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ORIGINAL ARTICLE

Indocyanine green fluorescence imaging to evaluate graft perfusion during liver transplantation

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Abstract

Background: Primary graft dysfunction (PGD) is a leading cause of graft loss after liver transplantation. There is no reliable method to anticipate this complication intraoperatively. Indocyanine green (ICG) fluorescence imaging is a technique used in hepatobiliary surgery for detection of liver malignancies, but has never been reported in the setting of liver transplantation (LT) for function assessment. We hypothesized that there could be an association between the type of fluorescence and the occurrence of PGD.

Methods: We retrospectively analyzed 72 patients who underwent LT at our center. An assessment of the liver graft with the ICG fluorescence technique was carried out. A classification comprising 3 types of fluorescence was created after evaluation of the recorded images. We assessed the relationship between the type of fluorescence and the occurrence of PGD.

Results: Crosstabulation analysis of the fluorescent types and occurrence of PGD yielded a statistically significant association ($p = 0.002$). Univariate analysis showed that an abnormal ICG fluorescence pattern was a risk factor for the occurrence of PGD after LT.

Conclusions: Our findings suggest that there could be an association between ICG fluorescence imaging and graft function. This intraoperative tool could be useful to detect patients at risk of developing PGD after LT.

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Introduction

Early graft function is a major factor influencing clinical outcomes after liver transplantation (LT).[1] Because of the existing discrepancy between the number of patients awaiting LT and the limited supply of liver grafts, marginal donors are being used more often in an attempt to minimize this imbalance. One potential negative consequence of expanding the pool of donors is the risk of an increased incidence of graft dysfunction following LT. Primary graft dysfunction (PGD) is a clinical situation defined by the presence of biological or clinical abnormalities reflecting liver injury, impaired function, or both, which exhibit varying degrees of severity. There are two main forms of PGD: initial poor function (IPF) being a reversible situation in most cases, and primary non-function (PNF) which is an aggravated form of reperfusion injury resulting in irreversible graft failure without detectable technical or immunological complications,[2,3] leading to death or re-transplantation.[4] The incidence of IPF is approximately 23%,[5] while PNF is a relatively more uncommon complication, with a reported incidence ranging from 2% to 8%.[1,3,4,6–8] Despite the available data, there is no reliable method for assessing and predicting graft dysfunction intraoperatively after graft implantation.[9] Indocyanine green (ICG) is a fluorescent agent that has been described as a marker of liver function, due to its capacity to be exclusively excreted into the bile.[10] This fluorescent imaging technique, used in other surgical fields before, has been recently introduced in hepatobiliary surgery mostly for visualization of biliary tract anatomy,[11,12] and detection of superficial liver tumors.[13] Recent reports suggest that this imaging technique could be used for evaluation of liver parenchymal perfusion,[14] as a tool to early detect vascular and, perhaps, functional complications. To the best of our knowledge

this dye has never been used in the LT setting as a predictor of graft function.

The goal of the present study is to describe ICG fluorescence patterns of liver grafts after intraoperative intravenous injection of ICG immediately after LT, and to assess whether the presence of an abnormal pattern is associated with a higher risk of presenting PGD.

Material and methods

Patients

From October 2013 to May 2016, 72 patients who underwent LT at our institution were enrolled for this study. An assessment of the liver graft parenchyma by using a near-infrared (NIR) ICG fluorescence camera was performed in these patients. All LTs included in this study were performed by the same senior surgeon (EV). Before biliary anastomosis, a Doppler ultrasound was systematically carried out for all patients, using a ProSound Alpha 7 ultrasound system (Hitachi Aloka Medical, Tokyo, Japan). All recipients were followed-up until hospital discharge or in-hospital death. Patient's age and gender, donor's age and body mass index (BMI), graft macrosteatosis, cold ischemia time (CIT) and warm ischemia time (WIT) were evaluated. These parameters were chosen based on prior reports on risk factors for PGD.[3,15–17] No long-term data was recorded. Written informed consent was obtained from all patients in the study before surgery.

