Assessment of liver function: pre- and peritransplant evaluation

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Liver transplantation has been demonstrated to be a successful therapeutic modality for patients with end-stage liver disease. The high rate of survival for an otherwise terminal condition has resulted in significant expansion of the indications and diseases treated by this procedure, and is hampered only by the limited numbers of organs available for transplantation. Efforts in clinical and laboratory medicine should be directed to identify candidates who would benefit most from this procedure, to provide better means for accurate assessment of liver reserve and the appropriate timing for transplantation, to identify quality liver grafts that would have the potential to tolerate cold preservation and reperfusion injury, and to assist in accurate monitoring of graft function immediately after transplantation. The aim of this manuscript is to describe the existing pathways for clinical and laboratory assessment of pretransplant residual liver function, the donor liver graft, and immediate posttransplantation function.

INDEXING TERMS: liver transplantation • end-stage liver disease • reperfusion injury • liver reserve

Liver transplantation has become the treatment of choice in the developed world for end-stage liver disease. The wider application is limited by an inadequate supply of organ donors and by cost. Over 4000 liver transplants are performed in the US annually, as part of a worldwide total of ~8000. The 1-year survival in many of the centers is ~80%, after which there is a slight attenuation annually. This significant initial mortality, and the relatively low numbers of organs available, requires that the procedure be restricted to patients with life-threatening disease. Under such circumstances, judging the appropriate time for placement on the waiting list becomes a critical issue, and is totally dependent on the clinical assessment of the patient’s condition, as well as laboratory evidence of residual liver function. The relative shortage of organs for transplantation has resulted in multiple attempts to utilize donors’ organs that otherwise would have been rejected for either medical reasons or on the basis of donor social history. Obviously, the extended criteria to use organs defined as marginal, i.e., those retrieved from older donors, unstable donors, etc. are critical for expansion of the donor pool. The inability of some of these grafts to withstand the injuries associated with procurement, cold preservation, and reperfusion necessitates the development of liver donor-specific assays that will assure that all the grafts will be functioning after transplantation. The relatively high rate of nonfunction of donor grafts (5–10%) judged to be acceptable for transplantation indicates that clinical evaluation coupled with a few basic biochemistry tests is not sufficient to estimate whether immediate posttransplant graft function will recover. The issue of residual liver function and reserve was addressed before the days of liver transplantation, and continues to be studied by many hepatology and transplant groups. There are numerous tests aimed toward quantitative and qualitative analysis of liver reserve. The principle of many of these assays is to define metabolic/energy-dependent pathways that can be easily measured, and reflect the functioning hepatic mass under normal circumstances and stress. This review will concentrate on the current laboratory assays that are commonly used to identify the status of the recipient liver reserve before transplantation, the quality of the donor liver before procurement, and the function of the graft immediately after reperfusion.

PRETRANSPLANT ASSESSMENT OF END-STAGE LIVER DISEASE

Determining the prognosis of liver disease and using this information to time liver transplantation is difficult. Apart from specific examples such as primary biliary cirrhosis, there are no well-documented prognostic models for chronic liver disorders [1, 2]. The Child–Pugh scale is the easiest prognostic instrument to use [3]. It combines synthetic function [serum bilirubin, prothrombin time
The administered substance falls. Substances used to calculate clearance. A decrease in clearance to a significant degree, whereas changes in liver cells functionally able metabolize the substance have a large effect on clearance. Substances are administered and the loss of the substance from blood or the appearance of a metabolic product in blood or expired air is determined.

The aminopyrine breath test has been investigated extensively as a simple and reliable index of hepatic microsomal enzyme reserve [10]. [14C]Aminopyrine is administered orally and the labeled carbon removed by hepatic microsomal enzyme activity. Expired 14CO2 is collected 2 h after oral administration and activity is determined. Marked decreased isotope activity occurs in cirrhotic patients compared with controls [11].

