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Original Article

Diagnosis and treatment of allograft rejection in islet transplantation

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ABSTRACT

Islet transplantation stabilizes glycemic control in patients with complicated diabetes mellitus. Rapid functional decline could be due to islet allograft rejection. However, there is no reliable method to assess rejection, and treatment protocols are absent. We aimed to characterize diagnostic features of islet allograft rejection and assess effectiveness of high-dose methylprednisolone treatment. Over a median follow-up of 61.8 months, 22% (9 of 41) of islet transplant recipients experienced 10 suspected rejection episodes (SREs). All first SREs occurred within 18 months after transplantation. Important features were unexplained hyperglycemia (all cases), unexplained C-peptide decrease (ΔC-peptide, 77.1% [−59.1% to −91.6%]; ΔC-peptide:glucose, −76.3% [−49.2% to −90.4%]), predisposing event (5 of 10 cases), and increased immunologic risk (5 of 10 cases). At 6 months post-SRE, patients who received protocolized methylprednisolone (n = 4) had significantly better islet function than untreated patients (n = 4), according to C-peptide (1.39 ± 0.59 vs 0.14 ± 0.19 nmol/L; $P = .007$), Igls score (good [4 of 4 cases] vs failure [3 of 4 cases] or marginal [1 of 4 cases]; $P = .018$) and β score (6.0 [6.0-6.0] vs 1.0 [0.0-3.5]; $P = .013$). SREs are prevalent among islet transplant recipients and are associated with loss of islet graft function. Timely treatment with high-dose methylprednisolone mitigates this loss. Unexplained hyperglycemia, unexpected C-peptide decrease, a predisposing event, and elevated immunologic risk are diagnostic indicators for SRE.

Abbreviations: BMI, body mass index; CP/G, C-peptide to glucose ratio; DSA, donor-specific antibody; HbA_{1c}, hemoglobin type A_{1c}; HLA, human leukocyte antigen; IAK, islet-after-kidney transplantation; ITA, islet transplantation alone; ITx, islet transplantation; IU, international unit; IV, intravenously; NA, not applicable; PRA, panel-reactive antibody; SRE, suspected rejection episode.

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1. Introduction

Pancreatic islet transplantation is a treatment option for patients with severely complicated type 1 diabetes mellitus. Indications include a history of recurrent (severe) hypoglycemia, impaired awareness of hypoglycemia, or progressive complications despite optimal medical management.[1,2] The goal of islet transplantation is to improve glycemic control and stability, thereby improving quality of life and reducing diabetes-related complications such as severe hypoglycemia.[2-6]

As in any allogeneic solid organ transplantation, allograft rejection may occur in islet transplant recipients.[7] However, in contrast to most solid organ transplantations, no consensus exists on diagnosis of islet allograft rejection. This can in part be explained because the gold standard of diagnosing rejection is through tissue biopsy, which is not possible in islet graft rejection.[8,9] Because islets are diffusely dispersed throughout the liver after intraportal infusion, a liver biopsy has a low chance of sampling a sufficient number of grafted islets for diagnosis.[10] For this reason, standardized clinical diagnostic criteria to reliably diagnose ongoing islet allograft rejection are needed.

While islet allograft rejection could be an important cause of islet graft function loss, there are currently no established treatment protocols. First-line treatment in kidney allograft rejection consists of high-dose methylprednisolone. This treatment regimen is well-established and has proven to be effective in restoring kidney graft function after rejection.[11,12] This treatment may potentially halt the ongoing rejection in islet allograft rejection as well. However, high-dose steroid therapy itself is thought to be associated with functional islet graft deterioration.[13-15]

Thus, there is a paucity of evidence regarding both diagnosis and treatment of islet allograft rejection, with only 2 cases previously reported.[7,16] For this reason, we studied a cohort of patients with a suspected rejection episode (SRE) in order to characterize diagnostic features for islet allograft rejection and to assess the effectiveness of high-dose methylprednisolone treatment in preventing rejection-related loss of islet graft function.

2. Materials and methods

2.1. Design and patients

We studied patients who received 1 or more allogeneic islet transplantations (ITx) between 2008 and 2019 for severely complicated insulin-deficient diabetes mellitus at the Leiden University Medical Center. A subset of these patients experienced SREs. Indications for ITx in patients without prior solid organ transplantation included severe or recurrent hypoglycemia and/or impaired awareness of hypoglycemia despite optimal conventional diabetes treatment and care, or problematic glycemic instability in patients who received a previous solid organ transplantation.[1,2] Immunosuppressive induction and maintenance regimens have previously been reported[1] and are discussed in detail in Supplementary Method 1.

