Nuclear imaging techniques for the assessment of hepatic function in liver surgery and transplantation.
Introduction

Surgical resection is the most effective treatment for patients with primary or secondary hepatic malignancies. Owing to the improved surgical techniques and perioperative care, extended resections are now performed with greater frequency. Extended resections can result in a small postoperative remnant liver, thereby increasing the risk of postoperative liver failure, especially in patients with chronic liver disease. Despite intensive care treatment, the mortality of liver failure is substantial. Although the causes of liver failure are multifactorial, the function of the postoperative remnant liver is main contributing factor. Therefore, preoperative evaluation of the future remnant liver (FRL) function is important when deciding the operative limits of safe liver resection. The availability of preoperative portal vein embolization (PVE) has further increased the importance of preoperative assessment of hepatic function. PVE induces atrophy of the embolized, tumor bearing liver segments while compensatory hypertrophy occurs of the non-embolized segments, hereby increasing volume and function of the FRL. The individual response to VPE is variable. Consequently, the quantification of hypertrophy of the non-embolized liver segments after PVE is a necessity.

After partial liver resection, the liver has a unique ability to regenerate. This capacity is influenced by multiple factors, including several underlying parenchymal liver diseases. Consequently, it is important to evaluate the regeneration process after partial hepatectomy. In the last decades, several applications have been developed for the assessment of hepatic function including CT volumetry, numerous blood clearance tests and clinical classification systems. Recently, technetium-99m (99mTc) labeled –diethylenetriamine pentaacetic acid galactosyl human serum albumin (99mTc-GSA) scintigraphy and hepatobiliary scintigraphy (HBS) with 99mTc-labeled iminodiacetic acid (IDA) have been developed as non-invasive alternatives for the evaluation of quantitative liver function. This review discusses in detail the biochemical and technical background, as well as the clinical applications of 99mTc-GSA scintigraphy and HBS with 99mTc IDA analogues in the assessment of hepatic function in liver surgery and transplantation.

The development of new techniques for assessment of liver function

The Conventional, preoperative selection of patients with cirrhosis is based on the clinical grading system of Child-Pugh (CP). This system is based on five parameters, including the presence or absence of encephalopathy and ascites, serum level of total bilirubin, serum level of albumin and prothrombin time. The CP provides only indirect information about the FRL function and can be unreliable for predicting the outcome of liver resections.

Blood clearance tests, such as indocyanine green clearance (ICG), galactose elimination capacity (GEC) and, caffeine clearance, have been employed as quantitative liver function tests. In Eastern Asia, ICG clearance is frequently used to evaluate preoperative liver function. ICG is a tricarbocyanine dye, exclusively cleared from plasma by hepatocyte transporters located on the baso-lateral membrane and excreted into the bile without intrahepatic conjugation. The ICG clearance test can be expressed as the retention time at 15 minutes (ICGR15), ICG clearance rate (ICGK), and ICG clearance at 15 minutes (ICGK15).
Galactose elimination capacity (GEC) measures the rate of galactose elimination from the blood which depends mainly on phosphorylation of galactose by galactokinase within the hepatocyte cytoplasm. Both ICG clearance and GEC provide prognostic information in liver diseases such as fulminant hepatic failure and primary biliary cirrhosis. Some studies have shown the ability of preoperative ICG and GEC to predict morbidity and mortality after partial hepatectomy. However, ICG clearance is dependent on hepatic blood flow and does not always correlate with postoperative outcome. Furthermore, all blood clearance tests measure global liver function and do not provide information on the distribution of function among the liver segments. Monoethylglycinexylidide (MEGX) test has been introduced as a quantitative liver function test to measure hepatic cytochrome p450 activity. The hepatic metabolism of intravenously administered lidocaine, through the cytochrome p450 pathway, results in the formation of MEGX. A correlation between decreased plasma MEGX values and increased risk of liver insufficiency after hepatic resection in patients with HCC has been demonstrated. However, the MEGX test is affected by changes in the hepatic blood flow and by drugs that interfere the cytochrome p450 pathway. Furthermore, similar to other blood clearance tests, MEGX test only provides information on global liver function.

Volumetric assessment of the liver is frequently used as an indirect measurement of liver function. The FRL volume can be measured preoperatively by 3-D volumetric computer tomography (CT) reconstruction. Preoperative volumetric assessment of the liver is widely used as a guideline to exclude patients from liver resection or to select patients who will benefit from PVE. In patients with a non-compromised liver parenchyma, a safe resection can be performed if the FRL volume is larger than 25-30% of total preoperative liver volume. However, in patients with parenchymal liver disease, the safety limit is more variable and is around 40% of total liver volume. Parenchymal liver diseases, such as cirrhosis, fibrosis and steatosis can affect total and segmental liver function. Preoperative liver biopsy is currently the most reliable method to assess the liver parenchyma. However, invasive liver biopsies bear a risk of severe complications, and are therefore not routinely performed. Furthermore, multiple biopsies are required for a reliable assessment of the liver parenchyma due to potentially unequal distribution of the liver disease. Consequently, the histopathology of the liver parenchyma is often unknown before liver surgery. As a result, the assessments of FRL volume by CT volumetry can be unreliable and unrepresentative for the actual liver function.

