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ORIGINAL ARTICLE

Title: Beta Cell Death by Cell-Free DNA and Outcome after Clinical Islet Transplantation

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Authorship: B.L.G-L., B.G., R.S., Y.D. and A.M.J.S. designed research studies; B.L.G-L, T.K., P.S., D.O., A.J.M. and A.M.J.S. performed transplant procedures; D.N. and S.P processed plasma and analyzed cfDNA; B.L.G-L., T.K, A.R.P., P.C., B.G., R.S., Y.D. and A.M.J.S. acquired and analyzed data; and B.L.G-L., B.G., R.S., Y.D. and A.M.J.S. wrote the paper.

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Abbreviations

AIHS, Alberta Innovates Health Solutions

AUC, Area Under the Curve

A1C, Glycated Hemoglobin

cfDNA, Cell Free DNA

CNTRP, Canadian National Transplant Research Program

IBMIR, Instant Blood-Mediated Inflammatory Response

IE, Islet Equivalent

ISI, Islet Size Index

IT, Islet Transplantation

JDRF, Juvenile Diabetes Research Foundation

OCR, Oxygen Consumption Rate

PCV, Packed Cell Volume

ROC, Receiver Operating Characteristic

TPV, Total Plasma Volume

T1DM, Type 1 Diabetes Mellitus

USAID, The United States Agency for International Development

cPRA, Calculated Panel Reactive Antibodies (cPRA)

DSA, donor-specific antibodies

Abstract

Background: Optimizing engraftment and early survival after clinical islet transplantation is critical to long-term function, but there are no reliable, quantifiable measures to assess beta cell death. Circulating cell free DNA (cfDNA) derived from beta cells has been identified as a novel biomarker to detect cell loss, and was recently validated in new-onset type 1 diabetes and in islet transplant patients.

Methods: Herein we report beta cell cfDNA measurements after allotransplantation in 37 subjects and the correlation with clinical outcomes.

Results: A distinctive peak of cfDNA was observed 1hr after transplantation in 31/37 (83.8%) of subjects. The presence and magnitude of this signal did not correlate with transplant outcome. The 1hr signal represents dead beta cells carried over into the recipient after islet isolation and culture, combined with acute cell death post infusion. Beta cell cfDNA was also detected 24hrs post-transplant (8/37 subjects, 21.6%). This signal was associated with higher 1-month insulin requirements ($p=0.04$), lower 1-month stimulated C-peptide levels ($p=0.01$) and overall worse 3-month engraftment, by insulin independence (ROC:AUC=0.70, $p=0.03$) and Beta 2 score (ROC:AUC=0.77, $p=0.006$).

Conclusions: cfDNA-based estimation of beta cell death 24hrs after islet allotransplantation correlates with clinical outcome and could predict early engraftment.

Introduction

Despite the success of clinical islet transplantation (IT), outcomes may still be hampered by cell death occurring early after intraportal infusion. Following transplantation, islets are hypoxic, and exposed to the instant blood-mediated inflammatory response (IBMIR) and alloimmunity [1]. As a consequence, a significant portion of the graft is lost at this early stage often necessitating multiple subsequent donor islet infusions to achieve insulin independence [2,3]. An inability to accurately determine cell death after intraportal islet transplantation remains a limiting factor in predicting early and long-term graft function, and in identification of modifiable factors that could enhance early engraftment. In most cases graft loss can only be measured by its functional consequence at a stage where a potential therapeutic window has been closed [4].

Assessing engraftment and graft function is not a straightforward process and normally relies on complex metabolic tests of insulin secretory profiles, which are both time consuming and expensive [4]. Multiple scoring systems have been implemented to measure post-transplant function more accurately including the Beta 2 Score recently described by our group [4], which can be obtained without using a stimulant to successfully discriminate between glucose intolerance and insulin independence after IT. A limitation to this functional approach is the lack of quantifiable evidence of graft loss and its corresponding clinical impact.

