

#### 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

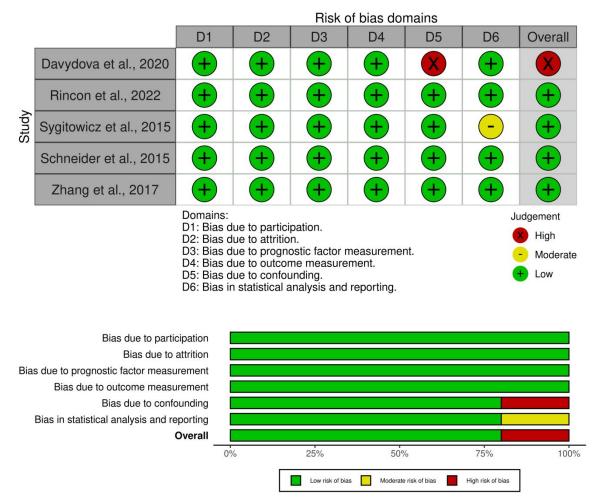
### **Scopus**

163 results

( TITLE-ABS ( "miR21" ) OR TITLE-ABS ( "hsa-mir-21" ) OR TITLE-ABS ( "miR-21" ) OR TITLE-ABS ( "miRNA-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    | D1 Participation   | Low      | Consecutive AHF admissions;  |
| 2020†       |                    |          | clear eligibility            |
| Davydova    | D2 Attrition       | Low      | Follow-up complete or        |
| 2020†       |                    |          | described                    |
| Davydova    | D3 Prognostic      | Low      | Plasma miR-21 measured per   |
| 2020†       | factor             |          | protocol                     |
| Davydova    | D4 Outcome         | Low      | Hospitalization/death        |
| 2020†       | measurement        |          | definitions provided         |
| Davydova    | D5 Confounding     | High     | Limited adjustment for key   |
| 2020†       |                    |          | covariates                   |
| Davydova    | D6                 | Low      | HRs reported with CIs        |
| 2020†       | Analysis/reporting |          |                              |
| Davydova    | Overall            | High     | High confounding risk        |
| 2020†       |                    |          | dominates                    |
| Rincon 2022 | D1 Participation   | Low      | Multicenter cohort; clear    |
|             |                    |          | inclusion                    |
| Rincon 2022 | D2 Attrition       | Low      | Minimal loss to follow-up    |
| Rincon 2022 | D3 Prognostic      | Low      | Pre-specified miR-21 assay   |
|             | factor             |          |                              |
| Rincon 2022 | D4 Outcome         | Low      | Standardized outcome         |
|             | measurement        |          | definitions                  |
| Rincon 2022 | D5 Confounding     | Low      | Multivariable adjustment     |
|             |                    |          | adequate                     |
| Rincon 2022 | D6                 | Low      | Appropriate models/reporting |
|             | Analysis/reporting |          |                              |
| Rincon 2022 | Overall            | Low      | _                            |
| Sygitowicz  | D1 Participation   | Low      | Clear sampling frame         |
| 2015        |                    |          |                              |
| Sygitowicz  | D2 Attrition       | Low      | Attrition described          |
| 2015        |                    |          |                              |
| Sygitowicz  | D3 Prognostic      | Low      | Assay procedure detailed     |

