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This study was conducted in accordance with the revised Declaration of Helsinki. All persons implicated in the research provided informed consent.

Abstract

Background

After heart transplantation, calcineurin inhibitors (CNI) (cyclosporin A and tacrolimus) are key immunosuppressive drugs to prevent graft rejection. Whole-blood concentration (C_{blood})-guided therapeutic drug monitoring (TDM) is systematically performed to improve graft outcomes. However, some patients will still experience graft rejection and/or adverse events despite CNI C_{blood} within the therapeutic range. Other pharmacokinetic parameters, such as the intra-graft, or intracellular concentration at the CNI site of action could refine their TDM. Nonetheless, these remain to be explored. The objective of the INTRACAR study was to describe the relationship between whole blood, intra-graft, and intracellular CNI concentrations as well as their efficacy in heart transplant recipients (HTR).

Methods

In a cohort of HTR, protocol endomyocardial biopsies (EMB) were collected to assess rejection by anatomopathological analysis. Part of the EMB was used to measure the intra-graft concentrations of CNI (C_{EMB}). C_{blood}, and the concentration inside peripheral blood mononuclear cells (C_{PBMC}), a cellular fraction enriched with lymphocytes, were also monitored. Concentrations in the three matrices were compared between patients with and without biopsy-proven acute rejection (BPAR).

Results

Thirty-four HTR were included, representing nearly 100 pharmacokinetic (PK) samples for each CNI. C_{blood}, C_{EMB} and C_{PBMC} correlated for both CNI. BPAR was observed in 74 biopsies (39.6 %) from 26 patients (76.5 %), all except one of low-grade. None of the PK parameters (C_{blood}, C_{EMB}, C_{PBMC}, $C_{EMB/blood}$ and $C_{PBMC/blood}$) was associated with BPAR.

Conclusion

In this cohort of well-immunosuppressed patients, no association was observed for any of the PK parameters including C_{blood}, with the occurrence of BPAR. However, a trend was noticed for the C_{EMB} and $C_{EMB/blood}$ of cyclosporin A. Further studies in higher-risk patients may help optimize the use of C_{EMB} and C_{PBMC} for CNI TDM in HTR.

Abstract length: 1989 characters

Keywords

Heart transplantation; cyclosporin A; tacrolimus; therapeutic drug monitoring; alternative matrix; personalized medicine.

1 Introduction

The only effective long-term treatment for end-stage cardiac failure is heart transplantation. [1] This procedure yields fairly positive results despite surgical, pharmacological, and immunological issues. The heart is obtained from a brain-deceased donor, who is genetically different from the recipient. Thus, a normal immunological reaction, from the recipient's immune system against the graft, is expected in heart transplant recipients (HTR). This phenomenon may lead to heart lesions and contribute to the relapse of heart failure and/or coronary lesions. [2] To prevent this potentially life-threatening reaction, immunosuppressive drugs (ISD) are systematically administered to HTR. The usual treatment is composed of an induction (e.g., rabbit anti-thymocyte globulin (rATG)) and triple maintenance immunosuppressive treatment: an anti-metabolite (e.g., mycophenolate mofetil (MMF)), a corticosteroid (CS) (e.g., prednisone), and a backbone calcineurin inhibitor (CNI). [3] CNIs, tacrolimus (TAC) and cyclosporin A (CsA), are the keystone immunosuppressive drugs after heart transplantation. Despite their remarkable efficacy, [4] these drugs can cause problematic adverse drug reactions (ADR), the most deleterious being nephrotoxicity. [5] Additionally, CNIs display a narrow therapeutic index and large inter-individual pharmacokinetic (PK) variability, making these difficult to use. Therefore, therapeutic drug monitoring (TDM) is necessary by measuring pre-dose whole blood drug concentrations (C_{blood}) in HTR to adjust the CNI drug dosage. [3]

CNI whole-blood TDM has improved the outcomes of heart transplantation. [3] However, the drug remains less effective or causes ADR in some patients despite adequate C_{blood}. [6] This finding can be explained by two factors. First, as the site of the rejection is the graft itself, the concentrations measured within the graft, that is, the intra-tissular concentration, might be more directly related to treatment efficacy. [7] A closer relationship between intra-tissular concentrations and graft rejection has been evidenced in previous studies in liver transplantation. [8,9]

Secondly, the calcineurin enzyme is located inside T lymphocytes. As a limited correlation exists between whole blood and intra-lymphocyte concentrations, [6,10,11] measuring the latter could be more

informative than the first, as shown for other transplanted organs. [12,13-16] In practice, the T lymphocyte concentration is approached by the peripheral blood mononuclear cells (PBMC) concentration. PBMCs are a blood cellular fraction enriched with lymphocytes and monocytes.