When cells die, fragments of their genomic DNA are released to circulation, where they travel shortly before being cleared by the liver. In a recent breakthrough, Akirav and Herold showed that hypomethylation in the insulin gene

promoter, which is unique to beta cells, can be used to detect DNA derived specifically from dying beta cells [5]. Indeed, studies from their group and other investigators showed that unmethylated insulin promoter circulating cell free DNA (cfDNA) can be detected in the blood of patients recently diagnosed with type 1 diabetes mellitus (T1DM), raising the exciting possibility that this novel type of biomarker can be used to precisely monitor beta cell death [6-8]. We have recently reported a new version of this technology, based on next generation sequencing, for the assessment of beta cell death as well as multiple other tissues based on tissue-specific methylation markers. We used the method to assess the levels of beta cell-specific cfDNA in serum and plasma of healthy individuals, in T1DM patients and in islet graft recipients [9]. While healthy individuals had extremely low concentrations of beta cell-derived cfDNA, a clear signal was observed in recently diagnosed T1DM patients. Specifically in islet graft recipients, we observed significant levels of beta cell-specific cfDNA as early as 1hr after islet infusion, and a second less intense beta-cell cfDNA signal 24hrs after transplantation, which could be an objective expression of early graft loss [9]. Since the clinical utility of this novel technology is not defined, we sought to characterize cfDNA measurements in a clinical islet allotransplantation setting, and to correlate findings with clinical outcomes as a method to complement the initial assessment of islet engraftment and function. Here we report the outcome of these studies, pointing to beta cell cfDNA as a promising prognostic biomarker for clinical islet transplantation.

Materials and Methods

Study design

Clinical islet isolations and allotransplants were performed at the University of Alberta, Canada over a 14-month period using defined standard of care. Only those subjects receiving a first intraportal allotransplant or a re-transplant after a remote (>1yr) previous infusion were included in the analysis (n=37) to avoid multiple confounding factors derived from previous transplants. This study was part of the on-going review of islet transplant patients at the University of Alberta (protocol number Pro00001120) in collaboration with JDRF and the Hebrew University (study number RES-0024003). This investigation is approved by the University of Alberta Health Research Ethics Board and conducted in accordance with the principles endorsed by the Declaration of Helsinki.

Human islet isolation, purification and culture

Human islet preparations were isolated from deceased donor pancreata, as previously described using a collagenase/thermolysin enzyme mixture (Roche Diagnostics, Laval, QC, Canada) and the resulting digest suspension was purified using a modified COBE 2991 cell processor (Terumo BCT, Inc., Lakewood, CO, United States) with continuous density gradients [2,10-12]. The purified islet fraction(s) were assessed for clinical suitability and cultured in CMRL 1066-based medium at 22°C (5% CO2) for up to 72 hours prior to transplantation.

Islet product characterization

Islet dose was calculated using standard methods [12]. Briefly, islets >50 μm in diameter were enumerated by manual count with an inverted light microscope and classified into size ranges in increments of 50 μm. The number of islets particles in each size range was converted to islet equivalent (IE) to account for size difference. Furthermore, islet size index (ISI) was calculated by dividing the total number of IE in a preparation by the total number of islet particles to reflect the average particle size in a preparation [12]. After the culture period, islets were assessed for viability and functionality. Viability was assessed by fluorescent membrane integrity assay with counterstains using SYTO 13 green fluorescent nucleic acid stain (Life Technologies, Burlington, ON, Canada) and ethidium bromide (Sigma-Aldrich, ON, Canada) [13-15]. Samples were manually assessed using fluorescent microscopy and reported as a percentage of viable to all cells. Hormonal islet secretory function was assessed by static glucose-stimulated insulin secretion (s-GSIS), sequentially performed at low (2.8 mmol/L) and high (28.0 mmol/L) glucose concentrations. The amount of insulin released was measured using an ELISA (Mercodia Insulin ELISA, Mercodia, Uppsala, Sweden) and a stimulation index was calculated as the ratio of stimulated to basal insulin secretion.

Oxygen consumption rate (OCR) is a real-time, operator-independent method of assessing fractional cell viability. OCR was measured as described previously [16-19]. OCR was normalized to the DNA content per chamber by collecting the islets

and assessing for DNA by using a double-strand DNA fluorescent dye (Quant-iT PicoGreen dsDNA Assay Kit, Invitrogen, Life Technologies Corporation, Grand Island, NY) resulting in OCR/DNA (nmol O2/min ● mg DNA). To further characterize the preparation, the mean OCR/DNA value of an islet preparation was normalized to Islet Size Index (ISI) (OCR/DNA/ISI) and the islet dose (IE/kg), as previously reported [20].

