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MR Imaging Monitoring of Iron-Labeled Pancreatic Islets in a Small Series of Patients: Islets Fate in Successful, Unsuccessful, and Autotransplantation

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Islet transplantation is one of the most promising and effective therapies for restoring normoglycemia in type 1 diabetic (T1D) patients, but islet engraftment is one of the main obstacles hampering long-term success. Monitoring graft loss, caused either by immunological or nonimmunological events, occurring in the first phase after transplantation and at later stages of a patient's life is a very important issue. Among the imaging approaches previously applied, magnetic resonance imaging (MRI) monitoring of islet fate following labeling with superparamagnetic iron oxide agents yielded promising results. The aim of this study was to translate into patients the method of islet labeling and MRI monitoring developed in our preclinical setting and to compare imaging results with graft clinical outcome.Three T1D patients and one nondiabetic patient undergoing autotransplantation following subtotal pancreatectomy received Endorem®-labeled islets. Patients were monitored by MRI and metabolically (HbA1c, exogenous insulin requirement, and C-peptide, TEF) at 1, 3, and 7 days following transplantation and once a month up to 10 months. Labeled transplanted islets appeared as hypointense areas scattered within the liver parenchyma, whose absolute number at 24 h after transplantation reflected the labeling efficiency. In Pt#1 and 3 with good midterm graft function, MRI follow-up showed an important early loss of hypointense spots followed by a slow and progressive disappearance at later timepoints. Graft loss of function in Pt#2 4 weeks after transplantation was associated with the complete disappearance of all hypointense signals. The autotransplanted patient, stably insulin free, showed no significant signal reduction during the first 3 days, followed by loss of spots similar to a Pt with good mid-term graft function. These results suggest that MRI monitoring of islet transplantation at early time points could represent a meaningful readout for helping in predicting transplant failure or success, but its relevance for mid-/long-term islet function assessment appears evanescent.

Key words: Pancreatic islet transplantation; T1 diabetes patients; Autoimmunity; Autologous islet transplantation; Superparamagnetic iron oxide particles (SPIO); Magnetic resonance imaging (MRI); Inflammation; Cytokines

INTRODUCTION

Despite technological advancements in the treatment of type 1 Diabetic (T1D) patients (14,23), islet transplantation (Tx) stands out as the most effective and promising procedure for restoring normoglycemia and for preventing long-term complications (3,15). Despite recent advances in achieving insulin independence following islet Tx (2,35), the mechanisms involved in islet engraftment are still poorly defined.

Monitoring graft loss, caused either by immunological or nonimmunological events, occurring in the first phase after Tx and at later stages, is a relevant issue. Considerable efforts have been made in recent years toward the development of noninvasive in vivo imaging methods to follow islet fate after intraportal infusion (10).

Several studies demonstrated the effectiveness of labeling islets with superparamagnetic iron oxide (SPIO) for in vivo detection of transplanted islets by magnetic

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resonance imaging (MRI) (5,11,17,20). The presence of labeled islets can be monitored noninvasively and repeatedly over time by MRI, and graft rejection was shown to be associated with disappearance of hypointense regions of interest (ROIs) representing labeled islets in animal models (12,22). After preclinical development in rodent models, this imaging approach was translated into the clinic, demonstrating its feasibility and safety of monitoring labeled islets up to 6 months after Tx (34,36). Since immunological events are thought to play a crucial role in loss of transplanted islets (24), herein we extend previous work with the aim of monitoring up to 10 months beside allotransplanted T1D patients and also, for the first time, an autotransplanted subject and to verify by quantitation of hypointense MRI signals following Tx of SPIO-labeled islets, whether their persistence/disappearance correlates with metabolic data reflecting graft function.

MATERIALS AND METHODS

Study Design

The clinical nonrandomized pilot study was approved by the IRB of the San Raffaele Scientific Institute (Prot N. EFSD/MSD ISLET MRI). Eligible participants were all T1D patients (Pts) on the local waiting list for hypoglycemia unawareness syndrome based on the published inclusion criteria (24). Exclusion criteria were basal liver iron overload (e.g., Pts with hemochromatosis or under iron treatment) and the presence of liver steatosis or focal lesions that could compromise adequate detection of labeled pancreatic islets. Pts were treated according to the standard Edmonton immunosuppression protocol (24). Since one of the objectives of the study was to assess safety, only the first islet infusion for each Pt was performed with labeled islets. All Pts received an MR scan of the abdominal region before and at 24, 48, and 72 h and 7 days post-islet Tx and subsequently once a month up to 10 months.

Human Islet Isolation and Labeling With Endorem®

Pancreatic islets were isolated essentially according to Ricordi's method (32). Pancreata were digested in a Ricordi chamber (31) and purified on continuous Ficoll gradients by Cobe 2901 (Cobe Laboratories Inc., Lakewood, CO, USA). Islets were cultured overnight in a modified CMRL (Mediatech, Herindon, VA, USA) as previously described (26). "First Layers" of islet preparations, with a purification degree of ≥70%, were subjected to SPIO labeling (Endorem®, Guerbet, S.p.A., Genoa, Italy). Except for the autoTx subject, one third of total islet equivalents (IEQ) to be infused (see Table1) were subjected to labeling for 18–24 h according to a standard operative procedure as previously described (25). Labeled islets were pooled with unlabeled of the first and the second layers and resuspended in an appropriate volume for Tx.

Table 1. Characteristics of Transplanted Patients and Islet Preparations

Pt	Age	Gender	Diabetes Duration (years)	Retinopathy	Hypoglycemia Awareness	Peripheral Neuropathy	Nephropathy	1st Islet Infusion	IEQ N.	Purity (%)	SPIO treated	Duration of Labeling (h)	Estimated Labeled Islets Transplanted (%)	IEQ/kg
#1	68	M	33	No	Yes	Yes	No	HP884	390,505	48	130,300	20	10	6,733
#2	52	F	39	Yes	Yes	Yes	No	HP886	327,400	55	109,000	20	12	5,037
#3	43	M	23	Yes	Yes	Yes	No	HP894/HP895	444,135	77	148,120	24	26	6,618
Auto-Tx8	31	M	No Diab	No	No	No	No	AutoTx8	78,575	60	58,575	24	n.d.	1,007

IEQ, islet equivalent; SPIO, superparamagnetic iron oxide.

In Vitro Quality Control Tests

Labeling efficiency, viability assays, insulin content, and secretion following Endorem® labeling along with determination of inflammatory cytokines are described in the supplementary material (SM) section with reference to the employed methods http://www.fcm.in.cnr.it/research/malosio/p1/sm/celltransplantation.pdf.

Islet Tx

Each T1D Pt received only the first infusion with labeled islets. Pt#1 and Pt#3 received the second infusion 106 days and 174 days following the first, respectively. Islet Tx was performed intrahepatically, by portal vein infusion, in Pts under local anesthesia as previously described (37).