Fluorescence imaging and ICG fluorescence score

After vascular reconstruction of the graft was completed (venous and arterial anastomoses), an intraoperative injection of ICG (Infracyanine[tm], SERB laboratories, Paris, France) was performed. ICG (25 mg vials) was re-suspended in 10 cc of sterile water for injection to yield a 2.5 mg/mL stock solution. Of this stock solution, 2 or 4 mL, corresponding to doses of 5 or 10 mg, was administered intravenously as a bolus. Five minutes after the dye injection was made, fluorescence imaging recording was undertaken, systematically capturing images of all the anterior liver surface, for around 2 min. This delay allowed us to observe all the consequences of hemodynamics features (arterial and portal perfusions, portosystemic shunts) and intracellular transports. Biliary excretion could not be studied at 5 min but our study was not designed to diagnose anastomotic stricture or leakage.

The Fluobeam[tm] (Fluoptics SAS; Grenoble, France) fluorescence system for intraoperative imaging was used to capture the images. This system activates ICG with emitted light at a wavelength of around 750 nm and filters out light with wavelengths below 820 nm. The light source was a light-emitting diode (LED), and the detector was a charge-coupled device camera. The fluorescence signals were sent to a digital video processor for display on a computer monitor and registered into a hard drive for further analysis. The camera head of the fluorescent imaging system, held by the primary surgeon, was positioned 20 cm above the liver surface, with the OR surgical lights turned off.

The overall population was divided in three groups (by one operator - RF) according to a newly developed classification of graft perfusion fluorescence imaging (Fig. 1), based on the parenchymal uptake of ICG ("perfused areas") and whether this uptake was homogeneous or not. All images were interpreted separately and grouped into one of the three categories described above, irrespective of grafts outcomes. This new classification was validated by a second senior surgeon (EV) who reviewed the recorded images in order to minimize potential biases.

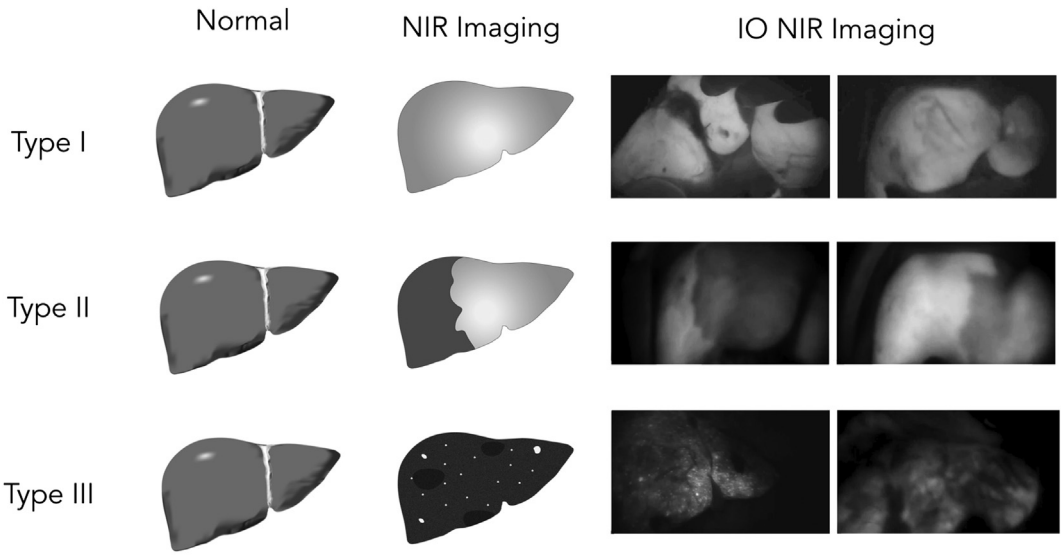

Figure 1 Schematic representation of the different fluorescence types according to our classification (See description of each category in the manuscript). Abbreviations: NIR, near infrared; IO NIR, intraoperative near infrared

Fluorescence patterns:

***Type 1*: *Homogeneous fluorescence*.** The whole graft takes the ICG in a similar fashion, and NIR light reveals a homogenous aspect of the liver surface (Fig. 1).
***Type 2*: *Non-perfused areas*.** There is a homogeneous uptake of ICG except for some regions. NIR light reveals some dark areas, surrounding homogenous fluorescent areas (Fig. 1).
***Type 3*: *Non-homogeneous fluorescence*.** The ICG uptake is irregular with a "patchy" fluorescence pattern. NIR light reveals some fluorescent spots on a dark background (Fig. 1).