Islet transplantation procedure

Islet transplant procedures were performed using a previously reported procedure [21]. Islets were suspended in 100 mL of CMRL-based transplant media supplemented with human serum albumin and HEPES buffer into an infusion bag. Patients received the infusion via a catheter placed intraportally, performed under local anesthetic and with combined ultrasonography and radiology guidance. Final vascular track ablation with Avitene paste (Davol, Inc., Warwick, RI, United States) was done following the procedure.

Immunosuppression consisted of T depletional induction therapy with alemtuzumab (MabCampath, Genzyme Corp.) and anti-inflammatory therapy included etanercept (Enbrel; Amgen Canada Inc., Mississauga, ON.) and anakinra (Kineret, Amgen Canada Inc., Mississauga, On.). Maintenance twice daily tacrolimus (Prograf, Astellas Pharma Canada Inc., Markham, ON.) was adjusted to provide target trough levels of 10 – 12 µg/L, together with mycophenolate mofetil (CellCept®, Hoffmann-La Roche Ltd., Mississauga, ON.). Three subjects received basiliximab (Simulect®, Novartis Pharmaceutical

Canada Inc. Dorval, QC) induction instead of alemtuzumab. All subjects received peritransplant insulin-heparin infusions as per our standard protocol and following the procedure; their insulin requirements were adjusted according to functional indicators [22]. All recipients and donors were HLA typed by low to medium resolution typing (LabType® SSO One Lambda A Thermofisher Scientific at HLA A, B, C,DRB1, DRB345, DQA1, DQB1, DPA1 and DPB1. Pre-transplant antibody testing was performed with single antigen bead testing (LabScreen® One Lambda. Calculated panel reactive antibodies (cPRA) was calculated using the Canadian cPRA calculator (https://ctr2.transplantregistry.ca/otd-cpra-client/ctr2.jsp). Flow crossmatches were performed on pre-transplant serum [23].

Measurement of cfDNA

Beta cell-specific cfDNA was measured in plasma from study subjects as reported by Lehmann-Werman and collaborators [9]. Blood samples were collected from patients at 1hr, 24hrs, 7 days and 1 month after IT. Cell-free DNA was extracted from 4 mL of plasma using the QIAsymphony liquid-handling robot (Qiagen) and was treated with bisulfite (Zymo Research). DNA concentration was measured using Qbit single-strand molecular probes (Invitrogen). Bisulfite-treated DNA was PCR amplified, using insulin promoter primers specific for bisulfite-treated DNA but independent of methylation status at monitored CpG sites. Sequencing was performed on PCR products using MiSeq Reagent Kit v2 (MiSeq, Illumina method). Sequenced reads were separated by a barcode, aligned to the target sequence (insulin gene promoter), and analyzed using

custom scripts written and implemented in Matlab. Reads were quality filtered based on Illumina quality scores. Reads were identified by having at least 80% similarity to target sequence and containing all the expected CpGs in the sequence. To calculate the concentration of cfDNA derived from beta cells, we multiplied the fraction of beta cell-specific cfDNA (as determined from the frequency of molecules carrying a beta cell-specific methylation pattern) by the concentration of cfDNA measured in each particular patient. The concentration was expressed in copies DNA/mL and a value of 50 copies/mL was considered as the minimum threshold for positive cell death.

Absolute beta cell loss was estimated multiplying the number of DNA copies by the patient's total plasma volume (TPV). Calculation of TPV was done using the total body water and the extracellular fluid [24-26], using the formula:

TPV = Extracellular Fluid – Interstitial Fluid

With the following assumptions:

- Total Body Water = 60% of Body Weight (For Male)
- Total Body Water = 50% of Body Weight (For Female)
- Extracellular Fluid = Total Body Water / 3
- Interstitial Fluid = Total Body Water * 0.25

Cell loss was estimated relative to the original cell count present in the islet preparation, assuming an average of 1140 beta cells/IE [27].

Metabolic studies after transplantation

Post-transplant graft function was measured as previously reported [3,4,28]. It consisted of sequential clinical and metabolic assessments including the results of several metabolic tests such as the record of hypoglycemia events, fasting blood glucose, basal and stimulated C-peptide levels, hemoglobin A1C, oral and intravenous glucose tolerance tests, mixed meal stimulation tests, glucagon and arginine, as well as requirements for exogenous insulin. Beta 2 scores were calculated three months after transplant as recently described by Forbes and collaborators [4], as a more accurate indicator of islet engraftment.