Functional Studies

Pts were subjected to blood tests for fasting glycemia and C-peptide, glycated hemoglobin (Hb1Ac), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cross-linked fibrin degradation product (XDP), C reactive protein (CRP) on admittance, every day for the first 7 days after Tx, then once a month concomitantly with MRI follow-up. Daily exogenous insulin requirement (EIR) and transplant estimated function (TEF) were calculated as previously described (8). Islet transplant outcome was measured and defined as stated earlier (29). Serum C-peptide levels were assayed by radioimmunoassay using commercial kits (Dako, Cambridgeshire, UK); HbA1c was measured by Bio-Rad Variant II HbA1c analyzer (Bio-Rad Laboratories, Munich, Germany); autoantibodies and donor-specific alloantibodies were measured and evaluated as previously described (29); serum AST and ALT and CRP were measured with the *ADVIA 2400* Chemistry System (Bayer Healthcare, Tarrytown, NY, USA); XDPs were measured with an immunoturbidimetric method (STA-Liatest, Diagnostica Stago, France).

Magnetic Resonance Imaging

MR scans were performed with a 1.5 T magnet (Achieva Nova, Philips Medical Systems, Best, The Netherlands; release 2; maximum gradient strength: 33 mT/m; maximum gradient slew rate: 180 mT m−1 sec−1) equipped with a four-channel sensitivity encoding (SENSE)-body coil applying T2*w fast field echo breath-hold sequences [relaxation time (TR) = 220 ms; echo time (TE) = 18 ms; flip angle (FA) = 250). For each Pt, two expert radiologists in agreement defined the number of MRI hypointense spots referring to labeled islets. In particular, all hypointense ROIs with a round/elliptic shape, recognizable on T2* images acquired 24 h after Tx, that were not visible in the pre-Tx MRI study, were considered as labeled islets. Usually these ROIs were evident in a single slice, but in no more than two to three adjacent image slices, contrary to intrahepatic vessels that appear as long hypointense structures detectable in several subsequent slices.

In each Pt, the absolute number determined at 24 h post-Tx was considered as 100%, and results of subsequent MRI scans were expressed as a percentage of the initial value.

Statistical Analysis

Data were represented as mean ± SD or SEM. Statistical analysis of the data including linear regression calculation has been performed with Prism_V5 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Patients

Three Pts with brittle T1D (Table 1) enrolled in the study were transplanted with Endorem®-labeled islets (Pt#1–3). One nondiabetic Pt (Auto-Tx8), suffering from recurrent pancreatitis was transplanted with his own labeled islets after subtotal pancreatectomy. Table 1 reports the characteristics of Pts and of the first infusions. Endorem®-labeled islets represented 30% of total infused in T1D Pts, while 75% of islets were labeled for the autologous Tx.

Quality Control of Labeled Islets

Based on the scored labeling efficiency (SM:http://www.fcm.in.cnr.it/research/malosio/p1/sm/celltransplantation.pdf; Materials and Methods) (Fig. S1A) labeled transplanted islets were 10%, 12%, and 26% of total infused islets for the three T1D Pts (Table 1).

The average iron incorporation for the first two islet preparations (HP884 and HP886) was 0.528 ± 0.166 and 1.5 ± 0.158 pg Iron/10^3 IEQ, respectively. The two islet preparations (HP894/895) used for the third T1D patient incorporated 5.88 ± 0.358 and 3.22 ± 0.066 pg Iron/10^3 IEQ, respectively.

Viability of labeled islets at 24 h and 48 h after labeling was not different compared to unlabeled ones for HP884 and HP886, while labeled HP894/895 preparations showed a viability of 69 ± 6% at 48 h (SM:Fig.S1B). Moreover, labeled islets exposed for 24 h to proinflammatory cytokines following labeling did not show a noteworthy viability decrease compared to unlabeled ones (SM:Fig. S1C).

Insulin content was not affected by iron labeling (SM:Fig. S2A). Secretion index (stimulated/basal release) after static insulin stimulation (SM:Fig. S2B) was similar in all labeled and unlabeled islet preparations. Dynamic insulin secretion studies on two labeled and unlabeled islet preparations (HP884 and HP886) showed similar performances (SM:Fig. S2C, D). Moreover ADP/ATP ratio and insulin content (measured by two independent ways), tissue factor,

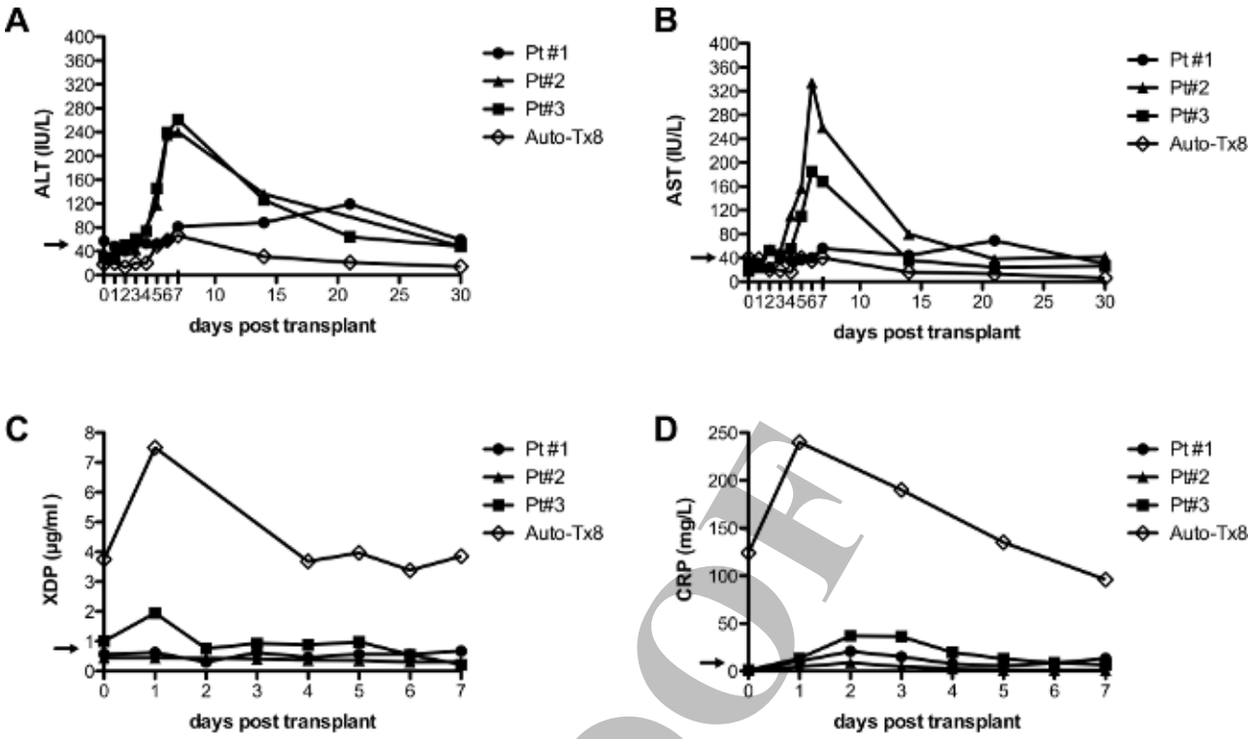

Figure 1. Liver enzymes, XDP, and CRP levels in transplanted Pts. Liver enzyme levels: (A) ALT and (B) AST measured up to 30 days in Pts following Tx with labeled islets. (C) Cross-linked (X) fibrin degradation product (XDP) measurements (µg/ml) during the first week after Tx in Pts receiving labeled islets; (D) CRP levels (mg/L) during the first week after Tx. Arrows on the left of the Y-axes indicate the threshold for each parameter.

monocyte chemotactic protein-1, interleukin-6 (IL-6), and IL-8 were unchanged upon labeling (SM:Supplementary Table 1). Owing to the overall small amount of transplanted islets destined to the auto-Tx8 Pt, islets were not withdrawn for quality control analyses.

