



Pharmacological Conditioning of Brain Dead Donor Hearts with Erythropoietin and Glyceryl Trinitrate: Clinical Experience

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Abstract

Background: With the increasing success of heart transplantation, older and higher-risk donors and recipients are being accepted for transplantation. The risk of primary graft dysfunction (PGD) is thus increased. We investigated a 'pharmacological conditioning' strategy, where Celsior preservation solution supplemented with glyceryl trinitrate (GTN) and erythropoietin (EPO) was used for cardioplegia and hypothermic storage, and determined graft recovery and patient survival after cardiac transplantation.

Methods: Donor hearts retrieved between August 2010 and November 2013 were arrested and stored with supplemented Celsior (n = 61). Historical comparisons were made with hearts stored in Celsior (April 2005 to July 2010; n = 104) and modified St. Thomas' solution (STS, January 2000 to March 2005; n = 100). Donor, recipient and procedural risk factors for PGD were determined, and post-transplant use of mechanical circulatory support (MCS), length of stay (LOS) and 12-month survival compared between groups.

Results: Fewer hearts stored in STS came from donors aged > 50 years (p < 0.01). Recipients of hearts stored in supplemented Celsior had increased use of MCS pre-transplant (36%, p < 0.0001), and increased cross clamp times during implantation (111min, p < 0.0001). Use of MCS post-transplant was 32.0%, 31.7% and 24.6% in STS, Celsior and supplemented Celsior groups respectively. There were no differences in LOS. Survival at 1-month was 92%, 95% and 98% and at 12-months 86%, 89% and 90% respectively.

Conclusion: Despite an increased donor and recipient risk profile, supplemented Celsior was associated with excellent operative and 12-month survival. There was a trend towards decreased need for post-transplant MCS, however the incidence of PGD remains high.

Keywords

Primary graft dysfunction (PGD), Donor heart preservation, Pharmacological conditioning, Ischemia-reperfusion injury (IRI), Hypothermic organ preservation

Introduction

The ongoing success and progress in heart transplantation has led to the acceptance of higher risk candidates for transplantation. These include patients of older age, with comorbidities, prior malignancies, redo sternotomies, and candidates on mechanical circulatory support (MCS) [1,2]. The rising demand for transplantation has also led to liberalization of donor acceptance criteria, with a significant increase in the use of extended criteria or 'marginal' donor hearts, in particular from older donors [1].

Transplanting higher risk recipients and using marginal donor organs increases the risk of post-transplant complications such as primary graft dysfunction (PGD) [3-7]. Particular emphasis has been placed on the additive effect of combining older donor age and prolonged ischemic time on the risk of PGD [1,8,9]. Certainly, longer ischemic times are encountered in the current era of transplantation, due to use of non-local donors and longer procedural times associated with technically complex recipients [1].

International Society for Heart and Lung Transplantation (ISHLT) registry data reveal that 39% of deaths in the first 30 days post-transplantation are due to PGD, with a further 18% attributed to secondary causes such as multi-organ failure [1,3]. A multi-center survey of autopsy results identified ischemia-reperfusion injury (IRI) as the main histopathological finding in 48% of deaths attributed to PGD [3]. Other findings such as myocyte necrosis (28%), multifocal edema and/or hemorrhage (14%) and freeze injury (7%) may also be attributed to injury incurred following prolonged hypothermic storage. Improving donor heart preservation is therefore of utmost importance, with organ retrieval a potential opportunity for therapeutic intervention.

Research in our institution has focused on applying myocardial 'conditioning' strategies to minimize IRI of the donor heart. The addition of the nitric oxide donor, glyceryl trinitrate (GTN), the

glycoprotein hormone erythropoietin (EPO), and sodium-hydrogen exchange (NHE)-inhibitors to cardioplegia and hypothermic storage has demonstrated significantly improved post-storage recovery of cardiac output, myocardial contractility and reduced biochemical and histological markers of IRI in several preclinical studies [10-19]. The mechanisms of action of pharmacological conditioning are through activation of pro-survival signaling cascades mediated by ERK1/2, Akt of the reperfusion injury salvage kinase (RISK) pathway [20,21], STAT 3 of the survivor activating factor enhancement (SAFE) pathway [22,23], and cytoprotective effects of nitric oxide [24,25], that prevent oxidative stress, cellular edema, intracellular calcium overload and preserve mitochondrial integrity to prevent cellular apoptosis and necrosis due to IRI [26-32].

