

Comparator-Dependent Discrimination of sST2, Galectin-3, and Copeptin in Pediatric Chest Pain: A Prospective Exploratory Study

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Abstract

Purpose

To determine whether the apparent discrimination of soluble suppression of tumorigenicity 2 (sST2), galectin-3, and copeptin in children with chest pain depended on comparator selection. The primary comparison was clinically suspected myocarditis/myopericarditis versus noncardiac chest pain within the symptomatic cohort; the patient-control comparison was secondary.

Methods

This prospective, single-center exploratory cohort included 66 children aged 6–17 years with nontraumatic chest pain, enrolled sequentially on predefined sampling days, and 22 contemporaneous clinically well controls. Research biomarker results were unavailable to clinicians and adjudicators. Directional areas under the receiver operating characteristic curve (AUCs) with outcome-stratified bootstrap 95% confidence intervals (CIs; 2,000 resamples) were estimated without cutoff optimization or formal between-contrast AUC testing.

Results

Seven symptomatic participants (10.6%) were classified as having clinically suspected myocarditis/myopericarditis and 59 as having noncardiac chest pain. In the primary comparison, AUCs were 0.47 (95% CI, 0.25–0.70) for sST2, 0.39 (0.20–0.61) for galectin-3, and 0.38 (0.18–0.60) for copeptin. In the secondary patient-control comparison, the corresponding AUCs were 0.98 (0.95–1.00), 0.98 (0.95–1.00), and 0.99 (0.97–1.00).

Conclusion

The biomarkers provided no evidence of clinically useful within-symptom discrimination in this small cohort. Near-perfect patient-control separation reflected comparator dependence and cannot be interpreted as diagnostic or triage accuracy for myocarditis.

Clinical trial registration

Not applicable this was a noninterventional observational study.

Introduction

Chest pain is common in pediatric emergency and outpatient practice, but cardiac causes are uncommon in unselected children. Most presentations are musculoskeletal, idiopathic, respiratory, gastrointestinal, or anxiety-related. The clinical challenge is to recognize the smaller group with myocarditis, myopericarditis, arrhythmia, coronary anomalies, or another important cardiac condition without exposing low-risk children to unnecessary testing [1–4].

Electrocardiography, cardiac troponin testing, and echocardiography are valuable when the history, examination, or initial clinical picture raises concern. Indiscriminate testing in low-risk children, however, can increase cost, incidental findings, and downstream evaluation [1, 3, 5–7]. This has prompted interest in blood biomarkers that might add early objective information.

We examined three nonspecific cardiovascular and stress-related biomarkers. Pediatric reference-interval data are available for sST2, and increased concentrations have been reported in children with fulminant myocarditis, but these settings differ from undifferentiated chest-pain evaluation [8, 9]. Galectin-3 is associated with inflammation, fibrosis, and remodeling, although its pediatric cardiovascular role remains unsettled [10, 11]. Copeptin reflects vasopressin-system activation and systemic stress and has primarily been studied in adult chest-pain pathways [12, 13].

Comparator selection was treated as a design variable rather than an analytic afterthought. Clinically well controls can inflate apparent discrimination through spectrum contrast, whereas the point-of-care question is whether a marker distinguishes suspected myocarditis/myopericarditis from competing noncardiac causes among children with the same symptom [14, 15]. We hypothesized that discrimination would be weaker within the symptomatic cohort than in the patient-control contrast. The two defined estimands were interpreted descriptively; no formal between-contrast AUC test was performed because they addressed different clinical questions and populations. The primary objective was within-symptom discrimination; the secondary objective was comparator dependence.

Materials and methods

Design, setting, participants, and ethics

This prospective, single-center exploratory biomarker study was conducted at Kütahya City Hospital, Türkiye, from March 1, 2024, to February 28, 2025. The report was prepared with reference to STARD 2015, where applicable to diagnostic-accuracy elements, and BRISQ for biospecimen reporting [16–18]. The study was approved by the Non-Interventional Clinical Research Ethics Committee of Kütahya Health Sciences University (approval no. 2024/02–06; February 15, 2024). Written parental consent and age-appropriate assent were obtained.