Clinical Outcome

Transplanted Pts did not experience major procedural complications. Liver enzymes ALT (reference range 10–40 IU/L) and AST (reference range 6–40 IU/L) were monitored immediately after islet infusion. A peak increase was observed at day 7 in Pt#2 and 3, while Pt#1 showed only a mild increase at day 21 declining to pre-Tx levels at day 28 (Fig. 1A, B). XDP (reference range 0.27–0.77 µg/ml) determinations in T1D patients showed peri-Tx levels above threshold only in Pt#3 (Fig. 1C). CRP (reference value ≤5–10 mg/L) was transiently increased in T1D Pt#1 and 3, but was not altered in Pt#2 (Fig. 1D).

The C-peptide, MRI ROIs, and insulin dependency are shown for each patient in Figure 2. Pt#1 and Pt#3 showed beside C-peptide levels >0.3 ng/ml (Fig. 2A and C), lower EIR < 50% 3 months after transplantation (Fig. 3A), reduced Hb1Ac (Fig. 3B), and a TEF around 1 from month 1 to month 6 post-Tx (Fig. 3C). Pt#1 became insulin free for 6 months after the second islet infusion (Fig. 2A). Pt#3 received a second islet infusion at 6 months, but never became insulin free, despite showing a progressive decline in EIR (from 0.79 to 0.21 Ins U/die/kg) (Fig. 3A). In Pt#2, experiencing graft failure at 1 month following Tx, some C-peptide production was detected 7 and 14 days following islet infusion (0.59, 0.51 ng/ml, respectively) (Fig. 2B) along with a mild reduction in EIR values (−38%) compared to pre-Tx (Fig. 3A). TEF in this patient was below 0.2 at 1 month and became negative at 6 months (Fig. 3C). Islet transplant survival defined as the number of days with C-pep > 0.3 ng/ml were 300 (Pt#1), 7 (Pt#2), and 300 (Pt#3).

Concerning autoimmunity, Pt#2 was highly gutamic acid decarboxylase autoantibodies (GADA)-positive before Tx but showed no further increase in the autoimmune response thereafter. Pt#3, who was negative for GADA, insulinoma-associated protein 2 autoantibodies, and zinc transporter 8 autoantibodies before Tx, showed a serum conversion above threshold [5.2 AU (29)] for GADA 15 days following Tx and oscillated thereafter between negative and positive values. Pt#1 was autoantibody negative before Tx and along the entire follow-up post-Tx. None of the three T1D patients showed donor-specific alloantibodies.

Auto-Tx8 receiving autologous islets had a C-peptide value of 0.61 ng/ml after pancreatectomy before Tx and

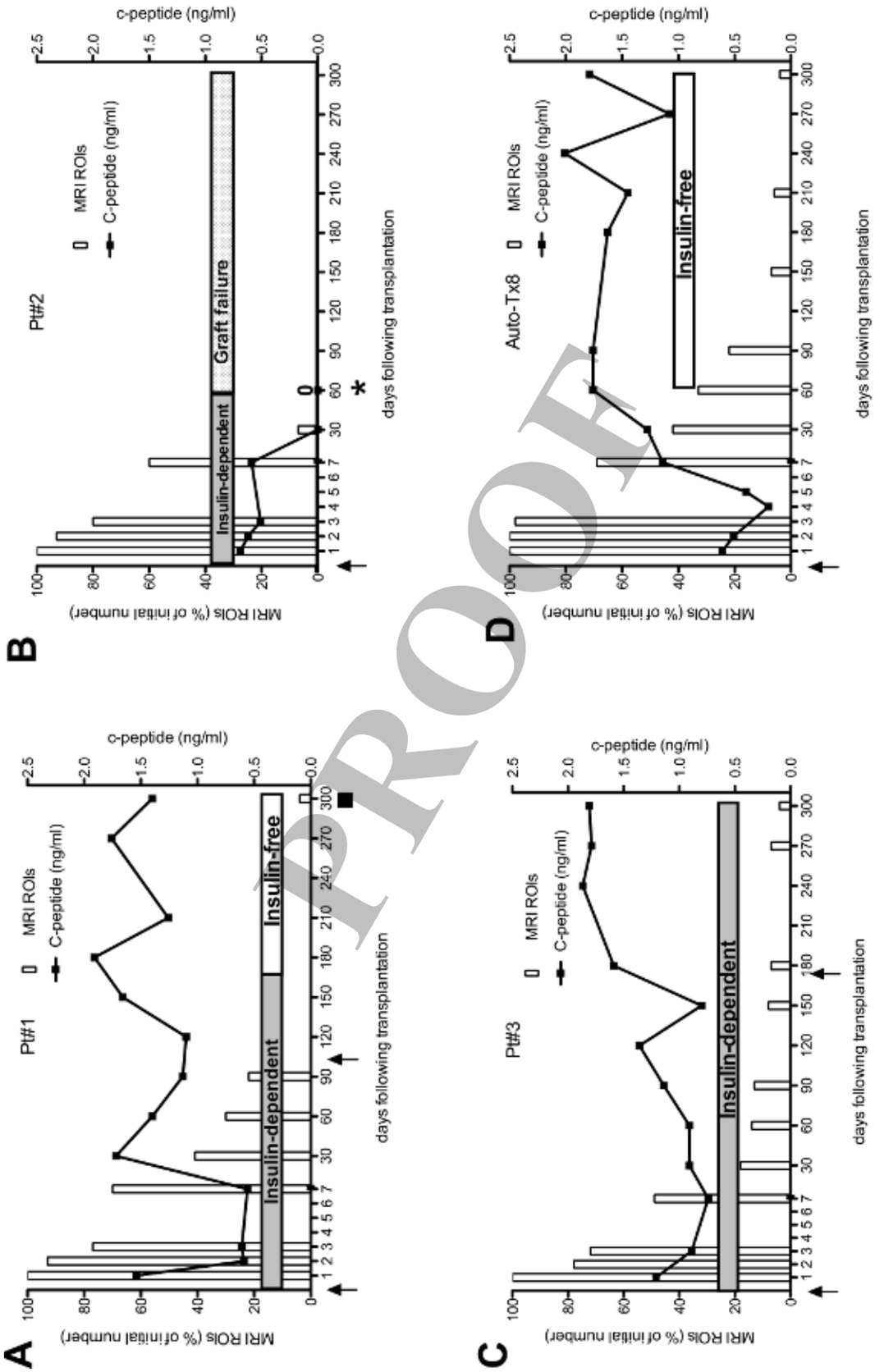

Figure 2. Corepresentation of C-peptide values and hypointense spots measured by MRI in the three T1D Pts and in the autotransplanted subject receiving labeled islets. (A) Pt#1; (B) Pt#2; (C) Pt#3; (D) Auto-Tx8. Arrows at the *X*-axis represent time point of islet infusions. The asterisk denotes islet transplant failure, 0 indicates no more detectable hypointense spots in MRI. The black square indicates withdrawal from the immunosuppressive regimen. The horizontal bar shows the insulin requirement. Right axis shows corresponding C-peptide levels.

