

• CLINICAL RESEARCH •

Accuracy of indocyanine green pulse spectrophotometry clearance test for liver function prediction in transplanted patients

Chung-Bao Hsieh, Chung-Jueng Chen, Teng-Wei Chen, Jyh-Cherng Yu, Kuo-Liang Shen, Tzu-Ming Chang, Yao-Chi Liu

Chung-Bao Hsieh, Chung-Jueng Chen, Teng-Wei Chen, Jyh-Cherng Yu, Kuo-Liang Shen, Yao-Chi Liu, Division of General Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, China

Tzu-Ming Chang, Department of Surgery, Tungs' Taichung Metroharbor Hospital, Taichung, Taiwan, China

Correspondence to: Dr. Chung-Bao Hsieh, 325, sec 2, Cheng-Kung Rd, Taipei, Taiwan, China. albert0920@yahoo.com.tw

Telephone: +886-2-87927191 **Fax:** +866-2-87927372

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Abstract

AIM: To investigate whether the non-invasive real-time Indocyanine green (ICG) clearance is a sensitive index of liver viability in patients before, during, and after liver transplantation.

METHODS: Thirteen patients were studied, two before, three during, and eight following liver transplantation, with two patients suffering acute rejection. The conventional invasive ICG clearance test and ICG pulse spectrophotometry non-invasive real-time ICG clearance test were performed simultaneously. Using linear regression analysis we tested the correlation between these two methods. The transplantation condition of these patients and serum total bilirubin (T. Bil), alanine aminotransferase (ALT), and platelet count were also evaluated.

RESULTS: The correlation between these two methods was excellent ($r^2=0.977$).

CONCLUSION: ICG pulse spectrophotometry clearance is a quick, non-invasive, and reliable liver function test in transplantation patients.

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INTRODUCTION

Monitoring of liver function is important in patients undergoing liver transplantation. Exact liver function and the extent of the remaining liver function at end-stage liver disease can indicate the appropriate timing of liver transplantation^[1-3]. During liver transplantation, immediate liver function evaluation can resolve primary non-functioning of the liver and technical complications^[4]. Moreover, post-transplantation early liver function evaluation can detect delayed graft malfunction or acute/chronic graft rejection.

Indocyanine green (ICG) is a synthetic dye that has been used for many years to measure hepatic blood flow and as a test of liver function^[5]. It was also used by Makuuchi and his colleagues, based on their experience, as a guide for selecting

the type of resection to be performed^[6,7]. During liver transplantation, ICG clearance rate has been used for evaluation of the recipient liver^[3,8], the donor liver^[9,10] and the post-transplant liver graft^[3]. Conventional ICG clearance is determined by measuring the rate of elimination using 3 mL venous samples at 0, 5, 10, 15, and 20 min after administering ICG. This invasive technique produces patient discomfort through repeated peripheral venous sampling at exact time intervals. Pulse spectrophotometry is an infrared-based non-invasive technique originally developed for monitoring tissue oxygenation. It has been previously used for the measurement of hepatic ICG concentration by Shinohara *et al.*^[11]. In a recent case report, infrared spectrophotometry was sufficiently sensitive to measure the ICG clearance in surgical patients^[12].

Even though the ICG clearance test has been used worldwide to predict liver function, there are some limitations for end-stage liver disease in patients with hyperbilirubinemia, intrahepatic shunt or capillarization^[13].

ICG pulse spectrophotometry (LiMON) has not been tested in transplanted patients previously.

The aim of this study was to determine the accuracy of ICG spectrophotometry, compared with the conventional invasive method, in predicting liver function in waiting and transplanted patients.

MATERIALS AND METHODS

Thirteen patients who underwent liver transplantation and follow-up at the Tri-Service General Hospital were evaluated in the current study. Eleven male patients were transplanted for chronic liver disease of various etiologies (1 hepatitis C, 2 hepatitis B, and 8 hepatitis B with hepatoma) and 2 patients with end-stage liver disease awaiting transplantation. Patients 7 and 11 were studied in acute rejection during follow up (d36 and 90) and proved by liver biopsy. Patients 3, 4 and 13 were studied immediately post-liver transplantation (within 24, 27 and 12 h) (Table 1).

Invasive ICG measurement

An intravenous (IV) bolus of 0.5 mg/kg ICG was injected rapidly through a central venous catheter or large peripheral venous line, and samples were obtained from another peripheral venous line at 0, 5, 10, 15, 20 min thereafter and kept in an EDTA-treated tube at room temperature until centrifuged at 3 000 r/min for 10 min. Absorbance was measured by a Perkin Elmer spectrophotometer at 805 nm. Direct ICG retention rate at 15 min (ICG_{15D}) and the elimination rate constant (ICG(K)_D) values were calculated using a commercial computer program (V-500 Spectra Manager, Jasco, Japan).

