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SUPPLEMENTAL DATA

Diagnostic and prognostic value of circulating microRNA-21 in heart failure: A systematic review and meta-analysis

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Full article is available at the following link: Diagnostic and prognostic value of circulating microRNA-21 in heart failure: A systematic review and meta-analysis

Supplementary material 1.

Search strategy

PUBMED

77 results

((("miR21"[tiab] OR "hsa-mir-21"[tiab] OR "miR-21"[tiab] OR "microRNA-21"[tiab] OR "miRNA-21"[tiab] OR "miR21a"[tiab] OR "miR-21-3p"[tiab] OR "miR-21-5p"[tiab])) AND "Heart Failure"[Mesh]

Web of Science

204 results

TS=("miR21" OR "hsa-mir-21" OR "miR-21" OR "microRNA-21" OR "miRNA-21" OR "miR21a" OR "miR-21-3p" OR "miR-21-5p")

AND

TS=(heart failure)

Limit to: article

Embase

129 results

('mir21':ti,ab OR 'hsa-mir-21':ti,ab OR 'mir-21':ti,ab OR 'microrna-21':ti,ab OR 'mirna-21':ti,ab OR 'mir21a':ti,ab OR 'mir-21-3p':ti,ab OR 'mir-21-5p':ti,ab) AND ('human'/exp OR 'human':ti,ab)

AND

'heart failure'/exp OR 'heart failure'

AND

'article'/it

Scopus

163 results

(TITLE-ABS ("miR21") OR TITLE-ABS ("hsa-mir-21") OR TITLE-ABS ("miR-21") OR TITLE-ABS ("microRNA-21") OR TITLE-ABS ("miRNA-21") OR TITLE-ABS ("miR21a") OR TITLE-ABS ("miR-21-3p") OR TITLE-ABS ("miR-21-5p")) AND (TITLE-ABS ("heart failure"))

Supplementary material 2.

