Assessment and preservation of liver function in hepatic ischemia and reperfusion
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Chapter 9


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Submitted
Abstract

**Background:** Preoperative assessment of liver function is used to estimate the outcome of major liver surgery. The indocyanine green (ICG) clearance test is the most frequently used test for liver parenchymal function but has its limitations. The aim of this study was to investigate the correlation between the liver uptake of $^{99m}$Tc-Mebrofenin measured with hepatobiliary scintigraphy and the ICG clearance test.

**Patients and Methods:** 54 patients were diagnosed as hepatocellular carcinoma (n=9), hilar (Klatskin) tumours (n=20) and 25 patients with non-parenchymal tumours (NPT) including colorectal metastasis (n=15) and miscellaneous tumours (n=10). Hepatobiliary $^{99m}$Tc-Mebrofenin scintigraphy was performed after intravenous injection of 85 Mbq $^{99m}$Tc-Mebrofenin, and hepatic uptake rate was calculated. $^{99m}$Tc-Mebrofenin hepatobiliary scintigraphy, the 15-minute clearance rate of ICG (ICG-C15) and conventional plasma liver function tests were carried out one day prior to operation.

**Results:** The mean ICG-C15 was 86.86 ± 1.19 % (SEM). The mean $^{99m}$Tc-Mebrofenin uptake rate was 12.87 ± 0.52 %/min. A significant correlation was obtained between $^{99m}$Tc-Mebrofenin uptake rate by scintigraphy and ICG-C15 (r = 0.73, P < 0.0001). The mean $^{99m}$Tc-Mebrofenin clearance capacity of the right liver segments (79.83 ± 1.63, range 47.75-95.97 %) was larger than that of the left segments (mean 20.24 ± 1.55, range 6.51 to 52.51 %).

**Conclusions:** These data show that $^{99m}$Tc-Mebrofenin uptake rate as assessed by scintigraphy is an efficient method for determining liver function and correlates well with ICG clearance. At the same time, $^{99m}$Tc-Mebrofenin scintigraphy provides information of segmental functional liver tissue which is of additional use when planning liver resection.
ICG clearance compared with $^{99m}$Tc-Mebrofenin uptake

**Introduction**

Hepatic resection is the therapy of choice for malignant and symptomatic benign, hepatobiliary tumours. Recent years have shown a marked decrease in morbidity and mortality rates after major liver resections $^{1,2}$. Refinements in operative techniques, better selection of patients and advances in peri-operative care are thought to be responsible for this improvement $^3$. Nevertheless, peri-operative blood loss and post-operative liver failure have remain the most significant complications after liver resections, particularly in patients with suboptimal liver function due to parenchymal liver disease, such as cirrhosis or steatosis $^4$. The major cause of mortality after liver resection consequently, is liver failure $^1$. For this reason it is important to estimate total and regional liver function before planning partial resection of the liver in order to predict function of the remnant liver.

To date, the most frequently used test for evaluating preoperative liver function is the indocyanine green (ICG) clearance test $^{5-8}$. ICG is a tricarbocyanine dye, exclusively removed by the liver and excreted into the bile $^9$. ICG clearance, requiring intravenous injection and multiple blood samples, provides indirect measurement of global liver function only.

Alternatively, Technetium-99m-labeled Iminodiacetic acid (IDA) analogues, transported in blood by binding to albumin in the same manner as ICG, can be used for hepatobiliary scintigraphy in the assessment of liver function $^{10}$. In liver transplant patients, hepatobiliary scintigraphy has been performed to obtain information about the functional and morphological status of the graft $^{11}$. Hepatobiliary scintigraphy, requiring a single intravenous injection, provides visual and quantitative information of global and regional liver function as well as excretory function (intrahepatic and extrahepatic bile transport). Both ICG and $^{99m}$Tc-Mebrofenin are excreted in bile by the hepatocytes $^{12}$ by the ATP-dependent export pump multidrug-resistance associated protein 2 (MRP 2), without undergoing biotransformation during their transit through the hepatocyte $^{13,14}$. Therefore, these agents are well suited for the study of hepatic transport. The aim of this study is to examine correlation of the ICG clearance test with the uptake of $^{99m}$Tc-Mebrofenin as determined from the blood and by scintigraphical assessment.

