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Postoperative assessment of donor's hepatic asialoglycoprotein receptor function using Tc-99m GSA scintigraphy in living donor liver transplantation

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Postoperative assessment of donor’s hepatic asialoglycoprotein receptor function using Tc-99m GSA scintigraphy in living donor liver transplantation

（Tc-99m GSA シンチグラフィを用いた生体肝移植ドナーにおける術後のアシアロ糖タンパク受容体機能評価に関する研究）
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本研究の一部は以下の論文に発表した。
1. Kentaro Kobayashi, Naoya Hattori, Osamu Manabe, Kenji Hirata, Keiichi Magota, Tsuyoshi Shimamura, Nagara Tamaki
   Postoperative Assessment of Hepatic Asialoglycoprotein Receptor Function with Tc-99m GSA: The Safety Margin of Resection Size in Living Donor Liver Transplantation
   Annals of Transplantation, in Press (2015年1月発行予定)

本研究の一部は以下の学会に発表した。
1. Kentaro Kobayashi, Naoya Hattori, Osamu Manabe, Kenji Hirata, Keiichi Magota, Tsuyoshi Shimamura, Nagara Tamaki
   GSA can predict future regeneration of resected liver.
   Society of Nuclear Medicine and Molecular Imaging 2013
   June 8-12, 2013, Vancouver, BC, Canada

2. 小林健太郎, 服部直也, 真鍋治, 平田健司, 孫田恵一, 嶋村剛, 玉木長良
   生体肝移植ドナーの肝切除後早期におけるTc-99m-GSAの有用性の検討
   第53回日本核医学会学術総会
   2013年11月8日-10日 福岡市

3. Kentaro Kobayashi, Naoya Hattori, Osamu Manabe, Kenji Hirata, Keiichi Magota, Tsuyoshi Shimamura, Nagara Tamaki
   Risk assessment of subclinical hepatic insufficiency immediately after partial hepatectomy in healthy living donors
   Society of Nuclear Medicine and Molecular Imaging 2014
   June 7-11, 2014, St. Louis, Missouri, USA
Background

Liver transplantation is the sole radical treatment for patients with severe liver failure. Liver transplantation needs a liver graft donated from either living or deceased donor with equivalent survival rates between two procedures\(^1\). In geographic regions where deceased donor rates are low, living donor liver transplantation improves survival from the time of listing compared to deceased donor liver transplantation\(^2\). However, adult living liver donation is associated with significant donor complications. Despite technical advances and considerable experience with liver resection at specialized centers, partial hepatectomy is still burdened by relatively high rates of postoperative morbidity (4.09–47.7\%) and mortality (0.24–9.7\%)\(^3\). A more cautious approach should be taken regarding use of this procedure, particularly in right-lobe living donor surgery as the right lobe has the larger volume than the left lobe\(^4\).

The rates of hepatocellular dysfunction and serious complications significantly increase as the volume of the resected liver increases\(^5\). In practice, however, recipient factors determine the size of the liver to be transplanted\(^6\). Therefore, the resectable size and its safety margin should be defined for the safety of donors undergoing partial hepatectomy. The volume of liver that can be safely resected is not well established, although several studies have suggested that the parenchymal hepatic resection rate measured by computed tomography (CT) is a significant predictor of postoperative liver failure.

In general, resection resulting in a 25\% volume of remnant liver or a volume of 250 mL/m\(^2\) was considered to be the safe limit for living human transplantation surgery\(^7,8\). Since experimental resection in humans is not possible, postsurgical risk assessment is a practical alternative to investigate the safety margin of the resected size for living human transplantation surgery.

Scintigraphy using technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin (Tc-99m GSA) is a widely used for in-vivo imaging to quantify liver function\(^9\). More specifically, Tc-99m GSA is a ligand to
asialoglycoprotein receptor (ASGPR) that is expressed on the cell membrane of hepatocyte. After intravenous injection, Tc-99m GSA quickly (within minutes) accumulates in the liver and thus its clearance from blood and its uptake in the liver reflect liver function. We performed Tc-99m GSA scintigraphy for the donors after the transplantation surgery and correlated its results with resected size and regeneration volume of the remnant liver.

The purpose of Chapter 1 was to determine if the current partial hepatectomy procedures are completed under the safety margin of the resectable size. We used the GSA scintigraphy results immediately (within 7 postoperative days) after the hepatectomy. I will demonstrate the results of analysis regarding the correlation between classical parameter of GSA clearance (HH15) vs. regeneration volume of the liver. To the best of our knowledge, this is the first imaging study to investigate postoperative ASGPR function immediately after partial hepatectomy in living human donors.

