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Heterogeneity of Human Pancreatic Islet Isolation Around Europe: Results of a Survey Study

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Background. Europe is currently the most active region in the field of pancreatic islet transplantation, and many of the leading groups are actually achieving similar good outcomes. Further collaborative advances in the field require the standardization of islet cell product isolation processes, and this work aimed to identify differences in the human pancreatic islet isolation processes within European countries. **Methods.** A web-based questionnaire about critical steps, including donor selection, pancreas processing, pancreas perfusion and digestion, islet counting and culture, islet quality evaluation, microbiological evaluation, and release criteria of the product, was completed by isolation facilities participating at the Ninth International European Pancreas and Islet Transplant Association (EPITA) Workshop on Islet-Beta Cell Replacement in Milan. **Results.** Eleven islet isolation facilities completed the questionnaire. The facilities reported 445 and 53 islet isolations per year over the last 3 years from deceased organ donors and pancreatectomized patients, respectively. This activity resulted in 120 and 40 infusions per year in allograft and autograft recipients, respectively. Differences among facilities emerged in donor selection (age, cold ischemia time, intensive care unit length, amylase concentration), pancreas procurement, isolation procedures (brand and concentration of collagenase, additive, maximum acceptable digestion time), quality evaluation, and release criteria for transplantation (glucose-stimulated insulin secretion tests, islet numbers, and purity). Moreover, even when a high concordance about the relevance of one parameter was evident, thresholds for the acceptance were different among facilities. **Conclusions.** The result highlighted the presence of a heterogeneity in the islet cell product process and product release criteria.

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INTRODUCTION

Cell therapy is a field of medicine that involves injecting intact, living cellular material directly into a patient for the treatment of disease. Often the inherent biological variability and rapid growth of research and development of cell therapy hindered harmonizing standards for components, processes, or products. This problem is particularly relevant in the field of islet transplantation.[1,2] Over the past 3 decades,

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The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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the methods for large-scale isolation and purification of human pancreatic islets have substantially improved, thanks to major collaborative efforts among centers in Europe and North America.[3,4] However, the lack of standardization of islet processing methods may have contributed to the variability of outcomes across centers and reduced the ability to compare results, even when the same clinical protocol and immunosuppressive regimen were used. A site to site variation in the clinical outcome and isolation processes was observed in all multicenter studies performed in the field of islet transplantation,[5-8] and it is well known that the quality of the islets is affected by organ recovery and preservation, donor characteristics, islet isolation process, and culture methods before transplantation.[1,9-11] To address this issue, 8 North American manufacturing facilities and the Nordic Network for Clinical Islet Transplantation (NNCIT) participating in the National Institutes of Health–sponsored Clinical Islet Transplantation (CIT) Consortium have recently undertaken a major effort to optimize and standardize processes, criteria, and test methods across participating processing centers.[12] This effort resulted in the implementation of a defined set of critical process parameters and in-process controls for islet isolation and release criteria. To our knowledge, this experience is unique and has been facilitated by the fact that the National Institutes of Health has made available dedicated resources. When applied to all 324 pancreata processed for CIT clinical protocols, the success rate among the centers in obtaining purified human pancreatic islet lots that met predefined release criteria ranged from 24.5% to 89.5% (mean 52.5%).[12] Islet lots that met release criteria in the centers and were transplanted (75 total; 4–15 per center) in the CIT protocol showed no primary nonfunction, unlike the center effect in other multicenter trials.[5-8] Europe is currently the most active region in the field of CIT. In spite of this, there are no ongoing initiatives aimed to optimize and standardize processes, criteria, and test methods across islet transplantation centers. By using a structured questionnaire to survey 11 processing facilities, we aimed to identify differences in the human pancreatic islet isolation processes within the European Countries. The result highlighted the presence of a heterogeneity in the islet isolation process and predefined release criteria. The reduction of this heterogeneity is required to enable future robust comparison of graft outcomes among centers and ensure meaningful multicentric clinical studies.

MATERIALS AND METHODS

Design

A web-based questionnaire about donor selection criteria, pancreas processing, pancreas perfusion and digestion, islet counting and culture before transplantation, islet quality assessment, microbiological contamination testing, and release criteria of islets for transplant was completed by 11 processing facilities participating at the Ninth International European Pancreas and Islet Transplant Association (EPITA) Workshop on Islet-Beta Cell Replacement held in Milan on September 24–26, 2018.