ICG pulse spectrophotometry

Pulse-dye densitometry (LiMON, Stahigruburring, Munich, Germany) was used to measure the blood ICG concentration non-invasively in real time. This apparatus makes such measurements possible by continuously monitoring the optical absorption at 805 nm and 890 nm, via an optical probe attached to the patient's finger. During the first 5-10 min after

Table 1 The demographic data of all patients

Diagnosis	Condition (d)	T. Bil	ALT	Cr.	PLT	ICG _{15D}	ICG _{15E}	ICG(K) _D	ICG(K) _E
1 Alcoholism	Waiting	1.6	121	0.8	16.4	27.2	28.4	0.05	0.08
2 HBV+HCC	Waiting	1.4	47	0.9	12.6	36	38.3	0.03	0.06
3 HBV	During-OLT-(2)	1.6	126	0.8	7.4	3	3.9	0.24	0.22
4 HBV	During-OLT-(2)	1.1	102	1.0	11.8	1	2.5	0.28	0.29
5 HCV+HCC	Post-OLT (43)	0.7	233	2.3	15.1	4	3.7	0.18	0.22
6 HBV	Post-OLT (212)	0.5	8	2.9	12.1	1	2.3	0.35	0.29
7 HBV	Post-OLT (36)	9.1	131	1.6	7.2	26	24	0.10	0.10
	Ac. rejection								
8 HBV+HCC	Post-OLT (142)	0.8	15	1.3	11	6.5	6.8	0.21	0.25
9 HBV+HCC	Post-OLT. (166)	1.1	37	0.7	24.9	2	4	0.32	0.23
10 HBV+HCC	Post-OLT (312)	0.5	16	1.0	18.8	4	4.5	0.24	0.21
11 HBV+HCC	Post-OLT (90)	2.0	50	2.2	11.5	18	13.7	0.16	0.13
	Ac. rejection								
12 HBV+HCC	Post-OLT (367)	1.3	46	3.4	6.4	10	11.2	0.28	0.27
	Ac. renal failure								
13 HBV+HCC	During OLT (1)	3.6	213	3.4	8.5	5.1	5.4	0.31	0.33

HBV: Hepatitis B virus; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; OLT: Orthotopic liver transplantation; Ac.: Acute; T. Bil: Total bilirubin; ALT: Alanine aminotransferase; Cr.: Creatinine; PLT: Platelet.

Table 2 The transplanted condition and laboratory data

Patients condition (n)	T. Bil	ICG _{15D}	ICG _{15E}	ICG(K) _D	ICG(K) _E
ESLD (2)	1.50±0.10	29.6±4.4	34.4±4.9	0.04±0.01	0.07±0.01
During-OLT (3)	1.35±0.25	2.0±1.0	3.2±0.7	0.26±0.02	0.25±0.04
Post-OLT (6)	0.86±0.30	3.6±2.1	3.9±0.8	0.26±0.08	0.24±0.03
Acute rejection (2)	5.60±3.60	22.0±4.0	18.9±5.2	0.13±0.03	0.12±0.02

ESLD: End-stage liver disease on waiting list; During-OLT: immediately after liver transplantation within seven days; Post-OLT: > one month after liver transplantation in stable condition; Acute rejection: proved by liver biopsy; the number in brackets means the case number.

ICG was injected, blood ICG concentrations were monitored at every pulse interval via pulse spectrophotometry. The elimination rate constant ICG(K)_E was calculated automatically by the time course of blood ICG concentration. The estimated ICG₁₅ retention rate (ICG_{15E}) was obtained via pulse spectrophotometry computer analysis during the first 5-10 min. As the usual measurement time of the Limon is between 5 and 10 min, the (ICG_{15E}) is simply calculated from the ICG plasma disappearance rate^[14].

Statistical analysis

Linear regression analysis was used to test the correlation. Statistical significance was considered at $P < 0.05$. The statistic software was S-Plus®2000 for Windows (CANdiensten, Amsterdam, The Netherlands).

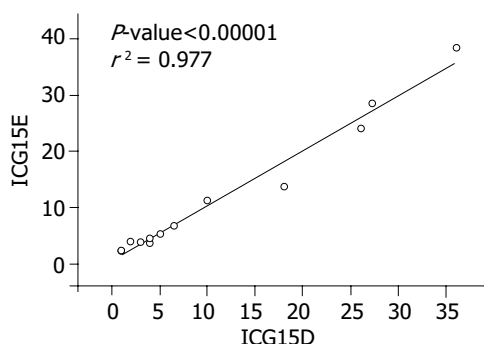


Figure 1 Correlation between the ICG_{15E} and ICG_{15D}. The correlation is excellent, $r^2 = 0.977$.