Therefore, the objective of the INTRACAR study was to describe the relationship between whole blood, intra-tissular (graft), and PBMC CNIs concentrations, and efficacy in HTR.

2 Materials and Methods

2.1 Consumables and reactants

CsA and ascomycin were obtained from Sigma-Aldrich (St. Louis, MO, US). Acetonitrile (ACN) was obtained from Carlo Erba (Val-de-Reuil, France), $ZnSO_4$ from Merck (Darmstadt, Germany), and NH_4SO_4 from EMSURE (Batch, Germany). The human granulocyte depletion cocktail, Lymphoprep™, and SepMate™ tubes were purchased from Stemcell Technologies (Grenoble, France), and phosphate-buffered saline from Thermo Fisher Scientific (Life Technologies Corporation, Waltham, MA, USA). Mass spectrometry grade water from Thermo Fisher Scientific (Waltham, MA, US) was used for all experiments.

2.2 Study design

INTRACAR was a prospective, observational, single-center study. This study was conducted in accordance with the revised version of the Declaration of Helsinki, and the ethics committee of Rennes University Hospital approved the study protocol (Decision 19.148).

2.3 Inclusion and non-inclusion criteria

All CsA- or TAC-treated HTR at our institution within the first two years after the transplantation were included. Non-inclusion criteria were CNI contraindications, multi-organ transplantations, liberty deprivation, and pregnancy or breastfeeding for women. All patients provided informed consent in accordance with French law on medical research.

2.4 Study procedures

As INTRACAR was an observational study, neither study-dedicated visit nor examination was planned. HTR follow-up is planned at our center according to a predefined protocol. After hospital discharge, patients are seen every other week until 4 months post-procedure, then monthly during the first year, and every 3 months thereafter. During each visit, patients undergo routine monitoring, including the determination of CNI C_{blood}. The leftover of the ethylenediaminetetraacetic acid-coated blood tube was used to isolate the PBMCs according to a previously published method. [17] During these routine visits, patients also undergo an endomyocardial biopsy (EMB) to detect a possible histological rejection. The histopathological parameters were graded routinely by trained medical pathologists and according to the International Society for Heart and Lung Transplantation (ISHLT) criteria. [18] A part of the EMB was immediately snap-frozen in a liquid nitrogen tank and stored at the Centre de Ressources Biologiques (CRB) Santé of Rennes BB-0033-00056. EMB were then conserved at – 80°C in a dedicated tissue library.

2.5 PBMC isolation and CNI extraction

The concentration of TAC or CsA inside the PBMC (C_{PBMC}) of the patients was determined using a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method, validated and published by our team, and previously described according to international guidelines. [15,19] Briefly, PBMC were isolated from whole blood using a ficoll gradient method. After centrifugation at 1200 × g for 15 min, the upper layer was discarded, mononuclear cells were collected, and cell count was performed using a previously published method. [20] PBMC pellets of 1 million cells were cryopreserved at − 80°C in 1 mL of methanol until the assay. On the day of analysis, the samples were ultrasonicated for 10 min, and methanol was evaporated under azote flux. Six hundred µL of ACN/$ZnSO_4$ in LC-MS/MS-grade water (0.05 M) (1:1 v/v) and then 100 µL of NH_4SO_4 in LC-MS/MS-grade water (40:100 w/v) (after 5 min agitation) were added. After another round of agitation on a

vortex, samples were centrifuged at 1700 × g, 10°C for 10 min, and 5 µL of the supernatant was injected into the LC-MS/MS apparatus.

2.6 Intra-EMB CNI extraction

Using the leftover EMB, the CNI concentration inside the graft was determined by adapting for TAC, a validated method for CsA. [21] After gentle thawing, EMB were crushed in LC-MS/MS grade water using a Polytron PT-MR 2100, 230 V (Kinematica, Lucerne, Switzerland). The tissue concentration was adjusted to 1 mg of tissue per mL of water. Homogenized EMB (200 µL) in LC-MS/MS-grade water was vortexed for 1 min with 75 µL ACN, 400 µL $ZnSO_4$ in LC-MS/MS-grade water (0.05 M), and 160 µL NH_4SO_4 in LC-MS/MS-grade water (40:100, w/v). The samples were then centrifuged at 3000 × g for 10 min at 4 °C. Finally, 5 µL of the supernatant was injected into the LC-MS/MS system.

