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Comparison of hepatoprotective effect from ischemia-reperfusion injury of remote ischemic preconditioning of the liver vs. local ischemic preconditioning of the liver during human liver resections

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Abstract:

Aim: To compare and evaluate the hepatoprotective effect of remote ischemic preconditioning (RIPC) with local ischemic preconditioning (LIPC) of the liver during human liver resections.

Methods: A prospective, single-center, randomized control trial was conducted in the Clinical Hospital "Dubrava" from April 2017 till January 2018. A total of 60 patients, who underwent liver resection due to colorectal cancer liver metastasis, were randomized to one of three study arms: 1) an RIPC group, 2) a LIPC group and 3) a control group (CG) in which no ischemic preconditioning was done before liver resection. The hepatoprotective effect was evaluated by comparing serum transaminase levels, bilirubin levels, albumin, and protein levels, coagulograms and through pathohistological analysis. The trial was registered on ClinicalTrials.gov (NCT03130920).

Results: Significant differences were found in serum levels of liver transaminases and bilirubin levels between the groups, the highest level in the CG and the lowest level in the LIPC group. Levels of cholinesterase were also significantly higher in the LIPC group. Pathohistological findings graded by the Rodriguez score showed favorable changes in the LIPC and RIPC groups versus the CG.

Conclusion: Strong evidence supports the hepatoprotective effect from ischemia-reperfusion injury of RIPC and LIPC preconditioning of the liver. Better synthetic liver function preservation in these two groups supports this conclusion.

Introduction:

Liver resection is the first line of treatment for primary and secondary liver malignancies (1-2). The major surgical problem during liver resections is intraoperative blood loss. Intermittent portal triad clamping associated with low central venous pressure achieved during the procedure decreases intraoperative blood loss during liver resection (3-4). The sequence of hepatic ischemia and reperfusion has been associated with ischemia/reperfusion (IR) injury of the liver. After major liver resection under partial or total vascular exclusion, IR injury of the remnant liver may be a serious complication, leading to postoperative liver dysfunction and increased morbidity and mortality (5). IR results in reduced perfusion of the liver and the induction of the inflammatory cascade involving the adhesion of leukocytes to endothelial cells and transmigration into the sinusoids. The IR injury correlates with the severity and duration of ischemia (6). Hepatic IR injury has an early and late phase. In the early phase, the Kupffer cells are most responsible for the activation of the inflammatory cascade, release of free radicals and cytokines and endothelial injury. In the late phase of IR liver injury neutrophils release free radicals and cause parenchymal injury (7).

The benefit of preconditioning in liver surgery has been well-known. Experimental and clinical evidence suggests that preconditioning can prevent or decrease IR injury, especially after long ischemic periods (8). There are several preconditioning techniques (mechanical and pharmacological), neither of which has been established as a "gold standard". In this trial two different mechanical techniques of ischemic preconditioning were analyzed.

Local ischemic preconditioning (LIPC) is a process during which a short period of ischemia is followed by a period of reperfusion prior to the prolonged ischemia, which seems to render organs more tolerant to IR injury (9). Local ischemic preconditioning is protective for different tissues, including skeletal muscles (9), brain (10), retina (11), spinal cord (12), kidney (13), intestine (14) and liver (15). The precise mechanism by which LIPC confers hepatoprotection is not fully understood yet. It is postulated that LIPC suppresses cytokine release, enhances the production of hepatoprotective adenosine, and increases adenosine triphosphate (ATP) availability by slowing the rate of ATP depletion, thus leading to upregulation of the process of cellular ATP production and liver regeneration, and also reduction of the liver apoptotic response (15-17).

