Comparison of HTK-Custodiol and St. Thomas Solution as Cardiac Preservation Solutions on Early and Midterm Outcomes Following Heart Transplantation

Filip Dulguerov[a], Tamila Abdurashidowa[b], Emeline Christophel-Plathier [c], Lucian Ion[a], Ziyad Gunga [a], Valentina Rancati[b], Patrick Yerly[b], Piergiorgio Tozzi[a], Adelin Albert[d], Zied Ltaief[e], Samuel Rotman[f], Philippe Meyer[g], Karl Lefol[h], Roger Hullin[b], and Matthias Kirsch[a]

[a]Department of Cardiac Surgery, Lausanne University Hospital (CHUV), Lausanne, Switzerland
[b]Department of Cardiology, Lausanne University Hospital (CHUV), Lausanne, Switzerland.
[c]Department of Anesthesiology, Lausanne University Hospital (CHUV), Lausanne, Switzerland
[d]Biostatistics and Research Methods (B-STAT), University Hospital of Liège, Liège, Belgium.
[e]Department of Intensive Care, Lausanne University Hospital (CHUV), Lausanne, Switzerland
[f]Institute of Pathology, Lausanne University Hospital (CHUV), Lausanne, Switzerland
[g]Cardiology, Department of Medical Specialties, University Hospitals of Geneva (HUG), Geneva, Switzerland
[h]Department of Cardiology, Organ Transplant Centre, Lausanne University Hospital (CHUV), Lausanne, Switzerland.

Word Count 5481 (Key words, disclosure, abstract, abbreviations, main text, figure legends, tables and references, excluding title page)

Key words: heart transplantation, cardiac preservation solution, inotropic score, acute cellular rejection, all-cause mortality

Disclosure: none

Corresponding author:
Filip Dulguerov M.D.
Cardiac Surgery
Department of Cardiovascular Medicine
University Hospital of Lausanne
Rue du Bugnon 46, 1011 Lausanne, Switzerland
E-mail: filip.dulguerov@chuv.ch
Phone: 0041 79 833 20 79

Abbreviations

ATP-Adenosine Triphosphate

CF-MCS-Continuous-flow Mechanical Circulatory Support

CI-Confidence Interval

CPB -Cardiopulmonary Bypass

CPS-Cardiac Preservation Solution

ECC -Extracorporeal circulation

ECMO-Extracorporeal Membrane Oxygenation

HR-Hazard Ratio

HTx-Heart Transplantation

ISHLT-International Society of Heart and Lungs Transplant

KM-Kaplan-Meier

LR-Logistic Regression

NO-Nitric Oxid

OR-Odds Ratio

OLR-Ordinal Logistic Regression

ROS-Reactive Oxygen Species

VAD-Ventricular Assist Device

VIS-Vasoactive Inotropic Score

Abstract

Objectives

The choice of the cardiac preservation solution for myocardial protection at time of heart procurement remains controversial and uncertainties persist regarding its effect on the early and midterm heart transplantation outcomes. We retrospectively compared our adult heart transplantations performed with two different solutions, in terms of hospital mortality, mid-term survival, inotropic score, primary graft dysfunction and rejection score.

Methods

From January 2009 to December 2020, 154 consecutive heart transplantations of adult patients, followed up in pre- and post-transplantation by two different tertiary centers, were performed at the University Hospital of Lausanne, Switzerland. From 2009 to 2015, the cardiac preservation solution used was exclusively St-Thomas, whereafter an institutional decision was made to use HTK-Custodiol only. Patients were classified in two groups accordingly.

Results

There were 75 patients in the St-Thomas group and 79 patients in the HTK-Custodiol group. The two groups were comparable in terms of preoperative and intraoperative characteristics. Postoperatively, compared to St-Thomas group, the Custodiol group patients showed significantly lower inotropic scores [median (interquartile range): 35.7 (17.5-60.2) vs. 71.8 (31.8-127), p<0.001], rejection scores [0.08 (0.0-0.25) vs. 0.14 (0.05-0.5), p= 0.036] and 30-day mortality rate (2.5% vs. 14.7%, p=0.007) even after adjusting for potential confounders. Microscopic analysis of the endomyocardial biopsies also showed less specific histological

features of subendothelial ischemia (3.8% vs. 17.3%, p=0.006). There was no difference in primary graft dysfunction requiring postoperative extracorporeal membrane oxygenation. The use of HTK-Custodiol solution significantly improved midterm survival (Custodiol vs St-Thomas: HR=0.20, 95%CI: 0.069 -0.60, p=0.004).

Conclusion

This retrospective study comparing St-Thomas solution and HTK-Custodiol as myocardial protection during heart procurement showed that Custodiol improves outcomes after heart transplantation, including postoperative inotropic score, rejection score, 30-day mortality and midterm survival.

Introduction

Over the last decades, Heart Transplantation (HTx) has become the gold standard of care for well-selected end-stage heart disease patients[1]. Nowadays, HTx still remains the treatment of choice despite the increasing number of Continuous-Flow Mechanical Circulatory Support (CF-MCS) devices and their favorable results in different clinical settings[1].

Successful organ preservation is a key element of transplantation since its goal is to maintain the viability of the organ until its implantation into the recipient. Two issues are important in this process: the type of preservation solution used to obtain the diastolic cardiac arrest and the duration of the cold ischemic storage. The duration of the latter should be limited to 4-6 hours, and it is well known that longer preservation data alter outcomes[2], although ischemic times as long as 13 hours have been reported[3]. In that perspective, the 2017 registry of the International Society for Heart and Lung Transplantation (ISHLT) reported that allograft ischemic time between 2 and 4 hours is associated with considerably higher survival and better early outcomes than allograft ischemic time of more than 4 hours[4]. More than one hundred preservation solutions[2] have been developed and applied worldwide, but there is no consensus on the choice to use Cardiac Preservation Solution (CPS), and uncertainties persist regarding its effect on early and mid-term HTx outcomes, including a potential survival benefit.

The goal of this work was to report our two-center (University Hospital of Lausanne and University Hospitals of Geneva, Switzerland) experience of HTx over a period of 12 years with two different CPS (St-Thomas and HTK-Custodiol). Based on unchanged patient profiles in the cohort of HTx recipients, we investigated the impact of these two CPS on hospital mortality (30-day mortality) and mid-term mortality, inotropic score, primary graft dysfunction requiring Extracorporeal Membrane Oxygenation (ECMO) and one-year post transplant rejection score.