2.7 LC-MS/MS conditions

We adapted and cross-validated the methods to different LC-MS/MS apparatuses: a Finnigan™ TSQ® Quantum Discovery Max, an UltiMate 3000 UPLC TSQ Quantis (ThermoFisher, San Jose, CA, USA), and an Acquity H class Xevo TQXS (Waters, Milford, MA, USA). The samples were injected onto a C18 Hypersil™ Gold column (30 x 2,1 mm, 3 µm) maintained at 60°C and fitted with a guard column (10 x 2.1 mm, 3 µm) (Thermo Electron, Dreieich, Germany). The mobile phase consisted of 5 % ammonium acetate in ACN with an isocratic elution mode. The total runtime was 2.5 minutes. Manufacturers-provided softwares were used for data acquisition and analysis. C_{PBMC} was corrected to cell counting and adjusted to one million cells, and C_{BEM} was corrected using biopsy weight in mg.

2.8 Endpoints

The primary endpoint was C_{EMB} according to the occurrence of a biopsy-proven acute rejection (BPAR). The secondary endpoints were the C_{blood}, the C_{PBMC}, the $C_{EMB/blood}$ and the $C_{PBMC/blood}$ according to the occurrence of BPAR and the correlations between the different PK parameters. In this study, any rejection, regardless of the severity, was interpreted as a BPAR.

2.9 Statistical analysis

In the text, qualitative results are presented as classes and percentages, and quantitative results are presented as medians [interquartile ranges]. In the figures displaying the qualitative/quantitative data, the horizontal lines represent the median and interquartile range. The coefficients of variation (CV) were calculated as the standard deviation divided by the mean, multiplied by 100, and expressed as percentages. Differences between C_{EBM}, C_{PBMC} and C_{blood} in patients with and without graft rejection were analyzed using the Mann–Whitney U test. Relationships between the different PK parameters were analyzed using non-parametric Spearman's correlation. Non-parametric tests were used because of the non-normal distribution of all the data. Prism 5.0 (GraphPad, San Diego, CA, USA) was used to plot the graphs. A *p*-value inferior to 0.05 was considered significant.

3. Results

3.1 Baseline characteristics

Patients were included between March 2020 and October 2021. Two firstly included patients were finally excluded because of a lack of at least one time point with an analyzable C_{blood}, C_{PBMC} and C_{BEM}. In total, 34 patients were analyzable, representing 191 time points (median number of visits: 5; interquartile range: [3;8]; range: [1;14]).

The patients were mostly men (73.5 %) with a median age of 54.5 years. Fourteen patients (41 %) underwent transplantation for ischemic cardiomyopathy. All patients underwent preoperative rATG induction. At the time of inclusion, 14 patients (41.2 %) were TAC-treated, 20 (58.8 %) were CsA-treated, 33 (97.1 %) received MMF, 32 (94.1 %) received CS, and 8 (23.5 %) received everolimus. Detailed information regarding baseline characteristics is shown in Table 1.

3.2 Pharmacokinetics

For CsA, 94 time points from 20 patients were analyzed. There were 97 time points from 22 patients for TAC (including the eight patients who switched from CsA to TAC throughout the study). The CsA-treated patients had C_{blood} of 153.50 [118.00;190.75] ng/mL (n = 94), C_{EMB} of 411.70 [278.49;724.14] pg/mg of tissue (n = 68), and C_{PBMC} of 721.34 [550.36;968.60] pg/10^6 cells (n = 92). TAC-treated patients had concentrations of 7.10 [5.80;8.30] ng/mL (n = 97), 21.65 [18.30;27.55] pg/mg of tissue (n = 85), and 35.40 [26.20;46.76] pg/10^6 cells (n = 94) for C_{blood}, C_{EMB} and C_{PBMC}, respectively. Anatomopathological analysis was prioritized in this observational study in case of insufficient material. Therefore, missing data is explained by the small size of the EMB fragments. As displayed in Fig. 1, all PK parameters (C_{blood}, C_{EMB} and C_{PBMC}) correlated for both of the CNI while being quite loosely, except for the correlation between CsA C_{blood} and C_{PBMC} (all $p < 0.05$).

With respect to the fact that the CPBMC is expressed as a quantity per million cell, for CsA, $C_{EMB/blood}$ was 2.7 [2.0;4.2] and $C_{PBMC/blood}$ was 5.1 [3.8;6.2]; the respective values for TAC were 3.4 [2.3;3.9] and 4.6 [3.5;6.5]. Detailed information regarding the PK parameters can be found in Table 2.

3.3 Pharmacodynamics

One hundred and eighty-seven biopsies were analyzed. Among these samples, 74 (39.6 %) from 26 patients (76.5 %) displayed a rejection. All rejection episodes were mild, except for one case of moderate rejection in a CsA-treated patient.