In Chapter 2, I will show the chronological data of liver volume and GSA scintigraphy that were taken at 1, 2, 4, 12 weeks after hepatectomy. In addition, I will introduce a new parameter to quantify ASGPR per unit volume of the liver. Using these data, more analyses of GSA uptake per unit volume will be presented and I will propose a hypothesis regarding liver regeneration potential.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>ASGPR</td>
<td>asialoglycoprotein receptor</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>BSA</td>
<td>body surface area</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CECT</td>
<td>contrast-enhanced x-ray computed tomography</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>GSK</td>
<td>glycogen synthesis kinase</td>
</tr>
<tr>
<td>GTP</td>
<td>glutamyltranspeptidase</td>
</tr>
<tr>
<td>HEC</td>
<td>hepatic endothelial cell</td>
</tr>
<tr>
<td>HGF</td>
<td>hepatocyte growth factor</td>
</tr>
<tr>
<td>HU</td>
<td>Hounsfield units</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>LV</td>
<td>liver volume</td>
</tr>
<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
</tr>
<tr>
<td>POW</td>
<td>postoperative week</td>
</tr>
<tr>
<td>POY</td>
<td>postoperative year</td>
</tr>
<tr>
<td>RDI</td>
<td>receptor density index</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SLV</td>
<td>standard liver volume</td>
</tr>
<tr>
<td>Tc-99m GSA</td>
<td>Technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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</tbody>
</table>
Chapter 1

Background

In Chapter 1, I will show data to determine if the current partial hepatectomy procedures are completed under the safety margin of the resectable size. For this purpose, GSA scintigraphy was performed immediately (within 7 postoperative days) after the hepatectomy. I will demonstrate the results of analysis regarding the correlation between classical parameter of GSA clearance (HH15) vs. regeneration volume of the liver.

Methods

Subjects and study design

The study population retrospectively included 84 living donors who underwent Tc-99m GSA scintigraphy after hepatectomy between March 2004 and December 2012. According to a standard institutional protocol, all of the donors underwent a postoperative evaluation using Tc-99m GSA scintigraphy and x-ray CT at 1 week after the hepatectomy. All donors who completed the required postoperative evaluation were included in the present study population, but the patients for whom an injection leakage of Tc-99m GSA (n=10) was suspected were excluded. The final study population consisted of a total of 74 donors (47 men and 27 women), and mean age of 35.4±10.9 years, ranging from 18 to 62 years old (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Left lobectomy</th>
<th>Lateral lobectomy</th>
<th>Right lobectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>46</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Age (years old)</td>
<td>37.7 ± 17</td>
<td>31.9 ± 3.7</td>
<td>31.6 ± 11.1</td>
</tr>
<tr>
<td>Sex (M : F)</td>
<td>34 : 12</td>
<td>1 : 10</td>
<td>12 : 5</td>
</tr>
</tbody>
</table>

Tc-99m GSA scintigraphy was scheduled 1 week after hepatectomy to
determine the clearance of GSA from the blood pool soon after the transplantation. We measured morphological liver volumes using contrast-enhanced x-ray computed tomography (CECT). The volume measurements were done 3 times: 1) before the operation, 2) one week after the operation, and 3) one year after the operation to evaluate the postoperative changes associated with partial hepatectomy. We analyzed the sizes of the resected livers in relation to GSA clearance and the regeneration volume of the donors’ residual liver. The Institutional Review Board at Hokkaido University Hospital, Sapporo, Japan approved this retrospective research and allowed the publication of the study results (#012-0032).

Operative procedures

Living donor liver transplantation was indicated for the treatment of cholestatic liver disease, chronic parenchymal liver disease (caused mainly by viral hepatitis), fulminant liver failure, metabolic liver disease, and hepatobiliary malignancy. Donors were selected according to the following criteria: second degree relation to or spouse of the recipient, 18 years old or older (to 65 years old), the donor’s blood type fit or agreed with the recipient’s blood type, the donors whose mind and body were healthy, the donors who were free from liver disease, and the donors whose intention to donate liver tissue was clear.

The surgical procedure was selected as follows. If the recipient was an adult (i.e., 18 years old or older), the liver graft was larger than 35% of the standard liver volume (SLV) calculated from the recipient’s body surface area (BSA) (SLV [mL]=706.2*BSA+2.4). If the recipient was a child, the liver graft was smaller than 150% of the SLV in addition to the condition mentioned above. There were 3 types of hepatectomy: left lobe hepatectomy (n=46), lateral lobe hepatectomy (n=11), and right lobe hepatectomy (n=17).