Methods

From January 2009 to December 2020, 165 consecutive HTx for end stage heart failures from all etiologies were performed in our institution (Lausanne University Hospital, Switzerland). The patients were followed-up pre- and post-operatively by two different tertiary centers, respectively Lausanne University Hospital and Geneva University Hospitals, Switzerland. After excluding patients under 18 years of age, the study population included 154 adult patients. From 2009 to 2015, the cardiac preservation solution used was exclusively St-Thomas, whereafter the institution made a decisive switch to HTK-Custodiol only. Thus, patients were classified in two groups according to the solution used, St-Thomas or HTK-Custodiol.

The study was approved by the Ethic Committee of the Lausanne University Hospital (Switzerland) in March 2018 (CER-VD2019-704) after a thorough scrutiny of the study protocol as well as an analysis of a sample of patients from the study population. We requested and obtained a written informed consent for all patients.

Operative strategy

During organ procurement, CPS administration varied according to the type of CPS used. HTK-Custodiol was perfused at the dose of 30 ml/kg (of donor body weight) to achieve a total infusion time of 7 minutes. St-Thomas was administered at the dose of 20ml/kg (of donor body weight). In both groups, topical cooling with ice-slush was also employed during harvest and transport. If allograft ischemic time exceeded 150 min, 500 ml of CPS were re-administered upon graft arrival in the operating room (St-Thomas or HTK-Custodiol depending on the first solution administered).

Clinical evaluation and follow-up data

Patients' demographic and clinical data recorded prior to surgery by the physician in charge were retrieved from electronic patient records without alteration. Operative, in-hospital postoperative and follow-up data were collected by the intensive care team and the heart failure cardiologists in charge of the patient from the time of the surgery. Primary outcomes of interest were hospital vasoactive inotropic score (VIS), rejection score, primary graft dysfunction requiring ECMO, and 30-day mortality. Overall mid-term survival was considered as secondary outcome.

The ISHLT histological rejection score [5] was obtained by endomyocardial biopsies every week for the first month, every 2 weeks for the next 6 weeks, monthly biopsies for 3 to 4 months, and every 3 months until the end of the first year. The rejection score was calculated as the average of the scores obtained from the first five endomyocardial biopsies. The VIS score was calculated according to Gaies et al. [6] formula, as a predictor of poor outcomes after cardiac surgery (death, cardiac arrest, need for mechanical circulatory support, renal replacement therapy and/or neurological injury) [6]. Hourly doses of all vasoactive medications were recorded and the maximum level of each medication through the first 48h carefully noted. The first three post-transplantation endomyocardial biopsies were analyzed in search of histological features of subendothelial ischemia to evidence potential ischemia reperfusion injuries. The cardiac allograft vasculopathy was also scrutinized one year after the surgery.

Statistical methods

Results were expressed as mean and standard deviation (SD) for quantitative variables or median and interquartile range (IQR) for non-normally distributed variables. Frequency tables (numbers and percent) were used for summarizing categorical data. Normality of distributions was assessed by the Shapiro-Wilk test. A log-transform was used to normalize non-normal distributions (VIS, waiting time). For quantitative variables, groups (St-Thomas vs. HTK-

Custodiol) were compared by the Kruskal-Wallis test while the chi-squared test for qualitative variables. Multiple linear regression was used to assess the relationship between a quantitative outcome and several covariates. For a binary (ordinal) outcome, (ordinal) logistic regression was applied to the data. Results were expressed as regression coefficient or odds ratio (OR) with 95% confidence interval (95%CI). The E-value was estimated to measure the effect of potential hidden biases on the association between the exposure (CPS) and outcome (30-day mortality). A high E-value suggests that uncontrolled confounders have to be strongly related to exposure and outcome to completely explain the association. The Kaplan-Meier (KM) method was used to estimate survival functions. The relationship between a survival outcome variable and covariates was assessed by Cox regression analysis. Results were then expressed by the hazard ratio (HR) and its 95%CI. Statistical calculations were always done on the maximum number of data available. Missing values were neither replaced nor imputed. Results were considered significant at the 5% critical level ($p<0.05$). All calculations were done with SAS version 3.4 (SAS Institute, Cary, NC, USA) and R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

Of the 154 adult patients who underwent HTx for end stage heart failure from all etiologies, 75 (48.7%) received St-Thomas and 79 (52.3 %) HTK-Custodiol as CPS. The overall percentage of missing data was 8.3%, respectively 6.3% (St-Thomas) and 10.1% (Custodiol). The mean number (range) of missing values per patient in St-Thomas group 1.3 (0-5) was significantly lower than in the Custodiol group 2.4 (0-7). However, for most variables, data were either complete or only barely missing in each group. Baseline patient (recipient and donor)

characteristics are displayed in **Table 1.** Recipients did not differ by age, etiology of the heart failure, presence of a VAD preoperatively, mean ejection fraction, CPB time, gender-, height- or weight-mismatch, previous cardiac surgery, and previous biventricular failure. By contrast, there were more women in the Custodiol group than in the St-Thomas group (29.2% vs. 13.3%, p=0.017) and the ischemic time was shorter (172 ± 45.5 vs. 144 ± 40.2 min, p<0.001). As for donors, they were perfectly comparable with respect to cause of death (p=0.39) and gender (p=0.75), but were slightly older in Custodiol group than in St-Thomas group (43.5 ±14.9 vs. 49.2 ± 14.4 years, p=0.038).

Outcomes

As seen in **Table 2**, the two groups differed for inotropic score [median (IQR): 71.8 (31.8-127) vs. 35.7 (17.5-60.2), p<0.001] **(Figure 1),** rejection score [0.14 (0.05-0.25) vs. 0.08 (0.0-0.25), p=0.036] **(Figure 2)**, and for 30-day mortality rate (14.7% vs. 2.5%, p=0.0068). The groups were similar for primary graft dysfunction requiring postoperative ECMO, immediately at the end of the surgery or within the first 24 hours (16.0% vs. 16.5%, p=0.94). The microscopic analysis of the first 3 endomyocardial biopsies revealed specific histological features of subendothelial ischemia in 13 (17.3%) patients of the St-Thomas group and 3 (3.8%) in the Custodiol group (p=0.006). One year after HTx, there was no significant difference between groups regarding the cardiac allograft vasculopathy.

