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Analysis of the Liver Effluent as a Marker of Preservation Injury and Early Graft Performance

E.G. Pacheco, O.D.C.E. Silva Jr, A.K. Sankarankutty, and M.A.F. Ribeiro Jr

ABSTRACT

In liver transplantation, the effluent solution, which represents the washout of residual preservation solution, can be collected before reperfusion to determine the release of the markers of endothelial cell injury and damage to the liver. The enzyme activities detected in the washout solution may allow the development of an index that could be clinically valuable for the prediction of early posttransplant graft function. In the present study, we collected liver effluents from 47 livers at the time of graft rinsing to measure liver enzymes (aminotransferases and lactate dehydrogenase) as well as the serum enzyme levels of the recipients for correlation with early postoperative graft viability (1-month survival). The patients were divided into two groups: death (D) and survival (S). Nonparametric statistical analysis was used with the level of significance set at $P < .05$. Aminotransferases and lactate dehydrogenase levels higher among the D group ($P < .05$ for all measurements), leading us to conclude that the effluent represents a good marker of preservation injury and early graft performance.

LIVER TRANSPLANTATION (OLT) has become the treatment of choice for patients with end-stage liver diseases as well as acute liver failure for whom conservative treatment has proven ineffective. The objective of the procedure is to improve patient survival as well as quality of life. There are several factors that limit the success of OLT, the two most feared ones definitely being rejection and primary graft dysfunction, which can contribute 15% to 25% to the mortality rate during the first year posttransplantation.[1] These complications are also related to preservation injury, which aggravates the tissue effects of ischemia and reperfusion (I/R).[2,3] The period of ischemia is divided into two phases, cold ischemia time (CIT) and normothermic ischemia time. CIT is more prolonged; its effects are alleviated due to the low temperature at which the graft maintained; although normothermic ischemia time is shorter, it can produce more deleterious effects. Experimental evidence suggests that the endothelial sinusoids are more susceptible to hypothermia and hepatocytes, to normothermic ischemia.[4] Cellular changes secondary to hypoxia have been well documented in the medical literature.[5–9] The damage to the plasma membrane provokes electrolyte changes that, accompanied by the loss of mechanisms of adenosine triphosphate production, lead to the interference with cell synthesis. Calcium deposition provokes mitochondrial membrane dysfunction and possibly irreversible damage, followed by autolysis secondary to ischemia: lysosome edema, endoplasmic reticulum vacuolization, enzyme and protein leakage, and loss of characteristic cellular compartments. Membrane integrity cannot be maintained; the cell dies.[10]

With reperfusion, the energy supply is restored and toxic metabolites removed. However, reperfusion has severe metabolic consequences, and causing greater tissue injury than ischemia. During this period, there is extensive release of free radicals, loss of endothelial viability in the sinusoids, activation of Kupffer cells, as well as the release of substances such as tumor necrosis factor (TNF), bradykinins, and proteolytic enzymes.[11]

Calmus et al[12] observed a correlation with graft failure, of increased concentrations of some enzymes in the ex vivo preservation fluid of the organ to be transplanted: metallo-

From the Special Liver Transplantation Unit (E.G.P.), Departments of Surgery and Anatomy (O.D.C.E.S., A.K.S.), Ribeirão Preto University of São Paulo, São Paulo, Brazil; and Department of Surgery (M.A.F.R.), Universidade de Santo Amaro-UNISA, São Paulo, Brazil.