Sixteen out of the 20 CsA-treated patients at baseline (80.0 %) experienced at least one rejection episode, including 15 while on CsA. Throughout the study, eight of the 20 CsA-treated patients (40.0 %) were converted to TAC, and none of the TAC-treated patients were converted to CsA. All 8 CsA-to-TAC converted patients experienced at least one rejection episode (one only after the conversion, one only before, and 6 on both CNI). Ten out of the 14 TAC-treated patients at baseline (71.4 %) experienced at least one rejection episode as well.

As shown in Fig. 2 and 3, no PK-PD relationship was observed in this study. For both CNI, neither C_{blood}, C_{EMB} nor C_{PBMC}, as well as $C_{EMB/blood}$ or $C_{PBMC/blood}$, were associated with BPAR (all p = N.S.). However, a tendency was observed with a numerical difference for overall CsA C_{EMB} (385.6 [232.1;525.5] with and 420.2 [290.0;1074.0] without BPAR; p = 0.2044; Fig. 2B) and C_{EMB} controlled with C_{blood} ($C_{EMB/blood}$ was 2.3 [2.0;3.2] with BPAR and 3.1 [1.9;5.7] without BPAR; p = 0.1496; Fig. 3A).

3.4. Longitudinal follow-up

Some patients underwent repeated concentration measurements during the study period. Data was plotted for every individual patient for whom at least three consecutive visits occurred while on a single CNI (n = 15 for CsA; n = 18 for TAC). Fig. S1 and S2 in the supplementary digital content show the measured C_{blood}, C_{EMB} and C_{PBMC} of these patients throughout the study. None of the patients died during the study.

4. Discussion

To the best of our knowledge, this is the first study aiming at describing the concentration-effect relationship of graft CNI concentrations in HTR. The large dataset for heart transplantation represented nearly one hundred time points for each CNI. However, despite the large amount of data collected, we did not evidence a relationship between C_{EMB} and graft rejection. This conclusion regarding the primary endpoint was supported by the visual analysis of the longitudinal follow-up. Nevertheless, our C_{EMB} parameter was non-inferior to the standard-of-care, *i.e.,* C_{blood}.

In this study, C_{EMB} was within the range previously observed during the method development. [21] Data presented in this article support the use of the linearity range for the chromatographic method, which ranges from 25 to 15000 pg/mg of tissue for CsA, and from 5 to 3000 pg/mg of tissue for TAC. Although the results of the dosages were low, especially for TAC, all the points fell within the validated linearity range. Thus, our method is adapted to large-scale studies of CNI C_{EMB} measurements.

Regarding intra-graft CNI concentrations, other studies have used kidney biopsies. Two of these analytical methods were developed using rat kidney tissue and were applied to human samples for CsA [22] and TAC. [23] Other methods have been developed for TAC using human kidney samples, [24,25] with clinical application, [26] or associated with liver biopsies. [27] Studies using liver biopsies are scarce, but observations have been reported for both CNI. [8,9,12,23,28] For heart biopsies, Robertsen and colleagues reported C_{EMB} values of CsA ranging from 216 to 833 pg/mg of heart tissue (n = 19 biopsies from 7 patients), with no data on rejection. [29] These values were consistent with the findings of the present study. Recently, a chromatographic method for quantifying TAC in EMB was published. [30] This method employs enzymatic digestion to homogenize heart tissue, while we used mechanical disruption. The authors reported 5 samples from 2 patients, with TAC C_{EMB} exceeding those observed in our study. The first patient had C_{EMB} between 15 and 45 pg/mg of tissue, and the second between 55 and 95 pg/mg of tissue. This discrepancy could be explained by higher C_{blood} observed by Molinaro et al.. The values indeed were higher than in this study, ranging between 10 and 15 ng/mL for both patients, whereas only 10.3 % of TAC C_{blood} in our study were above 10 ng/mL. Consequently, $C_{EMB/blood}$ appeared comparable between the two studies on visual analysis. Both studies met the EMA guidelines for validation of bioanalytical assays. [31]

The whole blood concentrations were within the therapeutic range (153.50 [118.00;190.75] ng/mL for CsA and 7.10 [5.80;8.30] ng/ml for TAC), as expected from stable patients at treatment steady-state. Regarding variability, the CV of C_{blood} was modest (< 40 %) for both CNI. However, the variability of the C_{PBMC} was higher than that of C_{blood} (CV of 63.5 % for CsA and 65.2 % for TAC), and similar to another study. In that study of our team, the CV was 56.7 % in 30 stable kidney transplant patients and 69.4 % in 30 stable liver transplant recipients treated with TAC (author's personal data). In contrast, TAC median $C_{PBMC/blood}$ was 2.2 (kidney) and 2.0 (liver) in that study, whereas in the current study, median $C_{PBMC/blood}$ was 5.1 and 4.6 for CsA and TAC, respectively. This suggests a more than two-fold greater CNI accumulation in PBMCs of HTR than kidney or liver recipients, if we take into account that the lymphocyte size is not different between these patients. This discrepancy was

not due to the C_{blood} level, as HTR levels were between those of liver (C_{blood} = 5.5 [4.0;6.9] ng/mL) and kidney (C_{blood} = 8.8 [7.1;10.0] ng/mL) transplant recipients. Post-graft delay was also not the cause considering the values obtained in this study, compared to the liver and kidney transplant recipient data (2.3 [2.3;2.5] months for liver transplant recipients and 9.4 [3.1;13.3] months for kidney transplant recipients).