Due to the limitations of conventional liver function tests, new applications are being developed. A successful liver function test should be noninvasive, reliable, safe, inexpensive and it should provide quantitative information on total and regional liver function. Moreover, the test should be applicable both in patients with a normal liver as well as in patients with a compromised liver.

In the last decades, functional imaging techniques with 99mTc-GSA liver scintigraphy and HBS with different 99mTc-IDA analogues have been developed as simple, non-invasive methods for evaluating total and regional quantitative liver function.
99mTc-GSA scintigraphy

Background
The asialoglycoprotein (ASGP) receptor is only present in mammalian hepatocytes and is specific for the asialoglycoprotein. The ASGP receptor is composed of 2 subunits (human hepatic lectins 1 and 2) and is expressed in large quantities on the hepatocyte sinusoidal surface adjoining the extracellular space of Disse. A significant decrease in the expression of ASGP receptors is seen in patients with chronic liver diseases together with serum accumulation of asialoglycoproteins. Asialoglycoprotein binds to ASGP receptor and is subsequently transferred to the hepatic lysosomes where the receptor mediated endocytosis occurs. The liver is the only uptake site for 99mTc-GSA and it is therefore an ideal agent for functional liver scintigraphy.

First, technetium labeled galactosyl-neoglyoalbumin (99mTc-GNA) was developed as a synthetic asialoglycoprotein to visualize and quantify its hepatic binding to the ASGP receptor. For the clinical analysis of ASGP receptor, 99mTc-GSA was developed as an analogue ligand of the ASGP receptor. Since the ASGP receptor does not bind to bilirubin, 99mTc-GSA scintigraphy is also effective in assessing the hepatic function in patients with hyperbilirubinemia.

Kinetics and quantitative measurement of liver function
Similar to HBS, dynamic 99mTc-GSA scintigraphy images are obtained, after an intravenous bolus of 99mTc-GSA, by a gamma camera positioned over the heart and liver region. The blood clearance and hepatic uptake are obtained by generating ROIs from heart and liver. It is assumed that the ligand-receptor binding is a second-order process, in which a significant fraction of receptor is occupied during the course of the imaging study. For the actual kinetics of the 99mTc-GSA receptor binding, three models are commonly applied.

Figure 1: Planar 99mTc-GSA scintigraphy.

The hepatic uptake ratio (LHL15) and blood disappearance ratio (HH15) is calculated from the 99mTc-GSA time-activity curves from the heart (grey) and the liver (black) (A). Blood disappearance constant (KL) is calculated from the liver uptake curve, with the use of the disappearance halftime (T1/2) (B).
**Figure 2:** Hepatobiliary scintigraphy with $^{99m}$Tc-mebrofenin SPECT in a patient with liver metastasis.

The CT scan (A) shows a large colorectal metastasis in liver segments 2 and 3. The matching SPECT image (B) shows an inhomogeneous distribution of mebrofenin, with a decrease uptake in segment 2 and 3. Panel C shows the planar dynamic hepatobiliary scintigraphy.

**Figure 3:** Dynamic image of planar hepatobiliary scintigraphy (HBS).

Panel A shows an example of summed HBS image from 150-300 sec after intravenous injection of $^{99m}$Tc-mebrofenin. A region of interest (ROI) is drawn around the entire liver (red line). A second ROI is drawn around the mediastinum (blood pool) (yellow line). A third ROI is drawn around the future remnant liver (green line), indicating segments 2 and 3 after extended right hemihepatectomy.

Panel B shows a blood pool corrected liver-uptake time-activity curve. The liver uptake of mebrofenin is calculated as an increase of blood pool corrected $^{99m}$Tc-mebrofenin uptake (y-axis) per minute over a time period of 200 sec.

Vera et al. developed a three-compartment model of a bimolecular chemical reaction. Required for the calculations in this model are the time-activity curves of liver and heart, the patient's height, weight, hematocrit, and a portion of the injected dose from a blood sample. Five independent parameters are calculated; receptor concentration, receptor affinity (forward binding rate), hepatic plasma volume, extra hepatic plasma volume and hepatic plasma flow.

The receptor concentration proved to be the most accurate index for hepatic function. A five compartment model based on Michaelis-Menten-type kinetics for the receptor-ligand binding was introduced as a non-invasive approach, requiring no blood samples. Blood flow and maximal removal rate ($R_{\text{max}}$) of $^{99m}$Tc-GSA (mg/min) from plasma, is calculated from the time activity curve of the heart, liver and lung (background).
Miki et al introduced a seven compartment model in which the receptor mediated endocytosis and receptor recycling were included. It permits the quantitative measurement of the total receptor amount ($R_{tot}$) and hepatic blood flow, without blood samples. $R_{tot}$ correlates with the number of viable hepatocytes and can be used to assess the functional liver mass.

Although different parameters are calculated from the different kinetic models, many are highly complex and not widely used in the context of liver surgery. Table 1 and 2 summarizes the parameters used in liver surgery and transplantation.

The most commonly used parameters in planar, dynamic $^{99m}Tc$-GSA scintigraphy are the hepatic uptake ratio of $^{99m}Tc$-GSA (LHL15) and the blood clearance ratio (HH15) (fig1). The HH15 is calculated by dividing the radioactivity of the heart ROI 15 min after the injection by that after 3 min after injection. LHL15 is calculated by dividing the radioactivity of the liver ROI by the radioactivity of the liver plus heart ROIs 15 min after the injection. The modified receptor index (MRI) is determined by dividing the LHL15 by the HH15.