Statistical Analysis

To assess the significance of differences in isolation parameters and cell loss between groups with positive and negative cell death, we used a two-tailed Mann–Whitney test. Two-tailed Pearson's correlation was used to measure the linear dependence between cfDNA and immediate post-transplant function variables (1-month stimulated C-peptide and insulin requirements). Fisher's exact test was used for comparison of proportions. Relationships between beta cell death and graft function (3-month insulin independence and Beta 2 score) were examined using receiver-operating characteristic (ROC) analysis. The area under the ROC curve (AUC) was calculated from ROC curves generated for each method. All comparisons between groups were performed with a 95% confidence interval and a p-value <0.05 was considered significant. Analysis was performed using GraphPad Prism (GraphPad Software version 6, La Jolla, CA, USA).

Results

We defined selection criteria for the study as subjects receiving a first transplant or a re-transplant after a remote (>1yr) previous infusion (see material and methods). During the study period (2014-2016), 100 islet allotransplants were performed in 83 patients. Only 37 (44.6%) subjects fulfilled these selection criteria and were included in the analysis. Baseline characteristics of these subjects are comparable to excluded patients during the same period (**Table 1**).

Isolation parameters do not influence post-transplant beta cell cfDNA levels

Patient and isolation characteristics were comparable throughout the study cohort. The median level of cfDNA one hour after transplant was 852 copies/mL (range: 0 – 6647); assuming 3000 ml plasma per individual, an average islet transplant recipient had ~2.5 million beta cell genomes in the circulation at this stage. Positive beta cell-specific cfDNA was detected in 31/37 (83.8%) patients at 1 hour, while only 8/37 (21.6%) patients were positive at 24 hours post-transplant ($p<0.0001$). Levels of beta cell cfDNA were particularly high 1 hour after transplant. Based on these cfDNA measurements, we estimated that $5.2x10^6$ beta cells (range: 349,198 – $3.3x10^7$) were lost prior to the infusion or during the first hour after transplant (**Table S1 and Figure S1**). These levels of 1hr beta cell cfDNA were not associated with any of the islet preparation parameters including culture time, preparation purity and viability, islet size, dose and packed cell volume (PCV), as well as other functional markers such as static

glucose-stimulated insulin secretion (sGSIS), pure oxygen consumption rate (OCR) or OCR adjusted by islet size index or islet dose (**Table 2**). In principle, cfDNA shortly after transplantation could reflect material from beta cells that have died during islet isolation or culture, acute death of beta cells after transplantation, or a combination of both.

The levels of beta cell cfDNA measured at 24 hours were not associated with any isolation parameter but we observed increased beta cell cfDNA at this time point when islets were infused in a larger PCV. Patients with positive cfDNA were transplanted with a median PCV of 4.0 mL (range: 3.5 – 7.5) vs. 3.0 mL (range: 2.0 – 5.0) in patients with negative cfDNA (p=0.002, **Table 3**). These 24hrs-cfDNA measurement resulted in a significantly lower estimation of 427,991 cells (range: 297,815 – $1.6x10^6$) lost after transplant, corresponding to a significantly lower graft loss from the initial islet preparation compared to the earlier time point (24hrs: 0.09% cell loss, range: 0.06 – 0.3%) vs. 1hr: 1.2%, range: 0.06% – 8.9%, p<0.0001. **Table S1 and Figure S1**)

Beta cell-specific cfDNA measured at 24 hours is associated with immediate post-transplant graft function

Subjects with a signal of beta cell cfDNA at 1hr had similar initial graft function compared to those with no 1hr-beta cell cfDNA, expressed as comparative 1 month-exogenous insulin requirements (0.14 U/Kg/Day vs. 0.11 U/Kg/Day, p=0.55) and 1 month-stimulated C-peptide levels (0.92 nmol/L vs. 1.3 nmol/L, p=0.22). These represent the most relevant indicators of immediate graft function

after islet transplantation. Significant differences however, were found at the 24hrs time point. Patients with 24hrs positive cfDNA had significantly higher 1 month-insulin requirements (0.26 U/Kg/Day vs. 0.13 U/Kg/Day, p=0.04), higher 1 month-absolute insulin usage (15 U/Day vs. 8 U/Day, p=0.04) and significantly lower 1 month-stimulated C-peptide (0.79 nmol/L vs. 1.4 nmol/L, p=0.01). These findings were also supported by positive 24hrs-correlations between cfDNA concentration and 1 month-exogenous insulin requirements (r^2=0.26, p=0.001), and between cfDNA concentration and 1 month-stimulated C-peptide levels (r^2=0.15, p=0.02). These correlations indicate a possible significant association between these variables despite a low r-squared value, which may be a consequence of an inherently higher amount of unexplainable variability between subjects. It is possible however, that additional predictors can increase the true explanatory power of this particular model (**Figures 1 and 2**).