CPS and inotropic score

Linear regression of log-transformed inotropic scores on CPS confirmed that scores were lower for HTK-Custodiol compared to St-Thomas solution (regression coefficient: -0.69, 95%CI: -1.0 to -0.38, p<0.001). The significant relationship between inotropic score and CPS remained unchanged after adjusting for any of the patient characteristics, even for ischemic time and CPB

time both positively associated with the inotropic score (data not shown). Multiple linear regression confirmed that, when combined with ischemic time (0.0052, 95%CI: 0.0011 – 0.0093, p=0.017) and log-transformed CPB time (0.71, 95%CI: 0.16-1.3, p=0.015), the preservation solution remained significantly related to the inotropic score (-0.60, 95%CI: -0.99 to -0.21, p=0.003) (**Table 3**).

CPS and rejection score

The overall distribution of the rejection score could not be normalized, therefore the 131 patients with a rejection score were classified into three categories as follows: 41 (31.3%) had a score equal to 0, 49 (37.4%) had a score between 0 and 0.2, and 41 (31.3%) has a rejection score > 0.2. Ordinal logistic regression confirmed that the rejection score was significantly impacted by CPS in favor of Custodiol (OR=0.41, 95%CI 0.24-0.86, p=0.016). No patient-related characteristics was associated with the rejection score, except renal glomerular function (N=109 patients; OR=0.979, 95%CI 0.961-0.999, p=0.036). The effect of CPS on the rejection score remained significant after adjusting for any of the patient-related characteristics but only a tendency remained for renal glomerular function (p=0.098) (data not shown).

CPS and 30-day mortality

Overall, 13 (8.4%) died within 30 days in the patient series, significantly more in the St-Thomas group than in the Custodiol group as mentioned above (OR=6.62, 95%CI: 1.41–30.9, p=0.016). None of the other recipient-related preoperative or intraoperative characteristics was related to 30-day mortality rate (**Table 4**). CPS remained associated with 30-day mortality after adjusting for any of these covariates. The E-value to assess the potential effect of non-controlled confounders was 12.7 (lower limit 2.18) confirming the strong association of CPS and 30-day mortality. Of note, however, the association between CPS and 30-day mortality vanished

(p=0.86) when inserting the outcome variable log (VIS) in the logistic regression, emphasizing the strong relationship between CPS and VIS.

CPS and midterm survival

The follow-up for HTK-Custodiol patients was necessarily shorter than for St-Thomas patients (3.1±1.5 vs. 7.0±3.9 years). Globally, 26 (16.9%) patients died, 21 in the St-Thomas group and 4 in the Custodiol group. The Kaplan-Meier survival functions of both groups (**Figure 3)** differed significantly (log-rank test, p=0.001). Cox regression analysis applied to each patient-related characteristic showed than CPS was the only significant factor affecting overall survival (HTK-Custodiol vs. St-Thomas: HR=0.20, 95%CI 0.069 - 0.60, p=0.004) (**Table 5**). The impact of CPS on midterm survival remained unchanged after adjusting for any of the other patient-related factors.

Discussion

More than 50 years after the first human HTx by Christian Barnaard, HTx remains the preferred surgical option for selected patients with end stage heart disease. The fact that the number of patients on waiting list and the duration of their HTx candidacy are continuously growing in Europe and the USA is an indirect sign of this trend[1,7,8].

Despite major recent progresses in the field of HTx, organ preservation remains imperfect and still impacts patients' survival and outcomes [3]. The ex vivo period is the vulnerable stage during which the organ can undergo cellular damage that is further compounded by reperfusion injury after the implantation. The goal during procurement and preservation is to minimize these injuries and maintain the viability of the organ until its implantation in the recipient. Rapid diastolic cardiac arrest and subsequent cold ischemic storage (at 4°C) are the two cornerstones

of the cardiac procurement technique. Diastolic cardiac arrest preserves adenosine triphosphate (ATP) levels by comparison to ischemic myocardial contracture[9], and cooling down the organ to 4°C results in a 10-to-12-fold decrease in metabolic demand. However, the persistence of a level of metabolism at 5-10% of normal values explains why cooling alone does not prevent all cellular damages [10]. During cold ischemic storage, the only source of energy for the graft is anaerobic glycolysis, but the enzymes involved in this process are inhibited by the acidosis resulting from the ischemia. Therefore, it is necessary to use a CPS containing buffers to maintain the cellular pH stable and allow a minimal ATP production[11].

CPSs are classified as intracellular or extracellular according to their concentration in sodium and potassium. Intracellular CPSs contain high potassium and low sodium and tend to be like the intracellular milieu. As a result, they limit the movement of ions and water across the cell membrane. Extracellular CPSs contain low potassium and were initially developed to prevent hyperkalemia related to the infusion of intracellular CPSs[12]. However, this classification remains rather artificial and subjective given that each CPS is best defined by its own ionic concentration and mostly by the residual osmotic space for the addition of other substances. These other substances could reduce intra- and extra-cellular edema, limit intracellular acidosis, reduce Reactive Oxygen Species (ROS) generation, and increase ATP production. All these factors tend to decrease the myocardial injury and thus improve the outcomes after HTx[13].

HTK-Custodiol is a hyperpolarizing solution with low sodium concentration that allows a large osmotic space as well as the addition of numerous other highly concentrated substances[14]. Among these substances, there is a high concentration of histidine/histidine hydrochloride intracellular buffering system, which enhances buffering capacity during ischemic induced acidosis; amino-acid tryptophan alpha ketoglutarate, which protects cell membrane as a substrate for anaerobic metabolism; and mannitol, which is an osmotic agent that helps reducing cellular and tissue edema. It is also an excellent scavenger of ROS[13, 15]. HTK-Custodiol has

also been shown to maintain high levels of intra-cellular ATP after reperfusion and this is known to be directly correlated with low output syndrome, which usually develops a few hours after surgery and is the result of myocardial edema during the ischemic phase. The latter decreases coronary blood flow and thus intra-cellular ATP levels[14].

St-Thomas solution is an extracellular solution, which provides a rapid diastolic cardiac arrest by high potassium and magnesium concentration as well as by the membrane's stabilizing effect of procaine hydrochloride. Cellular edema is reduced by the extracellular sodium concentration, procaine, and a variable concentration of bicarbonates[16]. The increase in extracellular potassium concentration causes a progressive depolarization of the membrane potential for each level of potassium concentration. Solutions with a high concentration of potassium, such as St-Thomas, are however known to cause toxicity to the vascular endothelium. Carpentier was the first to demonstrate reduced viability and function of endothelial cells after exposure to high potassium concentration[17, 18]. The endothelium is however important as it locally regulates coronary perfusion and cardiac function through the secretion of Nitric Oxid (NO) and vasoactive peptides. Therefore, after administration of a high potassium concentration solution, endothelial dysfunction occurs, which could lead to myocardial dysfunction[19].