2.2. Diagnosis and treatment of suspected rejection

For the diagnosis of an SRE, the following protocol was used: patients with persistent hyperglycemia were clinically evaluated by a diabetologist with expertise in islet transplantation. Complete patient history and examination were performed, with routine laboratory testing to screen for abnormalities. Patients with infections such as cytomegalovirus and Epstein-Barr virus were excluded. Immunosuppressant trough levels (to detect high or low concentrations of tacrolimus), autoantibodies against β-cell antigens and (novel) donor-specific antibodies (DSAs) were also assessed. After exclusion of infectious, inflammatory, or medication-related causes, SRE was considered the most probable diagnosis.

In 2015, a standardized treatment protocol for SREs was implemented: all patients diagnosed with an SRE were hospitalized and treated with high-dose methylprednisolone 1000 mg/d intravenously (IV) for 3 consecutive days, initiated as soon as possible after diagnosis. Patients also received supportive care with intravenous insulin to attain and maintain normoglycemia (ie, blood glucose levels of 4-8 mmol/L). The dosage of immunosuppressive agents was increased when trough concentrations were low or when no apparent cause for the SRE was identified. In the early years of our islet transplant program (2007-2015), recipients diagnosed with SRE did not receive antirejection treatment because of the absence of guidelines or a treatment protocol. During this period, due to emerging insight and poor post-SRE outcomes in untreated patients, 2 patients were empirically treated with methylprednisolone in a nonprotocolized manner. Using a preimplementation/postimplementation approach, we assessed the effectiveness of the treatment regimen.

2.3. Outcome measures

The primary outcome measure was islet graft function based on the Igls score at 6 months post-SRE. The Igls score expresses graft function after ITx as optimal, good, marginal, or failure based on 4 variables: insulin dose, hemoglobin type A_{1c} (HbA_{1c}), severe hypoglycemic events, and C-peptide (Supplementary Method 2).[17] We also assessed β[18] and BETA-2[19] scores and stimulated C-peptide during a mixed meal tolerance test at 6 months post-SRE. β score reflects islet graft function on a scale of 0 to 8 (from no to optimal function), using insulin dose, fasting glucose, HbA_{1c}, and stimulated C-peptide (Supplementary Method 3),[18] whereas BETA-2 score reflects islet graft function in a range from 0 to 42 (from no to optimal function), using insulin dose, fasting glucose, HbA_{1c}, and fasting C-peptide (Supplementary Method 4).[19] Other secondary outcome parameters included, among others, measures of glycemic control (eg, HbA_{1c}, fasting glucose, and insulin requirements) and measures of immunization to human leukocyte antigen (HLA; eg, panel-reactive antibodies [PRAs] and specificity of HLA antibodies). Details on outcome parameters and mixed meal tolerance test procedure are provided in Supplementary Method 5.

2.4. Comparison of groups and time points

Primarily, patients with an SRE who had received protocolized high-dose methylprednisolone were compared to untreated

patients. Furthermore, all patients with SRE were compared to patients without SRE (reference group). Igls and β scores were assessed at several preset time points: the most recent measurement before hyperglycemia onset, at the time of the SRE (t = 0), and 1 to 2 months, 3 to 4 months, and 6 months after the SRE. Because the reference group did not experience an SRE, t = 0 for this group was set equal to the SRE group's mean time to rejection since the last ITx. For stimulated C-peptide and BETA-2 score, we only compared single pre-SRE and post-SRE values because mixed meal tolerance tests and fasting measurements are only performed once in each of these time frames at most. For patients who received an additional ITx within 6 months post-SRE, only the measurements up until the subsequent transplantation were included.

2.5. Data collection and analysis

Patient data were extracted from patient records and entered into a Castor database (Castor Electronic Data Capture; Ciwit BV). Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corporation). Normality of distribution was assessed by the Kolmogorov-Smirnov test and through visual histogram distribution evaluation. An unpaired *t* test was used for comparing normally distributed numerical variables in patients with and without methylprednisolone treatment; a paired *t* test for normally distributed numerical variables in patients pre- and postrejection. Mann-Whitney *U* test was used for comparing nonparametric numerical variables in patients with and without treatment; Wilcoxon signed rank test was used for nonparametric numerical variables in patients pre- and postrejection. For categorical variables, χ^2 test was used for comparing unpaired and Wilcoxon signed rank test for paired variables. Normally distributed numerical variables are expressed as mean ± standard deviation, nonparametric numerical variables as median (first quartile-third quartile). Calculated differences (Δ) are expressed as mean difference ± standard error of the mean difference. Categorical variables are expressed as number of cases (percentage of patient population). Percentages displayed were calculated for the total number of patients or SREs in the group, unless otherwise indicated (eg, in case of missing values). A *P* value of $<.05$ was considered statistically significant.