Given the success of these measures in multiple small and large animal studies including models that incorporate donor brain death, we aimed to assess the efficacy of pharmacological conditioning in reducing IRI in clinical cardiac transplantation. Two clinically available pharmacological conditioning agents EPO and GTN were added to cardioplegia and hypothermic preservation of the donor heart, with the aim of reducing primary graft dysfunction and improving patient survival. We report initial findings from this clinical study.

Methods

The study was conducted at a quaternary center specializing in cardiac transplantation and advanced heart failure care. Transplants performed between August 2010 and November 2013 used Celsior preservation solution supplemented with GTN and EPO for cardioplegia and hypothermic storage (n = 61). Data were compared with historic controls where Celsior alone was used (April 2005 to June 2010; n = 104) or modified St. Thomas' solution (STS, January 2000 to March 2005; n = 100).

Celsior preservation solution is classified as an unapproved therapeutic good in Australia. Approval to use Celsior for donor heart preservation was granted to one of the authors (PSM) in April 2005 by the Therapeutic Goods Administration (TGA) under Section 41HC of the Therapeutics Goods Act 1989. The TGA approval was endorsed by the St Vincent's Hospital Human Research Ethics Committee (HREC). TGA approval and HREC endorsement were renewed annually following submission of progress reports to both bodies. All potential heart transplant recipients provided written informed consent to the use of Celsior preservation solution and for their clinical information to be entered prospectively into a dedicated transplant database.

Donor, recipient and procedural data were obtained from a transplant database and medical records. Detailed data were collected to determine donor, recipient and procedural risk factors for PGD as outlined by Kobashigawa et al. in the 2013 Consensus Conference on Primary Graft Dysfunction after Cardiac Transplantation [3]. Transplants performed with organ retrieval by non-regional centers were excluded due to the use of other cardioplegic and hypothermic storage solutions.

Extended criteria (marginal) donor hearts

Donor hearts were classified as 'marginal' (extended criteria) according to the definition stated in the Transplantation Society of Australia and New Zealand Consensus Statement on Eligibility Criteria and Allocation Protocols for Organ Transplantation from Deceased Donors, 2010. That is, donor age \geq 50 years, ischemic time > 360 minutes, donor left ventricular ejection fraction (LVEF) < 50% or regional wall motion abnormalities, the use of high dose inotropes (noradrenaline > 0.2 μ g/kg/min or equivalent) or donor hepatitis B, C or high risk behavior.

Cardioplegia and hypothermic preservation

Antegrade cardioplegia was delivered via the aortic root in all transplants. Hearts were stored in hypothermic conditions (2-3 $^{\circ}$) during transport, using the same preservation solution as used in cardioplegia. All retrievals were performed by the study institution's transplant team.

Composition of preservation solutions

Celsior $^{\circ}$ was obtained commercially (Genzyme, Naarden, The Netherlands). Modified St. Thomas' Solution (STS) was prepared in-house, with the composition outlined in table 1. Both solutions were 'extracellular'-based preservation solutions with hyperkalemia and hypothermia the modes of induction of cardiac arrest. In the study group, Celsior $^{\circ}$ was supplemented with glyceryl trinitrate (Hospira Australia, Pty, Ltd., Mulgrave, AU) at a concentration of 0.1 g/L, and erythropoietin-alpha (Eprex; Janssen-Cilag, North Ryde, AU) at 5000U/L. Glyceryl trinitrate and EPO were added to Celsior $^{\circ}$ at the recipient institution, just prior to cardioplegia and hypothermic storage. The optimal concentrations of pharmacological supplements were determined from preclinical studies [10,18,19].

Post-transplant outcomes and survival

Immediate post-transplant outcomes were determined by analysis of the use of mechanical circulatory support (extra-corporeal membrane oxygenation [ECMO] or intra-aortic balloon pump [IABP]) within the first 24 hours of transplantation. The length of stay in the intensive care unit (ICU) and length of hospital admission were compared between groups. Data were obtained for survival for 1, 3 and 12-months post-transplantation, with survival outcomes for all recipients obtained for the total 12 month follow-up period.