Recruitment

Processing facilities that were located in Europe and with an isolation activity >10 pancreata per year within the previous 3 years were eligible for inclusion and invited to participate in the survey. The survey was open from July 30 until September 9, 2018. Facilities that failed to respond within 1 month were sent a second reminder. Eleven out of 12 invited facilities completed the questionnaire.

The Questionnaire

The questionnaire was developed starting from the Standard Operating Procedure of the CIT.[13] A panel comprising 3 experts in islet isolation field first reviewed it. Based on the feedback from the experts, the questionnaire was revised. The final questionnaire included 4 sections (see supplemental document 1). The first included questions about the activities before islet isolation, such as donor selection criteria and organ procurement. The second section asked about the islet isolation process, including enzyme solutions, organ perfusion, digestion and purification steps, islet counting, and culture before transplantation. The third section focused on islet quality assessment, microbiological contamination tests, and predefined release criteria. The final section summarizes their activity and CIT program.

Analysis

Data from the questionnaires were entered into a database and then double-checked by a second researcher. Free text responses were grouped into similar categories and coded. Where individual free text responses contained several comments, these were each coded individually. All analyses were performed using IBM SPSS Statistics for Windows, Version 24.0.

RESULTS

Section 1: Characteristics of Islet Isolation Facilities

A total of 11 processing facilities completed the questionnaire: 2 from Italy and one each from Belgium, Czech Republic, France, Germany, Netherlands, Norway, Sweden, Switzerland, and United Kingdom. Table 1 shows the characteristics of manufacturing facilities included in the study. Altogether, the facilities reported 445 and 53 islet isolations per year over last 3 years from deceased organ donors and pancreatectomized patients, respectively. This activity resulted in 120 and 40 infusions per year in allograft and autograft recipients, respectively.

Section 2: Donor Selection and Pancreas Procurement

Proper procurement, preservation, and predigestion preparation of the pancreas are very important components of the manufacturing process that can have significant effects on islet quality and yields. The organization model of pancreas procurement varies among the manufacturing facilities. Three out of 11 facilities have their own pancreas retrieval teams who specifically retrieve organs for their islet program or a coexistent whole pancreas transplant program. Two out of 11 centers rely on pancreata being retrieved from a number of teams unrelated to the manufacturing facilities. The remaining 6 facilities have a mixed model. Among the parameters considered critical for donor acceptance, the concordance among manufacturing facilities varies from 100% for age and cold ischemia

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TABLE 1.
Characteristics of islet processing facilities included in the study

ID	Country	Facility classification	People engaged		Allo TX		Auto TX		Islet for research	
			Academic	Technicians	Isolation/y[a]	Infusion/y[a]	Isolation/y[a]	Infusion/y[a]		
1	Academic Hospital and Diabetes Research Center, Vrije Universiteit Brussel, Brussels, Belgium	Class A, background Class C	2	4	50	10	<1	<1	Yes	Research consent is granted
2	Clinical and Experimental Medicine, Prague, Czech Republic	Partial cGMP	12	2	25	7	4	3	No	Not allowed
3	European Genomic Institute for Diabetes, University Hospital, Lille France	ANSM accredited facility	5	10	55	10	5	3	Yes	Research consent is granted
4	Department of Medicine III, University Hospital Carl Gustav Carus Dresden, Dresden, Germany	cGMP	4	1	5	3	12	8	Yes	Only from auto Tx (when research consent is granted)
5	Diabetes Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy	Class A, background Class B	2	2	40	7	12	8	Yes	Allowed by local IRB (when islet are not suitable for clinical purpose)
6	Diabetology Unit, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy	Class A, background Class B	3	0	30	5	2	2	Yes	Allowed by local IRB (when islet are not suitable for clinical purpose)
7	Leiden University Medical Center, Department of Nephrology, Leiden, Netherlands	cGMP	6	6	42	7	2	2	Yes	Research consent is granted
8	Oslo University Hospital, Oslo, Norway	cGMP, Class A background Class B	2	3	11	5	5	4	Yes	Research consent is granted
9	Uppsala University Hospital, Uppsala, Sweden	cGMP	6	6	100	25	3	3	Yes	Allowed by local IRB if consent for research is available (when islet are not suitable for clinical purpose)
10	Hôpitaux Universitaires de Genève, Genève, Switzerland	cGMP, Class A background Class C	7	5	47	26	4	3	Yes	Allowed by local IRB (when islet are not suitable for clinical purpose)
11	Oxford Centre for Diabetes, University of Oxford, Oxford, United Kingdom	cGMP	10	9	40	15	3	3	Yes	Research consent is granted
TOT (median)	–	–	59 (5)	48 (4)	445 (40)	120 (7)	52 (4)	40 (3)	91%	

[a]Mean over last 3 y.