Table 1. Child–Pugh classification.

<table>
<thead>
<tr>
<th>Points scored</th>
<th>Encephalopathy grade</th>
<th>Bilirubin, µmol/L (mg/DL)</th>
<th>Albumin, g/L</th>
<th>Prolongation of PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>&lt;25.6 (&lt;1.5)</td>
<td>&lt;35</td>
<td>1–4</td>
</tr>
<tr>
<td>2</td>
<td>I, II</td>
<td>25.6–39.3 (1.5–2.3)</td>
<td>28–35</td>
<td>4–6</td>
</tr>
<tr>
<td>3</td>
<td>III, IV</td>
<td>&gt;39.3 (&gt;2.3)</td>
<td>&lt;28</td>
<td>&gt;6</td>
</tr>
</tbody>
</table>

Child class A = 5–6, Child class B = 7–9, Child class C = 10–15

Table 2. Indication for placement on the waiting list.

| Progressive hyperbilirubinemia | Portal hypertension manifested by intractable ascites | Hypersplenism, and (or) bleeding varices | Uncontrolled encephalopathy | Poor synthetic function expressed by low albumin, fibrinogen, and prolonged PT | Inability to function and maintain normal activity | Metabolic diseases associated with chronic liver disease | Unresectable hepatic malignancy confined to the liver |
candidate for transplantation, surgery is performed on the basis of the availability of a matched organ.

**Assessment of Donor Graft Function**

The limited time between determination of brain death and procurement restrict the evaluation of the donor liver to the clinical judgment of the transplant surgeon, and to the use of a limited set of standard laboratory tests directed at liver synthetic function and injury. These data are used to determine the capacity of the organ to withstand the surgical insult during procurement, extended cold ischemia, and reperfusion injury. Currently, most surgeons rely on the donor’s previous medical history, mechanism of death and its potential effect on the liver, and the relative stability or instability of the donor for initial assessment. Important laboratory tests include liver-specific transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], and synthetic function is reflected by serum albumin and PT. Serology screening is used to indicate the presence of acute or chronic liver diseases such as hepatitis B and C, or other common types of viral infections.

Unfortunately, the accuracy of this assessment can be measured only by outcome analysis of graft survival. Obviously, it is impossible and unethical to demand that all available donor livers be transplanted for the sole purpose of obtaining nonbiased data regarding the variables affecting graft survival. In reality, all outcomes are correlated with either immediate function or malfunction of the transplanted allograft, excluding technical complications leading to posttransplant ischemic damage.

Multivariate analysis of large numbers of recipients was used to identify donor-related factors that have significant impact on graft survival [17]. In general, primary nonfunction (PNF) and delayed nonfunction (DNF) of the graft were best correlated with the age of the donor, mechanism of death, the development of significant hemodynamic instability requiring treatment with multiple vasopressors, and the length of cold ischemia time. In a limited number of studies, donor liver function was analyzed by using liver-specific enzymatic pathways, or assessment of mitochondrial viability.

A major variable affecting graft function is the donor age [18, 19]. Pattern of liver injury, synthetic function, and graft survival in recipients receiving liver grafts from donors older than age 50 years were compared with recipients transplanted with grafts from donors ages 20–30. Ischemic/reperfusion injury, reflected by AST and ALT, was more severe in older donors (Fig. 1). PNF occurred at similar frequencies for all recipients (7%). However, normal liver function was regained in only 76% of recipients of older liver grafts vs 92% in recipients of younger grafts. DNF was characterized by a rapid rise in bilirubin despite normalization of PT and liver transaminases. There is no explanation for the relatively similar number of PNF in both groups, and the more common development of DNF in the older liver grafts. This phenomenon may relate to a not-well-defined mutual relation between aging and liver function. The question of whether aging of the liver affects outcome after exposure to procurement-related injury, cold preservation, and reperfusion injuries remains to be determined. In an experimental model of rat liver transplantation, donor age was correlated with a histology finding of biliary casts, and increased ALT concentrations were significantly higher in recipients of old livers [20].

