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ORIGINAL ARTICLE

Clinical utility of C-peptide measurement after pancreas transplantation with especial focus on early graft thrombosis

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SUMMARY

Since the beginning of our pancreas transplant programme, plasma C-peptide was routinely measured daily during the postoperative period. We aimed to evaluate the clinical interest of the C-peptide in the follow-up of pancreas transplantation with a particular look on early graft failure. From 2000 to 2016, 384 pancreas transplantations were evaluated. We collected and compared C-peptide, glycaemia and adjusted C-peptide (aCP; calculated based on C-peptide, glycaemia and creatininaemia) in patients with and without pancreas failure within 30 days after surgery. Variations of glycaemia, C-peptide and aCP between the day before and the day of failure were also recorded. The difference of aCP was significant during the first week after transplantation between patients with thrombosis and those with functional allograft: 63.2 vs. 26.7 on day 1, $P = 0.0003$; 61.4 vs. 26.7 on day 3, $P < 0.0001$; 64.8 vs. 5.7 on day 7, $P < 0.0001$, respectively. Glycaemia had a median increase of 8% on the day of failure, whereas C-peptide and aCP had, respectively, a median decrease of 88% and 83%. C-peptide monitoring after pancreas transplantation may help to identify graft function and early failure. This sensitive biomarker could allow pre-emptive diagnosis of an early thrombotic event allowing the possibility of rescue interventions.

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Introduction

Pancreas transplantation can provide normal and long-lasting glycaemia, improve patient survival and ameliorate quality of life in selected patients with diabetes [1,2]. Despite medical and surgical improvements over decades and standard use of anticoagulants, early allograft failure due to thrombosis still remains a major complication. The incidence reported in the literature ranges from 1% to 40% [3–5]. Reasons are not always known, but the surgical procedure *per se* and the inflammatory status of the organ secondary to preservation may play an important pathogenic role [6,7]. The physiopathological mechanisms leading to thrombosis could be linked to the time of occurrence. Early after transplant, vascular thrombosis is multifactorial, but beyond

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the first months, inflammation or acute rejection predominate as the cause of thrombosis [8]. Multiple risk factors for thrombosis have been reported: both donor and recipient high body mass index (BMI), donation after cerebrovascular death, long stay of the donor in the intensive care unit, high doses of vasoactive drugs, donor hypernatremia, extended cold ischaemia time (CIT) and severe hypotension in the perioperative period [9–11]. In the absence of a reliable marker, there is no way to anticipate a total thrombosis and potentially rescue the pancreas allograft. In practice, radiological imaging is the only way to confirm diagnosis.

Prevention, as opposed to treatment, is the key and should focus on reducing these multiple risk factors. This will involve tactical donor selection, optimal surgical technique, and some form of anticoagulation. Close monitoring and early intervention will be crucial when treating thrombosis once preventative methods have failed. Intra-operative and postoperative thromboelastogram was used to evaluate the patient's coagulation status [4].

C-peptide and insulin are secreted in equimolar amounts in response to hyperglycaemia [12]. C-peptide has a half-life of about 30 min and is exclusively metabolized by the urinary system [13,14]. Plasma C-peptide level is used to monitor endogenous insulin secretion (even under exogenous insulin therapy), but its potential use in the field of pancreas transplantation is not well defined. Some studies have demonstrated that a positive C-peptide before pancreas transplantation was not associated with worse patient or graft survival [15]. In the field of islet transplantation, C-peptide is a component of the β-Score which is routinely used to estimate beta cell functionality [16–18].

Since the beginning of our pancreas transplant programme, we have routinely measured daily fasting plasma C-peptide level for all pancreas transplant recipients throughout the postoperative period, at each outpatient routine visit, when clinically required and yearly. We therefore searched for the purpose of this study a possible association of post-transplant C-peptide level and the occurrence of an early allograft failure. We hypothesized that C-peptide assay could help to diagnose thrombosis and guide timing of CT scan angiogram.

Materials and methods

Studied population

All patients receiving a pancreas transplant in our institution between 1 January 2000 and 31 December 2016 were included. This period was selected because computerized data of C-peptide were prospectively recorded, and afterwards we modified our immediate postoperative immunosuppressive regimen. We categorized pancreas transplant as simultaneous kidney–pancreas (SPK), pancreas after kidney (PAK) and pancreas transplant alone (PTA). Pretransplant positive C-peptide was not an exclusion criterion for transplantation. For the aim of the study, two comparative groups were designed: the Thrombosis Group, consisting of patients with allograft failure caused by proven thrombosis occurring during the first 30 days after transplantation, and the Success Group, consisting of patients with a functional pancreas allograft (insulin-free) one-month post-transplantation. Thrombosis was defined as the complete absence of pancreatic perfusion on CT scan angiogram.

Management of pancreas transplantation was similar for all types of categories (SPK, PAK and PTA). Intravenous insulin therapy was introduced preoperatively for all patients, and doses were then adapted according to blood glucose in order to impede any level above 1.5 g/l. Plasma C-peptide was measured immediately after pancreas reperfusion and daily thereafter though immunoassay test (Electrochemiluminescence) in fasting patients (normal range, 0.4–4 ng/ml). A significant increase was considered a 'normal' functioning pancreas, even if exogenous insulin was used. Levels above 30 ng/ml were interpreted as abnormal and suspected to be the consequence of the ischaemic-reperfusion organ damage. Glycaemia was measured simultaneous to C-peptide dosage. Parenteral nutrition was continued until resumption of intestinal transit. Induction therapy consisted of Thymoglobulin for five alternate days, associated with two intravenous pulses of 500 mg of corticosteroids. Maintenance immunosuppressive therapy associated calcineurin inhibitors (mainly tacrolimus) and mycophenolate mofetil or mycophenolic acid. Low-dose corticosteroids were administered only during the first week after transplantation. A routine IV contrast CT scan was intended to be performed on postoperative day 10 in event-free patients (before hospital discharge). Low molecular weight heparin was given within the first days after surgery (mostly 7 days) followed by 100 mg aspirin and 150 mg of dipyridamole, daily.

