# Supplementary Appendix

Supplement to: Dhar R, Marklin GF, Klinkenberg WD, et al. Intravenous levothyroxine for unstable brain-dead heart donors. N Engl J Med 2023;389:2029-38. DOI: 10.1056/NEJMoa2305969

This appendix has been provided by the authors to give readers additional information about the work.

Supplement to:

**Intravenous Levothyroxine for Unstable Brain-Dead Heart Donors**

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# Supplementary Methods

## Inclusion and Exclusion Criteria

Inclusion – must meet all four criteria at the time of screening/randomization, which was recommended to occur as soon as possible after authorization and initial fluid resuscitation:

1. Declared dead by neurologic criteria
2. Authorization for organ donation and research
3. Donor age 14-55 years (inclusive) and weight ≥ 45 kg.
4. On one or more vasopressor and/or inotropes (excluding vasopressin < 1 unit/hour)

Exclusion criteria:

1. Brain-death declared greater than 24 hours prior
2. Coronary artery disease or history of myocardial infarction (by history, EKG, prior cardiac catheterization) that would exclude heart transplantation
3. Significant valvular heart disease (by history or echo) that would exclude transplantation
4. Previous sternotomy or cardiac surgery
5. Donor managed in a Veterans Affairs hospital
6. Donor received intravenous or oral/enteral thyroid hormone in month prior
7. Known HIV positive
8. Other reason that would exclude heart from being allocated for transplant

## Prespecified Definitions of Adverse Events

| Adverse Event | Definition |
|---|---|
| **Severe Hypertension** | Systolic blood pressure above 200 mm Hg |
| **Tachycardia** | Heart rate above 150 bpm and increased by 20 bpm or more above baseline heart rate |
| **Arrhythmias** | New or worsened arrhythmia, including SVT, atrial fibrillation, ventricular tachycardia, or ventricular fibrillation |
| **Fever** | New temperature elevation above 102 degrees Celsius |
| **Rash** | New skin rash |

**Serious Adverse Events** were defined as hemodynamic instability leading to cardiac arrest or donor loss prior to organ recovery

The DSMB reviewed all adverse events at regular intervals and any serious adverse events within 72 hours of reporting.

## Non-Inferiority Analysis

Given preexisting concern that thyroid hormone treatment in donors could lead to a withdrawal effect and possibly more early graft failure in recipients,[1,2] we planned on testing non-inferiority of 30-day graft survival of hearts transplanted from levothyroxine donors for our primary prespecified safety analysis. Review of SRTR data over the past five years (2016-2020) suggested that the expected 30-day graft survival (a standard outcome reported by transplant centers) after heart transplantation would be 96%. We tested for a decrease of no greater than 6% in graft survival, applying a one-sided alpha of 0.025. This margin was determined to be the clinically significant minimum meaningful reduction in graft survival, selected with input from the DSMB, including a thoracic transplant surgeon and an ethicist. It aligns with that used in another recent large trial evaluating graft survival after heart transplantation, which used a similar baseline assumption of graft survival but allowed a more liberal ten percent liberal margin.[3] We believe that setting the lower limit of tolerance for this safety analysis at 90% graft survival is most appropriate. We constructed two-sided 95% confidence intervals for the difference in graft survival between groups, allowing us to test for inferiority at a one-sided alpha of 0.025.

### Data Cleaning

Data was reviewed by the coordinating center's data management team for inconsistencies or missing data values, which were resolved first by reaching out to site coordinators for clarification. Various internal data consistency checks were executed at the time of interim and final analyses, including verification of eligibility criteria (for example, comparing age of donor calculated from SRTR date of birth to age exclusion in REDCap and verifying time from brain death declaration to randomization was less than 24 hours). The randomization log was also reconciled with the REDCap eligibility / enrollment log to ensure consistency and that no donors had been randomized and not entered. All inconsistencies were reconciled and corrected. The primary outcome of heart transplanted was reported by the study personnel, using the REDCap database, but was verified against heart utilization data reported to SRTR. Inconsistencies between the vasopressor flowsheet (e.g. infusion rate at start and throughout study period) and whether infusion was started or stopped early were identified and resolved.

In 12 cases when vasopressors had been weaned off prior to starting study infusion, baseline VIS was set to 0 and time to event (for weaning off pressors) was set to 0.05 hr, representing half the shortest time to event following infusion start (0.1 hr). Similarly, in 45 cases where the first echo was ordered prior to infusion start but performed after, we treated these as if the event (time to order echo) occurred at 0.05 hr for purposes of survival analysis. However, if the infusion was never started (whether due to donor stability, instability, or other factors), we did not infer the "infusion start time" and therefore left time to wean vasopressors, baseline VIS, and time to order echo as missing. However, in 10 cases where we knew donors had been weaned off vasopressors after randomization and study infusion was never started, we did set the endpoints of VIS score at 12 hours to zero and whether the donor had been weaned off vasopressors by 12 hours to yes. In 16 cases where VIS was missing at the end of case and there was no data showing earlier weaning off vasopressors, we censored survival analysis at the last available time

with VIS score. In 41 cases where no specific vasopressor end time was entered but VIS was entered as 0 at/after a particular time point (e.g. end of case), we used that time as the event time.

## Secondary Outcome Analyses

Binary outcomes (lungs, liver transplanted, weaned off vasopressors by 12 hours) were analyzed using modified Poisson regression, clustered according to site. Left ventricular ejection fraction was analyzed using a linear mixed effects model. The vasopressor-inotrope score was natural log-transformed and then compared using a linear mixed model, adjusting for baseline values. Total number of organs transplanted and number of kidneys transplanted was compared using Poisson generalized estimating equation regression, clustered according to site. The time to weaning off vasopressors as well as time to order the first echocardiogram were analyzed using a Cox regression model to estimate hazard ratios. The proportional hazards assumption was assessed graphically by the standardized score process and numerically by supremum testing. The assumption held for all performed Cox analyses. All final data analyses were performed using SAS, version 9.4 (SAS Institute) with some preliminary descriptive analyses performed using SPSS version 29 (IBM Corp).

**Multiple Imputation** was performed for binary (weaned off vasopressors at 12 hours) and continuous (LVEF, VIS) secondary outcomes with missing data with chained equations, using linear regression for continuous variables and logistic regression method for binary variables, and creating 100 imputed datasets. We included the following variables to impute outcome data: age, gender, blood type, treatment group, cause of death, history of hypertension, PHS increased infectious risk, troponin, and natural log VIS score at baseline.

## As-Treated Analysis

Although our primary analysis was in the intention-to-treat (ITT) population, there were some donors who were randomized to levothyroxine (active drug) but never started on the assigned treatment. As shown in Table S4, this occurred in only eight (2%), primarily due to donor instability or due to the donors no longer being hemodynamically unstable by the time the drug arrived at the bedside. These donors were still included in the ITT analysis even though they were not treated with levothyroxine. Similarly, there were three donors amongst those assigned to the control group (normal saline) who, instead, were given levothyroxine (i.e. protocol violations). These three donors were very hemodynamically unstable, on multiple pressors, and the local sites felt that levothyroxine was needed to stabilize them. Nonetheless, per ITT, these were analyzed as if they had not received levothyroxine (i.e. in the saline group). Finally, control donors were permitted to receive open-label levothyroxine, per protocol, after the 12 hours of saline infusion had completed (reasons in Table S6, primarily for persistent hemodynamic instability, remaining on vasopressors). Once again, per ITT, these 50 donors who received quite a large cumulative dose of levothyroxine (not dissimilar to the doses given to those assigned to levothyroxine from the start) were analyzed as controls. If hearts were transplanted from such donors, it would count as a primary outcome for the control group but could potentially be attributed to a positive effect of levothyroxine treatment. Therefore, given the concern that the ITT analysis might dilute the true efficacy of thyroxine on potential heart donors, when given as intended, we undertook an as-treated analysis.

We assigned all those in the levothyroxine group who actually began on the infusion as well as those in the saline group who crossed-over to receive levothyroxine either as a protocol violation within 12 hours or those who received open-label levothyroxine per-protocol after 12 hours to the "levothyroxine-treated" group in this analysis. In contrast, we assigned all those donors, regardless of randomization group, who did not receive any levothyroxine to the "untreated" or control group for this analysis. This included those in the levothyroxine group who did not start treatment and those in the saline group who never crossed over to receive any levothyroxine. We further excluded from this analysis those donors were ineligible, but were otherwise included in the ITT (see Table S2), to evaluate the optimal efficacy of levothyroxine in the intended donor population.

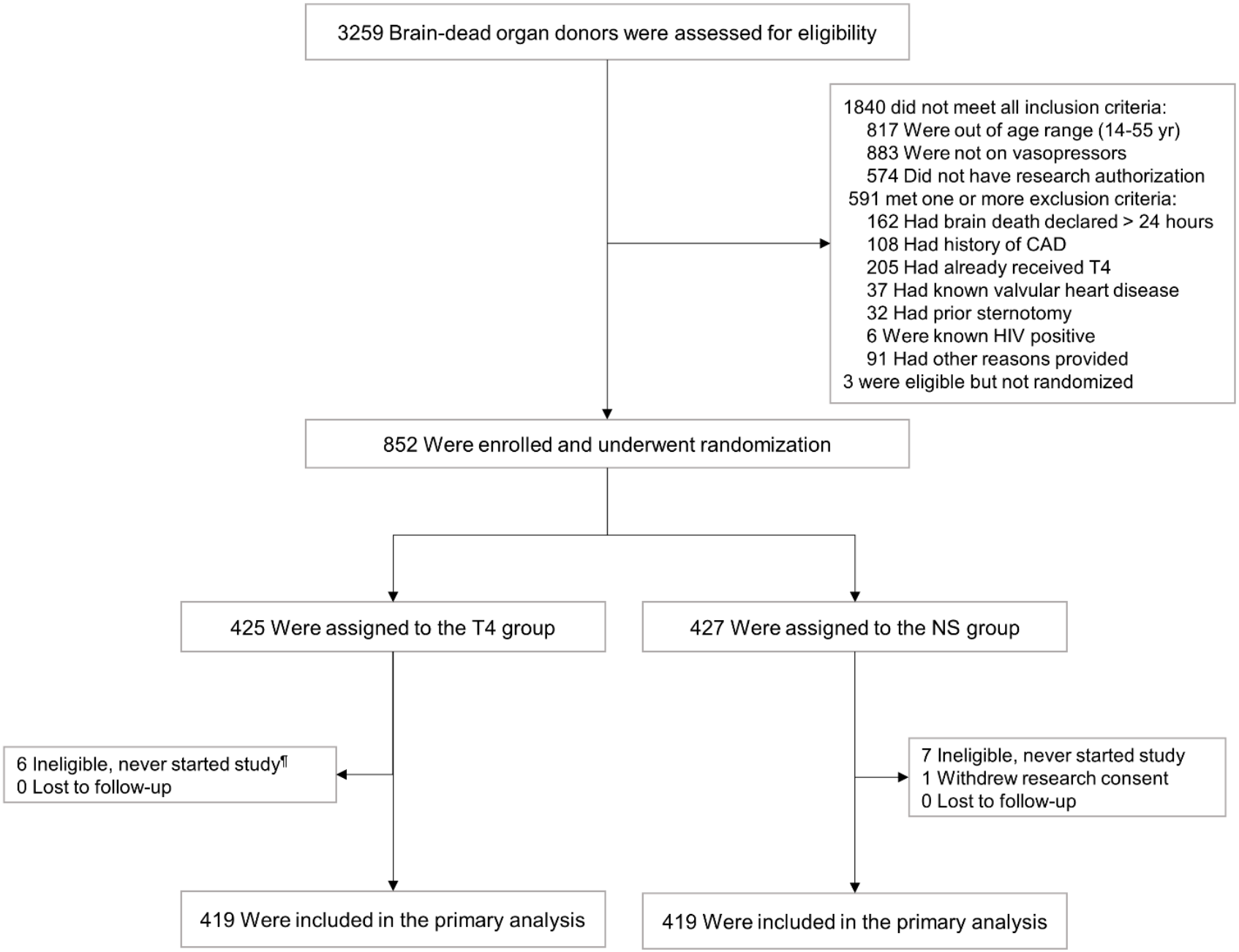
The results of the as-treated analysis are shown in Table S8. However, caution should be exercised in interpreting this post-hoc analysis as it is most likely to be subject to confounding due to covariate imbalance (i.e. those who were actually treated with levothyroxine are likely to be clinically different from those who did not receive levothyroxine, in important ways) and could be biased toward showing the most optimistic signal of treatment. Further, like the per-protocol analysis, it does not reflect the real-world effects of using levothyroxine in potential organ donors, where adverse events and non-adherence would inevitably occur.

## Interim Analysis

The DSMB charter proposed that an interim analysis would occur when 376 donors have been enrolled with evaluable data, as determined by the study data coordinator, Dean Klinkenberg. The DSMB will be provided data on primary outcome measures: hearts transplanted, recipient 30-day graft survival, by group. The DSMB statistician (Mark Schnitzler) will independently analyze this data for signs of superiority or inferiority of T4 for either endpoint (at $p<0.01$). The DSMB will meet and review the results of this analysis and forward their judgement on whether to stop or continue the trial. The trial will be terminated if either risk to the recipient is identified (i.e. inferior graft survival) or if there is clear superiority or inferiority of T4 treatment (at alpha 0.01) on hearts transplanted.

A total of 400 donors were accrued by the end of January 2022. Graft survival data was obtained in March 2022 for hearts transplanted and data was forwarded to the DSMB in mid-March. The interim data included 416 donors enrolled and randomized, including five that were mistakenly randomized and never eligible (excluded). Therefore 411 donors were included in the interim analysis, 208 in the NS group and 203 in the T4 group. Hearts were transplanted from 218 donors (53%), including 115 (56.7%) of those in the T4 group and 103 (50%) of the NS group. Unadjusted comparison of heart transplant rates using a chi-square test found a p-value of 0.18. After adjusting for age and blood type, study group was not associated with hearts transplanted (Odds Ratio 0.73, 95% confidence interval 0.46-1.18, $p=0.20$). Recipient heart graft survival at 30-days (excluding hearts transplanted in January 2022, for whom data was not available) was seen in 110 out of 112 hearts in the T4 group (98.2%) compared with 95 out of 98 hearts in the NS group (96.9%). There was no significant difference in the rate of 30-day graft survival ($p=0.67$). Based on review of these data and results, the DSMB voted unanimously to continue the study.

**Figure S1: Screening, Randomization, and Follow-up of Brain-Dead Donors**

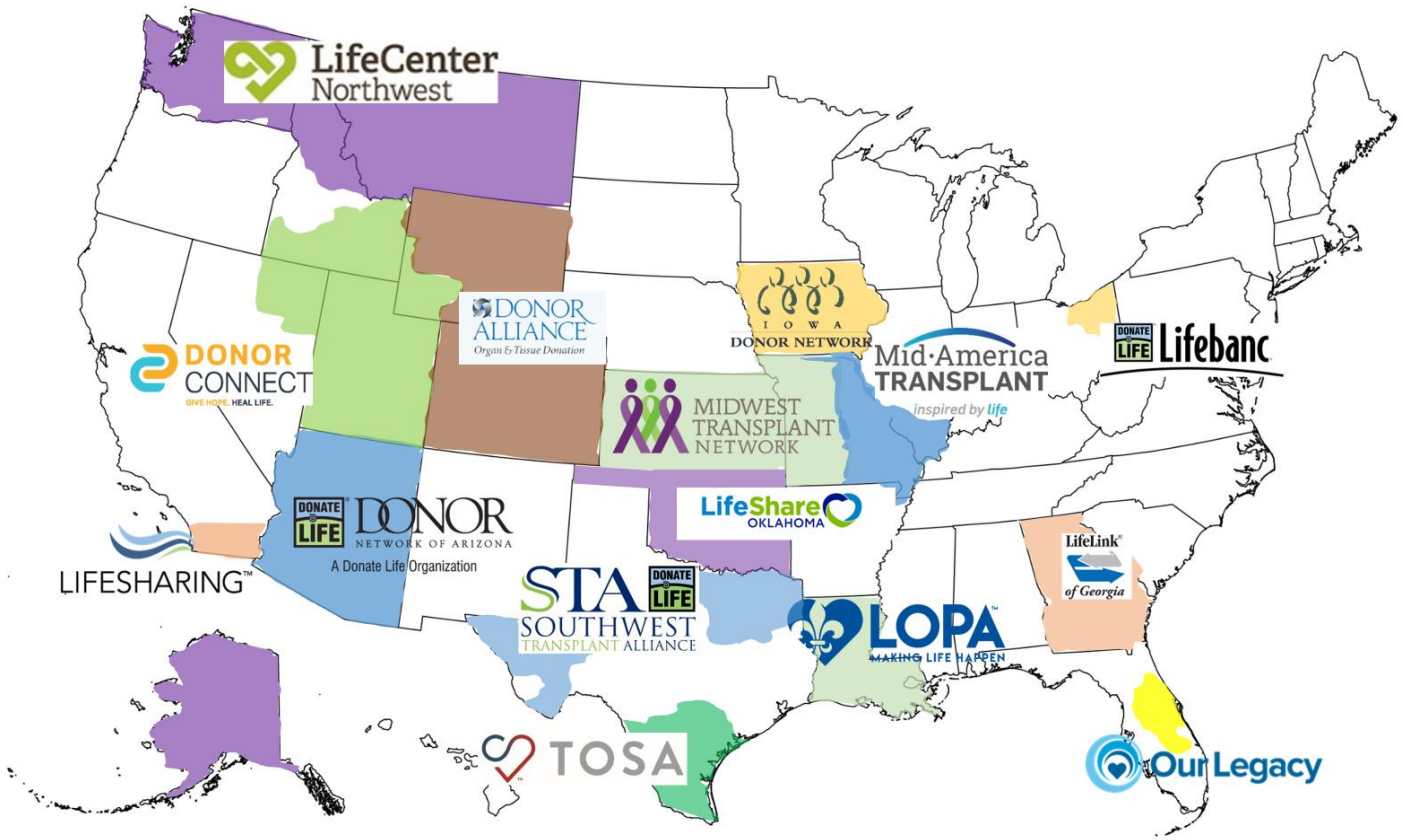


All brain-dead organ donors were screened at 15 participating organ procurement organizations. Donors who were screened could be ineligible for more than one reason (i.e., numbers for each ineligibility add up to more than the total of 1840 who did not meet all inclusion criteria and the number with each exclusion criteria add up to more than 591). The T4 group received levothyroxine infusion and the NS group received an equivalent volume of normal saline. Randomization was stratified by site. Only three eligible donors were not enrolled on review of screening logs.

¶ In thirteen cases (six in the T4 group and seven in the NS group), donors were randomized but sites realized that they were ineligible immediately after enrollment and randomization. The study protocol was never initiated in these donors and data was not collected. These mistakenly randomized donors are excluded from the intention-to-treat cohort. Twelve other donors were enrolled, received assigned study drug, but were subsequently determined to be ineligible on central adjudication of eligibility criteria. These donors were included in the intention-to-treat analysis. Reasons for these 25 donors' ineligibility is provided in Table S2. None of those enrolled were lost to follow-up but one family withdrew authorization for research and so this donor was removed from the analysis cohort. Three donors assigned to NS received levothyroxine instead; these crossovers occurred when sites overruled the assigned group due to severe donor instability. These crossovers were analyzed in the NS group by intention-to-treat.

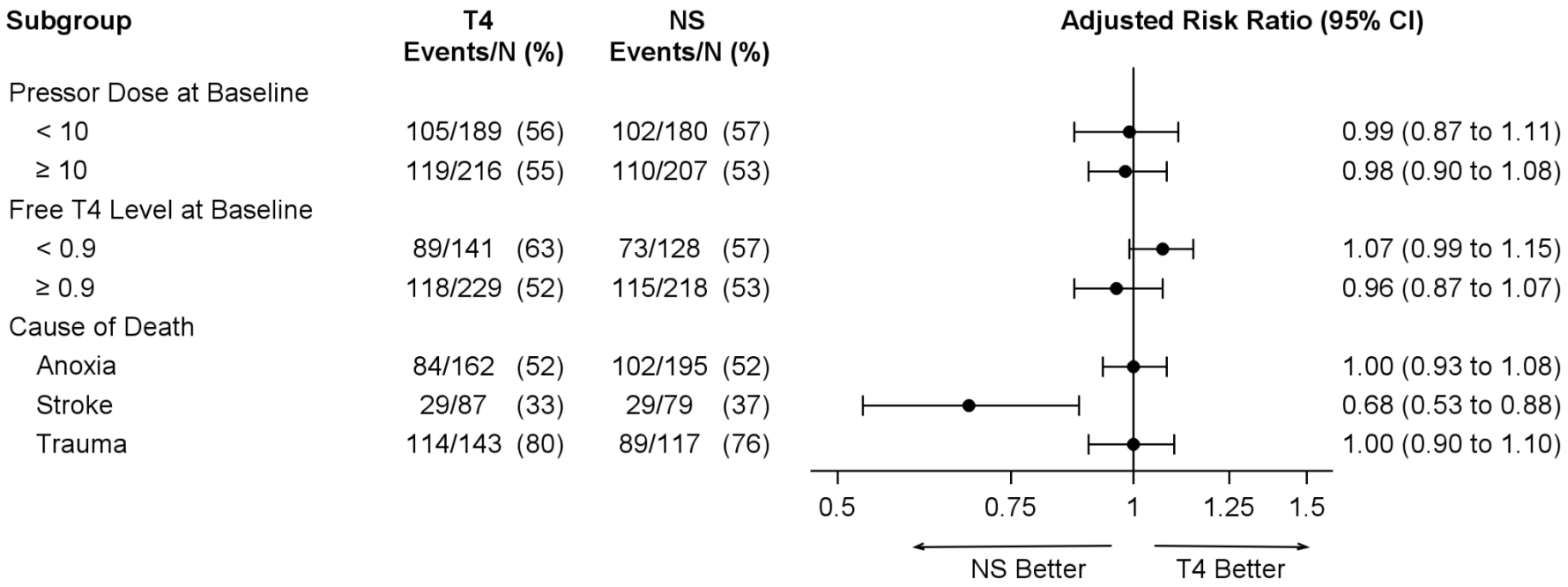
T4 denotes levothyroxine, CAD coronary artery disease, NS normal saline.

## Figure S2: Map of Participating Organ Procurement Organizations



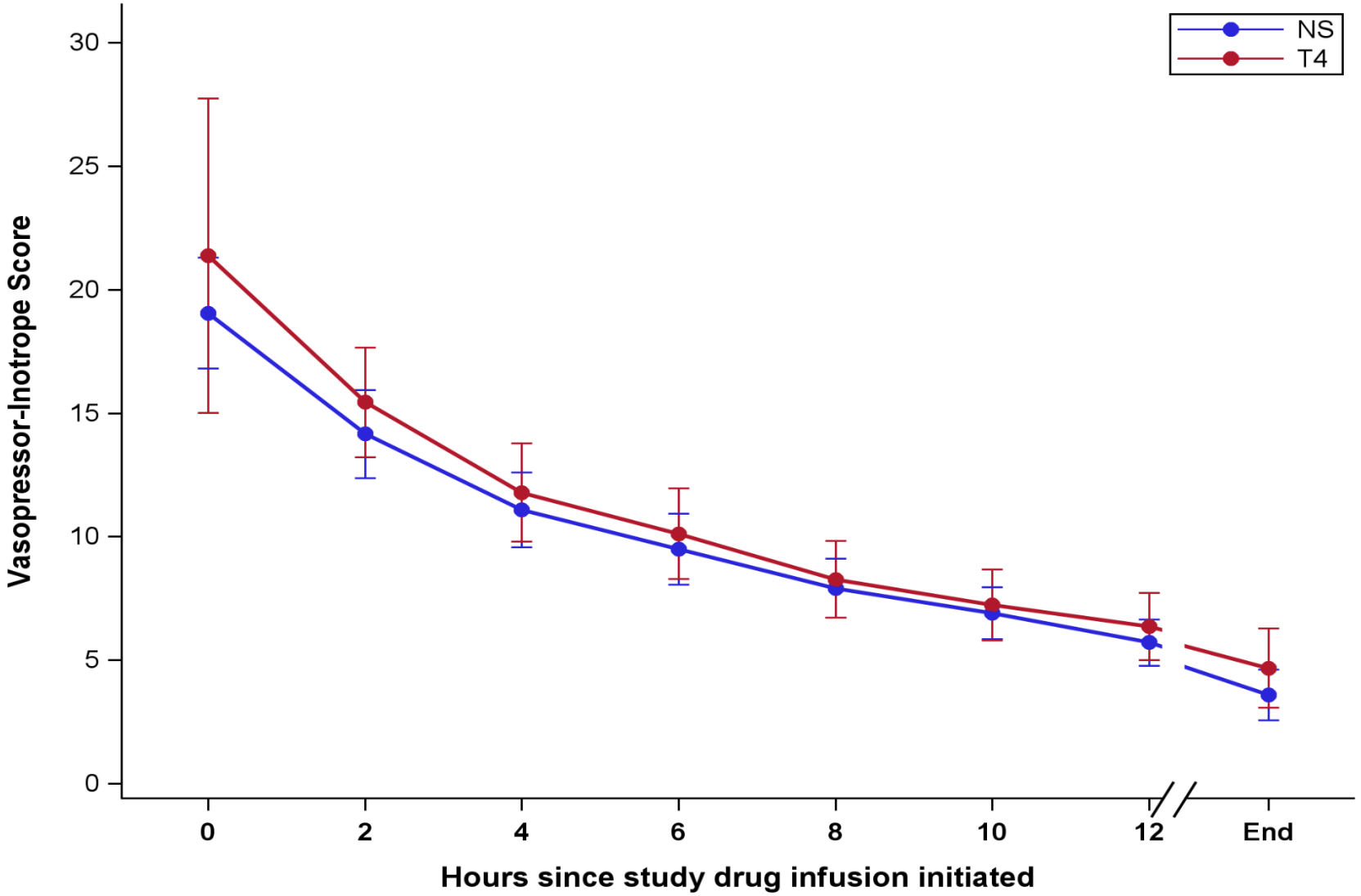

The donor service areas of the fifteen participating OPOs, with their logos.

## Figure S3: Post-Hoc Subgroup Analyses of Hearts Transplanted



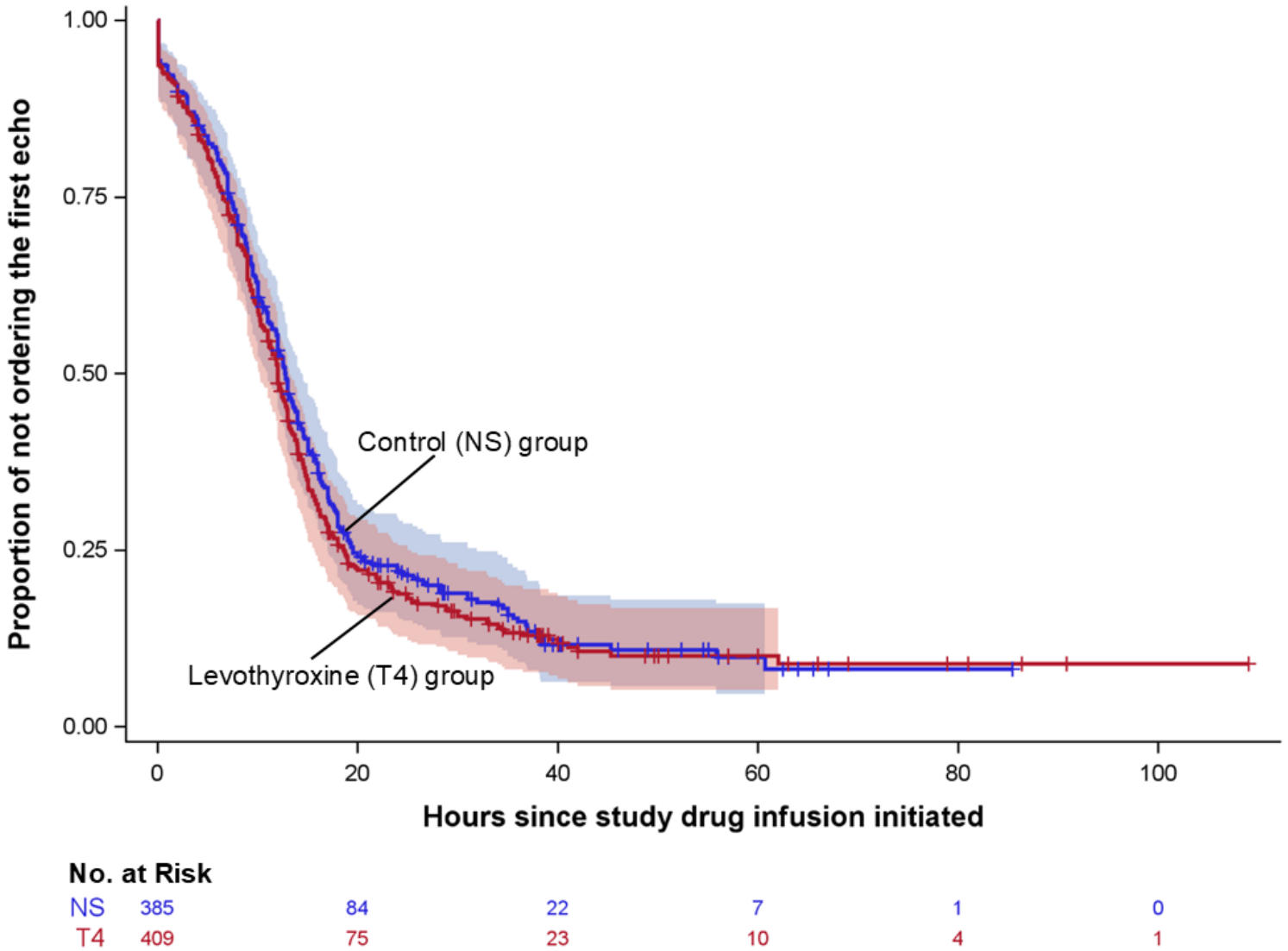

Forest plots showing analyses of post-hoc subgroups for primary outcome of hearts transplanted between treatment groups. The trial was not powered and had no prespecified correction for multiple comparisons; the widths of confidence intervals have not been adjusted for multiplicity and may not be used in place of hypothesis testing. T4 denotes levothyroxine and NS denotes normal saline.

**Figure S4: Vasopressor-Inotrope Score over Time**




Mean vasopressor-inotrope score (with 95% confidence intervals) for donors in each study group (by intention-to-treat) at serial study time points from start of infusion of study drug to the end of donor management. The widths of confidence intervals have not been adjusted for multiplicity and should not be used in place of hypothesis testing.

**Figure S5: Survival Curves for Time to Order First Echocardiogram**




Kaplan-Meier estimates for time to order first echocardiogram (as surrogate for donor hemodynamic stabilization) from start of study drug infusion, in the intention-to-treat population, by study group (NS denotes normal saline) with 95% equal-precision confidence bands. The proportional hazards assumption was satisfied. Hazard ratio was 1.09 (95% CI 0.93 to 1.27). The width of the confidence intervals have not been adjusted for multiplicity and should not be used in place of hypothesis testing.

## Table S1: Characteristics of Participating Organ Procurement Organizations

| OPO CODE | MOMA | TXSB | TXSA | CASD | MWOB | IAOP | AZOB | CORS | OHLB | FLFH | LAOP | UTOP | WALC | GALL | OKOP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCREENED** | 399 | 594 | 148* | 191 | 189 | 138 | 324 | 216 | 125 | 281 | 242 | 69 | 243 | 102 | 20* |
| **ENROLLED** | 116 | 105 | 49 | 26 | 85 | 41 | 41 | 70 | 46 | 88 | 54 | 14 | 80 | 35 | 2 |
| **THYROID HORMONE PRE-STUDY** | No | Yes T4 | Yes T4 | Yes T4 | Yes T4 | Yes T4 | Yes T4 | Yes T4 | Yes T4 | Yes T4 | No | No | Yes T4 | Yes T4 | Yes T4 |
| **T4 TO WHICH DONORS** | N/A | 2 + 3 or 4 | 2 + 3 or 4 | 1 | 1 | 4 | 2 + 4 | 1 | 2, 3 | 1 | N/A | N/A | 2 | 2 + 4 | 1 |
| **CORTICO-STEROID** | Low-dose HC | Low-dose HC | Low-dose HC | High-dose MP | High-dose MP | High-dose MP | High-dose MP | High-dose MP | High-dose MP | High-dose MP | Low-dose HC | High-dose MP | High-dose MP | MP 50 mg/hr | High-dose MP |
| **RECOVERY FACILITY** † | Yes Indep | Yes Indep | Yes Tx | No | Yes Indep | No | No | Yes Indep | Yes Indep | No | Yes Indep | Yes Tx | No | Yes Tx | No |
| **HEMODYNAMIC MONITORING** ¶ | PCA | PCA / NICOM | NICOM | PCA | PCA | -- | NICOM | NICOM | PCA / NICOM | PCA | NICOM | PCA | -- | PCA | -- |
| **TTE READ BY** ‡ | Expert | Remote | Remote | Remote | Local / Remote /Expert | Local | Local | Local / Remote | Local / Remote | Local /Expert | Remote | Local / Remote | Local | Local / Remote | n/a |

(OPOs listed using OPTN/UNOS abbreviations)

HC = hydrocortisone, low-dose is 300 mg then 100mg q8; high-dose MP = methylprednisolone 500-1000mg once or repeated at six-hourly intervals

T4 used for which donors: 1 = all braid-dead donors, 2 = only heart-eligible donors, 3 = donors with poor cardiac function, 4 = donors requiring vasopressors

† Recovery Facilities were either: Indep = Independent stand-alone facilities; Tx = Transplant-center associated facilities.

¶ Hemodynamic monitoring: PCA, pulse contour analysis (e.g. FloTrac®); NICOM, non-invasive cardiac output monitor (e.g. Starling™ SV by Cheetah Medical)

‡ TTE = transthoracic echocardiography: Local = read by cardiologist at local donor hospital; Remote = read by national service of centralized cardiologists; Expert = read by expert transplant cardiologist.

*TXSA participated in the study from January 2021 through May 2022 while OKOP participated for only two months (April-May 2021) before terminating their involvement, both due to logistical and staffing limitations during the COVID-19 pandemic.

## Table S2 Reasons for Ineligibility amongst Donors Randomized but Ineligible

| Reason | Mistakenly Randomized but not Studied (Excluded from Study) Total number of donors = 13 | | Included in Study but Ineligible (Included in Intention-to-Treat) Total number of donors = 12 | |
|---|---|---|---|---|
| | T4 Group | NS Group | T4 Group | NS Group |
| **Age out of range** | 1 | 1 | 1 | 0 |
| **Not on vasopressors** | 0 | 4 | 0 | 2 |
| **No research authorization** | 2 | 0 | 0 | 0 |
| **Heart ineligible** | 1 | 1 | 1 | 0 |
| **Time from BD > 24 hours** | 0 | 0 | 4 | 1 |
| **Received T4 prior** | 1 | 1 | 2 | 1 |
| **Allergy to T4** | 1 | 0 | 0 | 0 |
| **Total** | 6 | 7 | 8 | 4 |

## Table S3: Additional Baseline Donor Characteristics by Group

| Characteristic | T4 Group (N=419) | NS Group (N=419) |
|---|---|---|
| **Median time from hospital admission to declaration of death (IQR) – days** | 2.1 (1.2-3.3) | 2.0 (1.2-3.7) |
| **Coexisting conditions – no (%)** | | |
| **Hypertension** | 98 (24) | 104 (26) |
| **Diabetes mellitus** | 38 (9) | 31 (8) |
| **Substance use history – no (%)** | | |
| **Cigarette use** | 58 (14) | 76 (19) |
| **Cocaine use** | 92 (23) | 105 (26) |
| **Other drug use** | 229 (57) | 236 (59) |
| **PHS increased infectious risk – no (%)** | 90 (22) | 80 (20) |
| **Anthropometric measurements** | | |
| **Height – cm** | 173.4 ± 9.5 | 172.3 ± 10.8 |
| **Weight – kg** | 85.6 ± 24.1 | 85.4 ± 23.3 |
| **Body Mass Index – kg/m$^2$** | 28.4 ± 7.7 | 28.9 ± 7.7 |
| **Laboratory values ¶** | | |
| **Median bilirubin level (IQR) – mg/dL** | 0.7 (0.4-1.1) | 0.6 (0.4-1.1) |
| **Median ALT level (IQR) – IU/L** | 58 (28-159) | 61 (28-161) |
| **Median urea (IQR) – mg/dL** | 17 (12-27) | 19 (12-29) |
| **Median amylase (IQR) – U/L** | 70 (37-147) | 69 (35-166) |
| **Blood pressure – mm Hg** | | |
| **Systolic** | 120 ± 20 | 119 ± 19 |
| **Diastolic** | 69 ± 14 | 69 ± 14 |
| **Heart rate – bpm** | 97± 17 | 97 ± 18 |
| **On vasopressin – no (%)** | 202 (50) | 202 (52) |
| **On vasopressors alone – no (%)** | 380 (96) | 370 (96) |
| **On inotropes alone – no (%)** | 2 (0.1) | 1 (<0.1) |

Plus-minus values are means ± standard deviation; IQR represents the interquartile range, when presenting medians

Abbreviations: bpm, beats per minute; LV, left ventricular; PHS, U.S. public health service; T4, levothyroxine

The numbers with missing data are: time from admission to death, 2 in the T4 group, 6 in the NS group; history of hypertension, diabetes, cigarette/cocaine/drug use, PHS increased risk, height and BMI, 16 in the T4 group, 22 in the NS group; weight, none in the T4 group, 2 in the NS group; bilirubin, 5 in the T4 group, 14 in the NS group; ALT, 3 in the T4 group, 13 in the NS group; urea, 2 in the T4 group, 11 in the NS group; amylase, 67 in the T4 group, 93 in the NS group; blood pressure, heart rate, vasopressin use, 13 in the T4 group, 31 in the NS group; on vasopressors/inotropes, 9 in the T4 group, 27 in the NS group.

¶ The normal range for bilirubin is approx. 0.2-1.3 mg/dL, for ALT approx. 7-55 IU/L, for urea approx. 6-20 mg/dL, for amylase approx. 30-110 U/L.

## Table S4 Representativeness of Donors Enrolled in the Study

| Category | |
|---|---|
| **Disease, problem, or condition under investigation** | Potential heart donors after brain death who remain hemodynamically unstable despite initial fluid resuscitation |
| **Special considerations related to:** | |
| **Sex and gender** | More males become potential heart donors, likely due to a higher incidence of deaths from head trauma and anoxia in males; the proportion of females in this study (35%) is slightly but not substantially higher than the national data on all heart donors (29%; obtained from SRTR data, 2022). |
| **Age** | The vast majority of heart donors are adults under 50 years of age (fewer than five percent are over 50 and approx. ten percent are under the age of 18); in this study we enrolled from ages 14-55 years and had a similar distribution of ages to the national data except slightly more with age above 50 (13% vs. 4%), due to our inclusion of donors up to age 55 years. |
| **Race or ethnic group** | The majority of heart donors are white, with those of Hispanic ethnicity and those of black race each representing 15% of donors, similar to their distribution in the U.S. population; we enrolled a representative distribution of race and ethnicity. |
| **Geography** | Potential organ donors are evaluated and managed by 56 organ procurement organizations in the United States; we enrolled donors from 15 of these OPOs. As shown in Figure S1, we captured a wide geographic representation of the nation. Some regions (e.g. the Northeast, California) are likely under-represented. |
| **Cause of death** | A majority of brain dead potential heart donors die from head trauma or anoxia (e.g. overdose deaths). In recent years, anoxia has risen to represent the cause of almost half of all deaths amongst heart donors, with trauma representing 40% and stroke 10%. Our study had similar proportions, though a slightly higher proportion who died of stroke (likely due to inclusion of age to up to 55 years). |
| **Source of data** | These data were abstracted from the SRTR donor database, as reported to the OPO personnel by the patient's legal representative or extracted from the medical record based on patient self-report |
| **Other considerations** | We selected only hemodynamically unstable donors, which is a relatively large subgroup of all potential heart donors, but might introduce some bias (for example, less hearts are expected to be transplanted from the subset of unstable donors than all donors of a similar age); this may explain why our proportion of hearts transplanted (54%) was lower than the SRTR expected heart yield (approx. 60%), as that yield model does not take into account the vasopressor utilization/instability. |
| **Overall representativeness of this trial** | The donors enrolled in this trial are broadly representative of the potential heart donors nationwide, in terms of age, gender, and race/ethnicity. We captured a relatively representative geographic subgroup of all donors by enrolling many OPOs from different regions. It is difficult to comment on broader generalizability to organ donors globally, though we believe that the distribution of ages, genders, and causes of death would be similar. |

Abbreviations: OPO, Organ Procurement Organization; SRTR, Scientific Registry of Transplant Recipients

## Table S5 Reasons for Donors Randomized Not Starting on Assigned Treatment

| Reason | T4 Group<br>Number of donors = 8 | NS Group<br>Number of donors = 36 |
|---|---|---|
| **Stable, not requiring vasopressors**† | 4 | 7 |
| **Unstable, case aborted early** | 1 | 5 |
| **Unstable, tachycardia** | 1 | 0 |
| **Unstable, cross-overs** ‡ | 0 | 3 |
| **Missed / logistic delays** | 2 | 13 |
| **Case aborted for other reason** | 0 | 3 |
| **Volume overload / hypernatremia** | 0 | 5 |

† Eligible (on vasopressors) at time of randomization, but weaned off vasopressors by time study infusion was prepared, therefore infusion was not started

‡ Unstable at time of randomization, local OPO site decided to start T4 despite assignment to NS group

## Table S6 Reasons for Early Weaning and Discontinuation of Levothyroxine

| Reason | Weaning (n=47) | Discontinuation (n=58) |
|---|---|---|
| **Tachycardia** | 20 | 23 |
| **Hypertension** | 24 | 33 |
| **Atrial Fibrillation/Flutter** | 0 | 3 |
| **Supraventricular Tachycardia** | 1 | 1 |
| **Ventricular Tachycardia** | 0 | 2 |
| **Ectopy** | 1 | 1 |
| **Fluid Overload / Electrolytes** | 0 | 2 |
| **Logistic / Inadvertent** | 3 | 5 |
| **Case Aborted Early** | 0 | 4 |

Note: more than a single reason could be provided

Inadvertent represents cases where the infusion was changed or stopped without a clear reason for the change

## Table S7: Reasons for Open-Label or Extended Use of Levothyroxine

| Reason | Extension in T4 Group (n=209) | Open Label in NS Group (n=50) |
|---|---|---|
| **Remains on Vasopressors or Inotropes** | 102 | 40 |
| **OPO Preference** | 66 | 3 |
| **Transplant Center Request** | 0 | 2 |
| **Hypotension / attempting to avoid vasopressors** | 9 | 1 |
| **Doing well / stable on levothyroxine** | 3 | NA |
| **Cardiac wall motion defects** | 1 | 3 |
| **Trying to allocate heart** | 2 | 0 |
| **Logistic / Miscommunication** | 11 | 0 |
| **No reason provided** | 25 | 1 |

## Table S8: Primary Outcomes – Intention-to-Treat with additional covariate adjustment, Per-Protocol and As-Treated Analyses

### *Primary Outcome: Hearts Transplanted*

| Analysis | Overall ‡ | T4 Group ‡ | NS Group ‡ | Adjusted RR (95% CI) |
|---|---|---|---|---|
| **ITT (modified)*** | 453/838 (54.1%) | 230/419 (54.9%) | 223/419 (53.2%) | 0.98 (0.92 to 1.06) |
| **Per-Protocol** † | 390/725 (54.6%) | 192/351 (54.7%) | 204/374 (54.5%) | 0.98 (0.93 to 1.03) |
| **As-Treated** ¶ | 448/825 (54.3%) | 253/454 (55.7%) | 195/371 (52.6%) | 1.01 (0.93 to 1.11) |

All analyses were performed using modified Poisson regression, adjusting for age and blood type, clustered according to site with robust standard errors.

‡ Numbers represent those with hearts transplanted out of total number in each group

* The ITT analysis presented here adjusted for the additional covariates of gender, troponin level, cause of death, history of hypertension, and PHS (Public Health Service) increased infectious risk category.

### *Primary Safety Outcome: Recipient 30-day Graft Survival*

| Analysis | Overall ‡ | T4 Group ‡ | NS Group ‡ | Difference (T4-NS) (95% CI) |
|---|---|---|---|---|
| **Per-Protocol** † | 382/396 (96.5%) | 186/192 (96.9%) | 196/204 (96.1%) | 0.8% (-3.4% to 4.9%) |
| **As-Treated** ¶ | 432/448 (96.4%) | 245/253 (96.8%) | 187/195 (95.9%) | 0.9% (-3.1% to 5.0%) |

Recipient 30-day graft survival was assessed by estimating the between-group difference in proportion surviving and 95% confidence intervals to evaluate non-inferiority of the T4 group at a six percent margin. Non-inferiority was satisfied for both per-protocol and as-treated analyses.

‡ Numbers represent those with graft survival out of total number of heart recipients in each group

Footnotes for Both Tables:

† Per-Protocol groups represent those who were eligible and received at least six hours of their assigned intervention.

¶ As-Treated groups: those in the T4 column represent those who were eligible and who actually received levothyroxine (either as assigned or as cross-over or open-label from those assigned to normal saline) while those in the NS column represent those eligible for the study who did not receive any levothyroxine.

T4 represents the levothyroxine group and NS the normal saline (control) group.

Note: Widths of confidence intervals have not been adjusted for multiplicity and should not be used in place of hypothesis testing.

## Table S9 Reasons for Hearts not being Transplanted

| Reason | T4 Group (n=189) | NS Group (n=196) |
|---|---|---|
| Poor EF on Echocardiography | 52 | 59 |
| Coronary Artery Disease | 24 | 21 |
| LVH on Echocardiography | 13 | 14 |
| Valvular disease / anatomy | 10 | 6 |
| Size | 4 | 21 |
| Medical comorbidity | 29 | 28 |
| Limited/No Authorization | 4 | 5 |
| Positive serologies | 14 | 10 |
| COVID-19 positive | 3 | 4 |
| Increased risk donor | 7 | 6 |
| Donor instability (cardiac arrest) | 9 (1) | 9 (2) |
| Case aborted | 8 | 7 |
| Match list exhausted | 31 | 43 |
| Logistic issues | 3 | 5 |
| Recipient issues | 1 | 2 |
| Intra-operative decline | 9 | 4 |
| Surgical injury | 0 | 0 |

Abbreviations: EF, ejection fraction; LVH, left ventricular hypertrophy

## Table S10: Secondary Outcomes using Complete-Case Analysis

| Outcomes | T4 Group | NS Group | Treatment Effect (95% CI) |
|---|---|---|---|
| Weaned off vasopressors at 12-hours – no (%) | 143 (35) | 152 (39) | 0.91 (0.80 to 1.04) ‡ |
| VIS at 12 hours – geometric mean with 95% CI | 3.5 (3.0-4.1) | 3.3 (2.9-3.9) | 1.05 (0.92 to 1.2) ¶ |
| VIS at organ recovery – geometric mean with 95% CI | 2.1 (1.9-2.4) | 2.3 (2.0-2.6) | 0.93 (0.81 to 1.08) ¶ |
| LVEF, first echocardiogram -- % | 59 ± 11 | 58 ± 12 | 1.0 (-0.6 to 2.7) † |
| LVEF, maximum of all echocardiograms -- % | 60 ± 9 | 60 ± 10 | 0.6 (-0.9 to 2.0) † |

Plus-minus values are means ± standard deviations. 95% CI represents the 95 percent confidence interval

LVEF denotes left ventricular ejection fraction, VIS denotes vasopressor-inotrope score

‡ Adjusted Risk Ratio from modified Poisson regression model, clustered according to site with robust standard errors.

¶ Geometric mean ratio of vasopressor-inotrope score from linear mixed model, adjusting for baseline VIS and random site effect.

† Difference represents estimate from linear mixed model, adjusting for random site effect.

Note: Widths of confidence intervals have not been adjusted for multiplicity and should not be used in place of hypothesis testing.

## Table S11 Reported Adverse Events

| Type of Adverse Event | T4 Group (n=419) | Saline Group (n=419) | P value |
|---|---|---|---|
| Number of donors with adverse events – no (%) | 51 (12)* | 16 (4) | <0.001 |
| Severe Hypertension – no (%) | 26 (6)* | 5 (1) | <0.001 |
| Tachycardia, incl. SVT – no (%) | 16 (4)* | 3 (1) | 0.003 |
| Atrial Fibrillation – no (%) | 7 (2) | 3 (1) | 0.2 |
| Ventricular Ectopy – no (%) | 5 (1) | 3 (1) | 0.73 |
| Ventricular Tachycardia – no (%) | 4 (1) | 1 (<1) | 0.37 |
| Cardiac arrest -- no (%)<br>(number with donor loss)† | 2 (<1)<br>2 | 3 (1)<br>0 | >0.999 |
| Fever | 0 | 0 | NA |
| Rash – no (%) | 1 (<1) | 0 (<1) | >0.999 |
| Other reported events – no (%) ‡ | 3 (1) | 2 (<1) | >0.999 |
| Total number of adverse events | 64 | 20 | <0.001 |

Abbreviations: SVT, supraventricular tachycardia

* There was a significantly higher incidence of donors with adverse events (risk difference 8.4%, 95% CI 4.7 to 12%), as well as higher incidence of severe hypertension (risk difference 4.8%, 95% CI 2.3 to 7.3) and tachycardia (risk difference 3.1%, 95% CI 1.1 to 5.1); all $P < 0.01$

Additional notes: Of the cases with VT, one in each group required cardioversion (both occurred during a procedure, one during organ recovery and the other during heart catheterization)

† Cardiac arrest and/or donor loss due to hemodynamic instability or arrhythmia were considered **serious adverse events** for purposes of this study; of the two cardiac arrests in the levothyroxine group, neither was adjudicated to be related to the study infusion – one occurred prior to study commencing and the infusion was never started, in the other the donor was very unstable even prior to starting levothyroxine and had cardiac arrest after infusion was discontinued; in the NS group, one occurred prior to starting the protocol and the case was abandoned before the study was even started; in the other two, arrests occurred days after the protocol was completed (one was transient, only lasting a few minutes during a blood draw).

‡ Other adverse events reported included: sodium above 200 mEq/L, asthma exacerbation (both in T4 group), hypotension (1 in each group), flash pulmonary edema (1 in the NS group)

# References


1. Peled Y, Ram E, Klempfner R, Lavee J, Cherikh WS, Stehlik J. Donor thyroid hormone therapy and heart transplantation outcomes: ISHLT transplant registry analysis. J Heart Lung Transplant 2020;39(10):1070-1078. DOI: 10.1016/j.healun.2020.06.005.
2. Peled Y, Lavee J, Kassif Y, et al. Donor thyroid hormone therapy is associated with an increased risk of graft dysfunction after heart transplantation. Clin Transplant 2020;34(7):e13887. DOI: 10.1111/ctr.13887.
3. Ardehali A, Esmailian F, Deng M, et al. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. Lancet 2015;385(9987):2577-84. DOI: 10.1016/S0140-6736(15)60261-6.