Beta cell-specific cfDNA measured at 24 hours may be a predictor for islet engraftment

All patients were closely monitored for graft function and exogenous insulin requirements before receiving subsequent complementary islet infusions. We examined beta cell cfDNA levels at 1hr and 24hrs after the procedure to correlate beta cell death rate with graft function at a later time point. Three months after transplant 20/37(54.1%) patients were insulin independent and a favorable Beta 2 score (>15 points) was also calculated in these same subjects. These two indicators provide a more objective assessment of islet engraftment and may

predict the need for a supplementary transplant to facilitate long term benefits [4]. The 1hr beta cell-specific cfDNA failed to correlate with both, insulin independence (p=0.45) and with Beta 2 score (p=0.10), whereas 24hrs positive beta cell cfDNA was inversely associated with both outcomes. Only 4/20 (20%) subjects with positive 24hrs beta cell cfDNA were insulin independent at 3 months (p=0.04) and only one of those three patients (1/20, 5%) with detectable 24hrs beta cell cfDNA achieved a 3 month-Beta 2 score >15 points (p=0.008). The receiver-operating characteristic analysis supported utilizing the 24hrs beta cell cfDNA as an optimal model for insulin independence (ROC:AUC=0.70, p=0.03, sensitivity=75% and specificity=58.8%), and Beta 2 score (ROC:AUC=0.77, p=0.006, sensitivity=88.9% and specificity=52.6%) at 3 months, using a discrimination threshold of 50 copies/mL (**Figures 3 and 4**). Packed cell volume of the islet preparation was not correlated with these 3-month outcomes.

A positive beta cell cfDNA signal was only observed in 2 patients at 7 days post-procedure and in 2 different subjects, 1 month after transplant. This late beta cell mortality was not associated with unfavorable outcomes (**Table S2**). Moreover, 24hrs positive beta cell cfDNA failed to predict the time to second supplementary transplant, and there was no correlation between the cPRA or the presence of donor-specific antibodies (DSA) or the levels of cfDNA at any time point. Similarly, there was no relationship between cPRA/DSA and the 3-month insulin independence or Beta-2 score (data not shown).

Discussion

Assessing graft loss is a particularly challenging aspect of islet transplantation due to the many mechanisms eliciting islet injury and the lack of tools to measure beta cell mass in vivo [1]. We have described a 14% islet mass loss during culture associated with cell fragmentation and disintegration [29]. Another important islet loss event occurs immediately after infusion, largely due to cell hypoxia and inflammatory responses following transplantation. In particular, IBMIR accounts for significant graft attrition at this early stage and is reported to reach up to 70% of the initial preparation within the first 24hrs, when using the intraportal route [30,31]. The sum of all these events paired with sustained immune-related cell death, is the rationale for using more than one infusion per patient to achieve durable normoglycemia [32]. Nonetheless, implementation of new protocols in selected centers can result in high rates of single-donor insulin-independence [33].

To date, clinical islet transplantation lacks an accurate estimator for cell loss to support clinical and metabolic indicators from the early post-transplant phase. Beta cell-specific cfDNA has been identified as a novel biomarker to detect islet loss [9,34,35]. We have recently reported the use of a novel cfDNA measurement technology, based on next generation sequencing, to identify beta cell death in patients with new-onset T1DM and recent clinical IT patients [9]. Moreover, a recent publication used a similar technique to detect beta cell mortality after islet autotransplantation with a full characterization of islet loss throughout the process of pancreatitis, total pancreatectomy and transplant [35]. We herein report

the first observations of a correlation between beta cell-specific cfDNA after clinical allotransplantation and patient outcomes.