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

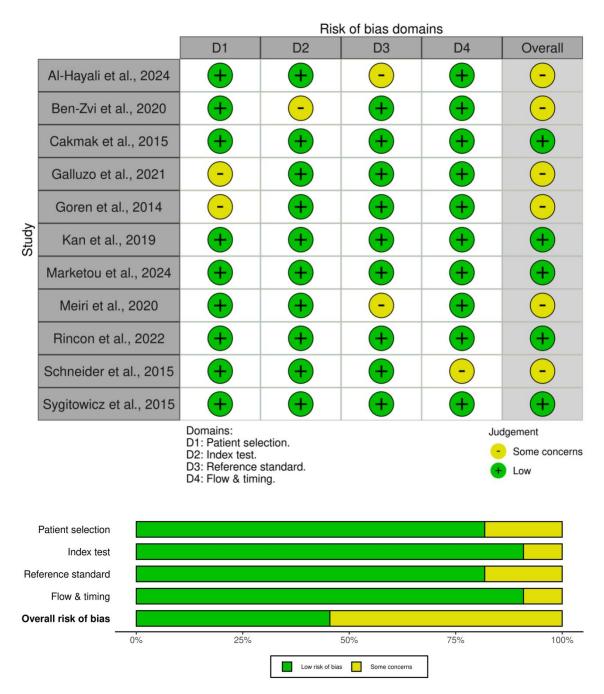
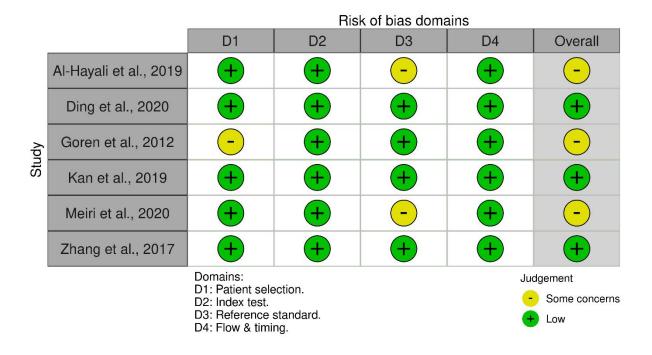


Figure S2. Risk-of-bias assessment of diagnostic accuracy studies included in the expression meta-analysis using the QUADAS-2 tool (n = 11). Most domains were judged at low risk of bias, with some concerns mainly in patient selection and flow and timing.



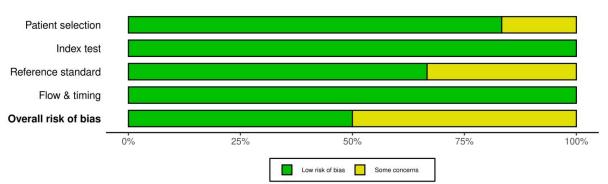


Figure S3. Risk-of-bias assessment of diagnostic accuracy studies included in the DTA meta-analysis using the QUADAS-2 tool (n = 6). Most domains were judged at low risk of bias, with some concerns mainly in patient selection and the reference standard.