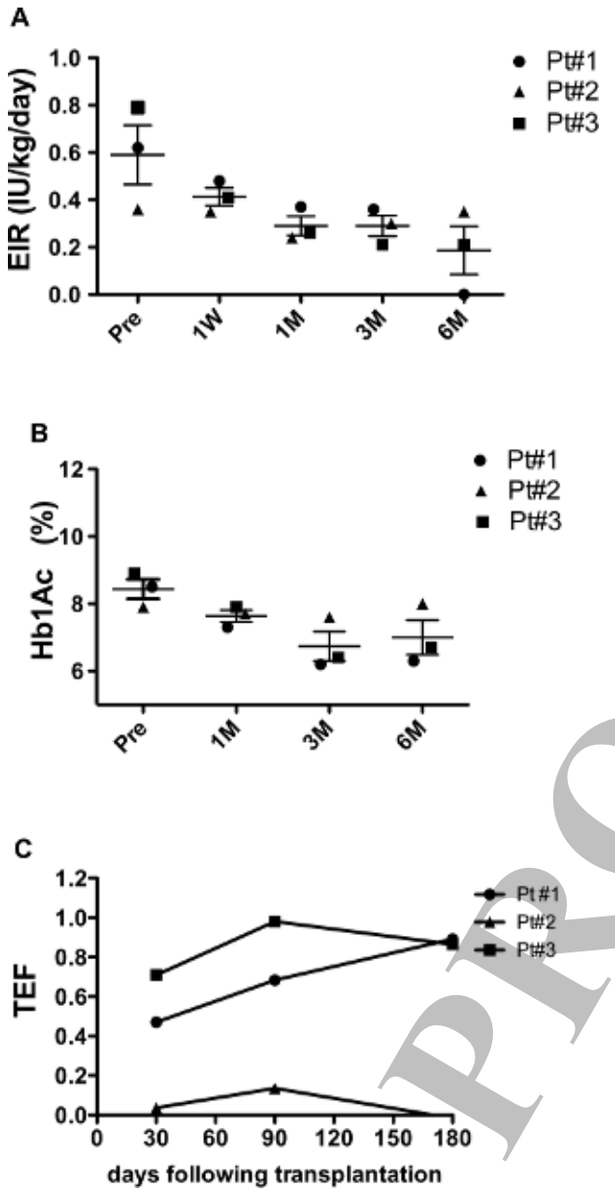

Figure 3. Metabolic parameters of the T1D Pts included in the study. (A) EIR (U/kg/day); (B) glycated hemoglobin (Hb1Ac, %); (C) TEF of Pt# 1–3 receiving labeled islets. Dot plots represent values from single Pt.

had an average C-peptide value of 0.22 ng/ml ± 0.13 SEM during the first 6 days following Tx, stably reaching values above 1 ng/ml from day 7 onward. This Pt received insulin during the first month (0.11 to 0.06 Ins U/die/kg) and was stably insulin-free thereafter (Fig. 2D). Auto-Tx8 Pt showed near normal values of ALT and AST enzymes along the entire follow-up following Tx (Fig. 1A, B), whereas the XDP and CRP values were significantly elevated following pancreatectomy and had a peak increase 1 day following Tx (Fig. 1C, D).

Quantitative Analysis of Endorem®-Labeled Islets in Transplanted Patients

Using T2*-weighted sequences and comparing post-Tx with pre-Tx MRI scans, labeled islets were clearly visible already at 1 day post-Tx as hypointense spots scattered within the liver of Pts, preferentially localized in the right lobe (Fig. 4). The number of ROIs scored in MRI images at this early time point plotted against the in vitro labeling efficiency of islet preparations showed a linear correlation (r^2=0.999; linear regression analysis; Pt#1: 27 spots, Pt#2: 41, Pt#3: 158) (Fig. 5A). This number was used as reference (100%) for each patient. The percentage of hypointense ROIs detected by MRI at later time points with respect to the reference value was plotted for each patient along with C-peptide values measured at the same time (Fig. 2). The cumulative decline in hypointense ROIs in all three T1D Pts receiving labeled islets (Fig. 5B) showed a similar pattern: within the first 3 days, a reduction in the ROI numbers to 76% ± 0.04% (avg ± SD) (Fig. 5B). The decline showed a higher degree of variability at day 7 post-Tx (residual signal in Pt#1 70%, in Pt#2 60%, and in Pt#3 49%, avg 60±0.1% SD). At the end of the first month, Pt#2 showed an almost complete loss of all visible MRI hypointense spots, which disappeared completely at 2 months, paralleling a drastic loss of graft function confirmed by undetectable C-peptide levels (Fig. 2B). The reduction in islet associated ROIs at 1 month was less severe in Pt#1 (41% of initial ROIs) and 3 (18% of initial ROIs), who showed functional grafts (Fig. 2A & C). During the subsequent months, a slow and progressive disappearance of the remaining hypointense ROIs was observed (about 2–10% of ROIs lost per month) with a persistence of only 4% of labeled islet-related signals after 10 months (Fig. 5B).

The fourth patient, receiving labeled autologous islets, despite receiving the lowest number of IEQ/kg body weight (Table 1) showed the highest number of hypointense ROIs at day 1 (Auto-Tx8: 220), most probably due to a higher proportion of islets labeled (75% of the infused number). Within the first 3 days, nearly no loss of hypointense signals was observed (96% of the initial number) (Fig. 2D); instead, subsequent MRI scans revealed a trend of signal loss similar to that observed in T1D patients with successful transplantation (Pt#1 and #3). In particular, at 1 week, 70% of the initial ROIs were detected, and at 1 month, they decreased to 42% (Fig. 2D). ROIs progressively decreased to 7% at 150 days. This patient was insulin free along the entire follow-up.

DISCUSSION

The possibility of monitoring islet persistence or disappearance by noninvasive imaging would undoubtedly represent an important asset to the islet Tx field. Previous studies have shown the feasibility both in animals (11,19,