After measuring cfDNA at 1hr, 24hrs, 7 days and 1 month, we observed two distinct signal peaks in our study population. One, at 1hr after infusion, very intense (934 copies/mL) and generalized (83.8% of patients) and another, less intense (93 copies/mL) and less frequent (21.6% of patients) at 24 hours. Although the 1hr signal is strong, it is transient, and given the limited number of sample time points and the rapid clearance of cfDNA, it likely represents a combination of beta-cell death carried from the islet isolation and culture procedures and acute cell loss early after infusion. These observations are consistent with the recent report on autotransplantation [35]. In contrast, cfDNA signal at 24hrs was more informative of future graft function. In our study this was not related to any isolation parameter, including well-established viability indicators like OCR/DNA, OCR/DNA/ISI or OCR Dose [16,19,20]. Surprisingly, only the PCV showed a significant association with 24hrs cfDNA. PCV is directly dependent on the islet fraction purity and increasing PCV (>5.5 mL) has been associated with high portal venous pressure, increased risk for portal thrombosis [21,36] and self-limited hepatocellular damage [37,38]. Our findings indicate that increased PCV may also be associated with early beta cell death. Despite this 24-hour cell mortality being relatively small compared with the signal measured at 1 hour, it correlated with less favorable post-transplant outcomes. The 24-hour beta cell-specific mortality was also a good predictor of islet engraftment as

subjects with no beta cell mortality at this time point were more likely to be insulin independent and have a favorable Beta 2 score at 3 months post-transplant.

A clear limitation of this study is that blood for early cfDNA measurements was only drawn at 1 and 24 hours post-transplant. As a consequence, our 24hrs beta cell cfDNA values represent a snapshot of graft cell death within that particular hour. Clearly the interpretation of our cell loss estimates is limited by the lack of more frequent time points within the first 24 hours and may be further influenced by unknown factors such as the islet death rate and the half-life of cfDNA, which is currently estimated between 15-120 minutes [39,40]. Additional studies with more frequent sampling may clarify the dynamics of graft loss in the critical hours and days after transplantation. Moreover, sampling for cfDNA at different stages of the islet isolation process could also provide more accurate information on the number of beta cells lost to digestion, purification and culture.

Islet culture performed at 22˚C has been the standard approach at our site for more than15 years to minimize islet loss prior to transplantation as oppose as a 37 ˚C. With the primary goal of clinical islet isolation being the recovery of the highest islet mass possible following purification, we often choose to compromise purity to maximize transplantable islet mass. This particular cfDNA assay may also serve as an evaluation to our current approach to islet isolation and culture leading to refinement of standard operating protocols.

Further clinical studies using cfDNA measurement paired with beta cell DNA kinetic may help estimate islet cell death rate before and after transplantation as a consequence of the process of islet isolation and post-transplant hypoxia, IBMIR or inflammatory response. All patients in this study received anti-inflammatory treatments (etanercept + anakinra) post-transplant. We have not carried out sub-analyses to evaluate cfDNA levels in the absence of these anti-inflammatory treatments, but this would be insightful if data were available. Only 10% of patients achieve and maintain insulin independence with single donor islet infusions across our entire experience. None of the study subjects fulfilled this criteria at one year. However, in a larger study group it would be interesting to look for associations between cfDNA levels in a subset that did maintain single donor islet transplant full function beyond one year versus others that did not.

In summary, we present a validation of our recently described method to detect beta cell death based on beta cell-specific methylation patterns in circulating DNA. Our results indicate that 24hrs estimation of beta cell death correlates with clinical allotransplantation outcomes and could predict islet engraftment at 3 months. This technique may represent a useful tool to accurately estimate the rate of cell loss after transplantation of islets and other organs, and potentially a means to monitor graft rejection and to optimize immunosuppression. Together with existing clinical and metabolic indicators of islet graft performance, it may

contribute to secure long-term graft survival by allowing adequate timely interventions and judicious planning of subsequent islet infusions.

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Tables

Table 1. Baseline characteristics of subjects and islet preparations included in the study compared to other transplanted patients during the same study period (2014-2016). Data are mean (range) or mean ± standard deviation or n.

Characteristic	Included patients (n = 37)	Excluded patients (n = 46)
Age (years)	44.1 (26 – 63)	46.2 (24 – 68)
Diabetes duration (years)	27.3 ± 9	28.7 ± 10.9
Male/Female	15/22	20/26
Weight (Kg)	70.5 ± 9.2	71.1 ± 11.8
Insulin requirements (u/kg/day)	0.60 ± 0.1	0.61 ± 0.19
Islet dose (IE/Kg)	7,475.6 (4,373 – 21,678)	7,265 (5,891 – 19,762)

IE: Islet equivalents.