To enable and improve the assessment of total and segmental liver function and functional volume, $^{99m}Tc$-GSA Single Photon Emission Computed Tomography (SPECT) was introduced. Both static SPECT and dynamic SPECT are clinically applied. Dynamic SPECT requires a fast rotating multidetector gamma camera, which is not available in every institution. Functional liver volume can be calculated from $^{99m}Tc$-GSA SPECT by the outline extraction method, in which specific cut-off values are used to automatically outline the liver. The cut-off method does not take into account the regional functional differences within the volume. Therefore, Satoh described a more precise method for calculating functional liver volume. The functional liver volume was calculated depending on the degree of $^{99m}Tc$-GSA radioactivity in each voxel. First, the voxel with maximal counts was determined. In a phantom study, it was determined that a voxel below 54% of the maximal count was regarded as a background. Voxels with counts above 80% of maximal were considered fully functional. The thickness of each voxel with counts above 80% of the maximum was considered as the maximal thickness (1.08). For each voxel between the 54% and 80%, the thickness was estimated according to the accumulated counts in that voxel.

Liver uptake ratio (LUR) and liver uptake density (LUD) can be calculated from dynamic SPECT acquisitions. LUR reflect the percentage of hepatic SPECT counts relative to the injected counts measured in the syringe, thereby calculating the dose that is incorporated in the liver. LUD is the liver uptake ratio divided by functional liver volume. Furthermore, the hepatic $^{99m}Tc$-GSA clearance ($K_u$ ml/min) can be calculated by Patlak plot analysis.

$^{99m}Tc$-GSA liver scintigraphy in experimental surgical research

Postoperative complications in patients with hilar cholangiocarcinoma can be substantial due to impaired liver function and the necessity of extended resections. Therefore, the preoperative assessment of the FRL function is critical in these patients. Cholangiocarcinomas are frequently associated with obstructive jaundice. Unlike ICG, $^{99m}Tc$-GSA uptake is not influenced by hyperbilirubinaemia. In a rat model of obstructive jaundice, $^{99m}Tc$-GSA scintigraphy proved to be a valuable method to evaluate liver function. A reduction in
the $^{99m}$Tc-GSA binding affinity, rather than a reduction of ASGP receptor per hepatocyte or a decrease in number of hepatocytes, was responsible for the impaired uptake of $^{99m}$Tc-GSA. In cirrhotic patients, there is evidence that the decreased uptake of $^{99m}$Tc-GSA is not caused by a reduction in the number of ASGP receptors per hepatocyte, nor in the decrease in affinity, but to a decrease in the number of hepatocytes. The mechanism of reduced $^{99m}$Tc-GSA uptake in other liver diseases such as steatosis is unknown.

The effect of liver regeneration and I/R injury on $^{99m}$Tc-GSA uptake and ASGP receptor expression has been investigated in some preclinical studies. A decrease in uptake of $^{99m}$Tc-GSA was described in the early phase after I/R injury, which correlated with increased hepatocellular necrosis. The decrease in uptake was followed by an increased $^{99m}$Tc-GSA uptake several days after I/R, which was consistent with liver regeneration. In experimental studies there is controversial data on the expression of ASGP receptor after hepatic injury and liver regeneration. Some studies described a decrease in surface ASGP receptors during liver regeneration. However, Kouda et al confirmed an increase of GSA uptake per hepatocyte suggesting a possible cellular compensation mechanism for the loss of hepatocellular mass.

**Clinical use of $^{99m}$Tc-GSA liver scintigraphy in liver surgery**

$^{99m}$Tc-GSA liver scintigraphy was developed in Japan as a non-invasive alternative to evaluate liver function. The parameters obtained from planar $^{99m}$Tc-GSA scintigraphy, including the modified receptor index (MRI) and LHL15, correlated with conventional function tests such as antithrombin III, total and direct bilirubin, prothrombin time, ICG clearance, Child-Pugh classification and histology (hepatic activity index (HAI) score), in cirrhotic patients. Planar $^{99m}$Tc-GSA scintigraphy was applicable for the evaluation of preoperative and postoperative liver function. However, a discrepancy between ICGR15 and $^{99m}$Tc-GSA scintigraphy parameters is described in 9-20% of the patients in whom the histological severity of disease is better reflected by $^{99m}$Tc-GSA scintigraphy.