This observational study implied no specific procedure in the case of an abnormal or unusual fluorescence aspect. Parameters associated with high risk of developing PGD were taken into consideration to determine whether they influenced or not the type of fluorescence pattern.

Definitions

PGD of the liver consists of IPF and PNF, which exhibit different stages of severity.[18]

1. **IPF:** was defined when one or more of the following variables were present:[5] bilirubin 10 mg/dL (171 μmol/L) on postoperative day 7, INR 1.6 on postoperative day 7 or aminotransferase level (alanine aminotransferase [ALT] or aspartate aminotransferase [AST]) >2000 IU/mL within the first 7 postoperative days.
2. **PNF:** was defined as the lack of normal graft function following transplantation, leading to death or retransplantation. These included not only apparent signs of hepatic failure (e.g., hypoglycemia, uncorrectable coagulopathy, coma, renal failure, and acidosis) but also other signs of irreparable damage to the organ (i.e., massive rises in transaminases along with daily rises in bilirubin).

Analysis

All quantitative data are expressed as median (range). Qualitative data are expressed as number (%). The Mann–Whitney *U* test for continuous variables and Pearson's chi-square test were used when applicable, or Fisher's exact test for categorical variables. Univariate risk factor analysis was performed using logistic regression for PGD occurrence. Receiver operating characteristics (ROC) curves and the area under the curve (AUC) were used to determine the accuracy of ICG fluorescence classification to predict the occurrence of PGD. Statistical analyses were performed using SPSS 20.0 (SPSS Inc, Chicago, IL, United States). A p-value of less than 0.05 was considered statistically significant.

Results

Patient characteristics and primary liver disease are summarized in Table 1 and Fig. 2. The overall incidence of PGD was 29% (21 patients): 19 patients (26%) presented with IPF, whereas 2 patients (3%) developed PNF. Table 2 reports the findings concerning the analysis of fluorescent imaging score. Eight patients (17%) in type I and 3 (30%) in the type II fluorescence group developed IPF; in the type III group, 10 patients (59%) presented PGD, including 2 PNF. These 2 patients underwent re-LT with a good graft outcome after the second operation. Fig. 3a shows one of the two patients with a type III fluorescence pattern who developed a PNF and had to undergo a re-LT; Fig. 3b shows the same patient after re-LT. Concerning the pathological assesment of these 2 grafts that presented PNF, none had findings of acute rejection; one had an 80% macrosteatosis and extensive parenchymal necrosis without any vascular alteration, and the other one received a left graft without any PNF identifiable cause in the pathologic examination.

The crosstabulation analysis of the three fluorescence types and the occurrence of PGD yielded a statistically significant association ($p = 0.002$), with a likelihood ratio of 12.5. None of the patients presented Doppler abnormalities.

Association between fluorescence patterns and patients and graft characteristics are summarized in Table 3. Contingency tables yielded no significant difference when comparing ICG fluorescent patterns with patient's age and gender, donor's BMI, graft macrosteatosis, CIT, and WIT. In order to identify if an abnormal fluorescence pattern represented a risk factor for PGD, an univariate analysis was carried out (Table 4). Univariate logistic regression analysis showed that an abnormal ICG fluorescence pattern was a risk factor for the occurrence of PGD post-LT (OR = 2.92; 95% CI: 1.56–5.44; $p < 0.001$). Out of the other parameters evaluated, even though WIT and macrosteatosis reached statistically significant differences, multivariate analysis showed that only WIT was independently associated with PGD.

