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Indocyanine Green Angiography for Quality Assessment of Renal Graft Before Transplantation: A Pilot Study

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Abstract

Objectives: **Criteria for donation have been expanded to meet the great demand for organ transplant, resulting in different tools and classifications to help physicians to better assess the quality of the transplanted kidney. In this study, we evaluated the use of indocyanine green angiography as an additional tool to evaluate the renal microcirculation and the quality of the potential kidney graft.**

Materials and Methods: **All kidneys from extended criteria donors or donors after cardiac death available for transplant underwent indocyanine green angiography before implantation and after reconditioning, when hypothermic perfusion was required. We performed fluorescent angiography with a 10-mm-view laparoscope connected to a high-definition camera system while a solution of indocyanine green and Celsior was injected into the renal artery. We compared fluorescence intensities with postoperative graft function and then analyzed increases in fluorescence intensity before and after hypothermic machine perfusion treatment.**

Results: **In transplanted kidneys preserved in traditional cold storage, we found a statistically significant difference in fluorescence intensity values between groups with early graft function and delayed graft function. Fluorescence intensity increased significantly in all perfused kidneys after hypothermic machine perfusion treatment, indicating that intensity was directly proportional to improved renal microcirculation. Among 21 kidneys retrieved for transplant that adhered to the inclusion criteria, 11 were examined histopathologically, with a Karpinski score ranging from 2 to 7. The kidney that scored 7 was immediately discarded. Five underwent hypothermic pulsatile perfusion since they came from donors after cardiac death. Fluorescence intensity increased significantly in all perfused kidneys (4/5 were closest to doubling). Histopathological evaluations and Karpinski scores of the grafts indicated that all 5 were considered suitable for transplant.**

Conclusions: **Indocyanine green angiography can be used in the future as an additional useful tool to help physicians to assess graft quality before implantation.**

Key words: *Fluorescence intensity, Hypothermic machine perfusion, Kidney transplant*

Introduction

Regardless of age, kidney transplant is the best treatment for patients with end-stage renal failure.[1] Compared with dialysis, this surgical treatment enhances the patient's quality of life, increases overall survival, and lowers health care costs.[2-4] In general, time on wait lists ranges from 3 to 5 years and can be even longer, depending on the geographic area.[5] Living donations, including crossover and ABO-incompatible protocols, donations from non-heartbeat donors, and donations from extended criteria donors (ECDs),[6] have been considered to expand the donor pool due to the scarcity of available organs. Extended criteria donors are also called marginal donors. The category includes donors over 60 years old and those older than 50

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years old with 2 of the following: personal history of high blood pressure, creatinine serum level equal to 1.5 mg/dL, or cerebrovascular cause of death.[6] The purpose of these strategies is to increase the availability of kidneys for transplant, so that wait list time and the mortality associated with long-term dialysis can be decreased. With donations from marginal organs, transplants are more likely to result in graft primary nonfunction (PNF) or delayed graft function (DGF).

Some chance of long-term graft survival would be reduced by the augmented immunogenicity of the transplanted organs, which is followed by an increased risk of rejection.[7] A number of factors, including those related to the donor and recipient, play a role in determining the risk of PNF or DGF. According to experimental studies,[6] ischemia and the recovery of blood flow after an additional hypothermic injury trigger an elaborate sequence of events that results in DGF. With the consideration that there are no specific remedies available, early detection of the underlying pathologic mechanisms can enable us to adopt techniques that reduce the risk of this complication. To this end, the quality of the graft before transplant must be assessed with clinical, instrumental, and histologic methods that are accurate and specific, regardless of donor and recipient characteristics, to prevent complications or to determine whether to transplant the graft as a single or dual kidney or to discard it.[6]

In this study, our goal was to propose adding an additional evaluation that analyzes microcirculation patency using indocyanine green (ICG) angiography. Along with pretransplant biopsy findings and renal resistive index, assessed after hypothermic perfusion following reconditioning when performed, quantitative assessment of fluorescence intensity could contribute to better defining of graft quality.