Liver volume measurements

Each CT examination was performed using 1 of the following 4 scanners:
the Somatom Volume Zoom + 4 (Siemens, Erlangen, Germany), the Sensation 64 (Siemens), the Aquilion Multi 64 (Toshiba Medical Systems, Tokyo), the LightSpeed VCT (GE Healthcare, Milwaukee, WI, USA), or the Aquilion ONE/ViSION Edition (Toshiba Medical Systems). All of the images were obtained during inspiration and covered the entire liver parenchyma. For the liver volume measurement, we used only contrast-enhanced CT images, which were obtained in either the dynamic or equilibrium-phase scanning manner. In the case of dynamic scanning, non-contrast-enhanced CT images were obtained first, and then repeated acquisition was conducted after a single intravenous (IV) bolus injection of 450 mgI/kg of nonionic iodocontrast media (iopamidol, iohexol, iomeprol, or ioversol) at a rate of 3.5 mL/s (adjusted according to the patient’s body weight) using a power injector (Nemoto Kyorindo Co., Tokyo).

Contrast-enhanced scanning of the liver in the arterial phase was initiated just after the density in the descending aorta reached 200 HU. The second scan (i.e., portal phase) covering the entire liver was initiated 15 seconds after the arterial phase, and the third scan (i.e., venous phase) covering from the chest to the pelvis was initiated 80 seconds after the injection of contrast medium. In cases of equilibrium-phase scanning, the images from the chest to the pelvis were acquired 80 seconds after the injection.

Each slice of liver was traced with a cursor, and the corresponding area and the morphological volume were calculated with the OsiriX application package (version 4.1.1, 32-bit; Pixmeo SARL, Geneva, Switzerland) installed in MacOS 10.6 (Figure 1)\textsuperscript{10,11}.

![Figure 1](image-url) 

Figure 1. Contrast-enhanced x-ray CT images of (A) Pre-hepatectomy, (B) one postoperative week, and (C) one postoperative year. Each slice of liver was manually contoured and integrated.
to calculate the liver volumes. This donor underwent right lobectomy of 545 mL in size, corresponding to 47.2% of the preoperative volume. One postoperative year CT (C) showed regeneration of the remnant liver to 99.5% of the preoperative volume.

The resection volume was calculated as the difference in liver volumes before and immediately after the operation, i.e., postoperative week (POW) 1. The regeneration volume was calculated as the difference in residual liver volume between POW 1 and postoperative year (POY) 1. The regenerated fraction was defined as the regenerated volume divided by the residual volume at POW 1, and the recovery fraction was defined as the residual volume at POY 1 divided by the preoperative original liver volume. In summary,

\[
\text{Regenerated fraction} = \frac{V_{1POY} - V_{1POW}}{V_{1POW}},
\]

\[
\text{Recovery fraction} = \frac{V_{1POY}}{V_{pre}},
\]

where \(V_{pre}\), \(V_{1POW}\), and \(V_{1POY}\) represent liver volumes before operation, 1 week after operation, and 1 year after operation, respectively.

**Measurements of liver function with Tc-99m GSA**

After a bolus IV injection of 185 MBq of Tc-99m GSA, dynamic scanning was performed with the patient in a supine position, using a large-field view gamma camera (E.CAM; Siemens Japan, Tokyo, Japan) in an anterior view equipped with a low-energy high-resolution collimator. The dynamic planar images were obtained for 30 min by 147 serial frames (60×1 s, 87×20 s) with a matrix size of 128×128. We estimated the hepatic ASGPR function with 2 established approaches. We calculated the blood clearance ratio of Tc-99m GSA using the radioactivity of the blood pool at the heart from 3 min to 15 min after the injection (HH15) (Figure 2).
Figure 2. Representative anterior planar images of Tc-99m GSA. (A) 3 min after injection. (B) 15 min after injection. This donor showed HH15 of 0.59 indicating mild reduced GSA clearance from the blood pool.

The quantitative results were used to investigate GSA clearance based on a previous study. We defined HH15 <0.55 as normal GSA clearance, and 0.55 ≤ HH15 <0.65 as mildly reduced clearance.

Statistical analysis

Data are expressed as mean ± SD. The changes in the donor liver volume and function were analyzed using a paired t test. P-values lower than 0.05 were considered significant. The correlations between the 2 continuous parameters were estimated using the Pearson product moment correlation coefficient. Comparison of continuous values between 3 or more groups were performed using ANOVA. All the statistical analyses were performed using JMP Pro 11.0.0 package (SAS Institute Inc., Cary, NC, USA).
Results

Operative procedures and resected sizes

The preoperative liver volume was 1204±245 mL, ranging from 817 to 1971 mL. The largest volumes were observed in the group of donors who underwent a left lobe lobectomy, followed by the right lobe lobectomy group and lateral lobe lobectomy group (p=0.0058, ANOVA). The average resected size was 337±170 mL, corresponding to 28±12% (range 5–54%) of the original liver volume. The resected size was largest in the right lobe lobectomy group (529±139 mL, range 284–769 mL), corresponding to 45±5% of the donors’ preoperative liver volume, whereas left lobe lobectomy and lateral lobe lobectomy resulted in relatively smaller resections. As a result, a donor who underwent a right lobe lobectomy showed the smallest residual volume at POW 1 (Table 2).