Definition of primary graft dysfunction

Primary graft dysfunction was defined by the use of ECMO or IABP in the first 24 hours after completion of cardiac transplantation surgery. Utilization of ECMO to treat severe PGD was instituted in our institution from 2005 onwards. Prior to 2005, all cases of PGD were managed by IABP support. Due to the retrospective nature of data collection, the use of inotropes and nitric oxide were not included in the analysis to determine mild left ventricular or right ventricular dysfunction. Echocardiography was routinely performed in the first hours post transplantation, however data were not used due to the absence of standardized reporting and recording in medical records. Reports of the first endomyocardial biopsy (week 1 post-transplant) were analyzed to exclude severe rejection as a cause of allograft dysfunction.

Table 1. Composition of preservation solutions.

	Modified STS	Celsior $^{\circ}$
Electrolytes		
Na $^{+}$	149 mmol/L	100 mmol/L
K $^{+}$	19 mmol/L	15 mmol/L
Ca $^{2+}$	2.0 mmol/L	0.25 mmol/L
Mg $^{2+}$	19 mmol/L	13 mmol/L
Cl $^{-}$	120 mmol/L	41.5 mmol/L
Metabolic substrates		
Glucose	39 mmol/L	0
Aspartate	24 mmol/L	0
Glutamate	0	20 mmol/L
Antioxidants		
Glutathione	0	3 mmol/l
Osmotic agents		
Lactobionate	0	80 mmol/L
Mannitol	0	60 mmol/L
Acid-base buffers		
Histidine	0	30 mmol/L
Bicarbonate	28 mmol/L	0
Osmolarity	400 mOsm/L	360 mOsm/L

The composition of St. Thomas' Solution (STS) and Celsior used are outlined above. Glyceryl trinitrate (GTN, 0.1 g/L) and erythropoietin (EPO, 5000 U/L) were added to Celsior for the groups of donor hearts arrested and stored with supplemented Celsior (Celsior+EPO+GTN).

Statistical analysis

Continuous variables are represented as mean \pm standard deviation and categorical data as the number of events and percentage. Differences between groups for continuous variables were calculated using one-way ANOVA with Tukey's post-hoc multiple comparisons test. Categorical data were analyzed using the Chi-square test. Survival data were analyzed using the Kaplan-Meier method. A p value < 0.05 was considered significant. All statistical analyses were performed using Prism 6 software (GraphPad Software, Inc., CA).

Results

Donor, recipient and procedural features

Donor, procedural and recipient risk factors for PGD are outlined for each group in [table 2](#). There was a significantly higher proportion of hearts from donors ≥ 50 years of age in the supplemented Celsior group (25%) and Celsior alone (23%) compared with STS (9%, $p < 0.01$). Donors with anoxic brain injury, cerebral edema and meningitis were higher in the supplemented Celsior group (28%, $p < 0.01$) compared with Celsior and STS groups (19% and 8.5% respectively).

A higher proportion of recipients were on mechanical circulatory

support prior to transplantation in the supplemented Celsior group (36%) compared with Celsior (22%, $p < 0.0001$) and STS (6.0%, $p < 0.0001$ supplemented Celsior; $p < 0.01$ Celsior). Procedural cross-clamp times were higher in the supplemented Celsior group (111 ± 36 minutes, $p < 0.0001$) compared with Celsior (83 ± 24 minutes) and STS (82 ± 20 minutes).

Other trends observed were an increase in the mean donor age in supplemented Celsior (38 ± 13 years) and Celsior groups (37 ± 14 years), compared with STS (33 ± 13 years, $p = ns$), and a higher proportion of redo-sternotomies in the supplemented Celsior group (48%) compared with Celsior (35%) and STS groups (35% and 34% respectively, $p = ns$). The proportion of donor hearts with left ventricular dysfunction at retrieval was similar in the three groups: supplemented Celsior (13%), Celsior (11%) and STS (10%, $p = 0.85$). There were no differences in mean recipient age or the presence of pulmonary hypertension across groups. Between 33 to 43% of ischemic times were > 240 minutes, with no significant differences across groups.

Post-transplant outcomes

Primary graft dysfunction, as defined by the use of ECMO or

Table 2: Donor, procedural and recipient risk factors for primary graft dysfunction.