Data were extracted from the French DIVAT cohort (www.divat.fr, approved by the CNIL, n°914184) and were restricted to recipients transplanted and monitored in our institution. The quality of DIVAT data base is validated by an annual cross-centre audit. All participants to our study gave their informed written consent at the time of the inscription on the waitlist.

Available data

Donor characteristics included age, gender, BMI, abdominal perimeter, stay in intensive care unit, use of vasoactive drugs, creatininaemia and glycaemia values. Recipient characteristics included age, gender, BMI, type and rank of pancreas transplantation, duration of diabetes, detection of nondonor and donor specific antibodies (DSA), creatininaemia, A1c Hb, glycaemia and C-peptide values before transplantation. Transplantation parameters evaluated were cold ischaemia time (CIT), crossmatches result and use of somatostatin. Biological follow-up (fasting glycaemia, fasting C-peptide and creatininaemia) was assessed for all patients after transplantation. For patients in the Thrombosis Group, time and cause of failure was reported, and biological follow-up was interrupted after allograft failure.

Biological values one-month post-transplantation were reported for all recipients in the Success Group. We also analysed biological values around occurrence of partial thrombosis (venous and/or arterial) for patients with a functional allograft.

Determination of the 'adjusted C-peptide'

Based on physiological properties of C-peptide, we established the adjusted C-peptide (aCP). aCP values were calculated according to the formula:

$$\text{Adjusted C}-\text{peptide} = 100 \times \frac{\text{C}-\text{peptide} \times \text{eGFR}}{90 \times \text{Glycemia}}$$

Indeed, we adapted the C-peptide value to renal clearance since its elimination is exclusively urinary and to glycaemia since its secretion depends on glycaemia value. eGFR was calculated according to the MDRD formula, and C-peptide values were reported in ng/ml and glycaemia values in mmol/l. The 90 in the denominator is based on the fact that a patient with a normal glomerular filtration rate (i.e. 90 ml/min) will not require C-peptide adaptation for the eGFR. Thus, we retrospectively calculated the daily aCP for all patients in order to compare post-transplant values between Thrombosis and Success groups.

Statistical analysis

Each donor and recipient characteristics in the Thrombosis and Success Groups were listed and compared. For continuous variables, Student's *t* tests were used, and for qualitative variables, we used chi-square test. Survival curves were estimated using a Cox model.

The evolution of glycaemia, C-peptide and aCP during the first week was evaluated and compared among groups. In the Thrombosis group, biological values were also evaluated retrospectively from the day of allograft failure. Indeed, daily average values were influenced by the variability of failure occurrence. Variations of glycaemia, C-peptide and aCP were considered between the day before thrombosis and the day of thrombosis for patients with complete thrombosis (Thrombosis group) but also for those with functional allograft presenting partial thrombosis. Finally, in Success group, C-peptide value at one-month post-transplantation was compared between patients with poor allograft survival (i.e. failure during the first year) and all remaining ones.

In the Thrombosis group, diagnostic performance of glycaemia, C-peptide and aCP to predict allograft failure over the next 24 h was evaluated using a logistic regression analysis. The areas under the ROC curves (AUC) and the corresponding two-sided 95% confidence intervals were estimated for the three models and compared to determine the best biomarker to predict allograft failure within the next 24 h. The optimal thresholds for each biomarker and their sensitivity and specificity were estimated using the Youden index. The same approach was used to assess the diagnostic performance of kinetic variation on allograft failure over the next 24 h. Kinetics were calculated as the percentage of evolution between two measurement times, taking the day of the graft failure as the reference.

The significance threshold was set at 0.05 (two-tailed), and analyses were performed using SAS software version 9.4.

Ethics statement

Following informed consent, all patients' data were extracted from the DIVAT database and de-identified in order to respect confidentiality.

Results

Description of the cohort

During the selected period, a total of 384 pancreas transplants were performed and all were included in our study. Five deaths with a functional pancreas and 3 allograft failures without thrombosis (haemorrhagic shock) occurring during the first month were excluded from analyses. Of the remaining 376 cases, 36 presented allograft failure during the first month (9.5%) and

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constituted the Thrombosis Group. Median occurrence of failure was 2 days after surgery. The 340 remaining patients (90.5%) constituted the Success Group. 326 of these patients (95.8%) had a functional pancreas allograft at one year (86.7% of the total cohort). In Thrombosis group, 11.1% were PAK, 11.1% PTA and 77.8% SPK. In Success group, 9.8% of patients received a PAK, 11.6% a PTA and 78.6% a SPK. Overall, there was no statistically significant difference between the Thrombosis and Success groups regarding the category of pancreas transplantation. Figure 1 shows the study flow chart. Survival curves of the overall cohort are represented in Figs S1–S3.

A large majority of transplants were first pancreas (83.8% in the Thrombosis Group, 90.9% in the Success Group, $P = 0.111$). Average recipient age was 40 years in both groups with a majority of men. The principal indication of transplantation was type-1 diabetes. Pretransplant C-peptide and pretransplant HbA1c did not differ between groups (0.5 ng/ml vs. 0.3 ng/ml, $P = 0.27$; 9.3% vs. 9.2%, $P = 0.95$). There was a trend to longer CIT in the Thrombosis Group compared to Success Group (761 vs. 731 min, $P = 0.09$). All transplants were from donation after brain death. Average donor age was 35 years in both groups, and majority were men. Donors in the Thrombosis Group tended to have higher BMI compared to those in the Success Group (23.8 vs. 22.7 kg/m^2, $P = 0.13$). Donor glycaemia and creatininaemia were comparable in both groups (8.2 vs. 7.9 mmol/l, $P = 0.38$; 76 vs. 79 μmol/l, $P = 0.61$, respectively). Table 1 summarizes these results.

All thrombosis episodes (complete and partial) were diagnosed by IV contrast CT scan. Indications for CT scan are reported in Table S1. During follow-up, 40 patients (11.7%) in the Success Group presented partial allograft thrombosis. All of them received curative anticoagulation by heparin immediately after diagnosis. 32 patients had partial venous thrombosis, occurring a median time of 8 days, 2 of them related to acute rejection. Eight patients had partial arterial thrombosis, occurring a median time of 580 days.

