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Copeptin as an Early Adjunct to High-Sensitivity Cardiac Troponin I in Initially Troponin-Negative Acute Myocardial Infarction: A Prospective Case–Control Study

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Abstract

Early diagnosis of acute myocardial infarction (AMI) remains challenging in patients with initially non-elevated high-sensitivity cardiac troponin I (hs-cTnI). We assessed the diagnostic contribution of copeptin, with endothelin-1 and chemerin as exploratory comparators. This prospective, single-center case–control study included 160 adults with suspected acute coronary syndrome: 40 controls, 40 with unstable angina, 40 with non-ST-elevation myocardial infarction (NSTEMI), and 40 with ST-elevation myocardial infarction (STEMI). Samples were obtained at presentation, and diagnoses were adjudicated blinded to investigational biomarkers. Twelve of the 80 AMI cases had initially non-elevated hs-cTnI (7 STEMI; 5 NSTEMI). Copeptin was positive in 8/12 and the three-marker panel in 9/12. Among the five initially troponin-negative NSTEMI cases, all positive on repeat hs-cTnI testing, copeptin was positive in 4/5 (80.0%; 95% confidence interval, 28.4–99.5%). The hs-cTnI OR copeptin strategy increased sensitivity from 85.0% to 95.0%, while specificity among controls decreased from 95.0% to 62.5%. Adding the biomarkers to a clinical model containing hs-cTnI changed the apparent area under the curve from 0.961 to 0.965, without significantly improving model fit (likelihood-ratio $p = 0.718$). These findings may support evaluating adjunctive copeptin within contemporary accelerated troponin pathways.

Keywords: myocardial infarction; copeptin; troponin I; emergency service; hospital; sensitivity and specificity

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1. Introduction

Chest pain is a frequent reason for emergency department evaluation, although only a minority of patients have acute coronary syndrome (ACS) [1,2]. Contemporary pathways integrate clinical assessment, electrocardiography, and high-sensitivity cardiac troponin (hs-cTn), with accelerated 0/1 h and 0/2 h algorithms recommended for suspected non-ST-segment elevation myocardial infarction (NSTEMI) [1,3]. Diagnostic uncertainty is

particularly relevant soon after symptom onset, when serial sampling may be needed despite a non-elevated initial troponin concentration [3,4]. Biomarkers reflecting complementary biological responses have therefore been investigated to improve assessment at presentation [5].

Copeptin, the stable C-terminal fragment of provasopressin, reflects acute endogenous stress and may complement the assessment of cardiomyocyte injury provided by troponin [6,7]. Its incremental diagnostic value in the hs-cTn era varies with the troponin assay and decision threshold [8,9]. These considerations motivate examination of both additional biomarker positivity among initially troponin-negative cases and the overall sensitivity–specificity trade-off.

Endothelin-1 and chemerin represent other potentially relevant pathways. Endothelin-1, an endothelium-derived vasoconstrictor, has been associated with impaired microvascular reperfusion after STEMI and adverse outcomes in coronary artery disease [10,11]. Chemerin is an adipokine implicated in inflammation, metabolic risk, and atherosclerotic biology, with heterogeneous findings in ACS [12–14]. We selected these biomarkers as exploratory comparators to assess whether vascular and inflammatory–metabolic signals complemented the acute stress response represented by copeptin.

We evaluated copeptin, endothelin-1, and chemerin alongside high-sensitivity cardiac troponin I (hs-cTnI) in adults with suspected ACS. We examined presentation-sample positivity in initially troponin-negative acute myocardial infarction (AMI), assessed each biomarker individually, and tested whether their combination improved discrimination beyond clinical variables and hs-cTnI.

2. Materials and Methods

2.1. Study Design, Setting, and Participants

We conducted a prospective, single-center, balanced case–control diagnostic accuracy study, reported in accordance with the Standards for Reporting Diagnostic Accuracy Studies (STARD) 2015 [15]. The study was performed in the Emergency Department of Inonu University Turgut Ozal Medical Center, a tertiary university hospital in Malatya, Türkiye, between 1 May and 1 August 2024. The protocol was approved by the Inonu University Health Sciences Scientific Research Ethics Committee (Decision No. 2024/58; 24 April 2024), and all participants provided written informed consent.

Adults aged 18 years or older presenting with chest pain or symptoms suggestive of ACS were eligible. Blood samples were collected at presentation from eligible patients. Recruitment followed a quota-based scheme targeting 40 participants in each final diagnostic group: control, unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI). Patients were assessed in order of presentation until 40 participants with complete clinical data and suitable specimens were included in each diagnostic group. Recruitment then stopped for that group, and subsequent otherwise eligible patients in that group were not enrolled. The final cohort comprised 160 participants, with an AMI prevalence of 50%.

Exclusion criteria were pregnancy, active infection, malignancy, chronic inflammatory or autoimmune disease, severe renal dysfunction (estimated glomerular filtration rate <30 mL/min/1.73 m^2), severe hepatic failure, surgery or trauma within the previous 3 months, alternative acute cardiovascular diagnoses including pulmonary embolism or aortic dissection, incomplete clinical data, and hemolyzed specimens. Of 180 patients assessed for eligibility, 20 were excluded (four declined participation, nine had incomplete clinical data, and seven had hemolyzed specimens), leaving 160 participants in the final analysis (Figure S1).

2.2. Clinical Assessment and Reference Standard

All participants underwent medical history, physical examination, 12-lead electrocardiography, and hs-cTnI testing. hs-cTnI was measured at presentation (0 h) and, when clinically indicated, at 2 h using a chemiluminescent immunoassay (Access hsTnI; Beckman Coulter, Brea, CA, USA). The assay limit of detection was 2.3 ng/L; the sex-specific 99th-percentile upper reference limits were 11.6 ng/L for women and 19.8 ng/L for men. Patients with ACS underwent bedside echocardiography after the diagnosis had been established.