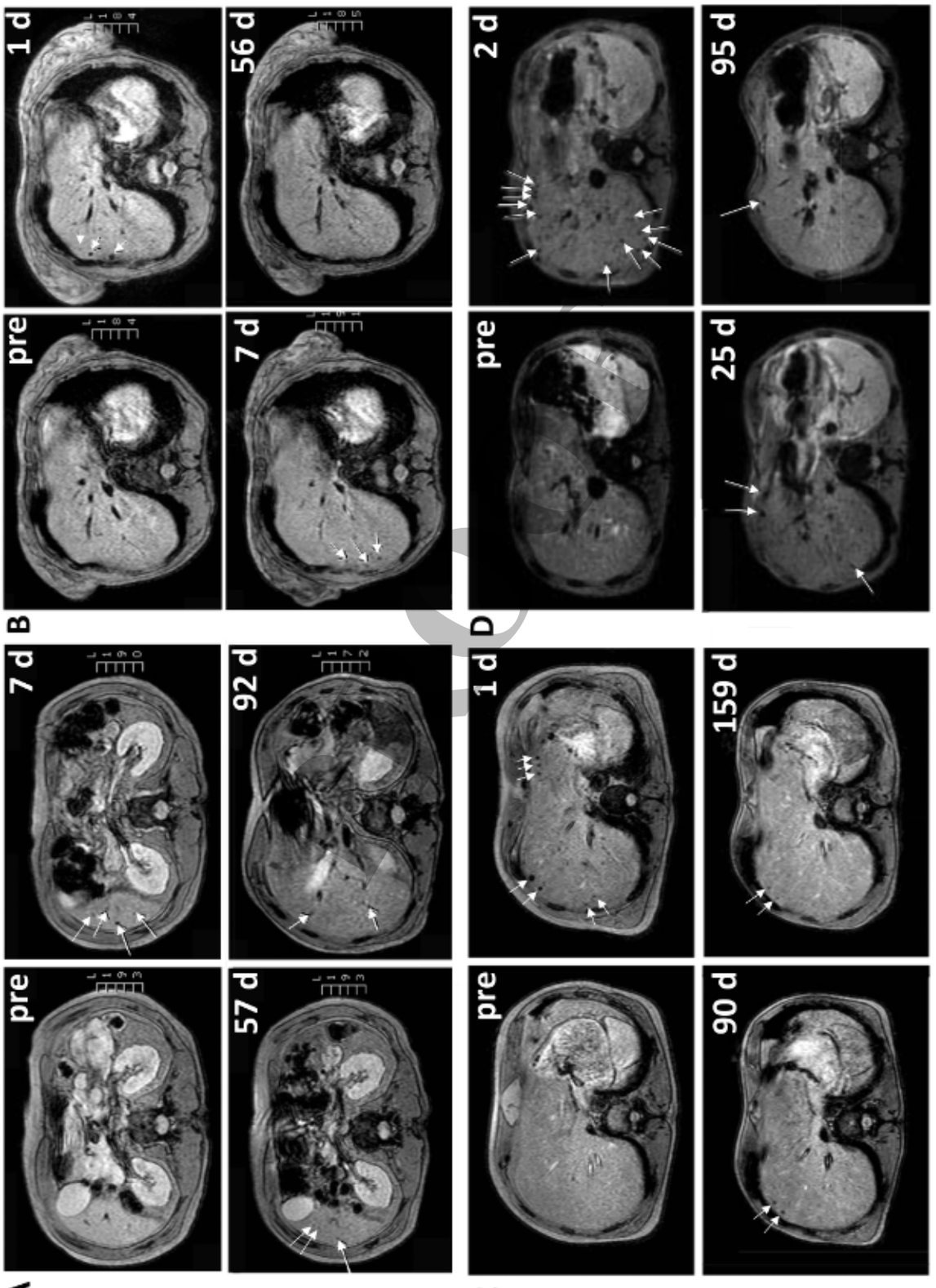

Figure 4. Representative axial T2* images acquired before (pre) and at different time points after labeled islet Tx. (A–D) Hypointense spots representing labeled islets transplanted within the liver are indicated by white arrows. (A) T1D Pt#1; (B) T1D Pt#2; (C) T1D Pt#3; (D) Auto-Tx8.

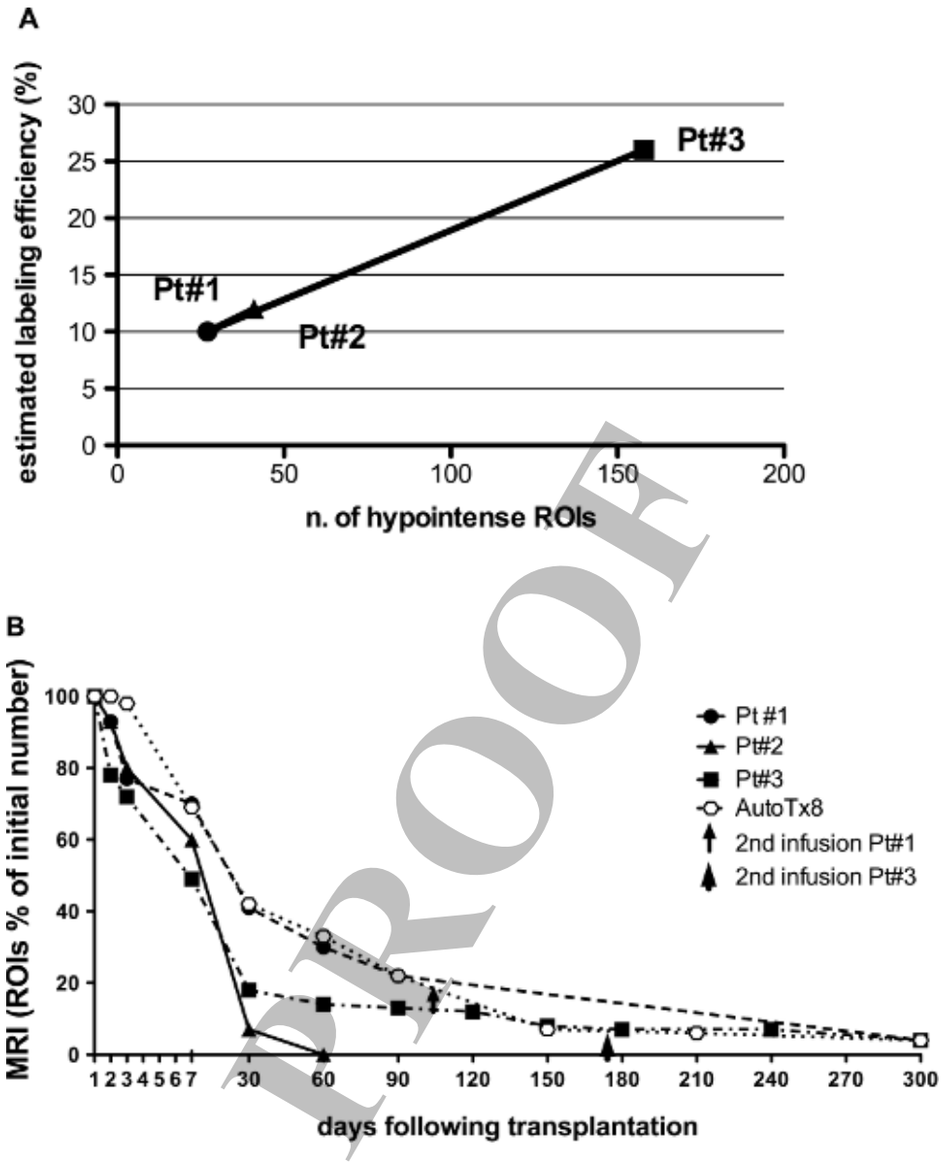

Figure 5. Correlation between islet labeling efficiency and number of MRI signals and their analysis over time. (A) Linear correlation between islet labeling efficiency and number of hypointense ROIs detected by MRI 24 h post-Tx in Pt#1–3. (B) Cumulative decline in MRI signals over time in the three T1D Pts transplanted with labeled islets and in the autotransplanted Pt (Auto-Tx8). Pt#1: filled circle; Pt#2: filled triangle; Pt#3: filled square; AutoTx8: open circle. The second infusion in Pt#1 and 3 is marked by arrows.

20,22,25) and in Pts (34,36) of MRI monitoring SPIO-labeled islets in the context of islet Tx. The safety and efficacy of this approach for midterm monitoring and comparison of the outcome in the context of auto-Tx had never been specifically addressed before.

