Acta Veterinaria (Beograd), Vol. 49. No. 2-3, 127–138, 1999.

UDK 619:612,357,131:636.2

THE CONCENTRATION OF TOTAL BILIRUBIN AND ACTIVITY OF AST, ALT, GLDH, CPK AND AP IN THE BLOOD OF COWS BEFORE AND AFTER CALVING

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(Received, 22. January 1999.)

The total bilirubin concentration and the activity of AST, ALT, GLDH, CPK, and AP were determined in the blood of cows (10 cows per group) after the first, third and fourth calvings, 15 days prior to the expected calving date and 3 and 10 days after calving.

In all three groups of cows, prior to calving, mean bilirubinaemia was within the physiological limits. In the puerperium it increased in all the three groups, to a peak on day 3 after calving. The increase was significant in the cows at the first calving (6,40 μmol/l) end very significant in the cows at the third calving (8,23 μmol/l) -i. e. hyperbilirubinaemia.

The mean AST activity was the lowest in the blood of all three groups of cows prior to calving (23,1-23,1-34,5 IU/l). On day 3 after calving AST activity was significantly increased in the cows at the first (47,0 IJ/l) and third calvings (45,9 IU/l) It was also above the upper physiological limit on day 10 after calving (63,5 IJ/l). At the time there was a very significant difference between the cows at the first calving (38,3 IU/l) and those at the third calving (63,5 IU/l), as well as between the cows at the third (63,5 IU/l) and fourth calvings (47,4 IU/l). All together, for the whole investigated period, in the cows at the third calving the correlation between AST activity and glucose concentration in the blood was very significant, and negative (r_{xy} =–0,75), and between AST and ALT very significant and positive (r_{xy}=0,49). As a whole, in all three groups of cows in late pregnancy the correlation between AST and GLDH was very significant and positive (r_{xy} = 0,57), whereas is was significant, and positive (r_{xy}=0,45) between AST activity and bilirubin concentration in the blood.

The mean activity of GLDH in the blood of cows at the first and third calvings was within physiological limits.In the cows at the fourth calving the GLDH activity was high on day 15 prior to calving (10,30 IU/l) and on day 3 after calving (12,40 IU/l), being significantly higher than in the blood of the cows at the third calving (6,88 IU/l).

During the investigated period the mean activities of ALT, CPK and AP in the blood of the cows remained within physiological limits.

Key words: total bilirubin, AST, ALT, GLDH,CPK, AP, blood,cows,calving

INTRODUCTION

With the termination of the dry period and the onset of lactation there is a significant change in the body needs for energy and nutrients. There is an increase in glucose metabolism as well as intensive mobilisation of energy resources (mainly triglycerides) from the body depots. As a result of lipomobilisation there is an accumulation of lipids in certain organs (the liver, muscles, kidney, adrenal glands etc) (Gall, 1983). Fat accumulation is most prominant in the liver, resulting in fatty liver syndrome. Such changes gi to disorders in the funciton of the liver, increasing at the same time the susceptibility of the organism to some diseases (ketosis, puerperal paresis, retentio secundinarum, mastitis, decreased fertility).

Alterations in hepatocyues membranes induce changes in the activity of certain enzymes in the liver and blood. In order to evaluate properly the changes in the activity of enzymes in the blood it is necessary to study the so-called "enzime profile of the liver" (Kraft and Schillinger, 1989) in high producing cows and assess the effect of late pregnancy, puerperium and the number of calvings on the changes in the activity of enzymes in the blood.

MATERIAL AND METHODS

The investigation was carried out on a dairy farm on 30 cows of the Holstein breed, after the first (N=10), third (N=10), and fourth calvings (N=10), 15 days prior to the expected calving date and 3 and 10 days after calving.

In fresh nonhaemolysed blood sera the concentration of bilirubin and aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GLDH), creatine phosphokinase(CPK) and alkaline phosphatase (AP) activity were determined by standard biochemical methods. During the investigation the diets were analysed. The obtained results were statistically evaluated using suitable methods.