Remote ischemic preconditioning (RIPC) involves the protection of an organ from prolonged ischemia by brief periods of ischemia and reperfusion to a remote organ. Previous studies have shown that RIPC improved parenchymal perfusion and oxygenation that reduced hepatocellular injury in the early phase of IR injury (18-19). Protective effects of RIPC are achieved owing to interactions between neural, humoral, and systemic pathways. These interactions lead to inhibition of the inflammatory response and activation of various hepatoprotective subcellular cascades (20). However, most of these studies have been performed in animals. There are not many studies conducted in humans which evaluate these methods of preconditioning.

Considering the results from published studies, we decided to carry out a prospective randomized control trial, which would clarify the effectiveness of RIPC or LIPC in preventing IR injury of the liver during liver resection, by evaluating the postoperative synthetic function of the liver remnant. Our hypothesis was that RIPC is effective in preventing IR injury during human liver resections. The aim of the study was to evaluate hepatoprotective effect from IR injury of RIPC of the liver against LIPC of the liver during human liver resections.

Patients and methods:

Sixty patients with colorectal cancer liver metastasis who underwent liver resection in the Clinical Hospital "Dubrava" from April 2017 until January 2018 were included in this study. The Clinical protocol was approved by Clinical hospital "Dubrava" Ethics Committee (no.25022016). All patients included in this study signed informed consent for participating in the study. The patients included in the study underwent resection of at least one liver segment under intermittent portal triad clamping. Hepatic tumors were detected preoperatively with multislice computed tomography (MSCT), magnetic resonance imaging (MRI) or positron tomography (PET–CT). Patients excluded from this study were those having any other underlying liver disease, or preoperative increased liver transaminase or bilirubin. Patients with ASA classification score higher than three, chronic cardiac, pulmonary and/or renal disease were also excluded from the study (Table 2). All patients were preoperatively classified by the guidelines of the American Society of Anaesthesiologists (ASA). All anaesthetic and operative procedures were performed by the same team of two experienced hepatic surgeons (M.R., L.P.) and two anesthesiologists (S.B., D.J.).

On the evening before their operation, all patients received antithrombotic premedication with low molecular weight heparin. The same standardized anesthetic protocol was used to manage all patients included in the study. The therapeutic strategy was fluid restriction strategy aimed to maintain a mean arterial pressure (MAP) over 65 mmHg and central venous pressure about 5 mmHg. Blood transfusions were administrated when hemoglobin was below 8.5 g/dL and fresh frozen plasma when the INR was more than 1.5. If the MAP was below 60 mmHg norepinephrine was administrated. After the operation, patients were transferred to the intensive care unit and extubated.

After anesthesia induction the patients were randomly assigned by computer program to three groups:

1 – group of patients which we preconditioned with RIPC of the right upper limb (three cycles of 5 minute ischemia of right upper limb by tourniquet up to 200 mmHg followed by 5 minute of reperfusion)(21-22);

2 – group of a patients who was preconditioned with LIPC of the liver (15 minute of portal triad clamping followed 10 minute of reperfusion)(23-24);

3 –control group (CG) of patients which was not ischemically preconditioned.

The type of laparotomy was a right subcostal "J"laparotomy. After laparotomy, we implemented one of the preconditioning protocols (21-24) and started to mobilize the liver. Intraoperative ultrasonography (US) was used to identify the exact localization of the liver tumor and its precise relationship with the liver vasculature. Next, the type of resection required was specified. Liver transection was performed with a blunt-clamp dissection technique, which allows visualization of intrahepatic vessels and individual ligation of major blood or bile vessels. It was performed with the use of the LigaSure device (Valleylab) (25). In all patients, the Pringle maneuver was used to avoid blood loss during liver transection.

Liver synthetic function was assessed by measurement of the laboratory liver tests preoperatively, on the first, third, and seventh day after resection. Residual synthetic liver function and liver ischemic-reperfusion injury was determined by levels of bilirubin, total proteins, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamic aminotransferase (GGT), cholinesterase, alkaline phosphatase (AP), prothrombin time (PV), activated partial thromboplastin time (APTV). We also recorded patients' clinical conditions, the type of liver resection, operative time, total warm liver ischemia time, blood loss and the need for transfusion. Doppler US of the hepatic artery and portal vein was performed intraoperatively before and after preconditioning in the RIPC and LIPC groups.