The present study was undertaken with the aim of extending previous work on the usefulness of islet labeling with a clinically approved SPIO-based contrast agent for the evaluation of short- and midterm islet Tx success. This is the first time that Endorem® was used for labeling islets in a clinical study. Despite a report indicating that Resovist™ is superior in labeling efficiency to Endorem® (33), we chose the latter because Resovist™ was not available on the Italian market for the entire study. Three T1D Pts receiving labeled islets on the first infusion were evaluated for efficacy and safety. Moreover, detectability of labeled islets by MRI was also investigated in a partially pancreatectomized Pt, who received autologous islets, in order to assess the burden of allogeneic immune response in the outcome of MRI detection of Tx islets. Despite the initial program to investigate more Pts, Endorem® was withdrawn from the market for commercial reasons while the study was ongoing thus preventing recruitment of more Pts for Tx with labeled islets.

All quality control tests performed in vitro on islet preparations infused into Pts showed no signs of functional impairment, loss of viability, secretion of proinflammatory cytokines, previously associated to negative Tx outcome (18,30), nor a different susceptibility to proinflammatory cytokine exposure compared to unlabeled ones, thus confirming that islet labeling is not disadvantageous. Monitoring liver function by ALT and AST levels, as markers of early post-Tx complications in T1D Pts receiving labeled islets, showed a transient increase, which was according to previous observations (6,27). The coagulation marker XDP never reached a pathological value indicating no further damage to the livers receiving labeled islets, further confirming also in vivo the safety of the procedure. Also CRP was moderately altered, but only transiently, in two T1D Pts out of three.

The relationship between islet detection by MRI and islet function deserves special attention. The total number of ROIs counted 24 h post-Tx in our T1D Pts is in the same range reported in the first clinical study (36), but it is significantly lower than that described in a second study performed by Saudek and colleagues (34). The latter discrepancy could be explained by the following differences between the latter and the present study: (i) the different amount of labeled islets; in the present study we have precautionarily exposed only 30% of the total infused islets to iron labeling, whereas Saudek et al. (34) labeled all islets to be transplanted; (ii) we used Endorem® as labeling agent, which could provide a labeling efficiency slightly lower than Resovist™ (33) used in the other study (34).

The first month following Tx is considered pivotal for islet engraftment. All Pts receiving islets from allogeneic donors showed a rapid decline (about 30%) in the number of hypointense ROIs 3 days post-Tx. The decline showed a higher degree of variability at 1 week in Pts with functional grafts, ranging from 35 (Pt#1, #2, and Auto-Tx8) to 50% (Pt#3) reduction (avg residual spots $60 \pm 0.1\%$ SD), similarly to what has been reported previously at 7 days post-Tx (34). Rapid ROI disappearance in Pt#2 at 1 month paralleled loss of islet function, confirming the effectiveness of MRI imaging in diagnosing short-term failure, whereas Pt#1 and #3 with functional grafts at 1 month showed residual ROIs, which were in one case (Pt#1; 41%) similar to what had been previously shown [45.5 ± 5.8 SD; (34)] in the other case lower (Pt#3; 18%). We cannot exclude that the difference in the number of hypointense spots observed at 3 months in our study compared to previously reported (34) might be due to the different labeling agents employed. Another possible explanation however could be attributed to recurrent autoimmunity (GADA), which was observed in Pt#3, who showed the lowest residual ROI number at day 7, as well as 1 and 3 months following Tx. This fits with Tx recipients showing recurrent autoimmunity having significantly lower probability of graft survival (29). Interestingly, the autotransplanted Pt showed no decline in the number of hypointense ROIs during the first 3 days following Tx. This patient lacks the immune reaction harnessing allogeneic islets and does not show signs of liver damage, not even the transient increase in ALT and AST levels observed in the T1D Tx recipients, suggesting that this could be related to the lack of inflammatory response elicited by the autologous islets or by the lower absolute number of islets infused in this patient. Thus the lower decrease in hypointense ROIs observed within the first days in Auto-TX8 Pt could be interpreted as a sign of higher early islet survival, a feature that is considered pivotal for the long-term success of islet Tx. Surprisingly enough, however, this Pt showed thereafter the same type of signal decline observed in Pt#1. Compared to T1D, Auto-Tx8 Pt showed higher XDP and CRP values, which were observed following pancreatectomy before and after Tx and could be most probably due to the surgery. These changes have been shown, however, not to correlate with graft function (6). Auto-Tx8 Pt suffers from a genetic condition causing recurrent pancreatitis, which was the reason why he underwent partial pancreatectomy; thus it could be interesting to investigate whether in such cases other still unknown factors could have a negative impact on Tx islet engraftment. Overall the important decrease in ROI number measured within the first month in all patients suggests that an important loss of islets occurs during engraftment. This loss of islets is not a trigger for autoimmunity recurrence as shown in Pt#2 experiencing massive early graft loss with no further increase in GADA titer or further serum conversion. Moreover islet loss in Pt#2 does not induce false positive hypointense signals as could have been predicted if Endorem® released from islets was unspecifically retained by liver cells (33). This is in agreement with previous observations produced in allogeneic transplantation experiments with islets labeled with Feridex®, the same reagent as Endorem®(12).

At later time points, despite functional islet grafts, monitoring hypointense ROIs in all subjects showed a slow, but steady decline starting from 1 month after Tx onward. This could be explained by dextran-coated SPIO particles—internalized by endocytic pathways (5,11) ending up in the lysosomal compartment—being unstable. In vitro studies evaluating the effects of low pH on the solubility of SPIO [Fe(III) structures] have demonstrated that pH 4.5 (typical of the lysosomal compartment) induces increase in reducible free iron [Fe(II)] suggesting nanoparticle dissolution (1). Slow solubilization of SPIO particles potentially occurring upon endosome fusion with lysosomes might thus contribute to MRI signal loss over time.

In the absence of a clinically validated automated scoring system for counting hypointense signals appearing following Tx of SPIO-labeled islets, applying identification

rules to the careful visual inspection of MRI images taken before and after Tx appeared to be the best approach to avoid overestimation errors. One might argue, however, that the discrepancy between the decline in MRI signals despite graft function might be related in part to insufficient sensitivity of MRI (16) and/or of the counting approach, which, relying on a visual score, might tend to include only signals coming from larger ROIs and miss multiple smaller ones. The development of a dedicated algorithm also able to identify weak signals significantly differing from the background could be very useful to tackle this issue. However small hypointense spots, which are missed by the visual scoring approach, have been recently shown to represent iron particles phagocytized by $CD68^+$ cells (19). Moreover, albeit only the purest fraction of infused islet preparations was exposed to SPIO, we cannot exclude that nonendocrine contaminants were labeled as well (13). Since also non-β endocrine cells can take up SPIO (5,12,25), the overall disappearance of iron signals could reflect in part the loss of both nonendocrine and non-β-cell components, accompanying islet engraftment (7,28).

Taken together, the study shows that (i) Endorem®-labeled islets proved overall safe both in vitro and in Pts; (ii) ROI number in the short term parallels the number of successfully engrafted islets; (iii) within the setting of allotransplantation, an average of 20–30% of the detectable ROIs are lost within the first few days post-Tx, whereas this is not evident in the autotransplanted Pt; (iv) the relevance of mid- to long-term islet survival monitoring by means of SPIO labeling is difficult to assess in the present study, probably also due to a suboptimal performance of Endorem® as a contrast agent.

