

## Evaluation of Liver Function Parameters by Tc-99m-GSA Using Multivariate Analysis: A Study of 47 Clinical Cases

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To investigate the correlation between nuclear medicine parameters determined by technetium-99m-DTPA-galactosyl-human serum albumin (Tc-99m-GSA) and liver function tests, canonical correlation analysis was performed. Tc-99m-GSA studies were performed on 47 patients with hepatocellular carcinoma (HCC) who had undergone transcatheter arterial embolization (TAE).

The nuclear medicine parameters LU15, HH15 and LHL15, which are results of nuclear imaging tests, were chosen in combination with the following liver function tests: the serum bilirubin level (T.Bil), the serum albumin level (Alb), serum cholinesterase activity (Ch-E), the clearance rate of indocyanine green (KICG), the hepaplastin test (HPT) and the prothrombin time (PT). The canonical correlation coefficient was 0.7345 and the upper tail probability was 0.00167. A significant correlation was observed between the two sets of variables.

The high structural coefficients of Ch-E, KICG and HPT indicated a close relationship with the nuclear medicine parameters, supporting the notion that these nuclear medicine parameters are useful for the estimation of liver damage. The structural coefficients of the nuclear medicine parameters were also high, with LU15 being a parameter as useful as both HH15 and LHL15. T.Bil may evaluate a liver function that is not measured by nuclear imaging techniques, so we should take T.Bil results into account before considering TAE.

**Key words:** Technetium-99m-DTPA-galactosyl-human serum albumin, hepatocellular carcinoma, transcatheter arterial embolization, multivariate analysis

**T** echnetium-99m-DTPA-galactosyl-human serum albumin (Tc-99m-GSA, Nihon Medi-Physics, Nishinomiya, Japan) is one of the newly synthesized neoglycoalbumins for clinical hepatic imaging. Due to properties such as adherence, it binds to asialoglycoprotein (ASGP) receptors on the hepatocyte membrane. These receptors are involved in the metabolism of serum glycoproteins and are located exclusively on the plasma membrane of mammalian hepatocytes, where they recognize and bind galactose-terminating glycoproteins by second-order kinetics (receptor-mediated endocytosis) (1, 2). The number of these receptors is decreased in patients with chronic liver damage, which in turn causes an increase in the serum ASGP levels in such patients (3). Hepatic imaging with Tc-99m-GSA reveals the level of ASGP receptor binding activity, and such images give an accurate indication of the state of liver function.

Several studies have already assessed the feasibility of evaluating liver function using Tc-99m-GSA, comparing the results with standard laboratory tests, but most of these studies employed simple regression analysis of a single nuclear medicine parameter calculated from the results of nuclear imaging tests and a single liver function test (4, 5).

So far, no study has evaluated these 2 kinds of liver function parameters as a group. Accordingly, we performed this study to evaluate the correlation between a group of nuclear medicine parameters and a group of liver function tests using canonical correlation analysis.

### Patients and Methods

**Patients.** Tc-99m-GSA studies were performed on 47 patients (37 men and 10 women aged 48-82 years,

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mean age: 65.3 years) with hepatocellular carcinoma (HCC) who had undergone transcatheter arterial embolization (TAE). Six patients had chronic hepatitis and 41 had liver cirrhosis as the underlying disease. The clinical stage was classified according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer (6), with 15 being in stage I, 30 in stage II, and 2 in stage III.