Eligible symptomatic participants were children aged 6–17 years with nontraumatic chest pain. On predefined sampling days, children were approached in presentation order until research-kit capacity was reached; enrollment was sequential within selected days, not continuous. Eligibility and order were determined before biomarker results and final adjudication. All 66 approached families consented; none

was excluded, and biomarker data and reference classification were complete. The primary analysis comprised 7 cardiologist-adjudicated clinically suspected myocarditis/myopericarditis cases and 59 noncardiac cases. The secondary analysis added 22 contemporaneous clinically well controls, frequency-balanced by broad age band and sex but not individually matched. Controls were used only for cross-spectrum context and not for target definition, threshold derivation, model training, or the primary estimand.

Biospecimen collection and research assays

During the index encounter and before disposition, 2–3 mL of venous blood was collected from symptomatic participants and controls into 5-mL serum-separator tubes (13 × 100 mm) containing separating gel. After arrival in the laboratory, samples were allowed to stand for 10–20 minutes and were centrifuged at 3,000 rpm for 20 minutes. The separated serum was transferred to microcentrifuge tubes and stored at – 80°C until analysis.

Serum soluble suppression of tumorigenicity 2 (sST2), galectin-3, and copeptin were measured by sandwich enzyme-linked immunosorbent assay using commercial BT Lab kits (Zhejiang, China) according to the manufacturer's instructions. Catalogue numbers, manufacturer-reported analytical sensitivities, and measurement ranges were E4287Hu, 0.23 ng/mL, and 0.5–150 ng/mL for sST2; E1951Hu, 2.49 pg/mL, and 5–2,000 pg/mL for galectin-3; and E1129Hu, 0.024 ng/mL, and 0.05–20 ng/mL for copeptin. Manufacturer-reported intra-assay and inter-assay coefficients of variation were < 8% and < 10%, respectively. Absorbance was measured at 450 nm, and concentrations were calculated from assay-specific standard curves. Results were reported in ng/mL for sST2 and copeptin and in pg/mL for galectin-3. Concentrations were analyzed on the reported scale without imputation, data-driven transformation, dichotomization, or post hoc reversal of direction. The assays were used for research purposes only, and results were unavailable to treating clinicians, pediatric cardiologists, and adjudicators.

Reference classification and follow-up

The target condition was a pragmatic, pediatric-cardiology-adjudicated classification of suspected myocarditis/myopericarditis, rather than any cardiac cause of chest pain. Classification incorporated structured history and examination, 12-lead electrocardiography, high-sensitivity cardiac troponin I, pediatric cardiology review, echocardiography, the emergency course, and 30-day outcome ascertainment. Follow-up was complete for all 66 symptomatic participants. Adjudication was completed without research biomarker results, which did not contribute to diagnosis, disposition, follow-up, or etiologic classification. The qualifier “clinically suspected” was retained throughout to reflect this reference standard.

High-sensitivity cardiac troponin I was measured with the ARCHITECT STAT High Sensitive Troponin-I assay (Abbott Diagnostics); 11.6 ng/L was the predefined laboratory threshold. High-sensitivity cardiac

troponin I and CRP were components of the routine clinical reference classification. Their inclusion in the composite reference process precludes interpreting either variable as an independent index test; accordingly, they are reported only as classification-support evidence and were not compared head-to-head with the research biomarkers. Cardiac magnetic resonance and endomyocardial biopsy were not performed systematically; the target labels therefore denote clinically suspected, cardiologist-adjudicated disease rather than CMR-confirmed or biopsy-proven myocarditis [7].

Statistical analysis

Continuous variables were summarized as median [IQR] and categorical variables as n (%). The participant was the unit of analysis, and each contributed one index sample. Analyses used complete observations; no research-biomarker data were missing. Primary estimands were single-marker AUCs for clinically suspected myocarditis/myopericarditis versus noncardiac chest pain. Chest-pain participants versus clinically well controls was a secondary cross-spectrum estimand. Biomarkers were continuous; no threshold, data-driven transformation, dichotomization, covariate-adjusted model, or multimarker combination was used.