Histologic evaluation of all resected liver specimens was done postoperatively. Liver paraffin-embedded, standard haematoxylin-eosin stained sections were analyzed. According to Rodriguez four elements of the liver

histology were analyzed: steatosis (micro vesicular and macro vesicular) in 4 grades, degree of sinusoidal congestion and dilatation in 3 grades, leukocyte infiltration in 3 grades and necrosis (focal, confluent, or zonal) in 3 grades (26). Each biopsy was evaluated by a single pathologist blinded to the treatment allocation.

Statistical analysis:

Sample and Instruments

Data collection for this study is:

- 20 patients in each of the three groups with all values for: AST, ALT, GGT, PV, APTV, leukocyte, erythrocyte, hemoglobin, hematocrit, urea, creatinine, serum proteins, albumins, cholinesterase, bilirubin at all periods of measurement

- 20 patients from RIPC and LIPC group of patients exposed to ischemic preconditioning for US assessment of flow through the hepatic artery and portal vein before and after preconditioning

- 20 patients from each group of patients exposed to ischemic preconditioning for histologic analysis (leukocyte infiltration, sinusoidal congestion, steatosis, hepatocyte necrosis).

Data analysis

Results are based on analyzed scores due to homogeneity. Although a relatively small sample, the distribution is in a normal range. According to *Kolmogorov-Smirnov and Shapiro Wilks* tests, the assumption of normality has been met for this sample and parametric tests could be used.

ANOVA and *Tukey HSD* tests were used to detect differences between the three groups of in all points of measurement for values of: AST, ALT, GGT, PV, APTV, leukocyte, erythrocyte, hemoglobin, hematocrit, urea, creatinine, serum proteins, albumins, cholinesterase and bilirubin.

Differences between the two groups of patients exposed to ischemic preconditioning for values of flow through the hepatic artery and portal vein was analyzed using *Summary independent samples test.*

The *Independent samples test* was used to detect differences between the two groups of patients exposed to ischemic preconditioning for values of pathohistological analysis.

The level of statistical significance was set at $P < .05$ for all analyses. The software used for statistical analysis was *IBM SPSS 21*.

Results:

There were 60 patients included in this study and all of them were available at all periods of measurements, 59.4% of patients were male and 40.6% were female (Figure 1). Their age range was between 48 and 79 years. The mean interval for occurrence of liver metastasis after operation of colorectal cancer was 37 months (SD 4 months) (Table 1). The average length of surgery was 165 minutes (SD 20 minutes). Average operating time length in the LIPC group was 191.4 minutes, in RIPC group was 159 minutes and in the CG group was 152 minutes (Figure 2). Averaged duration of the Pringle maneuver was 17 minutes (SD 4 minutes) for all groups, and average hospital stay was 10 days (SD 2 days) (Table 1).

There were no differences between the groups in preoperative measurements of AST, ALT, GGT, PV, APTV, leukocyte, erythrocyte, hemoglobin, hematocrit, urea, creatinine, serum proteins, albumins, cholinesterase and bilirubin. All postoperative measurements which showed significant differences between groups are shown with P values < 0.05 in Table 3.

The significant differences in results between groups are found in values of cholinesterase (P=0.044) and bilirubin (P=0.027) on the 7th postoperative day. The highest mean of cholinesterase value was on the 7th postoperative day in the LIPC group and the lowest in the CG of patients, just opposite for value of bilirubin (Figure 3, 4).

The significant differences between groups are also found on the first postoperative day in scores of AST (P=.029) and ALT (P=.017). Lowest means of scores for AST and ALT on the first postoperative day are in LIPC group of patients, and the highest in the CG (Figure 5, 6).

According to the Tukey HSD test, the differences are significant between all three groups in above mentioned measurements (Table 3).