**Tc-99m-GSA study.** After a bolus intravenous injection of 185 MBq of Tc-99m-GSA, the dynamic study was performed with the patient in a supine position under a large field-of-view gamma camera (Picker International, Inc., Prism-2000, Cleveland, OH, USA) with a low-energy high-resolution collimator. The digital images obtained ( $128 \times 128$  matrix) were transferred to an on-line nuclear data processor (Picker International, Inc., Odyssey) at 30 sec/frame for 30 min after injection. Time-activity curves for both the heart and liver were generated from regions-of-interest (ROI) set over the whole liver and heart. The following parameters were calculated from the time-activity curves (4, 7):

LU15: Cumulative liver uptake of the tracer from 15 to 16 min after injection.

$$\text{LU15} = \frac{\int_{15}^{16} L(t) dt}{\text{total injected dose}} \times 100 (\%)$$

HH15: Parameter representing retention of the tracer in the blood.

$$\text{HH15} = \frac{\text{count for the heart at 15 min}}{\text{count for the heart at 3 min}}$$

LHL15: Parameter representing uptake of the tracer in the liver.

$$\text{LHL15} = \frac{\text{count for the liver at 15 min}}{\text{sum of the counts for heart and liver at 15 min}}$$

**Statistical analysis.** We examined the relationships between tumor factors according to the TNM clinical classification and the nuclear medicine parameters using the Kruskal-Wallis test.

Correlations between the nuclear medicine parameters and liver function tests were investigated using Pearson's correlation analysis. A *P* value of less than 0.0001 was considered significant.

The explanatory variables (first variable group) comprised the following biochemical liver function tests: a) the serum bilirubin level (T.Bil) (mg/dl), b) the serum albumin level (Alb) (g/dl), c) serum cholinesterase activity (Ch-E) ( $\Delta\text{pH}$ ), d) the clearance rate of indocyanine green (KICG) ( $\text{min}^{-1}$ ), e) the hepaplastin test (HPT) (%)

and f) the prothrombin time (PT) (activity %). The criterion variables (second variable group) comprised the following nuclear medicine parameters: a) LU15, b) HH15 and c) LHL15. These variables were measured within two weeks prior to TAE.

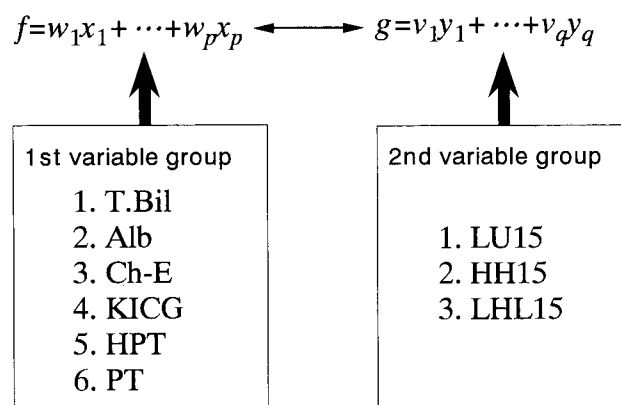
Canonical correlation analysis was used to compare the set of nuclear medicine parameters and the set of liver function tests. Canonical correlation analysis is a method used to determine the relationship between explanatory variables and criterion variables.

The canonical correlation coefficient represents the maximum correlation coefficient between two linear combinations of variables (Fig. 1). This analysis not only gives an estimation of the relationship between two sets of variables, but also can be used to assess the contribution of each variable to the other set of variables by determining the structural coefficient.

We conducted the analysis using the statistical software Stat View ver. 4.11 (Abacus Concepts, Inc., Berkeley, CA, USA) and HALBAU (High-quality Analysis Library for Business and Academic Users) ver. 3.0 (Gendaisugakusha, Kyoto, Japan).

## Results

Box plots of the nuclear medicine parameters (LU15, HH15 and LHL15) are shown in Fig. 2 (a, b and c). Based on the HH15 and LHL15 data, most of the



**Fig. 1** Canonical correlation analysis is a method to find the relationship between 2 sets of variables. The canonical correlation coefficient is the maximum correlation coefficient between 2 linear combinations of variable.

T.Bil: Serum bilirubin level; Alb: Serum albumin level; Ch-E: Serum cholinesterase activity; KICG: Clearance rate of indocyanine green; HPT: Hepaplastin test; PT: Prothrombin time.