Regarding the buffering system, St-Thomas solution contains only extracellular buffers, which are less effective than the intracellular buffers used in HTK-Custodiol and other CPS in preventing intracellular edema[20, 21]. Although St-Thomas is beneficial and still widely used in non-transplant cardiac surgery, our study, like others[13, 20], demonstrates that using St-Thomas solution leads to worse immediate outcomes after HTx, which likely explains its overall decreasing use. Concerning current trends in CPS use, most European centers moved from St-Thomas solution to HTK-Custodiol after 2010, and in the United States, in the past years, nearly half of the grafts were stored in the University of Wisconsin solution, one-fourth in Celsior and one-fourth in HTK-Custodiol[13].

Another salient element arising from our study is the difference in rejection score in favor of the Custodiol group, which to our knowledge, has not been described before. This could be interpreted as a reflection of improvement of the overall HTx patient care[21], given that the same trend has been observed in several other European countries during the last decades and seems to be related to the improvement of the immunosuppression monitoring[21, 22]. However, over the whole duration of our study, no changes in the immunosuppression protocol or its monitoring occurred. We therefore suggested that the integrity of the endothelial cells of the graft could be compromised by the different preservation and storage techniques and in particular by the type of CPS used. Indeed, it is now well known that endothelial cell damage leads to increased capillary permeability, cellular and tissue edema, vasospasm and microvascular hypoperfusion[23, 24]. As endothelial cell function is directly correlated to cardiomyocyte function, all these elements can lead to primary graft dysfunction [25, 26]. Moreover, different studies confirm that preservation related injuries in heart transplantation can be the cause of early complications but also of late events such as graft rejection and chronic transplant arteriopathy[27, 28].

To confirm our hypothesis, we reviewed the anatomopathological reports of the first 3 endomyocardial biopsies for each patient, in both groups. This time, we were interested not only in the overall rejection score but also in the microscopic analysis when it showed typical lesions of ischemia reperfusion phenomena. Specifically, the lesions found are infiltrates of mononuclear cells and granulocytes located in the endothelial layer and associated with interstitial oedema. These lesions are specifically different from rejection lesions and are interpreted as typical of ischemia-reperfusion phenomena by our pathologists.

Interestingly, we found that there were significantly more of these specific histological features in the St-Thomas group than in the Custodiol group.

Several factors can explain these endothelial lesions during the graft harvesting and storage process. At first, the duration of ischemia can directly affect the viability of endothelial cells through different pathways. These include reduced protein synthesis and ATP levels[28], increased anaerobic metabolism, and both intracellular and extracellular acidosis[25]. Under these conditions, the endothelium releases large quantities of proinflammatory chemoattractant cytokines (IL 1α, IL 8) and the availability of antioxidants is reduced[26]. All these elements lead to potassium efflux with membrane depolarization, cellular swelling, alteration of the endothelial barrier, and tissue edema. This in turn leads to abnormalities in the distribution of CPS but also in blood flow at reperfusion, which aggravates the phenomenon[13].

Secondly, reperfusion is accompanied by a real burst of ROS which occurs only 15 seconds after the onset of the reperfusion[15]. This increases the endothelial lesions and the previously mentioned inflammatory reaction. Usually 2-3 hours after reperfusion, activated neutrophils adhere to the endothelium, release large amounts of free radicals resulting in loss of endothelial barrier function, tissue edema and a functional impairment of both endothelial cell and cardiomyocytes[16].

It is likely that the difference in outcomes obtained, especially regarding the rejection score in favor of the Custodiol group, is explained by the response of the two CPSs to various lesional factors affecting the endothelium and consequently the cardiomyocytes, during graft harvesting and preservation.

As mentioned above, the St-Thomas solution is a high concentration of potassium solution, and it has been known since the 1980s and Carpentier[17] that solutions of this type induce vasoconstriction and an impairment of the endothelial function with a decrease in NO release and other factors including prostacyclin, endothelium derived hyperpolarization factor and adenosine[13]. In addition, potassium-induced depolarization is known to promote platelet adhesion, neutrophil activation, inflammation, and ROS generation, which could explain our

results. On the contrary, HTK-Custodiol is a low concentration potassium solution that contains different substances such as histidine, ketoglutarate, tryptophan and mannitol, whose role is to counteract the deleterious effects on the endothelium and the myocardium. Those differences in chemical composition may explain our results.

It is important to note that other studies have not found results similar to ours. For example, the study by Cannata et al.[29] reported retrospectively133 heart transplantations with 3 different CPS (Custodiol vs St-Thomas vs Celsior). Custodiol was mainly used. Outcomes included intraoperative biventricular dysfunction requiring ECMO and in-hospital mortality. There was no difference between groups. In comparison, our study confirms that there is no difference in biventricular dysfunction, but our mortality differs between the groups. However, our study was designed differently, the aim being to determine the patient's postoperative condition other than only by mortality (inotropic score, biventricular dysfunction) and to see whether the advantage of Custodiol based on its chemical composition is confirmed at histological level (rejection score, ischemia-reperfusion lesions).

Another interesting study written by Karduz et al.[30] aimed at evaluating the effect of HTK-Custodiol, St-Thomas and del Nido solutions functionally and biochemically in a rat model of donor heart. Custodiol administration led to reduced myocardial contraction, decreased ATP level, increased TNF-α and increased troponin-I levels. The results of this observational study run counter to several other studies on humans[14,15,16], especially regarding the ATP levels. However, the study is well conducted, and the results are very interesting.

It is likely that in the future, further studies, especially randomized control trials, could be necessary to confirm our data.

Limitations

This retrospective longitudinal study longitudinal of HTx patients suffers from the shortcomings of all retrospective observational studies, including selection biases, reliability, quality, and completeness of data collected from patient electronic records, even though a special effort was made in this study to eliminate erroneous data entry and avoid as much as possible missing data. In this respect, the data collection was complete, and the outcome measures were confirmed in our local database as well as in the Swiss Death registry and the Swiss Cohort Study.

Data availability statement

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

Competing interests statement

The authors have declared that no competing interests exist.

Conclusion

In our regional cohort of consecutive HTx recipients in pre- and post-transplant follow-up by two different tertiary centers, we observed that the use of HTK-Custodiol as myocardial protection during heart procurement leads to improved outcomes after HTx, including postoperative inotropic score, 30-days mortality, mid-term survival, rejection score and presence of specific ischemia-reperfusion lesions.