Risk of bias – QUADAS and QUIPS

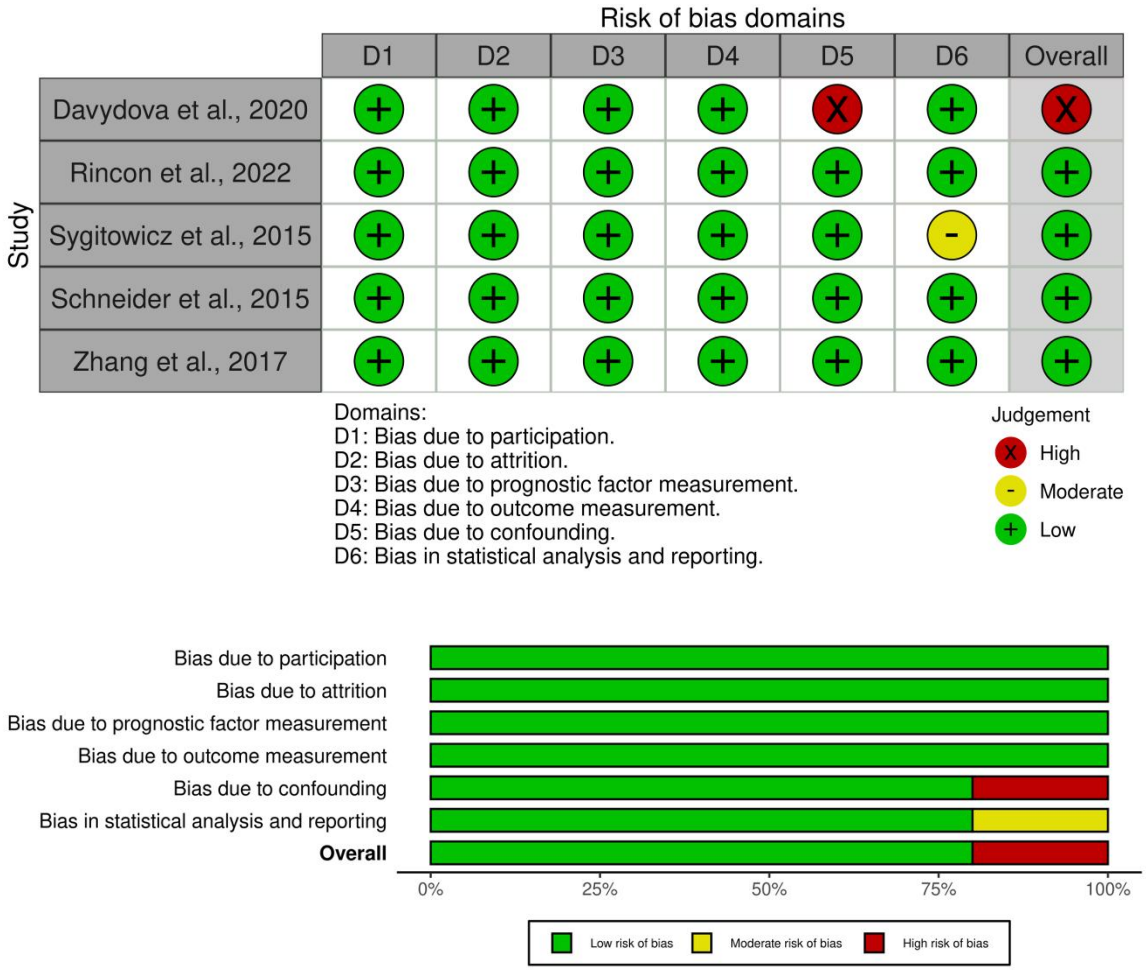


Figure S1. QUIPS for prognostic studies. Risk-of-bias assessment of prognostic studies on circulating miR-21 in heart failure was conducted using the QUIPS tool. Most domains were evaluated as having a low risk of bias, while increased risk was primarily associated with confounding factors and statistical analysis.

Table S1. QUIPS per-study risk-of-bias assessments (prognostic studies)

Study	Domain	Judgment	Justification (≤15 words)
Davydova 2020†	D1 Participation	Low	Consecutive AHF admissions; clear eligibility
Davydova 2020†	D2 Attrition	Low	Follow-up complete or described
Davydova 2020†	D3 Prognostic factor	Low	Plasma miR-21 measured per protocol
Davydova 2020†	D4 Outcome measurement	Low	Hospitalization/death definitions provided
Davydova 2020†	D5 Confounding	High	Limited adjustment for key covariates
Davydova 2020†	D6 Analysis/reporting	Low	HRs reported with CIs
Davydova 2020†	Overall	High	High confounding risk dominates
Rincon 2022	D1 Participation	Low	Multicenter cohort; clear inclusion
Rincon 2022	D2 Attrition	Low	Minimal loss to follow-up
Rincon 2022	D3 Prognostic factor	Low	Pre-specified miR-21 assay
Rincon 2022	D4 Outcome measurement	Low	Standardized outcome definitions
Rincon 2022	D5 Confounding	Low	Multivariable adjustment adequate
Rincon 2022	D6 Analysis/reporting	Low	Appropriate models/reporting
Rincon 2022	Overall	Low	—
Sygitowicz 2015	D1 Participation	Low	Clear sampling frame
Sygitowicz 2015	D2 Attrition	Low	Attrition described
Sygitowicz	D3 Prognostic	Low	Assay procedure detailed

2015	factor		
Sygitowicz 2015	D4 Outcome measurement	Moderate	Outcome assessment not clearly blinded
Sygitowicz 2015	D5 Confounding	Low	Adjusted for major confounders
Sygitowicz 2015	D6 Analysis/reporting	Moderate	Limited model diagnostics reported
Sygitowicz 2015	Overall	Low–Moderate	Driven by D4/D6
Schneider 2015	D1 Participation	Low	Prospective AHF cohort
Schneider 2015	D2 Attrition	Low	≥2 samples for most patients
Schneider 2015	D3 Prognostic factor	Low	Standard qPCR with controls
Schneider 2015	D4 Outcome measurement	Low	Objective outcomes predefined
Schneider 2015	D5 Confounding	Low	Multivariable models used
Schneider 2015	D6 Analysis/reporting	Low	Transparent statistics
Schneider 2015	Overall	Low	—
Zhang 2017	D1 Participation	Low	Defined HF population
Zhang 2017	D2 Attrition	Low	Follow-up described
Zhang 2017	D3 Prognostic factor	Low	Validated assay
Zhang 2017	D4 Outcome measurement	Low	Standard ascertainment
Zhang 2017	D5 Confounding	Low	Adjusted analyses
Zhang 2017	D6 Analysis/reporting	Low	Adequate reporting
Zhang 2017	Overall	Low	—

† Abstract only. Abbreviations: AHF: Acute heart failure; miR-21: MicroRNA-21; HR: Hazard ratio; CI: Confidence interval; CIs: Confidence intervals.