Multiple studies have described the use of preoperative planar dynamic $^{99m}$Tc-GSA scintigraphy for predicting postoperative complications. Preoperative GSA-Rmax and LHL15 proved to be reliable indicator for predicting the risk of postoperative complications in patients with HCC and chronic liver disease. Preoperative LHL15 or GSA-Rmax was significantly lower in patients with major postoperative complications than in patients with minor or no complications. Specific cut-off values were used to select patients with a high risk of complications and patients with complications. For the LHL15, cut-off values of 0.90 and 0.875 are described. The cut-off values from other parameters include; LHL15/preoperative liver volume of 0.76, total ASGP receptor concentration in the FRL of 0.05 μmoles. Multivariate analysis revealed that LHL15 was a preoperative predictor of postoperative complications, while ICGR 15 was not. Nanashima et al. compared $^{99m}$Tc-GSA scintigraphy with the ICG clearance test for selecting the extent of resection and predicting patient outcome. As describe before, a discrepancy between ICG 15 and LHL15 was seen in 8.6% of the 140 patients. In these patients, LHL15 was better in predicting postoperative complications. Postoperative complications were frequently observed in patients with a low LHL15 (< 0.875). However, liver failure was also observed in patients with a relatively normal liver function. This was explained by the
fact that LHL15 only measured preoperative total liver function and not the function of the FRL. For a more accurate assessment of the FRL, $^{99m}$Tc-GSA SPECT was introduced to estimate total as well as segmental liver function. Preoperative $^{99m}$Tc-GSA SPECT was more suitable for predicting the remnant liver function than CT volumetry. One explanation is the liver parenchymal damage which is seen around a tumor by mechanical compression or compression on bile ducts and/or blood vessels. This tumor compression has a large impact on the regional liver function, while liver volume is maintained over a longer period of time. In cirrhotic livers, advanced fibrosis is accompanied with a reduction of functional hepatocytes and therefore liver volume obtained by CT does not represent the hepatocyte functional mass in cirrhotic liver. On the other hand, functional volume measured by $^{99m}$Tc-GSA SPECT, reflected the functional hepatocyte mass.

The outline extraction method with specific cut-off levels is frequently used to calculate the functional hepatic volume. It is based on the assumption that the liver function is uniformly distributed in the tissue included within the cut off value. Especially in tumor bearing and compromised livers, function is not distributed homogeneously. Therefore, functional volume may not correlate with the intrinsic liver function measured with dynamic planar GSA scintigraphy.

To overcome this problem, dynamic SPECT was introduced. A study by Sugahara et al. demonstrated the advantage of dynamic SPECT for the assessment of regional liver function. Liver functional volume (calculated with the cut-off method), as well as liver uptake ratio (LUR) and liver uptake density (LUD) were calculated in patients with different severity of liver disease. Both LUR and LUD decrease with the severity of liver disease. Functional volume however, was only significantly decreased in patients with Child-Pugh C classification. The ratio between LUR and LUD of the left and right liver lobe changed with the progression of liver disease, confirming that the liver function in compromised patients is not distributed homogeneously.

Owing to the possibility to measure the FRL function, dynamic SPECT can be used to preoperatively predict postoperative complications. Patients with postoperative complications due to liver insufficiency had significantly lower hepatic FRL $^{99m}$Tc-GSA clearance ($K_u$ in ml/min) compared with patients without complications. Satoh et al used the predictive residual index (PRI) for evaluating the FRL function before resection. The PRI could be used to preoperatively predict postoperative complications (positive predicting value 71% and negative predicting value 100% with a cut-off value of 0.38). Conclusions in both studies however, are based on a relatively small amount of complications.

Postoperative liver regeneration is impaired in patients with chronic liver disease. Especially the severity of liver fibrosis correlates with impaired regeneration. Since $^{99m}$Tc-GSA scintigraphy correlates with the severity of liver fibrosis, it can be used as a preoperative non-invasive method to predict the rate of liver regeneration. Multiple studies have described a discrepancy between functional liver regeneration and volumetric regeneration. Tanaka et al. reports that functional recovery is impaired in large resections. However, data presented in this study showed that four weeks after a resection of two or three segments, the average LHL15 recovered to 95% of the preoperative measured value. The volume recovered to approximately 70%. Therefore, the functional recovery is greater then
the volumetric recovery indicating the exact opposite of the conclusions drawn by the authors.

Kwon et al described in two similar studies that the functional regeneration measured by $^{99m}$Tc-GSA SPECT is more rapid than the morphological regeneration measured by CT volumetry. The functional and volumetric liver regeneration was delayed in patients with underlying liver disease. Although, there was no direct comparison made between $^{99m}$Tc-GSA SPECT and CT volumetry, it was concluded that functional recovery was more rapid in patients with injured livers. However, again the data presented in these studies do not provide enough evidence for this conclusion. Although, $^{99m}$Tc-GSA scintigraphy is useful to assess regeneration, it is difficult to draw conclusions about the difference between functional and volumetric regeneration.

The functional hypertrophy after PVE can be evaluated with the use of $^{99m}$Tc-GSA scintigraphy. In two studies, the increase of liver function assessed with dynamic $^{99m}$Tc-GSA SPECT was compared with morphologic hypertrophy measured with CT volumetry after PVE in cirrhotic and non-cirrhotic patients. The increase in $^{99m}$Tc-GSA uptake (expressed in LUR, LUD, residual functional liver volume and PRI) was more extensive than the degree of morphologic hypertrophy after PVE. Therefore, it was concluded that $^{99m}$Tc-GSA scintigraphy was useful in evaluating the functional hypertrophy, which could not be evaluated with CT volumetry after PVE. Patients with a small liver volume and a low $^{99m}$Tc-GSA uptake in the non-embolized lobe after PVE had an increased risk of developing postoperative liver failure. So far no studies, regarding the use of $^{99m}$Tc-GSA scintigraphy in selecting candidates for PVE, have been published. Therefore, further research in this field is warranted.