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

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

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

| Galluzzo       D4 Flow timing       Some clearly reported       Timing/withdrawals not elearly reported       Low         Galluzzo       Overall timing       Some concerns       Driven by D4       —         2021       Patient selection       Done sampling       Low         Goren       D2 Index selection       Some threshold not prespecified       Low         Goren       D3       Low       Guideline-based standard       Low         2012       Reference standard       Some threshold not prespecified       Low         Goren       D4 Flow and timing       Low       Acceptable interval       Low         2012       Reference standard       Some one concerns       Driven by D2       —         Goren       Overall concerns selection       Some one consecutive/unclear exclusions       Low         Kan 2019       D2 Index test       Low       Protocolized assay       Low         Kan 2019       D3       Low       Guideline-based standard       Low         Kan 2019       D4 Flow and timing       Low       Verification complete concerns       Low         Kan 2019       Overall       Some concerns       Priven by D1       —  |          | standard  |          |                        |     |
|--|----------|-----------|----------|------------------------|-----|
| Galluzzo Galluzzo Overall Goren D1 Low Consecutive or random sampling  Goren D2 Index concerns D3 Low Goren Coren Selection  Goren D3 Low Guideline-based standard Goren D4 Flow timing Coren D9 D1 Some Concerns Standard Coren D1 Some Concerns Concerns Concerns Concerns Coren C | Galluzzo | D4 Flow   | Some     | Timing/withdrawals not | Low |
| Goren D4 Flow Low Acceptable interval timing  Goren Overall Some oncerns  Fan 2019 D1 Some oncerns  Kan 2019 D2 Index selection  Some D7 Fortication complete Kan 2019 D4 Flow and timing  Kan 2019 D4 Flow Low Reference standard  Kan 2019 D4 Flow Low Acceptable interval consecutive/unclear exclusions  Kan 2019 D5 Low Guideline-based standard  Low Acceptable interval Low consecutive/unclear exclusions  Kan 2019 D6 Index Low Guideline-based standard  Low Consecutive/unclear exclusions  Kan 2019 D7 Index Low Protocolized assay  Kan 2019 D8 Low Guideline-based standard  Kan 2019 D4 Flow Low Verification complete standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 D4 Flow Low Protocolized assay  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 D4 Flow Low Protocolized assay Verification complete Low and timing  Kan 2019 D4 Flow Low Protocolized assay Verification complete Low Protocolized assay  Kan 2019 D4 Flow Low Protocolized assay Standard   | 2021     | and       | concerns | clearly reported       |     |
| 2021concernsConsecutive or random<br>samplingLow2012Patient<br>selectionSome<br>concernsBlinding unclear /<br>threshold not pre-<br>specifiedLow2012testconcernsthreshold not pre-<br>specifiedGorenD3LowGuideline-based<br>standardLow2012Reference<br>standardstandardLow2012and<br>timingLowAcceptable intervalLowGorenOverall<br>2012Some<br>concernsDriven by D2—Kan 2019D1<br>Patient<br>selectionNon-<br>consecutive/unclear<br>exclusionsLowKan 2019D2 Index<br>testLowProtocolized assay<br>standardLowKan 2019D3<br>Reference<br>standardLowGuideline-based<br>standardLowKan 2019D4 Flow<br>and<br>timingLowVerification complete<br>standardLowKan 2019D4 Flow<br>and<br>timingLowVerification completeLow   |          | timing    |          |                        |     |
| Goren D1 Low Consecutive or random sampling  Goren D2 Index concerns threshold not prespecified  Goren D3 Low Guideline-based Low  2012 Reference standard  Goren D4 Flow Low Acceptable interval Low  2012 and timing  Goren Overall Some Driven by D2 —  2012 Concerns  Kan 2019 D2 Index test  Kan 2019 D4 Flow Low Guideline-based Low  Concerns Consecutive/unclear exclusions  Kan 2019 D4 Flow Low Guideline-based Low  Guideline-based Low  Concerns Consecutive/unclear exclusions  Kan 2019 D4 Flow Low Guideline-based Low  Standard  Kan 2019 D4 Flow and timing  Kan 2019 D4 Flow and timing  Kan 2019 D4 Flow and timing  Kan 2019 D6 Flow and timing  Consecutive or random Low  Guideline-based Low  Some Standard  Verification complete Low  Acceptable interval Low  Consecutive/unclear exclusions  Low  Consecutive/unclear exclusions  Low  Consecutive/unclear exclusions  Low  Consecutive/unclear exclusions  Consecutive/unclear exc | Galluzzo | Overall   | Some     | Driven by D4           | _   |