In our large sample of nearly 200 protocol-scheduled EMB for rejection diagnosis, we found almost 4 out of 10 biopsies with an objective rejection, in more than 3 quarters of our patients. All of these BPAR cases were mild, which was considered as "subclinical" and required no systematic treatment according to the clinicians in charge of the patients, except one. Moreover, none of our patients experienced graft failure (death of cardiac etiology or re-transplantation), and the overall survival was 100 %. The current cohort was composed of well immunologically-controlled patients, and the triple CNI/MMF/CS immunosuppression is currently the most effective treatment for acute rejection prevention in HTR. Therefore, the present study may have been under-scaled to reveal a difference on a PK basis. The situation where the greater deviation from the null hypothesis was observed, was for CsA C_{EMB} and even more $C_{EMB/blood}$. In the one hand, TAC is considered as a better option than CsA for the prevention of acute rejection in other transplanted organs. [3,32,33] In the other hand, 40.0 % of the CsA-treated patients of this study were converted to TAC, without any TAC patient being converted to CsA. It can thus be hypothesized that TAC is more effective than CsA in preventing BPAR in HTR. Considering this, and the numerical difference observed for CsA C_{EMB} regarding BPAR, this PK parameter could be of great informative value for graft preservation. Prospective studies aimed at providing sufficient exposure based on CsA C_{EMB} TDM, especially in high-risk patients, could be informative regarding HTR outcomes. This study supports the use of this the C_{EMB} PK parameter. The feasibility of the implementation of our LC-MS/MS method has also been proven.

5. Conclusion

Having previously developed a LC-MS/MS method to monitor CNI C_{EMB} in HTR, we included 34 patients, representing nearly 200 time points, to describe the PK-PD relationships. Having found a correlation between C_{blood}, C_{EMB} and C_{PBMC} for both CNI, our study, however, did not evidence a difference in the PK parameters regarding BPAR. However, the role of CsA C_{EMB} could be further evaluated as a biomarker in high-risk HTR.

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Figures legends:

Fig. 1: Correlation between the different PK parameters. C_{blood} versus C_{EMB} (A and D), C_{blood} versus C_{PBMC} (B and E) and C_{EMB} versus C_{PBMC} (C and F) are shown for CsA (A to C, rounds) and TAC (D to F, squares), in HTR. C_{EMB}: endomyocardial biopsy calcineurin inhibitor concentration; C_{blood}: whole blood pre-dose calcineurin inhibitor concentration; C_{PBMC}: peripheral blood mononuclear cells calcineurin inhibitor concentration; CsA: cyclosporin A; HTR: heart transplant recipients; TAC: tacrolimus.

Fig. 2: Concentration-effects relationships of CNIs in HTR. Rejectors versus non-rejectors' C_{blood} (A and D), C_{EMB} (B and E) and C_{PBMC} (C and F) are shown for CsA (A to C) and TAC (D to F). BPAR: biopsy-proven acute rejection; C_{EMB}: endomyocardial biopsy calcineurin inhibitor concentration; C_{blood}: whole blood pre-dose calcineurin inhibitor concentration; CNI: calcineurin inhibitor; C_{PBMC}: peripheral blood mononuclear cells calcineurin inhibitor concentration; CsA: cyclosporin A; HTR: heart transplant recipients; TAC: tacrolimus.