Both ICG clearance test and $^{99m}$Tc-Mebrofenin uptake and scintigraphy were sequentially performed in patients planned to undergo partial liver resection for primary or metastatic liver tumours or proximal bile duct malignancies. Special attention was paid to additional information obtained from the dynamic scintigraphy images such as possible, local differences in liver function or cholestasis of the left- and/ or right liver lobes.
Patients and methods

Patients

The study group consisted of patients planned to undergo partial liver resection for hepatobiliary tumours between March 2000 and September 2002 in the Academic Medical Center (AMC) in Amsterdam, The Netherlands. 54 patients were included (25 female, 29 male) with a mean age of 59.2 years (range 37-80 years). Nine patients were diagnosed as hepatocellular carcinoma, 20 as proximal bile duct cancer (Klatskin tumours) and 15 as colorectal metastasis and 10 as miscellaneous tumours. This study was approved by the local Medical Ethics Committee of the AMC (protocol 99/167) and was performed with informed consent of all patients. Patient characteristics are listed in table 1.

<table>
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<th>AGE (YEARS)*</th>
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<tr>
<td>POST-RESECTION MORTALITY (N)</td>
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<tr>
<td>UNRESECTABLE TUMOURS (N)</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 1

* Age is expressed as mean number of years ± SD (min-max range).
ICG clearance compared with $^{99m}$Tc-Mebrofenin uptake

**ICG clearance Test**

One day prior to surgery, bilateral intravenous Venflons™ were placed in the antecubital veins. After an overnight fast, 25 mg of ICG (Infracyanine, Laboratoires pharmaceutiques, Paris, France) was dissolved in 10 ml of 5% dextrose solution and injected rapidly into the antecubital vein. The clearance tests were performed after overnight fasting because food consumption stimulates hepatic function and bile flow. Blood samples were drawn before the administration of ICG (blank) and at 5, 10, 15 and 20 minutes after ICG injection. Plasma samples were read against the plasma blank at 805 nm by spectrophotometry to determine the concentration of ICG. The theoretical maximum concentration at zero minutes was estimated by using the least squares method. Results were expressed as the percentage ICG cleared at 15 minutes (ICG-C15 value).

**Hepatobiliary scintigraphy**

On the same day, all patients underwent hepatobiliary scintigraphy using the radiopharmaceutical agent $^{99m}$-Technetium-labeled (Tc-99m)-Mebrofenin. After intravenous administration of 85 MBq $^{99m}$Tc-Mebrofenin, dynamic image acquisition was performed with a gamma camera (Diacam, Siemens, Milwaukee, USA) with the liver and heart in the field of view (FOV), using a 128x128 matrix. Dynamic acquisition was performed in one hour at 10 seconds per frame for 60 frames (liver uptake sequence) followed by 50 frames of 1 minute (bile excretion sequence). Regions of interest (ROI) were drawn around the liver, the heart and large vessels within the mediastinum (serving as blood pool) and around the total FOV (indicative of total activity). Three different time versus radio activity curves were generated, based on the liver, blood pool and total FOV. Liver uptake was calculated in %/min as described by Ekman, based on these three parameters. Furthermore, ROI could be drawn around parts of the liver to calculate regional differences in $^{99m}$Tc-Mebrofenin uptake. Calculations of $^{99m}$Tc-Mebrofenin uptake by the liver were performed using scanned radio activity values acquired between 150 and 350 seconds post-injection, to make sure that calculations were made during a phase of homogenous distribution of the agent in the blood pool and before the rapid phase of hepatic excretion.

Since ICG clearance is measured in blood samples, and scintigraphic liver uptake function is measured on planar imaging, additional blood samples were taken to measure $^{99m}$Tc-Mebrofenin blood clearance. Blood samples were taken before the administration of $^{99m}$Tc-Mebrofenin and at 1, 2, 3, 5, 10, 15, 20 and 30 minutes after injection. By measuring radioactivity in the blood samples, the percentage clearance at 15 minutes was calculated in the same way as the ICG clearance was.
For calculating regional liver uptake on hepatobiliary scintigraphy the global liver ROI was manually divided into two parts referring to the right margin of the aorta as the border between the right and left liver lobes.

**Blood plasma analysis**

Blood samples were collected for routine laboratory biochemistry of liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) and of total and conjugated bilirubin. Routine laboratory chemistry tests were performed to obtain values of albumin, prothrombin time (PTT), antithrombin III (AT III) and activated partial thromboplastin time (APTT).

**Statistical analysis**

Commercial computer package was used for analysis of the data (GraphPad Prism; GraphPad Software, San Diego, California, USA). The relationship between ICG clearance at 15 minutes and liver uptake of $^{99m}$Tc-Mebrofenin was tested using the standard Pearson correlation coefficient r. A p-value < 0.05 was considered significant.