| Patient selection   Some   Blinding unclear /   Low  | 2021     |           | concerns |                        |     |
| Selection  Goren  D2 Index  Concerns  test  Concerns  Threshold not prespecified  Goren  D3  Low  Guideline-based  Standard  Goren  D4 Flow  2012  Acceptable interval  Low  2012  Goren  Overall  Some  Patient  Some  Concerns  Concerns  Consecutive/unclear  selection  Kan 2019  D2 Index  test  Low  Protocolized assay  Low  Reference  standard  Concerns  Kan 2019  D3  Low  Guideline-based  Low  Concerns  Consecutive/unclear  exclusions  Kan 2019  D3  Low  Reference  standard  Kan 2019  D4 Flow  and  timing  Kan 2019  D4 Flow  and  timing  Kan 2019  D4 Flow  Some  Consecutive/unclear  exclusions  Consecutive/unclear  exclusions  Low  Consecutive/unclear  exclusions  Low  Consecutive/unclear  exclusions  Low  Verification complete  Low  Acceptable interval  Low  Low  Concerns  Consecutive/unclear  exclusions  Low  Consecutive/unclear  exclusions  Consec | Goren    | D1        | Low      | Consecutive or random  | Low |
| Goren D2 Index test concerns threshold not prespecified  Goren D3 Low Guideline-based standard  Goren D4 Flow and timing  Goren Overall Some concerns  Kan 2019 D1 Some Non-consecutive/unclear exclusions  Kan 2019 D2 Index test Concerns standard  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow and timing  Kan 2019 D5 Index test Concerns standard  Kan 2019 D6 Flow and timing  Kan 2019 D7 Flow and timing  Kan 2019 D8 Flow and timing  Kan 2019 D9 Flow and timing  Kan 2019 Overall Some Driven by D1 —   | 2012     | Patient   |          | sampling               |     |
| 2012 test concerns threshold not prespecified  Goren D3 Low Guideline-based standard  Goren D4 Flow Low Acceptable interval Low  2012 and timing  Goren Overall Some Driven by D2 — concerns  Kan 2019 D1 Some Non-consecutive/unclear exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow Low Verification complete Individual standard  Kan 2019 D4 Flow Low Verification complete Low  Same Standard Standard Standard  Kan 2019 D4 Flow Low Driven by D1 —  |          | selection |          |                        |     |
| Goren D3 Low Guideline-based standard  Goren D4 Flow Low Acceptable interval Low  2012 and timing  Goren Overall Some Driven by D2  Concerns  Kan 2019 D1 Some Non- Consecutive/unclear exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow Low Verification complete  Kan 2019 D4 Flow and timing  Kan 2019 D5 Flow and timing  Kan 2019 D6 Flow and timing  Kan 2019 Overall Some Driven by D1 —  | Goren    | D2 Index  | Some     | Blinding unclear /     | Low |
| Goren D3 Low Guideline-based standard  Goren D4 Flow Low Acceptable interval Low  2012 and timing  Goren Overall Some concerns  Kan 2019 D1 Some Non- Patient concerns exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow and timing  Kan 2019 D4 Flow Low Verification complete  Kan 2019 D4 Flow and timing  Kan 2019 Overall Some Driven by D1 —  | 2012     | test      | concerns | threshold not pre-     |     |
| Reference standard  Goren D4 Flow Low Acceptable interval Low 2012 and timing  Goren Overall Some Driven by D2 — 2012 concerns  Kan 2019 D1 Some Non- Patient concerns consecutive/unclear exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —   |          |           |          | specified              |     |
| Goren D4 Flow Low Acceptable interval Low 2012 and timing  Goren Overall Some Driven by D2 — 2012 concerns  Kan 2019 D1 Some Non- Patient concerns consecutive/unclear exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —   | Goren    | D3        | Low      | Guideline-based        | Low |