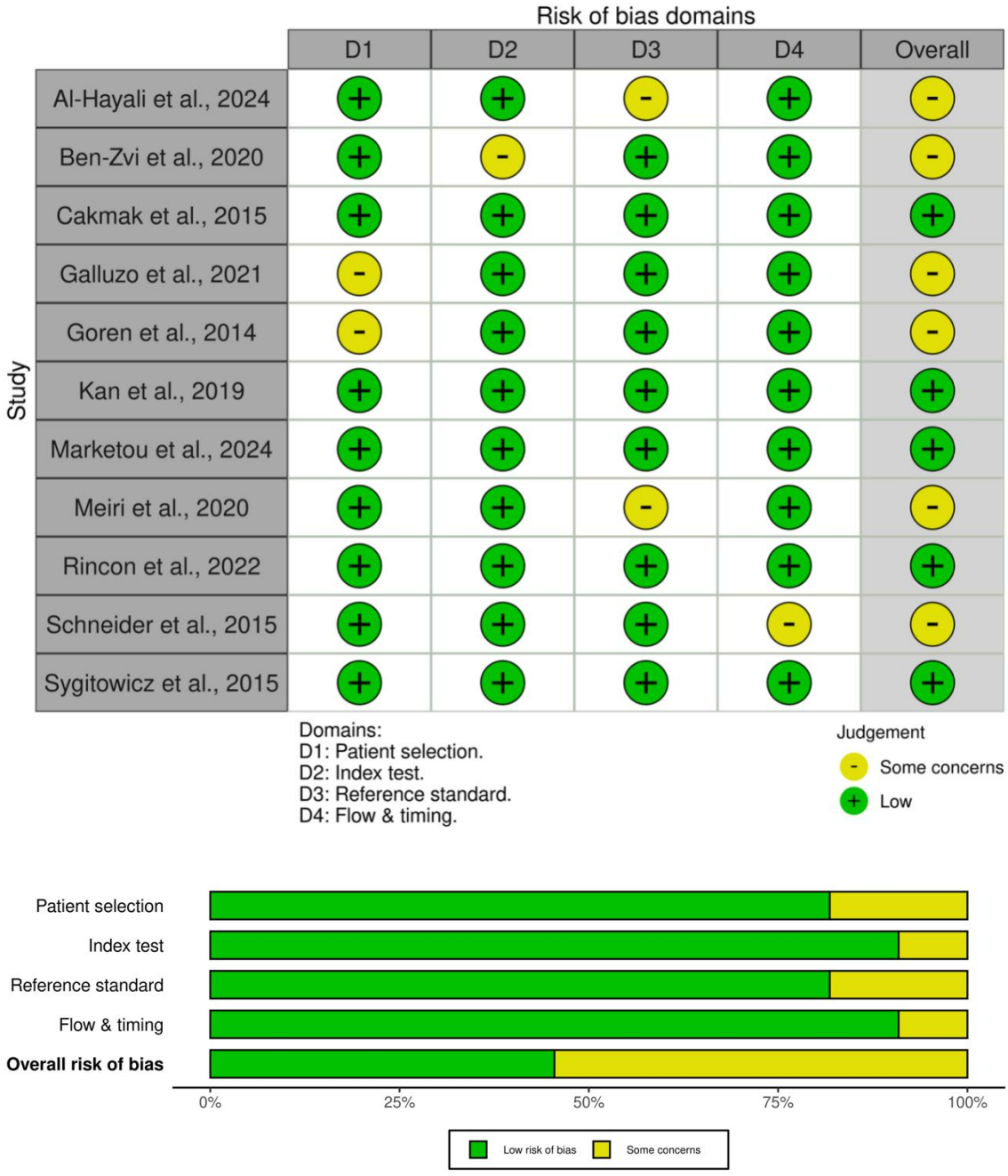


Figure S2. Risk-of-bias assessment of diagnostic accuracy studies included in the expression meta-analysis using the QUADAS-2 tool (*n* = 11). Most domains were judged at low risk of bias, with some concerns mainly in patient selection and flow and timing.