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time to 45% for BMI (Figure 1A). All facilities agree that, when considering inclusion criteria, the values of BMI and HbA1c should be >18 and <6.5%, respectively. On the contrary, the other parameter thresholds for the acceptance largely vary among facilities (Figure 1B). Five out of 11 facilities report a donor age of 18–65 years, while the others have different ranges: 16–70, 10–65, 0–75, 0–65, 16–60, 0–60 years. The maximum acceptable cold ischemia time (defined as the time interval between cross clamping the organ and starting intraductal pancreas perfusion) ranges from 8 to 24 hours (Figure 1B). The maximum acceptable intensive care unit length of stay varies from 5 to 10 days, or it is evaluated only when "extreme" or as part of a combination of factors. The maximum acceptable value of donor amylase is reported as 600 U/L, 300 U/L, 110U/L, and 3 times the normal range or as part of a combination of factors. Values of 10, 20, 30, and 60 minutes are indicated as maximum acceptable for the cardiac arrest duration and a threshold is not indicated by 2 facilities, which, however, suggests using biochemical markers of ischemic damage (ie, levels of creatinine, alanine and aspartate aminotransferases, gamma glutamine transferase) as exclusion criteria. Donation after circulatory death (DCD) is accepted in 6 out of 11 manufacturing facilities and 4 out of 6 uses more stringent criteria for DCD than for donation after brain death (lower age and shorter cold ischemia time). The preservation solutions vary among centers (Figure 1C). Additional actions are not performed before pancreas preservation except for one center that routinely performs an intraductal injection of the preservation solution with added insulin.

Section 3: Islet Isolation and Culture Before Transplantation

Despite the automated method described by Ricordi et al[14] that represents the backbone of islet isolation in all facilities, some differences emerge in the isolation procedure. Differences in the brand and concentration of collagenase for pancreas digestion are reported. Ten out of 11 manufacturing facilities have experience with Collagenase NB 1 from SERVA (Nordmark) and 5 out of 11 with Cl*zyme* from VitaCyte and/or Liberase Collagenase I/II MTF from Roche Diagnostics. The GMP grade enzyme is used by 6 out of 11 facilities. Collagenase is generally tailored to obtain a