In values of Doppler US flow through the hepatic artery significant differences between groups were also found. There was no difference between groups in point of measurement before ischemic preconditioning. After preconditioning there were significant differences between the groups (P=.044). The highest mean of Doppler US flow through the hepatic artery after ischemic preconditioning was found in LIPC group of patients (165.00 cm/s, SD 20.58), in contrast to the mean value of the RIPC group (140.00 cm/s, SD 26.16) as it is shown in Figures 7.

Significant differences between groups according to postoperative pathohistological evaluation by Rodriguez are found in scores of hepatocyte necrosis (P=.031). There were no findings of severe hepatocyte necrosis in the LIPC group of patients, in contrast to the RIPC and control group of patients (Figures 8).

Discussion:

The hepatoprotective effect of RIPC from ischemic reperfusion injury of the liver during liver resection was confirmed in this study, which confirms our hypothesis that RIPC is safe and feasible in patients undergoing liver resection for colorectal liver metastasis. The trial demonstrates significate reduction in postoperative serum transaminases and bilirubin in the RIPC and LIPC group.

Liver resection is regarded as the gold standard of treatment for resectable liver colorectal metastases (1-2). To avoid blood loss during these resections, portal clamping is commonly employed, which can induce IR injury of the liver (3-4). This IR injury can lead to insufficiency of the liver remnant and increased morbidity and mortality during liver resections. RIPC and LIPC can help to avoid and to attenuate IR injury of the liver. In recent years, a number of experimental trials have been designed to demonstrate effectiveness of ischemic liver preconditioning, but there is no study that compared liver's LIPC versus RIPC conducted on humans. To the best of our knowledge, this is the first studies of that type on the humans.

The results of previous studies are contradictory. In 2000. Clavien et al. (27) conducted a study that yielded the first clinical evidence of benefit in patients who received LIPC before they underwent hemihepatectomy. These result correlate with results in our study. In contrast, a few years later Azoulay et al. (28) found that LIPC by Pringle maneuver failed to protect the human liver against IR injury after major hepatectomy under continuous vascular exclusion with preservation of caval flow.

The first evidence of RIPC reported on 2008. by Pryzklenk et al. (29), showed that RIPC is effective in protecting the myocardium from IR injury. Several studies based on this concept have been conducted to investigate the effect of RIPC on the other organs, including the liver (30-32). These studies have shown that RIPC has a protective effect on the liver, but the study was conducted on rats. The exact mechanism of this hepatoprotective effect of RIPC is insufficiently understood, but studies have thus far proposed two mechanisms: humoral and neurogenic/neuroendocrine mechanisms (33-34). A hepatoprotective effect was achieved by reduction of cytokine release and neutrophil activation which decrease endothelial injury as the key factor in preserving hepatic microcirculatory flow with increasing vasoactive molecules such as nitric oxide

(NO) (35). Activation of some heat shock proteins inhibited apoptosis and also have a hepatoprotective effect (36). However, some recent clinical studies showed that RIPC on the recipient patient provided very limited effect (37).

The limitations of the study which must be considered include the sample population, however we to standardized the sample with the inclusion and exclusion criteria. Also, the size of the sample may be a problem but we consider the sizes of the samples of the other similar studies when we were doing power analysis. Another limitation of this study is that it has not investigated potential mechanisms of action of RIPC and LIPC.

According to our experimental data, a significant reduction was detected in liver synthetic activity in the group of patients which was not exposed to ischemic preconditioning, as is shown in Table 3. and Figures 3-6. Also, a significant increase in Doppler US flow through the hepatic artery after LIPC was found, which is in correlation with the better oxygenation of hepatocytes and better cytoprotective effect of LIPC, as is shown in Figures 7. Liver specimens of the LIPC group showed less necrosis on pathohistological examination than the control group, as is shown in Figures 8.