AUCs and 95% percentile CIs were estimated from 2,000 outcome-stratified nonparametric bootstrap resamples. Primary AUCs were oriented toward clinically suspected myocarditis/myopericarditis; 0.50 denoted no directional discrimination, and values below 0.50 were not inverted. Secondary AUCs were oriented toward chest-pain status. Emphasis was on AUCs and CIs. Mann–Whitney U tests were two-sided and descriptive; primary P values were unadjusted, whereas secondary P values were Benjamini–Hochberg adjusted. No formal primary-versus-secondary AUC test was performed because the contrasts represented different estimands. With only 7 target cases, no cutoff, sensitivity/specificity pair, multivariable or incremental-value model, multimarker score, or equivalence claim was estimated. All analyses were exploratory; bootstrap CIs describe uncertainty in this selected sample and are not validation intervals. Kit capacity determined sample size; no formal power calculation was performed. Figures were generated with Matplotlib 3.10.8.

Results

Participants and clinical diagnoses

The study included 88 participants: 66 with nontraumatic chest pain and 22 clinically well controls. All participants had complete research-biomarker data, 30-day follow-up was complete for all symptomatic participants, and no reference classification was indeterminate (Table 1;Fig. 1). Within the symptomatic cohort, etiologies were musculoskeletal in 42 (63.6%), pulmonary in 9 (13.6%), clinically suspected myocarditis/myopericarditis in 7 (10.6%), psychogenic or anxiety-related in 5 (7.6%), and gastrointestinal in 3 (4.5%).

Table 1
Baseline characteristics of chest-pain participants and clinically well controls.

Variable	Chest-pain participants (n = 66)	Clinically well controls (n = 22)
Age, years	13.5 [10.0–16.0]	13.0 [10.0–16.0]
Male sex, n (%)	36 (54.5)	12 (54.5)
Weight, kg	49.0 [33.5–59.3]	48.0 [32.0–59.0]
Height, cm	155 [142–168]	156 [140–168]
BMI, kg/m ²	20.2 [16.8–22.9]	19.9 [17.0–22.6]
Data are median [IQR] unless otherwise indicated. Controls were frequency-balanced by broad age band and sex; no between-group hypothesis tests were planned. BMI, body mass index.		

Primary within-symptom comparison

The three biomarker distributions overlapped extensively. Median values in suspected myocarditis/myopericarditis versus noncardiac chest-pain participants were 33.8 [28.9–45.5] versus 37.4 [29.1–47.0] ng/mL for sST2, 491 [405–514] versus 504 [458–580] pg/mL for galectin-3, and 5.08 [3.91–5.62] versus 5.80 [4.21–7.03] ng/mL for copeptin. Directional AUCs were 0.47 (95% CI, 0.25–0.70), 0.39 (0.20–0.61), and 0.38 (0.18–0.60), respectively; all three confidence intervals included 0.50 (Table 2; Fig. 2).

Table 2

Primary clinically relevant comparison: suspected myocarditis/myopericarditis versus noncardiac chest pain.

Research biomarker	Suspected myocarditis/ myopericarditis (n = 7)	Noncardiac chest pain (n = 59)	P value	Directional AUC (95% CI)
sST2, ng/mL	33.8 [28.9–45.5]	37.4 [29.1–47.0]	0.82	0.47 (0.25–0.70)
Galectin-3, pg/mL	491 [405–514]	504 [458–580]	0.36	0.39 (0.20–0.61)
Copeptin, ng/mL	5.08 [3.91–5.62]	5.80 [4.21–7.03]	0.31	0.38 (0.18–0.60)
Data are median [IQR]. P values are from unadjusted, two-sided Mann–Whitney U tests and are descriptive. AUCs were oriented toward clinically suspected myocarditis/myopericarditis; values below 0.50 were not inverted. All CIs include 0.50, which neither establishes equivalence nor proves absence of discrimination. CI, confidence interval.				

All 7 suspected myocarditis/myopericarditis cases had high-sensitivity cardiac troponin I above 11.6 ng/L; all 59 noncardiac participants and all 22 controls were below that threshold. CRP was higher in the suspected myocarditis/myopericarditis group than in noncardiac symptomatic participants and was not measured in controls. Because high-sensitivity cardiac troponin I and CRP contributed to the reference

classification, these observations are descriptive and cannot be interpreted as independent head-to-head test comparisons.