The reference standard was the final adjudicated diagnosis, integrating available serial hs-cTnI results, electrocardiographic findings, clinical course, and coronary angiography when performed. AMI definitions followed the Fourth Universal Definition of Myocardial Infarction [16]. Investigational biomarker results were unavailable to treating clinicians and adjudicators. STEMI was defined by ischemic symptoms and persistent ST-segment elevation meeting guideline-compatible electrocardiographic criteria, with angiographic correlation. NSTEMI required a rise and/or fall in hs-cTnI, with at least one value above the sex-specific 99th percentile, in a clinical presentation consistent with acute myocardial ischemia without persistent ST-segment elevation. UA was defined as ischemic symptoms occurring at rest or with minimal exertion without evidence of acute myocardial injury or necrosis. Presentations included prolonged rest angina (>20 min), new-onset severe angina, or worsening angina with increasing frequency or duration or a lower exertional threshold [3].

Controls presenting with chest pain were classified as non-ACS after clinical evaluation. Non-AMI participants with elevated presentation hs-cTnI retained their adjudicated diagnoses and were classified as hs-cTnI-positive in analyses using the sex-specific 99th-percentile thresholds.

Coronary angiography was performed in all 120 patients with ACS and one control.

2.3. Index Biomarkers

At the presentation blood draw, an additional venous sample was collected, centrifuged, and stored at −80 °C until batch analysis. Serum copeptin, endothelin-1, and chemerin were measured in duplicate with research-use enzyme-linked immunosorbent assay (ELISA) kits (Reed Biotech, Wuhan, China), following the manufacturer's instructions (Table S1). Manufacturer-stated minimum detectable concentrations were 18.75 pg/mL, 0.75 pg/mL, and 0.10 ng/mL, respectively, and served as limits of detection (LoDs) in sensitivity analyses. The corresponding calibration ranges were 31.25–2000 pg/mL, 1.25–80 pg/mL, and 0.16–10 ng/mL. The manufacturer reports a general coefficient-of-variation upper bound of ≤10% for intra- and inter-plate precision; separate numerical estimates are not provided (Table S1). Results became available after clinical classification and did not guide management. Primary analyses retained all reported concentrations, including six copeptin results entered as numerical zeros. There were 25 copeptin results below the lower calibration limit (including the six zeros), 11 endothelin-1 results below the lower limit, and seven chemerin results below and two above the range. Copeptin was the primary candidate adjunct; endothelin-1 and chemerin were exploratory comparators.

2.4. Test Positivity and Rule-Based Strategies

hs-cTnI positivity was defined by the sex-specific 99th-percentile limits. Investigational biomarker thresholds for rule-based strategies were derived by the Youden index in the control-versus-AMI comparison: copeptin ≥79.303 pg/mL, endothelin-1 ≥14.360 pg/mL, and chemerin ≤0.425 ng/mL. These exploratory thresholds were applied in both diagnostic comparisons. Chemerin ROC analyses used lower concentrations to indicate AMI; concentrations at or below its threshold were positive. In regression, chemerin remained

continuous in its original concentration direction. OR-rule strategies required hs-cTnI or the investigational biomarker component to be positive; AND-rule strategies required both. For the panel, the investigational component was positive if any of its three biomarkers met its threshold.

2.5. Data Completeness and Serial Troponin Sampling

All 160 participants had complete presentation biomarker and primary-model covariate data; no imputation was used. A 2 h hs-cTnI sample was available for all 40 NSTEMI patients, all 40 UA patients, and 28 controls. STEMI patients proceeded directly to reperfusion and were diagnosed by electrocardiography with angiographic correlation. Paired NSTEMI measurements identified cases with non-elevated presentation hs-cTnI and an elevated 2 h value, in whom presentation biomarker positivity was assessed.

2.6. Sample Size

Sample size was determined a priori for an omnibus comparison of biomarker concentrations across four groups. Sample size calculations were performed using WSSPAS (Web-Based Sample Size & Power Analysis Software, v3.5.0; Inonu University, Malatya, Türkiye). A one-way analysis of variance framework with $\alpha = 0.05$, power = 0.80, and Cohen's f = 0.2655 required 160 participants. This calculation targeted group differences in concentration rather than diagnostic accuracy endpoints.

2.7. Statistical Analysis

Analyses used R 4.5.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables are reported as median and interquartile range, and categorical variables as number and percentage. Group comparisons used the Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables. Pairwise comparisons used Dunn's test or Fisher's exact test, respectively, with Bonferroni adjustment. Trends across the ordered diagnostic categories were assessed by the Jonckheere–Terpstra test.

Diagnostic performance was assessed for AMI versus controls and AMI versus UA. Each investigational biomarker was evaluated individually by a receiver operating characteristic (ROC) analysis and jointly in logistic regression models. Empirical areas under the curve (AUCs) were reported with DeLong 95% confidence intervals (CIs). Pointwise 95% CIs for ROC sensitivity at fixed false-positive rates were obtained from 10,000 stratified nonparametric bootstrap resamples, resampling cases and reference participants separately and using identical participant indices across biomarkers. The 2.5th and 97.5th percentiles defined the interval limits. Rule-based combinations paired hs-cTnI with copeptin or with any investigational biomarker under OR and AND rules. Sensitivity and specificity were reported with exact Clopper–Pearson 95% CIs; paired differences relative to hs-cTnI alone were assessed by the exact McNemar test.

Incremental performance was assessed by comparing a model containing age, sex, creatinine, and hs-cTnI (M3) with the same model plus the three investigational biomarkers (M4). Biomarkers were transformed as $\ln(1 + x)$ and standardized using the original full-cohort mean and standard deviation of the transformed values, retained across sensitivity analyses; biomarker odds ratios therefore represent a one-baseline-standard-deviation increase. Apparent AUC differences were descriptive. The joint contribution of the three added terms was tested by a likelihood-ratio comparison of M4 with M3 (3 degrees of freedom), as appropriate for nested models fitted and evaluated in the same data [17]. Original M3 and M4 models underwent internal validation with 1000 stratified bootstrap resamples, refitting the models to estimate optimism.

Positive and negative predictive values were projected across assumed AMI prevalences using Bayes' theorem. Cumulative symptom-onset subgroup comparisons used the exact McNemar test. Covariate-adjusted correlations were calculated as Spearman correlations between residuals from linear models of ln(1 + biomarker concentration), adjusted for age, sex, recorded ejection fraction, blood urea nitrogen, creatinine, and diabetes. A proportional-odds model across the four ordered diagnostic categories was adjusted for age, sex, and the other biomarkers.