The results of this study are in accordance with the results of the study by Kanoria et al. where RIPC was done with three cycles of 10 minute ischemia of the lower limb by tourniquet up to 200 mmHg followed by 10 minute of reperfusion (39). Known tourniquet associated complications include pain, paresthesia of the limb, neither of which occurred in this study. We answered the hypothesis from Kanoria's study, that a longer period of limb ischemia can provide better protection from IR injury of the liver. This study's results contrast with that hypothesis, in that we showed that a shorter protocol of RIPC is safe and of equal effect.

These results show that the best liver remnant preservation from IR injury was achieved in the group of LIPC patients which are in line and complement with previous findings (on animal models). This is one of the first studies on human beings which shows hepatic cytoprotective effect of RIPC (39). This effect is slightly weaker than the one with LIPC but it is advisable in operations or in patients where portal congestion (Pringle maneuver) must be as short as possible. The RIPC group of patients had a shorter operative time which speaks in favor of this preconditioning method. It is recommended in synchronous operations of colorectal cancer and liver metastases, to avoid mesenteric congestion, because it can lead to increasing anastomotic leakage as well as prolonged operation time, or in patients which do not tolerate portal clamping (40-41). Strong evidence to conclude that RIPC may have a protective effect from ischemic reperfusion injury of the liver during liver resection is found in this prospective randomized controlled study. Mechanisms of this effect must be investigated in future studies; however this study gives us encouraging results which may help direct future studies.

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Table 1: Demographic characteristics of all patients included in the study

Variable	Patients n. 60
Age (years)	63.8 (48-79)
Gender (male/female)	59.4% / 40.6%
Height (cm)	172.7 (153-196)
Weight (kg)	78.4 (51-113)
BMI	26.8 (22-34)
Hospital stay (day)	10 (6-21)
Pringle maneuver (min.)	17 (11-32)
Occurrence of metastases (months)	37
Average operating time (min.)	
LIPC group	191.4
RIPC group	159
CG	152