Allograft was removed in all patients with allograft failure due to complete thrombosis.

Evolution of glycaemia, C-peptide, C-peptide/Glucose ratio and aCP among thrombosis and success groups

Average fasting glycaemia was relatively normal in the Success Group, whereas it remained high in the Thrombosis Group (6.7 vs. 10.2 mmol/l on day 1, $P < 0.0001$; 6.7 vs. 8.2 mmol/l on day 3, $P = 0.0111$; 6.2 vs. 7.6 mmol/l on day 7, $P = 0.3927$, respectively). In the Success Group, C-peptide immediately raised and then

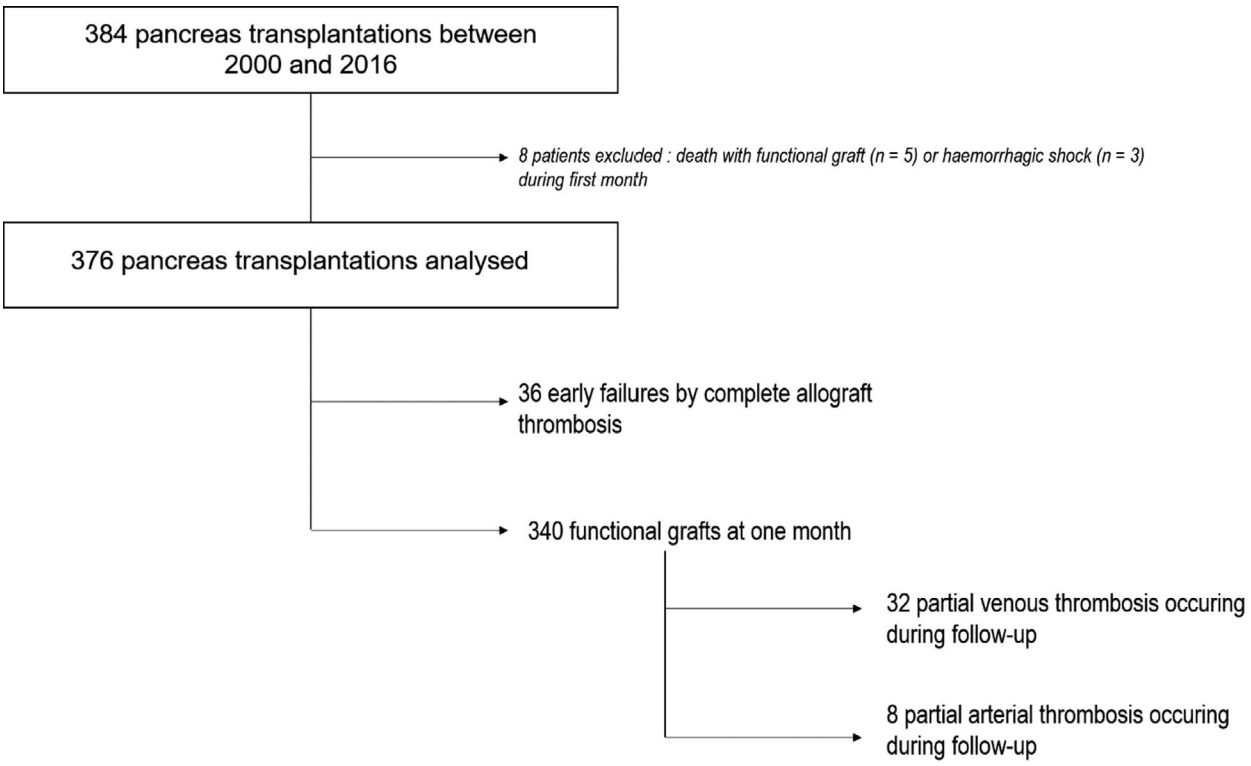

Figure 1 Flow chart of the study.

Table 1. Description of the cohort. Quantitative variables are expressed in mean with corresponding standard error.

	All	Thrombosis	Success	*P*
Pancreas category	*n* = 376	*n* = 36	*n* = 340	
PAK	9.9%	11.1%	9.8%	0.35
PTA	11.5%	11.1%	11.6%	0.78
SPK	78.6%	77.8%	78.6%	0.71
Rank of transplantation				
1	90.2%	83.3%	90.9%	0.11
2	9.5%	16.7%	7.8%	0.10
3	0.3%	0.0%	0.3%	>0.99
Recipient age (years)	40.4 ± 8.1	41.4 ± 9.2	40.3 ± 8.02	0.38
Recipient male gender	60.8%	66.7%	60.2%	0.27
Recipient BMI (kg/m^2)	23.0 ± 3.4	23.4 ± 3.7	23.0 ± 3.4	0.67
Duration of diabetes (years)	26.7 ± 7.9	28.2 ± 8.7	26.5 ± 7.8	0.18
Somatostatin use	14.1%	13.3%	14.2%	0.77
Cold ischaemia time (min)	734 ± 173	761 ± 197	731 ± 170	0.09
Pretransplant C-peptide (ng/ml)	0.3 ± 1.1	0.5 ± 1.4	0.3 ± 1.1	0.27
Pretransplant HbA1c (%)	9.2 ± 7.9	9.3 ± 2.5	9.2 ± 8.2	0.95
Donor age (years)	33.8 ± 10.9	35.2 ± 9.8	33.7 ± 11.0	0.28
Donor male gender	62.0%	66.7%	61.5%	0.41
Donor BMI (kg/m^2)	22.8 ± 2.8	23.8 ± 3.2	22.7 ± 2.8	0.13
Donor abdominal perimeter (cm)	82.6 ± 11.3	84.0 ± 11.20	82.5 ± 11.3	0.49
ICU stay (days)	3.6 ± 21.5	2.6 ± 1.7	3.8 ± 22.5	0.24
Use of vasoactive drugs	84.0%	81.2%	84.2%	0.29
Donor glycaemia (mmol/l)	7.9 ± 3.2	8.2 ± 3.1	7.9 ± 3.2	0.38
Donor creatininaemia (µmol/l)	79 ± 33	76 ± 28	79 ± 33	0.61
Pretransplant DSA	4.0%	5.5%	3.8%	0.66
Positive T-cell crossmatch	0	0	0	

