GAVIN PUBLISHERS

OPEN ACCESS

Journal of Urology and Renal Diseases

Research Article

Ietto G, et al. J Urol Ren Dis 06: 1230.

DOI: 10.29011/2575-7903.001230

Indocyanine Green (ICG) Angiography for Assessing Microcirculation Patency Improvement after Hypothermic Machine Perfusion (HMP) Re-Conditioning: A Pilot Study

Giuseppe Ietto[1*], Linda Liepa[1], Marika Morabito[1], Federica Masci[1], Elisa Bottazzoli[1], Davide Inversini[1], Marta Ripamonti[1], Natalia Palamara[1], Mattia Gritti[1], Davide Brusa[1], Enrico Ferri[1], Martina Pardo[1], Sara Marzorati[1], Cristiano Parise[1], Elia Zani[1], Domenico Iovino[1], Matteo Tozzi[2], Daniela Dalla Gasperina[3] and Giulio Carcano[1]

[1]Department of General, Emergency and Transplant Surgery, ASST-Settelaghi and University of Insubria, Varese, Italy

[2]Department of Vascular Surgery, ASST-Settelaghi and University of Insubria, Varese, Italy

[3]Department of Infectious Disease, ASST-Settelaghi and University of Insubria, Varese, Italy

[*]**Corresponding author:** Giuseppe Ietto, Department of General, Emergency and Transplant Surgery, ASST-Settelaghi and University of Insubria, Varese, Italy

Citation: Ietto G, Liepa L, Morabito M, Masci F, Bottazzoli E, et al. (2021) Indocyanine Green (ICG) Angiography for Assessing Microcirculation Patency Improvement after Hypothermic Machine Perfusion (HMP) Re-Conditioning: A Pilot Study. J Urol Ren Dis 06: 1230. DOI: 10.29011/2575-7903.001230

Received Date: 04 August, 2021**; Accepted Date:** 17 August, 2021**; Published Date:** 20 August, 2021

Abstract

Background: Nowadays, given the widening gap between organs available and patients on dialysis waitlisted for kidney transplant, many efforts are steered to expand the donor pool. Resorting consistently to marginal organs, clinical, instrumental and histological methods, that will be specific and accurate in assessing the quality of the graft before transplant, might be advisable in order to prevent any complications, helping to state if the graft has to be transplanted as a single or dual kidney or discarded. Our aim is to test indocyanine green angiography for the assessment of microcirculation patency improvement after Hypothermic Perfusion Machine (HMP) re-conditioning in order to contribute to defining graft quality, jointly with pretransplant biopsy findings and renal resistive index assessed at the end of hypothermic perfusion.

Methods: We conducted a prospective cohort study performing indocyanine green fluorescent angiography during back table surgery before and after hypothermic perfusion on all kidneys available for transplantation which required the treatment because retrieved from Extended Criteria Donors (ECDs) or Donors After Cardiac death (DCDs).

Results: From June 2020 to July 2021 we enrolled 5 grafts retrieved from DCDs selected for transplant and treated through HMP. All perfused kidneys showed a significant rise in terms of fluorescence intensity after HMP treatment: four out of five closes to doubling. Furthermore, statistical analysis demonstrated a moderate correlation (r = 0.39727998889383) between fluorescence intensity with the final resistance index scanned at the end of HMP treatment.

Conclusions: Our results establish how fluorescence can be a valid and cost-effective method for evaluating the graft before transplantation, jointly with histology and renal resistive index assessment. Further studies are needed to standardize this technique.

Keywords: Angiography; HPM; Graft; Fluorescence; ICG; Kidney; Transplant

Introduction

Kidney transplantation is the treatment of choice for end stage renal failure patients [1], even for older ones. Compared to dialysis, this surgical option improves patients quality life, increases overall survival and lowers healthcare costs [2-4]. Generally, waitlists can vary from 3 to 5 years, and even more depending on different areas of interest [5]. Due to scarcity of available organs, different ways have been thought to expand the donor pool: Living Donation (LD) - including crossover and AB0-incompatible protocols-, Non-Heart Beating Donors (NHBDs) and Extended Criteria Donors (ECDs) [6] The ECDs donors are also known as marginal donors. This category includes the donors older than 60 years of age and the ones older than 50 years with two of the following: personal history of high blood pressure, creatinine serum level equal or greater than 1.5 mg/dl, or cerebro-vascular

cause of brain death [6].