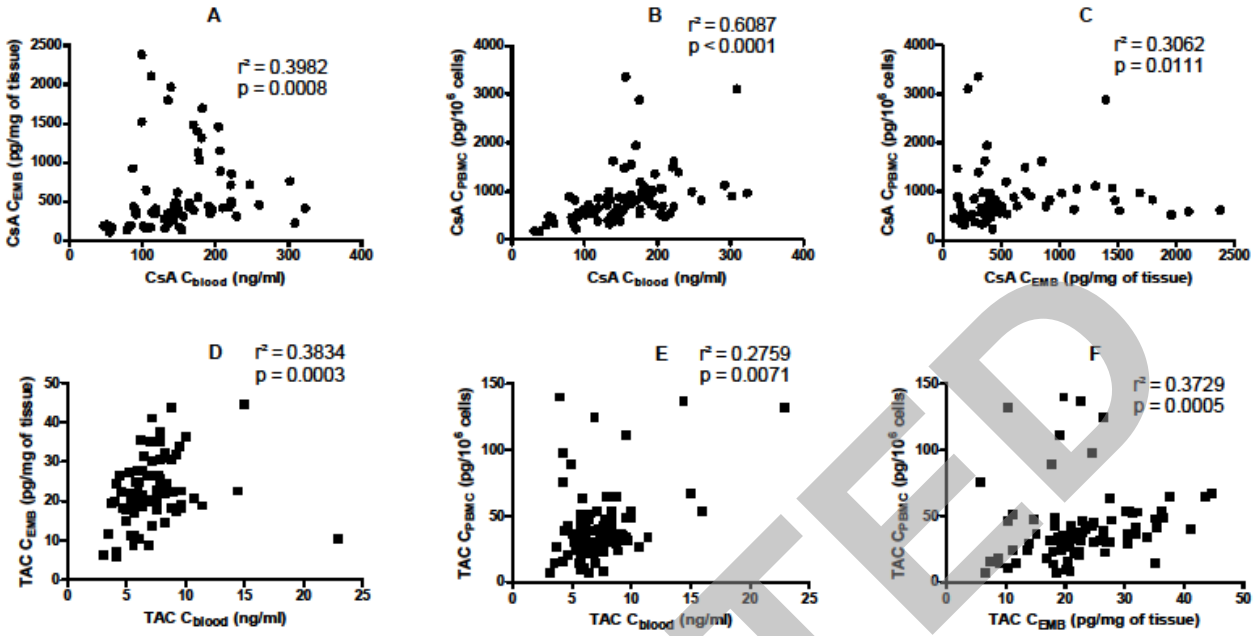
Fig. 3: Concentration-effects relationships of CNIs in HTR. Rejectors versus non-rejectors' $C_{EMB/blood}$ (A and C) and $C_{PBMC/blood}$ (B and D) are shown for CsA (A and B) and TAC (C and D). BPAR: biopsy-proven acute rejection; $C_{EMB/blood}$: endomyocardial biopsy over whole blood calcineurin inhibitor concentration ration; CNI: calcineurin inhibitor; $C_{PBMC/blood}$: peripheral blood mononuclear cells over whole blood calcineurin inhibitor concentration ratio; CsA: cyclosporin A; HTR: heart transplant recipients; TAC: tacrolimus.

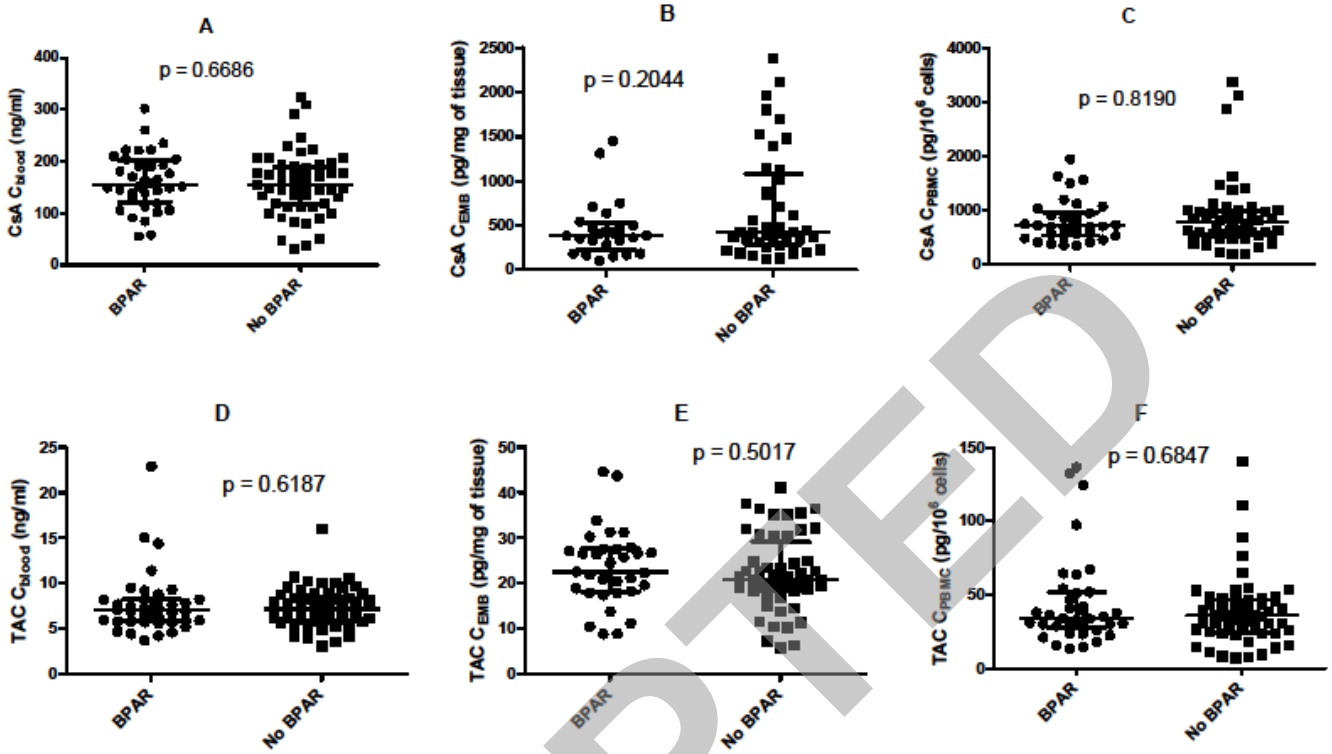
Age (years) (median [interquartile range])	54.5 [48.0;65.5]
Male gender (%)	74
Weight (kg)	70.4 ± 15.2
Transplantation indication (%)	
Ischemic cardiomyopathy	41
Dilated cardiomypathy	21
Genetic cardiomyopathy	18
Valvular disease	3
Other	17
Cold ischemia time (minutes)	211.3 ± 60.9
rATG induction (%)	100
Time since transplantation (months) (median [interquartile range])	5.3 [2.5;21.4]
Medication (%)	
Tacrolimus use	41
Cyclosporin A use	59
Mycophenolate use	97
Corticosteroid use	93
Everolimus use	24
Glomerular filtration rate (ml/min/1.73m²)	65.1 ± 26.6