<table>
<thead>
<tr>
<th>Table2. Morphological liver volume of the donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operation</td>
</tr>
<tr>
<td>LV (mL)</td>
</tr>
<tr>
<td>Left lobectomy</td>
</tr>
<tr>
<td>Lateral lobectomy</td>
</tr>
<tr>
<td>Right lobectomy</td>
</tr>
</tbody>
</table>

The perioperative course was stable except for 6 donors who presented with minor complications, including pylethrombosis (n=2), pneumonia (n=1), wound infection (n=1), subcutaneous abscess (n=1), and drug-induced liver injury (n=1). All donors left the hospital in good conditions. Blood examinations showed temporal increases of hepatic enzyme at POW 1, which were normalized by POW 2 (Table 3).
**Table 3.** Perioperative blood test results

<table>
<thead>
<tr>
<th></th>
<th>Pre-operation</th>
<th>1 POW</th>
<th>2 POW</th>
<th>1POY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.0 ± 1.4</td>
<td>12.1 ± 1.3</td>
<td>12.1 ± 1.3</td>
<td>14.3 ± 1.6</td>
</tr>
<tr>
<td>Total-bilirubin (mg/dL)</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>19.0 ± 5.0</td>
<td>62.9 ± 36.1</td>
<td>36.5 ± 14.7</td>
<td>20.6 ± 5.3</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>17.0 ± 7.0</td>
<td>103.7 ± 44.5</td>
<td>64.0 ± 33.5</td>
<td>16.5 ± 6.5</td>
</tr>
<tr>
<td>LDH(U/L)</td>
<td>167.6 ± 25.1</td>
<td>232.8 ± 44.3</td>
<td>186.3 ± 32.2</td>
<td>168.2 ± 32.3</td>
</tr>
<tr>
<td>γ-GTP(U/L)</td>
<td>19.0 ± 11.1</td>
<td>72.1 ± 41.9</td>
<td>69.7 ± 48.3</td>
<td>25.3 ± 30.5</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.0 ± 0.1</td>
<td>2.0 ± 2.7</td>
<td>0.4 ± 0.5</td>
<td>0.2 ± 0.6</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.6 ± 0.3</td>
<td>3.6 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Prothrombin time (sec)</td>
<td>11.4 ± 0.6</td>
<td>12.4 ± 0.8</td>
<td>12.0 ± 0.8</td>
<td>11.7 ± 0.6</td>
</tr>
<tr>
<td>Platelet (×10^9/L)</td>
<td>238.1 ± 45.7</td>
<td>235.0 ± 82.6</td>
<td>319.5 ± 80.7</td>
<td>200.6 ± 44.4</td>
</tr>
</tbody>
</table>

**Tc-99m GSA**

Overall, HH15 at POW 1 was 0.47 ± 0.06, ranging from 0.35 to 0.64. None of the donors showed HH15 greater than 0.65 to suggest moderate or severe delayed clearance, or clinical postoperative hepatic insufficiency. However, larger resection size was positively associated with higher HH15, suggesting a size-dependent delayed GSA clearance (R=0.53, p<0.001). In the present study population, mildly reduced GSA clearance (0.55 ≤ HH15 <0.65) was observed in 7 of the 19 (37%) donors who underwent larger resection (resected fraction ≥35%). In contrast, only 2 of the 55 (4%) donors who underwent smaller resection (<35%) showed mild reduced clearance (Figure 3).
Follow-up measurements demonstrated regeneration of the remnant liver (Figure 4). The trend was particularly clear in the donors who underwent larger partial resections, which showed a trend of positive correlation between the resected fraction and regenerated fraction (R=0.65, p=0.002). At POY 1, their remnant livers recovered the volume to reach 82.7±8.6% of the preoperative volume (ranging from 68.1% to 99.5%). The recovery fraction was not significantly associated with HH15 (Figure 5) in the present study population.
Figure 4. Scatter plot of remnant regeneration vs. resected fraction.

