

# Prediction of Clinical Outcome in Islet Allotransplantation

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**R**ecent progresses in the pancreas' enzymatic digestion process along with novel immunosuppression strategies have led to successful clinical trials of islet transplantation in humans. On the other hand, clinical outcome remains variable and unpredictable in centers with limited experience. The possibility of predicting in vivo islet graft function should allow the selection of preparations on the basis of their potential success, thus improving the overall results and making the processes more consistent and reproducible.

Graft function prediction is a work in progress. Initially, the best parameters representative of engrafted islet mass in recipients should be defined. Fasting C-peptide and exogenous insulin requirements are commonly used, although other methods for a more complete characterization of graft function (i.e.,  $\beta$ -score) have recently been described but not validated in a large number of patients. In addition, some of these data were shown to predict long-term graft function and might be used to establish whether recipients require further islet infusions.

Many pretransplant parameters representative of islet preparations and predictive for in vivo function have been proposed. C-peptide values as well as the exogenous insulin requirements of recipients were shown to be directly correlated with the number of transplanted islets, but there are many exceptions to this association. Other methods to define the quality of an islet preparation include analyses of islet morphology, cell compo-

sition, response to glucose, and viability and production of proinflammatory molecules. The most promising appear to be those that simultaneously analyze more than one aspect of islet physiology.

Islet transplantation has great potential for the normalization of the main parameters of glucose metabolism in diabetic patients. A sufficient number of islets can now be obtained from good quality pancreata more frequently than in the past, and diabetes can generally be reversed, normalizing A1C levels and eliminating severe hypoglycemic episodes (1). Clinical data produced by some centers on patients >1 year after the transplant report that the percentage of normal C-peptide secretion is 100% and that of insulin independence 80%, with an improvement of glycemic compensation (1,2).

In fact, data from the Islet Transplant Registry (Giessen, Germany) and the Collaborative Islet Transplant Center and the conclusions of a multicenter trial sponsored by the National Institutes of Health, produced to verify the reproducibility of the Edmonton Protocol, report that the comprehensive percentage of success (in terms of insulin independence) is only ~50% (3,4). This means that results are not reproducible to the same extent among different centers (5). Even in the centers with the highest percentage of transplant success, insulin independence is reached in only a few cases by a single transplant; in most cases it is necessary to repeat two or even three infusions (6,7). Therefore, it is clear that the efficacy of a

transplant preparation is variable and not always predictable.

The possibility of predicting whether a preparation can work in vivo represents a difficult goal. There are many factors that interfere with islet function in vivo, including quality and number of transplanted islets and their engraftment, pre- and posttransplant immunological conditions (both in terms of autoimmunity and alloimmunity), recipient immunological condition, and toxicity of administered drugs (1,8–10). The prediction of transplant success, however, represents an important objective to be reached. The possibility of predicting in vivo islet graft function should allow the selection of preparations on the basis of their potential success, thus improving overall results. Furthermore, it should result in more consistent, reproducible procedures and permit a proper evaluation of costs and benefits. Altogether, these represent prerequisites for the evolution of islet transplantation from a research procedure to a therapeutic option available to diabetologists. To define the relation between in vivo parameters and transplant function, it is necessary to define evaluation criteria for in vivo functionality.

## REPRESENTATIVE PARAMETERS OF ENGRAFTED $\beta$ -CELL MASS

The function of transplanted islets has often been defined on the basis of C-peptide values, the presence of which in before-transplant C-peptide-negative patients seems to be the best function marker for engrafted islets (6,11–15). C-peptide is also correlated with the reduction of exogenous insulin requirement (12), confirming that it may be representative of engrafted  $\beta$ -cell mass and function. This parameter is, however, influenced by several factors, e.g., inappropriate kidney functionality and increase of insulin resistance, which could overestimate the real engrafted  $\beta$ -cell mass. On the other hand, the exogenous insulin administration, which reduces the insulin and C-peptide secretion through a feedback mechanism, makes transplant functionality seem lower. The normalization of C-peptide on corresponding glycemia values (16) and creatinine should solve this problem.

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**Abbreviations:** IL, interleukin; MCP-1, monocyte chemoattractant protein-1; OCR, oxygen consumption rate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Considering that the true expectation of recipients of islet transplantation is independence from exogenous insulin administration, the transplant outcome has often been quantified through reaching insulin independence (1,2,17,18) or, when this is impossible, through the decrease of insulin requirement as percentage of initial need or reduction of absolute values of insulin units (12,19). However, this parameter too may not adequately represent the transplanted  $\beta$ -cell mass, especially the in case of multiple infusions, where it is necessary to discriminate the effects of various preparations.

According to interests of diabetologists for the real impact of the transplant on the overall metabolic recipient homeostasis, other parameters should be considered as representative of grafted  $\beta$ -cell mass. Glycemic normalization (basal or postprandial glucose levels), obtained both with and without the administration of exogenous insulin, has recently been proposed as a function parameter (20,21). The choice of this parameter is particularly justified for type 1 diabetic recipients proposed for transplant due to a serious problem of glycemic instability (brittle diabetes). Records of glycemic levels could also include the frequency of hypoglycemic episodes that have been described as greatly decreased after islet transplantation (20) as a further indicator of the quality of clinical care. As an indicator of glycemic instability, glycosylated hemoglobin (which could not adequately express postprandial glycemic excursions) or the mean amplitude of glycemic excursions (2,6,7,22) have been proposed. The former is commonly used by diabetologists, and the latter is more complex but, including the evaluation of the postprandial glycemic excursion, provides more complete information in a short time. The new parameter proposed by the Edmonton group ( $\beta$ -score) considers different criteria of function including A1C (23). Indeed, the  $\beta$ -score evaluates the following data from points 0 to 2: fasting glycemia ( $<100$ ,  $100$ – $126$ ,  $>126$  mg/dl), A1C ( $\leq 6.1$ ,  $6.2$ – $6.8$ ,  $>6.9\%$ ), insulin requirement ( $0$ ,  $0.01$ – $0.24$ ,  $>0.24$  units/kg), and stimulated C-peptide ( $>0.8$ ,  $0.3$ – $0.8$ ,  $<0.3$  ng/ml). Although this score must be validated through further studies, it currently appears to be very interesting, as it provides a simple scoring system that encompasses glycemic control, diabetes

therapy, and endogenous insulin secretion.