Prevalence-adjusted decision-curve analysis compared the rule-based strategies [18–20]. The control-versus-AMI comparison contained 80 AMI cases and 40 controls (66.7% AMI). Net benefit was projected from sensitivity (Se) and specificity (Sp) as $\pi \times Se - (1 - \pi) \times (1 - Sp) \times p_t/(1 - p_t)$, where π was the assumed AMI prevalence (5%, 10%, 15%, or 20%; reference, 10%) and p_t the threshold probability (0.5–20%). Differences from hs-cTnI alone were reported with 95% percentile intervals from 2000 bootstrap resamples drawn separately within cases and controls.

Sensitivity analyses replaced all investigational biomarker values below the manufacturer-stated LoD with LoD/2 or LoD/$\sqrt{2}$. Separate scenarios capped values above the upper calibration limit, combined upper capping with each lower-limit substitution, or clipped values to both calibration-range boundaries. Models were refitted using the same participants, covariates, transformations, and baseline standardization. Individual and model AUCs, odds ratios with Wald 95% CIs, and M4-versus-M3 likelihood-ratio tests were recalculated. Classification changes were assessed at the original thresholds, without re-optimization. All tests were two-sided, with $p < 0.05$ considered statistically significant.

In an exploratory analysis, we retrospectively applied the biochemical criteria of the ESC 0/2 h algorithm for the Access hsTnI assay to participants without STEMI who had paired measurements [3]. Rule-out required presentation hs-cTnI <4 ng/L with symptom onset >3 h earlier, or presentation hs-cTnI <5 ng/L with an absolute 2 h change <5 ng/L. Rule-in required presentation hs-cTnI ≥50 ng/L or an absolute 2 h change ≥20 ng/L; remaining participants were assigned to observe. The presentation-only rule-out branch was applied first. Presentation copeptin positivity was cross-tabulated against these categories using the existing threshold of ≥79.303 pg/mL, without re-optimization. Adjudicated diagnoses were retained. Participants lacking a second measurement were assessed separately using presentation criteria and remained unclassified when these were insufficient.

3. Results

3.1. Study Cohort and Baseline Characteristics

The cohort comprised 160 participants, with 40 in each diagnostic group. NSTEMI patients were older than controls and UA patients, and both AMI groups had lower left ventricular ejection fractions. The NSTEMI group had the highest proportions of diabetes and prior coronary artery disease. Sex distribution and time from symptom onset to sampling did not differ significantly between groups. Table 1 summarizes baseline characteristics, and Figure S2 shows biomarker distributions.

Six non-AMI participants (two controls and four with UA) had presentation hs-cTnI above the sex-specific 99th percentile (12.0–54.3 ng/L). All six had a 2 h sample, with observed absolute changes of 0–9.3 ng/L. These elevations were clinically attributed to causes other than AMI. Among controls with serial measurements, no diagnostically significant troponin rise was identified.

Table 1. Baseline demographic, clinical, and laboratory characteristics across diagnostic groups.

Characteristic	Control (n = 40)	UA (n = 40)	NSTEMI (n = 40)	STEMI (n = 40)	p
Age (years)	53.0 (45.0–69.0) [a]	53.0 (46.5–64.5) [a]	68.0 (59.0–74.0) [b]	62.0 (53.0–72.0) [ab]	0.001
Female	14 (35.0%)	8 (20.0%)	11 (27.5%)	10 (25.0%)	0.534
Dyspnea	14 (35.0%)	7 (17.5%)	8 (20.0%)	8 (20.0%)	0.270
Hypertension	17 (42.5%)	19 (47.5%)	23 (57.5%)	19 (47.5%)	0.614
Diabetes mellitus	6 (15.0%)	7 (17.5%)	16 (40.0%)	9 (22.5%)	0.048
Coronary artery disease (history)	14 (35.0%) [a]	16 (40.0%) [a]	30 (75.0%) [b]	14 (35.0%) [a]	<0.001
Chronic kidney disease	1 (2.5%)	4 (10.0%)	3 (7.5%)	2 (5.0%)	0.699
Ejection fraction (%)	60 (60–60)[a]	60 (52–60) [a]	50 (48–60) [b]	50 (45–50)[b]	<0.001
Creatinine (mg/dL)	0.9 (0.8–1.2)	1.0 (0.9–1.2)	1.1 (0.9–1.3)	1.0 (0.9–1.2)	0.095
BUN (mg/dL)	13.1 (11.0–17.3) [a]	15.0 (11.7–16.8) [ab]	18.0 (14.5–20.3) [b]	16.4 (12.2–22.2) [ab]	0.012
AST (U/L)	23.5 (18.5–31.5) [ab]	20.5 (17.0–27.0) [b]	25.0 (20.5–33.5) [ac]	30.0 (22.0–39.5) [c]	<0.001
ALT (U/L)	18.0 (14.5–31.0)	21.5 (15.0–27.0)	19.5 (16.0–24.5)	21.0 (14.0–30.5)	0.956
CK (U/L)	94.0 (65.5–134.0) [ab]	76.5 (57.5–114.5) [b]	112.0 (78.0–186.5) [a]	128.5 (92.0–294.0) [a]	<0.001
CK-MB (U/L)	22.0 (15.5–38.5) [a]	16.5 (11.0–22.0) [b]	18.0 (13.0–28.0) [ab]	24.5 (15.5–39.0) [a]	0.006
hs-cTnI (ng/L)	2.90 (1.87–5.22) [a]	3.40 (2.00–5.20) [a]	267.50 (72.62–788.75) [b]	174.50 (31.25–943.00) [b]	<0.001
Copeptin (pg/mL)	75.43 (31.77–139.68)	93.12 (59.94–125.02)	97.39 (51.07–129.05)	93.69 (64.38–167.02)	0.491
Endothelin-1 (pg/mL)	8.02 (3.47–12.93)	7.87 (3.52–13.47)	10.59 (4.04–16.23)	7.02 (4.53–13.41)	0.620
Chemerin (ng/mL)	0.59 (0.41–0.90)	0.56 (0.43–0.86)	0.41 (0.29–0.70)	0.46 (0.26–0.60)	0.019
Symptom onset to sampling (hours)	6.0 (4.0–12.0)	12.0 (4.0–24.0)	6.0 (4.5–24.0)	5.0 (3.0–12.5)	0.152

Data are presented as median (interquartile range) or n (%). Overall p values are from the Kruskal–Wallis test or Fisher's exact test. Superscript letters summarize pairwise Dunn or Fisher comparisons with Bonferroni adjustment: groups without a common letter differ at adjusted $p < 0.05$. Letters are omitted when no pairwise comparison is significant. UA, unstable angina; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; CK-MB, creatine kinase-MB; hs-cTnI, high-sensitivity cardiac troponin I.

