	Nemám konflikt zájmů	Mám konflikt zájmů	Specifikace konfliktu (vyjmenujte subjekty, firmy či instituce, se kterými Vaše spolupráce může vést ke konfliktu zájmů)
Zaměstnanecký poměr	X	X	Xxxxx
Vlastník / akcionář	X		
Konzultant	X		
Přednášková činnost	X		
Člen poradních sborů (advisory boards)	X		
Podpora výzkumu / granty	X		
Jiné honoráře (např. za klinické studie či registry)	X		

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Biomarkers of Right Ventricular Dysfunction in HFrEF Identified by Direct Myocardial Proteomics

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Why does RV dysfunction matter?

- Frequently observed in advanced HF
- Powerful indicator of mortality risk
- Essential for:
  - Assessing LVAD eligibility
  - Determining timing for heart transplantation
  - Monitoring progression of advanced HF
  - Currently, there is no specific circulating biomarker available

RV assessment still relies mainly on imaging and hemodynamics.

Study Hypothesis

RV dysfunction induces:

- myocardial remodeling
- extracellular matrix remodeling
- altered myocardial proteome

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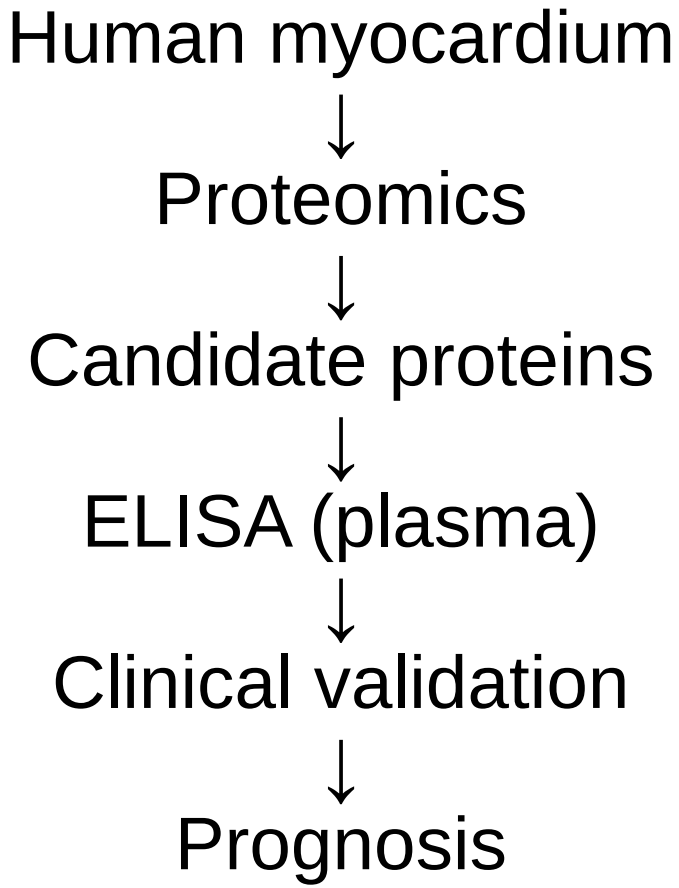
Some proteins may:

- enter circulation
- be measurable in plasma
- serve as biomarkers

Simple workflow diagram.



Discovery Cohort

Direct myocardial proteomics

Cohort 1

- **122 HFrEF hearts** screened

Selected:

- 10 non-failing donor hearts
- 10 preserved RV function
- 10 severe RVD

Matched for:

- age
- body size
- HF etiology
- comorbidities

Analyzed separately:

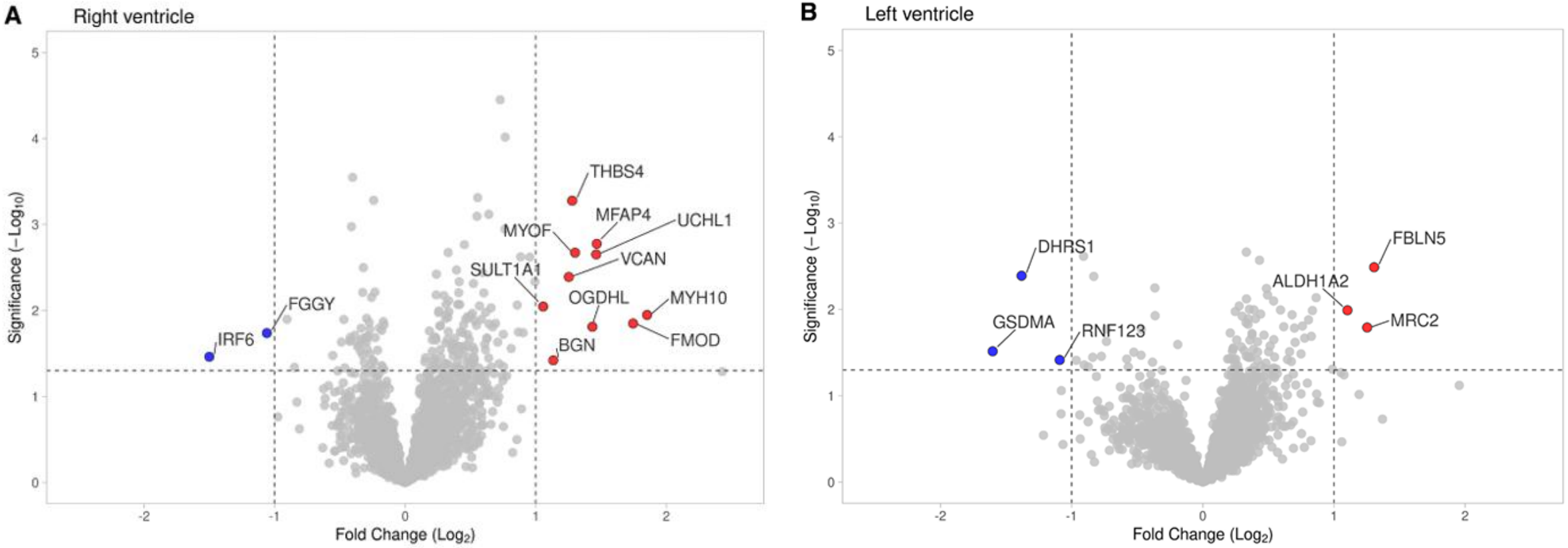
- RV myocardium
- LV myocardium

Proteomic Analysis

Label-free quantitative proteomics

RV: 3477 protein groups

LV: 3633 protein groups



Label-free quantification proteomic analyses of ventricular myocardium of patients with heart failure with reduced ejection fraction **with right ventricular dysfunction (RVD) and without RVD (noRVD).**

