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Indocyanine green clearance test in liver transplantation: defining cut-off levels for graft viability assessment during organ retrieval and for the prediction of post-transplant graft function recovery - the Liver Indocyanine Green (LivInG) Trial Study Protocol

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ABSTRACT

Introduction Viability assessment of the graft is essential to lower the risk of liver transplantation (LT) failure and need for emergency retransplantation, however, this still relies mainly on surgeon's experience. Post-LT graft function recovery assessment is also essential to aid physicians in the management of LT recipients and guide them through challenging decision making.

This study aims to trial the use of indocyanine green clearance test (IGT) in the donor as an objective tool to assess graft viability and in the recipient to assess graft function recovery after LT.

Methods and analysis This is an observational prospective single-centre study on consecutive liver transplant donors and recipients.

Primary objective To determine the capability of IGT of predicting graft viability at the time of organ retrieval. Indocyanine green will be administered to the donor and the plasma disappearance rate (PDR) measured using the pulsidensitometric method. Some 162 IGT donor procedures will be required (α, 5%; β, 20%) using an IGT-PDR cut-off value of 13% to achieve a significant discrimination between viable and non-viable grafts.

Secondary objective

IGT-PDR will be measured at different time-points in the LT recipient: during the anhepatic phase, after graft reperfusion, at 24 hours, on day 3 and day 7 after LT. The slope of IGT values from the donor to the recipient will be evaluated for correlation with the development of early allograft dysfunction.

Ethics and dissemination This research protocol was approved by Fondazione Policlinico Universitario Agostino Gemelli IRCCS Ethics Committee (reference number: 0048466/20, study ID: 3656) and by the Italian National Transplant Center (CNT) (reference number: Prot.11/CNT2021). Liver recipients will be required to provide written informed consent. Results will be published in international peer-reviewed scientific journals and presented in congresses.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first adequately powered prospective trial assessing indocyanine green clearance test (IGT) regarding graft viability assessment during liver retrieval surgery.
⇒ The pulsidensitometric method for IGT is easy to perform and transport to the donor hospital.
⇒ The application of IGT at different time points in the liver transplant recipient offers the possibility to highlight the modifications in liver graft function over time.
⇒ Limitations relate to the monocentric nature of the study that could cause a prolonged enrollment phase depending on the centre activity.

Trial registration number NCT05228587.

INTRODUCTION

Liver transplantation (LT) is the gold-standard treatment for end-stage liver diseases. The success of LT and the expansion of medical conditions that are successfully treated with LT have caused a growing gap between available organs and patients still dying while awaiting a transplantable organ.

Various attempts at fulfilling the gap continue to be made, including donation from live donors, split livers, and utilisation of extended criteria deceased donors (eg, elderly donors, steatotic grafts, donors after cardiac death).

Extended criteria grafts carry an increased risk of post-transplant failure which is difficult to quantify.[1] Yet, we rely on the donor surgeon's evaluation based on clinical aspects

and past experience. In selected cases, a liver biopsy can be used, however the limitations of liver biopsies in graft viability assessment are well known and extensively questioned in the scientific literature, to the point of being used only in selected cases by many transplant units.[2 3]

The adoption of an objective measure of graft viability is highly desirable to prevent from transplanting organs at high risk of failure. Similarly, the recovery of organ function after LT is not measured by means of an objective test. This is mainly monitored with laboratory tests, in some cases measuring bile production and monitoring the clinical evolution of patients condition.[1 4]

Indocyanine green clearance test (IGT) has been evaluated as a prognostic marker in patients with advanced cirrhosis or awaiting LT. In addition, it is used as a marker of portal hypertension in cirrhotic patients, as a prognostic factor in intensive care units and is commonly used as part of the preoperative work-up before liver resections.[5]