3.2. Copeptin and Early Troponin-Negative Myocardial Infarction

Twelve of 80 AMI patients had non-elevated presentation hs-cTnI: seven STEMI and five NSTEMI. Copeptin was positive in 8/12 and the panel in 9/12 (Figures 1c and S3). Panel positivity was 3/5 among those presenting within 3 h and 8/11 within 6 h (Table S2).

All 40 NSTEMI patients had paired troponin measurements; the five initially negative cases had elevated hs-cTnI at 2 h (Table S3). Presentation copeptin was positive in 4/5 (80.0%; exact 95% CI, 28.4–99.5%), and the panel in 5/5 (100%; exact 95% CI, 47.8–100%). No additional positive troponin results occurred among the 28 controls with a 2 h sample. In a retrospective presentation-only assessment, none of the five NSTEMI cases met the ESC single-sample rule-out criterion of hs-cTnI <4 ng/L with symptom onset >3 h [3]. Their presentation values ranged from 3.5 to 16.0 ng/L; the only value below 4 ng/L was obtained 1 h after symptom onset. Individual-marker AUCs for these 12 AMI cases versus controls or UA ranged from 0.560 to 0.627 (Table S4).

Among 108 participants with paired measurements, retrospective ESC biochemical classification assigned 43 to rule-out (18 controls and 25 UA), 24 to observe (10 controls and 14 UA), and 41 to rule-in (40 NSTEMI and one UA) (Table S18). All five initially troponin-negative NSTEMI cases met the 2 h change criterion for rule-in, with absolute changes of 22.5–197 ng/L. Presentation copeptin was positive in 23/43 rule-out and 12/24 observe participants, all of whom had adjudicated non-AMI diagnoses. Including participants without a second measurement yielded 51 rule-out, 24 observe, 41 rule-in, and four unclassified participants.

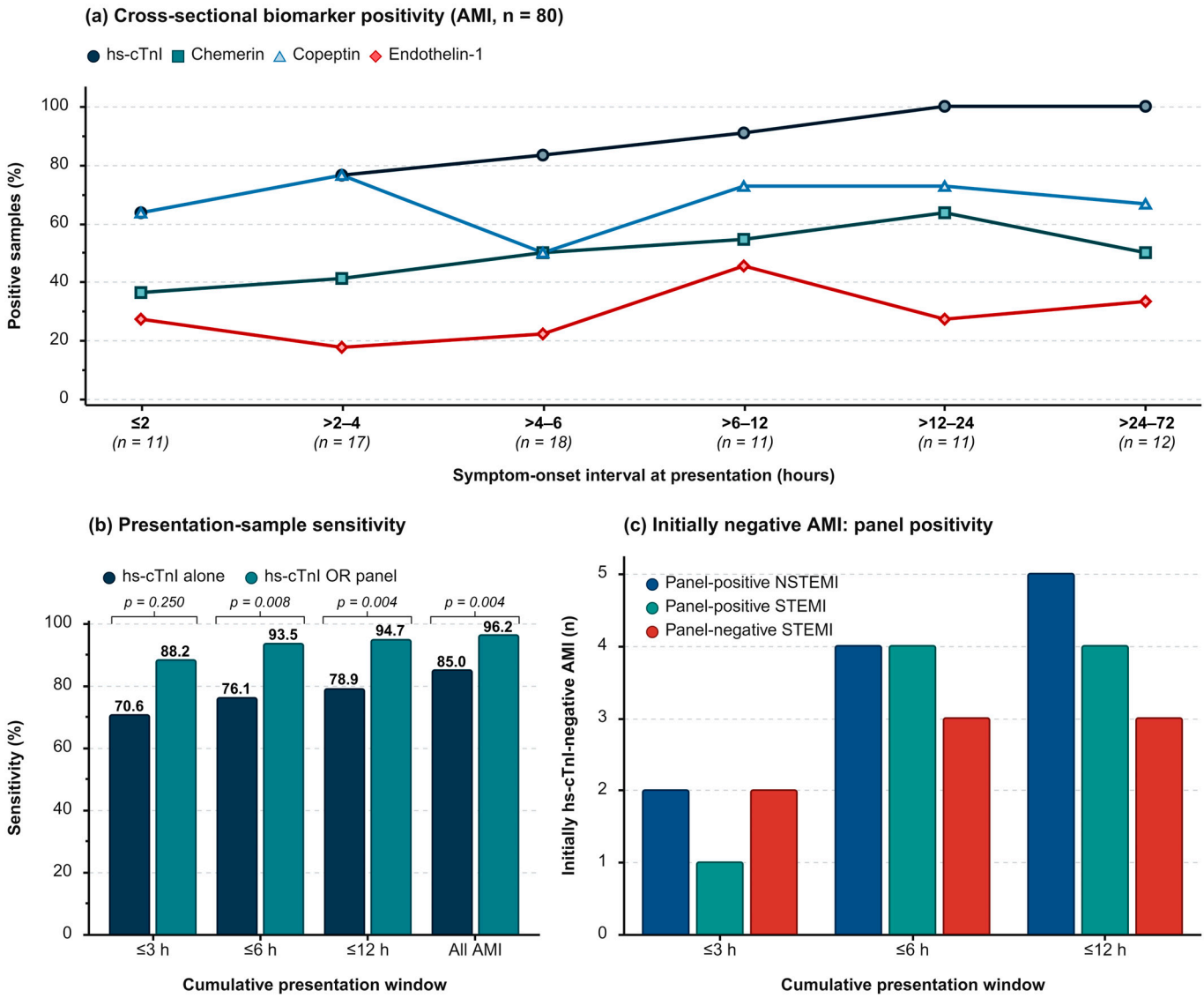


Figure 1. Presentation-sample biomarker positivity and sensitivity among patients with AMI. (**a**) Cross-sectional positivity within non-overlapping intervals from symptom onset to presentation; each patient contributes one sample. (**b**) Sensitivity of presentation hs-cTnI alone and the OR-rule combining hs-cTnI with the investigational panel, within cumulative presentation windows; *p* values are from the exact McNemar test. (**c**) Panel positivity among initially troponin-negative AMI cases within cumulative windows, shown separately for NSTEMI and STEMI; overall, the 12 initially troponin-negative cases comprised 5 NSTEMI and 7 STEMI, and the 3 cases not detected by the panel were all STEMI. Positivity thresholds: hs-cTnI, sex-specific 99th percentile; copeptin ≥79.303 pg/mL; endothelin-1 ≥14.360 pg/mL; chemerin ≤0.425 ng/mL. Panel positivity means at least one investigational biomarker is positive.