Even though, the present study is not a head-to-head comparison, our results suggest the superiority of HTK-Custodiol over the St-Thomas solution, in the context of very few differences in the baseline patient's characteristics, an unchanged pre- and posttransplant

follow-up and an unchanged national donor heart allocation system during the study period. Further studies, especially randomized control trials, are necessary to confirm these data.

References

1. Hullin R, Meyer P, Yerly P, Kirsch M. Cardiac surgery in advanced heart failure. *J Clin Med* 2022,11,773.
2. Jahania S, Sanchez JA, Narayan P, Lasley R, Mentzer R. Heart preservation for transplantation: principles and strategies. *Ann Thorac Surg* 1999;68:1983-7.
3. Latchana N, Peck JR, Whitson B, Black SM. Preservation solutions for cardiac and pulmonary donor grafts: a review of the current literature. *Journal of Thoracic Diseases* 2014,6(8):1143-1149.
4. Hund L, Khush K, Cherikh S, Goldfarb S, Kucheryavaya Y. The registry of the international society for heart and lung transplantation: thirty-fourth adult heart transplantation report-2017; focus theme: allograft ischemic time. *J Heart Lung Transplant*,Vol36, N°10,Oct 2017.
5. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. *Circulation*.2007 Nov 6;116(19):2216-33.
6. Gaies M, Gurney J, Yen A, Napoli M, Gajarski R. Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. *Pediatric Critical Care Medecine* 2010 Vol. 11,No. 2.
7. Stehlik J, Edwards LB, Kucheryavaya AY, Aurora P, Christie JD. The Registry of the International Society for Heart and Lung Transplantation: twenty-seventh official adult heart transplant report-2010. *J Heart Lung Transplant* 2011;29:1089-103.
8. Ganesh J, Rogers C, Baner M, Bonser N, steering group of the UK cardiothoracic transplant audit. Development and validation of a model to predict perioperative

mortality following heart transplantation in the UK. *Journal Heart Lung Transplant* 2004, 23:S18-9.

9. Stringham J, Southard J, Hegge J, Triemsta L, Fields B. Limitations of heart preservation by cold storage. *Transplantation* 1998;65:551-559.
10. Belzer F, Southard J. Principles of solid-organ preservation by cold storage. *Transplantation* 1988;45:673-6.
11. Churchill T, Kneteman N. Investigation of a primary requirement of organ preservation solutions: supplemental buffering agents improve hepatic energy production during cold storage. *Transplantation* 1998;65:551-559.
12. Hart N, Leuvenink H, Ploeg R. New solutions in organ preservation. *Transplantation Reviews*2002;16:131-141.
13. Carter K, Lirette S, Baran D, Creswell L, Panos A. The effect of cardiac preservation solutions on heart transplant survival. *Journal Surg Res* Oct 2019 (242):157-165.
14. Preusse C. Custodiol cardioplegia: a single-dose hyperpolarizing solution. *The journal of Extracorporal Technology* 2016; 48:P15-P20.
15. Bretschneider H, Hübner G, Knoll D, Lohr B, Nordbeck H. Myocardial resistance and tolerance to ischemia: physiological and biochemical basis. *J Cardiovasc Surg* 1975; 16:241-260.
16. Demertzis S, Wippermann J, Schaper J, Wahlers T, Schäfers HJ. University of Wisconsin versus St-Thomas' Hospital solution for human donor heart preservation. *Ann Thorac Surg* 1993;55:1131-7.
17. Carpentier S, Murawsky M and Carpentier A. Cytotoxicity of cardioplegic solution; evaluation by tissue culture. *Circulation.* 1981;64(supplII):90-5.

18. Sellke F, Shafique T, Johnson R, Dal H, Banitt P. Blood and albumin cardioplegia preserve endothelium dependant microvascular response. *Ann Thorac Surg.* 1993;55:977-85.
19. McCrystal G, Pepe S, Esmore D and Roosvelt F. The challenge of improving donor heart preservation. *Heart Lung Circ*. 2004;13:74-83.
20. Menasché P, Fabiani N. Protection myocardique. *Encyclopédie medico-chirurgicale 2008.* Doi:10.1016/S1241-8226(08)42510-6.
21. Berry G, Angelini A, Burke M, Winters G, Kobashigawa J. The international society for heart and lung transplantation working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: Evolution and current status (2005-2011). *J Heart Lung Transplant.* 2011;30(6):601-611.
22. Hullin R. Heart transplantation: current practice and outlook to the future. *Swiss Med Wkly.* 2014 Aug 7 ;144:w13977. doi:10.4414/smw.2014.13977. PMID : 25102016.
23. Parolari A, Rubini P, Cannata A, Bonati L, Alamanni F. Endothelial damage during myocardial preservation and storage. *Ann Thorac Surg* 2002;73:682-90.
24. Robson SC, Candinas D, Hancock WW, Wrighton CWinckler H. Roll of endothelial cells in transplantation. *Int Arch Allergy Immunol* 1995;106:305-22.
25. Maked PS, Amrani M, Rothery S, Devers NJ and Yacoub MH. Relative susceptibility of endothelium and myocardial cells to ischemia-reperfusion injury. *Acta Physiol Scand*.1997;161:103-12.
26. Cartier R, Hollman C, Dagenais F Buluran J, Pellerin M and Leclerc Y. Effects of University of Wisconsin solution on endothelium-dependant coronary artery relaxation in the rat. *Ann Thorac Surg*.1993;55:50-6.

27. Day JD, Rayburn BK, Gaudin PB. Cardiac allograft vasculopathy: the central pathogenetic role of ischemia-induced endothelial cell injury. *J Heart Lung Transplant* 1995;14(Suppl):S142-9.
28. Chomette G, Auriol M, Cabrol C: Chronic rejection in human heart transplantation. *J Heart Transplant.*1988;7:292-7.
29. Cannata A, Botta L, Colombo T, Russo C, Taglieri C. Does the cardioplegic solution have an effect on early outcomes following heart transplantation? *Eur J Cardiothorac Surg*.2012;41:e48-e53.
30. Karduz G, Yaman MO, Altan M, Sahin G, Toraman F. St-Thomas and del Nido cardioplegia are superior to Custodiol cardioplegia in a rat model of donor heart. Scand Cardiovasc J.2021 Apr;55(2):122-128.

Table 1. Baseline recipient and donor characteristics. Summary statistics are presented as mean (SD) or number (%).