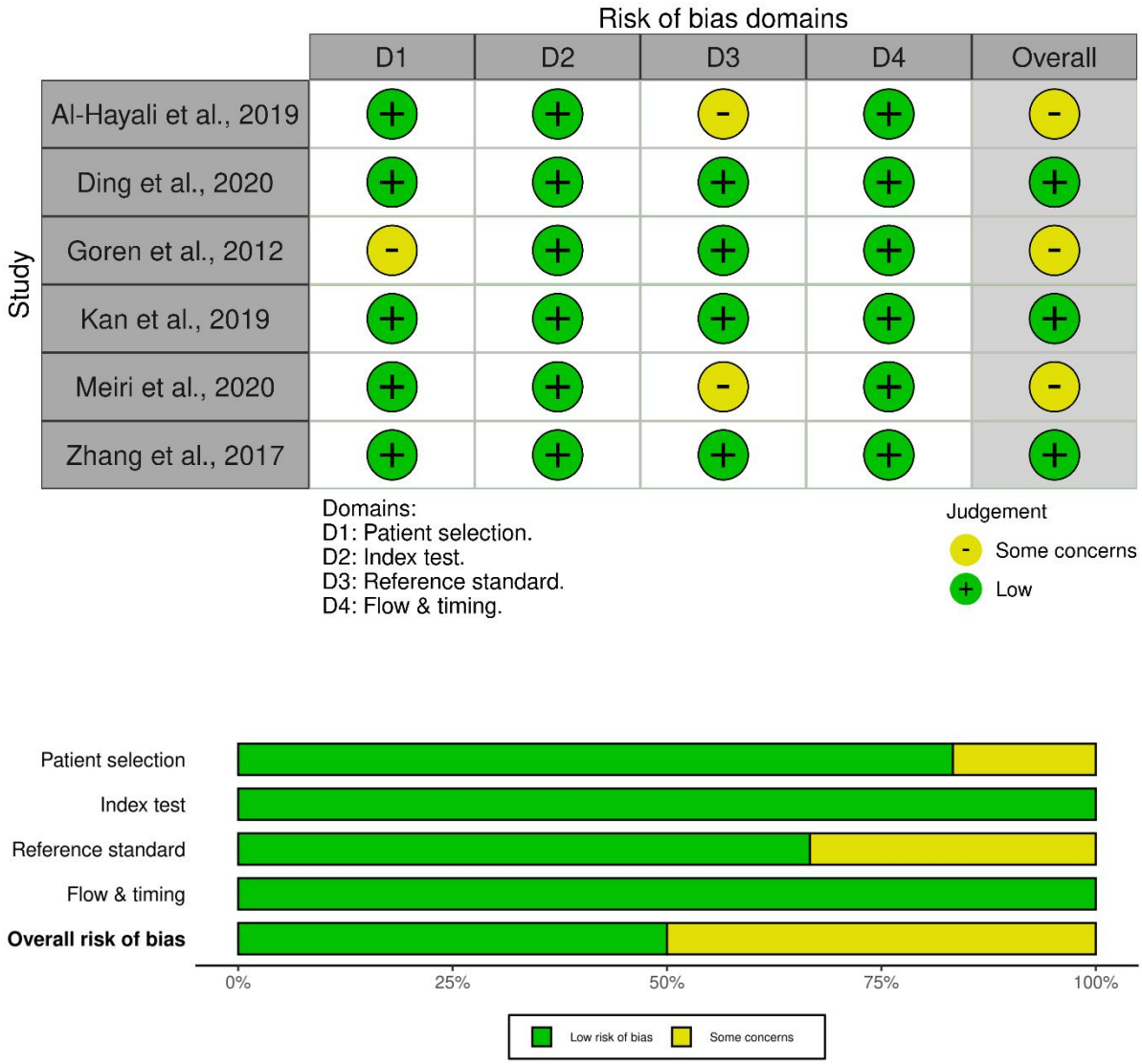


Figure S3. Risk-of-bias assessment of diagnostic accuracy studies included in the DTA meta-analysis using the QUADAS-2 tool (*n* = 6). Most domains were judged at low risk of bias, with some concerns mainly in patient selection and the reference standard.