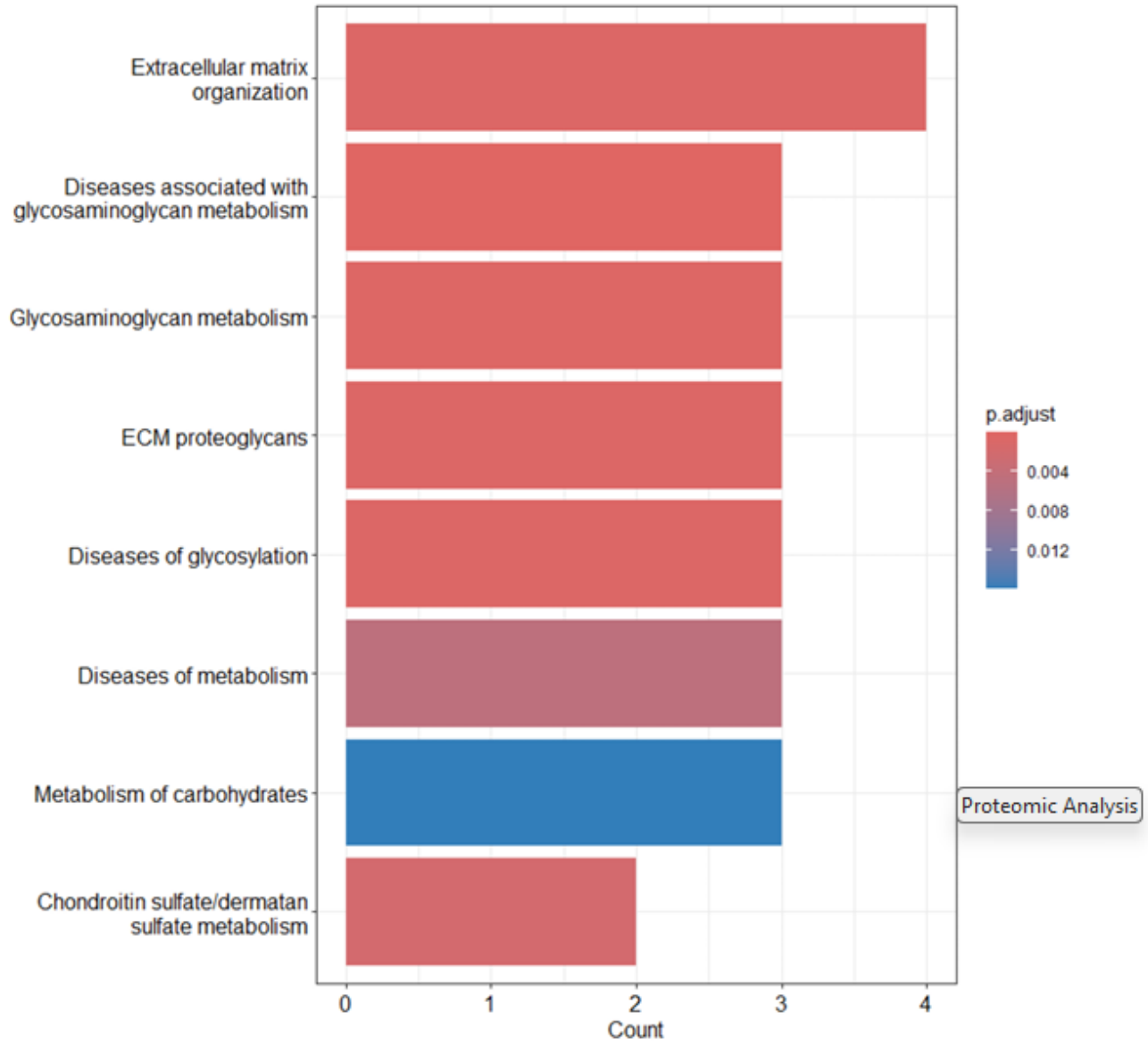


Figure S1. Gene Ontology (GO) enrichment analysis of proteins upregulated in right ventricles of HFrEF patients with RVD.