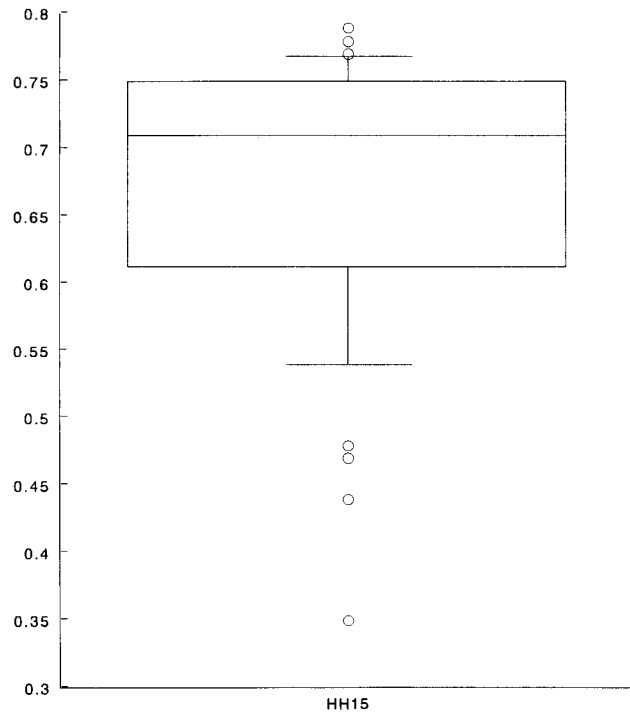
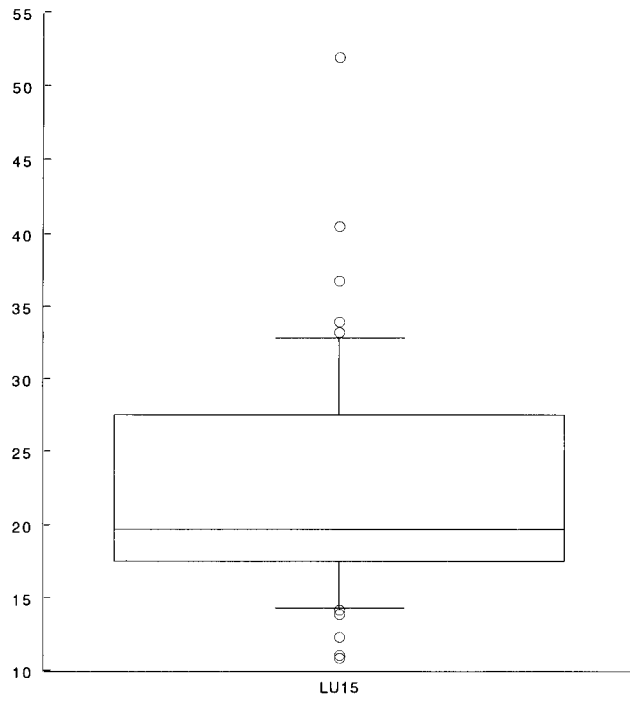