LIPC – local ischemic preconditioning

RIPC – remote ischemic preconditioning

CG – control group

Table 2: Diagram of flow

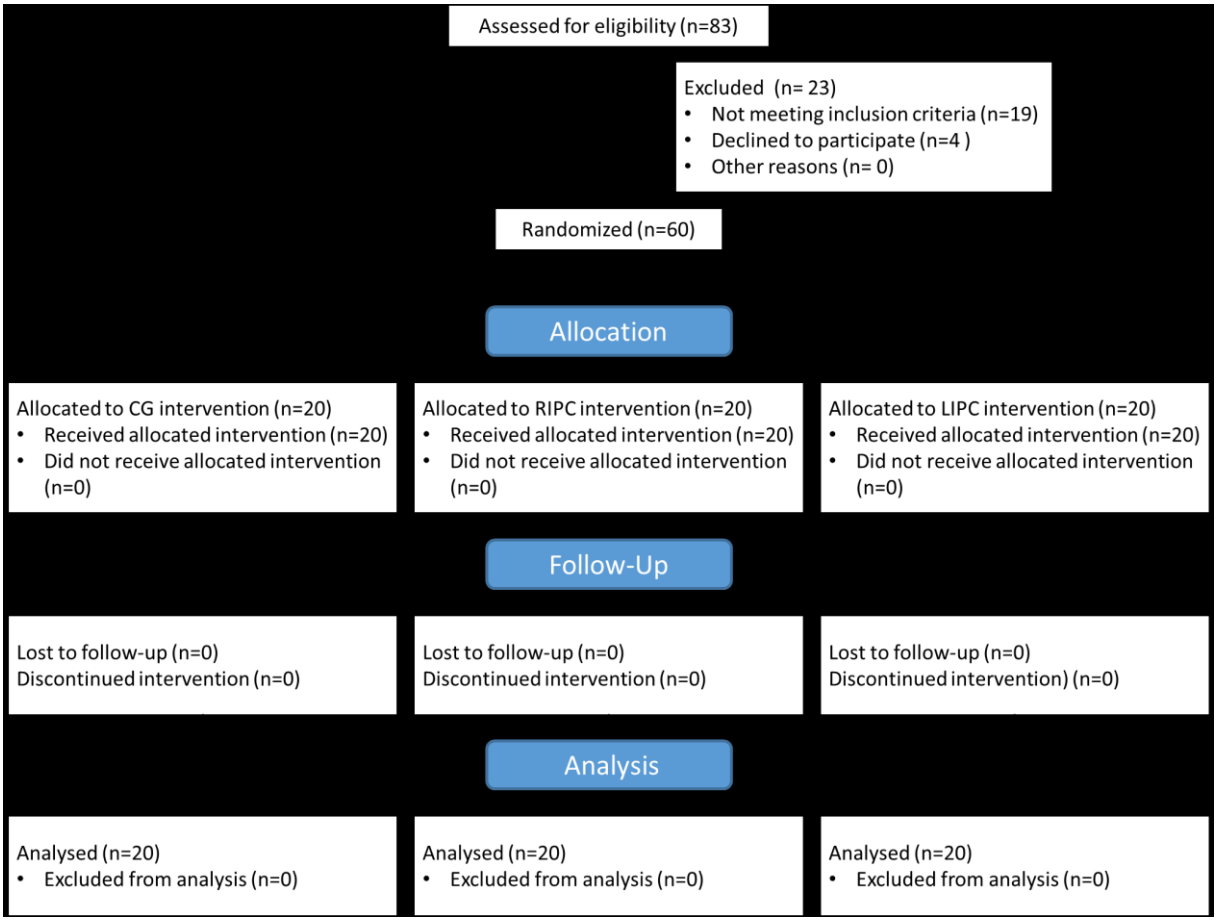


Table 3: Differences between groups in postoperative points of measurement

	Cholinesterase			Bilirubin			AST			ALT		
Period	**7th day**			**7th day**			**1st day**			**1st day**		
P	.044			.027			.029			.017		
Groups	LIPC/	RIPC/	CG/	LIPC/	RIPC/	CG/	LIPC/	RIPC/	CG/	LIPC/	RIPC/	CG/
	RIPC	CG	LIPC	RIPC	CG	LIPC	RIPC	CG	LIPC	RIPC	CG	LIPC
P	.038	.026	.041	.048	.031	.040	.041	.021	.026	.001	.005	.000

AST - aspartate aminotransferase

ALT – alanine aminotransferase

Figures:

Figure 1: Mean values of flow through the hepatic artery before and after preconditioning

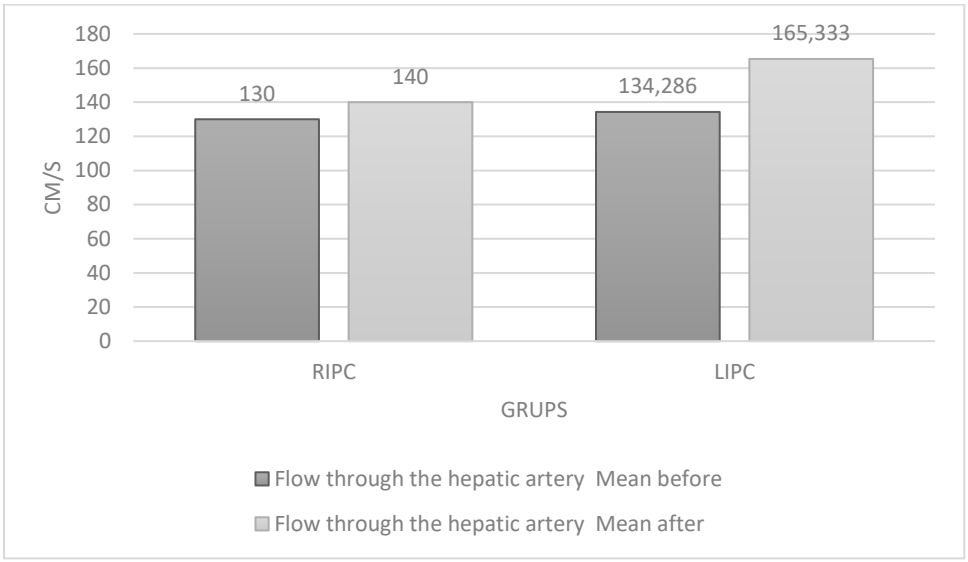
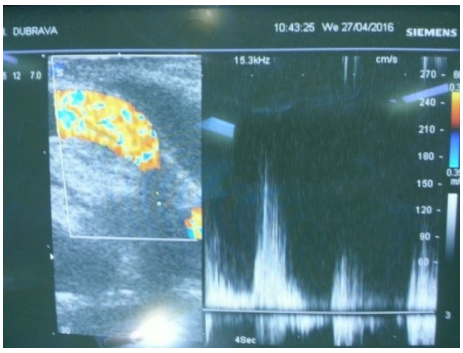