3. Results

3.1. Patients and characteristics

A total of 41 patients who received allogeneic islet transplantations from May 2008 to January 2019 were included (Fig. 1). The median follow-up period was 61.8 (21.2-92.4) months. Of these 41 ITx recipients, 9 (22%) experienced a first SRE within 18 months after the last islet transplantation. One of the patients experienced 2 SREs. Therefore, a total of 10 SREs were assessed in this study. Until the implementation of the standardized treatment protocol, 6 SREs occurred of which 4 remained untreated and 2 were treated with nonprotocolized methylprednisolone. Four SREs were treated according to protocol after implementation of the standardized treatment. The reference group consisted of the remaining 32 islet transplant recipients.

Mean time from the last received ITx to SRE was 9.4 months, which was set as t = 0 for the reference group. Prerejection islet function (according to Igls score, β score, and stimulated C-peptide) did not significantly differ between the groups ($P = .495$, $P = .249$, and $P = .565$, respectively) (Supplementary Table 1). Other baseline characteristics, with the exception of PRA ($P = .012$), were also similar.

Baseline islet graft function (according to Igls score, β score, and stimulated C-peptide) did not significantly differ between the patients who experienced an SRE with and without protocolized methylprednisolone treatment ($P = .285$, $P = .760$, and $P = .805$, respectively) (Table 1). Untreated patients had a higher baseline

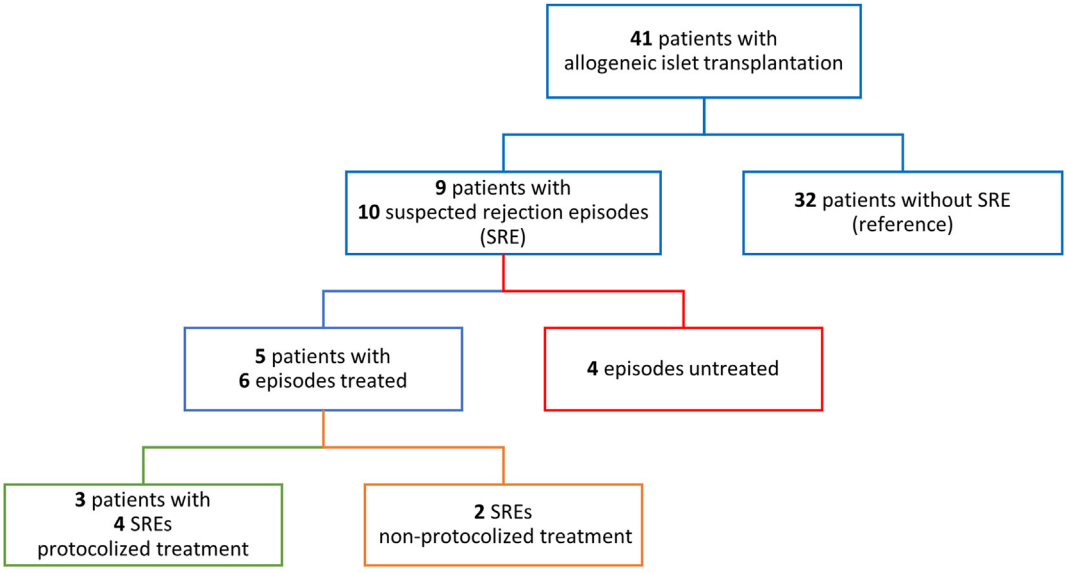

Figure 1. Flowchart illustrating the study population.

Table 1
Baseline characteristics—protocolized treatment vs untreated.

Baseline characteristics	Protocolized treatment	Untreated	*P*
Patients (n)	3	4	
Sex			
Male	1 (33.3)	4 (100.0)	.053
Female	2 (66.7)	0 (0.0)	
Age at time of SRE (y)	40.0 ± 8.7	51.5 ± 10.0	.174
BMI (kg/m^2)	19.9 ± 1.3	24.2 ± 2.4	**.037**
Duration of diabetes (y)	30.3 ± 3.8	33.0 ± 11.5	.721
Diabetic retinopathy[a]			
Not present	1 (33.3)	1 (25.0)	.809
Present	2 (66.7)	3 (75.0)	
Macrovascular complications[b]			
Not present	3 (100.0)	1 (25.0)	**.047**
Present	0 (0.0)	3 (75.0)	
Amputation			
No	3 (100.0)	2 (50.0)	.147
Yes	0 (0.0)	2 (50.0)	
Type of transplantation[c]			
IAK	3 (100.0)	3 (75.0)	.35
ITA	0 (0.0)	1 (25.0)	
No. of different islet donors per patient	2.0 (NA[d])	2.5 (1.0-4.0)	.354
No. of SREs	4	4	
Time since last islet transplantation (mo)	10.0 (7.8-23.5)	5.5 (2.8-15.0)	.146
Igls score pre-SRE[e]			
Failure	0 (0.0)	0 (0.0)	.285
Marginal	0 (0.0)	0 (0.0)	
Good	3 (75.5)	4 (100.0)	
Optimal	1 (25.5)	0 (0.0)	
β score pre-SRE[e]	6.0 (5.0-7.0)	6.0 (5.3-6.0)	.76
Maximum stimulated C-peptide pre-SRE[e] (nmol/L)	1.83 ± 0.86	2.38 ± 1.59	.805
CP/G ratio pre-SRE[e]	2.88 ± 1.56	2.65 ± 2.60	.884
Insulin dose per day pre-SRE[e] (IU/24 h)	6.6 ± 4.7	25.8 ± 6.8	**.004**
Insulin dose per kilogram per day pre-SRE[e] (IU/kg/24 h)	0.12 ± 0.09	0.33 ± 0.06	**.009**