A ROC curve was used to quantify the predictive strength of the different types of fluorescence patterns. As a predictor for PGD, NIR ICG imaging performed well, with AUC of 0.72 (95% CI 0.59–0.86; $p = 0.02$) (Fig. 4).

Table 1 Characteristics of the study group

Characteristic	Value
Age, years, median (range)	55 (16–71)
Gender, number (%)	
Male	47 (65)
Female	25 (35)
Weight, kg, median (range)	75,6 (44–180)
BMI > 25 kg/m^2	28
Macrosteatosis > 30%	4
CIT > 12 hs	3
WIT > 40 min	42

BMI, Body Max Index; CIT, Cold Ischemia Time; WIT, Warm Ischemia Time.

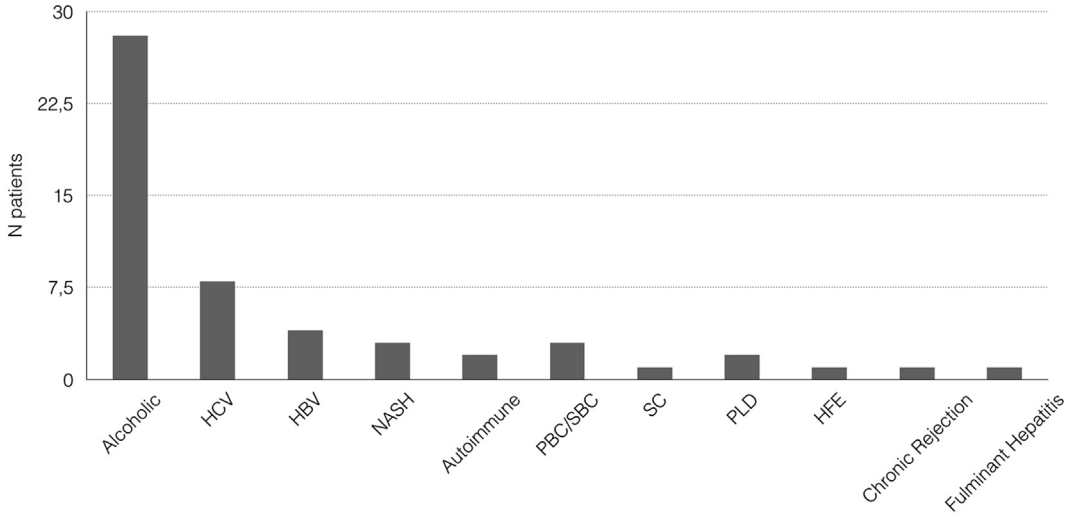

Figure 2 Primary liver disease for the patients undergoing LT in our study. Abbreviations: HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; NASH, Non-alcoholic Steatohepatitis; PBC/SBC, Primary/Secondary Biliary Cirrhosis; SC, Sclerosing Cholangitis; PLD, Polycystic Liver Disease; HFE, Haemocromatosis

Discussion

The present study on the intraoperative evaluation of liver grafts during LT using NIR fluorescence technology demonstrates for the first time that there is a correlation between ICG fluorescence patterns and post-LT graft (dys)function. A type III fluorescence pattern of the new ICG score herein reported was associated with an increased incidence of postoperative PGD.

Table 2 Correlation between fluorescence imaging and PGD

	IPF	PNF	Total
Type I (n = 45)	8	0	8 (17%)
Type II (n = 10)	3	0	3 (30%)
Type III (n = 17)	8	2	10 (59%)

PGD is still one of the leading causes of graft loss,[4] and includes two main biological and clinical settings varying in their degree of severity, IPF and PNF. In the present study, we adopted for IPF the definition proposed by Olthoff *et al.*[5] because the variables evaluated are objective and retrospectively obtainable. Aminotransferases on postoperative days 1–7 are more reflective of graft injury. Furthermore, using bilirubin levels and INR only at day 7, minimizes the contribution of preoperative elevated bilirubin and significant perioperative coagulopathy. In our series, the incidence of PGD and PNF was 29% and 3% respectively, which is similar to the overall incidence reported in the literature.[1,3–5,7,18,19] With the hypothesis that NIR fluorescence imaging could reflect immediate graft vascularization, and therefore could be a reliable method for predicting early allograft dysfunction, we analyzed all fluorescent images obtained with