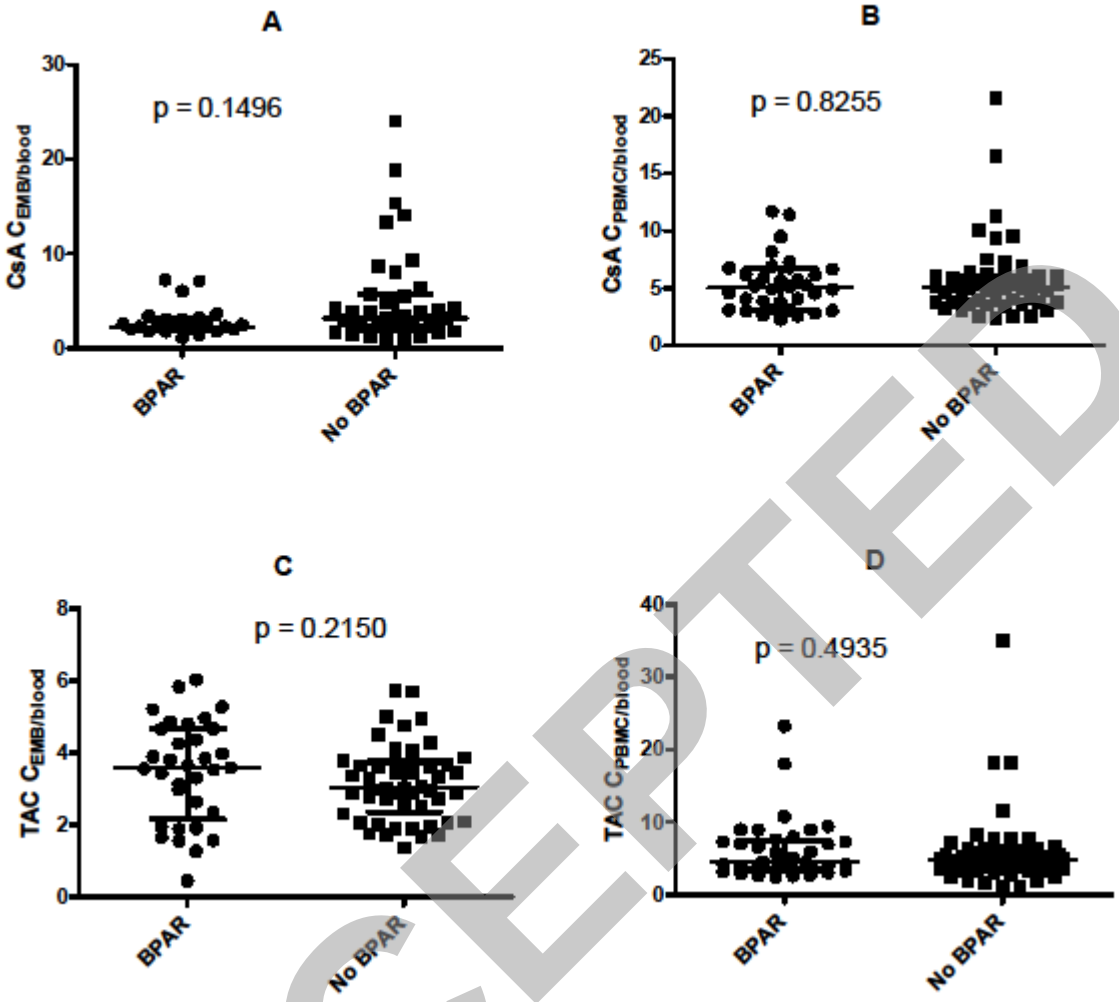
Table 1: Baseline characteristics of the cohort. rATG: rabbit anti-thymocyte globuline.

