Research Square

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Surgical complexity and perioperative cell-free Deoxyribonucleic Acid in children undergoing cardiac surgery

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Abstract

OBJECTIVES

Circulating cell-free Deoxyribonucleic Acid (cfDNA) rises after paediatric cardiac surgery and has been linked to adverse outcomes, but its relationship with procedural complexity is unknown. We examined whether total and cardiomyocyte-derived cfDNA are associated with the Society of Thoracic Surgeons–European Association for Cardio-Thoracic Surgery (STS-EACTS) mortality category throughout the perioperative period.

METHODS

This was a secondary analysis of a prospective cohort of 50 children undergoing cardiac surgery with cardiopulmonary bypass. Total and cardiac-specific (methylation-based) cfDNA were measured before surgery and 0, 6, 12 and 24 h after bypass. Associations with STS-EACTS category (1–5) were estimated with linear mixed-effects models of log-transformed cfDNA adjusted for age, supported by per-time-point regressions with false-discovery-rate correction.

RESULTS

STS-EACTS categories were 1–2 in 20 (40%), 3 in 9 (18%) and 4–5 in 21 (42%) patients. Each one-category increase was associated with 30–47% higher total cfDNA across time points (overall $P = 0.022$; time interaction $P = 0.89$), including before surgery (geometric mean ratio [GMR] 1.30, 95% CI 1.01–1.66). After adjustment for preoperative levels, postoperative total cfDNA remained 38% higher per category (GMR 1.38, 95% CI 1.13–1.68; $P = 0.001$), most clearly at 12 and 24 h. The immediate bypass-related rise was not associated with complexity. Cardiac-specific cfDNA was not associated with STS-EACTS category at any time point (overall $P = 0.90$).

CONCLUSIONS

Higher surgical complexity is associated with persistently higher total, but not cardiomyocyte-derived, cfDNA, from before surgery through 24 h after bypass. Studies of cfDNA as a perioperative biomarker should account for procedural complexity.

INTRODUCTION

Cardiac surgery with cardiopulmonary bypass (CPB) in children triggers a systemic inflammatory response and tissue injury whose magnitude varies widely between patients [1–3]. Quantifying this injury early, and understanding what drives it, remains a practical problem for perioperative care.

Circulating cell-free DNA (cfDNA) consists of DNA fragments released into plasma mainly by dying cells [4, 5]. Plasma cfDNA concentrations rise in critical illness and carry prognostic information for mortality [6, 7]. Because DNA methylation patterns are cell-type specific, the tissue of origin of cfDNA can be identified, allowing cardiomyocyte-derived cfDNA to be measured separately from the total pool [8–10]. cfDNA increases during and after cardiac surgery with CPB [11], and in infants and children, elevated nuclear cfDNA has been associated with death and other adverse events [12, 13]. In the cohort analysed here, cardiomyocyte-specific methylation markers revealed delayed cardiac cell death after open-heart surgery [14], and early postoperative cfDNA reflected renal and hepatic injury [15].

Procedural complexity is the principal determinant of risk after congenital heart surgery and is quantified with consensus-based and empirically derived tools, including the Risk Adjustment for Congenital Heart Surgery, the Aristotle score and the STS-EACTS mortality score and categories [16–20]. Whether cfDNA concentrations follow procedural complexity has not been examined. The question matters in two ways. If cfDNA largely mirrors complexity, its prognostic value must be judged against established risk scores rather than in isolation. Conversely, an association that persists after accounting for preoperative levels would suggest that more complex operations cause more cellular injury.

We therefore examined the association between STS-EACTS mortality category and total and cardiomyocyte-derived cfDNA at each perioperative time point, and distinguished differences present before surgery from those arising after CPB.

PATIENTS AND METHODS

Ethics statement

The study was approved by the Institutional Review Board of Sheba Medical Center (approval no. 4993-18-SMC). Written informed consent was obtained from the parents or legal guardians of all participants.

Study design and patients

This is a secondary analysis of a prospective observational cohort of 50 children (age < 18 years) who underwent cardiac surgery with CPB at the Edmond J. Safra International Congenital Heart Center, Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Israel, between August 2017 and August 2018. Earlier reports from this cohort addressed cardiomyocyte death kinetics and the relationship of cfDNA with renal and hepatic injury [14, 15]; the association between cfDNA and procedural complexity has not previously been reported. The study is reported according to the STROBE statement [21].

Surgical complexity

Each operation was assigned an STS-EACTS mortality category (1–5) [18]. The category was analysed as an ordinal variable and, for description, grouped as 1–2, 3 and 4–5.

Blood sampling and cell-free DNA measurement

Peripheral blood was sampled within 24 h before surgery and at 0 (completion of CPB), 6, 12 and 24 h after CPB. Plasma cfDNA extraction, methylation analysis and quantification of total and cardiomyocyte-derived cfDNA were performed as previously described, using cardiomyocyte-specific DNA methylation markers [10, 14]. Concentrations are expressed as copies/mL of plasma. Three derived indices were calculated: the postoperative peak (highest value 0–24 h); the area under the concentration–time curve from 0 to 24 h by the trapezoidal rule (carrying the 12-h value forward when the 24-h sample was missing); and the fold change from the preoperative to the 0-h value.

Statistical analysis

Continuous variables are presented as median (first–third quartile) and categorical variables as number (%). Characteristics were compared across STS-EACTS groups with the Kruskal–Wallis test or a permutation chi-squared test. cfDNA concentrations were right-skewed and were natural-log transformed for parametric models. Estimates were back-transformed to geometric mean ratios (GMRs) per one-category increase in STS-EACTS category.

The primary analysis was a linear mixed-effects model of log cfDNA across all five time points. It included fixed effects for time (categorical), STS-EACTS category, the time × STS-EACTS interaction and log age at operation, and a random intercept for each patient. Models were fitted by restricted maximum likelihood. The overall STS-EACTS effect (5 degrees of freedom) and the interaction (4 degrees of freedom) were assessed with likelihood-ratio tests on maximum-likelihood fits. Time-specific GMRs were obtained as linear contrasts. The model uses all available samples and is valid if data are missing at random. A second mixed model, restricted to postoperative samples and adjusted for the log preoperative concentration, estimated the association beyond baseline.

At each time point and for each derived index, we additionally calculated Spearman correlation coefficients (bootstrap 95% CI, 5000 resamples) and Jonckheere–Terpstra tests for ordered trend across the three groups (permutation *P*-values, 10 000 permutations). We also fitted linear regressions of log cfDNA on STS-EACTS category and log age with heteroscedasticity-robust (HC3) standard errors; postoperative values were further adjusted for the log preoperative concentration. *P*-values from these per-time-point analyses were corrected with the Benjamini–Hochberg false discovery rate (FDR) procedure, separately for the 10 time-point tests and the 6 derived-index tests [22].

Sensitivity analyses added CPB duration as a covariate (not in the primary model because it may lie on the causal pathway between complexity and cfDNA release). Further sensitivity analyses replaced log age with log weight (the two were highly collinear, r = 0.88); used generalized estimating equations with an exchangeable working correlation and robust standard errors; excluded in-hospital deaths; excluded observations with standardized residuals beyond ± 3; and modelled STS-EACTS as a three-level categorical variable. Residuals were heavier-tailed than normal, which motivated the robust and outlier-excluding analyses. Tests were two-sided with $\alpha = 0.05$. Analyses followed the journal's statistical

reporting guidelines [23] and were performed in Python 3.11 with statsmodels 0.15 and SciPy 1.17 [24, 25].

RESULTS

Patient characteristics

Of the 50 patients, 20 (40%) were in STS-EACTS categories 1–2, 9 (18%) in category 3 and 21 (42%) in categories 4–5 (category 1, n = 4; 2, n = 16; 3, n = 9; 4, n = 14; 5, n = 7). The median age at operation was 68 days (12–256), and 22 (44%) patients were neonates. Patients in higher categories were younger and more often neonates (5%, 67% and 71% in categories 1–2, 3 and 4–5; $P < 0.001$). They more often received preoperative prostaglandin E1 ($P < 0.001$) and mechanical ventilation ($P = 0.017$), and they had longer postoperative ventilation ($P < 0.001$) and higher vasoactive-inotropic scores ($P = 0.007$). All 4 in-hospital deaths occurred in categories 4–5 (Table 1).

Table 1
Patient characteristics, operative data and outcomes by STS-EACTS mortality category

Variable	All (n = 50)	Category 1–2 (n = 20)	Category 3 (n = 9)	Category 4–5 (n = 21)	*P*-value
Age at operation, days	68 (12–256)	250 (196–342)	11 (6–41)	25 (7–95)	< 0.001
Neonate (≤ 30 days)	22 (44)	1 (5)	6 (67)	15 (71)	< 0.001
Weight, kg	4.6 (3.2–7.6)	6.7 (5.0–7.8)	2.8 (2.7–3.7)	3.3 (3.1–4.5)	< 0.001
Female sex	20 (40)	8 (40)	2 (22)	10 (48)	0.44
Redo operation	16 (32)	10 (50)	2 (22)	4 (19)	0.11
Preoperative mechanical ventilation	12 (24)	1 (5)	2 (22)	9 (43)	0.017
Preoperative prostaglandin E1	23 (46)	2 (10)	7 (78)	14 (67)	< 0.001
Preoperative SpO2, %	85 (76–90)	82 (75–95)	85 (78–88)	85 (80–88)	0.96
Arch repair	9 (18)	6 (30)	0 (0)	3 (14)	0.14
CPB duration, min	70 (45–95)	52 (40–74)	98 (55–109)	77 (60–95)	0.059
Aortic cross-clamp duration, min	40 (21–56)	30 (0–48)	86 (36–89)	42 (35–51)	0.11
Maximal VIS	15.0 (5.6–25.0)	10.0 (5.0–15.0)	12.6 (12.5–15.0)	25.0 (15.0–30.0)	0.007
Mechanical ventilation, h	24 (7–96)	7 (4–18)	22 (20–25)	96 (48–168)	< 0.001
AKI (KDIGO stage 2–3)	16 (32)	6 (30)	1 (11)	9 (43)	0.24
ECMO	2 (4)	0 (0)	0 (0)	2 (10)	0.35
In-hospital death	4 (8)	0 (0)	0 (0)	4 (19)	0.056
Hospital length of stay, days	8 (6–16)	8 (6–12)	7 (7–7)	14 (6–18)	0.25

Values are median (first–third quartile) or n (%). *P*-values are from the Kruskal–Wallis test (continuous variables) or a permutation chi-squared test with 5000 permutations (categorical variables). AKI: acute kidney injury; CPB: cardiopulmonary bypass; ECMO: extracorporeal membrane oxygenation; KDIGO: Kidney Disease: Improving Global Outcomes; SpO2: peripheral oxygen saturation; STS-EACTS: Society of Thoracic Surgeons–European Association for Cardio-Thoracic Surgery; VIS: vasoactive-inotropic score.

Of 250 planned samples, 242 (97%) total and 241 (96%) cardiac cfDNA measurements were available; 6 patients lacked a 24-h sample. Across the cohort, total cfDNA rose from a median of 5068 (3309–9126) copies/mL before surgery to 32 934 (18 582–58 169) copies/mL at 0 h and then fell to 13 536 (7610–32 808) copies/mL at 24 h. Cardiac cfDNA rose from 44 (13–160) to 3686 (2047–8233) copies/mL and fell to 454 (330–2236) copies/mL at 24 h.

Total cell-free DNA and surgical complexity

Total cfDNA was highest in categories 4–5 at every time point (Fig. 1A). In the mixed-effects model, each one-category increase in STS-EACTS category was associated with 30–47% higher total cfDNA (overall $P = 0.022$; Table 2). The difference was already present before surgery (GMR 1.30, 95% CI 1.01–1.66; $P = 0.039$). Its size did not differ significantly between time points (time × STS-EACTS interaction $P = 0.89$), although point estimates rose from 0 h (GMR 1.32, 95% CI 1.04–1.69) to 24 h (GMR 1.47, 95% CI 1.14–1.89).

Table 2
Association between STS-EACTS mortality category and perioperative cell-free DNA (linear mixed-effects models)

Time point	Total cfDNA GMR (95% CI)	*P*-value	Cardiac cfDNA GMR (95% CI)	*P*-value
Preoperative	1.30 (1.01–1.66)	0.039	1.01 (0.67–1.52)	0.97
0 h (end of CPB)	1.32 (1.04–1.69)	0.023	1.04 (0.70–1.56)	0.83
6 h	1.39 (1.09–1.77)	0.008	1.06 (0.71–1.59)	0.76
12 h	1.43 (1.12–1.83)	0.005	1.18 (0.79–1.78)	0.42
24 h	1.47 (1.14–1.89)	0.003	1.21 (0.80–1.83)	0.36
Postoperative 0–24 h, adjusted for preoperative level	1.38 (1.13–1.68)	0.001	1.17 (0.89–1.55)	0.27
Overall STS-EACTS effect (likelihood-ratio test, 5 df)		0.022		0.90
Time × STS-EACTS interaction (likelihood-ratio test, 4 df)		0.89		0.86

GMR: ratio of geometric mean cfDNA per one-category increase in STS-EACTS mortality category (1–5). Estimates are from linear mixed-effects models of log cfDNA at all 5 time points, with fixed effects for time, STS-EACTS category, their interaction and log age, and a random intercept for each patient (total cfDNA, 242 samples; cardiac cfDNA, 241 samples; 50 patients). Mixed model restricted to postoperative samples with log preoperative cfDNA and log age as covariates. cfDNA: cell-free DNA; CI: confidence interval; CPB: cardiopulmonary bypass; df: degrees of freedom; GMR: geometric mean ratio; STS-EACTS: Society of Thoracic Surgeons–European Association for Cardio-Thoracic Surgery.

After adjustment for the preoperative concentration, postoperative total cfDNA remained 38% higher per category (GMR 1.38, 95% CI 1.13–1.68; $P = 0.001$). In per-time-point regressions, this baseline-adjusted association remained after FDR correction at 12 h (GMR 1.43, 95% CI 1.12–1.84; $q = 0.025$) and 24 h (GMR 1.54, 95% CI 1.18–2.02; $q = 0.016$), but not at 0 h (GMR 1.19, 95% CI 0.98–1.45; $q = 0.17$) (Supplementary Material, Table S1). The fold change from the preoperative to the 0-h value was not associated with STS-EACTS category (Spearman $\rho = 0.06$; $q = 0.81$). Among the derived indices, the 0–24 h area under the curve correlated most strongly with STS-EACTS category ($\rho = 0.42$, 95% CI 0.16–0.63; $q = 0.014$) (Table S2).

Patients in categories 4–5 drove the association. Their total cfDNA was approximately twice that of patients in categories 1–2 at every postoperative time point (GMR 1.95–2.32), whereas category 3 did not differ from categories 1–2 (Table S3).

Cardiomyocyte-derived cell-free DNA

Cardiac cfDNA followed a similar temporal course in all groups (Fig. 1B) but was not associated with STS-EACTS category at any time point (overall $P = 0.90$; interaction $P = 0.86$; Table 2). The postoperative baseline-adjusted GMR was 1.17, 95% CI 0.89–1.55 ($P = 0.27$). A nominal unadjusted correlation at 12 h ($\rho = 0.30$; $P = 0.033$) did not survive FDR correction ($q = 0.067$).

Sensitivity analyses

The total cfDNA association was similar in direction and size in all sensitivity analyses (Table S3). Adjustment for CPB duration modestly attenuated the preoperative and 0-h estimates (GMR 1.25, 95% CI 0.97–1.62 and 1.28, 95% CI 1.00–1.65) but not the 6–24-h estimates. Point estimates were similar when weight replaced age, with generalized estimating equations, after exclusion of the 4 deaths, and after exclusion of 2 outlying observations. The cardiac cfDNA association remained null in all analyses.

DISCUSSION

In this prospective cohort of children undergoing cardiac surgery with CPB, higher STS-EACTS mortality category was associated with higher total cfDNA from before surgery through 24 h after bypass. Cardiomyocyte-derived cfDNA showed no such association. The total cfDNA association had three features: it was present preoperatively; it persisted after adjustment for preoperative levels and was clearest 12–24 h after bypass; and it was not explained by the size of the immediate rise at the end of CPB (Graphical Abstract).

The preoperative difference probably reflects the clinical state of patients undergoing complex operations. In our cohort, these patients were predominantly neonates, and many required prostaglandin infusion or mechanical ventilation before surgery. These features are markers of physiological instability, impaired tissue perfusion and inflammation, all of which can increase cfDNA release [5, 6]. Thus, part of the association between complexity and total cfDNA predates the operation. Studies that relate postoperative cfDNA to outcome should therefore consider preoperative values and procedural complexity together, rather than treating cfDNA as independent of established risk scores [12, 13, 18].

In contrast, the size of the cfDNA surge at the end of bypass was similar across complexity groups. The immediate release with CPB likely reflects mechanisms common to all bypass procedures, including leucocyte activation with neutrophil extracellular trap formation, release of damage-associated molecular patterns, and surgical tissue trauma [3, 11, 26, 27]. Differences between complexity groups were, if anything, larger 12–24 h after bypass, with the clearest baseline-adjusted associations at these time points, although the time interaction was not statistically significant. A slower return towards baseline after complex operations would be consistent with ongoing cell death in non-cardiac tissues, reduced clearance of cfDNA by the liver and kidneys, or both [5]. In the same cohort, early postoperative cfDNA was associated with renal and hepatic injury [15], which supports a multi-organ source and a

possible contribution of impaired clearance. Distinguishing these mechanisms will require tissue-specific methylation markers for non-cardiac organs, as already applied to other tissues [8, 9].

The absence of an association between STS-EACTS category and cardiomyocyte-derived cfDNA is informative. Cardiomyocyte death after cardiac surgery depends on myocardial factors such as ischaemic time, myocardial protection and direct surgical injury, and it may follow a delayed course [10, 14]. These factors are only partly captured by a score developed to predict mortality across procedure types [18, 19]. In our cohort, for example, the longest aortic cross-clamp times were in category 3 rather than categories 4–5 (Table 1). Cardiac cfDNA therefore appears to provide information on myocardial injury that is largely independent of procedural complexity, whereas total cfDNA partly reflects the overall burden of illness associated with complex surgery.

These findings have practical implications. First, total cfDNA values after paediatric cardiac surgery should be interpreted against procedural complexity and preoperative levels. Second, studies testing cfDNA as a prognostic marker should adjust for complexity scores, so that any incremental value over existing risk stratification can be demonstrated. Third, the cardiomyocyte-specific fraction may be the more suitable target when the goal is to detect myocardial injury that is not anticipated from the planned procedure.

Limitations

This study has limitations. It was a single-centre secondary analysis of a modest cohort and was not designed or powered for this question; in particular, category 3 included only 9 patients, so group-level estimates for this category are imprecise. Complexity, age and preoperative condition were closely related, and despite adjustment for age (and weight in sensitivity analyses), residual confounding cannot be excluded. Sampling ended at 24 h, and 6 patients lacked a 24-h sample; the mixed-effects models used all available samples under a missing-at-random assumption. Non-cardiac tissue-specific cfDNA was not measured, so the cellular sources of the complexity-related excess remain speculative. Finally, model residuals were heavier-tailed than normal, although robust and outlier-excluding analyses gave consistent results. External validation in a larger multicentre cohort is required.

CONCLUSION

In children undergoing cardiac surgery with CPB, higher STS-EACTS mortality category was associated with higher total cfDNA before surgery and persistently higher postoperative levels, independent of preoperative concentrations. Cardiomyocyte-derived cfDNA was not associated with procedural complexity. Perioperative cfDNA should be interpreted against procedural complexity, and cardiomyocyte-specific cfDNA may offer information on myocardial injury beyond that captured by existing risk categories.

ABBREVIATIONS

CI
confidence interval
cfDNA
cell-free DNA
CPB
cardiopulmonary bypass
FDR
false discovery rate
GMR
geometric mean ratio
STS-EACTS
Society of Thoracic Surgeons–European Association for Cardio-Thoracic Surgery

DECLARATIONS

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This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

Conflict of interest: none declared.

DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

Statement on generative AI

During preparation of this work, the authors used ChatGPT to assist with language editing, grammar refinement, and improvement of manuscript readability. After using this tool, the authors carefully reviewed, revised, and approved all content and take full responsibility for the final version of the manuscript.

AUTHOR CONTRIBUTIONS

Eitan Keizman: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft.

Hiba Abuelhija: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft.

Asaf Mandel: Data curation; Formal analysis; Investigation; Writing – original draft.

Salmas Watad: Conceptualization; Resources; Writing – review & editing.

Hai Zemmour: Conceptualization; Resources; Writing – review & editing.

David Mishaly: Resources; Writing – review & editing.

Esther Arfi Levy: Conceptualization; Writing – review & editing.

Alain E. Serraf: Resources; Writing – review & editing.

Uri Pollak: Conceptualization; Methodology; Project administration; Supervision; Writing – original draft; Writing – review & editing.

All authors have read and agreed to the final version of the manuscript.

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Figures

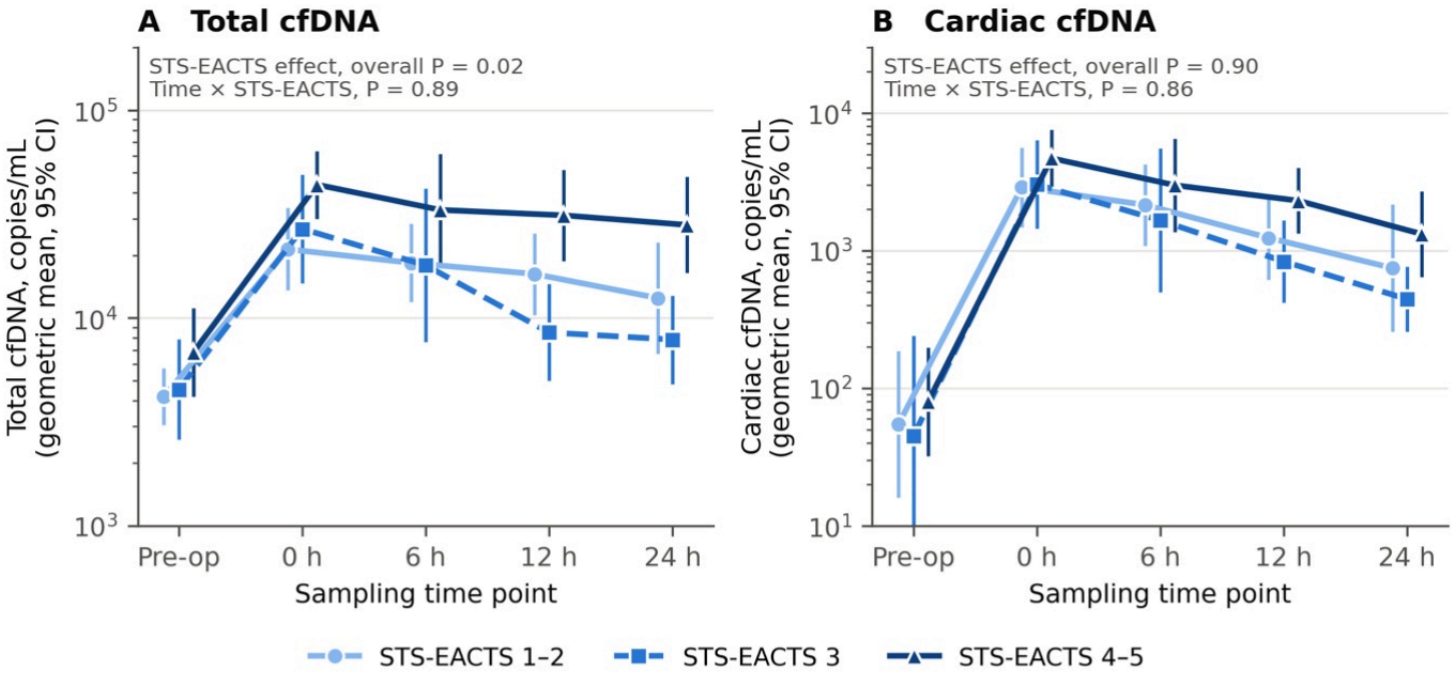


Figure 1

Perioperative total (A) and cardiomyocyte-derived (B) cell-free DNA by STS-EACTS mortality category. Points show geometric means and bars 95% confidence intervals on a logarithmic scale for categories 1–2 (n = 20), 3 (n = 9) and 4–5 (n = 21). *P*-values are from linear mixed-effects models adjusted for log age, with a random intercept for each patient, and test the overall STS-EACTS effect and the time × STS-

EACTS interaction. Points are offset horizontally to avoid overlap. cfDNA: cell-free DNA; CPB: cardiopulmonary bypass; STS-EACTS: Society of Thoracic Surgeons–European Association for Cardio-Thoracic Surgery.

Supplementary Files

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- GraphicalAbstract.docx
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