Qualitative variables are expressed in percentage.

slowly decreased, whereas in the Thrombosis Group, there was a moderated elevation followed by a rapid decreased (13.7 vs. 10.4 ng/ml on day 1, $P = 0.0023$; 10.6 vs. 6.8 ng/ml on day 3, $P = 0.0141$; 7.6 vs. 2.6 ng/ml on day 7, $P = 0.0007$, respectively). C-peptide/Glucose ratio was higher in the Success group compared to the Thrombosis group (2.2 vs. 1.7 on day 1, $P = 0.0002$; 1.7 vs. 1.0 on day 3, $P = 0.001$; 1.0 vs. 0.1 ng/ml on day 7, $P = 0.002$, respectively). Average aCP in the Success Group was nearly three times higher compared to the Thrombosis Group during the first week after transplantation (63.2 vs. 26.7 on day 1, $P = 0.0003$; 61.4 vs. 26.7 on day 3, $P < 0.0001$; 64.8 vs. 5.7 on day 7, $P < 0.0001$, respectively). Figure 2 shows the evolution of biological values during first week post-transplantation. Patients' biological follow-up is described in Tables S2–S5.

Evolution of glycaemia, C-peptide and aCP for patients presenting partial and complete thrombosis

Biological values were evaluated retrospectively from the day of thrombosis, meaning that we analysed variations of glycaemia, C-peptide and aCP between the day before thrombosis and the day of thrombosis.

For patients in the Thrombosis Group, glycaemia varied from 9.8 to 11.6 mmol/l, $P = 0.1772$. On the contrary, C-peptide, C-peptide/glycaemia and aCP dropped on the day of thrombosis compared to the previous day (5.3–0.6 ng/ml, $P < 0.0001$; 0.64–0.08, $P < 0.0001$ and 27.5–2.9, $P < 0.0001$, respectively). Globally, glycaemia had a median variation of 8% on the day of thrombosis, whereas C-peptide, C-peptide/glycaemia and aCP had, respectively, a median decrease of 88%, 86% and 86%, respectively.

Similarly, for patients with partial thrombosis, glycaemia varied from 6.3 to 7.4 mmol/l, $P = 0.3601$. We observed a variation of C-peptide values (6.3–5.3 ng/ml, $P = 0.9450$), C-peptide/glycaemia (1.12–1.09, $P = 0.5439$) and aCP (75.5–59.2, $P = 0.2897$). For these patients, median glycaemia variation on the day of partial thrombosis was 4%, C-peptide was 7%, C-peptide/glycaemia was 15% and aCP was 22%. Figure 3 and Table 2 represent variations of biological values between the day before thrombosis and the day of thrombosis.

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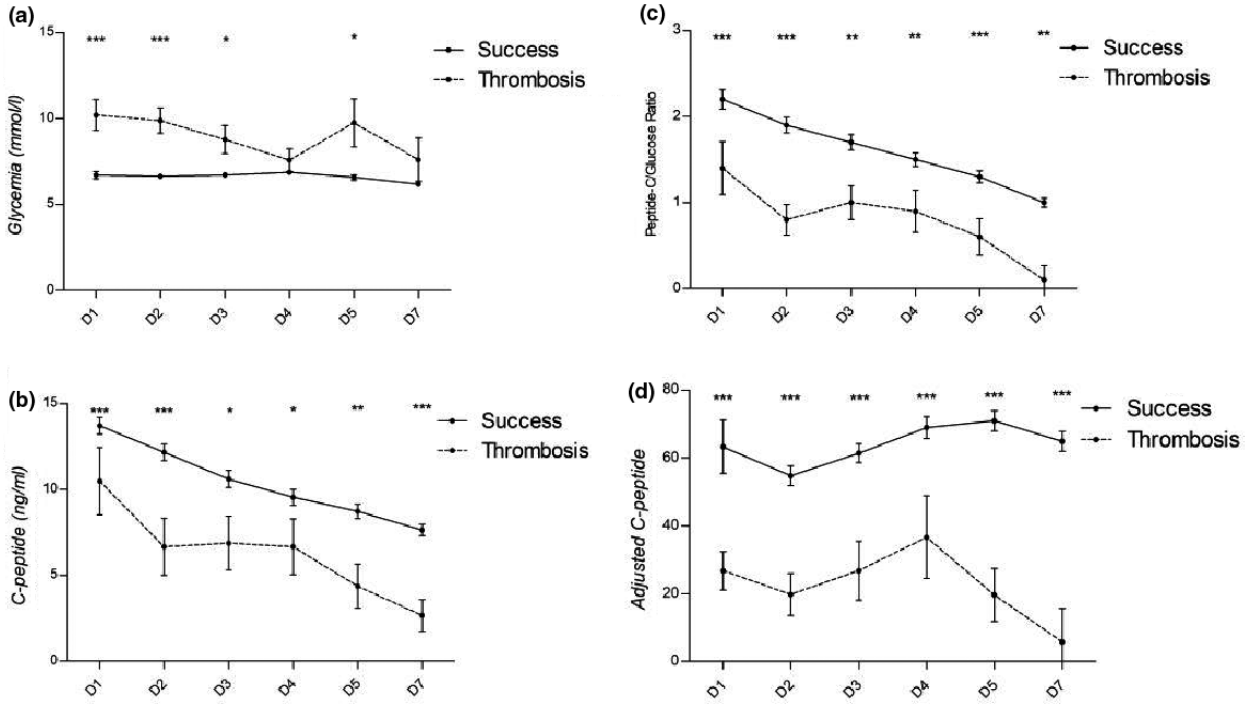

Figure 2 Glycaemia (a), C-peptide (b), C-peptide/glycaemia (c) and adjusted C-peptide (d) average values during the first week after transplantation with the corresponding standard error mean (SEM); *P value < 0.05, **P value < 0.01, ***P value < 0.001.