3.3. Rule-Based Sensitivity Gain and Specificity Trade-Off

The hs-cTnI OR panel strategy increased AMI sensitivity from 85.0% to 96.2% overall ($p = 0.004$), from 76.1% to 93.5% within 6 h ($p = 0.008$), and from 78.9% to 94.7% within 12 h ($p = 0.004$) (Figure 1b; Tables 2, S2 and S5). These cumulative windows overlap. hs-cTnI OR copeptin achieved 95.0% sensitivity overall and 91.3% within 6 h. The overall gains represented nine additional positive AMI cases for the panel and eight for copeptin among the 12 initially troponin-negative cases. In NSTEMI (n = 40), sensitivity increased from 87.5% (35/40; 95% CI, 73.2–95.8%) with hs-cTnI alone to 97.5% (39/40; 95% CI, 86.8–99.9%) with hs-cTnI OR copeptin ($p = 0.125$) and 100% (40/40; 95% CI, 91.2–100%) with hs-cTnI

OR panel ($p = 0.062$). Within 6 h (n = 21), the corresponding NSTEMI sensitivities were 81.0%, 95.2%, and 100%. In STEMI (n = 40), sensitivity increased from 82.5% (33/40) to 92.5% (37/40) with either OR strategy ($p = 0.125$). The within-subtype paired differences were not statistically significant (Table S5).

Table 2. Diagnostic performance of individual biomarkers and rule-based strategies for AMI.

Comparison	Biomarker/Strategy	Type	AUC (95% CI)	*p*	Cut-Off	Se (%)	Sp (%)	LR+	LR−
Control (40) vs. AMI (80)	Copeptin	Individual	0.577 (0.463–0.691)	0.185	≥79.303	66.2	62.5	1.77	0.54
	Endothelin-1	Individual	0.554 (0.444–0.664)	0.334	≥14.360	27.5	82.5	1.57	0.88
	Chemerin	Individual	0.633 (0.528–0.738)	0.013	≤0.425	48.8	75.0	1.95	0.68
	hs-cTnI alone	Strategy	–	–	Rule	85.0	95.0	17.00	0.16
	hs-cTnI OR copeptin	Strategy	–	–	Rule	95.0	62.5	2.53	0.08
	hs-cTnI AND copeptin	Strategy	–	–	Rule	56.2	95.0	11.25	0.46
	hs-cTnI OR any novel	Strategy	–	–	Rule	96.2	42.5	1.67	0.09
	hs-cTnI AND any novel	Strategy	–	–	Rule	73.8	95.0	14.75	0.28
UA (40) vs. AMI (80)	Copeptin	Individual	0.533 (0.426–0.640)	0.548	≥93.404	57.5	57.5	1.35	0.74
	Endothelin-1	Individual	0.556 (0.448–0.664)	0.310	≥8.795	55.0	62.5	1.47	0.72
	Chemerin	Individual	0.652 (0.553–0.752)	0.003	≤0.415	46.2	82.5	2.64	0.65
	hs-cTnI alone	Strategy	–	–	Rule	85.0	90.0	8.50	0.17
	hs-cTnI OR copeptin	Strategy	–	–	Rule	95.0	32.5	1.41	0.15
	hs-cTnI AND copeptin	Strategy	–	–	Rule	56.2	92.5	7.50	0.47
	hs-cTnI OR any novel	Strategy	–	–	Rule	96.2	17.5	1.17	0.21
	hs-cTnI AND any novel	Strategy	–	–	Rule	73.8	92.5	9.83	0.28

AMI includes NSTEMI and STEMI. Empirical AUCs are reported with DeLong 95% CIs; individual-marker *p* values test AUC = 0.5. Individual biomarker cut-offs (copeptin and endothelin-1, pg/mL; chemerin, ng/mL) were determined using the Youden index within each comparison and are exploratory. Chemerin ROC analyses used lower concentrations to indicate AMI; concentrations at or below the reported cut-off were coded as positive when calculating sensitivity, specificity, and likelihood ratios. hs-cTnI positivity was defined using sex-specific 99th-percentile upper reference limits (female 11.6 ng/L; male 19.8 ng/L). Rule-based strategies in both comparisons used the thresholds derived for the control-versus-AMI comparison (copeptin ≥79.303 pg/mL, endothelin-1 ≥14.360 pg/mL, chemerin ≤0.425 ng/mL). OR-rule strategies were positive if any component was positive; AND-rule strategies were positive only if hs-cTnI and the relevant investigational biomarker were both positive ("any novel": at least one of copeptin, endothelin-1, or chemerin). CIs and paired comparisons for the strategies are shown in Table S6, and predictive values projected across plausible prevalences in Table S7. AUC, area under the curve; CI, confidence interval; Se, sensitivity; Sp, specificity; LR+, positive likelihood ratio; LR−, negative likelihood ratio.

Specificity among the 40 controls was 95.0% for hs-cTnI alone, 42.5% for hs-cTnI OR panel, and 62.5% for hs-cTnI OR copeptin (both $p < 0.001$ versus hs-cTnI alone; Tables 2 and S6). Corresponding specificities among the 40 UA patients were 90.0%, 17.5%, and 32.5%. Among the 120 participants without STEMI, 39 were negative for both hs-cTnI and copeptin at presentation; 38 had non-AMI diagnoses (25 controls and 13 UA), and one had NSTEMI. Under the AND rule, sensitivity was 56.2% for hs-cTnI and copeptin and 73.8% for hs-cTnI and any investigational biomarker; specificity among controls remained 95.0%. Prevalence-adjusted predictive values are reported in Table S7.