Figure 5. Scatter plot of remnant regeneration vs. GSA clearance (HH15).
Discussion

Although the present results confirmed the safety of the partial hepatectomy of less than 54% of donors’ original liver volume, it demonstrated reduced GSA clearance, indicating an increased risk of postoperative liver failure in a subgroup of the living donors. The reduced GSA clearance function was associated with larger resection size (≥ 35% of the original liver volume), and this may warrant careful postoperative attention to prevent complications for donors undergoing larger partial hepatectomy. Among the 74 donors included in the present study, the postoperative reduced GSA clearance (i.e., high HH15) was not associated with poor prognosis, indicating that all procedures were done within the safety margin of partial hepatectomy.

Adult living liver donation is often associated with significant donor complications. Although most complications have been of low-grade severity, a significant proportion has been severe or life-threatening, including postoperative liver failure. Previous investigations reported incidences of liver failure after hepatectomy of 0.70–33.83%, and that the failure was related to inadequate residual liver tissue and functional capacity. In practice, recipient factors determine the size of the liver to be transplanted. Therefore, resectable size and its safety margin should be defined for the safety of the donors undergoing partial hepatectomy. Postoperative assessments of functional capacity may therefore serve as a practical approach to investigate the safety margin of resected size for living human transplantation where experimental resection is impossible. Precise assessments of postoperative risk may improve the informed consent process, perioperative planning, and donor care.

In the present study, higher postoperative risk was associated with larger resection size (≥ 35% of original liver volume). In general, right lobectomy is assumed to have a higher risk than left lobectomy due to larger resected size. It was also shown that morbidity and complications are significantly higher after right lobectomy (25%) compared to left or lateral lobe lobectomy (10%). However, there is still debate regarding the risk of right versus left lobectomy.
recent large multicenter study in Japan examined 3565 right and left lobe liver donations and reported similar morbidity rates after right lobe donation (9.4%) and left lobe donation (8.7%)\textsuperscript{20}. Since the prevention of liver failure is more important than treatment, careful preventive measures, including preoperative assessments of the liver’s functional reserve and the prevention of intraoperative bleeding and other perioperative complications, may compensate for the size-dependent risk at experienced institutions\textsuperscript{3}. The present results showed mildly reduced GSA clearance in donors who underwent larger resection. This may indicate the need for careful postoperative attention to prevent significant life-threatening complications.

In the present study, impaired postoperative ASGPR function (i.e., prolonged Tc-99m GSA clearance) did not predict the final outcome. All donors examined in the present study showed size-dependent regeneration of the liver. The recovery after POY 1 achieved 85.5±8.8% of the original liver, which is comparable to previously reported regenerated volumes\textsuperscript{21,22}. The resected size did not affect the final outcome, probably because the resected sizes in the present study were all under the safety margin of the partial hepatectomy. We also observed 8 donors who did not show regeneration of the residual liver, although they did not present with significant postoperative complications or prolonged Tc-99m GSA clearance. Their resected sizes were relatively smaller (18.7±7.7%, range from 4.6 to 26.2%), and thus the regeneration capacity might not be stimulated due to sufficient functional reserve in the remnant.

In the present study population, Tc-99m GSA scintigraphy was scheduled at POW 1 for all patients. We propose that the risk assessment of postoperative liver failure should be done immediately after the operation because of the relatively common perioperative complications associated with the postoperative risk of liver failure\textsuperscript{3}. In fact, postoperative liver failure is known to occur as early as postoperative day 5\textsuperscript{23}. Although postoperative liver failure may induce temporal hyperbilirubinemia, this would not affect the present results because the clearance
and uptake of Tc-99m GSA are known to be independent from hyperbilirubinemia\textsuperscript{24}. To the best of our knowledge, the present study is the first investigation of ASGPR function immediately after partial hepatectomy.

The major limitation of the present study is the lack of measurements of Tc-99m GSA clearance before surgery. Our healthcare insurance policy in Japan limits the use of Tc-99m GSA scintigraphy to healthy subjects. Preoperative assessments would allow the determination of the functional liver reserve, which may be correlated with the postoperative outcome. Clinically, however, risk assessment immediately after the operation may have more value, considering the relatively higher rate of perioperative complications.

Another limitation was the overlap of quantitative parameters. We used a cut-off value of HH15 >0.55 as the index of mildly impaired ASGPR function. This cut-off value corresponds to the lower limit of patients with impaired ASGPR function\textsuperscript{12} and thus may include false-positives in terms of diagnosing ASGPR dysfunction. However, our study was designed to investigate the safety margin, where sensitivity is considered to be more important than specificity.