RESULTS AND DISCUSSION

Analysis of the diets used in late pregnancy showed more protein and energy, and less dry matter, than recommended, whereas at the beginning of lactation there was more protein, and less energy and dry matter than needed. Many authors have found correlations between the bilirubin concentration and functional disorders, as well as pathological damage to the liver (Heidrich et al., 1962; Urbaneck and Rossow, 1963; Rosenberger, 1979; Krdžalić et. al., 1982; Hilary 1990) and thus the diagnosis of bilirubinaemia represents an important parameter in the assessment of the cows metabolic profile (Reid et al., 1977; Gregorović et al., 1980).

The obtained results show that bilirubinanemia was lowest during late pregnancy, for the first and third calvings. It was at that time, within the physiological limits in all the groups. On day 3 after calving there was a sudden increase in all the groups of cows which was significant at the first calving, and very significant at the third calving. In the cows at the third calving the mean value was above the upper physiciological limit and the differences between the mean values for each period were significant. (Table 1.)

The bilirubinaemia level in some cases during late pregnancy pointed to a disorder in liver function. There was a significant, positive, low correlation between the bilirubin concentration and the activity of AST in the blood (r_{xy}=0,45); as well as with the values of the AST/ALT quotient (r_{xy}=0,36) during this period.

Rosenberg (1979) pointed out that the bilirubin concentration in the blood of cows in the last week of pregnancy and in the puerperium can be increased up to 6,84 μmol/l. Such an increase in bilirubinaemia during the puerperium compared to late pregnancy has been noticed by many authors (Reid et al., 1983; Rosenberg, 1979; Jovanović et al, 1987; Kovačević, 1988; Lotthamer, 1991; Marković, 1991) and can be considered to be the result of functional damage to the liver during the puerperium.

Among the cows that had calved three times individual values of bilirubinaemia reached 15,56 μmol/l on day 3 after calving. A significant, low, positive correlation (r_{xy}=0,46) was found between the bilirubin concentration and the AST/ALT qoutient in the cows at the third calving for the whole period of investigation.

In the cows at the first calving a significant, low, positive correlation (r_{xy}=0,37) was observed between bilirubinaema and AST activity for the whole period. In those cows there was a very significant Highly positive correkation between bilirubinaemia and AST activity (r_{xy}=0,84) on day 3 after calving.

A significant, low, positive corelation existed between bilirubinaemia and AP activity in the blood (r_{xy}=0,37) in the cows at the fourth calving for the whole investigation.

AST is found in many organs and tissues (the cardiac muscles, liver, skeletal muscles, kidneys, brain) in the cytoplasm and mitochondria. Cell dammage results in release of enzyme and an increase in the activity in blood

Table 1. The concentration of total bilirubin and activity of AST, ALT, GLDH, CPK and AP in the blood of cows before and after calving