Preservation Solution	STS (n = 100)	Celsior (n = 104)	Celsior+EPO+GTN (n = 61)	p-value
DONOR FACTORS				
Donor age (years, mean \pm SD)	33 \pm 13	37 \pm 14	38 \pm 13	ns
Donor weight (kg, mean \pm SD)	79 \pm 14	78 \pm 15	81 \pm 15	ns
Cause of death (n, %)				
Head trauma	36 (36%)	29 (28%)	17 (28%)	ns
CVA or ICH	55 (55%)	53 (51%)	25 (41%)	ns
Cardiac arrest	1 (1%)	2 (2%)	2 (3%)	ns
Other ^a	8 (8%)	20 (19%)	17 (28%)	< 0.01
Valvular heart disease	3 (3%)	3 (3%)	3 (5%)	ns
Marginal donors (total)	44 (44%)	59 (57%)	34 (56%)	ns
High dose inotropes	30 (30%)	32 (31%)	20 (33%)	ns
Age ≥ 50 years	9 (9%)	24 (23%)	15 (25%)	< 0.01
LV dysfunction	10 (10%)	11 (11%)	8 (13%)	ns
Ischemic time > 360 min	4 (4%)	4 (4%)	1 (2%)	ns
PROCEDURAL FACTORS				
Ischemic time (min, mean \pm SD)	235 \pm 68	232 \pm 67	216 \pm 63	ns
Ischemic time > 240 min (n, %)	43 (43%)	42 (40%)	20 (33%)	ns
Cross-clamp time (min, mean \pm SD)	82 \pm 20	83 \pm 24	111 \pm 36	< 0.0001
Female to male transplants (n, %)	13 (13%)	16 (15%)	8 (13%)	ns
Donor : Recipient weight < 0.8 (n, %)	3 (3%)	10 (10%)	2 (3%)	ns
Urgent transplant (n, %)	2 (2%)	5 (5%)	3 (5%)	ns
RECIPIENT FACTORS				
Recipient age (years, mean \pm SD)	48 \pm 12	48 \pm 13	46 \pm 13	ns
Recipient weight (kg, mean \pm SD)	76 \pm 14	73 \pm 15	74 \pm 17	ns
MCS (n, %) (LVAD, BiVAD or TAH)	6 (6%)	23 (22%)	22 (36%)	< 0.0001
ECMO or IABP pre-transplant (n, %)	1 (1%)	0	0	ns
Congenital heart disease (n, %)	4 (4%)	6 (6%)	3 (5%)	ns
Redo sternotomy (n, %)	34 (34%)	36 (35%)	29 (48%)	ns
Multiorgan transplant				
Heart-Lung	1 (1%)	3 (3%)	2 (3%)	ns
Heart-Kidney	1 (1%)	1 (1%)	0	ns
Recipient PHT[#]	23 (23%)	22 (21%)	13 (21%)	ns
Retransplant	0	0	0	ns

^aAnoxic brain death (asthma, hanging), cerebral edema from overdoses and meningitis.

[#]Recipient pulmonary hypertension was defined by a transpulmonary gradient ≥ 12 mmHg.

STS indicates St. Thomas' Solution; EPO: Erythropoietin; GTN: Glycerol trinitrate; CVA: Cerebrovascular accident; ICH: Intracranial Hemorrhage; MCS: Mechanical Circulatory Support; LVAD: Left Ventricular Assist Device; BiVAD: Biventricular Assist Device; TAH: Total Artificial Heart; ECMO: Extra-Corporeal Membrane Oxygenation; IABP: Intra-aortic Balloon Pump; and PHT: Pulmonary Hypertension

IABP within the first 24 hours of transplantation, was present in 25% of supplemented Celsior, 32% of Celsior and 32% of STS transplants ($p = ns$, Table 3). Venous-arterial (VA) ECMO was used in all cases except one patient in the Celsior group in whom venous-pulmonary arterial (V-PA) ECMO and an IABP were used. In marginal hearts, PGD occurred in 29% of supplemented Celsior, 37% of Celsior and 39% of STS transplants ($p = ns$). Severe rejection was not identified as the reason for implementation of MCS in any of the transplants. There were no significant differences in the length of stay in ICU or hospital between groups (Table 4).

Of note, recipients in the STS group only received IABP support post-transplantation, which reflects practices in that era of transplantation (Table 3). The use of ECMO increased and the use of IABP decreased in Celsior and supplemented Celsior groups, also reflecting practices at the time.