At an assumed AMI prevalence of 10%, hs-cTnI OR copeptin had higher projected net benefit than hs-cTnI alone below a threshold probability of 3.3%; the corresponding crossover for hs-cTnI OR panel was 2.3% (Figure S5; Table S8). The copeptin strategy's net-benefit difference was 0.70 per 100 patients at a 1% threshold (95% interval, 0.06–1.40) and −2.25 per 100 patients at a 10% threshold (95% interval, −3.88 to −0.75). Across assumed AMI prevalences of 5–20%, its crossover threshold ranged from 1.6% to 7.1%.

3.4. Overall Diagnostic Discrimination and Incremental Value

hs-cTnI had the highest individual-marker AUC for AMI versus controls: 0.954 (95% CI, 0.921–0.987). Chemerin showed modest inverse discrimination, whereas copeptin and endothelin-1 AUCs were not significantly different from 0.5 (Figure 2; Table 2). Results across diagnostic contrasts and other copeptin analyses are reported in Tables S9 and S10 and Figure S6.

Adding hs-cTnI to clinical covariates improved discrimination. Further addition of the three investigational biomarkers increased the control-versus-AMI AUC from 0.961 to 0.965 (ΔAUC, 0.004); the likelihood-ratio comparison of M4 with M3 yielded $\chi^2 = 1.347$ (3 degrees of freedom; $p = 0.718$) (Table 3; Figure S7). The corresponding p value versus UA was 0.218. Bootstrap-estimated optimism was 0.008 for M3 and 0.017 for M4, giving optimism-corrected AUCs of 0.953 and 0.948, respectively (Figure S8).

Table 3. Diagnostic performance of clinical and biomarker models for AMI.

Comparison	Model	Variables	AUC (95% CI)	ΔAUC vs. M0	Brier Score
Control vs. AMI	M0	Clinical	0.696 (0.590–0.802)	–	0.1932
	M1	Investigational biomarkers	0.618 (0.510–0.727)	−0.077	0.2176
	M2	M0 + investigational biomarkers	0.724 (0.626–0.821)	0.028	0.1881
	M3	M0 + hs-cTnI	0.961 (0.933–0.989)	0.265	0.0769
	M4	M3 + investigational biomarkers	0.965 (0.938–0.991)	0.269	0.0750
UA vs. AMI	M0	Clinical	0.698 (0.597–0.798)	–	0.2005
	M1	Investigational biomarkers	0.594 (0.487–0.701)	−0.103	0.2192
	M2	M0 + investigational biomarkers	0.708 (0.609–0.808)	0.011	0.1985
	M3	M0 + hs-cTnI	0.945 (0.909–0.982)	0.248	0.0884
	M4	M3 + investigational biomarkers	0.950 (0.915–0.985)	0.252	0.0818

All models were fitted on the same complete-case dataset within each comparison (80 AMI and 40 reference participants). Biomarkers and hs-cTnI were ln(1 + x)-transformed and standardized. M0 = age, sex, creatinine; M1 = copeptin, endothelin-1, chemerin; M2 = M0 + M1; M3 = M0 + hs-cTnI; M4 = M3 + M1. All biomarker terms in these model definitions refer to the transformed, standardized continuous values. Chemerin was retained in its original concentration direction: an OR below 1 indicates lower AMI odds with increasing chemerin. Apparent AUCs and DeLong 95% CIs describe performance in the fitted sample; changes in AUC are descriptive. The joint contribution of the three biomarkers to M4 versus M3 was assessed by a 3-df likelihood-ratio test ($p = 0.718$ for controls; $p = 0.218$ for UA). Brier scores quantify prediction error in the enriched study sample. AUC, area under the curve; CI, confidence interval; OR, odds ratio.

Across six alternative assay-limit scenarios, M4 AUCs ranged from 0.965 to 0.966 versus controls and from 0.950 to 0.952 versus UA. M4-versus-M3 likelihood-ratio p values ranged from 0.668 to 0.757 and from 0.184 to 0.219, respectively. No fixed-threshold classification changed. Upper-range chemerin capping increased the biomarker-only M1 AUC from 0.618 to 0.655 versus controls and from 0.594 to 0.640 versus UA (Tables S15–S17).