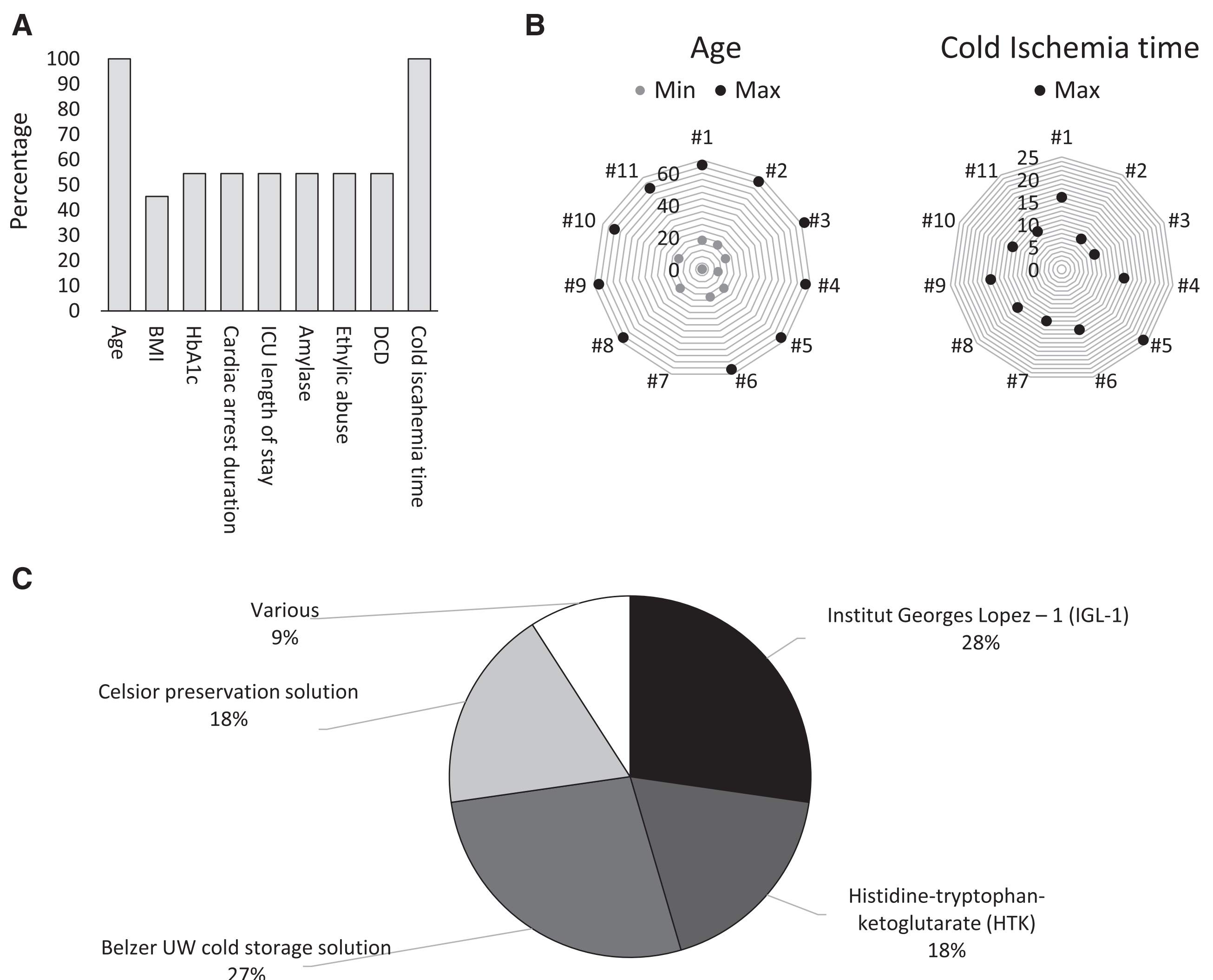

FIGURE 1. Critical steps in the human islet isolation procedure. A, percentage of concordance among 11 European processing facilities on parameters considered critical for donor acceptance. B, spider web graphs representing the values for age (left; units: y) and cold ischemia time (right; unit: h) reported as acceptable from the 11 European processing facilities. C, pancreas preservation solutions used. BMI, body mass index; DCD, donation after circulatory death.

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target concentration (range from 6 to 26 U/mL) or based on pancreas weight. The batch-to-batch collagenase variation is tested in 5 out of 11 facilities. Nine out of 11 manufacturing facilities select the enzyme concentration also based on donor characteristics like age. All the facilities add various proteolytic activities to the collagenase (Neutral Protease NB, Liberase Thermolysin or Clostripain) and various combinations of additive such as calcium, NaOH, DnAse, Hepes serine protease inhibitor, antibiotics, heparin, and glutamine. The collagenase solution (after filtration in 4 out of 11 facilities) is loaded into the pancreas by pump (8 out of 11, adjusting pressure) or by syringe (3 out of 11, manual) and different temperatures are used during the perfusion (from 4ºC to room temperature). Five out of 11 facilities maintain the organ in static digestion before starting the dynamic phase. Although the criteria for stopping the digestion are found to be specific for each facility (including percentage of free islets, number of islets, size of exocrine, islet morphology, presence of islet fragments, ratio islet/acinar cells, exocrine digested to the same size as the islets, and cloudy solution in the digestion chamber), the average digestion time reported is quite homogeneous: 15.6 ± 3.8 minutes. The maximum acceptable digestion time varies from 18 to 35 minutes. Six out 11 facilities use a stopping rule after digestion, mainly related to the number (<100 000 equivalent islets) and quality of islets (embedded or fragmented). Islet purification is performed with a continuous gradient by COBE 2991 Cell Processor except for one center that uses discontinuous gradient in tubes. Generally (9 out of 11), islets are soaked in an isotonic solution before purification, while a test gradient is performed only in 3 out of 11 facilities. The maximum tissue volume per purification run largely varies from 25 to 50 mL and in 7 out of 11 facilities, the COBE 2991 Cell Processor is cooled with different strategies during the purification (nitrogen vapor, cool pressurized medical air, ethanol cooling or via housing in cold room). Five out of 11 facilities use discontinuous gradient as rescue strategy in case of purification failure. All facilities count the islets after the purification step before transplant (cases of autologous) or culture with quantification made by the standard manual counting (5 out of 11) or by digital image analysis (6 out of 11). A large variability in the systems and conditions of culture is evident. Flasks (8 out of 11) or bags are used alternatively as culture dispositives. The suspension volume is decided on the basis of tissue volume (6 out of 11), purity (4 out of 11), or number of islets (1 out of 11) resulting in a wide range of tissue/medium volume ratio (from 0.5 to 45 µL tissue/mL medium) and islet equivalent number /medium volume ratio (from 500 to 2000 equivalent islet/mL). Different culture temperatures are reported: 37ºC from isolation until the transplant (5 out of 11), 37ºC for 24 hours then 25–26ºC until the transplant (3 out of 11), or 22–25ºC from isolation until the transplant (3 out of 11). The maximum acceptable culture time before transplant varies from 1 to >7 days. The loss of islets during the culture before transplantation is 25% as median (min 0, max 50%).