Table S2. QUADAS-2 per-study risk-of-bias assessments for DTA and expression study analysis

Study	Domain	Judgment	Justification (≤15 words)	Applicability concern
Al-Hayali 2019	D1 Patient selection	Low	Consecutive/recruitment clear; avoided case–control	Low
Al-Hayali 2019	D2 Index test	Low	qPCR protocol pre-specified; blinded to reference	Low
Al-Hayali 2019	D3 Reference standard	Low	Accepted HF criteria (guideline-based)	Low
Al-Hayali 2019	D4 Flow and timing	Low	Appropriate interval; complete verification	Low
Al-Hayali 2019	Overall	Low	No domain high risk	—
Ben-Zvi 2020	D1 Patient selection	Low	Consecutive or random sampling	Low
Ben-Zvi 2020	D2 Index test	Some concerns	Blinding unclear / threshold not pre-specified	Low
Ben-Zvi 2020	D3 Reference standard	Low	Guideline-based standard	Low
Ben-Zvi 2020	D4 Flow and timing	Low	Acceptable interval	Low
Ben-Zvi 2020	Overall	Some concerns	Driven by D2	—
Cakmak	D1	Low	Consecutive/recruitment	Low

2015	Patient selection		clear; avoided case–control	
Cakmak 2015	D2 Index test	Low	qPCR protocol pre-specified; blinded to reference	Low
Cakmak 2015	D3 Reference standard	Low	Accepted HF criteria (guideline-based)	Low
Cakmak 2015	D4 Flow and timing	Low	Appropriate interval; complete verification	Low
Cakmak 2015	Overall	Low	No domain high risk	—
Ding 2020	D1 Patient selection	Low	Consecutive/recruitment clear; avoided case–control	Low
Ding 2020	D2 Index test	Low	qPCR protocol pre-specified; blinded to reference	Low
Ding 2020	D3 Reference standard	Low	Accepted HF criteria (guideline-based)	Low
Ding 2020	D4 Flow and timing	Low	Appropriate interval; complete verification	Low
Ding 2020	Overall	Low	No domain high risk	—
Galluzzo 2021	D1 Patient selection	Low	Clear sampling frame	Low
Galluzzo 2021	D2 Index test	Low	Assay blinded or independent	Low
Galluzzo 2021	D3 Reference	Low	Appropriate and independent	Low

	standard			
Galluzzo 2021	D4 Flow and timing	Some concerns	Timing/withdrawals not clearly reported	Low
Galluzzo 2021	Overall	Some concerns	Driven by D4	—
Goren 2012	D1 Patient selection	Low	Consecutive or random sampling	Low
Goren 2012	D2 Index test	Some concerns	Blinding unclear / threshold not pre-specified	Low
Goren 2012	D3 Reference standard	Low	Guideline-based standard	Low
Goren 2012	D4 Flow and timing	Low	Acceptable interval	Low
Goren 2012	Overall	Some concerns	Driven by D2	—
Kan 2019	D1 Patient selection	Some concerns	Non-consecutive/unclear exclusions	Low
Kan 2019	D2 Index test	Low	Protocolized assay	Low
Kan 2019	D3 Reference standard	Low	Guideline-based standard	Low
Kan 2019	D4 Flow and timing	Low	Verification complete	Low
Kan 2019	Overall	Some concerns	Driven by D1	—

Marketou 2024	D1 Patient selection	Low	Consecutive/recruitment clear; avoided case–control	Low
Marketou 2024	D2 Index test	Low	qPCR protocol pre-specified; blinded to reference	Low
Marketou 2024	D3 Reference standard	Low	Accepted HF criteria (guideline-based)	Low
Marketou 2024	D4 Flow and timing	Low	Appropriate interval; complete verification	Low
Marketou 2024	Overall	Low	No domain high risk	—
Meiri 2020	D1 Patient selection	Low	Consecutive or random sampling	Low
Meiri 2020	D2 Index test	Some concerns	Blinding unclear / threshold not pre-specified	Low
Meiri 2020	D3 Reference standard	Low	Guideline-based standard	Low
Meiri 2020	D4 Flow and timing	Low	Acceptable interval	Low
Meiri 2020	Overall	Some concerns	Driven by D2	—
Rincon 2022	D1 Patient selection	Low	Clear sampling frame	Low
Rincon 2022	D2 Index test	Low	Assay blinded or independent	Low