**Clinical use of $^{99m}$Tc-GSA scintigraphy in liver transplantation**

After liver transplantation, the graft function is affected by many factors causing acute and chronic rejection and thus the evaluation of graft function after transplantation is of crucial importance.

In a study with seven liver transplant patients, the functional reserve of liver allografts was evaluated with $^{99m}$Tc-GSA scintigraphy. $^{99m}$Tc-GSA scintigraphy using the total amount of ASGP receptors ($R_{tot}$ as described by kinetic model of Miki) was compared with conventional liver function tests and histopathology evaluation of a biopsy. The histological liver damage of the graft had a good correlation with the $R_{tot}$ and even though cohort size was small, the study indicates the potential utility of $^{99m}$Tc-GSA scintigraphy as a noninvasive method to evaluate graft function after transplantation.

In a study with thirteen patients, $^{99m}$Tc-GSA scintigraphy was used to monitor both the graft and native liver function after auxiliary partial orthotopic liver transplantation (APOLT). In an APOLT procedure, the native liver is left partially in place and the donor liver graft is positioned orthotopically. The uptake of $^{99m}$Tc-GSA (calculated using the Patlak plot) was a better predictor for the actual graft function than the conventional morphological volume assessed by CT volumetry. Especially in patients with severely damaged liver grafts, the $^{99m}$Tc-GSA uptake corresponded better with the histopathological evaluation of liver biopsy than CT volumetry.
In 2004, Kwon et al. briefly described the necessity of accurate estimation of the FR L function in living donor operations. The authors conclude that the estimated FR L function by GSA SPECT is useful to select the procedure of hepatectomy in the setting of living donors. However, this study was performed in 152 patients resected predominantly for malignant tumors and not in LDLT procedures. Eighty-three percent of the patients were resected for hepatocellular carcinomas which are frequently associated with liver cirrhosis. Therefore, it is questionable if the patients included in this study are representative for living donors.

**Hepatobiliary scintigraphy with IDA analogues**

**Background**

99mTc-IDA agents were introduced in 1976 by Loberg et al. These lidocaine analogues are transported to liver predominantly bound to albumin. Dissociation between albumin and the 99mTc-IDA agents occurs in the hepatic space of Disse, after which it is taken up by the hepatocytes. The hepatic uptake of IDA analogues is similar to the uptake of organic anions such as bilirubin. IDA agents are excreted in the bile canaliculi similar to ICG, without undergoing biotransformation during their transport through the hepatocyte, and are therefore ideal tracers for the biliary tract. The list of suggested bile canicular transporters include bile salt excretory pump and multidrug resistance proteins. Liver uptake of IDA agents can be affected by high plasma levels of bilirubin. Of all IDA analogues, mebrofenin shows the highest hepatic uptake, minimal urinary excretion and resists strongly the displacement by high plasma bilirubin concentration. Therefore, mebrofenin is considered the most suitable agent for hepatic and biliary diagnostics. 99mTc-mebrofenin uptake can be hindered by hypoalbuminemia, as albumin is the main plasma carrier of mebrofenin. Hypoalbuminemia consequently decreases hepatic delivery of mebrofenin and increases renal excretion. Conversely, hypoalbuminemia in liver disease is a sign of impaired liver function and therefore decreased uptake of IDA in patients with hypoalbuminemia can still reflect liver function under these circumstances.

**The kinetics and quantitative measurement of liver function**

Measurement of hepatic uptake function by the clearance rate of the IDA analogue Iodida was first described by Ekman et al. After the intravenous injection of 99mTc-mebrofenin, a dynamic scintigraphy is obtained with the use of a gamma camera. 99mTc mebrofenin uptake of the liver is determined by drawing a region of interest (ROI) around the liver, the heart (serving as blood pool) and around the total field of view (fig. 2). Three different time-activity curves are generated based on these ROIs. With the 3 parameters, the hepatic 99mTc-mebrofenin uptake rate (%/min) can be calculated. Radioactivity values acquired between 150 and 350 sec post injection are used to ensure that the calculations are made during a phase of homogenous distribution of the agent in the blood pool and before occurrence of biliary excretion. Furthermore, ROIs can be drawn around parts of the liver to calculate regional differences in 99mTc-mebrofenin uptake (fig.2).
The regional uptake of mebrofenin can be assessed with a small intra- and interobserver variation\textsuperscript{88,90}. Alternative methods of determining liver function are the hepatic extraction fraction, the time at which maximal hepatic radioactivity occurs (\(T_{\text{peak}}\)), as well as the time acquired for peak activity to decrease by 50\% (\(T_{1/2\text{ peak}}\))\textsuperscript{91-93}. The hepatic extraction fraction is calculated by drawing ROIs around the left ventricle of the heart and the liver and is further calculated from the time-activity curve by a deconvolution analysis using the Fourier transform method\textsuperscript{94}.