Variable	St-Thomas N=75	HTK-Custodiol N=79	P-value
Recipient			
Age (years)	51.9 (12.1)	51.3 (12.9)	0.76
Female gender	10 (13.3)	23 (29.2)	0.017
Waiting time on list (days)*	170 (89-403)	209 (63-403)	0.87
Ischemic etiology	30 (40.0)	36 (45.6)	0.49
VAD	24 (32.0)	32 (40.5)	0.27
Diabetes	13 (17. 3)	21 (26.6)	0.17
RF (ml/min/1,73m^2)	50.8 (14.3)	56.7 (20.3)	0.079
Ejection Fraction (%)	25.0 (19.1)	28.4 (14.4)	0.15
VO_2 Max (ml/min/kg)	14.1 (4.0)	18.3 (24.2)	0.17
PVR (WU)	2.4 (1.2)	2.3 (0.97)	0.55
Ischemic time (min)**	172 (45.5) (N=68)	144 (40.2) (N=49)	<0.001
CPB time (min)*	135 (110-188)	143 (103-180)	0.73
Gender mismatch	28 (37.3)	31 (39.2)	0.81
Height mismatch	1 (1.3)	1 (1.3)	1.0
Weight mismatch	18 (24.0)	23 (29.1)	0.47
Previous cardiac surgery	40 (53.3)	52 (65.8)	0.11
Emergency transplantation	18 (24.0)	19 (24.1)	0.99
Donor			
Cause of death			
Cerebral hemorrhage	33 (44.0)	35 (44.3)	0.39
Anoxia	12 (16.0)	10 (12.7)	
Trauma	23 (30.7)	26 (32.9)	
Cerebral edema	7 (9.3)	8 (10.1)	
Age (years)**	43.5 (14.9) (N=72)	49.2 (14.4) (N=50)	0.038
Female gender**	25 (40.3) (N=62)	11 (44.0) (N=25)	0.75

SD : standard deviation ; *VAD* : ventricular assist device ; *RF*: renal function; *VO_2 Max* : maximal oxygen consumption; *PVR*: pulmonary vascular resistance ; *WU* :wood units ; *CPB* : cardiopulmonary bypass
*Median (IQR)
**Actual sample sizes are given in parentheses.

Table 2. Comparison of outcomes according to preservation solution. Summary statistics are presented as median (IQR) or number (%).

Outcome	St-Thomas N=75	HTK-Custodiol N=79	P-value
Inotropic score	71.8 (31.8-127)	35.7 (17.5-60.2)	<0.001
Intra/Postoperative ECMO	12 (16)	13 (16.5)	0.94
Rejection score	0.14 (0.05-0.25)	0.08 (0.0-0.25)	0.036
30-day mortality	11 (14.7)	2 (2.5)	0.007

ECMO Extracorporeal membrane oxygenation

Table 3. Relationship between cardiac preservation solution and inotropic score* adjusted for ischemic time and CPB time as derived by multiple linear regression

Covariate	Regression (95%CI)	P-value
CPS (Custodiol vs. St-Thomas)	-0.60 (-0.99 to -0.21)	0.003
Ischemic time (min)	0.0052 (0.0011 to 0.0093)	0.017
CPB time (min)*	0.71 (0.16 to 1.3)	0.015

SE: standard error; *CPB*: cardiopulmonary bypass

*Log-transform

Table 4. Relationship between 30-day mortality rate and each recipient-related characteristics adjusted for CPS as derived by logistic regression analysis

Risk factor	OR (95% CI)	P-value
Recipient-related preoperative		
Age (years)	1.03 (0.97–1.08)	0.32
Gender (male vs. female)	1.55 (0.33-7.37)	0.58
Etiology (ischemic vs. other)	1.16 (0.37-3.62)	0.80
VAD	1.10 (0.34-3.55)	0.87
Diabetes	0.27 (0.034-2.18)	0.22
RF (ml/min/1,73m^2)	0.99 (0.95-1.03)	0.65
Ejection Fraction (%)	1.002 (0.96-1.04)	0.94
VO_2 Max (ml/min/kg)	0 .90 (0.75-1.09)	0.28
PVR (WU)	1.10 (0.65-1.88)	0.72
Waiting time on list (days)*	1.03 (0.67-1.59)	0.89
Recipient-related intraoperative		
Ischemic time (min)	1.00 (0.99-1.02)	0.61
CPB time (min)*	4.06 (0.85-19.3)	0.078
CPS (Custodiol vs. St-Thomas)	0.15 (0.032-0.71)	0.016

OR: odds ratio; *CI:* confidence interval; *VAD*: ventricular assist device; *VO_2 Max*: maximal oxygen consumption; *PVR*: pulmonary vascular resistance; *WU:* Wood units*; CPB*: cardiopulmonary bypass

*Log-transform

Table 5. Relationship between overall survival and each recipient-related characteristics adjusted for CPS as derived by Cox regression analysis

Risk factor	HR (95% CI)	P-value
Recipient related preoperative		
Age (years)	1.03 (0.99–1.07)	0.11
Gender (male vs. female)	1.38 (0.47-4.00)	0.56
Etiology (ischemic vs. other)	0.95 (0.43-2.06)	0.89
VAD	0.97 (0.43-2.18)	0.94
Diabetes	0.63 (0.22-1.82)	0.39
RF (ml/min/1,73m^2)	0.998 (0.971-1.03)	0.90
Ejection Fraction (%)	1.01 (0.98-1.04)	0.46
VO_2 Max (ml/min/kg)	0 .95 (0.86-1.06)	0.36
PVR (WU)	1.07 (0.77-1.50)	0.68
Waiting time on list (days)*	0.92 (0.71-1.20)	0.55
Recipient related intraoperative		
Ischemic time (min)	1.00 (0.99-1.01)	0.94
CPB time (min)*	2.16 (0.75-6.26)	0.16
Preservation solution (Custodiol vs. St-Thomas)	0.20 (0.069-0.6)	0.004

HR: hazard ratio; *CI* : confidence interval ; *VAD* : ventricular assist device ; *VO_2 Max* : maximal oxygen consumption; *PVR*: pulmonary vascular resistance ; *WU* :wood units ;*CPB* : cardiopulmonary bypass