Parameter (Physiological limits)	Time of investigation	First calving (A)			Third calving (B)			Fourth calving (C)			Significance of differences between groups	
		M	SD	VR	M	SD	VR	M	SD	VR	P<0.05	P<0.01
Total Bilirubin	1.	3.88	1.24	2.20 – 6.06	3.14	0.70	1.90 – 4.28	5.24	1.36	3.42 – 8.63		
(0.85–6.84)	2.	6.40	3.66	1.16 – 13.56	8.23	3.27	3.68 – 15.56	6.31	2.61	3.10 – 10.98		
mmol/l	3.	5.95	2.83	0.38 – 9.80	5.65	0.96	4.09 – 8.07	4.88	2.12	1.01 – 8.84		
Significance of differences	P<0.05	1 : 2			1 : 3, 2 : 3							
	P<0.01				1 : 2							
AST (10–50 IU/l)	1.	23.1	7.37	13 – 38	23.1	7.65	7 – 35	34.5	17.80	15 – 72		
	2.	47.0	13.71	21 – 67	45.9	18.89	10 – 74	37.4	7.42	22 – 48		
	3.	38.3	15.28	11 – 62	63.5	17.60	38 – 93	42.4	14.88	22 – 73		A : B, B : C
Significance of differences	P<0.05	1 : 3										
	P<0.01	1 : 2			1 : 2, 1 : 3, 2 : 3							
ALT (5–20 IU/l)	1.	7.5	2.46	2 – 11	6.5	1.96	4 – 9	6.7	1.27	5 – 9		
	2.	6.6	1.96	3 – 10	6.7	2.87	2 – 13	6.4	2.58	4 – 13		
	3.	7.5	3.29	3 – 13	8.5	3.69	5 – 16	9.4	3.38	6 – 16		
Significance of differences	P<0.05							1 : 3, 2 : 3				
	P<0.01											
GLDH (3.5–10 IU/l)	1.	7.64	4.87	2.85 – 15.86	7.22	2.90	3.80 – 14.90	10.30	6.01	3.49 – 22.52		
	2.	8.45	5.89	2.05 – 16.81	6.88	3.22	3.17 – 14.28	12.40	8.09	5.40 – 33.32	B : C	
	3.	8.02	5.77	2.22 – 20.94	7.66	5.55	3.17 – 23.16	9.34	5.45	1.58 – 19.99		
Significance of differences	P<0.05											
	P<0.01											
CPK (10–50 IU/l)	1.	24.27	11.28	6.00 – 45.00	20.60	8.21	10.00 – 34.00	20.10	16.80	6.00 – 65.00		
	2.	38.71	24.25	4.00 – 86.00	19.82	11.33	0.00 – 42.00	30.44	14.93	12.00 – 58.00		A : B
	3.	29.65	10.81	20.83 – 44.33	28.68	19.88	6.00 – 63.00	23.10	11.33	8.00 – 42.99		
Significance of differences	P<0.05	1 : 2										
	P<0.01											
AP (10–30 IU/l)	1.	20.94	8.02	8.00 – 34.00	27.30	9.65	9.00 – 40.00	22.50	5.43	12.00 – 31.00		
	2.	25.45	9.57	7.00 – 37.00	19.85	5.94	14.00 – 36.00	28.29	7.50	14.20 – 38.00	B : C	
	3.	21.06	830	8.00 – 35.49	23.77	12.21	9.00 – 47.00	26.65	7.22	13.00 – 38.22		
Significance of differences	P<0.05											
	P<0.01											

Key: 1 = 15 days before calving
2 = day 3 after calving
3 = day 10 after calving

M = Mean
SD = Standard Deviation
VR = Variation Range

Significance of differences
$p < 0.05$ significant
$p < 0.01$ very significant

serum. Although it is not particularly specific, in combination with other enzyme metabolites it can give important diagnostic results pertaining to the health of the cows (Tietz, 1988; Holod and Ermolaev, 1988: Rosenberger, 1979, Kauppinen, 1983).

The mean activity of AST was, on the lowest in the blood of all the cow groups on day 15 prior to calving remaining within physiological limits. On day three after partus there was an increase in AST activity, which was very significant in groups A (47,0 IU/l) and B (45,9 IU/l). During this period in 50% of the cows from group A and 40% of the cows from group B there was increased activity of AST in the blood. On day 10 after calving in group A the mean AST activity was still, significantly higher ($p<0,05$) than the activity 15 days before calving. In the cows at the fourth calving (C group) the mean AST activity on day 10 after calving was the highest (42,4 IU/l) but the differences during the experiment were not significant. In the cows at the third calving the mean activity of AST increased in the puerperium and on day 10 after calving was above the upper physiological limit (63,5 IU/l). The difference in mean activities between days 10 and 3 after calving, as well as that between day 15 before calving and 10 after calving were very significant ($p<0.01$). During this period 20% of the cows at the first and fourth calvings and 80% of those at the third calving had increased AST activity. On day 10 after calving there was significant difference between the cows at the first calving (38,3 IU/l) and those at the third calving (63,5 IU/l) as well as between those at the first calving (63,5 IU/l) and those at the fourth calving (47,4 IU/l).