Islet function over time for SREs with protocolized treatment vs untreated SREs, represented by Igls and β score. Numerical variables were all nonparametric and presented as median (first quartile-third quartile); categorical variables were presented as number of cases (percentage of total population).
IQR, interquartile range; NA, not applicable; SRE, suspected rejection episode.
Baseline characteristics of the islet transplant recipients who experienced an SRE and received protocolized treatment compared with those of islet transplant recipients who experienced an SRE but did not receive treatment (untreated). Normally distributed numerical variables are presented as mean ± standard deviation, nonparametric numerical variables as median (first quartile-third quartile), and categorical variables as number of cases (percentage of total population).
BMI, body mass index; CP/G, C-peptide:glucose; IAK, islet-after-kidney transplantation; ITA, islet transplantation alone; IU, international unit; NA, not applicable; SRE, suspected rejection episode.

[a] Diabetic retinopathy was defined as having underwent laser photocoagulation.

[b] Macrovascular complications were defined as having experienced a cardiac event, cerebrovascular accident, or peripheral vascular complication, which required intervention (eg, stenting and coronary artery bypass graft).

[c] All patients who experienced (pre)terminal kidney failure received a kidney transplantation prior to islet transplantation (IAK). No patients with preterminal or terminal kidney failure were given an islet transplantation. All patients with islet-alone transplantation had a creatinine clearance of >60 mL/min.

[d] IQR: first and third quartile not calculable with only 3 values.

[e] For all reported pre-SRE outcomes, the last available measurement before the SRE was taken.

BMI, higher insulin requirements, and more macrovascular complications ($P = .037$, $P = .004$, and $P = .047$, respectively).

3.2. Diagnosis of rejection

All patients with an SRE presented at the hospital with persistent hyperglycemia and increased insulin requirement (Supplementary Table 2). In 5 of the 10 SREs, a predisposing event could be identified that precipitated the SRE, including reduced immunosuppression (1 SRE), infection within 2 weeks prior to the event (2 SREs), or both (2 SREs). In 8 of 9 SREs, C-peptide was significantly lower at the time of SRE than that pre-SRE (median ΔC-peptide, 77.1% [−59.1% to −91.6%]). A reduction in C-peptide:glucose (CP/G) ratio was present in all (8 of 8) SREs (median ΔCP/G, −76.3% [−49.2 to −90.4%]). Newly detected HLA antibodies were found in 3 of 10 SREs, which included DSA in 2 cases and HLA antibodies specific to previous nonislet donors in 1 case. In 3 of 10 SREs, patients were already substantially immunized before the SRE (PRA levels of 60%, 88%, and 88%, respectively), whereas this was much lower in the reference group (1 of 32; 3.1%; $P = .012$) (Supplementary Table 1). An increase in PRA levels after rejection was found in 2 of the 10 SREs. There were no significant changes in prerejection vs postrejection anti-GAD65 (6 of 10 SREs) or islet antigen 2 (7 of 10 SREs) levels.

3.3. Treatment with methylprednisolone

All 4 patients in the protocolized treatment group received 1000 mg methylprednisolone IV daily for 3 consecutive days. Treatment commenced within 3 days after first noticing hyperglycemia in all patients. Of the 2 patients who had an SRE before implementation of the protocol but received nonprotocolized treatment, one was treated with a lower dose of methylprednisolone (500 mg IV, 3 boluses spread out over 5 nonconsecutive

Table 2
Islet function—β score and Igls score.