Indocyanine green is administered intravenously, up-taken almost exclusively by hepatocytes and excreted unprocessed in the bile ducts. The disappearance rate from the bloodstream is measured either on a blood sample (ie, retention rate 15 min after injection) or—more recently—with a pulsidensitometric method (ie, plasma disappearance rate, PDR). Lower PDR values correlate with worse liver function. A cut-off PDR level of >14%/min has been reported to allow safe major liver resections.[6] The role of IGT in LT has not been investigated extensively yet, in particular for the assessment of graft viability during donation.[7] A correlation between graft steatosis and IGT in the donor has been observed[8] while an increased incidence of graft failure has been reported with PDR <11%/min.[9] Conversely, there is more evidence in the recipient setting, with IGT correlating with the occurrence of post-LT complications (PDR cut-off level for increased risk of post-LT complications of <12.85%/min or graft loss and/or patient death of <9.6%/min).[10 11]

However, a correlation between the changes in the values (ie, the slope) of IGT and graft function recovery has not been studied yet. Since recent technology enables PDR to be measured non-invasively at the bedside, this parameter is an attractive addition to liver function assessment. However, the current state-of-the-art as concerns this technology remains at a low level of evidence and thorough assessment is required.

Retrospective data correlating IGT values with graft function post-LT exist[12] while there is no prospective study adequately powered to demonstrate its role in graft viability assessment.

Data regarding the use of IGT in both liver donors and recipients are lacking in the current literature. There is no study analysing variations in IGT values starting from the donor, through the transplant, ending 7 days post-LT.

This study aims to assess the ability of IGT to discriminate between viable and non-viable liver grafts for solid organ transplantation. Secondarily, we aim to evaluate the correlation between the slope of IGT-PDR values and the development of early allograft dysfunction (EAD).

METHODS AND ANALYSIS

This protocol conforms to the recommendations outlined in the Standard Protocol Items: Recommendations for Interventional Trials statement guidelines.[13]

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Study design

This is an observational, prospective, single-centre study.

Setting

The study will take place at the Liver Transplant Center of Fondazione Policlinico Universitario Agostino Gemelli IRCCS, in Rome, Italy, beginning in April 2022. The donor procedures and the IGT will take place in the donor hospitals.

Performing IGT will be taught to the whole transplant team (six staff surgeons and four residents) and tutorials will be organised in advance before the start of the study to minimise the risk of learning curve effect. IGT blood tests are already performed in our unit for the assessment of liver function in prevision of liver resections in cirrhotic patients. Such expertise will be expanded and transmitted to as many members of the team as possible.

Participants

Inclusion criteria

- All consecutive liver donors included in the study period.
- All consecutive liver recipients transplanted in the study period with a graft from a donor undergone IGT.

Exclusion criteria

- Donor or recipients with history of allergy to iodine.

Experimental design: primary endpoint: liver donors

Primary endpoint of the study is to identify a PDR cut-off level below which the liver graft is not viable for solid organ transplantation.

Organ donors will be managed according to the Italian National Transplant Centre (CNT) policy and the current study will not require any change to standard practice. Indocyanine green 0.25 mg/kg will be administered intravenously to the multiorgan donor on arrival in the operating room. The IGT-PDR will be measured using the pulsidensitometric method (LiMON System, Impulse Medical System, Munich, Germany—or alternative/equivalent device), recorded and secured inside a specially designed 'IGT Study Box'. The value obtained will not be revealed to the surgical retrieval team who will carry out the operation without any deviation from standard

practice because of the current study (ie, surgical team blinded).

Research hypothesis

Based on the cut-off values available in the existing literature, we hypothesised an IGT-PDR cut-off inferior to 13%/min for predicting non-viability of the graft for solid organ transplantation.

Power calculation based on the primary endpoint

Based on our liver transplant centre, organ retrieval activity during 2017 and 2018 years, 162 organ retrieval procedures will be necessary for achieving 80% power (alfa 0.05) using IGT for graft viability assessment. Our current activity ranges between 60 and 70 organ retrievals per year and we plan to complete the enrolment in 30–32 months. Interim analysis at 50% enrolment will be carried out to compare hypothesis (IGT cut-off level <13%/min for liver graft viability) with actual results. Study sample size might be amended accordingly.