Address reprint requests to Eduardo Garcia Pacheco, MD, PhD, Special Liver Transplantation Unit, Department of Surgery and Anatomy, Faculty of Ribeirão Preto University of São Paulo, Avenida Bandeirantes, 3.90—CEP 14049-900, São Paulo, Brazil. E-mail: egpacheco@rca.fmrp.usp.br

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and aspartate proteases, and (isoleucine, cysteine, methionine, threonine, and mainly valine) amino acids. These observations suggested that the proteolytic activity of the fluid may relate to graft dysfunction during the immediate postoperative period. Failure also occurs due to primary graft dysfunction, complications of the surgical procedure, arterial thrombosis, and infections, among others, but the effects of preservation injury are inevitable for any transplanted liver.[13–15] Thus, several efforts are being made to minimize injury during preservation, mainly concerning the composition of the preservation solutions during ischemia in order to prevent its effects, and the supply of antioxidants and substrates that reduce adenosine triphosphate depletion and inhibit proteolysis.[16]

The term "hepatic effluent of the graft" is defined as the solution collected from the infrahepatic inferior vena cava during graft washing with Ringer's lactate before graft reperfusion, seeking to extract the preservation solution as well as excess components and residues, especially potassium.

The hepatic effluent has been studied for many years. In 1993, Mitsuo and Katsuhiko[17] measured ammonia and lactic acid in this effluent, observing that they were significantly more elevated among livers with a longer CIT. The transplant results were worse than for organs whose effluents contained lower levels of these substances.

Suehiro et al[18] in 1997 reported the possible value of differences in the levels of substances in the effluent, interpreting such levels as a predictive factor for hepatic function of grafts in human beings. They related the contents of cytokines, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), TNF, and hyaluronic acid, to the tissue injury provoked by I/R. In that study, the authors demonstrated that the levels of substances in the effluent were similar to serum levels and could therefore be used as markers.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), intracellular enzymes mainly synthesized in the liver and universally defined as good markers of acute hepatic injury,[19] can also be measured in effluents. Similarly, lactic dehydrogenase (LDH), an enzyme present in the cell cytosol of almost all tissues, acts on the glycolytic pathway, catalyzing the reversible reaction that transforms pyruvic acid to lactic acid in the presence of low cellular oxygen levels or cell injury.[20,21]

Thus, the aim of the present study was to correlate the relation of amino transferase and LDH levels in hepatic effluents and in the bloodstream of liver graft recipients to the patient's course during the first postoperative days. We also compared other factors, such as donor and recipient ages, time of cold and normothermal graft, ischemia, Child-Pugh classification, and patient score using the Model for End-stage Liver Disease (MELD) scale, as well as the histological injury suffered by the graft before and after reperfusion.

MATERIALS AND METHODS

We evaluated the hepatic effluents of 47 OLTs from cadaveric donors, including 37 patients (78.72%) who survived (S group) and 10 (21.27%) who died (D group). The S group consisted of patients who survived more than 30 days after transplantation and the D group, those who died within 30 days from causes related to the procedure. We excluded transplants performed for patients with acute liver failure, those preserved with substances other than University of Wisconsin (UW) solutions and those in recipients who died of undetermined causes or causes not exclusively related to the period after OLT surgery. After fulfilling the clinical criteria for OLT, patients included on the waiting list were classified according to blood group and MELD score.

The donor was sent to the operating room for harvesting with macroscopic evaluation by the surgeon, taking into account the liver appearance and the presence of possible anatomic variations. Via cannulation of the distal abdominal aorta and of the portal vein, we delivered two preservation solutions at 0°C to 4°C: 2000 mL of the UW solution into the portal vein and Eurocollins solution. The removed liver was placed on a tray where it was perfused with an additional 1000 mL UW divided between the portal vein and the hepatic artery.

At the end of the portal vein anastomosis and immediately before reperfusion, only a small space was left for the placement of a tube coupled to an infusion flask, which was connected to two flasks of ice-cold Ringer's lactate (a total amount of 1000 mL) for graft washing, while the suprahepatic vena cava was still clamped. The residue of this wash, or effluent, which extravasated through the infrahepatic vena cava, was collected into five sequential duly identified test tubes (approximately 20 mL each). After a complete wash of the graft, the anastomosis of the portal vein was completed and the vena cava and portal vein unclamped for venous reperfusion of the graft. The arterial and biliary anastomoses were then performed consecutively.