The length of cold ischemia has also been demonstrated to affect graft function. Currently, livers can be successfully preserved in University of Wisconsin (UW) preservation solution for 24 h. The UW solution is effective because it has a number of agents (lactobionic acid, raffinose, hydroxyethyl starch) that prevent cell swelling, as well as glutathione and adenosine, which may stimulate recovery by augmenting antioxidant capacity and high-energy phosphate generation [21].

The findings that a selected number of older donor grafts exposed to the same length of cold ischemia display more profound ischemic/preservation injury as manifested by increased DNF and poorer initial graft function indicate that specific biochemical/ enzymatic circuits are affected beyond recovery. Furthermore, preservation methods and length of cold ischemia appear to contribute to graft injury. Laboratory medicine must identify these pathways, provide rapid tests to determine which livers of older donors will have acceptable liver function early and late after transplantation, and correlate the length of cold ischemia with graft function in these older donors. Developing these tests may greatly encourage the use of many livers that are currently discarded.

**Biochemical Analysis of the Donor Liver**

The relatively high rate of PNF of liver grafts seen in 5–10% of the recipients after transplantation initiated the search for objective indicators to identify the quality of the
procured graft and predict its capacity to withstand cold storage and reperfusion injuries.

**Energy-dependent metabolic pathways.** Selecting energy-sensitive metabolic processes that may reflect the integrity of the various oxidative pathways within the hepatocyte was logical. Previous studies have found that lidocaine disposition is a highly sensitive indicator of hepatic dysfunction. Specifically, the kinetics of the lidocaine metabolite monoethyglycinexylidide (MEGX) can be used to assess hepatocyte injury [22–24]. MEGX is formed from lidocaine via oxidative N-deethylation by the hepatic cytochrome-P450 primarily by an enzyme identified as CYP34A. The product can be measured in the blood within 15–30 min after administration, and the metabolite can be detected via an automated immunoassay specially designed for this test. Prospective studies have debated the efficacy of this test in determining the donor liver graft survival after transplantation [25, 26]. However, there was a high rate of false-positive results, as reflected by good graft function after transplantation of liver grafts from donors with low MEGX in almost 50% of the recipients [27]. Furthermore, the test was associated with some incidence of normal results at the donor site, while the graft failed to function after transplantation, indicating poor correlation with prognosis.

**Function of endothelial cells.** The uptake of hyaluronate by the vascular endothelium can serve as a marker for cell injury, and may predict PNF of the graft secondary to reperfusion injury to the microcirculation. The hyaluronate content of reperfusion effluent has been found to inversely correlate with ultimate graft function. Because hyaluronate uptake by the microvascular endothelial cell is significantly greater than production, high concentrations in failing livers reflect decreased uptake by the injured cells [28].

These assays serve as representatives of a large number of tests that may be used to determine the quality of the donor liver before procurement. They are limited by either low sensitivity and (or) unrealistic application (since some are done immediately after reperfusion). More sophisticated and clinically feasible methods will be essential for more accurate assessment of the donor liver.

**ASSESSMENT OF POSTTRANSPLANT LIVER GRAFT FUNCTION**

Immediate and long-term function of the liver graft after transplantation is directly correlated to the quality of the donor liver, as well as multiple host-related variables that may affect the intraoperative course and the initial post-transplant recovery period. Previous studies have linked the pretransplant United Network for Organ Sharing status with short-term graft and patient survival, indicating that conditions reflecting the severity of the liver failure and (or) the presence of other organ system failure(s) have a major impact on the ability of the graft to function in the new surrounding [29]. Furthermore, the magnitude of the alloimmune response may result in a severe destruction of the hepatic parenchyma, leading to a significant decrease in hepatic reserve and subsequent graft failure [30].