Secondary patient-control comparison

All three research biomarkers were higher in the chest-pain cohort than in clinically well controls (Table 3). AUCs, oriented so that higher values indicated chest-pain status, were 0.98 (95% CI, 0.95–1.00) for sST2, 0.98 (0.95–1.00) for galectin-3, and 0.99 (0.97–1.00) for copeptin (Table 4; Fig. 2). These estimates quantify separation between two selected clinical spectra; they do not estimate diagnostic accuracy for myocarditis/myopericarditis, and no direct statistical comparison with the primary AUCs was made.

Table 3
Routine-care variables incorporated into the composite clinical reference classification.

Clinical variable	Suspected myocarditis/ myopericarditis (n = 7)	Noncardiac chest pain (n = 59)	Controls (n = 22)	Role in study
hs-cTnI, ng/L	426 [43–954]; 7/7 above 11.6	4.2 [3.5–5.5]; 59/59 below 11.6	22/22 below 11.6	Classification-support variable
CRP, mg/L	53.8 [44.4–78.1]	3.6 [1.7–7.0]	Not measured	Classification-support variable
High-sensitivity cardiac troponin I and CRP contributed to the routine clinical reference classification; these values are descriptive and are not independent index-test comparisons. CRP was not measured in controls. hs-cTnI, high-sensitivity cardiac troponin I; CRP, C-reactive protein.				

Table 4
Secondary cross-spectrum comparison: chest-pain participants versus clinically well controls.

Research biomarker	Chest-pain participants (n = 66)	Clinically well controls (n = 22)	FDR-adjusted P value	AUC (95% CI)
sST2, ng/mL	37.1 [29.1–47.0]	14.3 [12.5–16.6]	< 0.001	0.98 (0.95–1.00)
Galectin-3, pg/mL	500 [450–579]	299 [271–321]	< 0.001	0.98 (0.95–1.00)
Copeptin, ng/mL	5.63 [4.14–7.01]	2.18 [2.13–2.55]	< 0.001	0.99 (0.97–1.00)
Data are median [IQR]. P values were Benjamini–Hochberg adjusted. Secondary AUCs were oriented toward chest-pain status; 95% CIs used 2,000 outcome-stratified bootstrap resamples. These AUCs quantify cross-spectrum separation, not myocarditis diagnostic accuracy. No formal comparison with primary AUCs was performed.				

Discussion

The central finding was comparator dependence, not validation of a new cardiac biomarker. All three markers showed extensive overlap and no evidence of clinically useful discrimination within the symptomatic cohort, yet nearly perfectly separated selected symptomatic participants from clinically well controls. A patient-control design alone would therefore have produced a qualitatively different—and potentially misleading—interpretation. These data do not show that the biomarkers are universally uninformative; they show that this cohort does not support their use to resolve the symptomatic differential diagnosis.

This pattern is biologically coherent because sST2, galectin-3, and copeptin reflect broad myocardial, inflammatory, remodeling, vasopressin, or systemic-stress pathways rather than myocarditis-specific biology. Prior pediatric studies examined healthy reference intervals, established disease, or fulminant myocarditis, whereas adult copeptin studies focused on acute coronary syndromes [8–13]. A marker may therefore separate symptomatic physiology from wellness without resolving the differential among children with chest pain.

The secondary analysis addressed cross-spectrum group separation, not the clinical differential diagnosis. Although AUC is not determined by prevalence alone, it remains sensitive to case mix and spectrum; these secondary AUCs are therefore not transportable clinical-performance estimates. The contrast illustrates how spectrum composition can materially change apparent test performance [14, 15].

Only 7 target cases were available, so estimates are imprecise; CIs cannot exclude modest discrimination or establish equivalence. AUCs below 0.50 reflect lower observed values in target cases, but wide intervals, overlap, and lack of validation preclude inverse-test interpretation. The inference is absence of evidence of clinically useful discrimination in this cohort, not proof of no association. Cutoffs, modeling, and clinical implementation would be unsupported.