Fig. 2(a) Box plot of LU15

Fig. 2(b) Box plot of HH15

Fig. 2(c) Box plot of LHL15

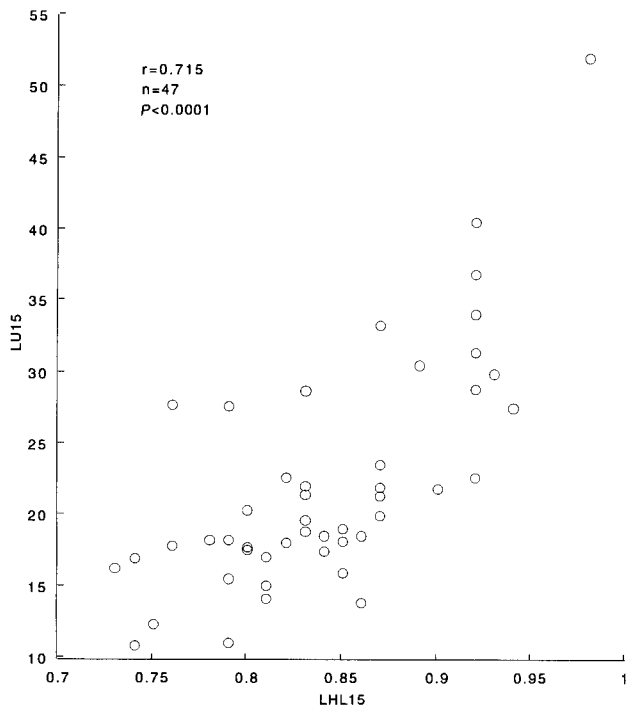
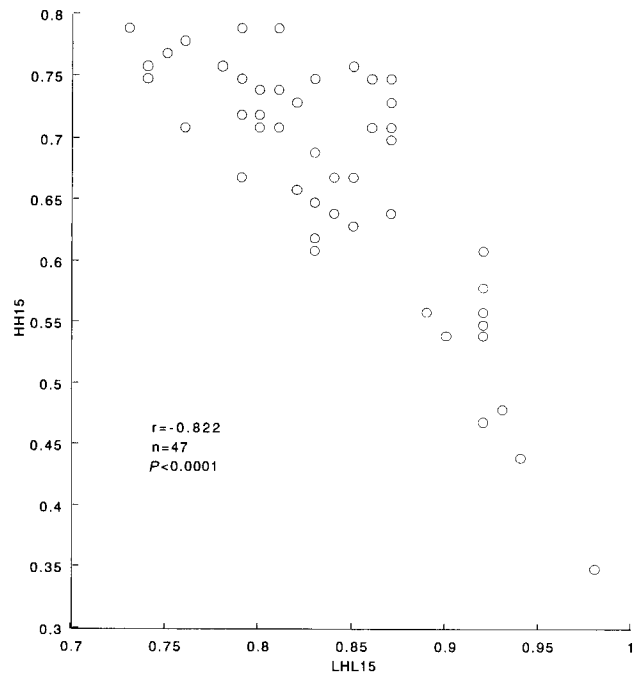