Many authors (Gould and Grimes, 1960; Stockl et. al., 1965; Herak and Herak, 1966; Hilary, 1990; Lothamer, 1991; Marković, 1991; Bostedt, 1976; Jovanović and Salas, 1985; Kovačević, 1988) have observed that the level of AST activity increased immediately prior to partus and up to 12 days after partus. Stockl et al., (1965) explained this phenomenon by an increased load to the liver of fats in the course of the period immediately prior to and after calving. The level of the AST activity in the blood of the investigated cows at the first and fourth calvings was very similar to the findings of Sommer (1985), Hilary (1990), Bostedt (1974), and lower than the findings of Kovačević (1988), Jovanović and Salas (1985) and Marković (1991). The results for the cows at the third calving were higher than those obtained by the above mentioned authors. Reid et al., (1983) found the mean AST activity to be 62,3 IU/l in cows with fatty liver syndrome. Our results obtained on the tenth day after calving for the cows at the third calving were higher then those obtained by Reid et al (1983). This lead us to conclude that cows with fatty liver syndrome exist in the group. According to Rosenberg (1979) the serum activity of AST increases even after mild damage to the liver. The author stated that among fast, timely enzyme tests the only one of any diagnostic value in cattle is AST. However, the best indicator of liver damage is simultaneous determination of the activity of several enzymes in the blood sera. Increases in the activity of AST and GLDH are a sign of liver damage; whereas increased activity of AST and CPK point to damage to the musculature (Rich and Dunavant, 1972; Rosenberg, 1979 and Kraft, 1989).

In cows at the first calving, on day 3 after calving, 50% of the cows had increased AST activity; in 30% of the cows there was simultaneous increased activity of AST and CPK, and in 10% of the cows increased activity of AST, CPK and GLDH.

Already in late pregnancy, simultaneous increases at the fourth calving in the activity of AST and CPK were observed in 10% of the cows, and of AST and GLDH in 20% of the cows at the fourth calving. Increases in AST and GLDH activity were observed in 10% of the cows on day 10 after calving.

Among the cows at the third calving, on days 3 and 10 after calving, 20% had simultaneously increased AST and GLDH activities in the blood sera, whereas an increase in AST and CPK, was noticed in 20% of the cows on day 10 after calving.

There was a very significant negative correlation between AST activity and glucose concentration in the blood (r_{xy}=–0,75), as well as a very significant, positive correlations between AST and ALT activities (r_{xy}=0,49), and between AST activity and the AST/ALT quotient (r_{xy}=0,57) in the cows at the third calving, for the whole period of the investigation. The results obtained confirm opinions about the existence of a disorder in liver function of the investiated cows. Taken in total, for all the cow groups in late pregnancy, there was a very significant, positive correlation between AST and GLDH activities (r_{xy}=0,57), and between AST activity and the AST/ALT quotient (r_{xy}=0,65).

Schroter et al., (1983) observed that the activity of AST in bovine blood sera increases with age. In cows, aged 2,5 years, it was 30 IU/l, but in older cows it reached 40 IU/l. However, Peterson and Waldern (1981) noticed a decrease in AST activity with age. In our case, the observed differences between cows at the first, third and fourth calvings, on day 10 after calving, must be the result of damage to hepatocytes, because no differences in AST activity in the blood related to the number of calvings and age were observed.

The mean ALT activity in all the groups of cows remained, during the experiment, within physiological limits. On day10 after calving (the fourth calving) there was an increase in ALT activity up to 9,4 IU/l, ($p<0,05$) in comparison to the first two periods. Kovačević (1988). Bostedt (1974) observed a mild drop in the serum activity of ALT in the puerperium compared to late pregnancy.

GLDH is a cellular enzyme, that is found exclusively in mitochoundria. The level of activity in blood sera coresponds to the severity of pathological processes in the liver (degeneration, toxic hepatitis, hepatocyte necrosis), namely those that are of longer duration (Rich and Dunavant, 1972; Graf, 1981; Lotthamer, 1981). In the cows at the first and third calving the mean GLDH activity remained within the physiological limits.

In the cows at the fourth calving, already in late pregnancy, the mean GLDH activity was above the physiological limit (10,30 IU/l) to be increased to 12,40 IU/l on day 3 after calving. This was significantly higher than in the blood sera of the cows at the third calving (6,88 IU/l). Hilary (1990) found somewhat lower GLDH activity in the blood sera of cows than our results. During late

pregnancy GLDH activity was 3,9±0,42 IU/l, and in the puerperium 5,2±1,34 IU/l. Holod and Ermolaev (1988) also had lower physiological values for the serum activity of GLDH in cows, namely 4 and 4-5,8 IU/l. Cote and Hoff (1991) found a much wider range of GLDH physiological activities in the blood sera of cows; 6-38 IU/l, with an average of 20 IU/l. The majority of authors (Grunder, 1979; Baumgartner and Schlerka, 1983; Kraft et al., 1989; Lotthamer, 1991) consider that the upper physiological limit of GLDH serum activity in cows is 10 IU/l.