Strengths and limitations

Strengths were prospective sampling, a clinically relevant primary comparator, blinding, complete 30-day follow-up, fixed AUC orientation, separation of classification-support variables from research index tests, and reporting of available specimen and assay details.

The single-center, selected-day, kit-limited design and 7 target cases restrict precision and generalizability; the sampling scheme does not support prevalence estimates and may affect AUCs through case mix. The pragmatic reference standard may misclassify cases because CMR and biopsy were not systematic, although research biomarkers were blinded and excluded. Troponin and CRP were incorporated and therefore were not independent index tests. Selected clinically well controls supplied only secondary context. Missing study-specific assay precision, lot, duplicate-well, run-level quality-control, storage, freeze–thaw, and relative-centrifugal-force records limit reproducibility of absolute

concentrations. These constraints preclude clinical-utility, incremental-value, equivalence, or transportability claims. Missing study-specific assay precision, kit lot numbers, duplicate-well procedures, run-level quality-control data, storage-duration records, freeze–thaw information, and relative centrifugal force values limit the reproducibility of absolute biomarker concentrations.

Clinical implications and next steps

Future studies should use multicenter consecutive enrollment, a screening log, source-locked reference criteria, blinded independent adjudication, standardized CMR reporting when clinically indicated, and complete BRISQ-aligned assay documentation. Adequate target-case accrual is needed to estimate precision and incremental value beyond clinical assessment, electrocardiography, and troponin. Clinically well controls may provide context but should not be the sole comparator for clinical claims.

Conclusion

In this prospective exploratory cohort, the biomarkers provided no evidence of clinically useful discrimination between clinically suspected myocarditis/myopericarditis and noncardiac chest pain. Near-perfect separation from clinically well controls was comparator dependent and cannot be interpreted as myocarditis diagnostic or triage accuracy. The study does not validate a diagnostic test; it shows why nonspecific biomarkers require evaluation against symptomatic alternatives.

Declarations

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Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Author contributions: All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Sarper Şahin, İbrahim Işıkman, Fatmagül Can, Batuhan Berk Demir, and Hüsam İbrahimoglu. The first draft of the manuscript was written by Sarper Şahin, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval: This study was performed in accordance with the principles of the Declaration of Helsinki and its later amendments. Approval was granted by the Non-Interventional Clinical Research Ethics Committee of Kütahya Health Sciences University (February 15, 2024; approval no. 2024/02-06).

Consent to participate: Written informed consent was obtained from the parents or legal guardians, together with age-appropriate assent from participating children.

Consent to publish: Not applicable; the manuscript contains no identifying individual participant information, images, or videos.

Data availability: The de-identified data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Clinical trial registration: Not applicable; this was a non-interventional observational study.