	CsA			TAC		
	C_{blood} (ng/mL)	C_{EMB} (pg/mg of tissue	C_{PBMC} (pg/10^6 cells)	C_{blood} (ng/mL)	C_{EMB} (pg/mg of tissue	C_{PBMC} (pg/10^6 cells)
n =	94	68	92	97	85	94
Median	153.50	411.70	721.34	7.10	21.65	35.40
Interquartile range	[118.00;190.75]	[278.49;724.14]	[550.36;968.60]	[5.80;8.30]	[18.30;27.55]	[26.20;46.76]
Mean ± SD	157.4 ± 58.0	612.0 ± 529.5	848.2 ± 538.7	7.5 ± 2.7	22.7 ± 8.4	40.8 ± 26.6
CV (%)	36.8	86.5	63.5	36.0	37.0	65.2
$C_{EMB/blood}$ (median [interquartile range])	2.7 [2.0;4.2]			3.4 [2.3;3.9]		
$C_{PBMC/blood}$ (median [interquartile range])	5.1 [3.8;6.2]			4.6 [3.5;6.5]		

Table 2: PK parameters of the cohort. C_{EMB}: endomyocardial biopsy concentration; $C_{EMB/blood}$: endomyocardial biopsy over blood concentration ratio; C_{blood}: whole blood pre-dose concentration; C_{PBMC}: peripheral blood mononuclear cells concentration; $C_{PBMC/blood}$: peripheral blood mononuclear cells over blood concentration ratio; CsA: cyclosporin A; CV: coefficient of variation; SD: standard deviation; TAC: tacrolimus.


A
r² = 0.3982
p = 0.0008
CsA C_EMB (pg/mg of tissue)
CsA C_blood (ng/ml)
B
r² = 0.6087
p < 0.0001
CsA C_PBMC (pg/10⁶ cells)
CsA C_blood (ng/ml)
C
r² = 0.3062
p = 0.0111
CsA C_PBMC (pg/10⁶ cells)
CsA C_EMB (pg/mg of tissue)
D
r² = 0.3834
p = 0.0003
TAC C_EMB (pg/mg of tissue)
TAC C_blood (ng/ml)
E
r² = 0.2759
p = 0.0071
TAC C_PBMC (pg/10⁶ cells)
TAC C_blood (ng/ml)
F
r² = 0.3729
p = 0.0005
TAC C_PBMC (pg/10⁶ cells)
TAC C_EMB (pg/mg of tissue)


A
p = 0.6686
CsA C$_{blood}$ (ng/ml)
400
300
200
100
0
BPAR
No BPAR
B
p = 0.2044
CsA C$_{EMB}$ (pg/mg of tissue)
2500
2000
1500
1000
500
0
BPAR
No BPAR
C
p = 0.8190
CsA C$_{PBMC}$ (pg/10^6 cells)
4000
3000
2000
1000
0
BPAR
No BPAR
D
p = 0.6187
TAC C$_{blood}$ (ng/ml)
25
20
15
10
5
0
BPAR
No BPAR
E
p = 0.5017
TAC C$_{EMB}$ (pg/mg of tissue)
50
40
30
20
10
0
BPAR
No BPAR
F
p = 0.6847
TAC C$_{PBMC}$ (pg/10^6 cells)
150
100
50
0
BPAR
No BPAR


A
p = 0.1496
CsA C EMB/blood
30
20
10
0
BPAR
No BPAR
B
p = 0.8255
CsA C PBMC/blood
25
20
15
10
5
0
BPAR
No BPAR
C
p = 0.2150
TAC C EMB/blood
8
6
4
2
0
BPAR
No BPAR
D
p = 0.4935
TAC C PBMC/blood
40
30
20
10
0
BPAR
No BPAR