Islet graft functional outcomes	Protocolized treatment (n = 4)	Untreated (n = 4)	*P*
Before SRE			
β score	6.0 (5.0-7.0)	6.0 (5.3-6.0)	.76
Igls score			
Failure	0 (0.0)	0 (0.0)	
Marginal	0 (0.0)	0 (0.0)	
Good	3 (75.0)	4 (100.0)	
Optimal	1 (25.0)	0 (0.0)	.285
At the time of SRE			
β score	2.5 (2.0-3.8)	3.0 (2.3-3.8)	.647
Igls score			
Failure	0 (0.0)	1 (25.0)	
Marginal	3 (75.0)	3 (25.0)	
Good	1 (25.0)	0 (0.0)	
Optimal	0 (0.0)	0 (0.0)	.368
1-2 mo after SRE			
β score	5.0 (5.0-5.0)	2.5 (0.5-3.8)	**.014**
Igls score			
Failure	0 (0.0)	4 (100.0)	
Marginal	0 (0.0)	0 (0.0)	
Good	4 (100.0)	0 (0.0)	
Optimal	0 (0.0)	0 (0.0)	**.005**
3-4 mo after SRE			
β score	5.5 (IQR NA[a])[b]	1.0 (0.0-3.5)	**.031**
Igls score			
Failure	0/3 (0.0)	2 (50.0)	
Marginal	0/3 (0.0)	2 (50.0)	
Good	3/3 (100.0)	0 (0.0)	
Optimal	0/3 (0.0)	0 (0.0)	**.03**
6 mo after SRE			
β score	6.0 (6.0-6.0)	1.0 (0.0-3.5)	**.013**
Igls score			
Failure	0 (0.0)	2 (50.0)	
Marginal	0 (0.0)	2 (50.0)	
Good	4 (100.0)	0 (0.0)	
Optimal	0 (0.0)	0 (0.0)	**.018**

Islet function over time for SREs with protocolized treatment vs untreated SREs, represented by Igls and β score. Numerical variables were all nonparametric and presented as median (first quartile-third quartile); categorical variables were presented as number of cases (percentage of total population).
IQR, interquartile range; NA, not applicable; SRE, suspected rejection episode.
Baseline characteristics of the islet transplant recipients who experienced an SRE and received protocolized treatment compared with those of islet transplant recipients who experienced an SRE but did not receive treatment (untreated). Normally distributed numerical variables are presented as mean ± standard deviation, nonparametric numerical variables as median (first quartile-third quartile), and categorical variables as number of cases (percentage of total population).
BMI, body mass index; CP/G, C-peptide:glucose; IAK, islet-after-kidney transplantation; ITA, islet transplantation alone; IU, international unit; NA, not applicable; SRE, suspected rejection episode.
[a] IQR not calculable with only 3 values.
[b] Data of 1 SRE missing; displayed data are based on 3 episodes.

days), which was started 14 days after hyperglycemia onset. The other was treated with the normal dose (3 boluses of 1000 mg IV over 3 consecutive days), but treatment was initiated 23 days after hyperglycemia onset.

3.4. Islet graft function after rejection

Islet function according to Igls criteria was better in the patients who received protocolized treatment than that in the untreated patients at 6 months after the SRE (scoring good [4 of 4] vs failure [3 of 4] or marginal [1 of 4]; $P = .018$) (Table 2; Fig. 2). Also, β score was better (6.0 [6.0-6.0] vs 1.0 [0.0-4.3]; $P = .013$). BETA-2 score could be calculated only for 3 patients. Postrejection BETA-2 score (measured at median 5 months post-SRE) was higher in the 2 treated patients (17.35 [15.58-19.12]) than that in the 1 untreated patient in whom BETA-2 score decreased to 0.0 ($P = .221$).

Compared to baseline, islet graft function at 6 months post-SRE showed a near-complete recovery of function in the treated patients, with similar Igls score (good [4 of 4] vs good [3 of 4] or optimal [1 of 4]; $P = .317$) and β scores (6.0 [6.0-6.0] vs 6.0 [5.0-7.0]; $P = 1.000$). Within the untreated group, both Igls score (failure [2 of 4] or marginal [2 of 4] vs good [4 of 4]; $P = .063$) and β score (1.0 [0.0-3.5] vs 6.0 [5.3-6.0]; $P = .068$) were nonsignificantly lower than those at baseline.

Stimulated C-peptide did not significantly change after rejection in the protocolized treatment group (-0.44 ± 0.56 nmol/L; $P = .224$) but decreased significantly in the untreated group (-1.55 ± 0.62 nmol/L; $P = .015$) (Table 3), resulting in a mean post-SRE stimulated C-peptide concentration of 0.14 ± 0.19 nmol/L ($P = .007$). Similarly, CP/G ratio post-SRE was not significantly different from that pre-SRE in the protocolized treatment group (-0.01 ± 0.79; $P = .980$), while in untreated patients, CP/G was significantly lower with a mean post-SRE CP/G ratio of 0.13 ± 0.16 ($P = .037$).