The main characteristics of a well-functioning graft are the relative hemodynamic stability of the recipient immediately after reperfusion, continuous urine output, and intact neurological function. PNF of the graft can be defined as nonrecoverable hepatocellular function necessitating emergency retransplantation within 72 h, whereas DNF may be defined as initial marginal graft function necessitating retransplantation within 1 month. PNF or DNF of the graft are usually associated with persistent tachycardia, decreased urine output and renal shutdown, and disturbances in mental status culminating in hepatic coma. Production of bile appears to be one of the most useful predictors of graft failure, and both quantity and quality of the bile are correlated with the development of PNF and DNF of the graft. Progressive increase in output, as well as a darker appearance, are signs of recovery. Investigating better biochemical means to define the quality of bile to more accurately determine the fate of the graft will be important.

**BIOCHEMICAL ASSESSMENT OF POSTTRANSPLANT GRAFT FUNCTION**

**Correction of acidosis.** Hemodynamic changes occurring during the anhepatic stage, coupled with initial release of various substances from the reperfused liver, result in the development of metabolic acidosis. In the presence of a functioning graft, the correction of the acidosis is possible with repeated injection of sodium bicarbonate, and is further prevented by better tissue perfusion and production of bicarbonate by the kidneys. Furthermore, the conversion of the citrate (used as preservative in packed red blood cells) to bicarbonate by the liver indicates the recovery of hepatocellular function. In contrast, persistence of the metabolic acidosis is an indicator of impaired liver graft function [31, 32].

**Serum markers and coagulation profile.** Liver-specific transaminases are commonly used to determine the extent of procurement/preservation/reperfusion injuries to the graft [33]. The serum concentrations of lactate dehydrogenase, AST, and ALT are good markers of hepatocyte loss, and are present in the systemic circulation within a short time after reperfusion. Continuous increases of serum transaminases coupled with persistent coagulopathy are signs of global injury and graft nonfunction, whereas recovery of these biochemical indices indicates improvement in graft function. Serial measurements of PT at different intervals within 24 h after reperfusion will indicate whether the new liver is capable of producing factors that are necessary for maintenance of normal coagulation. Typically, a trend toward normalization of the PT will be seen within the first 24 h after surgery and
may require a few days for full recovery. In contrast, a nonfunctioning graft will present with prolonged PT, which may be difficult to correct with infusion of fresh frozen plasma.

**Arterial ketone body ratio (AKBR).** Measurement of the AKBR is the best demonstration of how the clinical laboratory can assist in accurate determination of hepatic function and posttransplant recovery of the graft. The oxidation-reduction theory is based on the hypothesis that the energy charge of the liver is determined by the liver mitochondria energy production, and is reflected by the ratio of NAD+/NADH. The concept of an adenylate energy charge regards the adenine nucleotide system as the energy currency of the cell, which is a balance of energy-generating and energy-consuming reactions. Under normal aerobic conditions, the energy charge of the cell is maintained at a high and constant level. When energy-generating sequences are insufficient, the energy charge decreases. Thus, the energy charge is a convenient indicator by which to determine the intracellular energy status as well as the organ energy charge at any given time and condition.

The mitochondrial NAD+/NADH ratio was shown to correlate with the concentration of the ketone bodies’ acetoacetate and that of β-hydroxybutyrate [34]. In the liver mitochondria, acetoacetate is produced in the matrix compartment and undergoes reduction to β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase localized in the mitochondrial inner membrane [35]. Because the β-hydroxybutyrate dehydrogenase concentration is exceptionally high in the liver [36], and these two ketones penetrate cell membranes, the ketone body ratio in the hepatic venous blood can reflect that in the liver mitochondria. Subsequent studies proved that the changes in the ketone body ratio in the arterial blood are consistent with the changes observed in the hepatic veins [37]. In contrast, venous blood sampling for ketone body ratio will not reflect the actual energy status of the liver, since the equation should consider the use of ketones in the peripheral tissue.