RESULTS

The mean levels of the ALT, AST, and LDH in the hepatic effluent collected into the five flasks are sequentially illustrated in Fig 1. These effluent levels were similar to the serum levels of these enzymes. There was a significant difference between groups for all of them ($P < .05$); a marked peak was observed in the first flask, mainly among recipients who subsequently died.

For all enzymes, the difference between groups was greater in the first flask of effluent collection and was reduced in the subsequent flasks, where the substances were more diluted probably due to the end of graft washing.

After significant differences in both serum and effluent levels were detected between groups, we determined whether there was a correlation between the highest levels by correlating ALT, AST, and LDH levels in the first flask of collected effluent with the serum levels determined 48 hours after transplantation. We observed a significant correlation in serum and effluent levels for the three enzymes regardless of group.

Thus, the enzyme levels in the first flask showed a behavior similar to the serum levels of these enzyme 48 hours after transplantation, with the D group showing a larger number of elevated enzymes in both cases (Fig 2).

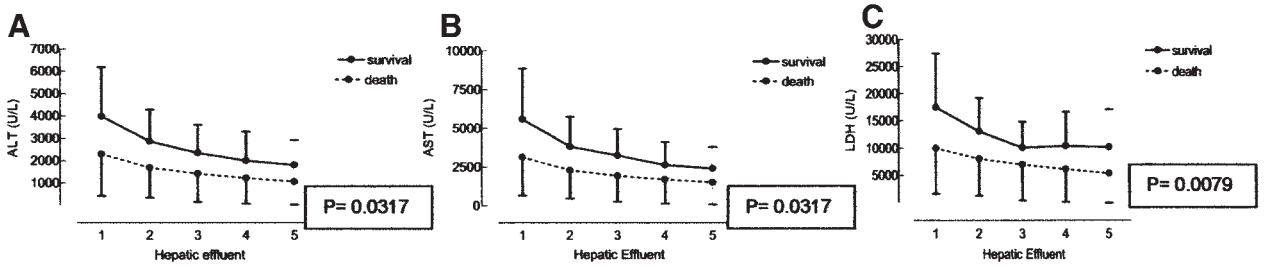

Fig 1. **(A)** Mean alanine aminotransferase (ALT) levels in each flask of the effluent collected during graft washing and divided for the groups of surviving and dead recipients. **(B)** Mean aspactate aminotransferase (AST) levels in each flask of the effluent collected during graft washing and divided for the groups of surviving and dead recipients. **(C)** Mean lactic dehydrogenase (LDH) levels in each flask of the effluent collected during graft washing and divided for the groups of surviving and dead recipients.

DISCUSSION

In most cases, injury due to organ preservation is believed to be the main factor influencing organ dysfunction, for it aggravates the effects of I/R.[13,14] In the present study, the cold and normothermal ischemia times to which the graft was exposed during preservation were similar regardless of the group. Calmus et al[12] reported that proteolysis may persist during cold ischemia of a graft preserved at 4°C in UW solution. They determined the levels of amino acids, such as alanine, cysteine, leucine, isoleucine, methionine, lysine, and threonine, in the fluid of the tray containing the preservation solution for the organ to be transplanted at the beginning and at the end of the preservation period. They observed an increased concentration of these substances at the end of organ preservation, which was directly proportional to the time of graft exposure to cold ischemia. However, the same authors observed a correlation of higher concentrations, mainly of valine, with graft failure, suggesting that proteolytic activity was related to graft dysfunction during the immediate postoperative period and was influenced by the CIT.[18] In contrast to Calmus et al,[12] we did not determine the substances present in the tray containing the preservation solution, but did not detect a statistically significant difference in the cold and normothermal ischemia times that might cause different levels of proteolysis among the grafts.

Jaeschke[13] described the phases of injury due to graft preservation and observed hypothermia to be less aggressive, although it reduces cell metabolism and produces cell edema. However, the author stated that UW solution contains a lactobinate that protects cells against edema; this solution was used herein.