Materials and Methods

We conducted a prospective cohort study between June 2020 and July 2021 in a high-volume kidney transplant center in Northern Italy. All kidneys from ECDs or donors after cardiac death (DCDs) available for transplant underwent ICG angiography during back table surgery before the implantation and after the reconditioning when hypothermic perfusion was required. In our cohort, there was only 1 exclusion criterion: allergy to ICG. Informed consent was obtained from each patient before the procedure. Preoperative investigations, organ removal, preservation, hypothermic perfusion, perioperative pharmacological therapy, and graft implant surgery were all carried out according to the protocol of our transplant center. An assessment of the Karpinski score was performed on all marginal kidney donors in accordance with international guidelines for donor risk management.

To perform fluorescence angiography, we used a 10-mm, 30-degree-view laparoscope connected to a full high-definition camera system (IMAGE 1 SPIES; Karl Storz GmbH) endowed with a particular filter for near-infrared (NIR) fluorescence and white light automatic optical detection. The xenon bulb is a powerful light source (D-Light P SCB; Karl Storz GmbH) for visible and NIR spectroscopy applications. By switching from white light to ICG mode via a pedal control, the surgeon can compare the images captured by the 2 modalities. Presently, most operating rooms can afford laparoscopic instruments, and fluorescence-guided procedures are performed for abdominal, lymph node, and breast surgery[8-12]; therefore, this technique is cost-effective since the hospital does not need to purchase another device. An assistant surgeon places the camera at the correct distance and covers the camera with a coat while analyzing grafts in a sterile environment. To obtain an accurate image of the kidney, the entire organ is included in the image.

In our tests, we melted 5 mg of ICG (ICG PULSION 25 mg/50 mg; PULSION Medical Systems) with 1 liter of Celsior solution and injected it directly into the renal artery of the graft with a catheter during back table surgery. The procedure was repeated after hypothermic machine perfusion (HMP) treatment when reconditioning was required. On the basis of previous research,[13] we selected the highest dose capable of producing a lifelike global microperfusion, despite limitations in the definition of maximum flow for the graft. A smaller amount of solution would have made it impossible to detect differences in perfusion, particularly in the case of compromised kidneys. The operating room lights were turned off every time the ICG was injected to record a 60-second video in complete darkness. We recorded the mean value of fluorescence in an HTML encoding system that combines the contributions of 3 colors (red, green, and blue) from none (value = 0) to full intensity (value = 255). Data were calculated in

3 regions of interest with a 100-pixel maximum diameter in the upper, medium, and lower poles, to minimize possible bias.

We analyzed the relationship between the mean fluorescence intensity values detected and the outcome of the graft in terms of DGF or PNF development. We also compared the mean fluorescence intensity values detected before and after HMP treatment and analyzed the relationship between fluorescence intensity and renal resistance at the end of perfusion. We performed statistical analysis using the program SPSS version 21 (SPSS Inc). Continuous variables are presented as mean and standard deviations, and ordinal variables are presented as median and ratio. We used Pearson correlation coefficient r analysis to find the degree of association between variables. A Mann-Whitney U test was conducted to compare the different groups, with $P < .05$ indicating statistical significance.

Results

Over 13 months beginning in June 2021, we analyzed 21 kidneys that were retrieved for transplant that adhered to the inclusion criteria. In total, 14 grafts were preserved using the "cold storage" method; the remaining 5 underwent hypothermic pulsatile perfusion since the graft donations were from DCDs. This procedure was not carried out on ECD grafts or on grafts from donors after brain death. Among the DCD donors, all met Maastricht III and II criteria: 2 died from postanoxic encephalopathy and 3 from cerebral hemorrhage, with a mean age of 61.5 years (minimum = 50 years; maximum = 79 years); 4 were men and 1 was a woman. Eleven kidneys were examined histopathologically, with a Karpinski score ranging from 2 to 7. The kidney that scored 7 was immediately discarded. Detailed demographic infor-mation about our population can be found in Table 1.

We conducted ICG angiography during bench surgery and after hypothermic perfusion when reconditioning was performed and completed (Table 2). When we analyzed transplanted kidneys preserved in traditional cold storage, there was a statistically significant difference in fluorescence intensity values between kidneys that immediately recovered function (9/13) and those that did not (4/13) ($P = .003$).