\textbf{Conclusion of Chapter 1}

Liver dysfunction defined by GSA clearance was observed in 7/19 (37%) of larger resection group (≥35%) and in only 2/55 (4%) of smaller resection group. Thus, we suggest that 35% may be the cut-off of safe hepatectomy.
Chapter 2

Background

In Chapter 1, I showed that the donors who received larger resection tended to have impaired GSA clearance from blood, indicating impaired liver function, at POW-1. On the other hand, the donors who received larger resection often present larger volume recovery (Figure 4 in Chapter 1). As described in Chapter 1, HH15 is a ratio of the heart count at 15 minutes over the heart count at 3 minutes, which originally means the GSA clearance from blood. Although HH15 has been known to be a good parameter to evaluate liver function (or dysfunction), HH15 represents the function of the whole liver. Because of the different sizes of residual liver by different surgical procedures, the parameter may need to be normalized to the volume in order to estimate liver function per unit volume. We hypothesized that this new parameter may reflect the activity of each hepatocyte and thus may be associated with the liver regenerated volume or fraction one year after the operation.

After 2006, we started to record the pre- and post-injection injector counts in order to measure the uptake fraction in the liver (i.e., %uptake of injected dosage). In Chapter 2, I will explain the new parameter we propose, and then will show its effect on liver regenerated fraction.

Methods

Subjects and study design

In this retrospective study, we reviewed the medical records of 45 living donors of the liver who underwent Tc-99m GSA scintigraphy 4 times (i.e., 1, 2, 4, and 12 weeks) after hepatectomy between April 2006 and December 2012. The donors’ characteristics are summarized in Table 4. Note that this study subjects were all included in the study population of 74 patients in Chapter 1. Because we did not take the injector images before April 2006, we could not calculate the new parameter for the old population.
Table 4. Characteristics of the 45 living human liver transplantation donors

<table>
<thead>
<tr>
<th>Operation procedure</th>
<th>Left lobectomy</th>
<th>Lateral lobectomy</th>
<th>Right lobectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 ± 12</td>
<td>31 ± 2.7</td>
<td>29 ± 10</td>
</tr>
<tr>
<td>Sex (M : F)</td>
<td>23 : 5</td>
<td>1 : 7</td>
<td>5 : 4</td>
</tr>
</tbody>
</table>

*Estimating ASGPR receptor density with Tc-99m GSA*

For this study population, GSA scintigraphy was performed four times after operation, including 1, 2, 4, and 12 POW. We first calculated liver uptake at 15 minutes (LU15) as follows. As planar images of scintigraphy are affected by photon attenuation in soft tissue, the tracer accumulated farther from the gamma camera is underestimated. This degrades the quantification of tracer uptake. Thus, to minimize the absorption effects, LU15 was calculated based on a geometric mean manner. More specifically, GSA scintigram was acquired with both anterior and posterior detectors. The corrected liver count was calculated as the square root of the product of anterior and posterior liver counts. Thus, LU15 is expressed by this formula.

\[
LU15 = \frac{\sqrt{A \cdot P}}{D_{\text{pre}} - D_{\text{post}}}
\]

where A is the liver count acquired from the anterior detector, P is the liver count acquired from the posterior detector, and \(D_{\text{pre}}\) and \(D_{\text{post}}\) are pre- and post-injection injector counts. LU15 is a unitless number that can theoretically range from 0 to 1. LU15 has been introduced by Koizumi and its standard value from healthy subjects has been reported to be 31.4±4.2%(range 24.7-37.0%)\(^2\)

Because our interest here is ASGPR function per unit volume of the liver, we introduced a new parameter or receptor density index (RDI, ml\(^{-1}\)) as follows:

\[
\text{RDI} = \frac{LU15}{V}
\]

where V is the liver volume (ml) measured with CT. The unit of RDI is thus ml\(^{-1}\.

Figure 6 demonstrates example anterior and posterior images of the liver (Figure 6A, B) and pre- and post-injection injector images (Figure 6C, D).
Figure 6. Representative anterior planar images of Tc-99m GSA. A: 15 min after injection. B: syringe count before (left) and after (right) injection. LU15 of this donor was calculated as 0.40.

Operative procedures

Operative procedures included left lobectomy, lateral lobectomy, and right lobectomy. For more detail regarding donor criteria and procedure selection criteria, please see Chapter 1.

Liver volume measurements

Contrast-enhanced CT was performed repeatedly (i.e., before, 1-POW,
2-POW, 4-POW, 12-POW, and 1-POY after the surgical procedure). The abdominal CT images were used to measure the liver volume by manually tracing the liver edge, as described in Chapter 1.