Experimental design: secondary endpoint: liver recipients

Secondary endpoint is to identify PDR cut-off level(s) below which post-LT organ recovery is impaired (EAD). IGT will be performed at different time points post-LT: during the anhepatic phase, postreperfusion, on day 1, 3 and 7. Each time point measurement will be analysed for correlation with EAD.[14] Finally, we will define distinct classes of EAD risk based on the slope of IGT values, starting from the donor IGT, ending on day 7 post-LT.

LT will take place as per our standard protocol and IGT-PDR will be measured with the pulsidensitometric method at different time points:

- T.0(zero): during the anhepatic phase (at completion of total hepatectomy) to calculate potential disappearance of indocyanine green via non-hepatic mechanisms (mainly extravasation in the interstitium as known from available literature).[15] The anhepatic disappearance rate will serve as a correction factor of IGT values until the recipient has evidence of fluid overload >10 L from their pre-LT weight.
- T.LT: after haemodynamic stability is obtained for at least 1 hour (usually after completion of bile duct reconstruction).
- IGT will take place also 24 hours after LT (T.1), on day 3 (T.3) and day 7 (T.7) after LT.

All post-LT IGT-PDR values will be recorded on the patient chart and will be accessible to the clinical staff managing the patient.

EAD will be defined according to the Olthoff criteria by the presence of one or more of the following: INR >1.6 on day 7; bilirubin >10 mg/dL on day 7; Alanine aminotransferase (ALT) > 2000 UI/L within the first 7 days.[14]

Each time point IGT-PDR value will be analysed for correlation with the development of EAD.

As per primary endpoint power calculation, out of the 162 donor cases enrolled, we expect to enrol approximately 120 liver transplant recipients.

Considering an incidence of EAD in 23% of LT recipients, 14 we expect 28 LT recipients experiencing EAD to be compared with 92 cases with normal graft function recovery.

Data analysis according to statistical methods described below will permit the creation of distinct EAD risk classes depending on the slope of IGT-PDR values.

Long-term follow-up will be carried out for each recipient lifelong as per our centre policy and the prospective database (currently in place) will be updated accordingly.

Graft and patient survival will be analysed at 1-year, 3-year and 5-year post-LT and any correlation with IGT slope risk classes will be analysed and used in long-term survival studies.

IGT measurements

IGT measurements will be conducted with the LiMON system (or equivalent): each patient is monitored with an IGT finger clip, which is connected to the liver function monitor via an optical probe.

Injected indocyanine green is detected from fractional pulsatile changes in optical absorption. The optical peak absorption at 805 and 890 nm allows continuous measurements of IGT.

For each measurement, 0.25 mg/kg indocyanine green is given through a peripheral or central vein as a bolus and immediately flushed with 10 mL of normal saline. The dose to be used was chosen on the basis of reports demonstrating that a dose between 0.25 and 0.50 mg/kg is accurate for the transcutaneous measurement of IGT in critically ill patients. The monitor automatically determines the PDR by monoexponential transformation of the original indocyanine green concentration curve and backward extrapolation to time point zero (100%), describing the decay as a percentage change with time (ie, PDR).

Informed consent

LT recipients will receive special informed consent to participate in the study and a dedicated leaflet will be produced to inform patients regarding study aims, possibility to withdraw from the study at any time, possible side effects related to indocyanine green and no financial implication neither for the patient nor for the researchers.

Statistical analysis

For the descriptive analyses, continuous variables will be presented as the medians plus interquartile ranges, and categorical variables will be presented as percentages and frequencies. The Kolmogorov-Smirnov test will be used to verify a normal distribution.

For the primary endpoint: the study groups (divided depending on IGT values) will be compared using the Mann-Whitney U test for continuous variables; Fisher's exact test will be used for categorical variables. To identify the independent risk factors associated with donor acceptance, a univariate logistic regression analysis will be conducted. Variables with a p<0.20 in the univariate

analysis will be included in the multivariate logistic regression analysis via the forward stepwise method; the results will be presented as ORs with 95% CIs. Receiver operating characteristic (ROC) curves will be plotted for identifying the best IGT-PDR threshold value for the diagnosis of graft non-viability.