Recently, some pharmacological treatments have been shown to improve islet Tx success both in mouse and in humans (4,9,21), possibly preventing islet death at early time points following Tx. An imaging approach providing a readout for early islet survival could be a valuable tool for validating novel therapeutic strategies improving early islet survival post-Tx, thus potentially leading to a lower need of IEQ/Pt to achieve insulin independence (actually 10,000 IEQ/kg of body weight). This would imply that more Pts could benefit from Tx.

The small number of cases studied certainly represents a limitation of our observations. This is due to the fact that Endorem® stopped being produced while the study was ongoing, and no replacement for it was available. Thus our observations would need a validation with more preclinical and clinical studies, with newer contrast agents potentially available for clinical and preclinical approaches.

In conclusion, the present study supports with detailed functional analyses that MRI-based monitoring of SPIO-labeled islets after Tx within Pts is a safe procedure, which does not alter islet function, neither induces additional stress to the liver of transplanted Pts, nor was associated to autoimmune recurrence. MRI-based monitoring of SPIO-labeled islets appears a promising way for monitoring short-term Tx success. If validated, it could be a very useful approach for testing the efficacy of novel immunomodulatory regimens preventing early islet loss.

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REFERENCES

1. Arbab, A. S.; Wilson, L. B.; Ashari, P.; Jordan, E. K.; Lewis, B. K.; Frank, J. A. A model of lysosomal metabolism of dextran coated superparamagnetic iron oxide (SPIO) nanoparticles: Implications for cellular magnetic resonance imaging. NMR Biomed. 18(6):383–389; 2005.
2. Barton, F. B.; Rickels, M. R.; Alejandro, R.; Hering, B. J.; Wease, S.; Naziruddin, B.; Oberholzer, J.; Odorico, J. S.; Garfinkel, M. R.; Levy, M.; Pattou, F.; Berney, T.; Secchi, A.; Messinger, S.; Senior, P. A.; Maffi, P.; Posselt, A.; Stock, P. G.; Kaufman, D. B.; Luo, X.; Kandeel, F.; Cagliero, E.; Turgeon, N. A.; Witkowski, P.; Naji, A.; O'Connell, P. J.; Greenbaum, C.; Kudva, Y. C.; Brayman, K. L.; Aull, M. J.; Larsen, C.; Kay, T. W.; Fernandez, L. A.; Vantyghem, M. C.; Bellin, M.; Shapiro, A. M. Improvement in outcomes of clinical islet transplantation: 1999–2010. Diabetes Care 35(7):1436–1445; 2012.
3. Bassi, R.; Fiorina, P. Impact of islet transplantation on diabetes complications and quality of life. Curr. Diab. Rep. 11(5):355–363; 2011.
4. Bellin, M. D.; Barton, F. B.; Heitman, A.; Harmon, J. V.; Kandaswamy, R.; Balamurugan, A. N.; Sutherland, D. E.; Alejandro, R.; Hering, B. J. Potent induction immunotherapy promotes long-term insulin independence after islet transplantation in type 1 diabetes. Am. J. Transplant. 12(6):1576–1583; 2012.
5. Berkova, Z.; Jirak, D.; Zacharovova, K.; Kriz, J.; Lodererova, A.; Girman, P.; Koblas, T.; Dovolilova, E.; Vancova, M.; Hajek, M.; Saudek, F. Labeling of pancreatic islets with iron oxide nanoparticles for in vivo detection with magnetic resonance. Transplantation 85(1):155–159; 2008.
6. Bertuzzi, F.; Marzorati, S.; Maffi, P.; Piemonti, L.; Melzi, R.; de Taddeo, F.; Valtolina, V.; D'Angelo, A.; di Carlo, V.; Bonifacio, E.; Secchi, A. Tissue factor and CCL2/monocyte chemoattractant protein-1 released by human islets affect islet engraftment in type 1 diabetic recipients. J. Clin. Endocrinol. Metab. 89(11):5724–5728; 2004.
7. Cantarelli, E.; Melzi, R.; Mercalli, A.; Sordi, V.; Ferrari, G.; Lederer, C. W.; Mrak, E.; Rubinacci, A.; Ponzoni, M.; Sitia, G.; Guidotti, L. G.; Bonifacio, E.; Piemonti, L. Bone marrow as an alternative site for islet transplantation. Blood 114(20):4566–4574; 2009.
8. Caumo, A.; Maffi, P.; Nano, R.; Bertuzzi, F.; Luzi, L.; Secchi, A.; Bonifacio, E.; Piemonti, L. Transplant estimated