Key Differentially Expressed Proteins

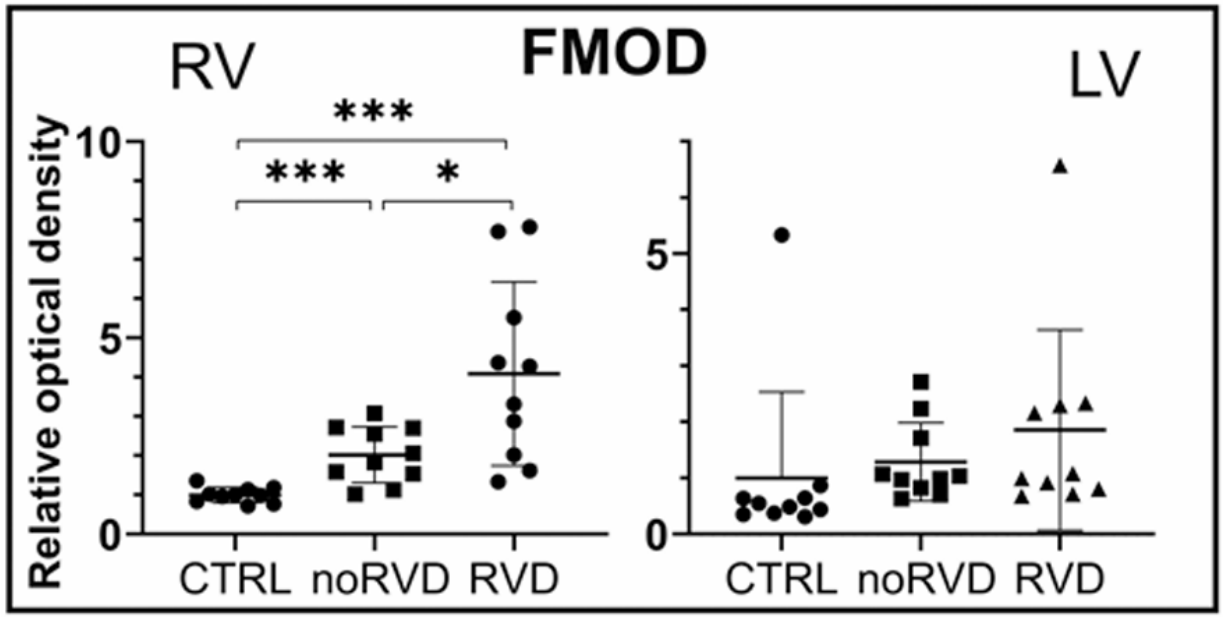
RV upregulated proteins

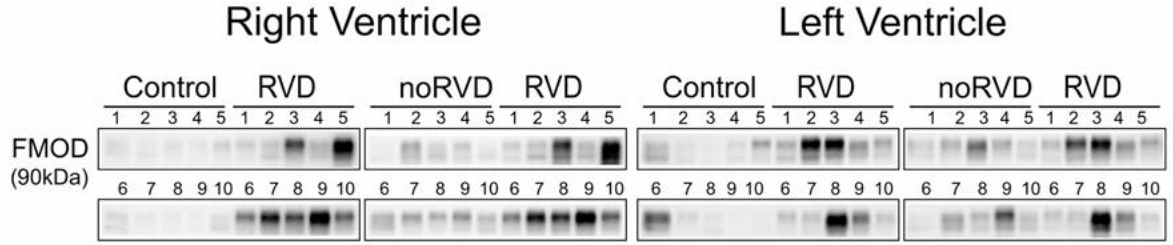
Protein	Fold change
FMOD	**3.35×**
MFAP4	2.76×
THBS4	2.43×
VCAN	2.38×