Even though these strategies could increase kidneys availability for transplant reaching the goal of decreasing the average waitlisting time and the following mortality associated with long term dialysis therapy, it has been emphasize how transplants performed with these marginal organs are burdened by a greater risk of graft Primary Non Function (PNF) or Delayed Graft Function (DGF), which is associated with major transplanted organ immunogenicity, increased risk of rejection and a lower long-term graft survival [7]. Many factors relating to the donor and the recipient too, play a role in determining the raised risk of PNF or DGF. Experimental studies highlighted that ischemia and the restoration of blood flow after hypothermic storage activate a complex sequence of events resulting in DGF [6]. An accurate knowledge of these mechanisms allows adopting strategies useful to reduce the risk of this complication, even if no specific treatments are available. On the other hand, clinical, instrumental and histological methods that will be specific and accurate in assessing the quality of the graft before transplant, regardless of the characteristics of the donor and the recipient, might be able to prevent any complications, helping to state if the graft has to be transplanted as a single or dual kidney or to discard it [6].

Our aim is to test indocyanine green angiography for the assessment of microcirculation patency improvement after Hypothermic Machine Perfusion (HMP) re-conditioning. The intensity value of fluorescence might contribute to defining graft quality, jointly with pretransplant biopsy findings and renal resistive index assessed at the end of hypothermic perfusion.

Materials and Methods

We conducted a prospective cohort study between June 2020 and July 2021 in a high-volume kidney transplant centre in Northern Italy. All kidneys available for transplantation from ECDs or DCDs which required hypothermic perfusion underwent indocyanine green angiography during back table surgery before and after the treatment. There was only one exclusion criterion: allergy to ICG stain of the recipients selected for graft implant, moreover involving none in our cohort. Before starting, the physician completely informed every patient and written consensus was obtained. We performed all the steps according to the protocol of our transplant center: preoperative investigation, organ removal and their preservation, hypothermic perfusion treatment, perioperative pharmacological therapy and graft implant surgery. According to International Guidelines for donor risk management, in all cases of marginal kidney donors a biopsy of the graft was performed to estimate the histopathological Karpinski Score.

We used a 10 mm with 30 degree view laparoscope connected to a full high definition camera system (IMAGE 1 SPIES; Karl Storz GmbH) endowed with a particular filter for NIR fluorescence and white light (WL) automatic optical detection. The xenon bulb is a powerful light source (D-Light P SCB; Karl Storz GmbH) for visible and NIR spectroscopic applications. For the surgeon it is easy to switch from WL to ICG mode using a pedal control and compare the images captured by these two modalities. Nowadays the majority of the operating theatres can afford laparoscopic instruments and fluorescence-guided surgery is used for abdominal, lymph node, and breast surgery [8-12]; so, this technique results to be cheap, because the hospital doesn't need to buy another new device. The graft analysis is performed in a sterile way, with the camera covered by a coat and placed at the right distance by the assistant surgeon, in order to include into the images the whole kidney, from the upper to the lower pole. In our tests, we melted ICG (Indocyanine green PULSION 25 mg/50 mg; PULSION Medical Systems, Feldkirchen, Germany) 5 mg with 1 Litre of Celsior® solution, then the surgeon injected the solution through a catheter directly into the renal artery of the graft during back table surgery before and after HMP treatment. Based on previous studies, we have chosen the highest dose capable of showing a lifelike global microperfusion, although there were limitations in the graft's maximal flow definition; differences in perfusion would have been impossible to detect with a smaller amount of solution, especially in case of compromised kidneys.

Everytime we injected the ICG, the lights in the operating room were turned off to record a 60" video in complete darkness. For our purpose we registered the mean value from a HTML encoding system which combines the contribution of three colours (RGB: Red, Green, Blue) from none (0) to full intensity (255). This data was calculated in three Regions of Interest (ROI) with a 100 pixels maximum diameter in the upper, medium and lower pole, to ensure a reduction of possible bias. The mean fluorescence intensity values detected before and after HMP treatment were compared. In addition, a relationship between fluorescence intensity and renal resistance reached at the end of perfusion treatment was analysed. Lastly, a statistical analysis of all the data was carried out using the program SPSS 21 (SPSS Inc., Chicago, IL, USA). Mean value and standard deviation defined continuous levels variables, instead median and ratio the ordinal ones. Pearson's correlation coefficient r analysis was used to demonstrate the variables association degree. A Mann-Whitney U test was carried out to compare the different groups, using a p value < 0.05.