Section 4: Quality Evaluation and Release Criteria for Transplant

Regarding the quality controls to be considered, and definition of what is critical for islets before transplantation, the survey reveals a low agreement among facilities (Figure S1A, SDC, http://links.lww.com/TP/B747). The most consistent parameter of the evaluation is the islet viability by fluorescein diacetate/propidium iodide staining with an acceptable value of at least 80% (a single facility reports Trypan blue/dithizone staining or acceptable value of 70% or 90%). The Glucose-Stimulated insulin Secretion tests, in particular the dynamic one, are less used and, more importantly, each facility indicated the protocol of their center (Figure S1B, SDC, http://links.lww.com/TP/B747) with variable numbers of islets tested (range 20–300 equivalent islets), different lengths of stimulation (range 45–60 min static and 10–60 min dynamic), different basal glucose concentrations (range 1.67–3.3 mmol/L) and different glucose concentrations for stimulation (range 15–22 mmol/L). Concordantly, acceptable secretion indexes reported were equally heterogeneous: ≥1, >1.5, ≥2, and >2. Oxygen consumption rate, ATP/ADP ratio, and apoptosis assay were not routinely performed. Few facilities perform in vivo quality assessment of the islet (transplant in immunodeficient mouse model), and the protocols are center-specific with differences in the number of transplanted islets, mouse recipient conditions (nondiabetic or diabetic mice), and readouts (C-peptide, glycaemia, weight). There is a concordance amongst all the facilities on the need for microbial surveillance of the islet isolation process: bacteriological tests (aerobic bacteria, anaerobic bacteria, and fungi) are performed on the final islet preparations (11 out of 11), transport medium (9 out of 11), and culture medium (8 out of 11). The utilization of other tests like the endotoxin content and gram staining was variable.

Regarding the release criteria for the transplantation, there was unanimity in considering the number of equivalent islet (IEQ) as a criterion (Figure S1C, SDC, http://links.lww.com/TP/B747). On the contrary, the cut off value varied largely: 2×10^6 beta cell/kg, >300 000 IEQ, >3500 IEQ/kg or 200 000 IEQ, ≥5000 IEQ/kg, ≥10 000 IEQ/kg first transplant and 5.000 IEQ/kg for subsequent transplant, ≥3000 IEQ/kg and >10 000 IEQ/kg in total, ≥4000 IEQ/kg, ≥5000 IEQ/kg and >8000 IEQ/kg in total. Similarly, a general agreement was evident in considering minimum purity, maximum culture time, and maximum tissue volume as release criteria, but a broad range of values were reported: from 20% to 50% for purity, 2 to 7 days for culture time, and 5 to 12 mL for tissue volume. Less consistent among the processing facilities were other criteria like a negative gram staining on media, the secretion index after glucose-stimulated insulin secretion test (range of value from 1.5 to >10), or the endotoxin content of the final product (value <5 EU/kg recipient). According to the survey (Figure 2), many steps of the islet allotransplantation are at least partially reimbursed with the exception of the pancreas recovery and islet isolation costs. Reimbursement of the procedure is reported to be less homogenous in autotransplantation than in allotransplantation. Finally, all the processing facilities were asked to indicate, which was the most important action to increase the islet transplant activity in their own country and in Europe? The results are summarized in Table S1 (SDC, http://links.lww.com/TP/B747): increasing the quality and quantity of pancreas donors and the reimbursement to cover all the procedure costs are classified in first and second position, respectively.