The analyses of glycemic levels post-transplant as markers of graft function should avoid recipient exposure to prolonged hyperglycemia that may be responsible for glucotoxicity. In fact, the maintenance of normoglycemia in recipients early after transplantation is critical for graft function: Hyperglycemia was described to desensitize human islets to further glucose stimuli (24) and might be deleterious for islet survival early after transplantation (25).

At present, there are no univocal data on the need to use other tests to measure graft function, such as oral or intravenous glucose test, arginine test, glucagon test, hyperglycemic clamp, disposition index, or mixed-meal tolerance test. Indeed, data in this regard are contrasting and inconclusive (3,26,27), even though the first step of insulin response to an arginine test or the first and the second step to a test for intravenous charge of glucose have been described to correlate with the functioning  $\beta$ -cell mass in several studies (17,19,28,29). In particular, the first phase and the area under the curve for insulin in response to intravenous glucose administration were closely related to glycemic control (29), but inversely correlated with the posttransplant insulin requirement and proinsulin levels (17), thus representing a useful test for islet graft function follow-up.

It is known that islet transplant function is variable with the passing of time, with a slow and progressive decrease of secretory reserve (17,30,31). Therefore, it is very important to decide at which time of follow-up islet function should be evaluated. It is reported in the literature that the first representative data on the engrafted mass are at 1 week after transplant (32), although data at 1 month seem more reliable (19). In fact, the definitive evaluation should be carried out at least 1 year after the transplant, due to the clinical importance for patients.

At this point, C-peptide currently represents the best method, although with some limitation, representative of the functioning transplanted  $\beta$ -cell mass in a pretransplant C-peptide-negative patient. The other three parameters, A1C, basal glycemic levels, and frequency of hypoglycemic episodes, should also be considered with the aim of understanding whether islet graft may or may not normalize metabolic control in transplanted patients. All together they are clear, basic,

and recognized parameters in the hands of diabetologist to measure the quality of the care for diabetic patients. The values of other methods have been proposed but need to be further assessed or mainly have only academic value.

## IN VITRO PREDICTIVE PARAMETERS OF GRAFT FUNCTION: ISLET PREPARATION QUALITY

### $\beta$ -Cell mass

Transplant function is in direct relation to the number of transplanted islets. It has been demonstrated that increasing the number of transplanted islets leads to better in vivo function in terms of C-peptide and decreased insulin requirement, with a higher probability of insulin independence (12,19,28). However, there is a significant dispersion of data concerning this correlation showing that transplanting many islets is not sufficient to obtain good transplant function. The reevaluation of islet number after a brief (at least overnight) culture period provides a better evaluation of the real number of islets available for transplant. Furthermore, the relative decrease in their number may be an indirect parameter of their viability, although it was shown to not be correlated with graft function (33).

The number of islet  $\beta$ -cells rather than the number of islets was proposed to be more representative of the transplanted tissue and hence correlated with grafted tissue (34). A group from Minnesota University in particular has observed that human islet  $\beta$ -cells seem to be a better predictor than the number of human islets of sustained insulin independence in both mouse and human recipients (B. Hering, personal communication).

However, also in this case, a large variability between transplanted islet cell mass and graft function remains (34). This data could be partially explained by a difference in quality of islets. At the moment, there are no absolute criteria to define whether an islet is suitable for transplantation in diabetic patients; thus, there are no means of predicting in vivo function. Islet quality can be defined not only as a level of viability or a three-dimensional structure conservation, but also as secretory reserve in response to glucidic stimulation (35,36).

### Islet viability

Islet viability is often difficult to assess. Several approaches have been tested to study islet viability: vital probes or stains

on fixed tissue for apoptotic/necrotic/viable cells, oxygen consumption rate, or ATP-to-ADP assessment as measurement of mitochondrial activity.

There are many vital stains available for human islets (37,38), and SYTO-13/ethidium bromide and calcein AM/ethidium homodimer seem more sensitive to islet cell damage than fluorescein diacetate/propidium iodide (37). Also, the assessment of intracellular calcium concentration in human islets was proposed as an indirect marker of islet cell viability (17). Indeed an islet is a cluster of cells where the most internal cells are not easily reached by the colorants designed to stain singular cells; therefore, common optical analysis instruments are not adequate. The possibility of using a confocal to study islet viability has been proposed by some authors (39–41), but none of them have correlated the information collected by this method with in vitro or in vivo islet functional data, yet. Therefore, the use of a confocal to assess islet viability remains interesting, potentially feasible, and requires further study. The dispersion of islets into singular cells should optimize their coloration and study but would be damaging because of further enzymatic digestion and could result in an underestimation of integrity. Recently, the method for cell dispersion proposed by the University of Miami appears to be quite optimized and reproducible (42), but its true standardization has yet to be proven.