**Results**

The mean ICG-C15 was 86.86% ± 1.19% (SEM) with a range of 58.51-98.32%. The mean $^{99m}$Tc-Mebrofenin clearance at 15 minutes was 83.70% ± 1.72% (n= 36, range 51.80-93.20% in 15 min). The mean $^{99m}$Tc-Mebrofenin uptake rate was 12.87 %/min. ± 0.52 (SEM) with range of 3.2- 24.4 %/min. The ICG-C15 value and the $^{99m}$Tc-Mebrofenin clearance rate in blood in 15 min showed a significant, positive correlation (r = 0.81, P < 0.0001, Figure 1).
A significant, positive correlation was obtained between the $^{99m}$Tc-Mebrofenin clearance rate at 15 minutes and $^{99m}$Tc-Mebrofenin uptake by scintigraphy ($r = 0.76$, $P < 0.0001$, Figure 2). Also, the ICG-C15 value and the $^{99m}$Tc-Mebrofenin uptake (as evaluated by scintigraphy) showed a significant, positive correlation ($r = 0.73$, $P < 0.0001$, Figure 3).
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Figure 3
Correlation between ICG clearance at 15 minutes and $^{99m}$Tc-Mebrofenin uptake as measured by scintigraphy ($y = 0.2925x - 12.745; r = 0.73, P < 0.0001, N=54$).

In total, 26 measurements with ICG-C 15 above 90 % were found. Eleven patients had ICG-C15 values between 86 and 90 % and 18 patients had values lower than 86 %. The obtained correlation between ICG C-15 and AST was strong. No significant correlation was found between the ICG-C15 values and LDH, APTT and AT III.

Moderate correlation was obtained between the ICG-C15 values and conjugated bilirubin ($r = -0.49$), total bilirubin ($r = -0.53$), ALT ($r = -0.50$) and albumin ($r = -0.60$) in the blood (Table 2).

The median values for $^{99m}$Tc-Mebrofenin uptake in the patients with various liver malignancies are shown in figure 4. The median values for $^{99m}$Tc-Mebrofenin uptake were significantly different between patients in the non-parenchymal tumour (NPT) group (n=25) and patients with Klatskin tumours (n=20), as well as between patients in the NPT group and patients with hepatocellular carcinoma (HCC, n=9; p<0.05).

The mean $^{99m}$Tc-Mebrofenin clearance capacity of the right liver lobes (79.83% ± 10.80, range 47.75-95.97 % in 15 min) was larger than that of the left lobes (mean 20.24% ± 10.26, range 6.511 to 52.51 % in 15 min; figure 5). Atrophic right or left segments as was apparent from CT images, had values lower than the mean (data not shown).
ICG clearance compared with $^{99m}$Tc-Mebrofenin uptake

<table>
<thead>
<tr>
<th>ICG-C15.</th>
<th>n</th>
<th>Correlation coefficient $r$</th>
<th>Qualification of correlation*</th>
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<tbody>
<tr>
<td>ALT</td>
<td>54</td>
<td>-0.50</td>
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<tr>
<td>AST</td>
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<td>Total bilirubin</td>
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<td>Conjugated bilirubin</td>
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<td>PTT</td>
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<tr>
<td>AT III</td>
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Table 2
Correlations between the ICG-C15 and the plasma parameters for hepatocellular damage (ALT, AST, LDH), bile duct epithelium damage (ALP, GGT) or cholestasis (total and conjugated bilirubin) and hepatic synthetic function (albumin, APTT, PTT and AT III).
Abbreviation: ns; not significant.
n, number of patients evaluated.
* According to Swinscow, Statistics at square one. 9th ed. (1997)

Figure 4
Box-Whisker plot of median values for $^{99m}$Tc-Mebrofenin uptake in patients with non-parenchymal tumor (NPT), Klatskin tumor or hepatocellular carcinoma (HCC). Whiskers represent the min-max range of $^{99m}$Tc-Mebrofenin uptake, boxes represent the interquartile range (*, p<0.05 by Mann Whitney U test).
Figure 5
Box-Whisker plot of an estimation of median (range and interquartile range) values of $^{99m}$Tc-Mebrofenin uptake in left and right liver lobes expressed as percentages of total liver uptake. (***, p < 0.05 by Mann Whitney U test).
Discussion

Prevention of postoperative hepatic insufficiency is important for improvement of the outcome after partial liver resection in patients with malignant liver disease. Patients with hepatic insufficiency after major liver resection have low chances for survival. Therefore, adequate preoperative estimation of liver function potentially improves postoperative morbidity and mortality after major liver resections.