**HBS in experimental surgical research**

The measurement of liver function in small animal models remains a challenge. Many quantitative liver function tests require repetitive blood samples, and are therefore difficult to use in small animal models. Hepatic extraction fraction, as well as \(T_{1/2\text{ peak}}\) measured by HBS is used as a non-invasive method to evaluate hepatic function after ischemia reperfusion (I/R) injury and it is used to quantify the protective effect of new interventions on I/R injury\textsuperscript{95,96}. The application of HBS for the measurement of liver function in small animals was confirmed in experimental models of acute and chronic liver damage\textsuperscript{93,97}. For the evaluation of functional regeneration in small animals, serial measurements are needed over a long time period. The use of the hepatic \(^{99m}\text{Tc}\)-mebrofenin uptake rate measured by dedicated pinhole HBS in different rat models of liver regeneration has recently been validated\textsuperscript{91}. HBS proved to be an accurate, non-invasive tool for the measurement of liver function in the rat, and enabled serial measurements within the same animal\textsuperscript{91}.

**Clinical use of HBS in liver surgery**

\(^{99m}\text{Tc}\)-labeled IDA analogues were first used in cholecintigraphy for the diagnosis of multiple biliary diseases\textsuperscript{82,98,99}. More recently, the application of HBS has been proposed for the assessment of liver function\textsuperscript{100}. The use of \(^{99m}\text{Tc}\)-mebrofenin HBS for the preoperative assessment of liver function in patients undergoing liver surgery, was first described by Erdogan et al\textsuperscript{89}. In 54 patients scheduled for liver resection, \(^{99m}\text{Tc}\)-mebrofenin uptake assessed by HBS strongly correlated with the ICG clearance test. HBS provided both quantitative as well as visual information about total and regional liver function. The morphological information obtained from HBS provided information on localization of liver segments with inferior function. The biliary excretion could be used to preoperatively determine segmental cholestasis as well as postoperative biliary complication such as bile leakage and biliary obstructions. These features make HBS a valuable liver function test in the context of liver resection.

Owing to the possibility to determine regional liver function, HBS was validated as a tool to determine the preoperative liver functional reserve and to estimate the FRL function\textsuperscript{88}. In this small patient study, the preoperative estimated FRL function correlated well with the actual remnant liver function one day after resection.

For a surgeon, an important aspect of a liver function test is the possibility to accurately predict postoperative complications before partial hepatectomy. The predictive value of FRL uptake function measured preoperatively by HBS for the short-term outcome after
partial liver resection was investigated by Dinant et al. Forty-six patients with and without parenchymal disease were included in this study. Preoperative measurement of FRL function by planar dynamic $^{99m}$Tc-mebrofenin HBS proved more valuable than measurement of FRL volume by CT for the risk assessment of postoperative liver failure and liver failure related mortality. A safe resection could be performed in patients with a FRL uptake above 2.5 %/min/BSA (Body Surface Area), with a 3% chance of developing postoperative liver failure and liver failure related mortality. However, in patients with a FRL uptake below 2.5 %/min/BSA, the risk of postoperative liver failure increased to 56%.

Although to date there are no studies on the use of HBS for the selection of candidates for PVE, HBS has the ability to select patients with a high risk of postoperative liver failure. Furthermore, because of the ability to measure regional liver function, HBS could possibly be useful for the evaluation of the functional increase of the FRL after PVE.

Postoperative liver regeneration is frequently evaluated by CT volumetry. Bennink et al. compared the volumetric regeneration three months after partial liver resection with the functional regeneration measured by HBS and ICG-15. There was a significant correlation between the ICG clearance and HBS. Only a weak association between functional liver regeneration measured with both HBS and ICG and liver volume regeneration measured with CT volumetry was observed. This discrepancy indicates that the functional recovery may be an independent mechanism in addition to the volumetric regeneration. Recently, $^{99m}$Tc-mebrofenin SPECT was introduced to measure functional volume. In a study with 18 patients, it was demonstrated that the functional volume measured by SPECT, correlated with the liver volume measured by CT. Furthermore, the preoperative estimated FRL functional volume had a strong correlation with actual postoperative functional volume. A combination of low dose CT scan with HBS combined with SPECT, provided accurate information on segmental liver function and enabled a more precise measurement of FRL functional volume and function (fig.3).

**Clinical use of HBS in liver transplantation**

Biliary complications, as well as hepatic dysfunction due to graft rejection, are major causes of postoperative morbidity and mortality in liver transplant recipients. Many studies have shown that HBS is an accurate, noninvasive technique for the diagnosis of biliary complications including segmental and total biliary obstruction, as well as bile leakage in both adult and pediatric transplanted patients.

The application of HBS for the detection of graft dysfunction due to rejection is unclear. Liver biopsy is frequently used to detect graft rejection. Brunot et al. demonstrated a close relation between early biopsy results and liver uptake function measured by HBS suggesting the usefulness of quantitative HBS in distinguishing graft rejection from cholestasis. In contrast, others report that HBS can distinguish between normal grafts and those suffering from rejection and/or cholestasis, but not between biliary complications and rejection.

In heterotopic liver transplantation the native liver is left in situ, and a graft is transplantated elsewhere in the abdominal cavity. In some patients, the native liver recovers and regains function. Individual assessment of the graft and the native liver is difficult since most
function test measure total liver function. HBS has the unique ability to separate the functional assessment of the graft from the native liver.\textsuperscript{108,109} Owing to the increased shortage of cadaver livers, living donor transplantation is used to expand the organ pool. In living related liver transplantation (LRLT), a left or right hepatectomy is performed in a living donor. After transplantation, the liver has the ability to regenerate to its predetermined size. The regeneration capacity in donors after LRLT was investigated with the use of HBS.\textsuperscript{110} As described by others, this study indicates that acceleration of organ function is an early compensatory mechanism after reduction of organ volume.\textsuperscript{111} To date, no studies have been published on the use of HBS for the preoperative assessment of the liver function of the donor in LRLT.