There were 8 men and 5 women among the recipients. All transplanted kidneys were derived from deceased donors (10 from brain death, 2 from cardiovascular arrest, and 1 from postanoxic encephalopathy). The mean age among recipients with early graft function (EGF) was 53.88 ± 13.48 years. In the group with DGF, mean age was 57.5 ± 8.54 years. There were 3 men (75.0%) and 1 woman (25.0%) in the DGF group, and 5 men (55.5%) and 4 women (44.4%) in the EGF group. In the DGF group, the average period of dialysis before transplant was 3.25 ± 1.70 years, whereas, in the EGF group, the average period was 3.88 ± 1.90 years. The average body mass index (in kilograms divided by height in meters squared) of recipients was 26.41 ± 4.38 in the DGF group and 26.66 ± 4.3 in the EGF group. The average age of the donor was 62.25 ± 16.68 years in the DGF group and 56.44 ± 14.91 years in the EGF group.

Table 1. Donor Demographics of the Kidneys Preserved With Cold Storage (N = 13 Kidneys)

	Delayed Graft Function Group (4/13)	Early Graft Function Group (9/13)
Age, years	62.25 ± 16.68	56.44 ± 14.91
Male/female	3/1	5/4
Weight, kg	77.5 ± 19.46	81.22 ± 12.57
Final serum creatinine, mg/dL	0.81 ± 0.17	0.86 ± 0.55
Cold ischemia time, h	18.55 ± 4.57	12.83 ± 3.57

Only the transplanted kidneys are listed; a kidney was discarded and excluded because of Karpinski score = 7.

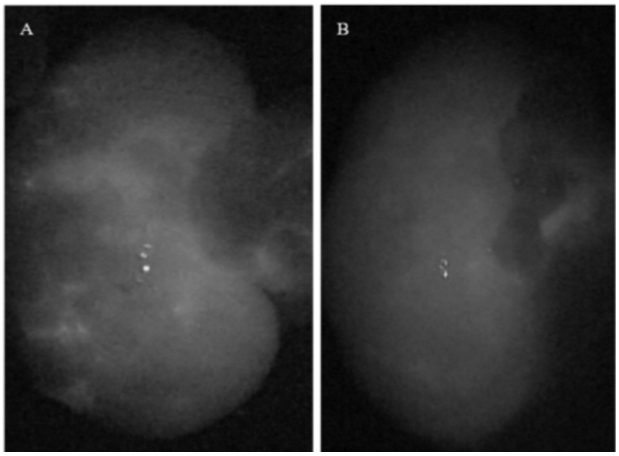
Table 2. Donor Demographics of the Kidneys Preserved With Hypothermic Machine Perfusion (N = 5 Kidneys)

	Delayed Graft Function Group (4/13)	Early Graft Function Group (9/13)
Age, years	65	54.75 ± 6.65
Male/female	1/0	4/0
Weight, kg	94.7	80.12 ± 17.21
Final serum creatinine, mg/dL	3.99	2.45 ± 0.44
Cold ischemia time, h	15	18.16 ± 4.19

Causes of death among the DGF donors included 3 (75%) who died from cessation of brain function and 1 who died from cardiocirculatory arrest (25%). Among the 9 donors of the EGF group, 7 (77.8%) died from brain death, 1 (11.1%) from cardiovascular arrest, and 1 (11.1%) from anoxia. The mean serum creatinine level of the donors was 0.81 ± 0.17 mg/dL for the DGF group and 0.86 ± 0.55 mg/dL for the EGF group. Cold ischemia time was 18.55 ± 4.57 hours for DGF kidneys and 12.83 ± 3.57 hours for EGF kidneys.

After HMP treatment, fluorescence intensity increased significantly in all perfused kidneys, with 4 of 5 close to doubling (Figure 1). In addition, we compared fluorescence intensity with the final resistance index measured after HMP treatment. This index was less than 0.6 for every kidney. After histopathological evaluation and measurement of Karpinski score of the grafts, all 5 kidneys were considered suitable for transplant: 3 were implanted as single kidney transplants and 2 were transplanted into the same recipient as double kidney transplants. Recipients were men with an average age of 54 years (minimum = 48 years, maximum = 63 years). The mean body mass index was 25.47 ± 3.25; 2 of 4 patients had high blood pressure, and none had a history of diabetes. Three patients experienced early recovery of renal function, immediately after transplant. One patient developed DGF. The mean creatinine value on postoperative day 7 was 5.46 ± 2.69 mg/dL.

