- Jan-Feb 2001.- 56(1). – p.51-55.

15. A Dierkes-Globisch, R. Schäfer, HH Mohr / Asymptomatic diclofenac-induced acute hepatitis // Review Dtsch Med Wochenschr.- 2000.- Jun. 23;125(25-26). – p.797-800.

16. JS Lill, T. O'Sullivan, LA Bauer et al. / Pharmacokinetics of diclofenac sodium in chronic active hepatitis and alcoholic cirrhosis // J. Clin. Pharmacol. – 2000.- Mar;40(3). – p. 250-257.

17. Bhogaraju A., Nazeer S., Al-Baghdadi Y. et al. / Diclofenac-associated hepatitis // Case Reports South Med J.- 1999.- Jul;92(7). – p.711-713.

18. Hackstein H., Mohl W., Püschel W. et al. / Diclofenac-associated acute cholestatis hepatitis // Case Reports Z Gastroenterol.- 1998.- May; 36(5). – p.385-389.

19. Farrell GC / Drug-induced hepatic injury // Review J. Gastroenterol. Hepatol. - 1997. - Oct; 12 (9-10). – p.242-250.

20. Matveev AV, Koniaeva EI / Diclofenac sodium in osteoarthritis. Is there a risk of hepatotoxicity? A systematic review // Review Eksp. Klin. Gastroenterol. – 2010.- (10). – p.93-100.

21. Naomi Jessurun, Eugene van Puijenbroek / Relationship between structural alerts in NSAIDs and idiosyncratic hepatotoxicity: an analysis of spontaneous report data from the WHO database // Comparative Study Drug Saf.- 2015.- May;38(5).- p.511- 515.

22. Saravanakumar Selvaraj, Jung-Hwa Oh, Jürgen Borlak / An adverse outcome pathway for immune-mediated and allergic hepatitis: a case study with the NSAID diclofenac // Arch Toxicol.- 2020.- Aug;94(8).- p.2733 -2748.

23. Swain MG / Fatigue in liver disease: pathophysiology and clinical management // Can. J. Gastroenterol.- 2006. - 20. - p.181-188.

24. D'Mello C., Swain MG / Liver-brain interactions in inflammatory liver diseases: implications for fatigue and mood disorders // Brain Behav. Immun. – 2014.- 35. – p. 9-20.

25. Jones EA / Fatigue complicating chronic liver disease / Metab. brain. Dis. – 2004.- 19. – p. 421-429.

26. Yeoh SW, Holmes ACN, Saling MM et al. / Depression, fatigue and neurocognitive deficits in chronic hepatitis C // Hepatol. Int.- 2018. – 12. – p. 294-304.

27. Wessely S., Pariante C. / Fatigue, depression and chronic hepatitis C infection // Psychol. Med.- 2002. - 32. - p. 1-10.

28. Golabi P., Sayiner M., Bush H. et al. / Patient-Reported Outcomes and Fatigue in Patients with Chronic Hepatitis C Infection / Clin. Liver Dis.- 2017. - 21. - p.565-578.

29. Seaman K., Paterson BL, Vallis M. et al. / Future directions for investigation of fatigue in chronic hepatitis C viral infection // Chronic Illn.- 2009.- 5. - p.115-128.

30. Kumar D., Tandon RK / Fatigue in cholestatic liver disease--a perplexing symptom // Postgrad. Med. J. - 2002. - 78. - p.404-407.

31. Jopson L., Dyson JK, Jones DE / Understanding and Treating Fatigue in Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis / Clin. Liver. Dis.- 2016.- 20.- p. 131-142.

32. Newton JL / Fatigue in primary biliary cirrhosis // Clin. LiverDis. – 2008.- 12. – p.367-83.

33. Zakharia K., Tabibian A., Lindor KD, Tabibian JH / Complications, symptoms, quality of life and pregnancy in cholestatic liver disease // Liver Int. - 2018. - 38. - p.399-411.