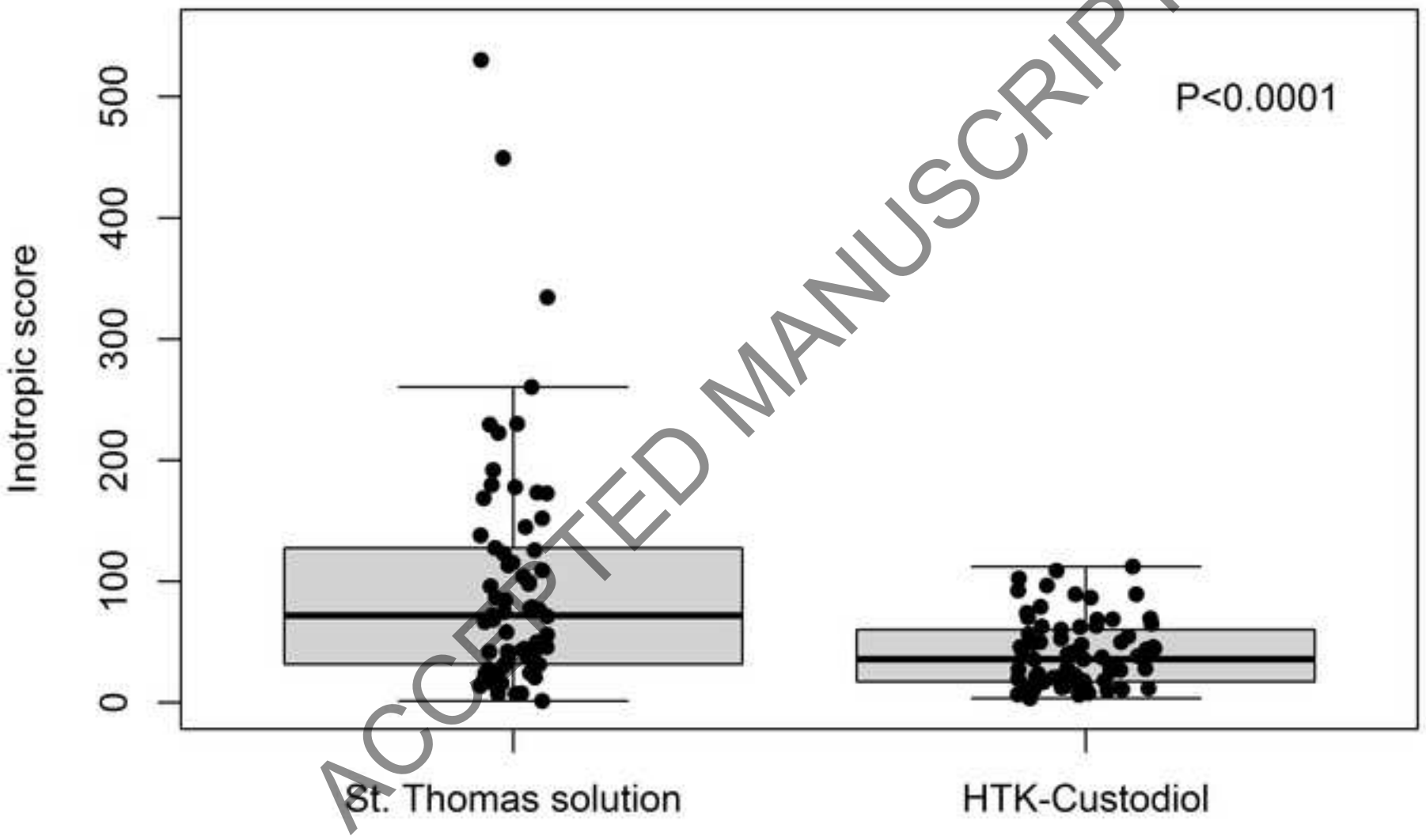
*Log-transform

Figure 1: Distribution of the inotropic score in St-Thomas (N=66) and HTK-Custodiol (N=74) solution groups

Figure 2: Distribution of the rejection score in St-Thomas (N=62) and HTK-Custodiol (N=69) solution groups

Figure 3: Kaplan-Meier post-transplant survival curves with censoring marks and 95%confidence limits in St-Thomas and HTK-Custodiol solution groups

Graphical Abstract – Central Image: Kaplan-Meier post-transplant survival curves with censoring marks and 95%confidence limits in St-Thomas and HTK-Custodiol solution groups