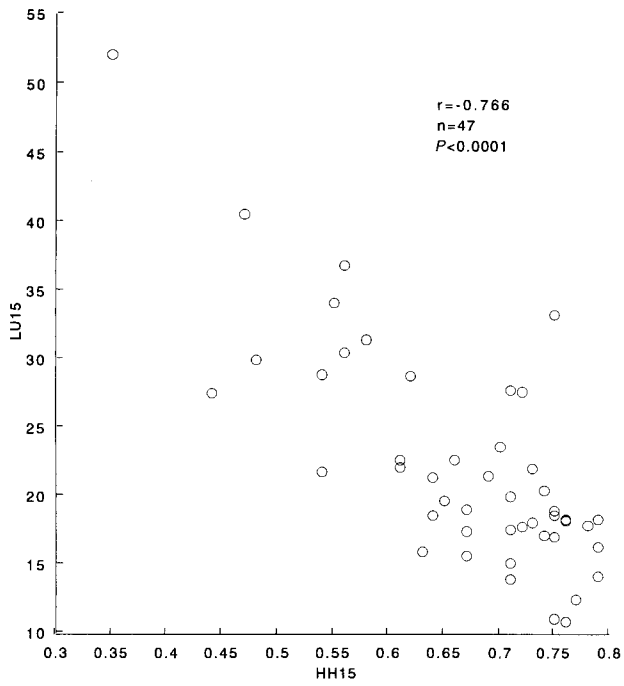
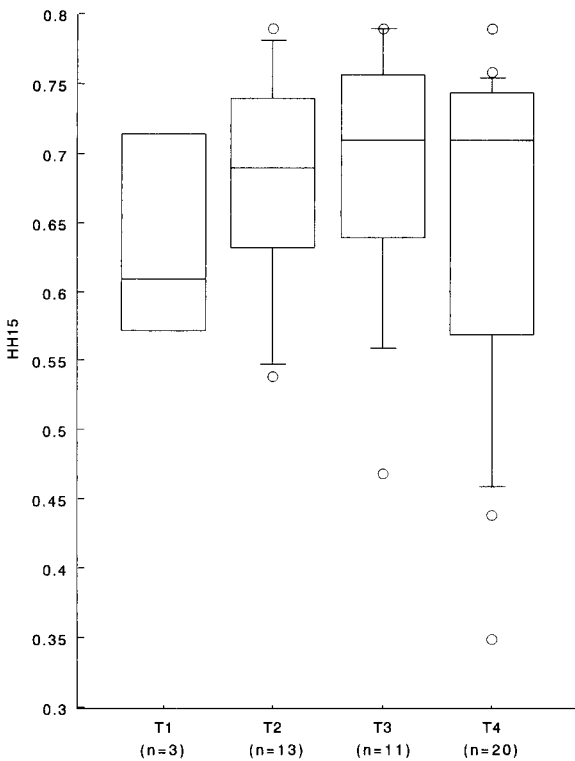
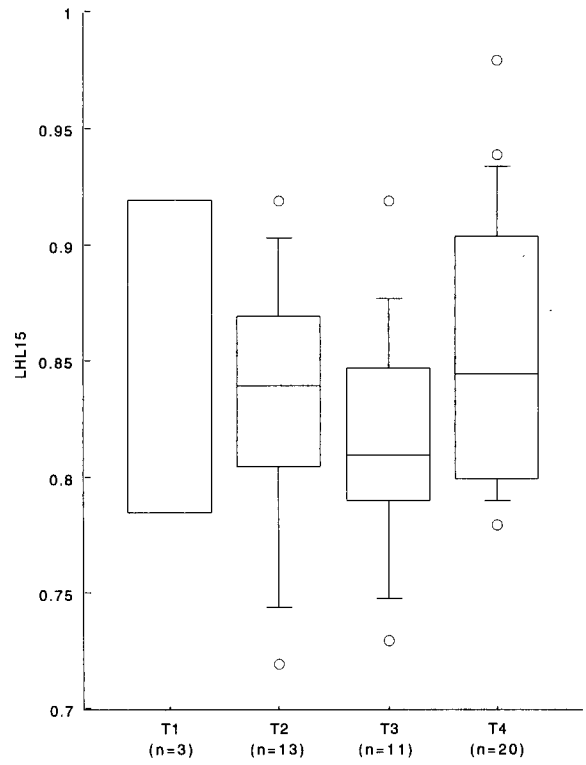
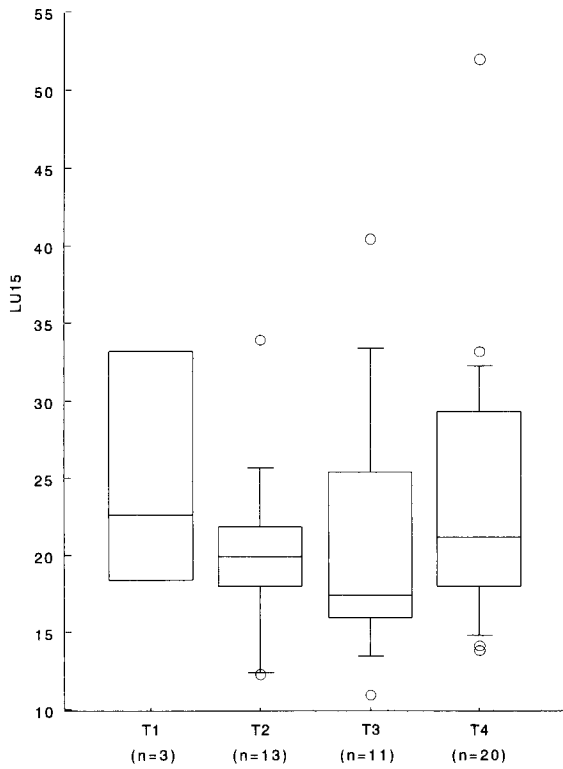