34. Abbas G., Jorgensen RA, Lindor KD / Fatigue in primary biliary cirrhosis // Nat. Rev. Gastroenterol. Hepatol.- 2010. - 7. - p.313-319.

35. Swain MG, Jones DEJ / Fatigue in chronic liver disease: New insights and therapeutic approaches // Liver Int. - 2019. - 39. - p. 6-19.

36. Fisk JD, Ritvo PG, Ross L. et al. / Measuring the functional impact of fatigue: initial validation of the fatigue impact scale // Clin. Infect. Dis. - 1994. - 18.- Suppl 1. - S79-S83.

37. Smets EM, Garssen B., Bonke B., De Haes JC / The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue // J. Psychosom. Res.- 1995. - 39. - p. 315-325.

38. Webster K., Cella D., Yost K. / The Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System: properties, applications, and interpretation // Health Qual Life Outcomes. - 2003. - 1. - p. 79.

39. Lee KA, Hicks G., Nino-Murcia G. / Validity and reliability of a scale to assess fatigue // Psychiatry.- Res.- 1991. - 36. - p. 291-298.

40. Krupp LB, LaRocca NG, Muir-Nash J., Steinberg AD / The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus // Arch. Neurol.- 1989. - 46. - p. 1121-1123.

41. Chalder T., Berelowitz G., Pawlikowska T. et al. / Development of a fatigue scale // J. Psychosom. Res. - 1993. - 37. - p. 147-153.

42. Belza BL / Comparison of self-reported fatigue in rheumatoid arthritis and controls // J. Rheumatol. - 1995. - 22. - p. 639-643.

43. Lai JS, Cella D., Choi S. et al. / How item banks and their application influence can measurement practice in rehabilitation medicine: a PROMIS fatigue item bank example // Arch. Phys. Med. Rehabil.- 2011.- 92. - S20-S27.

44. Michielsen HJ, De Vries J., Van Heck GL / Psychometric qualities of a brief self-rated fatigue measure: The Fatigue Assessment Scale. // J. Psychosom. Res. – 2003.- 54. – p. 345-352.

45. Younossi ZM, Guyatt G., Kiwi M. et al. / Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease // Gut.- 1999. - 45. - p.295-300.

46. Chang-Hsing Lee, Jung-Der Wang, Pau-Chung Chen / Increased risk of hospitalization for acute hepatitis in patients with previous exposure to NSAIDs // Pharmacoepidemiol Drug Saf.- 2010. - Jul;19(7). – p.708-714.

47. Suresh Kumar Nayudu, Shanti Badipatla, Masooma Niazi et al. / Cholestatic hepatitis with small duct injury associated with Celecoxibum // Case Rep Med. - 2013. - 315479.

48. O'Beirne JP, Cairns SR / Drug Points: Cholestatic hepatitis in association with Celecoxibum // Case Reports BMJ.- 2001.- Jul 7;323(7303). – p.23.

49. Antonio Grieco, Luca Miele, Andrea Giorgi et al. / Acute cholestatic hepatitis associated with Celecoxibum // Case Reports Ann Pharmacother.- 2002.- Dec;36(12). – p.1887-1889.

50. Galan MV, Gordon SC, Silverman AL / Celecoxibum-induced cholestatic hepatitis // Case Reports Ann Intern Med.- 2001.- Feb 6;134(3). – p.254.

51. Fernando Bessone, Nelia Hernandez, Marcelo Gabriel Roma et al. / Review Expert Opin Drug Saf // Hepatotoxicity induced by coxibs: how concerned should we be?.- 2016.- Nov;15(11).- p.1463-1475.

52. Borovnikov V.P. / STATISTICA - Statistical analysis and data processing in the Windows environment / V.P. Borovnikov, I.P. Borovnikov / M.: Information and publishing house "Filin", 1997. - 608 p.