Table 2. Comparison of baseline islet preparation characteristics between patients with positive vs. negative beta cell-specific free circulating DNA, 1 hour after clinical transplantation

	Positive 1hr cfDNA	Negative 1hr cfDNA	p-value
Sample size	31	6	-
Pre-transplant culture period (hr)	40 (14.2 – 69.5)	31 (17.5 – 54.5)	0.50
Purity (%)	50 (30 – 90)	46 (45 - 65)	0.98
Viability (Syto/EtBr) (%)	85 (76 - 98)	83 (76.5 - 98)	0.51
Islet Equivalent (IE)	484,751 (307,913 – 1,018,889)	565,447 (381,461 – 636,700)	0.25
Islet Size Index (ISI)	1.1 (0.6 – 2.4)	1.0 (0.8 – 1.7)	0.49
sGSIS (Stimulation Index)	2.4 (0.5 – 9.4)	3.6 (1.3 – 4.1)	0.82
OCR/DNA (nmol O2/min•mg DNA)	108 (61 - 203)	116 (80 – 198)	0.52
OCR/DNA/ISI (nmol O2/min•mg DNA)	91 (29.7 – 199)	109 (53 – 240)	0.32
Islet dose (IE/Kg)	6,718 (4,373 – 21,678)	7,622 (5,338 – 10,621)	0.79
OCR Dose (nmol O2/min•kg)	7.7 (3.0 - 25)	8.3 (6.3 – 17)	0.53
Packed Cell Volume (mL)	3.0 (2.0 – 7.5)	3.3 (2.5 – 4.0)	0.81

Two-tailed Mann-Whitney, 95% confidence interval. cfDNA (circulating free DNA), OCR (oxygen consumption rate).

Table 3. Comparison of baseline islet preparation characteristics between patients with detectable or undetectable beta cell-specific free circulating DNA, 24 hours after clinical transplantation

	Positive 24hrs cfDNA	Negative 24hrs cfDNA	p-value
Sample size	8	29	-
Pre-transplant culture period (hr)	36 (16 – 70)	36 (14 – 67)	0.65
Purity (%)	50 (30 – 90)	46 (36 – 80)	0.86
Viability (Syto/EtBr) (%)	80 (77 – 93)	85 (76 – 98)	0.55
Islet Equivalent (IE)	505,559 (390,068 – 591,890)	487,220 (307,913 - 1,018,889)	0.84
Islet Size Index (ISI)	1.1 (0.95 – 2.4)	1.1 (0.6 – 1.7)	0.39
sGSIS (Stimulation Index)	3.1 (0.5 – 4.2)	2.3 (1.3 – 9.4)	0.41
OCR/DNA (nmol O2/min•mg DNA)	95 (73 – 203)	111 (61 – 198)	0.82
OCR/DNA/ISI (nmol O2/min•mg DNA)	82 (30 – 169)	91 (38.8 - 240)	0.23
Isle dose (IE/Kg)	6,718 (5,569 – 10,621)	7,205 (4373 – 21,678)	0.76
OCR Dose (nmol O2/min•kg)	6.9 (5.1 – 18)	8.1 (3.0 – 25)	0.94
Packed Cell Volume (mL)	4.0 (3.5 – 7.5)	3.0 (2.0 – 5.0)	0.002[a]

[a] Significant p-value (two-tailed Mann-Whitney, 95% confidence interval). cfDNA (circulating free DNA), OCR (oxygen consumption rate).

Table S1. Estimated beta cell loss at 1hr and 24hrs after clinical islet allotransplantation based on blood circulating free beta cell-specific DNA and estimated patient's total plasma volume.

	1 hour	24 hours	p-value
Number of patients	31	8	-
Median DNA copies/mL (range)	934 (105 – 6647)	93 (64 – 476)	<0.0001
Estimated absolute beta cell loss (number of cells)	$5.2x10^6$ (349,198 – $3.3x10^7$)	427,991 (297,815 – $1.6x10^6$	<0.0001
Estimated relative beta cell loss (percentage from original preparation)	1.2% (0.06% – 8.9%)	0.09% (0.06 – 0.3%)	<0.0001

cfDNA (circulating free DNA).

Table S2. Patients with late beta cell mortality at 7 days and 1 month after clinical islet allotransplantation based on blood circulating free beta cell-specific DNA.

