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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-center 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.

Trial registration number: NCT05228587

Keywords: Liver transplant, Liver failure, Organ donation, Indocyanine green clearance, Liver ischemia-reperfusion injury.

Strengths and limitations of this study

- This is the first adequately powered prospective trial aiming to demonstrate the possibility of graft viability assessment using the indocyanine clearance test (IGT) during liver retrieval surgery; the pulsidensitometric method for IGT is easy to perform and transport to the donor hospital.
- The addition of IGT to routine practice organ retrieval may provide an objective tool to assess graft viability, increase the chances of success of LT, and aid the retrieval surgeon in the decision whether to accept organs for LT.
- Donor IGT might be performed before the surgical team is mobilized, potentially optimizing resources.
- Recipient IGT might allow to quantify the chances of organ function recovery and enable the establishment of tailored management strategies by providing prognostic information**.**
- Some limitations rely in the pharmacokinetics of indocyanine green (excreted unmodified in the bile ducts, therefore not a measure of hepatocyte metabolism) and in the potentially long enrolment time (single-center prospective study design).

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 utilization of extended criteria deceased donors (e.g., elderly donors, steatotic grafts, donors after cardiac death, etc.).

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 liver transplantation. 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 (i.e., retention rate 15 minutes after injection) or - more recently - with a pulsidensitometric method (i.e., 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] whilst 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 (i.e., 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] whilst 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 analyzing 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-center 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 (6 staff surgeons and 4 residents) and tutorials will be organized in advance before the start of the study to minimize 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 Center (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 upon 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 (i.e., surgical team blinded).

Research hypothesis

Based on the cut-off values available in the existing literature, we hypothesized 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 Center 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 enrollment

in 30-32 months. Interim analysis at 50% enrollment 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 (early allograft dysfunction). IGT will be performed at different time-points post-LT: during the anhepatic phase, post-reperfusion, on day 1, 3 and 7. Each time-point measurement will be analyzed 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.

Liver transplantation 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 hemodynamic stability is obtained for at least one 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.

Early allograft dysfunction 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 > 10mg/dL on day 7; ALT >2000 UI/L within the first 7 days.[14]

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

As per primary endpoint power calculation, out of the 162 donor cases enrolled, we expect to enroll approximately120 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 Center policy and the prospective database (currently in place) will be updated accordingly.

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

Indocyanine green clearance test 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 plasma disappearance rate (PDR) by mono-exponential 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 (i.e. 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 value of <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 odds ratios (ORs) with 95% confidence intervals (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 value of <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 odds ratios (ORs) with 95% confidence intervals (CIs). Receiver operating characteristic curves 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 analyzed using the Kaplan-Meier method, and group comparisons will be conducted using the log-rank test. Statistical analyses will be performed using the Statistical Package for the Social Science (SPSS) 22.0 (IBM, USA). 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 the present 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 a 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 (i.e., 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 (i.e., 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% enrollment 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.

Authors' contributions

GB, AC and GS conceptualized and designed the protocol, drafted the initial manuscript and reviewed the final manuscript. QL planned the data extraction and statistical analysis. GM and MC provided critical insights. GS and SA applied for ethical and regulatory approvals. All authors have approved and contributed to the final written manuscript.

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Conflict-of-interest statement

All the Authors have no conflict of interest related to the manuscript.

References

1. Avolio AW, Franco A, Schlegel A, et al. Development and Validation of a Comprehensive Model to Estimate Early Allograft Failure Among Patients Requiring Early Liver Retransplant. *JAMA Surg*. 2020 Dec 1;155(12):e204095.

2. Lo IJ, Lefkowitch JH, Feirt N, et al. Utility of liver allograft biopsy obtained at procurement. *Liver Transplant*. 2008 May;14(5):639–46.

3. Frongillo F, Lirosi MC, Sganga G, et al. Graft steatosis as a risk factor of ischemic-type biliary lesions in liver transplantation. *Transplant Proc*. 2014 Sep;46(7):2293–4.

4. Agopian VG, Harlander-Locke MP, Markovic D, et al. Evaluation of Early Allograft Function Using the Liver Graft Assessment Following Transplantation Risk Score Model. *JAMA Surg*. 2018 May 1;153(5):436–44.