Islet cell viability is largely tested on DNA-binding dyes. While these tests identify cells that have lost selective membrane permeability, they do not allow us to recognize apoptotic cells, which do not yet stain with DNA-binding dyes. The simultaneous cell staining with probes for apoptosis and necrosis (i.e., tetramethylrhodamine ethyl ester and 7-aminoactinomycin D, ref. 42) is a practical and complete method for the assessment of cell conditions. Alternatively, the mitochondrial oxygen consumption rate (OCR) has been proposed as a dynamic indicator of cellular viability (43–45). The central hypothesis is that the OCR of cells is directly proportional to viable tissue volume and that its normalization to DNA content is a measure of fractional viability. In addition, the increase of OCR by glucose administration might be considered a parameter representative of the fractional  $\beta$ -cell mass of the analyzed tissue. At this time such tests are still research tools and have not yet been applied

as product release criteria in clinical islet transplantation. Finally, the ATP-to-ADP ratio in  $\beta$ -cells has been shown to represent the metabolic condition of islets and therefore to be an indirect marker of islet viability (33,46). In addition, although biochemical markers of islet cell function do not really reflect actual metabolic function of the islet  $\beta$ -cells, especially upon their graft, the increasing evidence of their correlation (in particular ATP-to-ADP ratio) with graft function deserves some attention (33).

#### Islet integrity

It has been demonstrated that the preservation of islet morphology as a representative parameter of morphostructural integrity is important for a prediction of islet function subsequent to transplantation (12,47). Morphostructural integrity is defined as the right interactions and rapports (three-dimensional architecture) among various cytotypes into islets (47).

A new method for the assessment of islet quality, recently proposed by the University of Miami, calculates fractional  $\beta$ -cell viability in addition to cellular composition of the final islet cell products (42). It is therefore possible to obtain information concerning not only the characterization of the percentage of necrotic and apoptotic cells, but also of cellular components in the final preparation ( $\beta$ - and  $\alpha$ -cells, nonendocrine tissue); selective information on viable, nonapoptotic  $\beta$ -cell mass is also obtainable. This method appears to be correlated not only with graft function in model animals of islet allotransplantation, but also in allotransplantation in type 1 diabetic patients (48). In particular, the number of equivalent islets  $\times$  (%  $\beta$ -cell content)  $\times$  (% nonapoptotic  $\beta$ -cell)/kg of recipient correlates with the reduction rate (>60% or not) after the first infusion or with insulin independence.

#### Islet preparation composition

The preparation composition for transplants is another variable that could play a role in the success of islet allotransplantation. The contamination of preparations by exocrine and ductal tissue is assessed to define the preparation purity. The contamination by exocrine tissue may be like a mantle around the islets (embedded islets) due to incomplete pancreas digestion or, like free tissue, inefficacy of the purification procedure.

The presence of embedded islets does not seem to interfere with the transplant; moreover, it has been reported that it may, in some way, be a sign of insular

integrity and can protect islets during the initial phase of transplant (49).

The level of purification of a successful islet preparation is controversial. It is believed that the large volume of intraportal distribution of preparation during the transplant permits the infusion of islets and exocrine tissue together without interfering with islet engraftment. In addition, the production of chemo-attractive chemokines, which attract macrophages, seems for the most part to be produced by  $\beta$ -cells rather than exocrine tissue (50). Therefore, also in this case, the presence of exocrine tissue would not amplify the transplant inflammatory response. But partial thrombosis of portal vein branches (51) and, in the long-term, tissue remodeling and morphological alteration into the liver (13,52) have been described as complications of large tissue volume transplantation. In addition, in a univariate analysis, the islet purity level was directly correlated with the C-peptide value of recipients a month after transplant (12), although this was not confirmed by the multivariate analysis or other studies (12,53).

Even more controversial is the role of ductal contaminants in an islet preparation for transplantation. The ductal contaminants of preparations have been observed to be the only variable that correlates with the transplant function over a long period of time (54). This suggests a possible role of ductal cells in the process of  $\beta$ -cell regeneration (55) being able to prevent a functional exhaustion of transplanted islets. On the other hand, it has been observed that ductal cells have a strong proinflammatory connotation, both because they produce tissue factor and CD40 (the former able to activate the coagulation cascade, the latter to contribute in triggering rejection) and because they produce NO and tumor necrosis factor- $\alpha$ , which damage islets (56–59).

Therefore, highly purified islet preparation should be transplanted not only because there are few reports on the harmlessness of contaminating tissue but also because the eventual benefits of contaminant cells have not yet been proven.

#### Islet insulin secretion

The secretive capacity of isolated islets has long been considered a useful criterion for the selection for transplants expressed as absolute value after glucose stimulation or as secretion index, i.e., the ratio between basal and stimulated insulin levels both in static (static incubation) and dynamic perfusion (35,60). In any case, islets with

a well-preserved morphology, or constituted by viable cells, often present a deficit in their insulin responsiveness to stimuli (61), whereas *in vitro* lightly responsive islets may be capable of restoring the normoglycemia after transplantation in a diabetic patient (62). In addition, secretory defects should be reversible; therefore, it is not a strong parameter of islet quality. At this stage, the evaluation of insulin responsiveness to glucose in isolated islets is not justified as a control quality test predictive of graft function.

A final observation concerns the proposal to use animal models, such as immune-deficient mice, to evaluate human islet quality (63–65). This consists of transplanting human islets in nude mice as quality control for islets that are designed for transplant. This, in any case, does not permit the selection of preparations for transplant, as results would be obtained too late relative to the time available to keep islet preparations in culture without losing islet function. Moreover, it has been reported in a recent study (64) that the animal model has low sensitivity for the prediction of islet function in humans. Indeed, it has been observed that in animals, human islet function is strictly correlated with purification level and insulin content (63). The role of these parameters could be valid for the animal model only. The lack of purification worsens the oxygenation level and the inflammatory state under the renal capsule of mice but is different in humans because the preparation is dispersed in an ample vascular bed. Furthermore, in mice, the low insulin content could be responsible for an increase of glycemia immediately posttransplant, causing glucose toxicity in the freshly implanted islets, whereas in humans, the insulin treatment maintains a condition of rigorous normoglycemia. To overcome hyperglycemia, in animal models of transplantation a peritransplant maintenance of normoglycemia by exogenous insulin was shown to improve islet graft function (66), thus preserving islet by glucotoxicity. Also, in this case, the animal model remains an unsuitable method to assess islet quality before transplantation.