Results

Starting from June 2020, we carried out 31 kidney transplants over a year. 26 grafts were preserved through "cold storage" method; 5 underwent hypothermic pulsatile perfusion because they were considered marginal organs as they came from DCDs. Neither ECDs nor DBDs received hypothermic pulsatile perfusion. All DCDs donors match Maastricht III and II criteria: 2 died from post-anoxic encephalopathy and 3 from cerebral haemorrhage,

mean age was 61.5 (max 79, min 50) years, 4 were males and 1 female. Four kidneys underwent histopathological examination and the Karpinski score ranged from 2 to 5. All main demographic features of our population are summarized in table (Table 1).

Donor demographics	
Age	56.4±14.48 (79-18)
Male/Female	17/14
Diabetes	4 (13.3%)
Hypertension	10 (33.3%)
Karpinski score	3 (2-5)
Causes of death	
Brain death	22 (71%)
Cardiac death	3 (9.67%)
Post-anoxic encephalopathy	6 (19.35%)

Table 1: Donor demographics.

ICG angiography was performed during bench surgery as well as at the end of hypothermic perfusion for all HMP treated grafts (Table 2).

Graft	**Fluorescence intensity** Before HMP treatment	**Fluorescence intensity** Before HMP treatment	Renal resistance (RR)
1	25.68	26.5	0.47
2	8.85	15.68	0.55
3	12.03	24.35	0.29
4	9.20	14.79	0.29
5	9.22	20.72	0.15

Table 2: Fluorescence intensity before and after HMP; renal resistance.

All perfused kidneys showed a significant rise in terms of fluorescence intensity after HMP treatment: four out of five closes to doubling (Figure 1).

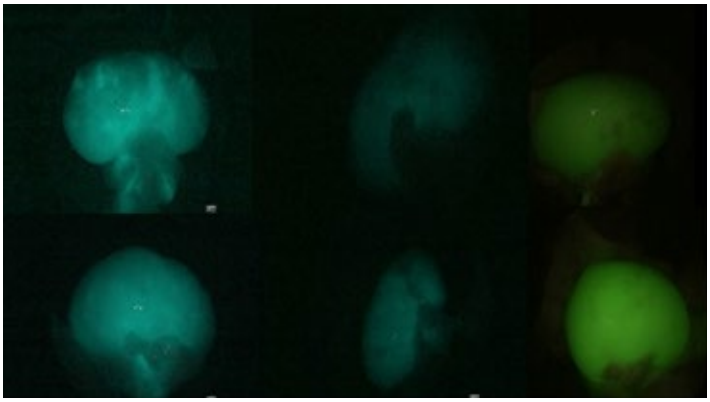

Figure 1: ICG fluorescence intensity on back table before HMP treatment (upper row) and after HMP treatment (lower row).

Furthermore, we compared fluorescence intensity with the final resistance index scanned at the end of HMP treatment. This index was less than 0.6 for every kidney. Statistical analysis demonstrates a moderate correlation (Pearson's coefficient r = 0.39727998889383) between the two values. Due to histopathological evaluation of the grafts and their Karpinski score, all 5 kidneys were considered fit for transplantation: three for single kidney transplantation, while two grafts were transplanted in the same recipient performing a Double Kidney Transplantation (DKT). All the four recipients were male with a mean age of 54 (min 48, max 63) years. Mean BMI was 25.47 ± 3.25; 2 out of 4 patients had high blood pressure, none of them had a history of diabetes. Three patients experienced early recovery of renal function, immediately after transplantation. Otherwise, only one patient developed DGF. The mean creatinine value on the 7th postoperative day was 5.46 ± 2.69.

Discussion

Current guidelines recommend performing a biopsy before transplantation for all patients over 60 years of age or with a history of diabetes, hypertension and vascular diseases, in order to assess graft quality by an anatomical-pathological score [13-15]. However, histopathological evaluation of the renal specimen can define chronic damage, but the histological picture tubular necrosis only can be revealed when an acute damage occurs, as for DCDs [16-18]. On the whole though, the histological analysis alone does not seem to be sufficient for the overall assessment of grafts quality. In fact, several biases have to be kept in count

such as the site where the biopsy is performed, the timing after the ischemic injury, the experience of the pathologist [19]. Plus, the real value of each histological data requires to be related to the patient's outcome. In a recent study, Mohan et al. demonstrated that 73.2% of the kidneys maintain their function for 5 years, even if the histological examination does not appear to be excellent.