Fig. 3(a) Correlation between LU15 and HH15

Fig. 3(b) Correlation between LU15 and LHL15

Fig. 3(c) Correlation between HH15 and LHL15



**Fig. 4(a)** Correlation between tumor factor and LUI5 (Kruskal-Wallis test,  $P = 0.3615$ )

**Fig. 4(b)** Correlation between tumor factor and HH15 (Kruskal-Wallis test,  $P = 0.7289$ )

**Fig. 4(c)** Correlation between tumor factor and LHL15 (Kruskal-Wallis test,  $P = 0.4204$ )

**Table 1** Correlation between nuclear medicine parameters and liver function tests

	T.Bil	Alb	Ch-E	KICG	HPT	PT
LU15	-0.252	0.306	0.416	0.567*	0.365	0.175
HH15	0.163	-0.289	-0.490	-0.521	-0.536*	-0.379
LHL15	-0.170	0.247	0.521	0.531*	0.559*	0.282

\* $P < 0.0001$ . T.Bil; Alb; Ch-E; KICG; HPT; PT: See legend to Fig. 1.

patients who underwent TAE had mild liver dysfunction; those with good liver function are usually treated surgically and those with severe liver dysfunction are usually unsuitable for TAE.

The mutual correlations of the nuclear medicine parameters are shown in Fig. 3 (a, b and c). A significant correlation was demonstrated in each case.

There was no significant relationship between tumor factor and the nuclear medicine parameters (Fig. 4 a, b and c).

Table 1 shows the correlation of the nuclear medicine parameters with the liver function tests. High correlations were observed between LU15 and KICG, HH15 and HPT, LHL15 and KICG, or HPT.

Canonical correlation analysis was conducted on the set of nuclear medicine parameters and the set of liver function tests (Table 2). The canonical correlation coefficient was 0.7345 and the upper tail probability was 0.00167. Substantial correlations were observed between the 2 sets of variables. The structural coefficients of Ch-E, KICG, and HPT were high, indicating that there was a close relationship with the nuclear medicine parameters.

## Discussion

Mathematical models and compartmental analyses of measurements obtained from radiolabeled liver-binding proteins have been proposed by several authors as a means of quantitatively analyzing hepatic function (8-10). However, none of these methods has become widely used because each requires a specific analysis program.

The 2 imaging parameters initially investigated, LHL15 and HH15, can be easily determined and fairly accurately reflect the functional status of the liver. In clinical studies, the correlation of these 2 parameters is strong, but not linear (4). However, the equation for calculating LHL15 indicates that this parameter reaches a plateau as liver function improves and it does not exceed 1.0. Thus, precise functional discrimination among indi-

**Table 2** Results of canonical correlation analysis

1st variable group	Structural coefficient
T.Bil	-0.2593
Alb	0.3933
Ch-E	0.7218
KICG	0.7738
HPT	0.7649
PT	0.4426
2nd variable group	Structural coefficient
LU15	0.8237
HH15	-0.9421
LHL15	0.9593
Canonical correlation coefficient	0.7345
$\chi^2$	40.7040
Degrees of freedom	18
Upper tail probability	0.00167

T.Bil; Alb; Ch-E; KICG; HPT; PT: See legend to Fig. 1.

viduals with almost normal liver function may not be possible. In contrast, HH15 seems to reach a plateau in patients with severe liver dysfunction.

The blood disappearance corrected hepatic uptake ratio (LHL/HH) was introduced to overcome the faults of LHL15 and HH15, but this parameter had no physiological significance in itself (11). Furthermore, the parameter LU15 proposed by Koizumi *et al.* (7) has a strong correlation with LHL15, HH15 and ICGR15. In addition, it does not seem to reach a plateau, and can be calculated rapidly.

In recent years, visual analysis of Tc-99m-GSA imaging as a simple means of evaluating liver function has been reported (12, 13). Since this is not a quantitative parameter, however, it can only be considered as a secondary method of evaluation. In our study, HH15, LHL15 and LU15 were selected as nuclear medicine parameters, because these parameters can all be calculated easily and are regarded as useful in the evaluation of hepatic function.