Finally, among the obstacles to defining a function predictive quality control for isolated islets, there is the lack of standardization of the before-mentioned procedures, rendering a comparison of results obtained from different laboratories difficult.

Among the parameters for an evalua-

tion of the purified islets in order to use them in recipients, it seems that donor age could represent a determinant of quality. This emerges from the experience of the group from the University of Minnesota (2), who include age among the criteria for the selection of preparations, resulting in one of the most successful clinical protocols. It is known that young age is associated with better islet insulin responsiveness to glucose and graft function in transplanted patients (67). Therefore, young age of donors should be considered an additional parameter predictive of islet transplant success.

In all, several are the methods that have been proposed to assess islet and many are the parameters used to describe islet quality. Data on islet cell viability and composition appear to be the most important parameters, not exclusive but complementary to one another.

### IN VITRO PREDICTIVE PARAMETERS OF GRAFT FUNCTION: ISLET PROINFLAMMATORY CONDITION

It has been demonstrated without a doubt that pancreatic islets can produce several molecules with proinflammatory activity. It has been observed that isolated islets present high mRNA expression of monocyte chemoattractant protein-1 (MCP-1), migration inhibitory factor, vascular endothelial growth factor, tissue factor, and thymosin  $\beta$ -10 (68). In addition, interleukin (IL)-8, IL-1 $\beta$ , IL-5R, and interferon- $\gamma$  antagonist were expressed in islets that had been cultured for 2 days. IL-2R was expressed in islets that had been cultured for >6 days. The production of these molecules seems to be associated with the donor's clinical conditions, mainly concerning cerebral death, or procedures of isolation, due to the exposition of the tissue to lack of oxygen and free radicals (69–71). The possibility that islets produce proinflammatory factors has stimulated the search for their effects upon the early steps after transplant (engraftment of islets) and upon transplant function in the short term. Graft function depends on several variables, such as donor condition, digestion, purification characteristics, and especially immunosuppression therapy, not only on molecules released by islets. Therefore, any attempts to evaluate the effects of the chemokines/cytokines released by islets on the fate of their transplant in patients should be carefully considered.

Some of these molecules may stimulate islet engraftment (e.g., vascular endothelial growth factor), whereas others have been considered responsible for causing coagulation cascade (tissue factor) or for amplifying the posttransplant inflammatory response (MCP-1). It has been demonstrated that the production of tissue factor activates a coagulation cascade in recipients (72) with negative effects on transplant function in terms of C-peptide 1 week after transplant (32), as well as on hepatocytes, as demonstrated by a correlation between tissue factor in islets and increase of transaminases during the 1st week posttransplant (73). In any case, a truly predictive role of tissue factor for *in vivo* islet function has yet to be confirmed, due to lack of data on its effect over the medium and long term. Furthermore, data concerning the consequences of MCP-1 production by islets on transplant function are more complex. In islets after kidney recipients were treated with cyclosporine and mycophenolate, a negative correlation has been observed between high MCP-1 level in transplanted islets and clinical success of the transplantation in the 1st year of follow-up (50). This correlation has not yet been confirmed in recipients transplanted using the Edmonton protocol (73). This suggests that the different immunosuppressive therapies might modulate the inflammation pathogenesis with consequent damage to  $\beta$ -cells caused by MCP-1. A further element that renders an understanding this phenomenon difficult is the possibility that recipients may receive more than one pharmacological treatment capable of modulating the inflammatory response. On the other hand, the variability of cellular culture conditions, as well, could interfere with a correct *in vitro* evaluation of the proinflammatory activity of islets.

Considering the above-mentioned data, although the role of proinflammatory molecules secreted by islets on their engraftment on the hepatic location appears undisputable, it is only possible to consider secretion as a predictive factor of the *in vivo* function of transplanted islets for MCP-1 and only in the case of recipients who receive the traditional immunosuppressive therapy with cyclosporine and micofenolate, but studies on these aspects are ongoing.

## IN VIVO PREDICTIVE PARAMETERS OF GRAFT FUNCTION

— The possibility of transplanting twice in the same recipient permits a repetition of the procedure in case the first infusion of islets proves insufficient for insulin independence. The decision to repeat the transplant should be made as soon as possible in order to avoid rendering the immunosuppressive therapy induction step too long. It is therefore important to define which parameters of in vivo function of transplanted islets could predict the transplant function in the long term in order to indicate the patients who need a new infusion. The benefits of islet transplant in recipients emerge progressively during the early months after transplantation. One of the peculiar characteristics of the recipients with the Edmonton protocol is the rapidity of action of transplanted islets, such that within 2 months it is already possible to reach the maximum reduction of insulin requirement (18). Conversely, the experience of islet after kidney transplant recipients treated with an immunosuppressive therapy with cyclosporine and mycophenolate presents a progressive reduction over the successive months (17). The most extreme example is a patient who became insulin independent 11 months after the transplant (17). In this situation the identification of precocious parameters of insufficient transplant function becomes extremely important in case a further transplant is required.