Rincon 2022	D3 Reference standard	Low	Appropriate and independent	Low
Rincon 2022	D4 Flow and timing	Some concerns	Timing/withdrawals not clearly reported	Low
Rincon 2022	Overall	Some concerns	Driven by D4	—
Schneider 2015	D1 Patient selection	Low	Consecutive/recruitment clear; avoided case–control	Low
Schneider 2015	D2 Index test	Low	qPCR protocol pre-specified; blinded to reference	Low
Schneider 2015	D3 Reference standard	Low	Accepted HF criteria (guideline-based)	Low
Schneider 2015	D4 Flow and timing	Low	Appropriate interval; complete verification	Low
Schneider 2015	Overall	Low	No domain high risk	—
Sygitowicz 2015	D1 Patient selection	Low	Consecutive/recruitment clear; avoided case–control	Low
Sygitowicz 2015	D2 Index test	Low	qPCR protocol pre-specified; blinded to reference	Low
Sygitowicz 2015	D3 Reference standard	Low	Accepted HF criteria (guideline-based)	Low
Sygitowicz 2015	D4 Flow and	Low	Appropriate interval; complete verification	Low

	timing			
Sygitowicz 2015	Overall	Low	No domain high risk	—
Zhang 2017	D1 Patient selection	Low	Consecutive/recruitment clear; avoided case–control	Low
Zhang 2017	D2 Index test	Low	qPCR protocol pre-specified; blinded to reference	Low
Zhang 2017	D3 Reference standard	Low	Accepted HF criteria (guideline-based)	Low
Zhang 2017	D4 Flow and timing	Low	Appropriate interval; complete verification	Low
Zhang 2017	Overall	Low	No domain high risk	—

Abbreviations: HF: Heart failure; qPCR: Quantitative polymerase chain reaction.

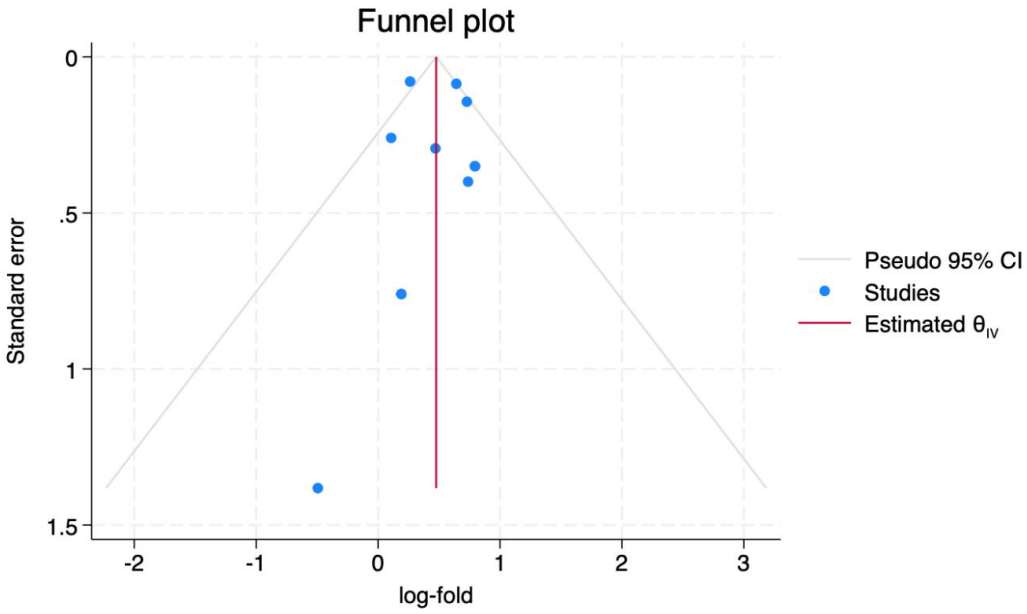
Supplementary material 3.

Table S3. Raw data for sensitivity and specificity in each study

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Al–Hayali et al., 2019	38	13	7	32	0.84 [0.71, 0.94]	0.71 [0.56, 0.84]
Ding et al., 2017	57	11	7	51	0.89 [0.79, 0.95]	0.82 [0.70, 0.91]
Goren et al., 2012	27	3	3	27	0.90 [0.73, 0.98]	0.90 [0.73, 0.98]
Kan et al., 2019	51	4	9	31	0.85 [0.73, 0.93]	0.89 [0.73, 0.97]
Meiri et al., 2020	5	0	3	11	0.63 [0.24, 0.91]	1.00 [0.72, 1.00]
Zhang et al., (CS) 2017	80	1	0	39	1.00 [0.95, 1.00]	0.97 [0.87, 1.00]
Zhang et al., (PV) 2017	80	1	0	39	1.00 [0.95, 1.00]	0.97 [0.87, 1.00]

Abbreviations: CS: Coronary sinus; PV: Peripheral vein.