It is also known that transplantation failure is mainly due to primary graft dysfunction, complications of the surgical procedure, arterial thrombosis, and infections, among other factors, but the effects of the injury caused by the preservation solution are inevitable for any transplanted liver.

In an extensive review of the effects of injury due to graft preservation in liver transplantation, Teoh and Farrell[20] stated that sinusoidal endothelial cells and hepatocytes are the main targets of damage caused by the period of graft ischemia. They concluded that damage to the microcirculation was aggravated by a disequilibrium between vasoconstrictors and vasodilators. Exaggerated production of nitric oxide is considered to be the cause of circulatory changes, such as hypotension and nonresponsiveness to vasoconstrictors. On this basis, dysfunction of a liver graft during the immediate postoperative period may be due to several factors involving the donor and the recipient, although in most cases it is accepted to be due to organ preservation.[20,21]

Several experimental and clinical studies support the hypothesis that substrate supply in the preservation solution

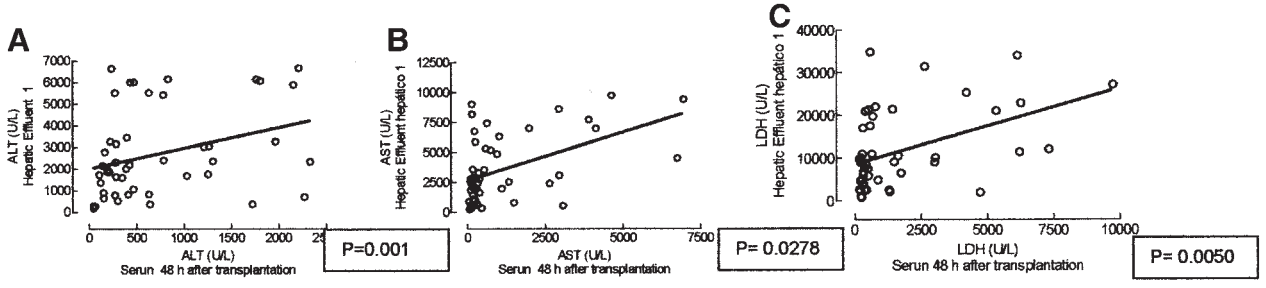

Fig 2. Panel **(A)** Correlation of serum alanine aminotransferase (ALT) levels 48 hours after transplantation with the levels in the effluent of the first flask collected ($P = .0278$; $r = .3211$). **(B)** Correlation of serum aspartate aminotransferase (AST) levels 48 hours after transplantation with the levels in the effluent of the first flask collected ($P = .005$; $r = .4877$). **(C)** Correlation of mean lactic dehydrogenase (LDH) levels for all patients calculated by relating serum levels 48 hours after transplantation to the level in the first effluent flask ($P < .002$; $r = .4460$).

during ischemia is important for the maintenance of ATP levels,[21,22] especially during prolonged ischemic periods. Other studies have suggested that the lack of these substrates reduces phagocytosis and TNF-α regeneration.[23]

We believe that, in the present study, the measurement of hepatic enzymes both in the effluent and in the blood stream expressed the degree of injury suffered by each graft and that determinations in the effluents before reperfusion could reflect the effects of cold ischemia and of preservation.

Lendoire et al[23] divided the hyaluronic acid concentrations in the hepatic effluents of 11 liver transplants into two groups: high hyaluronic acid levels (>400 μg/L) showing greater preservation aggression and the other below this concentration, considered to have lower aggression. They correlated these levels with serum AST and ALT levels as well as prothrombin times (PT) and CIT. They did not detect a correlation between the group with higher hyaluronic acid concentrations in the effluents and the measured substances. In contrast to Lendoire,[23] other investigators such as Rao et al[24] and Karayalçin et al[25] noted significant correlations between hyaluronic acid levels and graft outcomes, but the substances were not collected from effluents but rather from perfusates (ie, immediately after organ reperfusion through the portal vein). These authors noted that most patients who had high hyaluronic acid levels in the perfusate also had high serum hepatic enzyme levels but, in contrast to the present study, they did not divide the patients into death and survival groups for correlations.