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Figures

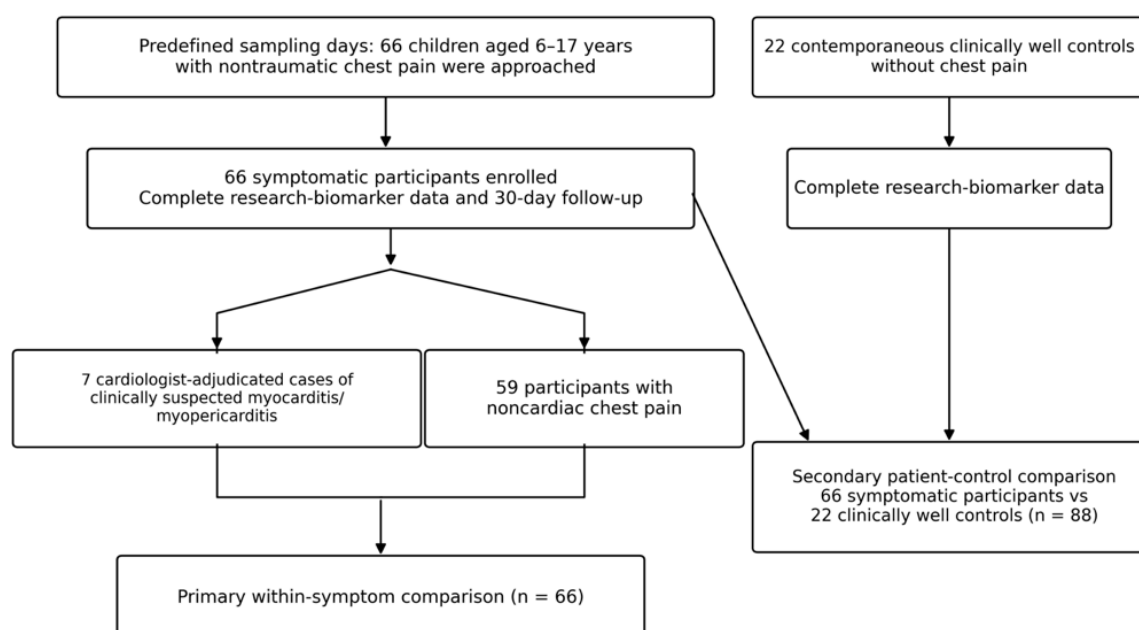


Figure 1. Participant flow and analysis populations. Enrollment was sequential on predefined sampling days and limited by research-kit capacity; all symptomatic participants had complete biomarker data and 30-day follow-up.

Figure 1

See image above for figure legend.

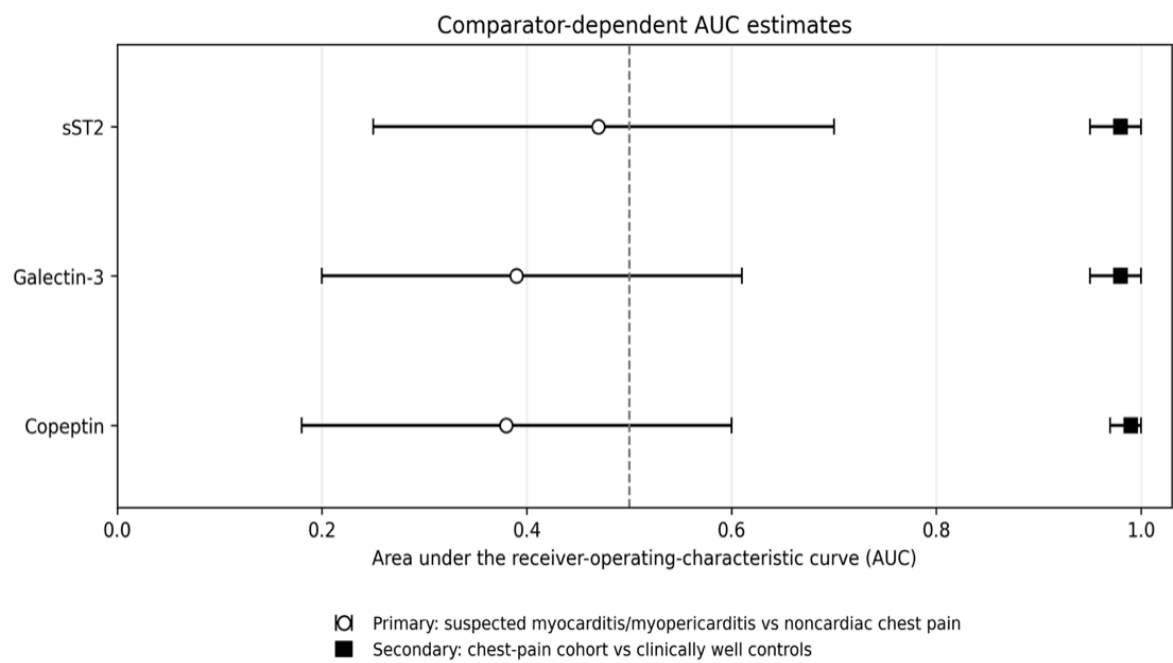


Figure 2. Comparator-dependent AUC estimates. Open circles show the primary clinically suspected myocarditis/myopericarditis-versus-noncardiac comparison; filled squares show the secondary chest-pain-versus-control comparison. Error bars are 95% outcome-stratified bootstrap CIs. Primary values below 0.50 were not inverted; the two estimands were not formally compared.

Figure 2

See image above for figure legend.