**Discussion**

Current guidelines for safe resections are based on preoperatively determined FRL volume by CT.\textsuperscript{28} Liver diseases such as cirrhosis, fibrosis and steatosis can affect total and segmental liver function. Therefore, FRL volume assessed by CT volumetry can be unreliable and unrepresentative for the actual liver function. Nuclear imaging techniques, including $^{99m}$Tc-GSA scintigraphy and $^{99m}$Tc-mebrofenin HBS are used as non-invasive quantitative methods for evaluating total liver function as well as FRL function. Both techniques are applicable in patients with parenchymal liver disease. This aspect forms a growing clinical challenge as the number of patients with parenchymal liver disease is expected to dramatically increase in the near future due to the close connection of several parenchymal liver diseases with aspects of Western lifestyle, i.e. obesitas and sexually transmitted diseases.

HBS is an accurate method for preoperative assessment of liver function and prediction of postoperative complications.\textsuperscript{89,90} Although dynamic HBS can be used to determine regional liver function, planar images are not suitable for the assessment of segmental liver function. $^{99m}$Tc-mebrofenin SPECT could improve the assessment of liver function on the segmental level and enable the assessment of functional volumes. Multiple studies have investigated the use of preoperative $^{99m}$Tc-GSA scintigraphy to determine the limits for safe resection.\textsuperscript{61,65,73} Liver function is expressed by many different, sometimes complex parameters (table 1 and 2), making it difficult to compare studies and cause extensive discrepancies of obtained data. However, there is enough evidence indicating that $^{99m}$Tc-GSA scintigraphy is an accurate technique to select patients with a high risk of developing postoperative liver failure. To date, there is no consensus on the indications for PVE.\textsuperscript{28} Although no studies have been performed on the application of $^{99m}$Tc-GSA scintigraphy and $^{99m}$Tc-mebrofenin HBS to select patients for PVE, both techniques have the capability to clarify the indications for PVE.

After PVE, hypertrophy of the non-embolized liver segments is traditionally assessed by CT volumetry. However, a discrepancy between volumetric hypertrophy and functional increase has been described. Some studies indicate that functional regeneration is more rapid and of greater magnitude than the volumetric gain.\textsuperscript{69,77} However, few methods are
available to measure liver function of the non-embolized liver segments. Both $^{99m}$Tc-GSA scintigraphy and $^{99m}$Tc-mebrofenin HBS have the unique ability to visualize and quantify regional liver function and can therefore be used to assess the functional increase of the FRL after PVE. In liver transplantation, HBS and $^{99m}$Tc-GSA scintigraphy are used to assess graft function. Additionally, HBS can diagnose biliary complications after liver transplantation.

$^{99m}$Tc-GSA scintigraphy and $^{99m}$Tc-mebrofenin HBS are based on two different principles. $^{99m}$Tc-GSA scintigraphy measures the binding of $^{99m}$Tc-GSA to its receptor on the hepatocyte, and therefore it is an indirect parameter of liver function. The amount of ASGP receptors is decreased in chronic hepatitis and liver cirrhosis. However, it is unknown if the receptors are decreased in patients with compromised livers due to steatosis or chemotherapy. Since $^{99m}$Tc-GSA is not excreted into the bile, $^{99m}$Tc-GSA scintigraphy is not suitable to visualize the biliary system. Therefore it can not be used to diagnose biliary complications after liver surgery or transplantation. HBS, on the other hand, measures the uptake and excretion of mebrofenin by the hepatocytes, and therefore has the ability visualize the biliary system. The uptake and excretion of $^{99m}$Tc-mebrofenin is similar to that of organic anions such as bilirubin $^{82}$. HBS is therefore a direct parameter of liver function. The uptake of $^{99m}$Tc-mebrofenin can be influenced by hepatic blood flow, hypoalbuminemia and high concentrations of bilirubin $^{85}$. As the hepatic uptake of many substances is influenced by the same factors, it can still be used as a functional test under these conditions.

Although many studies have proved the value of nuclear imaging in liver surgery and transplantation, they are not widely used. $^{99m}$Tc-GSA scintigraphy is used in Japan, but is not approved in Europe and the USA. $^{99m}$Tc-mebrofenin HBS technique is relatively new, and only few clinical trials have been performed. Clinical trials in larger populations are required to confirm the application of $^{99m}$Tc-mebrofenin HBS for the pre-operative assessment of liver function and postoperative evaluation of complications and regeneration.

Both GSA scintigraphy and HBS are simple techniques that can be implemented in every hospital with a nuclear medicine department. Since many conventional methods, including CT volumetry have limitations, additional HBS or GSA scintigraphy, can improve the selection of patients with increased risk of postoperative complications.