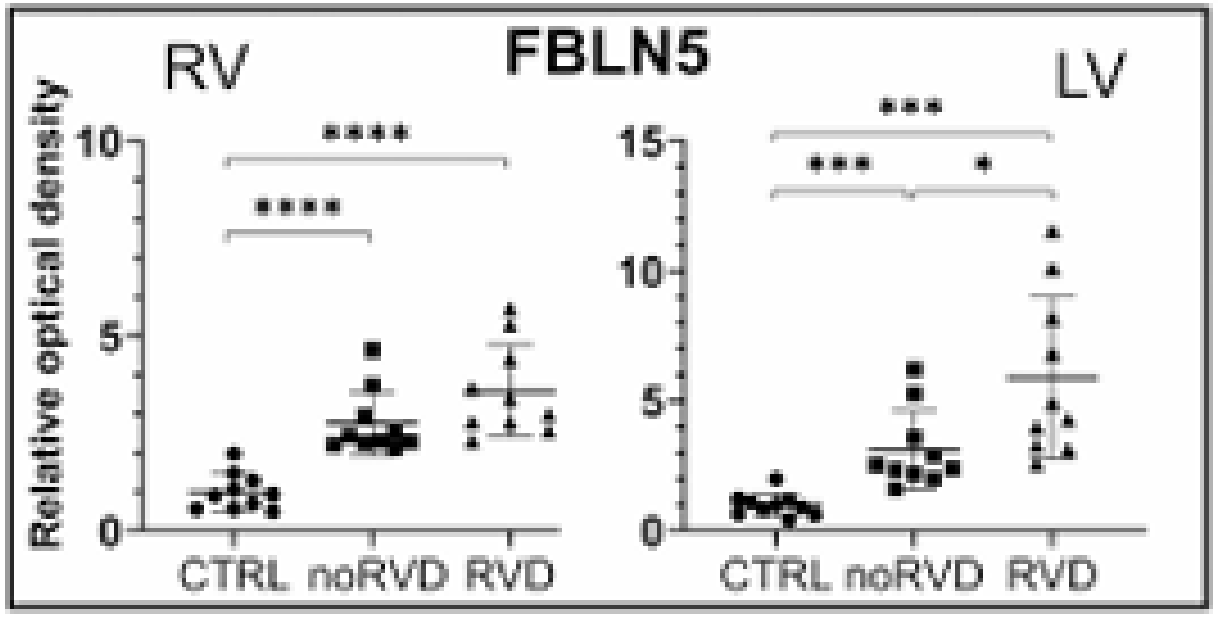
LV upregulated proteins

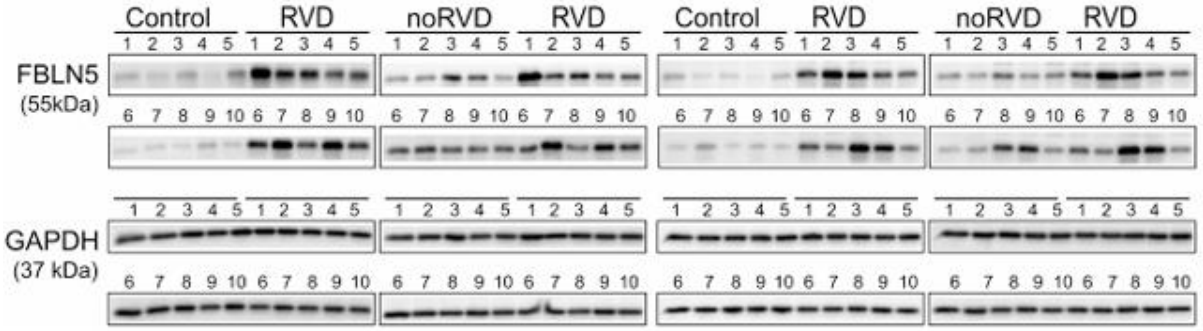
Protein	Fold change
FBLN5	**2.47×**

ECM remodeling emerged as the dominant molecular signature.


FMOD
RV
LV
Relative optical density
10
5
0
***
***
*
CTRL noRVD RVD
5
0
CTRL noRVD RVD


Right Ventricle
Left Ventricle
Control
RVD
noRVD
RVD
1 2 3 4 5 1 2 3 4 5
6 7 8 9 10 6 7 8 9 10
FMOD
(90kDa)


FBLN5
RV
LV
Relative optical density
10
5
0
****
****
CTRL noRVD RVD
15
10
5
0
***
***
*
CTRL noRVD RVD


Control
RVD
noRVD
RVD
1 2 3 4 5 1 2 3 4 5
6 7 8 9 10 6 7 8 9 10
FBLN5
(55kDa)
GAPDH
(37 kDa)

Tissue → Plasma Translation

Can myocardial remodeling be detected in plasma?

- Multiple candidate proteins were tested by ELISA using plasma from:
  - **the SAME patients** whose myocardium underwent **proteomics**

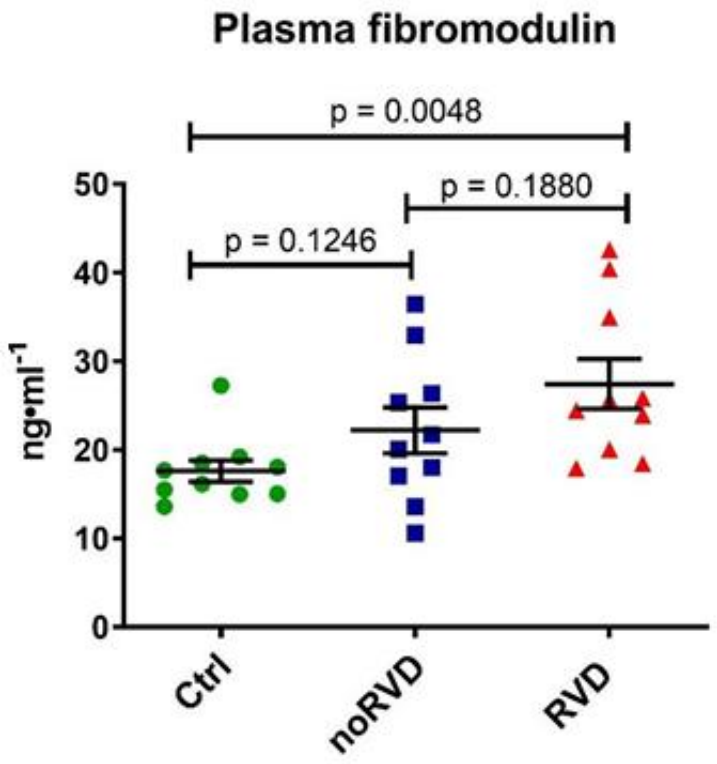
However:

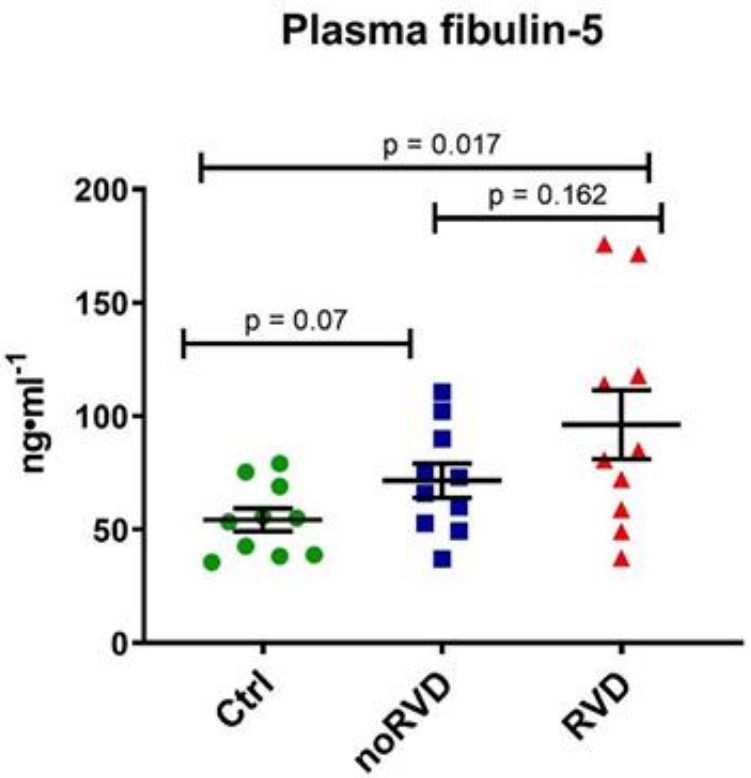
- **Only FMOD and FBLN5 showed meaningful plasma differences.**

↓

- Selected for large-scale clinical validation.

Initial Plasma Results





Group	FMOD	FBLN5
Controls	17.6 ng/mL	54.3 ng/mL
HFrEF noRVD	22.2 ng/mL	71.6 ng/mL
HFrEF + RVD	27.1 ng/mL	96.3 ng/mL

Biological Interpretation

What do FMOD and FBLN5 biologically represent?

FMOD

- collagen fibrillogenesis
- collagen cross-linking
- fibrosis remodeling

FBLN5

- elastic fiber assembly
- tissue stiffness
- TGF-β-related remodeling

RV dysfunction appears strongly linked to extracellular matrix remodeling.

Validation Cohort

Advanced HF population:

- mean LVEF: 21%
- NYHA III–IV: 86%
- moderate/severe RVD: 62%

Large clinical validation cohort

65 controls

232 HFrEF patients

- HFrEF, preserved RV function (n=47)
- HFrEF, mild RVD (n=43)
- HFrEF, moderate RVD (n=96)
- HFrEF, severe RVD (n=46)

RV Function Parameters

FMOD and FBLN5 correlate with RV dysfunction

Significant association with:

Parameter	FBLN5	FMOD
RV dysfunction grade	β = 0.17*	β = 0.36†
TAPSE	β = 1.92‡	β = 0.27†
Sm-TDI	β = 0.14*	β = 0.27†
PAPi	β = 0.25†	β = 0.35†
CVP/PCWP ratio	β = 0.19§	β = 0.30†

- FMOD: $p < 0.0001$
- FBLN5: $p < 0.0001$

Multivariable Analysis

- **Association with RV dysfunction is independent of major confounders**

Adjusted for:

- LVEF
- age
- BMI
- diabetes
- eGFR
- cardiac index
- pulmonary vascular resistance