Collapsed C-peptide is frequent in patients with complete allograft thrombosis

In the Success Group, an exceedingly small number of patients with a C-peptide ≤1 ng/ml was encountered. Considering all patients' measurements, only 3 out of 1883 were ≤1 ng/ml (0.15%), meaning that only 3 out of 340 patients (0.87%) had once a C-peptide ≤1 ng/ml. On the contrary, C-peptide measurements of patients in the Thrombosis group were ≤1 ng/ml in 40% of cases and nearly 80% of patients had at least once a C-peptide measurement ≤1 ng/ml. Finally, four patients in the Thrombosis group had a C-peptide >1 ng/ml before transplantation, and all of them had a C-peptide <1 ng/ml at allograft failure.

We then analysed outcomes of patients depending on C-peptide value on post-transplantation day one. Among patients with lowest values of C-peptide on day one (10% of patients with lowest C-peptide from the cohort), nearly half of them evolved towards allograft failure during the first month. Also, among the 10% of patients with highest C-peptide values at day one, 10% evolved towards allograft failure. Patients with intermediate C-peptide value on day one (between 3.5 and 26.5 ng/ml) were those with lower incidence of allograft failure (5%). Figure 4 represents these results.

Low C-peptide at one-month post-transplantation is associated with long-term functionality

From the 340 patients in the Success Group, 18 presented allograft failure between one-month and one-year post-transplantation. Causes of failure were varied, and none was related to thrombosis. Comparing C-peptide at one-month post-transplantation between patients with allograft survival superior to one year, we observed lower C-peptide in patients with allograft survival superior to one year (respectively, 4.7 ng/ml vs. 6.9 ng/ml, $P = 0.01$).

Ability of glycaemia, C-peptide, C-peptide/glycaemia and aCP to predict allograft failure in the next 24 h

The occurrence of allograft failure in the next 24 h was assessed using AUC of ROC curves (Table 3 and Fig. 5). AUC for glycaemia was 0.63 (95% IC = [0.45; 0.80]). Considering hyperglycaemia threshold of ≥9 mmol/l (determined by Youden index), we obtained a sensitivity of 47.1% and a specificity of 82.5%. C-peptide had an AUC of 0.84 (95% IC = [0.72; 0.97]), with a sensitivity of 76.5% and a specificity of 82.9% determined by the Youden index for a threshold <2.5 ng/ml. C-peptide/Glycaemia had an AUC of 0.85 (95% IC = [0.72; 0.97]), with a sensitivity of 76.5% and a specificity of

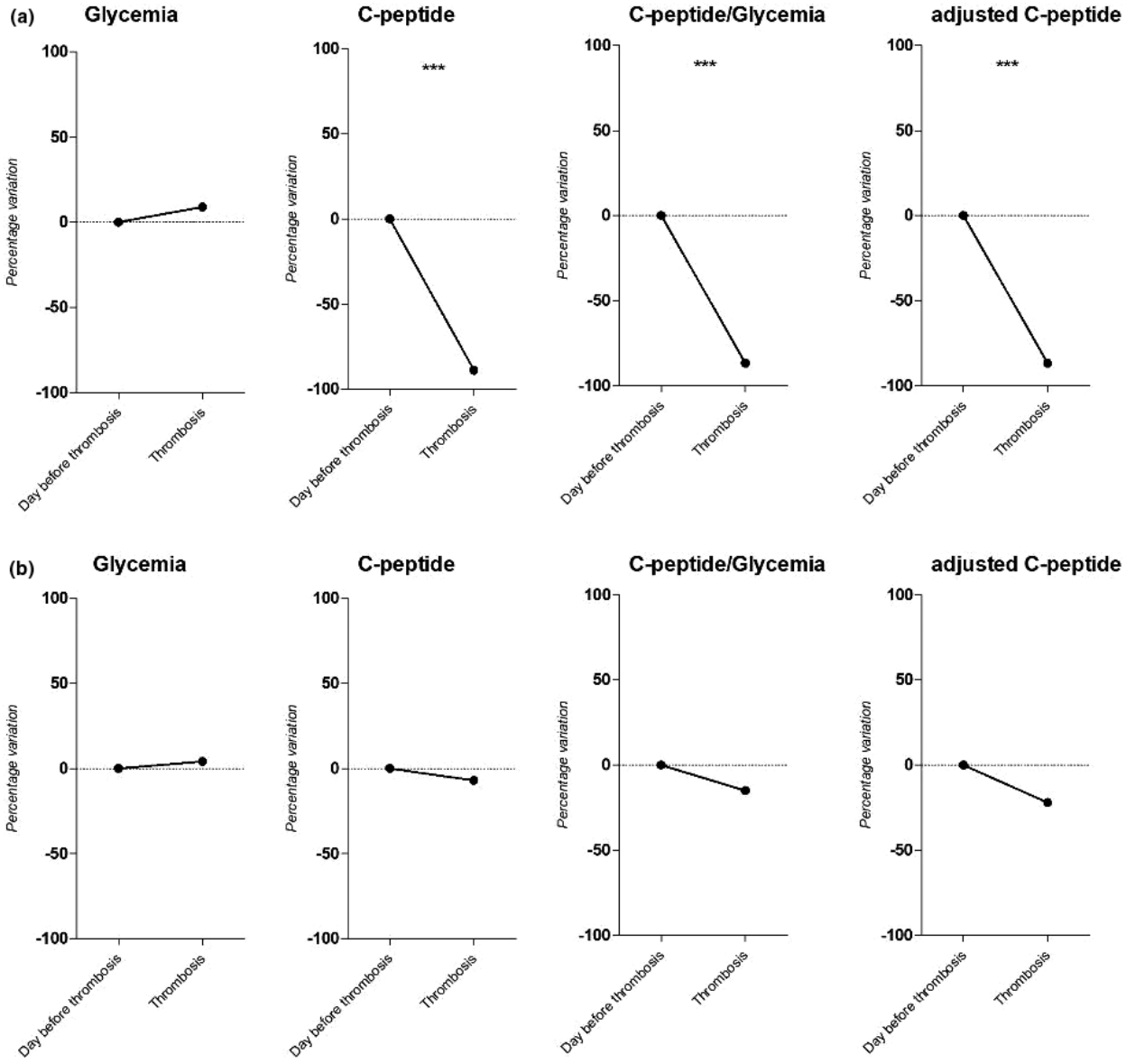

Figure 3 Variation of glycaemia, C-peptide, C-peptide/glycaemia and adjusted C-peptide between the day before thrombosis and the day of thrombosis. Results are expressed in median percentage of variation for patients with complete (a) and partial (b) allograft thrombosis. **P* value < 0.05, ***P* value < 0.01, ****P* value < 0.001.