**Statistical analysis**

Data are expressed as mean ± SD. The changes in the donor liver volume and function were analyzed with a paired t test. Difference in continuous values among two or more categories was assessed with ANOVA. In cases that the ANOVA results were statistically significant, the Tukey-Kramer method was used to identify the combinations showing significant differences. P-values less than 0.05 were considered significant. The correlations between the 2 continuous values were analyzed using the Pearson product moment correlation coefficient. All the statistical analyses were performed using JMP Pro 11.0.0 package (SAS Institute Inc., Cary, NC, USA).
Results

Time course of morphological liver volume

Figure 7 shows morphological liver volume changes over time based on CT grouped by operative procedures. Left lobectomy group showed a slow increase in liver volume (1-POW vs. 2-POW, P=NS; 2-POW vs. 4-POW, P<0.05; 4-POW vs. 12-POW, P=NS; 4-POW vs. 12-POW, P<0.0001). Lateral lobectomy group showed no significant volume change until 4-POW, but slight decrease at 12-POW, followed by slight increase at 1-POY (1-POW vs. 2-POW, P=NS; 2-POW vs. 4-POW, P=NS; 4-POW vs. 12-POW, P<0.01; 12-POW vs. 1-POY, P<0.001, 1-POW vs. 1-POY, P<0.05). In contrast, right lobectomy group showed continuous increase in liver volume (1-POW vs. 2-POW, P<0.01; 2-POW vs. 4-POW, P<0.05; 4-POW vs. 12-POW, P=NS; 12-POW vs. 1-POY, P<0.01). Regenerated fraction, defined as \( \frac{(V_{1POY} - V_{1POW})}{V_{1POW}} \), was higher for right lobectomy group (60.6 ± 19.7 %) than for left lobectomy group (11.3 ± 11.0 %, P<0.0001) or lateral lobectomy group (9.3 ± 9.2 %, P<0.0001). No significant difference in liver volume was observed among three groups at 1-POY (ANOVA, P=NS).

Figure 7. Morphological liver volume changes before and after hepatectomy.
Time course of RDI

At 1-POW, RDI was significantly higher in right lobectomy group (0.00058 ± 0.00014 ml⁻¹) than in left lobectomy group (0.00027 ± 0.00007 ml⁻¹, P<0.0001) and lateral lobectomy group (0.00033 ± 0.00009 ml⁻¹, P<0.0001), indicating that right lobectomy group had the highest liver function per unit volume among three groups at 1-POW (Figure 8). Right lobectomy group then started to decrease in RDI until 2-POW and remained stable after 2-POW (1-POW vs. 2-POW, P<0.05; 2-POW vs. 4-POW, P=NS; 4-POW vs. 12-POW, P=NS). Similar but less-steep trends were observed in the other groups (left lobectomy and lateral lobectomy groups, 1-POW vs. 2-POW, P<0.05; 2-POW vs. 4-POW, P=NS; 4-POW vs. 12-POW, P=NS). These results indicated that the most significant change in liver function per unit volume occurred in acute-phase (1~2 weeks).

Figure 8. Receptor density index (RDI) changes before and after hepatectomy. R, L, and Lat express right lobectomy, left lobectomy, and lateral lobectomy, respectively.
**RDI and regenerated fraction**

Correlation analysis was done between each time-point RDI vs. regenerated fraction (Figure 9). Among RDIs at different time points, RDI at 1-POW was found to be most strongly associated with regenerated fraction ($R=0.71$, $P<0.0001$), which was followed by RDI at 2-POW ($R=0.66$, $P<0.0001$), RDI at 12-POW ($R=0.60$, $P<0.0001$), and RDI at 4-POW ($R=0.52$, $P<0.0001$).

Figure 9. Correlations between each time-point RDI and regenerated fraction. (A) 1 post-operative week (POW), (B) 2 POW, (C) 4 POW, and (D) 12 POW.
Discussion

We found that donors who underwent larger resection had a larger RDI. In addition, a larger RDI was associated with larger regenerated fraction of the liver. Since RDI is considered to represent ASGPR function per unit volume, these results suggested that the ASGPR function per unit volume is upregulated after heptectomy. Also, the liver that has greater potential of proliferation has more active ASGRP receptor function per unit volume.

One of the advantages of nuclear imaging using Tc-99m-GSA is capability of quantification as well as qualitative assessment. Several indices have been introduced by researchers, such as HH15, LHL15, LU15, and more complicated parameters based on mathematical models and compartment analysis. Theoretically, compartment analysis can implement molecular mechanisms of GSA uptake and is able to more accurately quantify the ASGPR binding potential (e.g., $R_{\text{max}}$, tot) $^{28-31}$ In spite of accuracy, these complicated methods have been hardly used for routine clinical examinations because some require invasive arterial input function and some need dynamic SPECT acquisition.