5. Levesque E, Martin E, Dudau D, et al. Current use and perspective of indocyanine green clearance in

liver diseases. *Anaesth Crit Care Pain Med*. 2016;35(1):49–57.

6. Lau H, Man K, Fan ST, et al. Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. *Br J Surg*. 1997 Sep;84(9):1255–9.

7. Cherchi V, Vetrugno L, Terrosu G, et al. Association between the donor to recipient ICG-PDR variation rate and the functional recovery of the graft after orthotopic liver transplantation: A case series. *PloS One*. 2021;16(8):e0256786.

8. Wang W, Zhao Q, Hu A, et al. Application of Indocyanine Green (ICG) Detection in Evaluating Early Prognosis in Patients with Fatty Liver Disease After Liver Transplantation. *Ann Transplant*. 2017;22:208–14.

9. Tang Y, Han M, Chen M, et al. Donor Indocyanine Green Clearance Test Predicts Graft Quality and Early Graft Prognosis After Liver Transplantation. *Dig Dis Sci*. 2017;62(11):3212–20.

10. Levesque E, Saliba F, Benhamida S, et al. Plasma disappearance rate of indocyanine green: a tool to evaluate early graft outcome after liver transplantation. *Liver Transplant*. 2009;15(10):1358–64.

11. Schneider L, Spiegel M, Latanowicz S, et al. Noninvasive indocyanine green plasma disappearance rate predicts early complications, graft failure or death after liver transplantation. *Hepatobiliary Pancreat Dis Int*. 2011;10(4):362–8.

12. Cherchi V, Vetrugno L, Zanini V, et al. Indocyanine green dye clearance test: early graft (dys)-function and long-term mortality after liver transplant. Should we continue to use it? An observational study. *J Clin Monit Comput*. 2021 May;35(3):505–13.

13. Chan A-W, Tetzlaff JM, Gøtzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ 2013;346:e7586.

14. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transplant*. 2010 Aug;16(8):943–9.

15. Bruegger L, Studer P, Schmid SW, et al. Indocyanine green plasma disappearance rate during the anhepatic phase of orthotopic liver transplantation. *J Gastrointest Surg*. 2008;12(1):67–72.

Data sharing statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,11

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1,11
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1,11
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5,6
Objectives	#7	Specific objectives or hypotheses	6,7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5-8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	5,6

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6,7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	9,10
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9,10
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6,7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6,7
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	5,6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n/4
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6-8
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	5
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8,9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8,9

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8,9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	2
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	6
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9,10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9,10
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect	10

		confidentiality before, during, and after the trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	10,11
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Patient Consent
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Notes:

- 32: supplementary 2 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 16. March 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

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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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-center 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.

Trial registration number: NCT05228587

Keywords: Liver transplant, Liver failure, Organ donation, Indocyanine green clearance, Liver ischemia-reperfusion injury.

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 center activity.

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 utilization of extended criteria deceased donors (e.g., elderly donors, steatotic grafts, donors after cardiac death, etc.).

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 liver transplantation. 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 (i.e., retention rate 15 minutes after injection) or - more recently - with a pulsidensitometric method (i.e., 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] whilst 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 (i.e., 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] whilst 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 analyzing 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-center 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 (6 staff surgeons and 4 residents) and tutorials will be organized in advance before the start of the study to minimize 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 Center (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 upon 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 (i.e., surgical team blinded).

Research hypothesis

Based on the cut-off values available in the existing literature, we hypothesized 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 Center 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 enrollment

in 30-32 months. Interim analysis at 50% enrollment 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 (early allograft dysfunction). IGT will be performed at different time-points post-LT: during the anhepatic phase, post-reperfusion, on day 1, 3 and 7. Each time-point measurement will be analyzed 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.

Liver transplantation 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 hemodynamic stability is obtained for at least one 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.

Early allograft dysfunction 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 > 10mg/dL on day 7; ALT >2000 UI/L within the first 7 days.[14]

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

As per primary endpoint power calculation, out of the 162 donor cases enrolled, we expect to enroll approximately120 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 Center policy and the prospective database (currently in place) will be updated accordingly.