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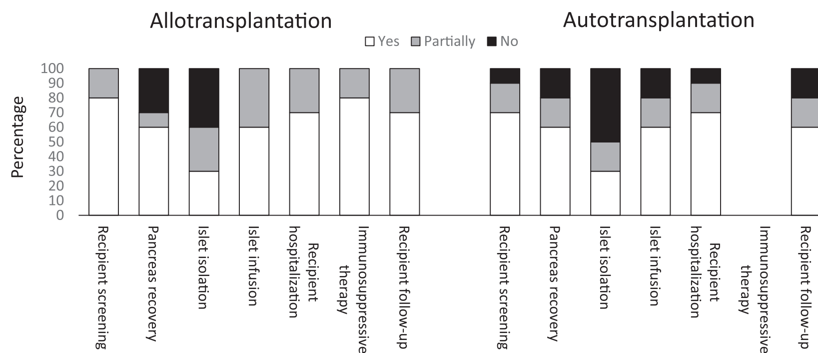

FIGURE 2. Reimbursement of islet transplantation around Europe. Reimbursement (classified as no, yes, or partial) of the different components of the human islet transplantation process. Data are expressed as percentage of facilities.

DISCUSSION

Europe is currently the most active region in the field of CIT, and many of the leading groups are actually achieving similar good/beneficial outcomes. Islet isolation and clinical management are key elements in any islet transplant program. Over the past 3 decades, the field of islet processing has progressively introduced the automation of islet isolation[14,15] and purification,[16] the use of purified enzyme blends[17-19] and new systems for pancreas perfusion,[20,21] the analysis of different factors able to influence islet isolation outcome,[1] and the development of standard operating procedures for guaranteeing the quality and safety of the procedures.[4,12] Despite this, a standardized method for islet isolation is not available in Europe, resulting in a significant challenge in the comparison of data coming from different clinical programs. It is true that the intricate nature of pancreatic islets and the limited number of reliable characterization assays for assessing the quality of the islets obtained make the standardization of the process complicated. Moreover, every center optimizes and standardizes their own procedures and processes within their center's unique framework of regulatory issues, donor organ availability and quality, local processing facility requirements, and financial considerations. Despite this the control of the source material, isolation process, and quality of the islets obtained are critical issues that need to be harmonized to enable future robust comparison of graft outcomes and ensure meaningful multicentric clinical studies. This effort was recently completed by 8 North America manufacturing facilities and the NNCIT participating in CIT.[12] Unfortunately, there are no similar initiatives across islet transplantation centers in Europe. Variability among facilities is evident in specific steps of the islet isolation process: donor selection, enzyme blends, digestion time and temperature, additives used during the islet isolation process, purification methods, the temperature and duration of the islet culture, as well as the release criteria. While this allows for significant flexibility in the islet isolation process, it prevents the development of a reproducible process, which can be compared and validated between various centers. This situation needs to be improved for different reasons. First, as islet graft function correlates with the number of transplanted functional islets,[22] and maximization of islet retrieval is the prerequisite for the clinical success. Second, the islet isolation procedure itself greatly affects in vivo islet graft functions such as viability, insulin secretion, and proinflammatory activities.[23] Third, the standardization of islet isolation can improve the cost efficacy of islet transplant programs, an element indicated among the major limiting factors for the wider application of the procedure. Fourth, the standardization of procedures is a "sine qua non" condition to enable future robust comparison of graft outcomes among centers and ensure valuable multicentric clinical studies. Fifth, there is a recent request endorsed by multiple members of the islet research ecosystem toward a better standardization of islets provided for experimental studies.[24,25] In fact, scientists and major journals in the field are asking to improve the reporting and, to the extent possible, standardization of the preparations and methods used by individual laboratories.[26,27] The analysis of this survey generates questions and issues that should be addressed, such as the contribution of biology or islet isolation techniques to human islet variability and the reproducibility of results in CIT. New efforts are required to address these questions and issues. Although the need for harmonization is clear, the question that arises is why it has not yet been done. Overall, the problem is not related to the lack of regulation. The process of islet isolation and storage has been regulated by the European Union Tissue and Cells Directive (EUTCD) since 2004. Islet product is considered a tissue therapy (it does not fall within the definition of an advanced therapy medicinal product), and specific technical requirements were dictated by subsequent EU Commission directives (2006/17/EC, 2006/86/EC, 2015/566 and 2015/565). They specify, among other things, the traceability, coding, preparation, culture, and distribution of human cells and tissue. The main problem is the lack of cross-validated scientific evidence among the various centers on key steps of the procedure. This lack is mainly related to the limited resources made available by the funding agencies to conduct these types of studies and their low "impact factor" in the publication phase. As recently it was done for the definitions of beta cell replacement outcomes,[28-30] a common effort should be accomplished to improve procedures, protocols, and communication related to human islet isolation. Since pancreas recovery and islet isolation costs are mainly not reimbursed by the system, any improvement will depend on willingness of funding agencies (mainly the European commission) to provide critical financial and organizational support.

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