Insulin requirement increased in all patients after an SRE, with a mean increase of 14.5 ± 10.0 international units (IU)/24 h ($P = .001$) or 0.21 ± 0.12 IU/kg/24 h ($P < .001$) (Table 1). In cases receiving protocolized treatment, insulin dose significantly increased by 6.0 ± 3.3 IU/24 h ($P = .035$) or 0.11 ± 0.06 IU/kg/24 h ($P = .029$), compared to 19.8 ± 11.2 IU/24 h ($P = .039$) or 0.25 ± 0.13 IU/kg/24 h ($P = .031$) in untreated cases (Table 3). The difference between untreated and treated cases did not reach statistical significance ($P = .057$ for IU/24 h or $P = .101$ for IU/kg/24 h, respectively).

3.5. Treatment side effects

Of the 6 treated patients, 2 (33.3%) reported side effects of methylprednisolone treatment. One patient experienced a feeling

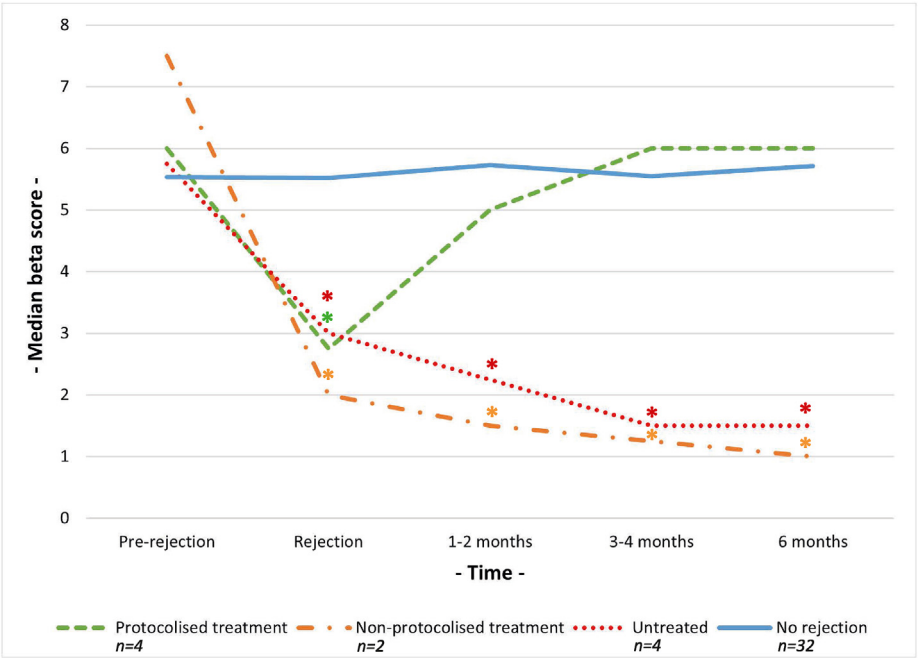

Figure 2. Progression of β score over time, according to β score of patients in the protocolized treatment group (green; n = 4), nonprotocolized treatment group (orange; n = 2), untreated group (red; n = 4), and reference population (no rejection; blue; n = 32). *Significantly different from reference group ($P < .05$).

of hyperactivity/restlessness. The other reported hyperactivity, anxiety/panic attacks, and fluid retention. No severe adverse events occurred.

4. Discussion

A rejection episode in islet allograft recipients is associated with a significant and sustained decline in graft function if left untreated. Surprisingly, there are no clear treatment guidelines for SREs in islet transplantation, which may be related to a paucity of data on diagnostic criteria and hesitation to use steroids after ITx. Here we show that timely treatment with high-dose methylprednisolone combined with supportive care is associated with a substantial 1-year islet graft function recovery after SRE. Over a median 5 years of follow-up, SREs occurred in 22% of our islet transplant recipients. Important features for diagnosing SRE

Table 3
Islet function—stimulated C-peptide and insulin requirement.