	1hr-cfDNA	24hrs-cfDNA	7 day-cfDNA	1 month-cfDNA	3 month-Insulin independence
Subject 1	2,060.6	2.3	57.3	10.7	Yes
Subject 2	1,748.6	45.5	62.3	0	No
Subject 3	382.6	0	1.8	80.1	No
Subject 4	454.2	0	0	73.3	Yes

cfDNA (circulating free DNA).

Figure Legends

Figure 1. Twenty-four -hour cfDNA is better correlated with immediate post-transplant exogenous insulin demand. A. 1-month insulin requirements in subjects with positive (n=31) and negative cfDNA (n=6) measured 1hr post-transplant (p=0.55). B. 1-month insulin requirements in subjects with positive (n=8) and negative cfDNA (n=29) measured 24hrs post-transplant (p=0.04*). C. Linear regression of cfDNA measured 24hrs post-transplant and 1-month insulin requirements (n=37, r^2=0.26, p=0.001). D. 1-month absolute insulin used in subjects with positive and negative cfDNA measured 24hrs post-transplant (p=0.04*). cfDNA (circulating free DNA). A greater value of cfDNA suggests greater beta cell death and lower insulin requirement reflect better post-transplant function. Summary data are reported as median (interquartile range), two-tailed Mann-Whitney, 95% confidence interval.

Figure 2. Twenty-four -hour cfDNA is better correlated with immediate post-transplant C-peptide levels. A. 1-month stimulated C-peptide blood levels in subjects with positive (n=31) and negative cfDNA (n=6) measured 1hr post-transplant (p=0.22). B. 1-month stimulated C-peptide blood levels in subjects with positive (n=8) and negative cfDNA (n=29) measured 24hrs post-transplant (p=0.01). C. Linear regression of cfDNA measured 1hr post-transplant and 1-month stimulated C-peptide blood levels (n=37, r^2=0.0002, p=0.94). D. Linear regression of cfDNA measured 24hrs post-transplant and stimulated C-peptide blood levels (n=37, r^2=0.15, p=0.02). cfDNA (circulating free DNA). A greater

value of cfDNA suggests greater beta cell death and higher stimulated c-peptide levels reflect better post-transplant function. Summary data are reported as median (interquartile range), two-tailed Mann-Whitney, 95% confidence interval.

Figure 3. Twenty-four -hour cfDNA can predict 3-months insulin independence after intraportal islet allotransplantation. A and B. Correlation of cfDNA measured 1hr post-transplant with clinical outcome expressed as 3-month insulin independence and its corresponding receiver-operating characteristic (ROC) curve. C and D. Correlation of cfDNA measured 24hrs post-transplant with clinical outcome expressed as 3-month insulin independence and its corresponding receiver-operating characteristic (ROC) curve. The area-under-the-curve (AUC) has been calculated and displayed for each group. cfDNA (circulating free DNA). A greater value of cfDNA suggests greater beta cell death.

Figure 4. Twenty-four -hour cfDNA can predict 3-months engraftment after intraportal islet allotransplantation. A and B. Correlation of cfDNA measured 1hr post-transplant with clinical outcome expressed as 3-month Beta 2 Score and its corresponding receiver-operating characteristic (ROC) curve. C and D. Correlation of cfDNA measured 24hrs post-transplant with clinical outcome expressed as 3-month Beta 2 Score and its corresponding receiver-operating characteristic (ROC) curve. The area-under-the-curve (AUC) has been calculated and displayed for each group. cfDNA (circulating free DNA). A greater value of cfDNA suggests greater beta cell. Beta 2 Score is a composite measure of beta

cell function after transplant [4]. A Beta 2 Score > 15 points reflects a functioning graft.

Figure S1. cfDNA measurements as an estimator for beta cell loss. A. cfDNA measurements (copies/mL, blue) at 1hr (n=31/37, positive cfDNA) vs. 24hrs (n=8/37, positive cfDNA) ($p<0.0001$), and estimation of absolute beta cell loss (number of cells, red) at 1hr vs. 24hrs ($p<0.0001$). B. Estimated relative beta cell loss from the original islet preparation at 1hr vs. 24hrs ($p<0.0001$). Figure is represented with log 2 scale and data points with cfDNA<0.06 are not represented. cfDNA (circulating free DNA). Summary data are reported as median (interquartile range), two-tailed Mann-Whitney, 95% confidence interval.