| Goren D4 Flow and timing  Goren Overall Some Driven by D2 — 2012  Kan 2019 D1 Some Non- Low Patient concerns selection  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow and timing  Kan 2019 Overall Some Driven by D1 —   Acceptable interval Low Driven by D2  ——  Concerns consecutive/unclear exclusions  Low Protocolized assay Low Guideline-based Low standard  Exam 2019 D4 Flow Low Verification complete Low and timing  Concerns Consecutive/unclear exclusions  Exam 2019 D3 Low Guideline-based Low Standard Complete Low Driven by D1 —   Exam 2019 D4 Flow Low Driven by D1 —   Exam 2019 Overall Some Driven by D1 —  | 2012     | Reference |          | standard               |     |
| 2012 and timing  Goren Overall Some Driven by D2 — 2012  Kan 2019 D1 Some Non- Low Patient concerns exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow and timing  Kan 2019 Overall Some Driven by D1 —  |          | standard  |          |                        |     |
| Goren Overall Some Driven by D2 —  2012 Concerns  Kan 2019 D1 Some Non- Patient concerns consecutive/unclear exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow and timing  Kan 2019 Overall Some Driven by D1 —   | Goren    | D4 Flow   | Low      | Acceptable interval    | Low |
| Goren 2012  Kan 2019  D1 Some Patient selection  Kan 2019  D2 Index test  Kan 2019  D3 Reference standard  Kan 2019  D4 Flow and timing  Kan 2019  Overall  Some Driven by D2  —  Low Consecutive/unclear exclusions  Low Protocolized assay Low Standard  Cuideline-based Standard  Low Verification complete Low  And Cuiming  Consecutive/unclear Exclusions  Low Consecutive/unclear Exclusions  Low Verification complete Low  Consecutive/unclear Exclusions  Low Consecutive/unclear Exclusions  Exclusions  Consecutive/unclear Exclusions  Low Consecutive/unclear Exclusions  Low Consecutive/unclear Exclusions  Consecutiv | 2012     | and       |          |                        |     |
| Concerns  Kan 2019 D1 Some Non- Patient concerns exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based standard  Kan 2019 D4 Flow and timing  Kan 2019 Overall Some Driven by D1 —   |          | timing    |          |                        |     |
| Kan 2019 D1 Some Non- Low Patient concerns consecutive/unclear exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based Low Reference standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —   | Goren    | Overall   | Some     | Driven by D2           | _   |
| Patient selection concerns consecutive/unclear exclusions  Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based Low standard  Reference standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —   | 2012     |           | concerns |                        |     |
| Kan 2019D2 Index testLowProtocolized assayLowKan 2019D3LowGuideline-based<br>standardLowReference standardStandardVerification completeLowKan 2019D4 Flow and timingDriven by D1—  | Kan 2019 | D1        | Some     | Non-                   | Low |
| Kan 2019 D2 Index test  Kan 2019 D3 Low Guideline-based Low standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —  |          | Patient   | concerns | consecutive/unclear    |     |
| test  Kan 2019 D3 Low Guideline-based Low Reference standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —  |          | selection |          | exclusions             |     |
| Kan 2019 D3 Low Guideline-based Low standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —  | Kan 2019 | D2 Index  | Low      | Protocolized assay     | Low |
| Reference standard  Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —  |          | test      |          |                        |     |
| Kan 2019 D4 Flow and timing   Kan 2019 Overall   Some Driven by D1   | Kan 2019 | D3        | Low      | Guideline-based        | Low |
| Kan 2019 D4 Flow Low Verification complete Low and timing  Kan 2019 Overall Some Driven by D1 —  |          | Reference |          | standard               |     |
| and timing  Kan 2019 Overall Some Driven by D1 —   |          | standard  |          |                        |     |
| timing   Compared timing   Com | Kan 2019 | D4 Flow   | Low      | Verification complete  | Low |
| Kan 2019 Overall Some Driven by D1 —   |          | and       |          |                        |     |
|  |          | timing    |          |                        |     |
| concerns   | Kan 2019 | Overall   | Some     | Driven by D1           | _   |
|  |          |           | concerns |                        |     |