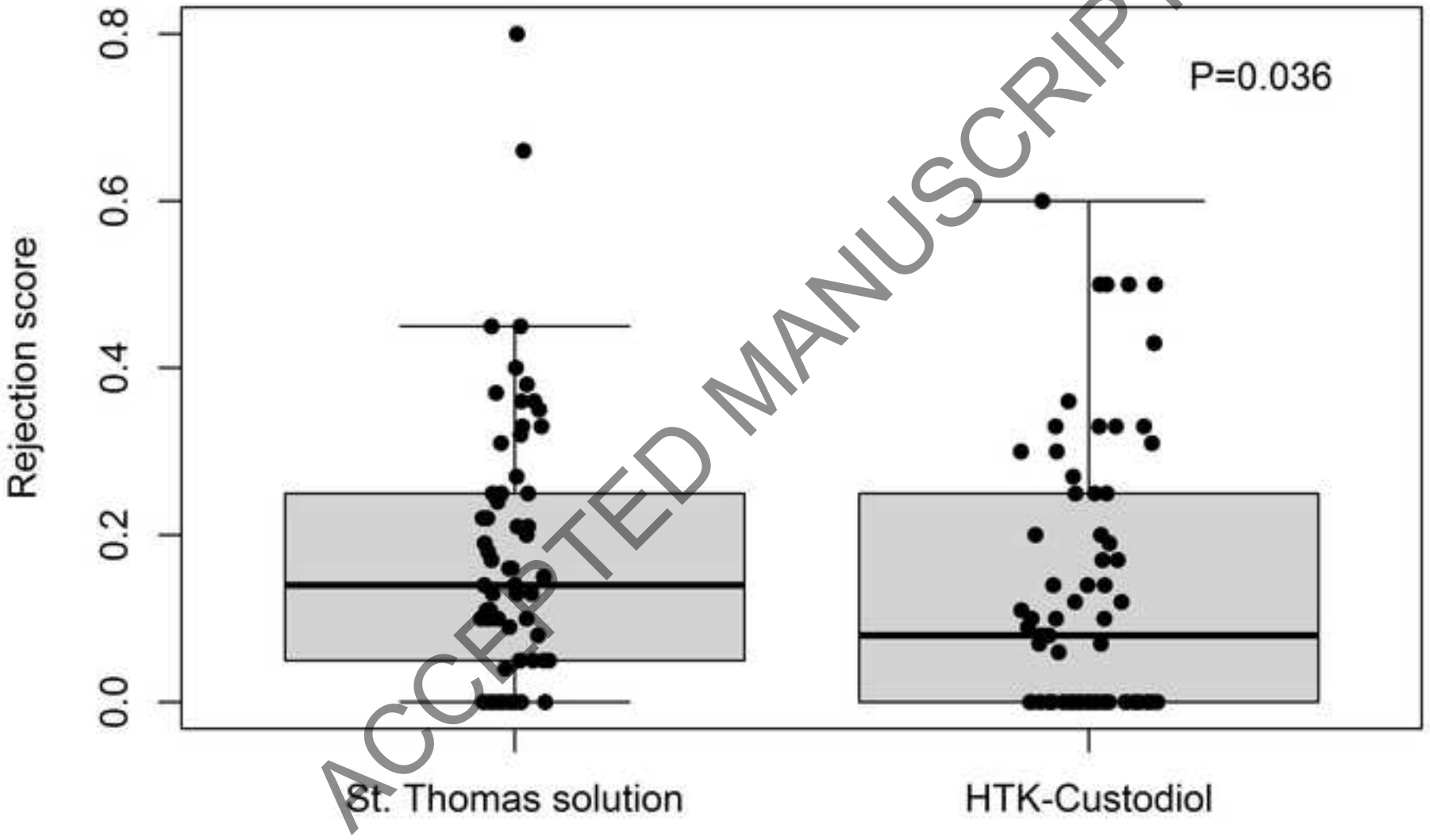
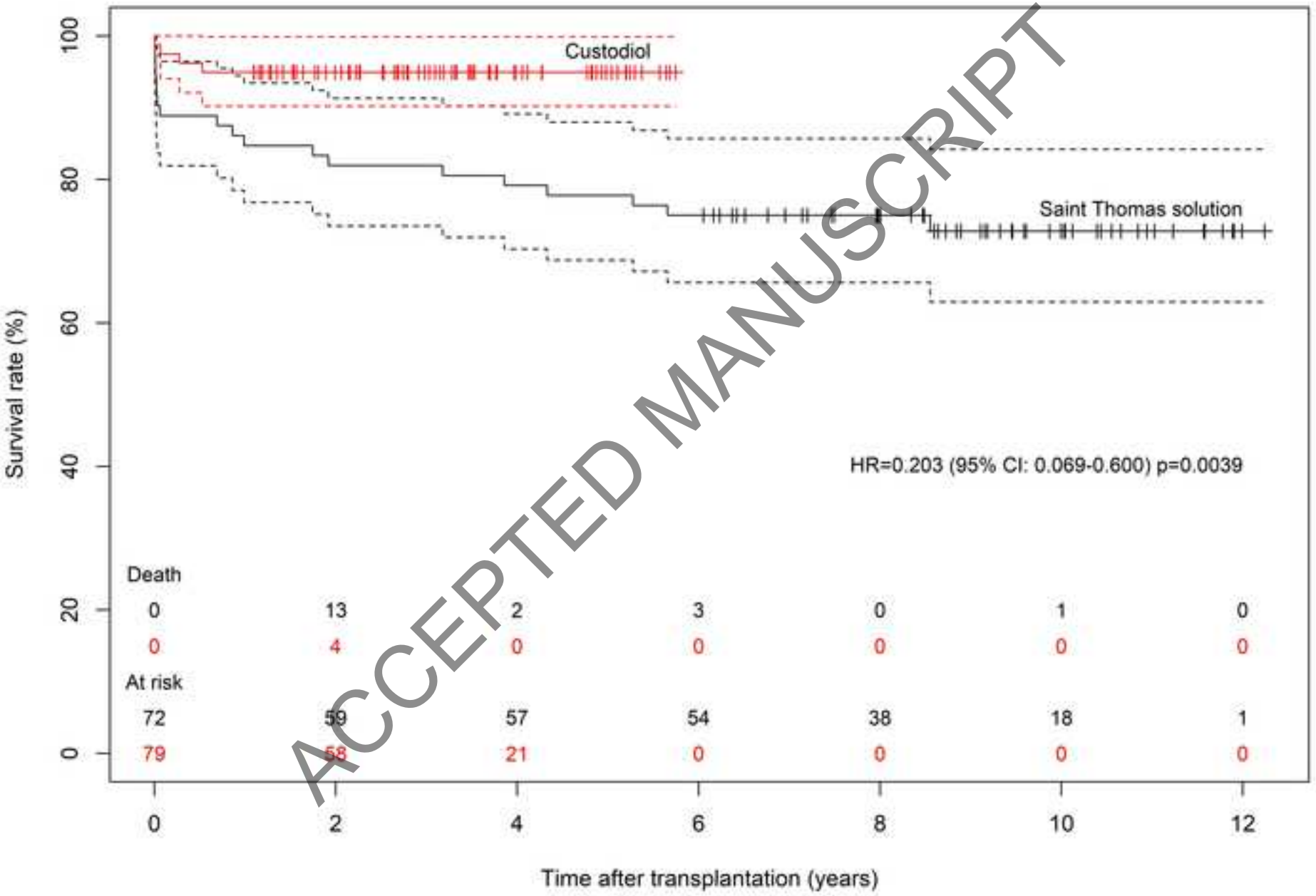


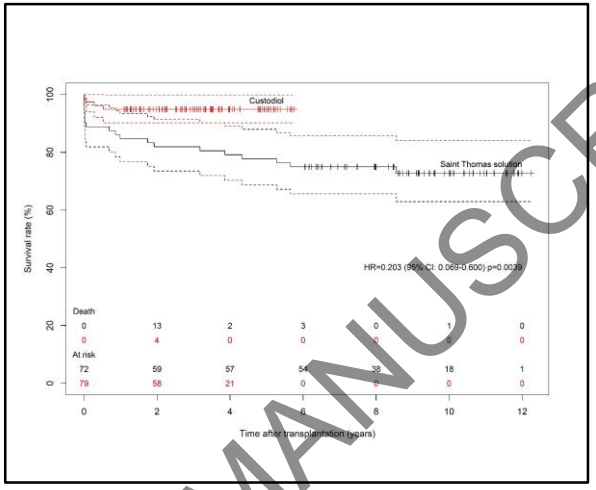
Figure 3



Comparison of HTK-Custodiol and St. Thomas Solution as Cardiac Preservation Solution on Early and Midterm Outcomes Following Heart Transplantation

Summary

In this retrospective study we analyzed 154 adult heart transplants according to the cardiac preservation solution received (St. Thomas solution vs HTK-Custodiol). Postoperatively, the Custodiol group showed lower inotropic score, mean rejection score, 30-day and mid-term mortality, but also less specific histological features of ischemia-reperfusion lesions.



Legend: Kaplan-Meier post-transplant survival curves with censoring marks and 95% confidence limits in St. Thomas and HTK-Custodiol solution groups