One month after transplant of islets in kidney recipients, the glycemia at fasting (measured suspending exogenous insulin from the previous evening) and proinsulin values correlated with the insulin requirement checked 1 year after the transplant (17). In islet recipients isolated according to the Edmonton protocol, the values of the area under the insulin curve in response to an endogenous charge of glucose or the acute insulin response to glucose were lower in patients who once again began to experience the need for exogenous insulin (19).

In a more general context, these parameters can be considered part of a metabolic condition that predicts a progressive functional exhaustion and includes a progressive increase of glycemic value and consequently of glycosilated hemoglobin, a reduction of the insulin responsiveness to a glucose charge, and perhaps later to an arginine charge and increase of proinsulin values; these pa-

rameters may therefore need to be evaluated together (17,31,32).

Finally, the prediction of graft failure could be obtained by analyses of immunological parameters anti-GAD and anti-IA2 or, better, their increase after transplantation, which has been shown to be associated with poor graft function (74–76).

## PREDICTION OF CLINICAL OUTCOME IN ISLET ALLOTRANSPLANTATION: THE “INTEGRATED APPROACH”

— Many are the parameters, formula, and methods proposed to predict graft function, and a final decision on the best method has not yet been established. In particular, it appears evident that single in vitro parameters are scarcely representative of the whole preparation and that single recipient values may not be representative of graft function.

The most experienced centers are therefore integrating data from various types of analyses. This method, we call the “integrated approach,” allows more detailed characterization of the quality of the islets available for the transplant and also the new metabolic condition of islet-transplanted patients.

Cell viability and composition appeared to be the most critical information of an islet preparation. This is why some of the most experienced laboratories integrate data on cell viability (apoptosis/necrosis for the University of Miami and fractional viability assessed by ATP-to-DNA and OCR-to-DNA ratios for the University of Minnesota) with those on islet cell composition ( $\beta$ -cell mass, assessed by laser scan cytometer for the University of Miami and by immunostaining for the University of Minnesota). Preliminary results in these laboratories provide evidence that this approach is predictive not only in animal models of transplantation but also in human islet recipients (B. Hering, personal communication). Our point of view is that the analysis of MCP-1 in the case of islet after kidney transplantation should also be evaluated, since islet graft function is lower in the case of transplantation of islets releasing a huge amount of this chemokine.

In recipients, an integrated evaluation of their metabolic conditions appears to better describe the effects of islet transplantation than single parameters such as C-peptide or exogenous insulin requirement. The  $\beta$ -score proposed by the Uni-

versity of Alberta is a clear example of this new approach, though not yet validated. We believe that C-peptide values should be recorded together with fasting glycemia and A1C values and the frequency of hypoglycemic episodes for a complete assessment of recipient medical care quality. Other parameters are informative but not essential at this stage.

## PREDICTION OF CLINICAL OUTCOME IN ISLET ALLOTRANSPLANTATION: AN ON-GOING SCIENCE

— Only a multicentric study in a large number of patients could be conclusive toward the prediction of clinical outcome in islet allotransplantation. Due to the limited number of transplants feasible per year, the possibility to soon have some final guidelines on this topic is not realistic. In addition, even with the large number of proposals for standardization of these procedures, the experience of the center remains the key factor in determining the success of the islet transplant (5), thus sometimes complicating comparisons between centers. To overcome these problems, the islet centers both in Europe and in North America have organized important meetings to share protocols and experiences specific to the unsolved matter related to in vitro and in vivo islet function characterization. In Europe the workshop of the Network for Islet Centre of Europe (NICE, in its 5th year) and in North America the Human Islet Isolation and Transplantation Techniques Training (HIITT, in its 6th year) are fruitful meetings aimed at updating training researchers on islet transplantation problems as well as favoring discussion and sharing of protocols and ideas between experienced researchers. An additional meeting that shares these aims is the Islet Cell Resource Center's (ICR) Consortium Annual Islet Workshop (in its 2nd year). These workshops should be an opportunity for the proposal of common protocols aimed at defining guidelines on parameters and strategies to predict clinical outcomes of islet allotransplantation.

**CONCLUSIONS** — The prediction of graft function in islet allotransplantation remains a challenging objective, although recently proposed parameters could be of assistance in standardizing reproducible procedures for an assessment of islet cell products before transplantation and also in providing useful product

release criteria for the prediction of post-transplant function.

The prediction of graft function must take into account many variables, from islet mass, viability, function, and proinflammatory conditions to the type of the immunosuppression therapy. In addition to these variables, recipient conditions and treatments may seriously interfere with any attempt to predict in vivo graft function. The identification of integrated and standardized pretransplant strategies capable of optimizing islet viability and function as well as reducing the proinflammatory profile of islet cell products are considered highly desirable.