In 1997, Suehiro et al[18] reported the possible value of differences in the levels of effluent substances of 29 patients by measuring cytokines, interleukins, HGF, VEGF, TNF, hyaluronic acid, thromboxane, and thrombomodulin. They related the levels to patients who had dysfunction during the first 3 days after transplantation (ie, patients with elevated serum levels of AST and ALT [>2500], as well as a longer PT [>16 minutes]. In that study, the authors detected higher levels of substances among patients who experienced dysfunction after surgery.

Aminotransferases and LDH are also considered to be excellent markers of hepatic tissue injury and were chosen in the present study to correlate with the groups studied by determining their concentrations in serum and in the effluent in the D and S groups. In contrast to the data cited above, we compared the same substances in both the effluent and in the bloodstream of surviving and succumbing patients, detecting significant differences between them. We then decided to calculate a possible correlation between the serum and effluent levels of these substances. In the present study, it was possible to determine that there was no influence on D versus S groups of multiple factors, such as donor or recipient ages, Child-Pugh classification, recipient MELD score, or cold and normothermic ischemia times.

The serum levels of ALT, AST, and LDH determined during the first 10 days were significantly different between the two groups ($P < .05$), showing a marked peak at 48 hours after transplantation, especially in the D group, suggesting that the major laboratory manifestation of the aggression suffered by the graft occured on the second postoperative day. Mean AST, ALT, and LDH levels in the hepatic effluent collected into the five flasks differed significantly between the two groups, a behavior also observed in serum, as mentioned earlier. In addition, a marked peak of these enzymes was observed in the first flask collected, especially in the D group, due to a greater preservation injury in this group. In the S group, the enzymes measured in the five effluent flasks and in serum during the first postoperative days were below the general mean in most patients, suggesting a lower aggression of the graft and consequently a better graft prognosis. In contrast, in the D group, most patients had enzyme levels above the general mean both in the effluent and in serum, probably due to greater preservation injury.

Serum and effluent levels were positively correlate on the second postoperative day. We noted that the substances in the effluent behaved similarly to those in the bloodstream most patients with elevated levels of these substances showed poor outcomes. Thus, by correlating these markers in both effluent and serum, we obtained an early indicator of patient course, since in the D group the levels were higher than in the S group.

This analysis may allow us to use prophylactic or preventive resources for the management of transplanted patients, such as antioxidants, or even to assess preservation solutions and the solutions used to wash the graft immediately before the execution of the anastomoses. In addition, measurements of aminotransferases and dehydrogenases in the collected effluents are easy to perform and low cost.

Bilzer and Gerbes[26] presented therapeutic perspectives for the prevention of cell aggression, especially with regard to the endogenous content of the antioxidant glutathione (GSH), which is more abundant in the hepatocytes and the bile when compared to extracellular and plasma concentrations. The authors demonstrated that GSH release by the hepatocytes attenuated the effects of I/R injury, thus showing that infusion of this antioxidant may be useful to prevent tissue aggression.

In the same study, the authors cited reports demonstrating a reduction of preservation aggression by the use of atrial natriuretic peptide, which acts by inhibiting the effects of superoxide activated by Kupffer cells.

Knowing that the graft suffers aggression during preservation and during ischemia, it is even more necessary to identify as soon as possible the severity of this injury by means of easily accessible exams. The effluent is a fluid rich in markers of tissue injury, which behave in a similar manner to that of serum liver enzymes, as demonstrated in the present study by determining correlations. Effluent analysis may become part of routine tests at liver transplant centers, providing the team with an early possibility to predict graft evolution and thus to attempt to minimize I/R damage.

In conclusion, substances measured in the effluent behaved in a similar manner to the bloodstream; most patients

who showed elevated levels displayed poor outcomes. The hepatic effluent can be considered to be a good indicator of graft damage due to preservation, which in turn is a predictive factor for the quality of the transplanted liver.

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