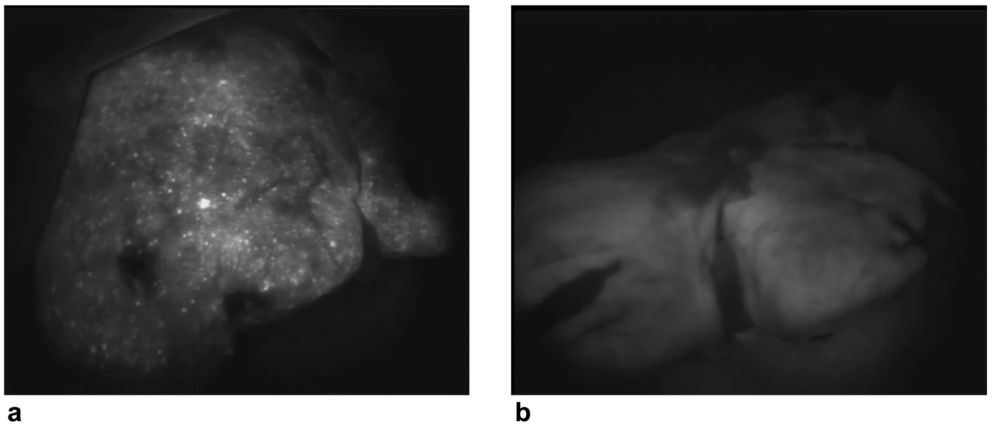

Figure 3 a) Type III fluorescence image of a patient who presented with a PNF. Neither anatomical nor doppler abnormality were detected during LT. The patient underwent re-LT the day after. b) Type I fluorescence image of the same patient after re-LT; postoperative course after the second LT was uneventful. Abbreviations: PNF, Primary Non function; LT, Liver Transplantation

Table 3 Correlation between fluorescence type and reported risk factors for PGD

Characteristic	Type I (n = 45)	Type II (n = 10)	Type III (n = 17)	
Age rec. (years)	53	54	53	*p = 0.8*
Gender (M/F)	29/26	6/4	11/6	*p = 0.66*
BMI > 25 kg/m^2	19	2	7	*p = 0.4*
Macrosteatosis > 30%	1	1	2	*p = 0.19*
CIT > 12 hs	2	1	0	*p = 0.5*
WIT > 40 min	24	6	12	*p = 0.5*

Abbreviations: BMI, Body Max Index; CIT, Cold Ischemia Time; WIT, Warm Ischemia Time.
A *p*-value of less than 0.05 was considered statistically significant.

ICG injection of reperfused liver grafts. Some of those grafts were not perfused in a uniform way and in the immediate post-operative course, some of these "unevenly-perfused" livers developed PGD. Thus, we created a novel classification in an attempt to group the grafts with similar fluorescent characteristics. Our results showed that most patients had a homogenous colorant distribution, whether complete (type I, 63%) or partial (type II, 14%), whereas a minority showed an inhomogeneous or patchy like perfusion (type III, 20%). Interestingly, almost 60% of type III patients developed PGD, and the only 2 PNF of the cohort also corresponded to this category. For type I and II the incidence of PGD was 17% and 30% respectively. Given that there is no evidence concerning the application of ICG fluorescence on donor grafts, little is known about the reason why some grafts present different types of fluorescence patterns. Nevertheless, in the absence of vascular abnormalities detected with the IOUS, we hypothesize, for type II, that it could be due to a problem in the venous outflow, creating congestive areas that yield a non-perfused region on fluorescence. For type III it is maybe due to impaired function at hepatocyte level, which causes an impaired ICG uptake by the cells after reperfusion. However, pathological reports could not provide any definitive explanations. In this study, we found a significant correlation between the type of fluorescent imaging and the likelihood of presenting PGD ($p = 0.002$). On the contrary, we found no significant association in crosstabulation analysis between fluorescence type and the classical risk factors for PGD. Moreover, our classification was a predictor of PGD occurrence, all together with macrosteatosis and an extended WIT, as seen in other reports.[16,17] The fact that multivariate logistic regression analysis did not show ICG fluorescence type as an independent predictor might be explained by the fact that the sample size of this cohort was small, and particularly due to the low incidence of PGD. Our findings suggest that there is a link between the ICG fluorescence imaging (as a surrogate of ICG uptake by the liver) and immediate graft function. According to our results, patients presenting an imaging fluorescence type III of our classification are at higher risk of developing PGD after LT and should be closely monitored in the immediate postoperative period.