LHL15 is a simple index that requires only planar scintigraphy. LHL15 represents liver uptake ratio to blood pool as follows.

$$LHL15 = \frac{L15}{H15 + L15}$$

where H15 and L15 represent heart and liver count, respectively, at 15 minutes after GSA injection. LHL15 has some disadvantages, however. LHL15 has significantly overlapped ranges between normal (0.942±0.017, mean±SD) and mildly impaired (0.909±0.044) liver function$^{12}$, which makes it difficult to distinguish these two. Heart is different in size among patients, which also degrades the accuracy of LHL15. In addition, inter-operator variability cannot be ignored for LHL15, while HH15 is less variable (i.e., ROI independent) because HH15 uses same ROI of the heart for 3-minute and 15-minute images. Thus, HH15 has been most frequently used in clinical settings. HH15 is a clearance index, where high HH15 corresponds to low clearance. By this nature, HH15 cannot be
simply normalized to liver volume, although we were interested in the ASGPR function per unit volume.

LU15 represents the amount of liver uptake at 15 minutes normalized to injected dosage of Tc-99m-GSA. LU15 is built on a similar idea of LHL15, but LU15 has been considered to be superior to LHL15\textsuperscript{32}. LU15 can be theoretically normalized to (i.e., divided by) the liver volume to create our original parameter RDI to estimate ASGPR function per unit volume of the liver. In the current study, we found that RDI was significantly associated with the liver regeneration potential, implying that RDI may reflect the physiological reactions occurring in hepatocytes in regeneration process against partial liver loss.

Because the current investigation was just a correlation analysis, the causal relationship is still unknown. In the orchestrated process of liver regeneration, little is known about the role of ASGRP. Many molecules, including interleukin (IL)-1, -6, tumor necrosis factor (TNF)-\(\alpha\), and hepatocyte growth factor (HGF), and many cells, including Kupffer cells, hepatic stellate cells and hepatic endothelial cells (HEC)\textsuperscript{33}, are known to be involved in liver regeneration process. Kupffer cells that are stimulated by TNF-\(\alpha\) and acetylcholine secret cytokines such as IL-6, TNF-\(\alpha\); these cytokines activate neighbor hepatocytes (paracrine) as well as Kupffer cells themselves (autocrine)\textsuperscript{34}. Hepatic stellate cells and hepatic endothelial cells are also stimulated after hepatectomy and they secret HGF, which promotes hepatic regeneration\textsuperscript{35}.

More recently, Sekiya et al. reported the important roles of Snail in controlling liver regeneration\textsuperscript{36}. Snail, a zinc-finger transcription factor, is activated by phosphorylation by glycogen synthesis kinase (GSK)-3\(\beta\) that is activated by HGF. In normal healthy liver, Snail suppresses hepatocyte proliferation. Once hepatectomy is performed, decrease in Snail results in elevated DNA synthesis in hepatocytes regarding liver regeneration. Finally, the liver recovers in size and Snail amount returns to normal level; the liver proliferation is inactivated.
It is known that HGF increases hepatic 99mTc-GSA uptake in a rodent model\textsuperscript{37}. Nakaya et al. described that IL-1 and 6 promoted ASGPR synthesis\textsuperscript{38}. Thus, it is reasonable that liver regeneration can be visualized indirectly and non-invasively by measuring ASGPR function using Tc-99m GSA scintigraphy.

We demonstrated that RDI is the parameter that can predict liver regeneration potential after hepatectomy of healthy donors. In clinical settings, however, it would be more useful if RDI can provide the information for patients. For example, the patients who undergo hepatectomy because of liver tumor would benefit by this prediction. The recipients of liver transplantation also need the information of liver regeneration. Further studies are needed to test the performance of GSA scintigraphy for these patient groups.

As a preclinical study, we think it is interesting to perform experimental liver resection for animal model and to correlate the exhaustive analysis of gene/protein expression vs. GSA uptake in the liver tissues. That investigation may help understand the entire process of liver regeneration.

**Conclusion of Chapter 2**

Donors who underwent larger resection had a larger RDI, which was associated with larger regenerated fraction of the liver. Estimating ASGPR function using Tc-99m-GSA and RDI may predict the liver regeneration potential.
Conclusions

In Chapter 1, I demonstrated postoperative reduced GSA clearance in a subgroup of living donors who underwent larger partial resection (≥35% of the original liver volume). In contrast, smaller resection (<35% of original liver volume) was considered to be under the safety margin of the hepatectomy.

In Chapter 2, I reported the usefulness of our original index of RDI, which was associated with larger regenerated fraction after hepatectomy. The data suggested that ASGPR function measured using Tc-99m-GSA and RDI may predict the liver regeneration potential. Not only donors, but also recipients and patients with hepatectomy would benefit by this prediction.
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