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

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

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

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

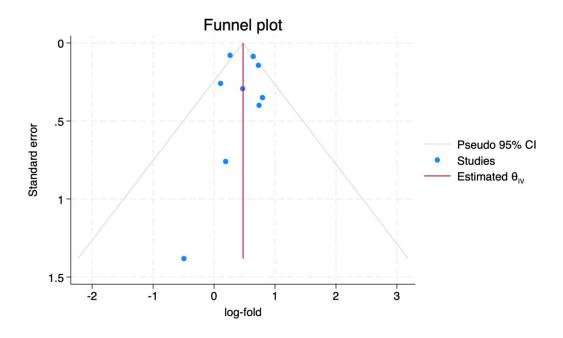
# **Supplementary material 3.**

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

| Study              | TP | FP | FN | TN | Sensitivity (95%  | Specificity (95%  |
|--------------------|----|----|----|----|-------------------|-------------------|
|                    |    |    |    |    | CI)               | CI)               |
| Al-Hayali et al.,  | 38 | 13 | 7  | 32 | 0.84 [0.71, 0.94] | 0.71 [0.56, 0.84] |
| 2019               |    |    |    |    |                   |                   |
| 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) | 80 | 1  | 0  | 39 | 1.00 [0.95, 1.00] | 0.97 [0.87, 1.00] |
| 2017               |    |    |    |    |                   |                   |
| Zhang et al., (PV) | 80 | 1  | 0  | 39 | 1.00 [0.95, 1.00] | 0.97 [0.87, 1.00] |
| 2017               |    |    |    |    |                   |                   |

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

## **Supplementary material 4.**



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).

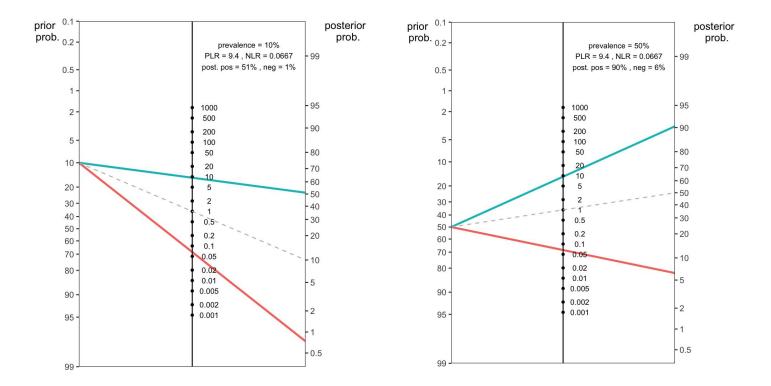
# **Supplementary material 5.**

| Omitted study            |   | fold change<br>with 95% Cl | p-value |
|--------------------------|---|----------------------------|---------|
| Ben-Zvi et al., 2020     |   | 1.61 [ 1.46, 1.78]         | 0.000   |
| Cakmak et al., 2015      |   | 1.61 [ 1.46, 1.78]         | 0.000   |
| Galluzzo et al., 2021    |   | 1.61 [ 1.46, 1.78]         | 0.000   |
| Goren et al., 2012       | - | —— 1.86 [ 1.64, 2.12]      | 0.000   |
| Kan et al., 2019         |   | 1.64 [ 1.48, 1.81]         | 0.000   |
| Marketou et al., 2024    | - | 1.60 [ 1.45, 1.77]         | 0.000   |
| Meiri et al., 2020       | - | 1.61 [ 1.46, 1.78]         | 0.000   |
| Rincon et al., 2022      | - | 1.56 [ 1.40, 1.73]         | 0.000   |
| Schneider et al., 2015 – | • | 1.48 [ 1.31, 1.67]         | 0.000   |
| Sygitowicz et al., 2015  |   | 1.61 [ 1.45, 1.77]         | 0.000   |
| Al-Hayali et al., 2019   |   | 1.60 [ 1.45, 1.77]         | 0.000   |
| 1.3                      | 1 | 2.12                       |         |

Fixed-effects inverse-variance model

**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          | DTA meta-     | Prognostic    | Expression                      |
|-------------------|---------------|---------------|---------------------------------|
| studies (n =      | analysis (n = | meta-analysis | <b>studies</b> ( <i>n</i> = 11) |
| 14)               | 6)            | (n=5)         |                                 |
| 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)         |               |               |                                 |