Two possible mechanisms may explain the fall in AKBR after orthotopic liver transplantation (OLT). The first is the inhibition of the electron transport system, due to relative deprivation of blood supply available to the liver mitochondria. The second is an enhancement of β-oxidation of fatty acids, since β-oxidation reduces the mitochondrial NAD+/NADH ratio. β-oxidation is unlikely a contributing factor in this system, since all the measurements were done under glucose load. In contrast, ischemic damage is more likely to occur after OLT, resulting in a decrease in AKBR as seen in the adult and pediatric recipients with PNF and DNF of the graft. This was also demonstrated in other systems where an acute decrease in arterial blood flow to the liver after hepatic artery embolization, or warm ischemic injury, resulted in falling AKBR [37, 38].

Laboratory and clinical studies have demonstrated the significance of AKBR as a means to estimate liver functional reserve. Animal experiments have shown that changes in AKBR reflect the energy charge of the liver and the liver mitochondrial function during endotoxic shock, jaundice, and after liver transplantation [39–41]. These experiments demonstrated that peripheral perfusion and acid–base balance affect the residual liver function. Clinical studies confirmed these observations, allowing a correlation between the residual hepatic function and the outcome of patients undergoing liver resection or transplantation, trauma, sepsis, and multisystem organ failure [42–44]. Recent studies by us and others have demonstrated that the dynamic changes in the AKBR pattern are useful in the diagnosis of PNF or DNF of adult liver recipients, and correlate with short-term graft and patient survival (Fig. 2). The assay requires 1 mL of heparinized arterial blood, and results can be obtained within 40 min. AKBR was determined by the enzymatic method of Williamson and Melanby [45, 46], using a commercially available kit (Ketorex Sanwa Kit; Sanwa Kagaku Kenkyusho Co., Kasugai, Japan). Acetoacetate and β-hydroxybutyrate are calculated by the decrease or increase in absorbance at a wavelength of 340 nm in the adult recipient. All measurements were done under glucose load [blood sugar >6.66 mmol/L (120 mg/dL)], since ketosis occurring during hypoglycemia was shown to affect the molar concentration of acetoacetate and β-hydroxybutyrate [47]. Rapid recovery was associated with 100% 1-month graft survival. In contrast, slow or no recovery pattern resulted in the loss of 50% and 100% of the grafts [48–52]. These studies also demonstrated that the synthetic function of the grafts, as reflected by the PT, correlated with the AKBR, and predicted graft survival.

![AKBR](image-url)  
**Fig. 2.** AKBR reflects the ability of the graft to recover after cold ischemia and reperfusion injury. Rapid recovery with ratio >1 within 12 h is associated with excellent graft function. In contrast, persistent low ratio of AKBR was seen in PNF grafts.
Peroxide and graft injury. The formation of prostacyclin, thromboxane, and lipid peroxide were postulated to reflect the severity of hepatocyte damage and anoxia/reperfusion injury to the microvasculature. Studies in human recipients demonstrated that prostacyclin production correlated with early postoperative graft function, whereas lipid peroxide production, as measured by thio-barbituric acid-reacting substances, was indicative of significant injury [53].

In summary, accurate assessment of residual liver function is based on a set of clinical criteria as well as specific liver-related laboratory tests. The current trend appears to be the development of assays that can challenge energy-dependent metabolic pathways within the hepatocyte, with an attempt to quantify the remaining hepatic mass as well as to predict whether the liver can withstand a stress such as procurement, cold-preservation, and reperfusion injuries. The most promising approach appears to target the liver mitochondria and the cytochrome-P450 system. The available assays such as the lidocaine test are more accurate in the assessment of already established liver disease, whereas the AKBR is more reliable in predicting the function of the procured graft before transplantation. More innovative approaches and sophisticated assays need to be developed to better define liver function and residual reserve.

References
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