Figure 2: Doppler ultrasound (Siemens Acuson P300) of flow through the hepatic artery of a patient from the LIPC group before and after preconditioning.

Before



After

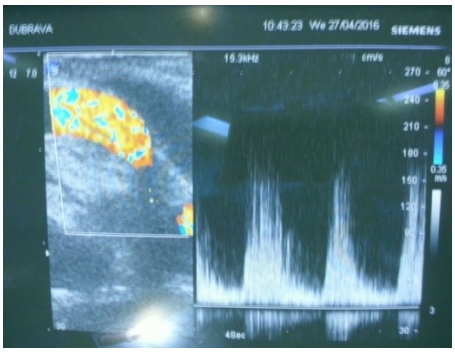
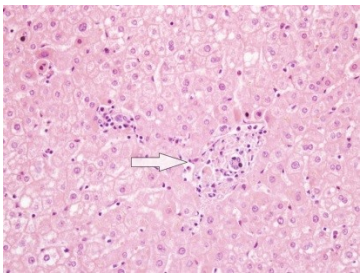


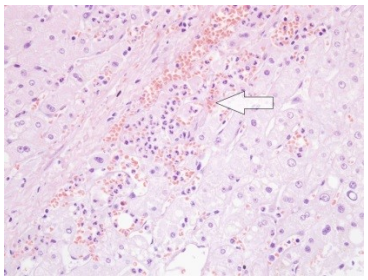
Figure 3: Mean values of histologic liver tests (Rodriguez score) for patients exposed to ischemic preconditioning

Test	Preconditioning	N	Mean	Std. Deviation	Mod
Hepatocyte necrosis	RIPC	20	0,1429	0,37796	0
	LIPC	20	0	0	0
	CG	20	0.1743	0.3915	0

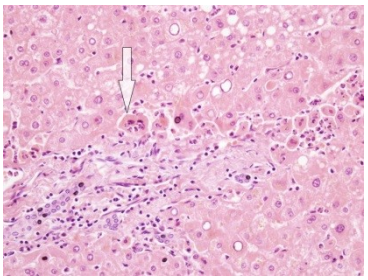
Figure 4: Preserved liver tissue structure with: mild (a), moderate (b) and severe (c) neutrophilic infiltrate and sinusoidal congestion and dilatation in and around portal spaces (arrow); Routine HE staning, magnification 400x



(a) LIPC



(b) RIPC



(c) CG

Declaration of authorship: MR designed the study, performed the acquisition, analysis, and interpretation of data, drafted and revised critically the manuscript for important intellectual content. MR and LP participated in the acquisition of data and drafting the manuscript. LP, IG and GA contributed to the development of study design, data analysis, interpretation of the results, drafting, preparation and critical revision of the manuscript. All authors critically reviewed the manuscript and gave their final approval of the version of the manuscript to be published.

The authors declare that there are no conflicts of interest in relation to this article.