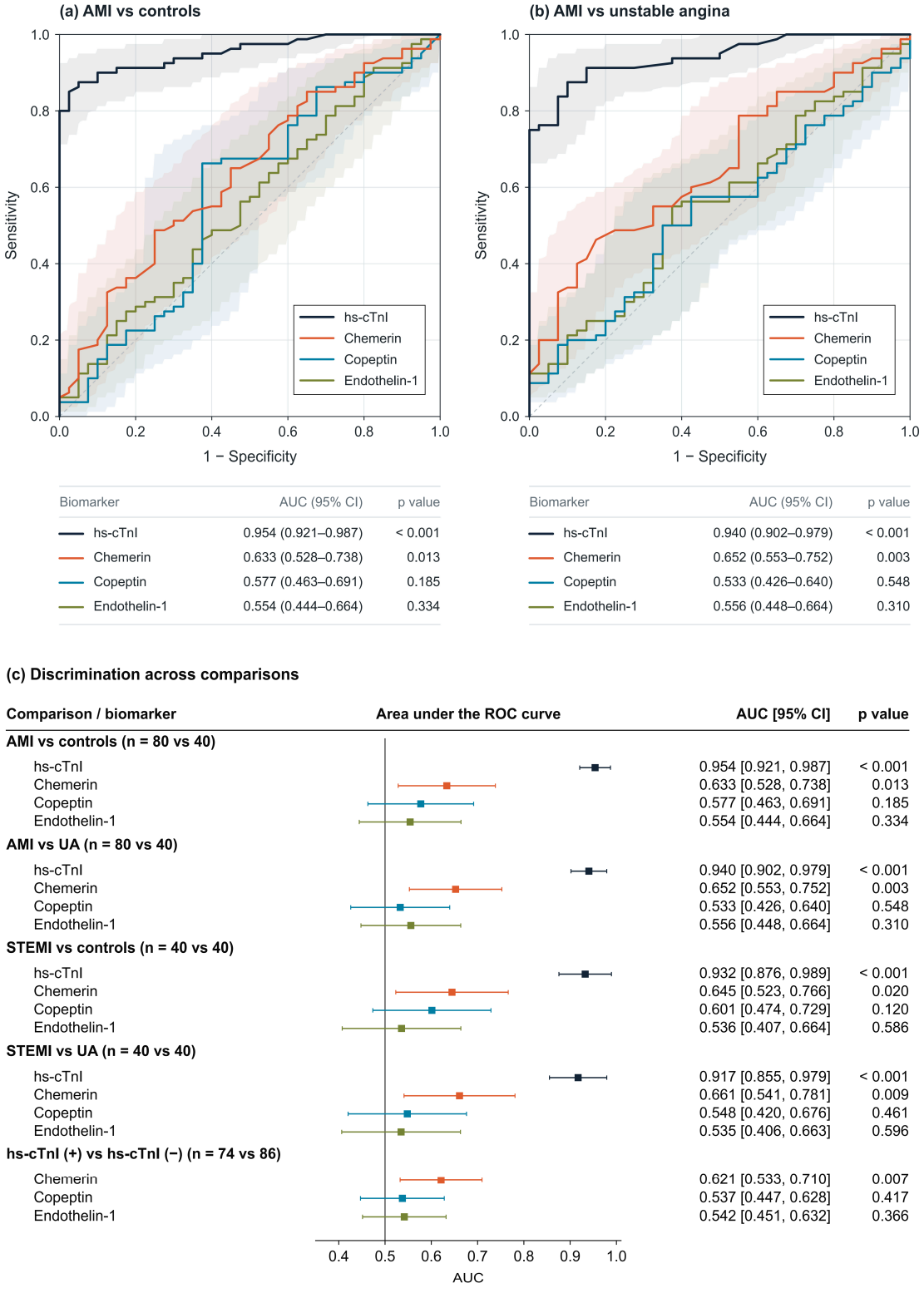


Figure 2. Diagnostic discrimination of hs-cTnI and the investigational biomarkers. (**a**,**b**) Empirical ROC curves for AMI versus controls (**a**) and UA (**b**), each with 80 AMI cases and 40 reference participants. Shading indicates pointwise 95% bootstrap CIs for sensitivity; tables report AUCs with DeLong 95% CIs. (**c**) AUC estimates (squares) with DeLong 95% CIs (horizontal lines) across five comparisons. The final comparison assesses presentation hs-cTnI positivity in the whole cohort (74 positive; 86 negative). Diagonal ROC lines and the vertical line at AUC = 0.5 indicate chance discrimination. Two-sided *p* values test AUC = 0.5 using DeLong standard errors. Lower chemerin concentrations indicate the positive group.

3.5. Endothelin-1 and Chemerin

Endothelin-1 concentrations did not differ significantly across diagnostic groups, and their standalone AUCs were not significantly different from 0.5 (Tables 1 and 2; Figures 2 and S2).

Median chemerin concentrations were lower in the AMI groups (Table 1; Figure S2). In the control-versus-AMI comparison (n = 120), AMI prevalence decreased from 75.0% in the lowest chemerin tertile to 55.0% in the highest (Table S11). Discrimination was modest (Tables 2, S9 and S12). The chemerin–hs-cTnI correlation attenuated after residual adjustment (ϱ, −0.250 to −0.104; Table S13); the adjusted ordinal odds ratio for chemerin was 0.78 per standard-deviation increase ($p = 0.081$; Table S14).

4. Discussion

Copeptin was positive at presentation in eight of 12 AMI cases with initially non-elevated hs-cTnI, including four of five NSTEMI cases whose hs-cTnI was elevated at 2 h. Combining hs-cTnI and copeptin with an OR-rule increased sensitivity from 85.0% to 95.0%, while specificity against controls decreased from 95.0% to 62.5%. Adding all three investigational biomarkers to the clinical model containing hs-cTnI changed the apparent AUC from 0.961 to 0.965, without a significant joint improvement in model fit ($p = 0.718$). These analyses address distinct questions: the OR-rule identifies additional positive presentation samples, whereas the regression models assess added information beyond clinical variables and hs-cTnI.

Earlier studies and meta-analyses showed that adding copeptin to troponin increased initial sensitivity and negative predictive value, often at the expense of specificity [6,21,22]. The incremental gain was generally smaller with hs-cTn assays [23]. In selected low-to-intermediate-risk patients with suspected ACS, BIC-8 increased direct emergency department discharge and shortened median length of stay from 7 to 4 h, meeting its prespecified 30-day major adverse cardiac event non-inferiority criterion [24]. In AROMI, prehospital copeptin plus arrival hs-cTnT shortened mean length of stay by 0.9 h among patients discharged within 12 h; 30-day major adverse cardiac event rates in the randomized cohort were non-inferior to a 0/3 h strategy [25]. Higher copeptin concentrations have also been associated with adverse outcomes after ACS [26,27], consistent with its role as a marker of acute physiological stress.

Contemporary assay-specific hs-cTn pathways provide the relevant clinical benchmark. The 2023 ESC guidelines recommend 0/1 h or 0/2 h algorithms for suspected NSTEMI and do not recommend additional biomarkers for routine ACS diagnosis when cardiac troponin is available [3]. The 2025 ACC/AHA ACS guideline recommends repeat hs-cTn measurement at 1–2 h when the initial result is nondiagnostic [1]. Across 20 cohorts, pooled sensitivities were 99.1% and 98.6%, with negative predictive values of 99.8% and 99.6%, respectively [28]. Evidence for adjunctive copeptin varies with the troponin assay, threshold, and comparator strategy [29–32]. In two cohorts, adding copeptin did not improve discrimination or sensitivity in patients eligible for ESC single-sample rule-out more than 3 h after symptom onset and reduced the proportion ruled out [33]. More recent dual-marker comparisons likewise found similar negative predictive values but fewer patients classified for rule-out than with serial hs-cTn strategies [34]. Our primary comparison used presentation values at the 99th percentile; the exploratory ESC reclassification provided a separate assessment using serial assay-specific thresholds.