Kloene (1974) and Hilary (1990) did not find a statistically significant difference in the GLDH activity between late pregnancy and the puerperium. According to Bostedt (1974) GLDH activity only slightly oscillates, this being much more marked in adult cows. Depke (1981), found different courses of the serum activity of enzymes in different groups of cows, during the first weeks of lactation. The same was evident during our experiment.

The very significant, positive correlation between GLDH and AST activities in late pregnancy (r_{xy}=0,57), for all the groups of cows, points to liver damage during late pregnancy; in which case calving only contributed to an otherwise bad condition.

Many authors (Bostedt, 1974; Kloene, 1974; Adam, 1983; Bostedt et al., 1976) stress the dependence of GLDH activity in blood sera upon the age of the cows. The high GLDH activity in the cows at the fourth calving can be considered as the result of parenchymatous liver demage, rather than the influence of age upon GLDH activity.

The increase in CPK activity, during the studied period, was most marked in the cows at the first calving, where a significant difference ($p<0,05$) was noted between the CPK activity on day 15 prior to and day 3 after calving.

Many authors (Frahm et al., 1978; Holod and Ermolaev, 1988; Hilary, 1990) point to considerable physiological changes in CPK activity in cow blood. Increased physical activity, movement, muscle damage, even the act of parturition can cause increased CPK activity, as shown here. Data concerning the physiological values of the serum activity of CPK in cows vary widely.Our results are similar to those obtained by Rosenberger (1970), Vrzgula and Sokol (1987), Kraft (1989) and Hilary (1990). Kaneko (1989) observed much lower values (7,4±2,4 or 4,8-12,1 IU/l) and Holod and Ermolaev (1988) and Cote and Hoff (1991) gave much higher physiological values (85-300 and 85-468 IU/l).

The mean CPK, values for the investigated groups on day 15 prior to and on day 10 after calving indicated decreased serum activity dependent on the calving number. On day 3 after calving there was a very significant difference ($p<0.01$) in CPK activity between the first (38,7 IU/l) and third calving (19,82 IU/l). In young animals the CPK activity is physiologically higher.

In cows at the first calving there was a significant,positive correlation between AST and CPK activities on day 10 after calving (r_{xy}=0,65). The results point to muscle damage, as well as a simultaneous increase in AST and CPK activities in 30% of cows on day 3 after calving..

Among the cows at the third calving there was a very significant, close, negative correlation between CPK activity ond bilirubin concentration (r_{xy}=-0,79). The results obtained show that on day 15 prior to and day 3 after calving the parenchyma of the liver was greatly damaged due to lipomobilisation, whereas there was no significant damage to the musculature.

In the cows at the fourth calving chronic damage of the liver was observed as well as damage to the musculature.This was signalled by the significant, positive correlation between the activities of CPK and GLDH (r_{xy}=0,44) and also there was a significant, positive correlation between the CPK and GLDH activities on day 3 after calving (r_{xy}=0,65) as well as between CPK and AST activities in late pregnancy (r_{xy}=0,76).

The mean values for AP in the blood of all the groups were within the physiological limits during the investigation.

Physiological oscillations in AP activity in bovine blood sera are pronounced, which makes many authors (Wurzner, 1964; Rich and Dunavant, 1972) challenge the diagnostic importance of this enxyme. From the relevant data in the literature it can be seen that our results agree with those of Rosenberg (1970) and Vrzgula and Sokol (1987). Thus, data concerning AP activity during late pregnancy and the puerperium in cows also vary. Umshelm and Flock (1967) found increased AP activity during the last months of lactation, and decreased activity at the end of pregnancy. Belyea and coworkers (1975) described increased AP activity prior to calving, with maximum values at the time of calving, followed by a downward trend.These changes in AP activity during the periparturient period point to increased resorption of calcium from the bones. Bostedt (1974) considered that this increase is result of changes occuring in the uterus during this period.

The mean AP activities in individual groups on day 3 after calving exhibited a significant difference ($p<0.05$) between the cows at the third calving (19,85 IU/l) and those at the fourth calving (28,28 IU/l).