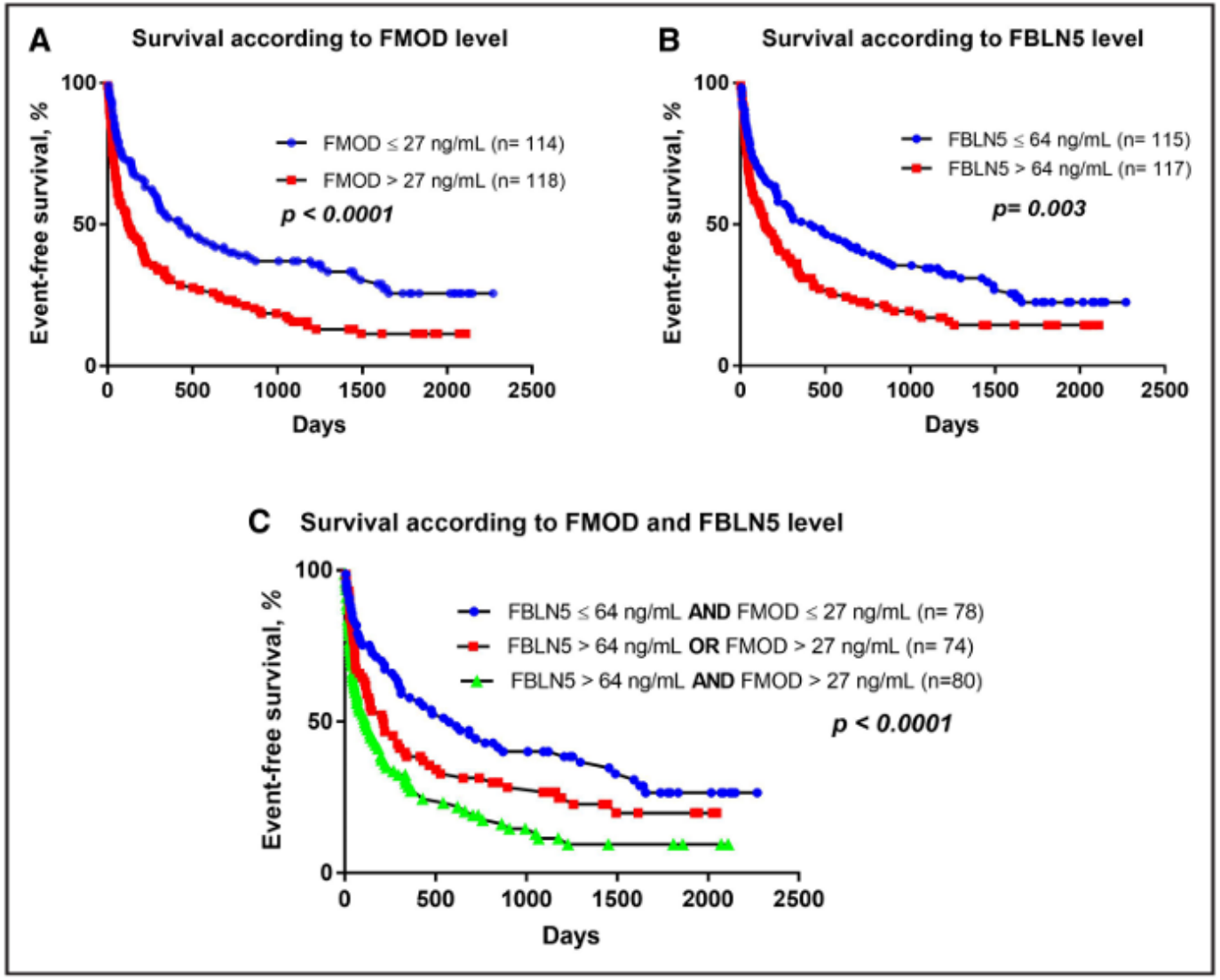
Findings:

FMOD:
highly RV-specific

FBLN5:
associated with RV dysfunction and RV afterload

Prognostic Value

Both biomarkers predict adverse outcomes



Higher FMOD and FBLN5 levels were associated with:

- significantly worse **event-free survival**

- higher risk of death, urgent HTx, or MCS implantation

- progressive risk stratification when **both biomarkers were elevated**

Median follow-up: 4.4 years

Prognostic Value

Incremental prognostic value

Cox regression

FMOD
HR 1.20 per SD
p = 0.005

FBLN5
HR 1.30 per SD
p = 0.0004

AUC improvement

MAGGIC + RV dysfunction grade
0.57

MAGGIC + FMOD + FBLN5:
0.669
p = 0.02

Biomarkers improved prognostic stratification beyond standard RV assessment.

Conclusions

- **RV dysfunction was associated** with a distinct **extracellular matrix remodeling signature.**
- **FMOD and FBLN5** emerged as the most **clinically relevant candidate biomarkers.**
- **Both proteins** progressively **increased with worsening RV dysfunction** and remained significant after multivariable adjustment.
- **Elevated FMOD** and **FBLN5** levels were associated with significantly **worse long-term outcomes**.
- **Combined biomarker assessment** improved **prognostic stratification** beyond standard RV assessment.