Endothelin-1 and chemerin contributed little additional discrimination beyond hs-cTnI. Despite the vascular rationale and reported associations in other settings [10,11,35], endothelin-1 showed neither significant differences across diagnostic groups nor significant discrimination of AMI. Chemerin concentrations were lower in the AMI groups in our

cohort, whereas several previous studies and a meta-analysis reported higher levels in ACS or AMI [12,13,36,37]. Differences in study populations and assay methods may contribute to this inconsistency; chemerin also reflects metabolic and inflammatory processes [14,38–40].

The additional presentation positivity in four of the five initially troponin-negative NSTEMI cases is the most clinically relevant finding. All 40 NSTEMI patients, including these five, met biochemical rule-in criteria in the retrospective ESC classification. Copeptin therefore provided complementary information in the initial sample without identifying additional NSTEMI cases beyond serial hs-cTnI. Its positivity among rule-out and observe participants illustrates the accompanying specificity trade-off. The seven initially troponin-negative STEMI cases were already identified by ECG. Decision-curve projections favored hs-cTnI OR copeptin within a narrow threshold range, reflecting the balance between additional sensitivity and false-positive results. A useful time advantage would require an actionable copeptin result before repeat rapid troponin testing; our batch-assay study did not assess this outcome or biomarker-guided management. Prospective evaluation should enroll consecutive patients with suspected NSTEMI, use predefined thresholds on a clinically available assay [41], and compare complete accelerated pathways, including time to disposition, downstream testing, missed AMI, and short-term events.

Limitations

The single-center, balanced case–control design created an enriched disease spectrum and artificial AMI prevalence, limiting transportability of predictive values, data-derived thresholds, and apparent rule performance. Use of hs-cTnI in both index testing and diagnostic adjudication creates a risk of incorporation bias, potentially favoring hs-cTnI [42]. Coronary angiography was performed in all ACS patients but in only one of 40 controls; this difference in diagnostic work-up may have introduced verification bias.

The initially troponin-negative subgroup comprised 12 AMI cases; estimates in the five NSTEMI cases had wide confidence intervals. Cumulative symptom-onset subgroups overlapped, and data-derived thresholds and subgroup findings were evaluated in the same cohort. Single presentation measurements of the investigational biomarkers precluded assessment of their kinetics, and the research-use ELISA cut-offs require validation on clinically available assays. Several concentrations lay outside calibration ranges; alternative numerical handling preserved the main hs-cTnI-containing model results, but biomarker-only models were sensitive to upper-range handling of chemerin. Type 1 versus type 2 AMI subclassification was not prespecified. Body mass index and waist circumference were unavailable, limiting adjustment for adiposity in the chemerin analyses.

5. Conclusions

Copeptin identified additional positive presentation samples among patients with initially non-elevated hs-cTnI, increasing sensitivity when combined with hs-cTnI at the cost of specificity. The three-biomarker panel added little discrimination beyond clinical variables and hs-cTnI. These findings support prospective evaluation of adjunctive copeptin within contemporary accelerated troponin pathways for suspected NSTEMI.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/jcdd13110574/s1. Figure S1: STARD 2015 flow diagram of participant enrollment and diagnostic assessment; Figure S2: Distribution of hs-cTnI, chemerin, copeptin, and endothelin-1 across diagnostic groups; Figure S3: Patient-level heatmap of additional detection by investigational biomarkers among troponin-negative AMI cases (n = 12), by AMI type; Figure S4: Biomarker-to-cut-off ratios according to time from symptom onset among patients with AMI; Figure S5: Prevalence-adjusted decision-curve analysis of rule-based strategies; Figure S6: Pairwise ROC curves of chemerin, copeptin, and endothelin-1 across diagnostic contrasts; Figure S7: Clinical and

biomarker model discrimination for AMI; Figure S8: Bootstrap distributions of AUC estimates for chemerin and nested diagnostic models; Table S1: Analytical characteristics and assay conditions of the ELISA kits used for biomarker measurements; Table S2: Time-stratified biomarker positivity, incremental sensitivity, and additional detection of troponin-negative AMI cases; Table S3: Presentation (0-h) and 2-h hs-cTnI results and presentation copeptin positivity in NSTEMI; Table S4: Diagnostic performance of investigational biomarkers for troponin-negative AMI; Table S5: Sensitivity of biomarker-based diagnostic strategies according to symptom-onset time window among patients with acute myocardial infarction, overall and separately for NSTEMI and STEMI; Table S6: Sensitivity and specificity of OR- and AND-rule strategies, with paired comparisons versus hs-cTnI alone; Table S7: Positive and negative predictive values of diagnostic strategies projected across plausible emergency department AMI prevalences; Table S8: Prevalence-adjusted decision-curve analysis: thresholds for added net benefit and net-benefit differences versus hs-cTnI alone; Table S9: Pairwise receiver operating characteristic analyses of individual biomarkers across diagnostic groups; Table S10: Exploratory analyses of copeptin across ordinal, multivariable binary, and standalone discrimination models; Table S11: Association between chemerin tertiles and AMI prevalence; Table S12: Bootstrap assessment of chemerin discrimination and threshold stability; Table S13: Spearman correlations of biomarker concentrations and covariate-adjusted residuals; Table S14: Ordinal logistic regression across four ordered diagnostic categories; Table S15: Model discrimination under alternative assay-limit handling scenarios; Table S16: Biomarker odds ratios under alternative assay-limit handling scenarios; Table S17: Individual biomarker AUCs and fixed-threshold classification stability; Table S18: Retrospective classification using ESC 0/2-h Access hs-cTnI thresholds and presentation copeptin positivity.

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Abbreviations

The following abbreviations are used in this manuscript:

ACS	acute coronary syndrome
AMI	acute myocardial infarction
AUC	area under the curve
BUN	blood urea nitrogen
CI	confidence interval
CK	creatine kinase
CK-MB	creatine kinase-MB
ELISA	enzyme-linked immunosorbent assay
ET-1	endothelin-1
hs-cTnI	high-sensitivity cardiac troponin I
LR	likelihood ratio
NPV	negative predictive value
NSTEMI	non-ST-elevation myocardial infarction
PPV	positive predictive value
ROC	receiver operating characteristic
STARD	Standards for Reporting Diagnostic Accuracy Studies
STEMI	ST-elevation myocardial infarction
UA	unstable angina

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