Survival

Survival at 1-month post-transplant was 92%, 95% and 98% in STS, Celsior and supplemented Celsior groups respectively ($p = ns$, Figure 1). Survival at 3-months was 89%, 91% and 93% ($p = ns$) and at 12-months was 86%, 89% and 90% respectively ($p = ns$).

Discussion

The introduction of brain dead donor heart conditioning with GTN and EPO at arrest, perfusion and storage at our institution has resulted in high patient survival despite increasing donor, procedural and recipient risk factors for PGD. The survival rate observed in our

series compares favorably with ISHLT registry data, which reported post-transplant survival of 92% at 1-month, 90% at 3-months and 85% at 1-year in contemporaneous years of 2006 to 2011 [1,3]. To our knowledge, this is the first clinical study assessing the efficacy of pharmacological conditioning at improving preservation of donor hearts, and has demonstrated encouraging early results.

The need for MCS to treat PGD in our series is relatively high compared with other published series [33-35]. We believe this is explained in part by our relatively high utilization of hearts from marginal donors in an era when more than one third of recipients were supported on a VAD at the time of transplant. Indeed, in a recent US registry analysis, Fudim et al. [36] reported that implantation of marginal donors into VAD-supported patients was an independent risk factor for early graft failure and death. In the most recent era, 56% of donors had one or more marginal characteristic. Of particular note, 25% of donors in the most recent cohort were 50 years of age or older, and another 13% had evidence of left ventricular systolic dysfunction at the time of retrieval. In addition, due to large retrieval distances, the average ischemic time for heart transplants in our institution is one hour longer than the international average. The advent of peripheral ECMO as a relatively simple and safe option for short-term mechanical support [37] may have introduced two biases in the more recent era, firstly a greater preparedness on the part of surgeons to utilize marginal donors and secondly a move towards earlier utilization of ECMO to allow the heart more time to recover from the transplant process rather than attempt repeated weaning of a heart that is clearly struggling at the first attempt.

Table 3: Use of ECMO or IABP post-transplantation.

	STS	Celsior	Celsior+EPO+GTN	p-value
ECMO or IABP (n, %)				
All transplants	32 (32.0%) (32 IABP)	33 (31.7%) (22 ECMO, 11 IABP)	15 (24.6%) (13 ECMO, 2 IABP)	ns
Marginal transplants	17/44 (38.6%) (17 IABP)	22/59 (37.2%) (16 ECMO, 6 IABP)	10/34 (29.4%) (9 ECMO, 1 IABP)	ns

The use of extra-corporeal membrane oxygenation (ECMO) or an intra-aortic balloon pump (IABP) within the first 24 hours post-transplant is shown above for all transplants and for marginal transplants.

Table 4: Length of stay: All transplants.

	STS (n = 48)	Celsior (n = 59)	Celsior+EPO+GTN (n = 34)	p-value
LOS ICU (days, mean \pm SD)	6 \pm 6	6 \pm 6	9 \pm 11	0.08
LOS hospital (days, mean \pm SD)	24 \pm 20	29 \pm 28	27 \pm 18	0.40

The length of stay (LOS, days) in the intensive care unit (ICU) and entire hospital admission are demonstrated above for patients transplanted with hearts stored in St. Thomas' Solution (STS), Celsior or Celsior supplemented with erythropoietin and glyceryl trinitrate (Celsior + EPO + GTN).

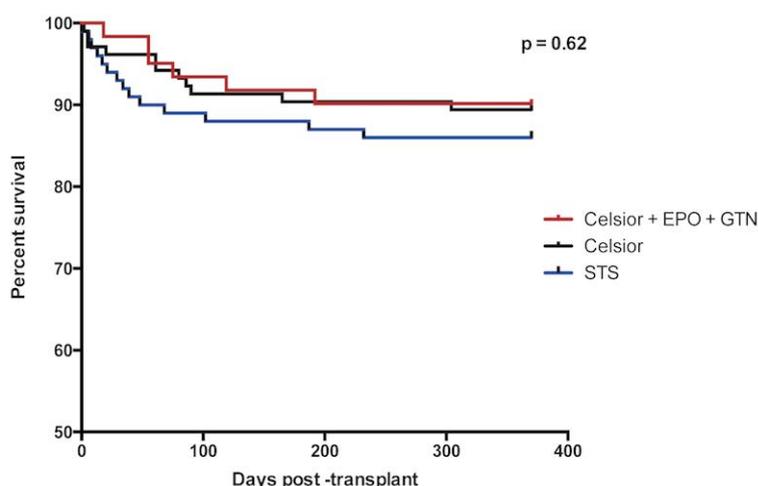


Figure 1: Survival (all hearts) at 370 days post-transplant.