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

Indocyanine green clearance test 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 plasma disappearance rate (PDR) by mono-exponential 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 (i.e. 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 value of <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 odds ratios (ORs) with 95% confidence intervals (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 value of <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 odds ratios (ORs) with 95% confidence intervals (CIs). Receiver operating characteristic curves 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 analyzed using the Kaplan-Meier method, and group comparisons will be conducted using the log-rank test. Statistical analyses will be performed using the Statistical Package for the Social Science (SPSS) 22.0 (IBM, USA). 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 the present 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 a 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 (i.e., 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 (i.e., 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% enrollment 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.

Authors' contributions

GB, AC and GS conceptualized and designed the protocol, drafted the initial manuscript and reviewed the final manuscript. QL planned the data extraction and statistical analysis. GM and MC provided critical insights. GS and SA applied for ethical and regulatory approvals. All authors have approved and contributed to the final written manuscript.

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Conflict-of-interest statement

All the Authors have no conflict of interest related to the manuscript.

References

1. Avolio AW, Franco A, Schlegel A, et al. Development and Validation of a Comprehensive Model to Estimate Early Allograft Failure Among Patients Requiring Early Liver Retransplant. *JAMA Surg*. 2020 Dec 1;155(12):e204095.

2. Lo IJ, Lefkowitch JH, Feirt N, et al. Utility of liver allograft biopsy obtained at procurement. *Liver Transplant*. 2008 May;14(5):639–46.

3. Frongillo F, Lirosi MC, Sganga G, et al. Graft steatosis as a risk factor of ischemic-type biliary lesions in liver transplantation. *Transplant Proc*. 2014 Sep;46(7):2293–4.

4. Agopian VG, Harlander-Locke MP, Markovic D, et al. Evaluation of Early Allograft Function Using the Liver Graft Assessment Following Transplantation Risk Score Model. *JAMA Surg*. 2018 May 1;153(5):436–44.

5. Levesque E, Martin E, Dudau D, et al. Current use and perspective of indocyanine green clearance in

liver diseases. *Anaesth Crit Care Pain Med*. 2016;35(1):49–57.

6. Lau H, Man K, Fan ST, et al. Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. *Br J Surg*. 1997 Sep;84(9):1255–9.

7. Cherchi V, Vetrugno L, Terrosu G, et al. Association between the donor to recipient ICG-PDR variation rate and the functional recovery of the graft after orthotopic liver transplantation: A case series. *PloS One*. 2021;16(8):e0256786.

8. Wang W, Zhao Q, Hu A, et al. Application of Indocyanine Green (ICG) Detection in Evaluating Early Prognosis in Patients with Fatty Liver Disease After Liver Transplantation. *Ann Transplant*. 2017;22:208–14.

9. Tang Y, Han M, Chen M, et al. Donor Indocyanine Green Clearance Test Predicts Graft Quality and Early Graft Prognosis After Liver Transplantation. *Dig Dis Sci*. 2017;62(11):3212–20.

10. Levesque E, Saliba F, Benhamida S, et al. Plasma disappearance rate of indocyanine green: a tool to evaluate early graft outcome after liver transplantation. *Liver Transplant*. 2009;15(10):1358–64.

11. Schneider L, Spiegel M, Latanowicz S, et al. Noninvasive indocyanine green plasma disappearance rate predicts early complications, graft failure or death after liver transplantation. *Hepatobiliary Pancreat Dis Int*. 2011;10(4):362–8.

12. Cherchi V, Vetrugno L, Zanini V, et al. Indocyanine green dye clearance test: early graft (dys)-function and long-term mortality after liver transplant. Should we continue to use it? An observational study. *J Clin Monit Comput*. 2021 May;35(3):505–13.

13. Chan A-W, Tetzlaff JM, Gøtzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ 2013;346:e7586.

14. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transplant*. 2010 Aug;16(8):943–9.

15. Bruegger L, Studer P, Schmid SW, et al. Indocyanine green plasma disappearance rate during the anhepatic phase of orthotopic liver transplantation. *J Gastrointest Surg*. 2008;12(1):67–72.

Data sharing statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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None.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,11

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1,11
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1,11
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5,6
Objectives	#7	Specific objectives or hypotheses	6,7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5-8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	5,6

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6,7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	9,10
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9,10
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6,7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6,7
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	5,6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n/4
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6-8
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	5
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8,9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8,9

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8,9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	2
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	6
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9,10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9,10
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect	10

		confidentiality before, during, and after the trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	10,11
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Patient Consent
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Notes:

- 32: supplementary 2 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 16. March 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai