Elevated Cardiac Troponin to Detect Acute Cellular Rejection After Cardiac Transplantation: A Systematic Review and Meta-Analysis

Zhengyang Liu1†, Luke A. Perry1†, Jahan C. Penny-Dimri2†, Michael Handscombe1†, Isabella Overmars3†, Mark Plummer4,5†, Reny Segal1,5† and Julian A. Smith2†

1Department of Anaesthesia, Royal Melbourne Hospital, Parkville, VIC, Australia, 2Department of Surgery, School of Clinical Sciences at Monash Health, Monash University, Clayton, VIC, Australia, 3Infection and Immunity Theme, Murdoch Children’s Research Institute, Parkville, VIC, Australia, 4Department of Intensive Care Medicine, Royal Melbourne Hospital, Parkville, VIC, Australia, 5Department of Medicine, University of Melbourne, Parkville, VIC, Australia

Cardiac troponin is well known as a highly specific marker of cardiomyocyte damage, and has significant diagnostic accuracy in many cardiac conditions. However, the value of elevated recipient troponin in diagnosing adverse outcomes in heart transplant recipients is uncertain. We searched MEDLINE (Ovid), Embase (Ovid), and the Cochrane Library from inception until December 2020. We generated summary sensitivity, specificity, and Bayesian areas under the curve (BAUC) using bivariate Bayesian modelling, and standardised mean differences (SMDs) to quantify the diagnostic relationship of recipient troponin and adverse outcomes following cardiac transplant. We included 27 studies with 1,684 cardiac transplant recipients. Patients with acute rejection had a statistically significant late elevation in standardised troponin measurements taken at least 1 month postoperatively (SMD 0.98, 95% CI 0.33–1.64). However, pooled diagnostic accuracy was poor (sensitivity 0.414, 95% CrI 0.174–0.696; specificity 0.785, 95% CrI 0.567–0.912; BAUC 0.607, 95% CrI 0.469–0.723). In summary, late troponin elevation in heart transplant recipients is associated with acute cellular rejection in adults, but its standalone diagnostic accuracy is poor. Further research is needed to assess its performance in predictive modelling of adverse outcomes following cardiac transplant.

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Keywords: heart transplantation, meta-analysis, systematic review, cardiac troponin, acute cellular rejection

Abbreviations: ANOVA, analysis of variance; AUC, area under the ROC curve; BAUC, Bayesian AUC; BSRGC, Bayesian summary ROC; CI, confidence interval; CrI, credible interval; EMB, endomyocardial biopsy; ISHLT, International Society for Heart and Lung Transplantation; MOOSE, meta-analysis of observational studies in epidemiology; QUADAS-2, quality assessment of diagnostic accuracy studies 2; ROC, receiver operating characteristic; SMD, standardised mean difference.
INTRODUCTION

The endomyocardial biopsy (EMB) has remained the gold standard for detecting acute allograft rejection after cardiac transplant since its introduction in the early 1970s (1). However, this diagnostic test is invasive, can be poorly concordant amongst grading pathologists (2), and repeat procedures are associated with small but significant risks of complications including tricuspid regurgitation, cardiac tamponade, arrhythmias, and haemorrhage (3–5).

In light of these challenges, various biomarkers have been explored as diagnostic alternatives to EMB, contributing to an emerging sphere of multidisciplinary interest in the predictive (both diagnostic and prognostic) ability of routine serum biomarkers for adverse outcomes in a variety of conditions (6–13). In particular, cardiac troponin, a sensitive and specific marker of myocardial injury, is of broad prognostic significance across a range of cardiovascular diseases (14, 15). Although most classically elevated in the context of acute coronary syndromes, elevated troponin levels are also associated with a range of other cardiac and non-cardiac conditions including atrial fibrillation, congestive cardiac failure, myocarditis, myocardial contusion, pulmonary embolism, sepsis, renal failure, and hypovolaemia (16). Both donor and recipient troponin have been associated with adverse outcomes following cardiac transplant (17, 18). We have previously found that troponin elevations in cardiac transplant recipients may be prognostic for primary graft failure, adverse cardiac events, coronary artery disease, and long-term mortality, but its prognostic value in the context of acute rejection up to 1 year after transplant was uncertain (19). Donor troponin elevations though, were not associated with increased 30-day, 1-year, or long-term mortality post cardiac transplant despite increasing the risk of graft rejection at 1 year (but not at 30 days) (20).

However, the diagnostic utility of elevated cardiac troponin is controversial, and this biomarker has yet to be routinely integrated into the diagnostic pathway for acute allograft rejection or recommended by international guidelines (21, 22). Hence, we conducted this systematic review and meta-analysis of elevated cardiac troponin in diagnosing acute allograft rejection in heart transplant recipients.

METHODS

Study Design and Registration

This systematic review and meta-analysis evaluated study level data, and was reported in compliance with the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines (23). Protocol details were prospectively registered on PROSPERO (CRD42021227861) and there were no major protocol deviations.

Eligibility Criteria

We included all original research studies which reported the diagnostic accuracy of elevated recipient troponin to detect adverse outcomes in heart transplant recipients. We excluded non-human studies, abstracts and conference presentations, case reports and series, editorials and expert opinions, review articles, and studies with incompletely reported data.
Search Strategy
We searched MEDLINE (Ovid), Embase (Ovid), and the Cochrane Library from inception to December 2020. Our search strategy included a comprehensive set of search terms for troponin and cardiac transplantation (Supplementary Material) (24). We placed no restrictions on language or publication period.

Study Selection
Two authors (ZL and MH) independently screened titles and abstracts of each search result for potentially relevant studies. The same two authors assessed full texts of shortlisted studies against eligibility criteria, with a third author (LAP) adjudicating any disagreements. We reviewed the reference and citation lists of included studies for further potentially relevant studies.

Data Extraction and Management
Two authors (ZL and LAP) independently extracted data from included studies using standardised spreadsheets. We recorded the following, where reported and applicable: study design, population baseline characteristics including comorbidities, operative details, troponin type and measurement details, troponin threshold, definitional threshold of significant rejection by the International Society for Heart and Lung Transplantation (ISHLT) acute cellular rejection grade (25), outcomes, and diagnostic performance measures. Where studies reported dichotomous measures of diagnostic performance, we standardised reported data in confusion matrices and calculated sensitivity and specificity values; where studies reported continuous measures of effect, we standardised data reported as mean and standard deviation and calculated standardised mean differences (SMDs) (26).

Assessment of Methodological Quality and Risk of Bias
Two authors (ZL and LAP) independently assessed the methodological quality of included studies using a modified version of the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool (27), with discrepancies resolved through discussion with a third author (MH). For this study, we expanded the grading of overall risk of bias to three categories (low, unclear, and high risk) from 2 categories (low risk and at risk). We used receiver operating characteristic (ROC) analysis we noted optimised cut-off values, areas under the ROC curve (AUCs), sensitivities, specificities, and associated 95% CIs. From these, we calculated true positive, false positive, false negative, and true negative rates, and generated Bayesian Summary ROC (BSROC) curves and summary sensitivity, specificity, and Bayesian AUC (BAUC) statistics with 95% credible intervals (Crl) using a bivariate Bayesian modelling approach (29).

We estimated statistical heterogeneity using the I² statistic for each meta-analysis. Where reporting of pre-specified covariates was sufficient across included studies, we used meta-regressions to explore possible sources of heterogeneity.

Where there were more than 10 included studies, we formally assessed publication bias with visual inspection of funnel plot skew and a regression test for funnel plot asymmetry (30). All analyses and figures were generated using Review Manager (RevMan) 5.4 (31) and the R statistical packages “metafor” (32) and “bamdit” (33).
### TABLE 1 | Characteristics of included studies.

| Study ID | Design          | Number of patients, number of samples, and demographic | Age (Mean ± SD, years) | Sex (% male) | Troponin type | Troponin measurement period post transplantation and early measurement exclusions | Troponin measurement method | Troponin threshold (ng/ml) and Selection method | Classification threshold for significant rejection and samples with significant rejection (%) | Outcome(s) and effect measure(s) | Modified QUADAS-2 risk of bias |
|----------|-----------------|---------------------------------------------------------|-------------------------|--------------|---------------|-------------------------------------------------|-----------------------------|-------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------|-------------------------------|
| Ahn (34) | Single Centre Retrospective | 47 252 Adult | 47.4 ± 15.8 | 68.1% | TnI, hsTnI Index<sup>a</sup> | 2 weeks postoperative onwards Exclusions: none and first 2 months after transplantation | ARCHITECT 0002r STAT TnI and hsTnI assay (Abbott Diagnostics, Abbott Park, Illinois, USA) | 1.17 (hsTnI Index) Receiver operating characteristic analysis | ISHLT 2004, 2R | Acute Cellular Rejection | high |
| Alexis (35) | Single Centre Prospective | 90 256 Adult | 48.0 ± 15.2 | 74.4% | TnT | 1 week to 72 months postoperative Exclusions: none and first 3 months after transplantation | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.1 Manufacturer’s recommendation | ISHLT 1990, 3A | Acute Cellular Rejection | high |
| Balduni (36) | Single Centre Prospective | 57 149 Adult | 37.5 (SD not reported) | Not reported | TnT | 1 month to 12 months Exclusions: first 1 month after transplantation | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | Not reported | ISHLT 1990, 1B | Acute Cellular Rejection | Continuous | unclear |
| Cauliez (37) | Single Centre Prospective | 56 100 Adult | Not reported | Not reported | TnI | 10 to 3,807 days (median 458 days) No exclusions | Stratus Cardiac Tnl fluorometric enzyme immunoassay (Dade-Behring, Newark, Delaware, USA) | 0.6 Manufacturer’s recommendation | ISHLT 1990, 2 | Acute Cellular Rejection | Continuous | unclear |
| Chance (38) | Single Centre Prospective | 145 704 Adult | Not reported | Not reported | TnT | 3 days to 206 months (median 29 months) Exclusions: none and first 1 month after transplantation | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | 0.1 Manufacturer’s recommendation | ISHLT 1990, 3A | Acute Cellular Rejection | Continuous | unclear |
| Dengler (39) | Single Centre Retrospective | 95 271 Adult | 48.2 ± 11.4 | 82.1% | TnT | 3 months–70 months (median 15 months) Exclusions: first 3 months after transplantation | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.015 Lower limit of assay detection | ISHLT 1990, 3A | Acute Cellular Rejection | Continuous | unclear |
| Dyer (40) | Single Centre Prospective | 42 53 Paediatric | 11.1 (SD not reported) | Not reported | hsTnT | 3 months onwards (median 24 months) Exclusions: first 3 months after transplantation | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | 0.014 99th percentile of healthy adult reference population | ISHLT 2004, 2R | Acute Cellular Rejection | Continuous | unclear |
| Faulk (41) | Single Centre Prospective | 68 151 Adult | 30.3 ± 14.2 | 60.3% | TnT | 6 months onwards Exclusions: first 6 months after transplantation | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.1 Manufacturer’s recommendation | ISHLT 1990, 3A | Acute Cellular Rejection | Dichotomous | high |
| Forri (42) | Single Centre Prospective | 114 385 Adult | 52.0 ± 6.0 | 86.0% | TnI | 15 to 1,740 days (median 640 ± 95 days) No exclusions | Dimension Rx L clinical chemistry system (Siemens Medical Solutions Diagnostics, Erlangen, Germany) | 0.1 Manufacturer’s recommendation | ISHLT 1990, 3A | Acute Cellular Rejection | Dichotomous | high |

(Continued on following page)
| Study ID | Design       | Number of patients, number of samples, and demographic | Age (Mean ± SD, years) | Sex (% male) | Troponin type | Troponin measurement period post transplantation and early measurement exclusions | Troponin measurement method | Troponin threshold (ng/ml) and Selection method | Classification threshold for significant rejection and samples with significant rejection (%) | Outcome(s) and effect measure(s) | Modified QUADAS-2 risk of bias |
|----------|--------------|-------------------------------------------------------|------------------------|--------------|---------------|-------------------------------------------------|-----------------------------|-------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-------------------------------------------------|---------------------------------|
| Garrido (43) | Single Centre Prospective | 21 Not applicable Adult | 60.0 ± 10.0 | 81.0% | TnT | 1 year onwards No exclusions | Electrochemiluminescence immunoassays with a Modular Analytics E170 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) | 0.026 | Receiver operating characteristic analysis | Not applicable | Cardiac allograft vasculopathy and continuous |
| Gleissner (44) | Single Centre Retrospective | 132 788 Adult | 58.5 ± 9.4 | 85.6% | TnT | 3 months–48 months (mean 13 months) Exclusions: first 3 months after transplantation | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.14 | Receiver operating characteristic analysis | SHL'T 1990, 3A 13% | Acute Cellular Rejection Dichotomous and continuous |
| Halwachs (45) | Single Centre Retrospective | 15 183 Adult | 49.8 ± 13.6 | 80.0% | TnT | 1 day to 2 years No exclusions | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.2 | Manufacturer’s recommendation | SHL'T 1990, 3A 1% | Acute Cellular Rejection Continuous |
| Hossein-Nia (46) | Single Centre Prospective | 15 85 Adult | Not reported | Not reported | TnT | Postoperative onwards No exclusions | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.2 | Manufacturer’s recommendation | SHL'T 1990, 2 16% | Acute Cellular Rejection Continuous |
| Hossein-Nia (47) | Single Centre Prospective | 29 Not reported Adult | 48.5 ± 7.8 | 83.9% | TnT | Postoperative onwards (mean 87 ± 32 weeks) No exclusions | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.2 | Manufacturer’s recommendation | SHL'T 1990, 2 Not reported | Acute Cellular Rejection Continuous |
| Hossein-Nia (47) | Single Centre Prospective | 17 214 Adult | Not reported | Not reported | TnI | Postoperative onwards (mean 61 ± 16 days) No exclusions | Tri Assay (Sanofi Diagnostic Pasteur Ltd., Guildford, United Kingdom) | Not reported | Not reported | SHL'T 1990, 2 4% | Acute Cellular Rejection Continuous |
| Hsu (49) | Single Centre Prospective | 51 71 Adult | 47.8 ± 11.3 | 80.0% | TnI | 1 week to 5 years No exclusions | R&D Systems ELISA (R & D Systems USA, Minneapolis, Minnesota, USA) | Not reported | Not reported | SHL'T 1990, 2 23% | Acute Cellular Rejection Continuous |
| Mendez (50) | Multicentre Prospective | 73 224 Adult | 54.0 ± 14.0 | 71.2% | hsTnT | Within 3 months of surgery to over 18 months, as needed No exclusions | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | 0.017 | Receiver operating characteristic analysis | SHL'T 2004, 2R 7% | Acute Cellular Rejection Dichotomous and continuous |
| Moran (51) | Single Centre Prospective | 37 85 Paediatric | Median 12.4, range 1.3–30.0 | Not reported | TnI, TnT | 2.05 ± 2.43 years (median, 0.9; range, 0.03–9.1) No exclusions | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | TnI: 0.5 Receiver operating characteristic analysis TnT: Not reported | SHL'T 1990, 3A 15% | Acute Cellular Rejection Dichotomous and continuous |

(Continued on following page)
| Study ID | Design | Number of patients, number of samples, and demographic | Age (Mean ± SD, years) | Sex (% male) | Troponin type | Troponin measurement period post transplantation and early measurement exclusions | Troponin measurement method | Troponin threshold (ng/ml) and Selection method | Classification threshold for significant rejection and samples with significant rejection (%) | Outcome(s) and effect measure(s) | Modified QUADAS-2 risk of bias |
|----------|--------|----------------------------------------------------------|------------------------|-------------|--------------|-------------------------------------------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------------|---------------------------|
| Mullen (52) | Single Centre Prospective | 29 173 Adults | 52.0 ± 5.4 | 79.3% | TnI, TnI<sup>b</sup> | 12–564 days (mean 129 ± 9 days) No exclusions | Not reported | Not reported | ISHLT 1990, 3A Acute Cellular Rejection | Continuous | low |
| Munoz-Esparrza (53) | Single Centre Prospective | 72 | 53.0 ± 13.0 | 75.0% | hsTnT | Within 1 year No exclusions | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | 0.035 Receiver operating characteristic analysis | ISHLT 2004, 2R Acute Cellular Rejection | Dichotomous and continuous | high |
| Ogawa (54) | Multicentre Prospective | 69 660 Adults | 50.0 ± 10.0 | 79.7% | TnT | 9–141 weeks (mean 53 ± 26 weeks) No exclusions | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | Not reported | ISHLT 1990, 3A Acute Cellular Rejection | Continuous | unclear |
| Patel (55) | Multicentre Retrospective | 98 418 Adults | 53.8 ± 12.1 | 83.0% | hsTnI | 1 week—long term (median 1522 (IQR 773–2160) days) No exclusions | ARCHITECT 2000x STAT high-sensitivity cTnI assay (Abbott Diagnostics, Abbott Park, Illinois, USA) | 0.015 Receiver operating characteristic analysis | ISHLT 2004, 2R Acute Cellular Rejection | Dichotomous and continuous | unclear |
| Siaplaouras (56) | Single Centre Retrospective | 25 | Mean 2 months, range 2 weeks–13 years | 40.0% | TnI | 3 weeks to 4 years No exclusions | Stratus Cardiac TnI fluorometric enzyme immunoassay (Dade-Behring, Newark, Delaware, USA) | 0.6 Manufacturer’s recommendation | ISHLT 1990, 3A Acute Cellular Rejection | Dichotomous | high |
| Vazquez-Rodriguez (57) | Single Centre Prospective | 62 259 Adults | Not reported | 85.5% | TnT | Postoperative onwards Exclusions: None and first 3 months after transplantation | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.1 Manufacturer’s recommendation | ISHLT 1990, 2 Acute Cellular Rejection | Dichotomous | low |
| Wåhlander (58) | Single Centre Prospective | 14 79 Paediatric | Not reported | Not reported | TnI | 1 month onwards Exclusions: first 1 month after transplantation | Elecsys Electrochemiluminescence Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA) | 0.1 Manufacturer’s recommendation | ISHLT 1990, 3A Acute Cellular Rejection | Continuous | unclear |
| Walpole (59) | Single Centre Prospective | 25 392 Adults | Not reported | Not reported | TnT | Postoperative to 2 years No exclusions | Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | 0.2 Manufacturer’s recommendation | Texas score, 4 Not reported | Acute Cellular Rejection | Dichotomous and continuous | unclear |
| Wang (60) | Single Centre Prospective | 186 358 Adults | Not reported | Not reported | TnI, TnI<sup>b</sup> | Postoperative onwards Exclusions: first 5 weeks after transplantation | TnI: Stratus Cardiac TnI fluorometric enzyme immunoassay (Dade-Behring, Newark, Delaware, USA) TnT: Enzymun-Test TnT enzyme immunometric assay (Boehringer Mannheim Diagnostics GmbH, Mannheim, Germany) | TnI: 1.7 Not reported TnT: 0.07 Not reported | ISHLT 1990, 3A Acute Cellular Rejection | Dichotomous and continuous | high |

<sup>a</sup>Where studies measured both conventional and high sensitivity troponin variants and underwent meta-analysis, high sensitivity troponin was included in quantitative analysis where appropriate.

<sup>b</sup>Where studies measured both troponin I and T subtypes and underwent meta-analysis, troponin I measurements was chosen for quantitative synthesis and a sensitivity analysis was performed by including troponin T measurements to determine the impact of this decision. TnI, Troponin I; TnT, Troponin T; hsTnT, High Sensitivity Troponin T; hsTnI, High Sensitivity Troponin I.
RESULTS

Search Results
We identified 1,927 results through the search, and one additional citation through reference lists. After automatic deduplication, we screened 1,499 titles and abstracts. We reviewed full texts of 68 potentially relevant studies, from which 27 were included in this review, with 20 in quantitative form (Figure 1).

Description of Included Studies
Twenty-seven studies (34–60) involving 1,684 cardiac transplant recipients were included. Detailed characteristics of included studies are reported in Table 1.

Methodological Quality
Methodological quality was variable. Five studies (44, 48, 50, 52, 57) were deemed low risk of bias, 12 studies (36–40, 45–47, 54, 55, 58, 59) unclear risk of bias due to no specific reporting of certain domain characteristics, and 10 studies (34, 35, 41–43, 49, 51, 53, 56, 60) high risk of bias. The full QUADAS-2 assessment can be found in the Supplementary Material.

Descriptive Analyses and Meta-Analysis

Acute Cellular Rejection

Adult
No Temporal Exclusion Criteria.
Dichotomous Measure of Diagnostic Accuracy. Eight studies (35, 38, 42, 50, 53, 55, 57, 60) with 840 participants reported sensitivity, specificity, and AUC values regarding the ability of troponin to diagnose acute cellular rejection in heart transplant recipients. We found a pooled sensitivity of 0.479 (95% CrI 0.190–0.783), specificity of 0.702 (95% CrI 0.395–0.910), and BAUC 0.584 (95% CrI 0.377–0.760) (Figure 2).

As one included study (60) measured both troponin I and T values, we performed a sensitivity analysis investigating the effects of including troponin T measurements instead of troponin I in quantitative synthesis. The result was not significantly different (pooled SMD 0.26, 95% CI –0.33–1.31) (Figure 3).

Wang 1996 (60) reported mean measurements in both troponin I and T between acute cellular rejection vs. non-rejection groups (0.216 vs. 0.707 and 0.134 vs. 0.088 ng/ml respectively); however, neither were statistically significant (p = 0.357 and p = 0.374 respectively). Contrary to this, Walpolth 1998 (39) reported statistically significant elevations (no measure of statistical significance reported) troponin T measurements between acute cellular rejection (0.77 ± 0.80 ng/ml) and non-rejection (0.02 ± 0.05 ng/ml) groups. Hossein-Nia 1993 (48) reported an elevated median troponin T in those with acute cellular rejection compared to without (0.370 vs. 0.300 ng/ml); however, statistical significance was not reported.

Between-study statistical heterogeneity was considerable (I² statistic 95%). We investigated potential sources of statistical heterogeneity with a meta-regression, and found that the troponin assay sensitivity and ISHLT rejection criteria (overlapping exactly; all studies using high sensitivity troponin also used ISHLT 2004 criteria), study year, troponin threshold, and standard deviation of age were significant SMD modifiers and accounted for up to 49% of heterogeneity on univariable analysis (Supplementary Table S2). Notably, the following were not significant SMD modifiers: ISHLT cut-off grade for definition of significant rejection (1R vs. 2R in ISHLT 2004; 2 vs. 3A in ISHLT 1990), prevalence of samples with significant rejection per cohort, troponin threshold, and study risk of bias.

A regression test for funnel plot asymmetry was unable to detect significant publication bias (p = 0.1023) (Supplementary Figure S5).

Early Postoperative Exclusion Criteria.
Dichotomous Measure of Diagnostic Accuracy. After exclusion of measurements from the early postoperative period (at least 1 month postoperatively), eight single centre studies (34, 35, 38, 39, 41, 44, 57, 60) with 825 participants reported sensitivity, specificity, and AUC values regarding the ability of troponin to diagnose acute cellular rejection in heart transplant recipients. We found a pooled sensitivity of 0.414 (95% CrI 0.190–0.696), specificity of 0.785 (95% CrI 0.567–0.912), and BAUC 0.607 (95% CrI 0.469–0.723) (Figure 4).
We investigated potential sources of statistical heterogeneity with a meta-regression, and found that the troponin assay sensitivity and ISHLT rejection criteria, and troponin type, and study design were significant AUC modifiers (Supplementary Table S3). In particular, use of high sensitivity troponin I assays by one study (34) corresponded exactly to use of ISHLT 2004 criteria, and was associated with a 0.257 increase in AUC ($p = 0.0270$) (Supplementary Figure S6). Of note, the length of early postoperative exclusion (from 1 month to 6 months) was not associated with significant changes to troponin’s diagnostic ability. Additionally, the following were not significant SMD modifiers: ISHLT cut-off grade for definition of significant rejection (1R vs. 2R in ISHLT 2004; 2 vs. 3A in ISHLT 1990), prevalence of samples with significant rejection per cohort, troponin threshold, and study risk of bias.

**Continuous Measure of Diagnostic Accuracy.** Five studies (34, 36, 38, 39, 44) with 476 participants reported troponin mean differences between those with and without acute cellular rejection. We found that the standardised troponin measurements were higher in those with acute cellular rejection, and that this was a large and statistically significant effect (SMD 0.98, 95% CI 0.33–1.64) (Figure 5).

Wang 1996 (60) reported mean measurements in both troponin I and T between acute cellular rejection vs. non-rejection groups (0.059 vs. 0.102 and 0.069 vs. 0.044 ng/ml respectively) after measurements during the first 5 weeks were
excluded; however, neither were statistically significant ($p = 0.713$ and $p = 0.382$ respectively).

Statistical heterogeneity was considerable ($I^2$ statistic 95%); however, meta-regression was not possible due to insufficient study numbers ($n = 5$).

### Paediatric

**No Temporal Exclusion Criteria.** Two studies (51, 56) with 62 participants investigated the association between troponin and adverse outcomes in cardiac transplantation recipients. Moran 2000 (51) found that troponin I values differed significantly across ISHLT 1990 grades 0, 1, 2, and 3 on analysis of variance (ANOVA) ($p = 0.034$), with a diagnostic sensitivity of 0.500 and specificity of 0.776. However, troponin T values were not significantly different across ISHLT 1990 grades 0, 1, 2, and 3 on ANOVA ($p = 0.16$)—sensitivity was 0.421 and specificity was 0.837. Siaplaouras 2003 (56) found a sensitivity of 0.750, but did not report a corresponding specificity.

**Early Postoperative Exclusion Criteria.** After exclusion of measurements from the early postoperative period (at least 1 month postoperatively), three studies (40, 56, 58) with 81 participants studied the association between troponin and adverse outcomes in cardiac transplantation recipients. Excluding measurements from the first 3 months after transplantation, Dyer 2012 (40) reported a statistically significant elevation in high sensitivity troponin T values in those with acute cellular rejection (SMD 2.44, 95% CI 1.51–3.37), and a sensitivity of 0.857 and specificity of 0.913. Wa’lander 2002 (58) found that conventional troponin T values were also elevated in those with acute cellular rejection (SMD 1.43, 95% CI 0.70–2.17), reporting a sensitivity of 0.556 and specificity of 0.768. Siaplaouras 2003 (56) found a sensitivity of 0.591, but did not report a corresponding specificity.

### DISCUSSION

In this systematic review and meta-analysis of 27 diagnostic observational studies involving over 1,600 patients, we provide the most up-to-date evidence of the value of troponin in diagnosing adverse outcomes in heart transplant recipients. We found that late troponin levels (measured at least 1 month postoperatively) were significantly elevated in adult recipients with acute cellular rejection. Diagnostic accuracy of plasma troponin was slightly higher for measurements taken after the early postoperative period compared to those taken across all postoperative periods; however, the diagnostic ability of both were poor.

The poor diagnostic utility of troponin in the immediate to early post-operative period in detecting acute cellular rejection is not surprising given the manifold pathologies that can drive elevated plasma troponin in this setting (61). Our results suggest that this “early” post-operative period is confined to 1 month, with no significant difference in diagnostic accuracy of troponins measured after 1 month compared to 6 months post-transplant. However, risk of rejection is also highest in the first months after transplant (62), coinciding with this period of poorer diagnostic utility. Biomarkers capable of distinguishing between early acute rejection and routine perioperative cardiac injury are needed.

Additionally, our meta-regressions suggest that the utility of troponin may be improving over time, with study year being positive effect modifier in multiple analyses. While this is possibly attributable to the transition to high-sensitivity troponin assays, these findings are also confounded by a perfect overlap with a transition to the ISHLT 2004 criteria for acute cellular rejection.

Our search revealed one other systematic review, without meta-analysis, on a similar topic (63). However, this literature search excluded key databases (Embase and the Cochrane Library) and therefore may have lacked sensitivity, with only 88 abstracts identified and 12 studies included in the final analysis; there was no formal assessment of methodological quality; and there was no quantitative meta-analysis or assessment and management of potential sources of heterogeneity. Hence, we believe our study adds to the existing knowledge base, and provides the most recent and high-quality synthesis regarding the diagnostic value of cardiac troponin in heart transplant recipients.
Our review should be interpreted with the following limitations. While five studies were identified to be at low risk of bias, the 22 remaining studies were at unclear or high risk of bias; reassuringly though, study risk of bias was not found to be a significant effect modifier in all meta-regressions where this was possible. Studies did not report timing of troponin sample
procurement—before vs. after EMB—despite this being a possible confounder as procedure related injury can occur. The majority of studies were single centre, raising potential concerns for external validity. Finally, despite our efforts in determining significant sources of statistical heterogeneity, we were not able to account for all observed statistical heterogeneity. Although our prespecified use of a random-effects model is a strength of our study design, our findings are nonetheless tempered by unaccounted heterogeneity—an inherent part of meta-analysis methodology—which may be attributable to systematic differences in unreported study baseline characteristics as well as other study and patient-level factors. Large, high quality, randomised studies would be needed to control for these unmeasured factors in particular.

In accordance with international guidelines (21, 22), our results do not support the routine use of troponin for surveillance or diagnosis of acute cellular rejection. However, our work identifies many opportunities for future research. The current gold standard diagnostic test for acute cellular rejection involves an invasive EMB which exposes patients to small but significant risks (3–5) and can be associated with poor pathological concordance (2); safer and more effective diagnostic tests are urgently needed. While numerous imaging parameters and biomarkers have been investigated for this purpose, donor-derived cell-free DNA has recently emerged as a promising, non-invasive marker of acute rejection detection (64). Not only is donor-derived cell-free DNA able to detect episodes of rejection with remarkable sensitivity and specificity, but it may also be able to distinguish between acute cellular rejection and antibody mediated rejection, at time points earlier than possible with EMBS (65). As accurate as any one diagnostic marker may be however, experience from multiple disciplines has supported the implementation of well-selected multi-biomarker diagnostic panels over any singular biomarker (66–68). Accordingly, opportunity exists to assess elevated high-sensitivity cardiac troponin—a sensitive and specific marker of the cardiomyocyte death which occurs during acute rejection—in conjunction with emerging biomarkers representing different pathophysiological aspects of acute cellular rejection to optimise the “liquid biopsy” approach and reduce uncertainty and clinical risk of the current EMB approach. While the diagnostic ability of troponin (in the early postoperative month in particular) as a single parameter is insufficient to warrant implementation, whether or not its diagnostic utility can be enriched by integration into sophisticated multivariable diagnostic models with other non-invasive haematological and clinical markers is a field with significant potential. High-sensitivity troponin in particular may possess sufficiently high negative predictive value aid in ruling out acute cellular rejection (55, 63). Additionally, in order to optimise methodological quality and minimise risk of study bias, future researchers should design and report diagnostic test accuracy studies in accordance with QUADAS-2, among other design and reporting guidelines.

CONCLUSION

In this systematic review and meta-analysis, we found a positive association between late troponin elevation and acute cellular rejection in adults, however diagnostic performance was insufficient to support its routine use in the diagnostic pathway. Further research is warranted to assess whether the addition of troponin to emerging biomarkers of acute cellular rejection, such as circulating cell-free DNA, could lead to an enhanced “liquid biopsy” capable of superseding invasive testing.

AUTHOR CONTRIBUTIONS

ZL—Data acquisition, analysis, and interpretation, drafting and critical revisions of manuscript. LP—Study conception, data interpretation, critical revisions of manuscript. JP—D—Study conception, data analysis, critical revisions of manuscript. MH—Data acquisition, analysis, and critical revisions of manuscript. IO—Data acquisition and critical revisions of manuscript. MP—Data interpretation and critical revisions of manuscript. RS—Data interpretation and critical revisions of manuscript. JS—Data interpretation and critical revisions of manuscript. All authors approve the version to be published.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10362/full#supplementary-material

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