In literature, the results of ICG tests are expressed as ICG-R15, which describes the percent of retention in 15 minutes. It has been reported that the ICG-R15 value is a better indicator of liver function than the Child-Pugh classification in patients who underwent cardiac surgery. In these patients a high ICG-R15 correlated with a high rate of mortality. ICG retention of 14 percent at 15 minutes, equalling a clearance of 86 percent at 15 minutes, has been suggested as the safe limit for patients undergoing major liver resection. Clinical use of ICG has been associated with a few cases of serious adverse reactions including anaphylactic shock, hypertension, and urticaria. We did not encounter any of these complications during our study.

Several studies have been performed to estimate the preoperative hepatic functional reserve by making use of $^{99m}$Tc-diethylenetriamine-penta acetic acid-galactosyl-human serum albumin ($^{99m}$Tc-GSA) liver scintigraphy. $^{99m}$Tc-GSA is a novel liver scintigraphy agent that binds to the asialoglycoprotein receptor (ASGPR) on hepatocytes. It was found that the total counts and counts per unit hepatic volume based on $^{99m}$Tc-GSA in the entire liver significantly decreased in patients with more extensive liver disease. The disadvantages of $^{99m}$Tc-GSA are that it does not provide any information on hepatic excretory function. For the present study $^{99m}$Tc-Mebrofenin was used because it has high hepatic uptake and fast excretion and it has been shown to have a very low renal excretion. Moreover, it can be used at higher plasma bilirubin levels than previous derivatives due to a halogen atom on the phenyl ring. It has been demonstrated that uptake of ICG is competitively inhibited by conjugated and unconjugated bilirubin. In our study this is of less importance because most of the patients underwent decompression of the biliary tract by stenting to reduce serum bilirubin levels prior to surgery. The patients who were not decompressed showed bilirubin values within normal range.

From the ICG blood-time curve and $^{99m}$Tc-Mebrofenin levels in blood samples taken during scintigraphy, it was concluded that a mono exponential fit rendered a suitable mathematical model to describe the concentration of circulating radioactivity in the chosen
interval. The blood concentration curves of ICG and $^{99m}$Tc-Mebrofenin showed the same decline, suggesting the same hepatic uptake rate for ICG and $^{99m}$Tc-Mebrofenin. (Figure 6)

![Graph of blood concentration against time](image)

**Figure 6**
ICG (■) and $^{99m}$Tc-Mebrofenin (▲) curves in blood after bolus injection.

To compare clearance of ICG and $^{99m}$Tc-Mebrofenin, the clearance rate at 15 minutes from blood was calculated with the same mono exponential regression analysis. A strong positive correlation coefficient was seen, indicating that the clearance function of the liver was equally represented by both agents.

When the $^{99m}$Tc-Mebrofenin clearance rate at 15 minutes was compared with the uptake rate calculated by using the scintigraphy images, a strong positive correlation was seen. Also, a statistically significant, positive correlation was seen between $^{99m}$Tc-Mebrofenin liver uptake by scintigraphy and ICG-C15 levels. These findings indicate that both the blood clearance rate and liver uptake by scintigraphy of $^{99m}$Tc-Mebrofenin give a similar estimation of liver function.

The results of this study confirm that the standard tests for liver enzymes and bilirubin plasma levels are not appropriate for describing actual liver function in pre-operative patients.
When ICG-C15 was compared with the liver enzymes as ALT, ALP, poor correlation was found. Between the ICG-C15 and hepatic synthetic function tests, a correlation was observed for albumin and PTT, but not for APTT. Furthermore, the degree of improvement in routine liver transaminase levels was not able to identify clinically improved or unimproved patients with alcoholic hepatitis.

It is known that unaffected liver segments may retain good function and that liver function in cancer-bearing segments tends to be decreased when compared with that of non-cancerous segments. Thus, although liver enzymes may be increased due to regional hepatocellular damage, other liver lobes may compensate and establish good liver function. The correlation coefficient between ICG-C15 and ALT plasma values was lower than between ICG-C15 and AST plasma levels. The discrepancy between the correlations of these transaminases with ICG-C15 can be explained by the localisation of the enzymes within hepatocytes. ALT is localised in the cytosol, whereas AST is found for 80% in mitochondria. Mitochondria are the site of oxidative phosphorylation and are specialised in the production of cellular ATP. A study showed that the hepatic ATP level is reflected by the amount of ICG and excretion of ICG in bile. Severe damage of hepatocytes leads to damage of mitochondria as reflected by the increase of AST in blood, and due to lack of ATP, a decrease of ICG clearance results.

In this study, patients with colorectal metastases (including other liver malignancies in the non-parenchymal tumour (NPT) group) showed a better $^{99m}$Tc-Mebrofenin uptake in liver than patients with HCC or patients with a Klatskin tumour. Hepatic changes develop very rapidly after bile duct obstruction. Hepatocyte function can decrease in late stages of biliary obstruction, as is the case in many Klatskin tumour patients, resulting in secondary decline of hepatocyte extraction. In long-standing cases biliary cirrhosis develops which in part explains the decreased $^{99m}$Tc-Mebrofenin uptake seen in this patient group. An advantage of hepatobiliary scintigraphy is that affected areas of the liver can be seen as not properly visualized areas on the dynamic images obtained during scintigraphy.

Another main advantage of scintigraphy over ICG clearance studies is the additional information that comes with the obtained dynamic images, e.g. visualisation of bile pooling in ducts of patients with cholestasis and heterogeneous uptake of the radiopharmaceutical agent in the presence of liver disease. Moreover, the images may be valuable in separating primary biliary from primary hepatocyte disease.
Hepatobiliary scintigraphy has high diagnostic sensitivity in the diagnosis of biliary obstruction during long-term follow-up after curative hepatic resection with biliary-enteric anastomosis. It has proven especially helpful in diagnosis of segmental biliary obstruction. Patients with biliary obstruction involving one or two segments may present with normal or minimally deviating liver function clearance tests. The rest of the liver may function normally. Scintigraphy allows distinguishing bile pooling in the uptake-image of the corresponding segment. In our study the dynamic images in some of the patients with segmental biliary obstruction showed clearly which parts were affected (figure 7).
ICG clearance compared with $^{99m}$Tc-Mebrofenin uptake

**Figure 7**
Dynamic hepatobiliary scintigraphy with 85 Mbq $^{99m}$Tc-Mebrofenin. Sequential 5 minutes images starting at 15 minutes post-injection. The images show homogenous tracer distribution in the liver (panel A), with excretion into large bile ducts and subsequently, gallbladder and bowel. In segment 7 and 8 stasis of tracer is visualized (panel C-I), corresponding with bile duct obstruction.

In our study, for calculating function of the left- and right liver lobes as a percentage of total liver uptake, the right margin of the aorta was taken as the border. This choice was based on the appearance of the aorta in the first dynamic images. In truth, the border is defined by the inferior vena cava. Scintigraphy showed that the right liver lobes had a higher uptake function in comparison with the left lobes. This is in accordance with the larger liver volume of the right liver lobes. It should be noted that the true border between the right and left liver lobes (i.e. the division of segments 5 and 8 on the right side and segment 4 on the left side) corresponds with the caval vein, lying right of the aorta, accounting for an underestimation of function of the left liver.

Affected uptake activity of liver segments could be detected by decreased function percentage. Affected liver segments showed decreased uptake activity when compared to the mean value. Livers with atrophy of the right lobes due to obstruction of the right portal vein as a result of tumorous ingrowth, showed a decrease in $^{99m}$Tc-Mebrofenin uptake when compared to non-atrophic livers. Cirrhosis present in particular segments of the liver showed decreased activity (figure 8). Other studies showed that in diseases like cirrhosis the hepatic extraction, i.e. the portion of the radiopharmaceutical removed during each circulatory pass from plasma, decreases $^{22}$.

Following the comparison between ICG-C15 and $^{99m}$Tc-Mebrofenin uptake by scintigraphy, an uptake rate of 12.6 %/minute could be regarded as the safe limit. It should be noted, that this is a theoretically calculated cut off value.

**Conclusion**
These data show that $^{99m}$Tc-Mebrofenin uptake rate as assessed by scintigraphy is a valid method for determining liver function and correlates well with ICG clearance. Additional morphological information obtained from hepatobiliary scintigraphy, provides valuable information on localisation of liver segments with inferior function and/or bile pooling in obstructed liver segments. Plasma levels of liver enzymes, bilirubin and tests that measure hepatic synthetic function do not correlate well with ICG clearance and are therefore less suitable for assessment of global liver function in patients in need of a liver resection.
Figure 8
Dynamic hepatobiliary scintigraphy with 85 Mbq $^{99m}$Tc-Mebrofenin. A decreased uptake is depicted in segment 7 and 8 (panel A-I). Gallbladder has been marked out for image quality purposes.
ICG clearance compared with $^{99m}$Tc-Mebrofenin uptake

References


ICG clearance compared with $^{99m}$Tc-Mebrofenin uptake