77.7% determined by the Youden index for a threshold <0.5. Finally, AUC for aCP was 0.91 (95% IC = [0.81; 1.00]). The threshold of aCP < 16 determined by the Youden index corresponded to a sensitivity of 88.2% and a specificity of 90.2%. Comparing AUC values of the ROC curves, aCP, C-peptide/glycaemia ratio and C-peptide were highly significantly better than glycaemia ($P = 0.0014$, $P = 0.0200$ and $P = 0.0341$, respectively).

The ability of biological value kinetic variation to predict occurrence of allograft failure in the next 24 h was assessed using AUC of ROC curves (Table 4 and Fig. 6). Considering the kinetic of glycaemia, we obtained an AUC of 0.55 (95% IC = [0.38; 0.73]), with a variation threshold of −4% (estimated by the Youden index) corresponding to a sensitivity of 62.5% and a specificity of 54.6%. C-peptide kinetic of variation had an AUC of 0.85 (95% IC = [0.73; 0.96]). The variation threshold was −71%, with a corresponding sensitivity and specificity of 64.7% and 97.5%, respectively. C-peptide/glycaemia kinetic of variation had an AUC of 0.78 (95% IC = [0.66; 0.91]). The variation threshold was −90%, with a corresponding sensitivity and specificity of 50.0% and 99.6%. Finally, the aCP kinetic of variation had an AUC of 0.79 (95% IC = [0.66; 0.93]). The

Table 2. Variation of biological values according to complete or partial thrombosis.

	D-1	D0	Median variation (%)	*P*
Complete thrombosis				
Glycaemia (mmol/l)				
Mean	9.8	11.7	+9	0.1772
SD	3.9	7.2		
n	22	31		
C-peptide (ng/ml)				
Mean	5.3	1.0	−88	<0.0001
SD	5.7	1.6		
n	25	31		
C-Pep/Gly				
Mean	0.64	0.08	−86	<0.0001
SD	0.72	0.11		
n	26	29		
aCP				
Mean	27.4	2.9	−86	<0.0001
SD	40.9	4.4		
n	23	29		
Partial thrombosis				
Glycaemia (mmol/l)				
Mean	6.3	7.4	+4	0.3601
SD	1.5	3.0		
n	31	34		
C-peptide (ng/ml)				
Mean	6.8	7.3	−7	0.9450
SD	3.6	4.7		
n	30	36		
C-pep/Gly				
Mean	1.12	1.09	−15	0.5439
SD	0.57	0.60		
n	28	33		
aCP				
Mean	74.4	65.1	−22	0.2897
SD	46.1	42.8		
n	28	33		

D0, day of thrombosis; D-1, day before thrombosis; SD, standard derivation.

variation threshold of –36% corresponded to a sensitivity of 62.5% and a specificity of 88.25%. Comparing the different AUC values, there was no difference between aCP and C-peptide ($P = 0.4548$), and a trend towards better AUC for aCP compared to glycaemia (= 0.0791). The C-peptide AUC was significantly better than the glycaemia AUC ($P = 0.0075$).

Discussion

The main finding of the present study was that a significant drop of plasma C-peptide and adjusted C-peptide values predicted all cases of complete thrombosis of the pancreas graft. This dramatic drop was absent in case of functional pancreas, and no variation was observed with strict monitoring of blood glucose.

Pancreas thrombosis is still considered the Achilles' heel of pancreas transplantation and remains the leading cause of early graft failure. It accounts for 29% of grafts lost within the first 6 months after transplantation [4,5]. The pancreas' inherently low microvascular flow state makes it vulnerable to vascular complications, as does the hypercoagulable blood of diabetic patients. Ultimately, the phenomenon is most definitely multifactorial. Despite universal use of all type of anticoagulation strategies, its high prevalence did not change over time indicating the lack of full knowledge of its physiopathology. Tools for the diagnosis of thrombosis are only restricted to radiological examinations [5]. The measurement of plasma C-peptide is part of our routine assessment after pancreas transplantation and has always been so. Indeed, C-peptide is secreted by the transplanted pancreas depending on blood glucose level, which can be influenced in postoperative days by multiple factors (insulin perfusion, steroids pulse, calcineurin inhibitors etc.). We questioned whether monitoring of daily C-

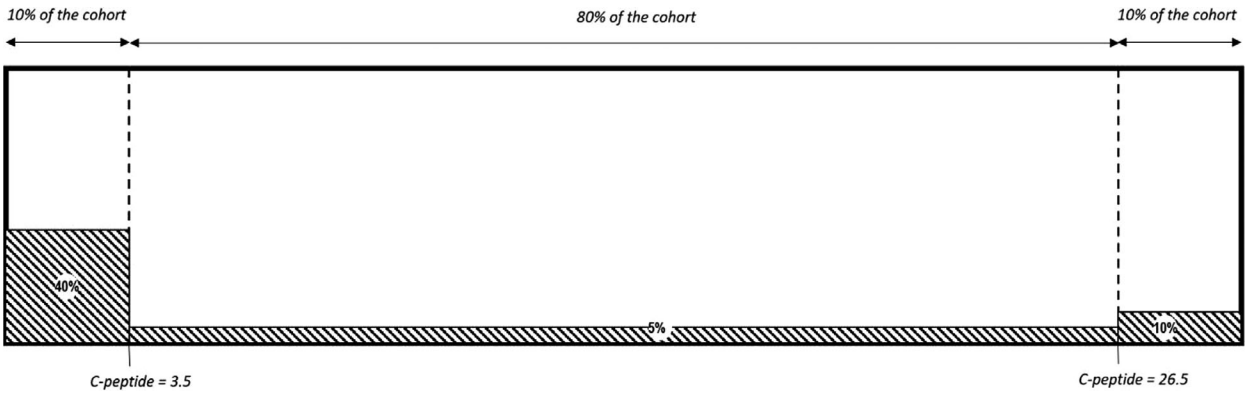

Figure 4 Percentage of early failure due to thrombosis according to C-peptide value on the first postoperative day.