Cut-off values will be measured using the highest Youden index (specificity +sensitivity − 1) obtained from the ROC curves.

For secondary endpoint: descriptive statistics as per primary endpoint. In addition, to identify the independent risk factors associated with EAD, a univariate logistic regression analysis will be conducted. Variables with a $p<0.20$ in the univariate analysis will be included in the multivariate logistic regression analysis via the forward stepwise method; the results will be presented as ORs with 95% CIs. ROCs will be plotted for identifying the best IGT threshold value for the diagnosis of EAD. Cut-off values will be measured using the highest Youden index (specificity +sensitivity − 1) obtained from the ROC curves.

All patients will be followed until death, graft failure, or last known follow-up visit. Graft survival will be analysed using the Kaplan-Meier method, and group comparisons will be conducted using the log-rank test. Statistical analyses will be performed using the SPSS V.22.0. All p values will be two-tailed, and $p<0.05$ will be considered to indicate significance.

Study current status

The recruitment phase of the study will start in April 2022.

DISCUSSION

With this study, we expect to validate the use of IGT in the setting of organ retrieval to aid the retrieval surgeon in the decision-making process of accepting a liver graft for solid-organ transplantation. This will expand the yet limited armamentarium of the retrieval surgeon for graft viability assessment.

We expect to identify cut-off levels of IGT-PDR at distinct time points after LT, which could predict the development of EAD or graft failure. In addition, we expect to describe EAD risk classes by evaluating the slope of IGT-PDR from the time of organ retrieval to day 7 post-LT.

With this, we will add an objective measure of liver function post-LT to better detect EAD not only by laboratory data or clinical observation, thus offering a useful tool to the transplant physicians managing complex clinical scenarios where there is uncertainty due to impaired graft function recovery.

Undiagnosed EAD or graft failure can lead to delayed indication for retransplantation and recipient's death due to overcoming complications.

Risk analysis, possible problems and solutions

Risks related to the administration of indocyanine green to the donor and to the recipient have been considered.

The drug is contraindicated in patients with hypersensitivity to iodine. All patients will be screened for allergy and excluded whenever there is history of allergy to iodine. Allergic reactions have been reported although frequency is not defined by the pharmaceutical companies.

A possible problem relates to non-hepatic clearance mechanisms of indocyanine green, which has been reported to happen especially in fluid overloaded patients. This has been taken into account and we have introduced an IGT-PDR measurement during the anhepatic phase to be used as a correction factor when measuring IGT until the fluid overload is present.

If the primary outcome expectations are not met (ie, identifying a cut-off PDR value to discriminate graft viability), the bulk of data obtained with our study will provide exceptional added knowledge to the field of assessing graft function recovery post-LT using IGT (ie, the objective of secondary outcome). In fact, this has been the objective of clinical research mainly based on retrospective studies and adequately powered prospective studies are still lacking.

If interim analysis at 50% enrolment demonstrates expected insufficient power to demonstrate the hypothesis, we will reassess the sample size and potentially expand the study enrollment.

Ethics and dissemination

This research protocol was approved by Fondazione Policlinico Universitario Agostino Gemelli IRCCS Ethics Committee (reference number: 0048466/20, study ID: 3656). In addition, this research protocol was approved by the Italian National Transplant Center (CNT) (reference number: Prot. 11/CNT 2021). Informed consent will be sought in all liver transplant candidates at the time of organ donation offer. All data are deidentified and no patient-related information will be revealed during analysis.

All data regarding patients included in this study are covered by strict confidentiality in accordance with the General Data Protection Regulation EU 2016/679 (GDPR) and D.lgs. 30.06.2003, n. 196, as modified from D.lgs. 10.08.2018, n. 101. The study is conducted in accordance with the national law and according to international guidelines for the conduction of clinical trials according to the Declaration of Helsinki and in the respect of the principles of the Good Clinical Practice.

Results will be published in international peer-reviewed scientific journals and presented in relevant congresses.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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