function: A simple index to evaluate beta-cell secretion after islet transplantation. Diabetes Care 31(2):301–305; 2008.
9. Citro, A.; Cantarelli, E.; Maffi, P.; Nano, R.; Melzi, R.; Mercalli, A.; Dugnani, E.; Sordi, V.; Magistretti, P.; Daffonchio, L.; Ruffini, P. A.; Allegretti, M.; Secchi, A.; Bonifacio, E.; Piemonti, L. CXCR1/2 inhibition enhances pancreatic islet survival after transplantation. J. Clin. Invest. 122(10):3647–3651; 2012.
10. Eich, T.; Eriksson, O.; Lundgren, T. Visualization of early engraftment in clinical islet transplantation by positron-emission tomography. N. Engl. J. Med. 356(26):2754–2755; 2007.
11. Evgenov, N. V.; Medarova, Z.; Dai, G.; Bonner-Weir, S.; Moore, A. In vivo imaging of islet transplantation. Nat. Med. 12(1):144–148; 2006.
12. Evgenov, N. V.; Medarova, Z.; Pratt, J.; Pantazopoulos, P.; Leyting, S.; Bonner-Weir, S.; Moore, A. In vivo imaging of immune rejection in transplanted pancreatic islets. Diabetes 55(9):2419–2428; 2006.
13. Evgenov, N. V.; Pratt, J.; Pantazopoulos, P.; Moore, A. Effects of glucose toxicity and islet purity on in vivo magnetic resonance imaging of transplanted pancreatic islets. Transplantation 85(8):1091–1098; 2008.
14. Fendler, W.; Baranowska, A. I.; Mianowska, B.; Szadkowska, A.; Mlynarski, W. Three-year comparison of subcutaneous insulin pump treatment with multi-daily injections on HbA1c, its variability and hospital burden of children with type 1 diabetes. Acta Diabetol. 49(5):363–370; 2012.
15. Fiorina, P.; Shapiro, A. M.; Ricordi, C.; Secchi, A. The clinical impact of islet transplantation. Am. J. Transplant. 8(10):1990–1997; 2008.
16. Heyn, C.; Bowen, C. V.; Rutt, B. K.; Foster, P. J. Detection threshold of single SPIO-labeled cells with FIESTA. Magn. Reson. Med. 53(2):312–320; 2005.
17. Jirak, D.; Kriz, J.; Herynek, V.; Andersson, B.; Girman, P.; Burian, M.; Saudek, F.; Hajek, M. MRI of transplanted pancreatic islets. Magn. Reson. Med. 52(6):1228–1233; 2004.
18. Johansson, H.; Lukinius, A.; Moberg, L.; Lundgren, T.; Berne, C.; Foss, A.; Felldin, M.; Kallen, R.; Salmela, K.; Tibell, A.; Tufveson, G.; Ekdahl, K. N.; Elgue, G.; Korsgren, O.; Nilsson, B. Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. Diabetes 54(6):1755–1762; 2005.
19. Kim, J. H.; Jin, S. M.; Oh, S. H.; Lee, S.; Oh, B. J.; Kim, S. K.; Suh, S.; Lee, J. H.; Jung, H. S.; Lee, M. S.; Lee, M. K.; Kim, K. W. Counting small hypointense spots confounds the quantification of functional islet mass based on islet MRI. Am. J. Transplant. 12(5):1303–1312; 2012.
20. Koblas, T.; Girman, P.; Berkova, Z.; Jirak, D.; Kriz, J.; Dovolilova, E.; Zacharovova, K.; Hajek, M.; Saudek, F. Magnetic resonance imaging of intrahepatically transplanted islets using paramagnetic beads. Transplant. Proc. 37(8):3493–3495; 2005.
21. Koulmanda, M.; Bhasin, M.; Awdeh, Z.; Qipo, A.; Fan, Z.; Hanidziar, D.; Putheti, P.; Shi, H.; Csizuadia, E.; Libermann, T. A.; Strom, T. B. The role of TNF-alpha in mice with type 1- and 2- diabetes. PLoS One 7(5):e33254; 2012.
22. Kriz, J.; Jirak, D.; Girman, P.; Berkova, Z.; Zacharovova, K.; Honsova, E.; Lodererova, A.; Hajek, M.; Saudek, F. Magnetic resonance imaging of pancreatic islets in tolerance and rejection. Transplantation 80(11):1596–1603; 2005.
23. Lombardo, F.; Scaramuzza, A. E.; Iafusco, D. Failure of glycated hemoglobin drop after continuous subcutaneous insulin infusion initiation may indicate patients who discontinue: A 4-year follow-up study in children and adolescents with type 1 diabetes. Acta Diabetol. 49(Suppl 1): S99–S105; 2012.
24. Maffi, P.; Scavini, M.; Socci, C.; Piemonti, L.; Caldara, R.; Gremizzi, C.; Melzi, R.; Nano, R.; Orsenigo, E.; Venturini, M.; Staudacher, C.; Del Maschio, A.; Secchi, A. Risks and benefits of transplantation in the cure of type 1 diabetes: Whole pancreas versus islet transplantation. A single center study. Rev. Diabet. Stud. 8(1):44–50; 2011.
25. Malosio, M. L.; Esposito, A.; Poletti, A.; Chiaretti, S.; Piemonti, L.; Melzi, R.; Nano, R.; Tedoldi, F.; Canu, T.; Santambrogio, P.; Brigatti, C.; De Cobelli, F.; Maffi, P.; Secchi, A.; Del Maschio, A. Improving the procedure for detection of intrahepatic transplanted islets by magnetic resonance imaging. Am. J. Transplant. 9(10):2372–2382; 2009.
26. Marzorati, S.; Antonioli, B.; Nano, R.; Maffi, P.; Piemonti, L.; Giliola, C.; Secchi, A.; Lakey, J. R.; Bertuzzi, F. Culture medium modulates proinflammatory conditions of human pancreatic islets before transplantation. Am. J. Transplant. 6(11):2791–2795; 2006.
27. Melzi, R.; Mercalli, A.; Sordi, V.; Cantarelli, E.; Nano, R.; Maffi, P.; Sitia, G.; Guidotti, L. G.; Secchi, A.; Bonifacio, E.; Piemonti, L. Role of CCL2/MCP-1 in islet transplantation. Cell Transplant. 19(8):1031–1046; 2010.
28. Melzi, R.; Sanvito, F.; Mercalli, A.; Andralojc, K.; Bonifacio, E.; Piemonti, L. Intrahepatic islet transplant in the mouse: Functional and morphological characterization. Cell Transplant. 17(12):1361–1370; 2008.
29. Piemonti, L.; Everly, M. J.; Maffi, P.; Scavini, M.; Poli, F.; Nano, R.; Cardillo, M.; Melzi, R.; Mercalli, A.; Sordi, V.; Lampasona, V.; Espadas de Arias, A.; Scalamogna, M.; Bosi, E.; Bonifacio, E.; Secchi, A.; Terasaki, P. I. Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. Diabetes 62(5):1656–1664; 2013.
30. Piemonti, L.; Leone, B. E.; Nano, R.; Saccani, A.; Monti, P.; Maffi, P.; Bianchi, G.; Sica, A.; Peri, G.; Melzi, R.; Aldrighetti, L.; Secchi, A.; Di Carlo, V.; Allavena, P.; Bertuzzi, F. Human pancreatic islets produce and secrete MCP-1/CCL2: Relevance in human islet transplantation. Diabetes 51(1):55–65; 2002.
31. Ricordi, C.; Lacy, P. E.; Finke, E. H.; Olack, B. J.; Scharp, D. W. Automated method for isolation of human pancreatic islets. Diabetes 37(4):413–420; 1988.
32. Ricordi, C.; Lacy, P. E.; Scharp, D. W. Automated islet isolation from human pancreas. Diabetes 38(Suppl 1):140–142; 1989.
33. Ris, F.; Lepetit-Coiffe, M.; Meda, P.; Crowe, L. A.; Toso, C.; Armanet, M.; Niclauss, N.; Parnaud, G.; Giovannoni, L.; Bosco, D.; Morel, P.; Vallee, J. P.; Berney, T. Assessment of human islet labeling with clinical grade iron nanoparticles prior to transplantation for graft monitoring by MRI. Cell Transplant. 19(12):1573–1585; 2010.
34. Saudek, F.; Jirak, D.; Girman, P.; Herynek, V.; Dezortova, M.; Kriz, J.; Peregrin, J.; Berkova, Z.; Zacharovova, K.; Hajek, M. Magnetic resonance imaging of pancreatic islets transplanted into the liver in humans. Transplantation 90(12):1602–1606; 2010.
35. Shapiro, A. M.; Lakey, J. R.; Ryan, E. A.; Korbutt, G. S.; Toth, E.; Warnock, G. L.; Kneteman, N. M.; Rajotte, R. V.

Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N. Engl. J. Med. 343(4):230–238; 2000.
36. Toso, C.; Vallee, J. P.; Morel, P.; Ris, F.; Demuylder-Mischler, S.; Lepetit-Coiffe, M.; Marangon, N.; Saudek, F.; James Shapiro, A. M.; Bosco, D.; Berney, T. Clinical magnetic resonance imaging of pancreatic islet grafts after iron nanoparticle labeling. Am. J. Transplant. 8(3):701–706; 2008.
37. Venturini, M.; Angeli, E.; Maffi, P.; Fiorina, P.; Bertuzzi, F.; Salvioni, M.; De Cobelli, F.; Socci, C.; Aldrighetti, L.; Losio, C.; Di Carlo, V.; Secchi, A.; Del Maschio, A. Technique, complications, and therapeutic efficacy of percutaneous transplantation of human pancreatic islet cells in type 1 diabetes: the role of US. Radiology 234(2):617–624; 2005.