Survival of patients with donor hearts stored in either St Thomas' Solution (STS), Celsior or supplemented Celsior (Celsior + EPO + GTN) are demonstrated for 370 days post-transplantation, with Kaplan-Meier analysis.

Future Directions

This first study on pharmacological conditioning of donor hearts in clinical cardiac transplantation has demonstrated safety and non-inferiority of this strategy of myocardial preservation. The translation of the pharmacological conditioning strategy was based on robust pre-clinical data which demonstrated significant improvements in the recovery of donor hearts in small and large animals [10-14,16-19,38]. These studies also demonstrated reduced biochemical markers of myocardial injury such as lactate dehydrogenase and troponin I, increased phosphorylation of key cell survival signaling enzymes ERK1/2, Akt, GSK-3 β and reduction of markers of apoptosis such as cleaved caspase 3. A limitation in the translation of our pharmacological conditioning protocol from animal studies to man was that only two pharmacological agents, GTN and EPO are approved for clinical use. The third agent, a NHE-inhibitor, either zoniporide or cariporide, was not used due to discontinuation of clinical development of these agents due to safety concerns following the EXPEDITION trial [39]. In that trial, repeated bolus intravenous doses of cariporide for myocardial preservation in high-risk coronary artery bypass grafts demonstrated a higher incidence of fatal cerebrovascular accidents despite significantly improved myocardial protection. In the context of cardiac transplantation, NHE-inhibitors would only be used in cardioplegia and hypothermic storage of the donor heart and not administered systemically in the recipient, hence similar adverse effects are unlikely. Based on our previous finding of a synergistic interaction between zoniporide, EPO and GTN in preclinical studies, we believe that the addition of an NHE inhibitor would result in further improved myocardial preservation and reduced PGD if this 'triple supplementation' strategy is able to be implemented clinically.

We have also added EPO and GTN to STS in clinical heart transplants from donation after circulatory death (DCD) donors conducted at our institution [40]. These transplants utilized normothermic *ex vivo* perfusion (NEVP) for transport of the donor heart. The decision to flush the donor heart with STS rather than Celsior was based on the recommendation of the NEVP manufacturer. Recipients in the present study, and DCD transplants using pharmacological conditioning will be followed to determine longer-term effects of donor heart preservation, such as the incidence of cardiac allograft vasculopathy.

Limitations

There are a number of limitations to our study. Although all data were prospectively entered into a dedicated database, the analysis was conducted retrospectively. This was a single center study and historical controls were used as a basis for comparison. The use of historical controls however does highlight the dramatic changes that have occurred in both donor and recipient populations in little more than a decade. This study did not use hemodynamic criteria or echocardiographic data, hence all mild and some moderate cases of primary graft dysfunction were not captured in our data.

An era effect was demonstrated in practices of IABP and ECMO use. The use of ECMO, as opposed to ventricular assist device use for PGD has been associated with improved survival and is now considered a safer and more effective method of maintaining circulatory support in severe PGD [41]. Of patients treated with ECMO in our study, 80% one-year survival was demonstrated. Furthermore, while a reduction in PGD and an increase in survival were noted in the supplemented Celsior group, statistical significance was unable to be demonstrated due to relatively small patient numbers and hence the lack of statistical power. A prospective multi-center randomized controlled trial would be required to address these limitations and provide further support for our hypothesis that pharmacological conditioning improves donor heart preservation in the current era.

Conclusion

Early results of pharmacological conditioning demonstrate excellent survival rates in the immediate and early post-transplant period. The rate of primary graft dysfunction remains high and

indicates an ongoing need for further improvements in donor heart preservation in an era of increasing donor and recipient risk profiles.

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Conflicts of Interest

The authors have no conflicts of interest to declare in relation to this manuscript.

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