**Conclusion**

Owing to the complexity of liver function, an ideal liver function test meeting all required criteria is yet to be discovered. Many liver function tests have limitations for the assessment of patients suitable for liver surgery. Both $^{99m}$Tc-GSA scintigraphy and $^{99m}$Tc-mebrofenin HBS are noninvasive, reliable techniques that provide visual and quantitative information of both total and regional liver function. Both tests are applicable in patients with normal liver as well as patients with a compromised liver. These features make both $^{99m}$Tc-GSA scintigraphy and $^{99m}$Tc-mebrofenin HBS useful liver function tests for the application in liver surgery and liver transplantation.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Parameter</th>
<th>Description</th>
<th>Mathematical Formula</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHL15</td>
<td>Hepatic uptake ratio of 99mTc-GSA</td>
<td>Liver counts at 15 min (L15) divided by the heart (H15) plus liver counts at 15 min</td>
<td>$LHL15 = \frac{L15}{L15 + H15}$</td>
<td>Total liver function, Regional liver function</td>
</tr>
<tr>
<td>HH15</td>
<td>Blood disappearance ratio</td>
<td>Heart counts at 15 min (H15) divided by heart counts at 3 min (H3)</td>
<td>$MRI = \frac{LHL15}{HH15}$</td>
<td>Total liver function</td>
</tr>
<tr>
<td>MRI</td>
<td>Modified Receptor Index</td>
<td>Liver uptake ratio (LHL15) divided by the blood disappearance ratio (HH15)</td>
<td>$HH15 = \frac{H3}{H15}$</td>
<td>Total liver function</td>
</tr>
<tr>
<td>KL</td>
<td>Blood disappearance constant</td>
<td>Calculated from the liver uptake curve, with the use of disappearance halftime (T1/2)</td>
<td>$K = \frac{\ln 2}{T_{1/2}}$</td>
<td>Total liver function</td>
</tr>
<tr>
<td>LU15</td>
<td>Liver uptake</td>
<td>Cumulative liver uptake from 15 to 16 minutes after injection from the liver time-activity curve (L(t))</td>
<td>$LU15 = \int_{15}^{16} L(t) dt \times total \ injected \ dose \times 100$</td>
<td>Total liver function</td>
</tr>
<tr>
<td>LHL15-V</td>
<td>Ratio of Liver uptake to liver volume</td>
<td>Liver uptake ratio (LHL15) divided by total liver volume</td>
<td></td>
<td>Total liver function</td>
</tr>
<tr>
<td>Rmax</td>
<td>Maximal removal rate of GSA</td>
<td>Calculation with the kinetic model of Hasekawa</td>
<td>Multiple equations</td>
<td>Total liver function</td>
</tr>
<tr>
<td>RO</td>
<td>Asialoglycoprotein receptor concentration</td>
<td>Calculation with the kinetic model of Vera et al.</td>
<td>Multiple equations</td>
<td>Total liver function</td>
</tr>
<tr>
<td>Abbreviation</td>
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<tr>
<td>FLV</td>
<td>Functional liver volume</td>
<td>Outline extraction method with specific cut-off level Sum of the product of the liver surface in each slice and slice thickness.</td>
<td>$\sum (\text{liversurface} \times \text{slicethickness})$</td>
<td>Total liver Function</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Regional liver function</td>
</tr>
<tr>
<td>Ku</td>
<td>Hepatic $^{99m}$Tc-GSA clearance</td>
<td>Patlak plot analysis $L(t)$ is the liver activity, $B(t)$ is the blood activity, $Vh$ is the hepatic blood volume.</td>
<td>$L(t) = K_v \times \int \frac{B(t) \times Vh}{B(t) + Vh}$</td>
<td>Total liver Function</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Regional liver function</td>
</tr>
<tr>
<td>LUR</td>
<td>Liver uptake ratio</td>
<td>Hepatic SPECT counts divided by injected syringe counts</td>
<td>$LUR = \frac{\text{Total SPECT counts}}{\text{counts syringe proportion}} \times 100$</td>
<td>Total liver Function</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Regional liver function</td>
</tr>
<tr>
<td>LUD</td>
<td>Liver uptake density</td>
<td>Liver uptake ratio divided by liver functional volume</td>
<td>$LUD = \frac{\text{LUR}}{\text{Functional liver volume}}$</td>
<td>Total liver Function</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Regional liver function</td>
</tr>
<tr>
<td>PRI</td>
<td>Predictive residual index</td>
<td>Sum of the product of $k_i$-value (blood disappearance constant) and functional liver volume (FLVi) in the FRL in each slide divided by the product of normal $k$-value (healthy volunteers) and total FLV</td>
<td>$PRI = \frac{\sum k_i \times FLVi}{Kn \times FLV}$</td>
<td>Regional liver function</td>
</tr>
<tr>
<td>FRR</td>
<td>Functional resection ratio</td>
<td>Counts in the expected resection volume divided by the counts in the total liver volume.</td>
<td>$FRR = \frac{\text{resection volume counts}}{\text{total liver volume counts}} \times 100$</td>
<td>Regional liver function</td>
</tr>
</tbody>
</table>
References


72. Sugahara K, Togashi H, Takahashi K et al. Separate analysis of asialoglycoprotein receptors in the right and left hepatic lobes using Tc-GSA SPECT. Hepatology 2003; 38:1401-1409.