Table 3. Ability of glycaemia, C-peptide, C-peptide/glycaemia and aCP to predict allograft failure in the next 24 h.

	AUC	Sensitivity	Specificity
Glycaemia < 9 mmol/l	0.63 (IC 95% = 0.45; 0.80)	47.1% (IC 95% = 22.9%; 72.2%)	82.5% (IC 95% = 80.5%; 83.9%)
C-peptide < 2.5 ng/ml	0.84 (IC 95% = 0.72; 0.97)	76.5% (IC 95% = 50.1%; 93.2%)	82.9% (IC 95% = 81.3%; 84.4%)
C-pep/Glyc < 0.5	0.85 (IC 95% = 0.72; 0.97)	76.5% (IC 95% = 50.1%; 93.1%)	77.7% (IC 95% = 75.7%; 79.6%)
aCP < 16	0.91 (IC 95% = 0.81; 1.00)	88.2% (IC 95% = 63.6%; 98.5%)	90.2% (IC 95% = 88.6%; 91.6%)

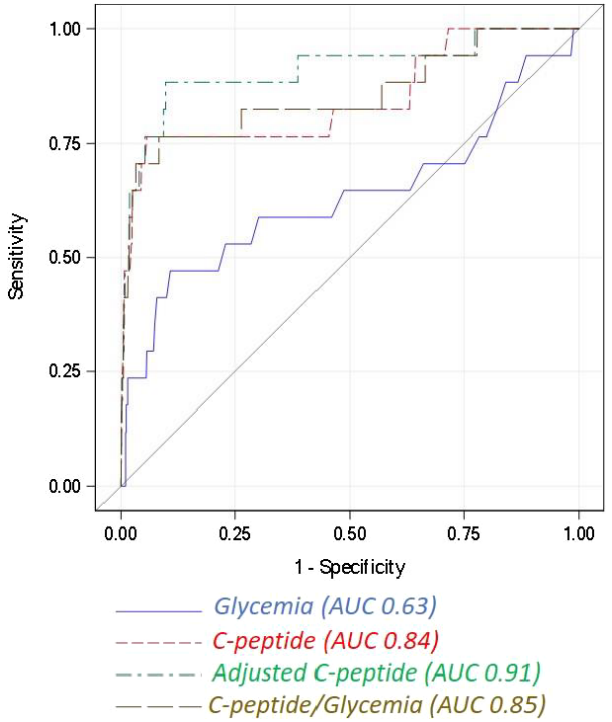

Figure 5 ROC curves representing ability of glycaemia, C-peptide, C-peptide/glycaemia and aCP to predict occurrence of allograft failure in the next 24 h and the corresponding AUC.

peptide secretion could have clinical relevance in the diagnosis of early graft failure due to thrombosis. Recent recommendations for pancreas transplantation monitoring follow-up did not include C-peptide measurement [19]. However, plasma C-peptide assay is the main criteria searched after an islet transplant [20]. Faradji *et al.* showed that after islet transplantation, the use of C-peptide/glucose ratio adapted to creatininaemia was also predictive of a high glucose rate at 90 min following a mixed meal tolerance test and thus could indicate islet dysfunction [21]. The discrepancy between organ and islet may lie in the fact that insulin independence is immediately obtain after pancreas transplantation whereas it requires weeks or months after islet transplantation [22,23].

In order to avoid average values variations in the group of patients with early failure, we evaluated biological values from the day of failure and retrospectively back. Indeed, median percentage of variation in C-peptide and aCP values was statistically significative (about 90%) whereas it was not the case for glycaemia. This can be the consequence of using corticosteroids, calcineurin inhibitors, parenteral nutrition and insulin infusion, which can all influence blood glucose but not C-peptide. Systematic insulin perfusion after pancreas transplantation is part of our transplant protocol as we believe that this approach helps the organ to overcome the surgical stress and insulin resistance induced by parenteral nutrition and immunosuppressive drugs during the first days after transplantation (i.e. resting pancreas). In fact, early insulin perfusion has been proven to protect long-term beta cell functionality in native pancreas [24,25], but also to reduce post-transplant diabetes after kidney transplantation [26].

Table 4. Ability of kinetic biological values variations to predict allograft failure in the next 24 h.

	AUC	Sensitivity	Specificity
Glycaemia variation <–4%	0.55 (IC 95% = 0.38; 0.73)	62.5% (IC 95% = 35.4%; 84.8%)	54.6% (IC 95% = 51.7%, 57.4%)
C-peptide variation <–71%	0.85 (IC 95% = 0.73; 0.96)	64.7% (IC 95% = 38.3%; 85.8%)	97.5% (IC 95% = 96.6%; 98.2%)
C-pep/Glyc variation <–90%	0.85 (IC 95% = 0.73; 0.96)	50.0% (IC 95% = 24.6%; 75.3%)	99.6% (IC 95% = 99.0%; 99.9%)
aCP variation <–36%	0.79 (IC 95% = 0.66; 0.93)	62.5% (IC 95% = 35.4%; 84.8%)	88.25% (IC 95% = 86.1%; 90.1%)