## References

- Ricordi C, Inverardi L, Kenyon NS, Goss J, Bertuzzi F, Alejandro R: Requirements for success in clinical islet transplantation. *Transplantation* 79:1298–300, 2005
- Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsuoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DE: Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 293:830–835, 2005
- Brendel MD, Hering BJ, Schultz AO, Bretzel RG: *International Islet Transplant Registry Newsletter*. No. 9, vol. 8, issue 1. Giessen, Germany, Third Medical Department Center of Internal Medicine University Hospital Giessen, 2001
- Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbitt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR: International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 355:1318–1330, 2006
- Shapiro AM, Ricordi C: Unraveling the secrets of single donor success in islet transplantation. *Am J Transplant* 4:295–298, 2004
- Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM: Five-year follow-up after clinical islet transplantation. *Diabetes* 54:2060–2069, 2005
- Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, Pileggi A, Poggioli R, Ichii H, Khan A, Ferreira JV, Pugliese A, Esquenazi VV, Kenyon NS, Alejandro R: Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. *Am J Transplant* 5:2037–2046, 2005
- Hering BJ: Achieving and maintaining insulin independence in human islet transplant recipients. *Transplantation* 79:1296–1297, 2005
- Korsgren O, Nilsson B, Berne C, Felldin M, Foss A, Kallen R, Lundgren T, Salmela K, Tibell A, Tufveson G: Current status of clinical islet transplantation. *Transplantation* 79:1289–1293, 2005
- Shapiro AM, Lakey JR, Paty BW, Senior PA, Bigam DL, Ryan EA: Strategic opportunities in clinical islet transplantation. *Transplantation* 79:1304–1307, 2005
- Luzi L, Perseghin G, Brendel MD, Terruzzi I, Battezzati A, Eckhard M, Brandhorst D, Brandhorst H, Friemann S, Socci C, Di Carlo V, Piceni Sereni L, Benedini S, Secchi A, Pozza G, Bretzel RG: Metabolic effects of restoring partial  $\beta$ -cell function after islet allotransplantation in type 1 diabetic patients. *Diabetes* 50:277–282, 2001
- Nano R, Clissi B, Melzi R, Calori G, Maffi P, Antonioli B, Marzorati S, Aldrighetti L, Freschi M, Grochowicki T, Socci C, Secchi A, Di Carlo V, Bonifacio E, Bertuzzi F: Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. *Diabetologia* 48:906–912, 2005
- Bhargava R, Senior PA, Ackerman TE, Ryan EA, Paty BW, Lakey JR, Shapiro AM: Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. *Diabetes* 53:1311–1317, 2004
- Warnock GL, Meloche RM, Thompson D, Shapiro RJ, Fung M, Ao Z, Ho S, He Z, Dai LJ, Young L, Blackburn L, Kozak S, Kim PT, Al-Adra D, Johnson JD, Liao YH, Elliott T, Verchere CB: Improved human pancreatic islet isolation for a prospective cohort study of islet transplantation vs. best medical therapy in type 1 diabetes mellitus. *Arch Surg* 140:735–744, 2005
- Alejandro R, Lehmann R, Ricordi C, Kenyon NS, Angelico MC, Burke G, Esquenazi V, Nery J, Betancourt AE, Kong SS, Miller J, Mintz DH: Long-term function (6 years) of islet allografts in type 1 diabetes. *Diabetes* 46:1983–1989, 1997
- Faradji RN, Monroy K, Cure P, Froud T, Baidal D, Pileggi A, Messinger S, Ricordi C, Alejandro R: C-peptide and glucose values in the peritransplant period after intraportal islet infusions in type 1 diabetes. *Transplant Proc* 37:3433–3434, 2005
- Bertuzzi F, Grohovaz F, Maffi P, Caumo A, Aldrighetti L, Nano R, Hengster P, Calori G, Di Carlo V, Bonifacio E, Secchi A: Successful transplantation of human islets in recipients bearing a kidney graft. *Diabetologia* 45:77–84, 2002
- Shapiro AM, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
- Ryan EA, Lakey JR, Rajotte RV, Korbitt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Warnock GL, Larsen I, Shapiro AM: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50:710–719, 2001
- Ryan EA, Shandro T, Green K, Paty BW, Senior PA, Bigam D, Shapiro AM, Vantyghem MC: Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. *Diabetes* 53:955–962, 2004
- Kessler L, Passemard R, Oberholzer J, Benhamou PY, Bucher P, Toso C, Meyer P, Penfornis A, Badet L, Wolf P, Colin C, Morel P, Pinget M, the GRAGIL Group: Reduction of blood glucose variability in type 1 diabetic patients treated by pancreatic islet transplantation: interest of continuous glucose monitoring. *Diabetes Care* 25:2256–2262, 2002
- Clayton HA, Davies JE, Pollard CA, White SA, Musto PP, Dennison AR: Pancreatectomy with islet autotransplantation for the treatment of severe chronic pancreatitis: the first 40 patients at the Leicester general hospital. *Transplantation* 76:92–98, 2003
- Ryan EA, Paty BW, Senior PA, Lakey JR, Bigam D, Shapiro AM:  $\beta$ -Score: an assessment of  $\beta$ -cell function after islet transplantation. *Diabetes Care* 28:343–347, 2005
- Davalli AM, Ricordi C, Socci C, Braghi S, Bertuzzi F, Fattor B, Di Carlo V, Pontiroli AE, Pozza G: Abnormal sensitivity to glucose of human islets cultured in a high glucose medium: partial reversibility after an additional culture in a normal glucose medium. *J Clin Endocrinol Metab* 72:202–208, 1991
- Makhoul L, Duvivier-Kali VF, Bonner-Weir S, Dieperink H, Weir GC, Sayegh MH: Importance of hyperglycemia on the primary function of allogeneic islet transplants. *Transplantation* 76:657–664, 2003
- Rickels MR, Schutta MH, Markmann JF, Barker CF, Naji A, Teff KL:  $\beta$ -Cell function following human islet transplantation for type 1 diabetes. *Diabetes* 54:100–106, 2005
- Bergman RN, Ader M, Huecking K, Van Citters G: Accurate assessment of  $\beta$ -cell function: the hyperbolic correction. *Diabetes* 51 (Suppl. 1):212–220, 2002
- Teuscher AU, Kendall DM, Smets YF, Leone JP, Sutherland DE, Robertson RP: Successful islet autotransplantation in humans: functional insulin secretory reserve as an estimate of surviving islet cell mass. *Diabetes* 47:324–330, 1998
- Ryan EA, Lakey JR, Paty BW, Imes S, Korbitt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AM: Successful islet

- transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–2157, 2002
30. McDonald CG, Ryan EA, Paty BW, Senior PA, Marshall SM, Lakey JR, Shapiro AM: Cross-sectional and prospective association between proinsulin secretion and graft function after clinical islet transplantation. *Transplantation* 78:934–937, 2004
31. Davalli AM, Maffi P, Socci C, Sanvito F, Freschi M, Bertuzzi F, Falqui L, Di Carlo V, Pozza G, Secchi A: Insights from a successful case of intrahepatic islet transplantation into a type 1 diabetic patient. *J Clin Endocr Metab* 85:3847–3852, 2000
32. Johansson H, Lukinius A, Moberg L, Lundgren T, Berne C, Foss A, Felldin M, Kallen R, Salmela K, Tibell A, Tufveson G, Ekdahl KN, 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* 54:1755–1762, 2005
33. Goto M, Holgersson J, Kumagai-Braesch M, Korsgren O: The ADP/ATP ratio: a novel predictive assay for quality assessment of isolated pancreatic islets. *Am J Transplant* 6:2483–2487, 2006
34. Keymeulen B, Ling Z, Gorus FK, Delvaux G, Bouwens L, Gruppig A, Hendriekx C, Pipeleers-Marichal M, Van Schravendijk C, Salmela K, Pipeleers DG: Implantation of standardized beta-cell grafts in a liver segment of IDDM patients: graft and recipients characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. *Diabetologia* 41:452–459, 1998
35. Ricordi C, Gray D, Hering B, Kaufman D, Warnock G, Kneteman N, Lake S, London N, Socci C, Alejandro R, Zeng Y, Sharp D, Viviani G, Tzakis A, Bretzel R, Federlin K, Pozza G, James R, Rajotte R, Di Carlo V, Morris P, Sutherland D, Starzl T, Mintz D, Lacy PE: Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 27:185–195, 1990
36. Ricordi C: Quantitative and qualitative standards for islet isolation assessment in humans and large mammals. *Pancreas* 6:242–244, 1991
37. Barnett MJ, McGhee-Wilson D, Shapiro AM, Lakey JR: Variation in human islet viability based on different membrane integrity stains. *Cell Transplant* 13:481–488, 2004
38. Miyamoto M, Morimoto Y, Nozawa Y, Balamurugan AN, Xu B, Inoue K: Establishment of fluorescein diacetate and ethidium bromide (FDAEB) assay for quality assessment of isolated islets. *Cell Transplant* 9:681–686, 2000
39. Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC: Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem* 53:1087–1097, 2005
40. Boffa DJ, Waka J, Thomas D, Suh S, Curran K, Sharma VK, Besada M, Muthukumar T, Yang H, Suthanthiran M, Manova K: Measurement of apoptosis of intact human islets by confocal optical sectioning and stereologic analysis of YO-PRO-1-stained islets. *Transplantation* 79:842–845, 2005
41. Hermann M, Pirkebner D, Draxl A, Margreiter R, Hengster P: “Real-time” assessment of human islet preparations with confocal live cell imaging. *Transplant Proc* 37:3409–3411, 2005
42. Ichii H, Inverardi L, Pileggi A, Molano RD, Cabrera O, Caicedo A, Messinger S, Kuroda Y, Berggren PO, Ricordi C: A novel method for the assessment of cellular composition and beta-cell viability in human islet preparations. *Am J Transplant* 5:1635–1645, 2005
43. Sweet IR, Gilbert M, Jensen R, Sabek O, Fraga DW, Gaber AO, Reems J: Glucose stimulation of cytochrome C reduction and oxygen consumption as assessment of human islet quality. *Transplantation* 80:1003–1011, 2005
44. Wang W, Upshaw L, Strong DM, Robertson RP, Reems J: Increased oxygen consumption rates in response to high glucose detected by a novel oxygen biosensor system in non-human primate and human islets. *J Endocrinol* 185:445–455, 2005
45. Janjic D, Wollheim CB: Islet cell metabolism is reflected by the MTT (tetrazolium) colorimetric assay. *Diabetologia* 35:482–485, 1992
46. Ishii S, Saito T, Ise K, Sato Y, Tsutiya T, Kenjo A, Kimura T, Anazawa T, Suzuki S, Terashima M, Gotoh M: Evaluation of energy state of islet independent of size using a newly developed ATP bioluminescence assay. *Transplant Proc* 37:3499–3500, 2005
47. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A: The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci U S A* 103:2334–2339, 2006
48. Ichii H, Wang X, Alvarez A, Baidal DA, Khan A, Pileggi A, Inverardi L, Alejandro R, Ricordi C: Pre-transplant assessment of fractional beta cell viability correlates with clinical islet transplantation outcomes. *Transplantation* 82 (Suppl. 2): 341–342, 2006
49. Ricordi C, Alejandro R, Rilo HH, Carroll PB, Tzakis AG, Starzl TE, Mintz DH: Long-term in vivo function of human mantled islets obtained by incomplete pancreatic dissociation and purification. *Transplant Proc* 27:3382, 1995
50. Piemonti L, Leone BE, 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: relevance in human islet transplantation. *Diabetes* 51:55–65, 2002
51. Shapiro AM, Lakey JR, Rajotte RV, Warnock GL, Friedlich MS, Jewell LD, Kneteman NM: Portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined human liver/islet allograft. *Transplantation* 59:1060–1063, 1995
52. Maffi P, Angeli E, Bertuzzi F, Paties C, Socci C, Fedeli C, De Taddeo F, Nano R, Di Carlo V, Del Maschio A, Secchi A: Minimal focal steatosis of liver after islet transplantation in humans: a long-term study. *Cell Transplant* 14:727–733, 2005
53. Eckhard M, Brandhorst D, Winter D, Jaeger C, Jahr H, Bretzel RG, Brendel MD: The role of current product release criteria for identification of human islet preparations suitable for clinical transplantation. *Transplant Proc* 36:1528–1531, 2004
54. Street CN, Lakey JR, Shapiro AM, Imes S, Rajotte RV, Ryan EA, Lyon JG, Kin T, Avila J, Tsujimura T, Korbitt GS: Islet graft assessment in the Edmonton Protocol: implications for predicting long-term clinical outcome. *Diabetes* 53:3107–3114, 2004
55. Bonner-Weir S, Weir GC: New sources of pancreatic beta-cells. *Nat Biotechnol* 23:857–861, 2005
56. Pavlovic D, Chen MC, Bouwens L, Eizirik DL, Pipeleers D: Contribution of ductal cells to cytokine responses by human pancreatic islets. *Diabetes* 48:29–33, 1999
57. Beuneu C, Vosters O, Movahedi B, Remmelink M, Salmon I, Pipeleers D, Pradier O, Goldman M, Verhasselt V: Human pancreatic duct cells exert tissue factor-dependent procoagulant activity: relevance to islet transplantation. *Diabetes* 53:1407–1411, 2004
58. Movahedi B, Van de Casteele M, Caluwe N, Stange G, Breckpot K, Thielemans K, Vreugdenhil G, Mathieu C, Pipeleers D: Human pancreatic duct cells can produce tumour necrosis factor-alpha that damages neighbouring beta cells and activates dendritic cells. *Diabetologia* 47:998–1008, 2004
59. Vosters O, Beuneu C, Nagy N, Movahedi B, Aksoy E, Salmon I, Pipeleers D, Goldman M, Verhasselt V: CD40 expression on human pancreatic duct cells: role in nuclear factor-kappa B activation and production of pro-inflammatory cytokines. *Diabetologia* 47:660–668, 2004
60. Bertuzzi F, Garancini P, Socci TC, Nano R, Taglietti MV, Santopinto M, Di Carlo V, Davalli: Lessons from in vitro perfusion of pancreatic islets isolated from 80 human pancreases. *Cell Transplant* 8:709–712, 1999
61. Pai GM, Slavin BG, Tung P, Volk BW, Johnson DG, Anderson DG, Levin SR:

- Morphologic basis for loss of regulated insulin secretion by isolated rat pancreatic islets. *Anat Rec* 237:498–505, 1993
62. Socci C, Davalli AM, Vignali A, Bertuzzi F, Maffi P, Zammarchi O, Di Carlo V, Pozza G: Evidence of in vivo human islet function despite a weak response to in vitro perfusion. *Transplant Proc* 24:3056–3057, 1992
  63. Migliavacca B, Nano R, Antonioli B, Marzorati S, Davalli AM, Di Carlo V, Bertuzzi F: Identification of in vitro parameters predictive of graft function: a study in an animal model of islet transplantation. *Transplant Proc* 36:612–613, 2004
  64. Sabek OM, Fraga DW, Minoru O, McClaren JL, Gaber AO: Assessment of human islet viability using various mouse models. *Transplant Proc* 37:3415–3416, 2005
  65. Bharat A, Benshoff N, Olack B, Ramachandran S, Desai NM, Mohanakumar T: Novel in vivo murine model to study islet potency: engraftment and function. *Transplantation* 79:1627–1630, 2005
  66. Merino JF, Nacher V, Raurell M, Biarnes M, Soler J, Montanya E: Optimal insulin treatment in syngeneic islet transplantation. *Cell Transplant* 9:11–18, 2000
  67. Ihm SH, Matsumoto I, Sawada T, Nakano M, Zhang HJ, Ansit JD, Sutherland DE, Hering BJ: Effect of donor age on function of isolated human islets. *Diabetes* 55:1361–1368, 2006
  68. Johansson U, Olsson A, Gabrielsson S, Nilsson B, Korsgren O: Inflammatory mediators expressed in human islets of Langerhans: implications for islet transplantation. *Biochem Biophys Res Commun* 308:474–479, 2003
  69. Contreras JL, Eckstein C, Smyth CA, Sellers MT, Vilatoba M, Bilbao G, Rahemtulla FG, Young CJ, Thompson JA, Chaudry IH, Eckhoff DE: Brain death significantly reduces isolated pancreatic islet yields and functionality in vitro and in vivo after transplantation in rats. *Diabetes* 52:2935–2942, 2003
  70. Melzi R, Piemonti L, Nano R, Clissi B, Calori G, Antonioli B, Marzorati S, Perseghin G, Di Carlo V, Bertuzzi F: Donor and isolation variables associated with human islet monocyte chemoattractant protein-1 release. *Transplantation* 78:1564–1567, 2004
  71. Balamurugan AN, He J, Guo F, Stolz DB, Bertera S, Geng X, Ge X, Trucco M, Bottino R: Harmful delayed effects of exogenous isolation enzymes on isolated human islets: relevance to clinical transplantation. *Am J Transplant* 5:2671–2681, 2005
  72. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Kallen R, Ostraat O, Salmela K, Tibell A, Tufveson G, Elgue G, Nilsson Ek-dahl K, Korsgren O, Nilsson B: Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* 360:2039–2045, 2002
  73. 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* 89:5724–5728, 2004
  74. Roep BO, Stobbe I, Duinkerken G, van Rood JJ, Lernmark A, Keymeulen B, Pipeleers D, Claas FH, de Vries RR: Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. *Diabetes* 48:484–490, 1999
  75. Jaeger C, Brendel MD, Hering JB, Eckhard M, Bretzel RG: Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. *Diabetes* 46:1907–1910, 1997
  76. Bosi E, Braghi S, Maffi P, Scirpoli M, Bertuzzi F, Pozza G, Secchi A, Bonifacio E: Autoantibody response to islet transplantation in type 1 diabetes. *Diabetes* 50:2464–2471, 2001