Islet graft functional outcomes	Protocolized treatment (n = 4)	Untreated (n = 4)	*P*
C-peptide post-SRE[a] (nmol/L)	1.39 ± 0.59	0.14 ± 0.19	**.007**
ΔC-peptide post-SRE vs pre-SRE (nmol/L)	−0.44 ± 0.56	−1.55 ± 0.62	**.040**
CP/G ratio post-SRE	2.87 ± 2.04	0.13 ± 0.16	**.037**
ΔCP/G ratio post-SRE vs pre-SRE	−0.01 ± 0.79	−2.52 ± 2.70	.124
Insulin dose post-SRE[b] (IU/24 h)	12.6 ± 2.6	45.5 ± 18.0	**.011**
ΔInsulin dose post-SRE vs pre-SRE (IU/24 h)	6.0 ± 3.3	19.8 ± 11.2	.057
Insulin dose post-SRE (IU/kg/24 h)	0.23 ± 0.08	0.57 ± 0.18	**.015**
ΔInsulin dose post-SRE vs pre-SRE (IU/kg/24 h)	0.11 ± 0.06	0.25 ± 0.13	.101

Stimulated C-peptide and insulin requirements after the SRE and the differences (Δ) between post-SRE vs pre-SRE are compared between SREs treated with protocolized treatment and SREs left untreated. All variables were numerical and normally distributed and presented as mean ± standard deviation. Differences (Δ) were presented as mean difference ± standard error of the difference.
CP/G, C-peptide:glucose; IU, international unit; IQR, interquartile range; SRE, suspected rejection episode.

[a] The median time of C-peptide measurement after rejection for these patients was 4.5 months (IQR, 3.0-6.0). Postrejection C-peptide was calculated in half of the cases (2 of the 4 patients were treated timely; 1 of the 2 patients was treated late; and 2 of the 4 patients were not treated).

[b] The median time of determining insulin requirement after rejection in these patients was 3.0 months (IQR, 2.5-3.0).

included unexplained hyperglycemia, considerable unexpected decrease in C-peptide, presence of a predisposing event, and high immunologic risk.

Allograft rejection may occur in any allogeneic transplant recipient and is an important cause for (acute) graft function deterioration. Although there is a scarcity of evidence,[7,16,20] there is no reason to assume that this would not be the case in islet transplantation. Indeed, we found that if an SRE was left untreated, islet graft function was almost completely lost. However, absence of protocols or guidelines regarding both diagnosis and treatment of SREs has hampered clinical care in islet transplant recipients with an SRE. This study is the first to thoroughly describe and analyze 10 SREs and, therefore, the first to report important features of SREs including incidence and timing after transplantation. Because of our preimplementation/postimplementation approach, we have been able to record both the natural course of islet function after SRE as well as compare this to the clinical course with high-dose methylprednisolone and intravenous insulin treatment.

Incidence of islet allograft rejection has not been described in existing literature. Our incidence of 22% in 5 years (with all first SREs occurring within 18 months) appears to be in line with the reported incidence in simultaneous pancreas-kidney transplant recipients of 21% over 5 years (and 18% over 1 year).[21] Incidence of rejection in kidney transplant recipients is reported to be slightly lower at 16% in 6 months.[22] A possible explanation could be the higher immunogenicity of pancreata and/or islets.[23,24]

Normally, allograft rejection is diagnosed through a tissue biopsy, and treatment depends on the type and severity of rejection.[8,9,25] Because islets are dispersed in the liver after intraportal infusion, it is not possible to obtain a representative biopsy. Random biopsies have a success rate of merely 31% to detect grafted islets, and only in approximately half of these, enough islets are sampled to make a diagnosis.[10] Also, liver biopsies are invasive and may lead to complications like bleeding.[26,27] There are also no reliable imaging modalities to assess (a reduction of) β-cell mass; thus, clinical features and laboratory parameters are assessed to diagnose islet allograft rejection. However, the diagnosis is often made tardily, when irreversible islet graft damage has already occurred. This emphasizes the need for standardized diagnostic criteria early in the clinical course. In this study, we describe the important early diagnostic features for SREs in our islet transplant population. In all SREs, recipients presented with unexplained hyperglycemia and increased insulin requirements, as a marker of (sub)acute islet graft failure. Importantly, other causes of hyperglycemia (eg, use of glucose-increasing medication, stress, inflammation, or current infection) should be ruled out. An abrupt decrease in C-peptide indicates sudden loss of islet graft function and can contribute to the diagnosis of islet rejection. In our patients, an unexpected decrease in C-peptide of >40% appeared indicative of islet rejection. All but 1 of the patients presented with an unexpected decrease in C-peptide, rendering it a robust diagnostic feature. The patient who experienced an increase in C-peptide presented to our hospital within a few hours after noticing hyperglycemia and had a history of chronic kidney disease (estimated glomerular filtration rate of 35 mL/min/1.73m^2 with creatinine of 152 μmol/L at the time of SRE). Acute β-cell destruction as a result of rejection may lead to an acute release of C-peptide from damaged cells, and C-peptide clearance is delayed due to impaired kidney function. This combination of factors could explain the increase in C-peptide in the early phase of rejection in this patient. CP/G ratio provided an even more robust diagnostic feature since a sudden CP/G ratio decrease of ≥33% was present in all cases, including the patient with increased C-peptide. Presence of a predisposing event, that is, a decrease in immunosuppression or a recent infection, which could stimulate crossreactive cellular alloimmunity, a phenomenon that has been associated with a higher risk of graft rejection in other organs,[28,29] also added to the probability of an SRE. T cell–mediated rejection may be even more important in islet transplant recipients than in kidney transplant recipients.[30] $CD4^+$ and $CD8^+$ T cell infiltrates are present in biopsies of transplanted islets.[10] Newly detected DSA, an increase in PRA or substantial baseline (prerejection) immunization (PRA >50%), was also an important diagnostic feature. Some evidence exists for a link between DSA and transplant rejection in islet transplant recipients, but only in selected case reports.[16,20] Broad development of DSA (both types I and II) has been shown in islet transplant recipients after failed islet transplantation.[31] In kidney transplantation, it is well-known that patients who are substantially immunized, that is, a high PRA at baseline, have a higher risk of developing graft rejection.[32] No significant increase in anti-GAD65 or anti–islet antigen 2 was found in this study, even though these are known to be elevated in autoimmunity and/or when (a large amount of) β-cell destruction is present.[33-35] Although autoimmunity cannot be ruled out, we therefore assume that recurrent autoimmunity did not play a major role in our patient population.