When deciding whether or not to implement TAE, we should give serious consideration to hepatic functional

reserve. Some authors have suggested that clinical stage should also be given serious consideration (14) and still others suggest that T.Bil and Ch-E be taken in account (15, 16). As for calculating the prognosis after TAE, Kanno *et al.* suggested that the esophageal varices, HPT, Alb and the ICG test are the most useful (17). Nakao *et al.*, however, argued that T.Bil, the ICG test, gamma globulin level and the pattern of OGTT were the most relevant factors (18). In this study, we found KICG to be the most important prognostic factor. Based on this finding, we decided to compare T.Bil, Alb, Ch-E, KICG, HPT and PT with nuclear imaging findings.

Some researchers have evaluated liver function using Tc-99m-GSA before and after hepatectomy (19, 20) or TAE (21, 22). Regarding the evaluation of liver function, some studies using multivariate analysis to predict liver failure and the prognosis after hepatectomy (23) and TAE (24) have already been reported. However, no study using nuclear medicine parameters as the variable has been published. Also, in the previous studies, which assessed the significance of nuclear medicine parameters, simple regression analysis was generally used, ignoring the possible benefits of multivariate analysis. In the present study, the canonical correlation coefficient was 0.7345, and the upper tail probability was 0.00167. This showed that there was a significant correlation between the two sets of variables. Judging from the structural coefficient, LU15, HH15 and LHL15 all showed high correlations. LU15 and LHL15 are parameters, which reflect receptor volume, and high values of these indicate good liver function. HH15 is a parameter of blood disappearance, so a low value reflects good liver function. There are fewer reports on LU15 than there are on HH15 and LHL15. The difference in the structural coefficients between these three parameters is small, so the usefulness of LU15 is supported by the present findings. With respect to the liver function tests, the structural coefficient of Ch-E, KICG and HPT were high enough to prove a significant relationship with the nuclear medicine parameters.

The uptake of indocyanine green by liver cells is a receptor-mediated process that occurs through ICG-receptor carrier proteins. Tc-99m-GSA recognizes and binds to galactose-terminating glycoproteins by second-order kinetics. With respect to estimating functional receptors in the liver, these two tests are similar (25, 26). Unlike Alb, Ch-E showed a significant correlation with the nuclear medicine parameters. The reason for this

could be that Ch-E is more sensitive than Alb with respect to protein synthesis. PT and HPT reflect quantitative changes of coagulation factor production in the liver, and are also an index of the capacity for protein synthesis. They are used as parameters of acute liver injury because their half-life is brief. HPT is thought to reflect the degree of liver damage more sensitively than PT, and there was a difference in their structural coefficients.

The correlation coefficient of T.Bil was the lowest in this study. Bilirubin and bile acids did not interfere with the measurement of serum asialoglycoproteins in cases of hyperbilirubinemia (27). In patients with cholangiocellular carcinoma, Tc-99m-GSA scintigraphy allows the physician to evaluate reserved hepatic function with the results unaffected by the presence of jaundice (26). T.Bil may evaluate a liver function that is not measured by nuclear imaging, so we should take T.Bil results into account when deciding whether to implement TAE.

There was no significant relationship between tumor factor and nuclear medicine parameters. It is well known that HCC has no ASGP receptor on its surface membranes (3, 28). Because ASGP ligand-receptor interaction is carried out only at the surrounding hepatocytes, the presence of HCC did not affect in this study.

In conclusion, group evaluation of the correlation between nuclear medicine parameters and liver function tests was carried out by canonical correlation analysis. This confirmed a close relationship between the two sets of measures, in particular those of Ch-E, KICG and HPT, which were closely correlated with nuclear medicine parameters. The structural coefficients of all three nuclear imaging parameters were high, and LU15 was a parameter as useful as LHL15 and HH15.

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Received February 1, 1999; accepted June 18, 1999.