Other authors, particularly Bissolati et al., investigated the prognostic role of renal resistive index during HMP based on outcome data and their relationship with the biopsy score: kidneys that reached a Renal Resistance (RR) ≤ 1 one hour later the perfusion beginning showed a lower rate of PNF and DGF as well as faster decrease of creatinine [20]. If the correlation between the renal resistance and histologic score will be confirmed their alternative use might be approved. Main advantage of RRs would be a shorter acquisition time in absence of well-known biopsy risks. In this study, we investigated the feasibility and validity of fluorescent angiography using ICG as a dye before and after hypothermic perfusion of the kidney. Our aim was to obtain a technique for immediately verifying the improvement of microvasculature after HMP treatment in renal graft parenchyma. Actually, we suppose that microvessels suffering from brain death, ischemia and reperfusion time that are behind renal function recovery course after transplantation, can directly be related to fluorescence intensity. Is well known that first microcirculation upheaval is due to endothelial cells destruction, resulting in renovascular resistance raising [21]. Other causes of tissue oxygenation deprivation and consequently tubular necrosis could be mechanical (due to sudden changes in blood vessel flow), prostaglandin and other regulatory peptides induced vasomotor twitch, free radical damage and cytokine release [22,23]. This extremely complex pathophysiology explains the proportional correlation with intraoperative cortical microcirculation assessed at least 45 minutes after graft reperfusion and the data obtained on the fluorescence intensity extensively described in one of our previous papers [24].

Due to its pharmacological characteristic, ICG was chosen as the most fitting stain. ICG is a water-soluble tricarbocyanine compound, belonging to the larger family of cyanine, a 776-Da disulfonated molecule with a negatively charged ion [25]. ICG was developed during World War II for photographic application and later used for NIR photography by the Kodak Research Laboratories in 1955. Firstly tested for clinical use in humans in 1956 at the Mayo Clinic, US Food and Drug Administration allowed its utilization in 1959 , primarily in liver function diagnostic tests [26]. Only ten years later was judged feasible for angiography use [27], mainly for retinal examination since the early 1970s [25,27,28]. After intravenous injection, ICG properly binds plasma proteins very quickly. ICG has the tendency to remain mainly in the intravascular space, yielding poor affinity with interstitium distribution. ICG has hepatic metabolism with a 3 to 5 half-life and bile excretion; its carrier is the glutathione S-transferase protein and its metabolites are unknown. It's a harmful nonionizing stain, and the highest dose tested in human (5 mg/kg of body weight) has not shown toxic effects. ICG results to be optimal for angiography use for several reasons: its affinity with vascular dissemination; good signal-to-noise ratio (tissues have little NIR self-fluorescence and low noise background) and its action into the portion of visible and infrared spectrum where living tissue absorbs the lights [NIR]) [27,28]. Our pilot study results suggest that ICG angiography correctly portrays graft microcirculation and its improvement thanks to HMP. A quantitative assessment of fluorescence intensity before and after HMP might clarify the contribution of hypothermic perfusion to reconditioning the graft. Data obtained in combination with RR can describe parenchyma state after the acute injury due to ischemia time and might contribute to guide the decision of performing a single vs a double transplantation or the graft refusal.

Conclusions

In conclusion, our study demonstrates that fluorescence angiography may be a useful tool for assessing microcirculation patency improvement after HMP re-conditioning. Further studies would be advisable to validate the method for evaluation of the quality of the graft before transplantation, jointly with histology and renal resistive index assessment.

References

1. RA W, VB A, EL M, AO O, RE E, et al. (1999) Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. The New England journal of medicine 1999: 341.
2. YS L, JL B, LR A, MH H-K, MG H (2007) Quality of life assessed with the Medical Outcomes Study Short Form 36-Item Health Survey of patients on renal replacement therapy: a systematic review and meta-analysis. Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research 10.
3. de Wit A (2002) Economic evaluation of end-stage renal disease treatment 2002.
4. Meier-Kriesche HU, Port FK, Ojo AO, Rudich SM, Hanson JA, et al. (2000) Effect of waiting time on renal transplant outcome. Kidney Int 58: 1311-1317.
5. SIT 2021.
6. FK P, JL B-G, RA M, DM D, BW G, et al. (2002) Donor characteristics associated with reduced graft survival: an approach to expanding the pool of kidney donors. Transplantation 2002: 74.
7. N P, D C, MH S, G R (2004) Delayed graft function in kidney transplantation. Lancet 364.
8. Ishizawa T, Kokudo N (2013) History and basic technique of fluorescence imaging for hepatobiliary-pancreatic surgery. Frontiers of Gastrointestinal Research 2013: 1-9.