Only 2 patients with SRE have previously been described in separate reports.[7,16] The authors of these 2 cases based their diagnosis on similar criteria: sudden hyperglycemia (>11 mmol/L), increased insulin requirements (from 17 to 30 IU/24 h[7] and from 0 to 10 IU/24 h[16]), and an unexpected decrease in C-peptide (from 1.2 ng/mL [0.4 nmol/L] to 0.3 ng/mL [0.1 nmol/L][7]). DSA was newly detected in 1 patient.[16]

Our data showed that treatment with high-dose methylprednisolone and intravenous insulin is effective in almost fully restoring islet graft function 1 year after an SRE, provided this treatment is started early. Islet recipients with an SRE who were treated adequately and timely within 3 days after first noticing hyperglycemia exhibited near-complete restoration of islet graft function. When treatment was started more than 2 weeks after hyperglycemia onset, islet graft function remained poor. Methylprednisolone treatment should, therefore, be initiated as soon as possible after hyperglycemia onset and confirmation of SRE. In the protocolized treatment group, graft function may also have been affected by the SRE, but this loss of graft function was not sufficient to be significant.

Treatment with high-dose corticosteroids appeared to be safe. No serious adverse events occurred, and the number of reported side effects was low. Timely methylprednisolone treatment with a similar dosage has been reported to be successful in another case of islet graft rejection in current literature.[7] This underlines

the effectiveness of timely high-dose methylprednisolone treatment, combined with intravenous insulin, when rejection is suspected.

Corticosteroids are generally avoided in islet transplant recipients due to possible diabetogenic and islet-toxic side effects.[36,37] On the other hand, corticosteroids have potent anti-inflammatory properties,[38] and beneficial effects of corticosteroids on islets in vitro and in vivo (in animals) have been shown. Human-isolated islets exposed to methylprednisolone for 48 hours showed higher insulin secretion than control islets.[39] Also, transplantation of the corticosteroid-treated islets in mice cured more diabetic mice compared with mice receiving untreated islets.[40] Several studies in which glucocorticoids were used in early type 1 diabetes also demonstrated β-cell protective effects.[41,42] These and our findings indicate the potential positive effect of methylprednisolone in islet transplant recipients with suspected islet graft rejection. Importantly, long-term effects of the 3-day course of high-dose steroid treatment on islet graft function are unknown. Although it is hypothesized that long-term steroid use has a detrimental effect on glycemic control, a Cochrane Systematic Review shows that the use of chronic low-dose prednisolone, as is also the case in our patients, is not associated with more posttransplant diabetes mellitus and reduces acute rejection in kidney transplant patients compared to withdrawal of steroids.[43]

We recognize that the number of patients included in this study is low. However, this is the largest cohort of patients with SRE to date. Even so, future prospective and larger (multicenter) studies are needed to validate our findings.

In conclusion, a relevant number of patients experience islet allograft rejection after islet transplantation. Important diagnostic features such as unexplained hyperglycemia, an unexpected decrease in C-peptide or CP/G ratio, a predisposing event, and elevated immunologic risk can help in establishing the diagnosis of islet allograft rejection. If left untreated, islet graft rejection results in significant and sustained loss of islet graft function. However, timely treatment with high-dose methylprednisolone and intravenous insulin is effective in mitigating this graft function loss, resulting in near-complete restoration of islet graft function after rejection. We recommend instructing patients to contact the hospital as soon as possible after noticing unexpected hyperglycemia in order to initiate treatment timely.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2023.05.035.

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