RESULTS

The correlation between ICG_{15D}, ICG_{15E}, ICG(K)_D, ICG(K)_E values for ICG₁₅ and ICG(K) by these two methods was excellent ($r^2 = 0.977, 0.855$) (Figures 1, 2). Two patients had poor liver function, elevated T. Bil, and higher ICG₁₅ retention rates, and were on the waiting list for transplantation. Three patients who had good liver function immediately post-liver transplantation showed elevated T. Bil, and lower ICG₁₅ retention rates. Two patients who were in acute rejection had poor liver function with elevated ALT and higher ICG₁₅ retention rates. Five patients, who were in a stable condition with good liver function post-liver transplantation, had low T. Bil. and better ICG₁₅ retention rates.

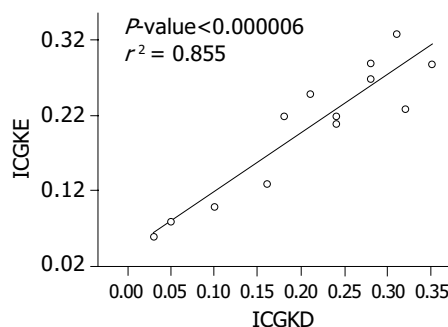


Figure 2 Correlation between ICG (K)_E and ICG (K)_D. The correlation is excellent, $r^2 = 0.855$.

DISCUSSION

ICG is a water-soluble tricarbo-cyanine dye that is extracted by

hepatic parenchyma cells and excreted almost entirely into bile. Hepatic clearance of ICG occurs by two major processes: by uptake across the sinusoidal plasma membrane with a high extraction ratio; and by removal from the hepatocytes via cytoplasmic transport and exclusive biliary excretion with neither intrahepatic conjugation nor enterohepatic circulation^[15]. The concentration in the plasma can be measured by spectrophotometry and its rate of elimination has been widely used as a measure of liver blood flow and liver function^[16]. Most of the studies assessing liver function before hepatectomy and the postoperative outcome were carried out using the ICG clearance test^[17].

Pulse spectrophotometry has been developed in recent years to enable the blood ICG concentration to be measured easily, less invasively, and continuously. The previous method for measuring the ICG concentration by spectrophotometry did not show sufficient accuracy. The new approach, based on pulse spectrophotometry, is able to continuously measure the arterial ICG concentration because of its ability to detect the pulse wave, greatly improving its accuracy.

Pulse spectrophotometry measurements of ICG concentration with each pulse could provide information on ICG liver uptake and excretion. From the concentration, a time curve of the ICG uptake and excretion rates were calculated under different experimental conditions. The experimental study by El-Desoky *et al.*^[5] showed the sensitivity of measurement of ICG uptake and excretion rates in hepatic artery occlusion, portal vein occlusion, ischemia-reperfusion injury, hepatic microcirculation, colchicines treatment, and bile duct ligation.

Assessment of liver function remains difficult in transplanted patients because of etiological complexity. The differential diagnosis includes primary non-function of the graft, rejection, virus re-infection, drug intoxication, and thrombosis of hepatic blood vessels. After liver transplantation, liver function tests are often difficult to interpret and non-specific. The diagnosis at present relies on a combination of biochemical, hemodynamic, clinical markers, and occasionally liver biopsy. However, serial observations up to 72 h are required. Measurement of ICG clearance appears to be a simple and safe test to assess early liver graft function. The development of non-invasive pulse spectrophotometry has enabled bedside assessment of the elimination of ICG with immediate results, therefore increasing its clinical usefulness.

A study by Jalan *et al.* showed the ICG measured at 24 h after liver transplantation accurately reflected graft function and predicted graft survival and outcome. In other studies, ICG excretion was a sensitive index of ischemia/reperfusion injury during the early stages post-liver transplantation^[17].

The traditional ICG clearance test is invasive and labor-dependent. The whole procedure requires more than 30 min at the bedside for loading dosage and venous puncture. Failure may occur as a result of the patient's underlying disease and medication such as gout, arthritis, and anti-TB drugs. Technical failure may be due to blood hemolysis or laboratory errors. The real-time pulse spectrophotometry ICG clearance test is non-invasive and machine-dependent. Technical failure may be due to detecting probe malpositioning, and patient movement.

In this study, we have chosen two ICG clearance test methods to measure end-stage liver disease, immediately post-liver transplantation or in acute rejection patients. In patients with hyperbilirubinemia, before and after successful liver transplantation, the ICG₁₅ clearance test may be very useful in

evaluating the new liver's function. ICG pulse spectrophotometry appears to be a sensitive and specific test to predict graft function in ischemia/reperfusion and acute rejection patients. The correlations between ICG(K) and ICG₁₅ using these two methods were excellent.

In conclusion, in this study, the correlation between conventional and pulse spectrophotometry ICG clearance tests is excellent in transplanted patients. The ICG pulse spectrophotometry clearance test is a quick, non-invasive, easy, inexpensive, and reliable liver function test in transplantation patients.

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