In the cows at the first calving a singificant, negative correlation (r_{xy}=-0,39) betweeen AP and ALT activities was observed for the whole period of investigation, as well as a significant, negative correlation (r_{xy}=-0,65) on day three after calving. There were also significant, negative correlations between the activities of AP and GLDH for the whole period of investigation and on day 10 after calving (r_{xy}=-0,64). On day 3 after calving there was a very significant, negative correlation between the samme parameters (r_{xy}=-0,79).

A significant, positive correlation (r_{xy}=0;51) between AP and GLDH activities existed among the cows at the third calving for the whole period of investigation, and a very significant, positive correlation (r_{xy}=0,78) on day 10 after calving.

In the cows at the fourth calving there was a signifiant, positive correlation (r_{xy}-0,37) between the acivity of AP and the bilirubin concentration.

The obtained results show that more or less marked disturbances in the functioning of the liver are present in the investigated cows during the peripartum. This is diagnosed on the basis of the findings of hyperbilirubinaemia, increased serum activity of AST and GLDH, positive correlations between bilirubinaemia and AST activity (significant), AST and GLDH activities (very significant), AST and ALT activities (very significant), and a negative correlation between AST activity and glycaemia (very significant).

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KONCENTRACIJA UKUPNOG BILIRUBINA I AKTIVNOST AST, ALT, GLDH, CPK I AP U KRVI VISOKOGRAVIDNIH I TEK OTELJENIH KRAVA KOD PRVOG, TREĆEG I ČETVRTOG TELENJA

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SADRŽAJ

Koncentracija ukupnog bilirubina i aktivnost AST, ALT, GLDH, CPK i AP ispitivani su u krvi po deset krava prvog, trećeg i četvrtog telenja 15 dana pre i 3. 10. dana posle telenja.

Bilirubinemija u sve tri grupe krava pre telenja u proseku je u fiziološkim granicama. U puerperijumu bilirubinemija se u svih grupa krava povećava i to najviše 3. dana posle telenja, što je kod prvotelki značajno (6,40 μmol/l), a kod trećetelki vrlo značajno (8,34 μmol) i označava hiperbilirubinemiju.

Aktivnost AST u proseku je najniža u krvi svih grupa krava pre telenja (23,1-23,1-34,5 UJ/l). Trećeg dana posle telenja aktivnost AST vrlo značajno raste kod prvotelki (47.0 UJ/l i trećetelki (45,9 UJ/l), kod kojih je 10. dana posle telenja iznad fiziološke granice (63,5 UJ/l). Tada postoji vrlo značajna razlika između prvotelki (38,1 UJ/l) i trećetelki (63,5 UJ/l), kao i između trećetelki (63,5 UJ/l) i četvorotelki (47,4 UJ/l). Zbirno za ceo ispitivani period kod trećetelki razlika između aktivnosti AST i koncentracije glukoze je vrlo značajna, srednja, negativna (r_{xy} =–0,76), a između AST i ALT vrlo značajna, niska, pozitivna (r_{xy}=0,49). Zbirno za sve grupe krava u visokom graviditetu korelacija između aktivnosti AST i GLDH je vrlo značajna, srednja, pozitivna (r_{xy} =0,57), a između aktivnosti AST i koncentracije bilirubina u krvi značajna, niska, pozitivna (r_{xy}=0,45).

Prosečna aktivnost GLDH u krvi prvotelki i trećetelki kretala se u fiziološkim granicama. Kod četvorotelki aktivnost GLDH je povećana 15. dana pre telenja (10,30 UJ/l) i 3. dana posle telenja (12,40 UJ/l), kada je značajno veća od aktivnosti u krvi trećetelki (6,88 UJ/l).

Prosečna aktivnost ALT, CPK i AP u krvi svih grupa krava nalazi se u ispitivanom periodu u fiziološkim granicama.

U ispitivanih grupa krava u peripartalnom periodu postoje više ili manje izraženi poremećaji funkcionalnog stanja jetre, što se ustanovljava na osnovu hiperbilirubinemije, porasta aktivnosti AST i GLDH u krvi, postojanja korelacije između koncentracije bilirubina u krvi i aktivnosti AST (značajna, pozitivna), aktivnost AST i glikemije (vrlo značajna, negativna), aktivnosti AST i GLDH (vrlo značajna, pozitivna), aktivnost AST i ALT (vrlo značajna, pozitivna).