9. Cahill RAaRFaMNJ (2011) Near-infrared laparoscopy for real-time intra-operative arterial and lymphatic perfusion imaging. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland 7: 12-17.

10. Kitai TaITaMMaST (2005) Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. Breast cancer (Tokyo, Japan) 12: 211-215.

11. Ietto G, Amico F, Soldini G, Chiappa C, Franchin M, et al. (2016) Real-time Intraoperative Fluorescent Lymphography: A New Technique for Lymphatic Sparing Surgery. Transplant Proc 48: 3073-3078.

12. Ietto G, Iovino D, Soldini G, Zani E, Parise C, et al. (2019) Indocyanine Green as a Beacon Light in Laparoscopy: A New Application in Transplant Surgery: A Case Report. Transplantation Proceedings 51: 532-537.

13. G R, J G, P R, M B, EH C, et al. (1999) Early experience with dual kidney transplantation in adults using expanded donor criteria. Double Kidney Transplant Group (DKG). Journal of the American Society of Nephrology : JASN 1999: 10.

14. J K, G L, D C, S F, J Z, et al. (1999) Outcome of kidney transplantation from high-risk donors is determined by both structure and function. Transplantation 67.

15. Lee CY, Zhang JX, Jones Jr JW, Southard JH, Clemens MG (2002) Functional recovery of preserved livers following warm ischemia: Improvement by machine perfusion preservation. Transplantation 74: 944-951.

16. SC H, RL K, S A, MA S, DE K, et al. (2002) Molecular and immunohistochemical characterization of the onset and resolution of human renal allograft ischemia-reperfusion injury. Transplantation 2002: 74.

17. P C, M Z, L C, E B, A M, et al. (2013) Impact of the pre-transplant histological score on 3-year graft outcomes of kidneys from marginal donors: a single-centre study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 28.

18. P D, A K, A P-P, M W, L P, et al. (2009) Assessment of kidneys procured from expanded criteria donors before transplantation. Transplantation proceedings 2009: 41.

19. I G, G G, C G, S B, A C, et al. (2020) Pre-implantation kidney biopsy: value of the expertise in determining histological score and comparison with the whole organ on a series of discarded kidneys. Journal of nephrology 2020: 33.

20. M B, PG G, R C, G G, O A, et al. (2018) Renal Resistance Trend During Hypothermic Machine Perfusion Is More Predictive of Postoperative Outcome Than Biopsy Score: Preliminary Experience in 35 Consecutive Kidney Transplantations. Artificial organs 2018: 42.

21. M A, T K, M W, O H, U H, et al. (2003) Assessment of renal graft function by perioperative monitoring of cortical microcirculation in kidney transplantation. Transplantation 75.

22. Shoskes DA, Halloran PF (1996) Delayed graft function in renal transplantation: etiology, management and long-term significance. J Urol 155: 1831-1840.

23. Yin M, Kurvers HA, Tangelder GJ, Booster MH, Daemen JH, et al. (1995) Intravital microscope studies of the ischemically injured rat kidney during the early phase of reperfusion. Transplant Proc 27: 2847-2848.

24. G I, E Z, F B, C P, V I, et al. (2021) Indocyanine Green Angiography for Quality Assessment of the Kidney During Transplantation: An Outcome Predictor Prospective Study. Transplantation proceedings 2021.

25. Mishra (2000) Cyanines during the 1990s: A Review. Chemical reviews 100: 1973-2012.

26. Kamisaka KaYYaYHaKH. (1974) The binding of indocyanine green and other organic anions to serum proteins in liver diseases. Clinica chimica acta; international journal of clinical chemistry 53: 255-264.

27. Kang YaLJaKKaCC (2010) Dynamic fluorescence imaging of indocyanine green for reliable and sensitive diagnosis of peripheral vascular insufficiency. Microvascular research 80: 552-555.

28. Alander JTaKIaLAaP (2012) A review of indocyanine green fluorescent imaging in surgery. International journal of biomedical imaging 2012: 940585.