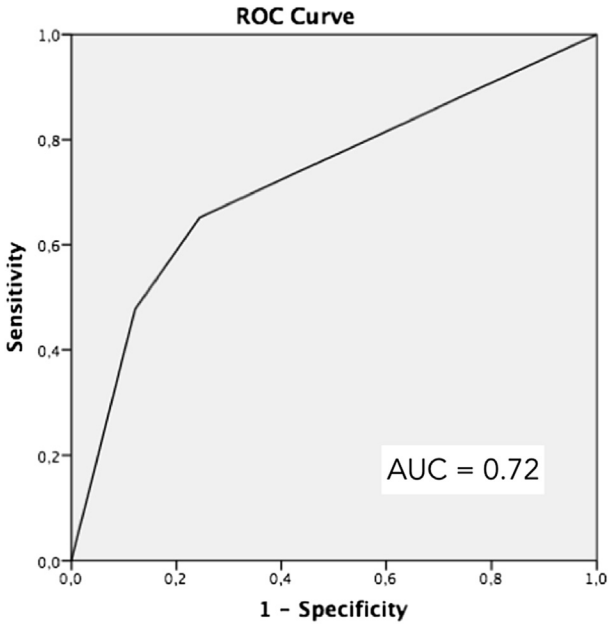

Figure 4 Receiver operating characteristic (ROC) curve used to determine the accuracy of ICG fluorescence imaging to predict graft dysfunction after liver transplantation

This study presents several limitations, mainly associated with the small number of patients as well as its retrospective design.

Table 4 Predictive factors for primary graft dysfunction: Logistic regression model

Variable	OR (95% CI)	P-value	OR (95% CI)	P-value
Fluor. Pattern	2.92 (1.56–5.44)	0.001	1.86 (0.89–3.88)	0.09
CIT		0.27		
WIT	1.03 (1.00–1.07)	0.03	1.03 (1.00–1.07)	0.05
Donor BMI		0.22		
Macrosteatosis	1.06 (1.00–1.13)	0.04	1.06 (0.99–1.14)	0.07
Microsteatosis		0.17		
Recipient's age		0.45		

Abbreviations: CI, Confidence Interval; OR, Odds Ratio; CIT, Cold Ischemia Time; WIT, Warm Ischemia Time; BMI, Body Mass Index.

The new fluorescence score used to group the graft imaging, albeit subjective, was validated separately by two surgeons. Nevertheless, it remains a subjective classification that should be further validated. Modern fluorescence imaging software will be shortly available, which will be able to quantify intensity zones and will add objectivity to our classification. With french mathematicians and engineers, we established close prospective collaboration to analyze liver intensity dynamics in the very early post-ICG injection period (before 2 min).[20] Ongoing works will soon correlate early infusion patterns (delay and type of vascular and parenchymal fluorescence increases) and graft outcomes (vascular complications and PGD). However, these data are still being analyzed and we were not able to show early fluorescence data for these 72 patients presented here.

In conclusion, ICG fluorescence technique is a promising intraoperative navigation tool that could be useful to detect patients at risk for developing PGD after LT. Prospective validation studies should be carried out in order to support our findings.

Conflicts of interest

None declared.

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