Figure 1. Fluorescence Became More Intense and Homogeneous After Hypothermic Perfusion Treatment



(A) Before treatment. **(B)** After treatment.

Discussion

According to current guidelines, patients over 60 years of age or with diabetes, hypertension, and vascular diseases should be screened for graft quality by an anatomical-pathological score before transplant.[14-16] Nevertheless, histopathological evalu-ation of the renal specimen can determine chronic damage, but the histologic picture of tubular necrosis only becomes apparent when acute damage occurs, as shown for DCDs.[17-19] Although the histologic analysis alone does not appear adequate for assessing the quality of grafts in general, it does seem to be an important component of the assessment. In fact, several biases have to be considered, such as the site where the biopsy is performed, the timing after the ischemic injury, and the experience of the pathologist.[20] Furthermore, the value of each histologic test must be linked to the patient's outcome.

As demonstrated by Mohan and colleagues, even when histologic examinations do not appear excellent, 73.2% of kidneys work for 5 years.[21] Based on outcome data and their relationships with renal resistance index and biopsy score, other authors, especially Bissolati and colleagues,[22] investigated the prognostic role of the renal resistive index during HMP: kidneys that reached renal resistance ≤1 hour after perfusion began showed a lower rate of PNF and DGF AND a faster decrease in creatinine.[22] There might be a possibility for their alternative use if a correlation would exist between renal resistance and histologic score. In addition to the absence of the well-known risks related to biopsy, renal resistance would have the advantage of a quicker acquisition time.

In this study, we examined the feasibility and validity of fluorescent angiography with ICG dye before the graft was implanted and after hypothermic renal perfusion in predicting the outcome of the transplanted kidney. Through the evaluation of microcirculation patency and its improvement after HMP treatment, we aim to develop a technique for assessment of graft quality. We hypothesize that the intensity of fluorescence is directly associated with microvessels affected by brain death, ischemia, or reperfusion injury after kidney transplant. Upheaval of microcirculation could be due to the initial destruction of the endothelial cells, resulting in an increase in renovascular resistance.[23]

In addition to mechanical causes (due to changes in blood vessel flow), prostaglandins and other regulatory peptides can induce vasomotor twitches, free radical damage, and release of cytokines.[24,25] In one of our previous papers,[13] we extensively described fluorescence intensity data obtained intraoperatively and assessed 45 minutes after graft reperfusion and its proportional correlation with intraoperative cortical microcirculation. As a result of its pharmacological properties, ICG was selected as the most appropriate stain.

An ICG molecule has a negative charge and is a water-soluble tricarbocyaninic compound, which belongs to the larger family of cyanines. It was developed during World War II for photographic

purposes and was later used by Kodak Research Laboratories for NIR photography in 1955. It was tested for clinical use in humans for the first time in 1956 at the Mayo Clinic, and the US Food and Drug Administration approved its use in 1959, primarily for liver function tests. Since the early 1970s, angiography has been considered feasible, mainly for retinal examinations.[26-28] As soon as the ICG is injected intravenously, it binds to plasma proteins[29] and remains confined to the intravascular space, resulting only in a small spreading within the interstitium. In addition, ICG is metabolized by the liver with a half-life of 3 to 5 hours and excreted in the bile. Its carrier protein is glutathione S-transferase, but its metabolites are unknown. Despite its harmful nonionizing properties, the highest dose tested in humans (5 mg/kg) has not been shown to cause toxic effects.

A number of factors make ICG a good angiography tool: its suitability for vascular dissemination, good signal-to-noise ratio (tissues have little self-fluorescence in the NIR and low background noise), and its action in the infrared and visible spectrum region, where living tissue absorbs NIR light.[27,28] According to our pilot study results, ICG angiography correctly depicts graft microcirculation and its improvement as a result of HMP therapy. Combined with the donor's medical history and biopsy scoring, a quantitative assessment of fluorescence intensity before grafting could guide the physician on graft quality. The data obtained, combined with renal resistance when available, can also aid in the decision regarding a single versus double transplant or graft refusal based on the overall state of the parenchyma after acute ischemia.

Conclusions

Our study results suggest that fluorescence angiography is a useful tool for assessing microcirculation patency, as well as its improvement, after HMP reconditioning. It would be beneficial to conduct further studies to validate the method for evaluating the quality of the graft before transplant, together with the assessment of renal resistive index and histology.

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