Supplementary material 4.




```
  Effect-size label: log-fold
        Effect size: logFC
          Std. err.: _meta_se

Nonparametric trim-and-fill analysis of publication bias
Linear estimator, imputing on the left

Iteration                           Number of studies =     11
  Model: Fixed-effects                      observed =     11
 Method: Inverse-variance                   imputed =      0

Pooling
  Model: Fixed-effects
 Method: Inverse-variance
```

Studies	log-fold	[95% conf.	interval]
Observed	**0.476**	**0.378**	**0.575**
Observed + Imputed	**0.476**	**0.378**	**0.575**

Figure S4. Assessment of publication bias through funnel plot analysis for expression studies. The funnel plot shows a symmetric distribution of study estimates with no imputed studies, indicating no evidence of publication bias or small-study effects (Egger's test intercept = 0.11, SE = 0.46; $p = 0.80$).

Supplementary material 5.

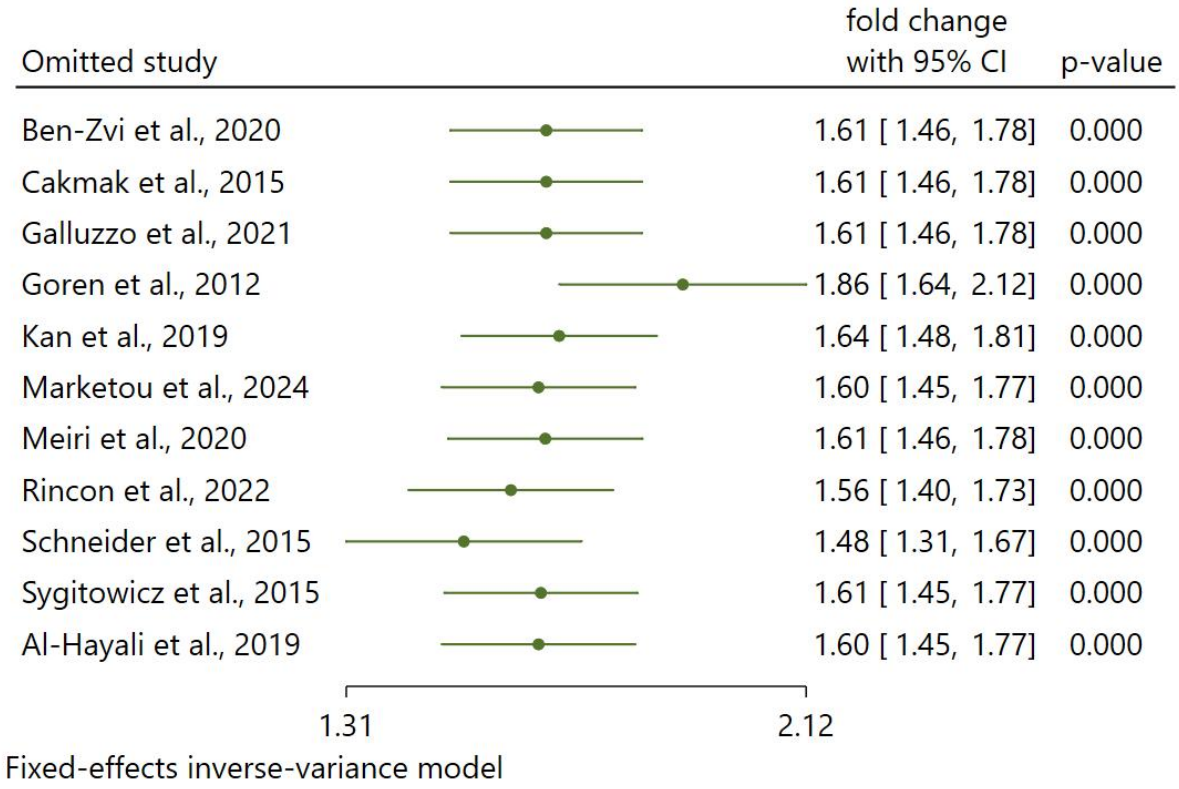


Figure S5. Sensitivity analysis for expression studies. Leave-one-out sensitivity analysis showing that omitting any single study does not materially change the pooled fold-change estimate.

Supplementary material 6.