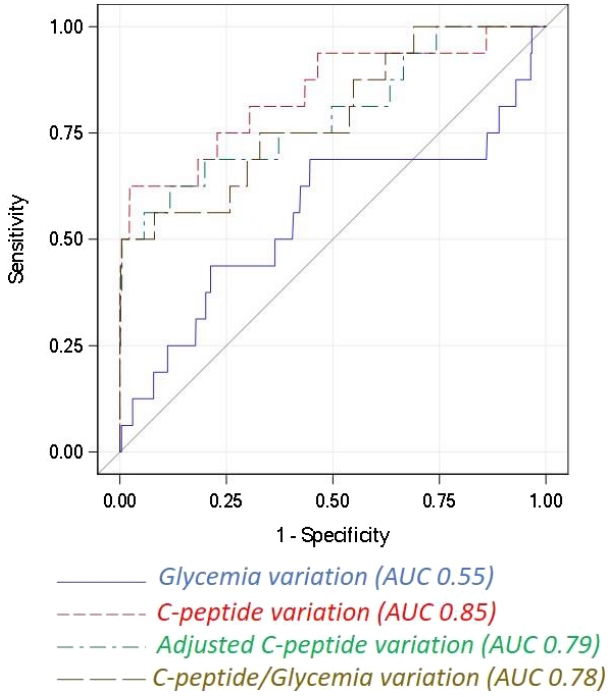

Figure 6 ROC curves representing the ability of the kinetic variation of glycaemia, C-peptide, C-peptide/glycaemia and aCP to predict occurrence of allograft failure in the next 24 h and the corresponding AUC.

To guide clinical practice of C-peptide and aCP monitoring, we determined that C-peptide <2.5 mg/l and aCP < 16 seemed to correlate with thrombosis within the next 24 h. Similarly, C-peptide decrease of 70% and aCP decrease of 36% seemed to be linked to acute thrombosis. We suggested that CT scan could be performed in these cases in order to diagnose early thrombosis. These thresholds are determined based on our retrospective cohort and need prospective evaluation to confirm their truly predictive values.

For patients with partial thrombosis (mostly diagnosed by routine CT scan before discharge), aCP varied about 20%, suggesting a moderate allograft function impairment. Blood glucose again did not change in these cases. Of interest, aCP was also useful in four patients with pretransplant positive C-peptide and early graft loss. We think that, due to postoperative stress and diabetogenic medications, the weak and residual secretion of C-peptide by the native pancreas may be abolished after surgery [27,28]. Moreover, the recovery of renal function by a simultaneous kidney graft shall impact C-peptide's clearance. Therefore, C-peptide secretion can be also monitored even in patients with residual C-peptide secretion from their native pancreas. Thus, data on aCP monitoring always corresponded to pancreas allograft function and not to native pancreas one.

Our result on the very early measurement of C-peptide following pancreas transplantation differs from that reported by Niederhaus *et al.* [19] who found that C-peptide remained elevated in cases of allograft failure. This difference is certainly due to the heterogeneous definition of pancreas failure as well as the timing of failure occurrence. Early allograft failure mainly results from acute thrombosis, whereas late failure may be related to CNI toxicity, rejection, diabetes recurrence, low vascular flow and many others. These chronic events are not always responsible for complete endocrine function exhaustion [8,29,30]. We also confirmed our early clinical observations that patients with very high C-peptide levels from the very beginning had more thrombotic events than those with intermediate values. Additional possible contributors to elevated C-peptide levels in the early post-transplant period included marked insulin resistance due to high-dose glucocorticoid use (varying by recipient differences in adiposity) and postoperative and postinduction inflammation. Unfortunately, we did not monitor inflammatory markers to confirm this hypothesis, but it could be a subject for a further study.

Our data suggest that C-peptide can be used as an additive and simple marker in clinical practice in order to diagnose 'pre-emptive' pancreas thrombosis. Currently, medical imaging including ultrasonography, CT scan and MRI are the only way to diagnose and confirm thrombosis. These radiological procedures cannot be used repeatedly and have limitations. Doppler ultrasound results can be uncertain, especially in the postoperative period and depend on physician expertise. CT scan or MRI needs contrast injection which can damage kidney allograft function. Thus, variations of C-peptide levels can represent an alert to prescribe medical imaging and detect potential thrombosis. Nowadays, timing of CT scan after pancreas transplantation remains variable [31]. We therefore suggest that multi-daily C-peptide monitoring during postoperative days could guide timing of CT scan. We can hypothesize that performing CT scan in the early phase of C-peptide decrease can allow to identify partial thrombosis prior to overt thrombosis and potentially lead to allograft salvage using anticoagulation or even thrombotic removal by radio-interventional procedure [32]. Obviously, prospective evaluation is needed to confirm this hypothesis and validate our observation.

Our study suffered from the relatively small number of patients with allograft failure (10%) rendering weaker

the statistical power. On the other hand, the distinctive and powerful feature of our cohort was the daily measurement of C-peptide, glycaemia and kidney function in all our patients. This joint monitoring provided new scientific data on the clinical utility of C-peptide after pancreatic transplantation.

In conclusion, prospective daily plasma C-peptide monitoring from the day of pancreas transplantation seemed to be a simple and reproducible tool to guide medical imaging to allow a precocious diagnosis of a thrombotic event and discuss possible rescue intervention.

Authorship

DC: elaborated design and research project, supervised analysis and helped in writing the manuscript and critically revising it. CM: collected the data, helped in the study analysis and wrote the manuscript. All authors participated in writing and revising the manuscript.

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Conflicts of interest

The authors have declared no conflicts of interest.

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Ethical approval

This study was performed using data extracted from the French DIVAT cohort (www.divat.fr, approved by the CNIL, n°914184). The quality of the DIVAT data bank is validated by an annual cross-centre audit and has been reviewed by the appropriate ethics committee in accordance with the ethical standards laid down in the Declaration of Helsinki 2000 as well as the Declaration of Istanbul 2008. All participants gave informed consent, and data were de-identified before analysis in order to respect confidentiality.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Indications for CT-scan in patients with complete and partial thrombosis.

Table S2. Glycemia (mmol/l) kinetic following pancreas transplantation (SD, standard deviation).

Table S3. C-peptide (ng/ml) kinetic following pancreas transplantation (SD, standard deviation)

Table S4. Kinetic of adjusted C-peptide following pancreas transplantation; SD, standard deviation.

Table S5. Kinetic of C-peptide/glycemia following pancreas transplantation; SD, standard deviation.

Figure S1. Patient survival: period 2000–2016.

Figure S2. Pancreas allograft: period 2000–2016.

Figure S3. Patient and pancreas allograft survival: period 2000–2016.

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