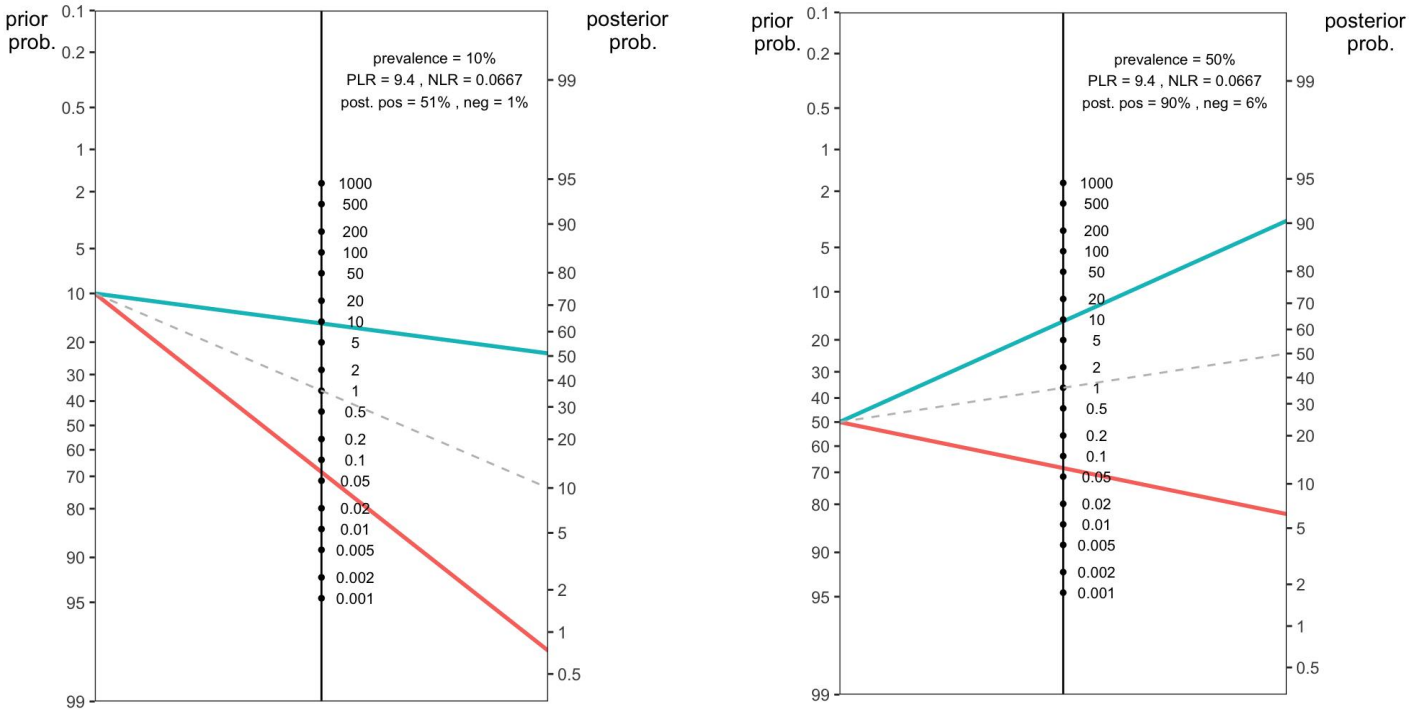


Figure S6. Plausible priors for Fagan nomogram. Fagan nomograms showing how a diagnostic test with positive likelihood ratio (PLR) = 9.4 and negative likelihood ratio (NLR) = 0.0667 converts pre-test probability into post-test probability for two assumed pre-test prevalences: 10% (left panel) and 50% (right panel); blue lines indicate post-test probability after a positive result and red lines after a negative result.

Supplementary material 7.

Table S4. Details of study-to-analysis mapping

Included studies (*n* = 14)	**DTA meta-analysis (*n* = 6)**	**Prognostic meta-analysis (*n* = 5)**	**Expression studies (*n* = 11)**
Al-Hayali et al., 2019 (16)			
Ben-Zvi et al., 2020 (17)			
Cakmak et al., 2015 (18)			
Davydova et al., 2020 †(19)			
Ding et al., 2020 (20)			
Galluzzo et al., 2021 (21)			
Goren et al., 2012 (22)			
Kan et al., 2019 (23)			
Marketou et al., 2024 (24)			
Meiri et al., 2020 (25)			
Rincón et al., 2022 (4)			
Schneider et al., 2018 (26)			
Sygitowicz et al., 2015 (27)			
Zhang et al., 2017 (28)			