Supplementary material

Methods

Transthoracic Echocardiography (TTE)

TTE was performed using commercially available systems and measurements calculated according to the American Society of Echocardiography guidelines.[17] TTE was interpreted by cardiologists blinded to the data on any participant. During examination, saline was agitated to create microbubbles which were injected into the peripheral vein. The appearance of microbubbles in the left atrium (LA) within 3 cardiac cycles was diagnosed as an intracardiac shunt.[18] Left ventricular ejection fraction (LVEF) was calculated using the biplane Simpson's formula or visually estimated when the endocardial definition was not well defined. Left ventricle (LV) mass was indexed to body surface area. Stroke volume was calculated as the product of the LV outflow tract cross-sectional area and velocity time integral in the LV outflow tract.[19] Cardiac output was calculated as the product of stroke volume and heart rate. E/A was calculated from the early diastolic and late diastolic blood flow velocity through the mitral valve. LV global longitudinal strain was quantified by 2D speckle-tracking echocardiography. Strain analysis was performed offline using specialized software. (TOMTECARENA.Version2.50.BuildNo.544347.UDI(01)04260361440181(11)211117(10)50.00)

RVSP was calculated using the formula 4 x (peak TRV)^2 + right atrial pressure.

Laboratory assays

Bile acid measurements: Performed using liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS) at Mayo Clinic laboratories (CLIA# 24D0404292). Primary bile acids reported

are cholic and chenodeoxycholic acids. Secondary bile acids reported include deoxycholic acid and ursodeoxycholic acid. Total bile acids are a sum of both primary and secondary bile acids.

Vascular factors measurements: Blood samples were collected and aliquoted in multiple microcentrifuge tubes to avoid repeated freeze-thaw cycles during or within 3 days of TTE for analysis. All assays were performed in bulk at the conclusion of the study. Enzyme-Linked Immunosorbent Assays (ELISAs) for human vascular cell adhesion molecule 1(VCAM1), [abcam, cat # ab223591], angiopoietin 2 (ANG2) [abcam, cat # ab99971] and von Willebrand factor (vWF) [abcam, cat # ab223864] were performed using the manufacturer's instructions. Absorbances were measured using a microplate reader (SpectraMax i3x MiniMax 300 Imaging Cytometer, Molecular Devices) and SoftMax Pro 7.0 (Molecular Devices). Concentrations were quantified via Four Parameter Logistic Regression (4PL) using GraphPad Prism Version 9.5.1.

Supplementary Table 1 Cause of Noncirrhotic portal hypertension (NCPH)

NCPH (n=32)	preclinical NCPH (n=10)
Pre-hepatic: none Hepatic: PSVD (n=30) Congestive hepatic fibrosis (n=1) Post-hepatic: Budd-Chiari syndrome (n=1)	Pre-hepatic: none Hepatic: PSVD (n=6) Congestive hepatic fibrosis (n=1) Patients at risk (n=3) Post-hepatic: none

PSVD-porto-sinusoidal vascular disease

Supplementary Table 2 Echocardiographic features associated with shunting

Characteristics	**Shunt present (N = 17)**	**Shunt absent (N = 21)**	**P value**

Left ventricular ejection fraction (%)	61 [60 - 65]	62 [59 - 64]	0.7
Cardiac output (L/min)	5.2 [3.6 - 6.3]	4.4 [3.7 - 5.3]	0.4
Cardiac index (L/min/m^2)	2.6 [2.4 - 3.5]	2.6 [1.9 - 2.9]	0.3
LV diastolic volume (mL)	124 [99 - 137]	116 [96 - 138]	0.8
LV diastolic volume index (mL/m^2)	62.6 [57.0 - 68.6]	60.9 [53.8 - 71.7]	0.9
LV mass index (gm/m^2)	73.6 [16.4]	81.1 [22.1]	0.2
LV global longitudinal strain (%)	-22.4 [-23.9 - -20.3]	-20.9 [-23.4 - -19.6]	0.4
RV free wall strain (%)	-27.6 [-29.8 - -25.8]	-25.3 [-27.0 - -24.4]	0.1
TRV (m/s)	2.4 [2.2 - 2.6]	2.3 [2.1 - 2.5]	0.2
RVSP (mmHg)	29 [24 - 36]	25 [23 - 29]	0.1
LA volume (ml)	56 [45 - 74]	50 [46 - 72]	0.8
LA volume Index (mL/m^2)	28.8 [24.8 - 36.8]	28.0 [23.2 - 35.0]	0.7
RA volume (mL)	36 [28 - 55]	43 [29 - 60]	0.3
RA volume index (mL/m^2)	20.5 [16.3 - 27.0]	20.2 [15.8 - 34.0]	0.4
Septal MV E/e’	9.7 [6.8 - 11.2]	7.7 [7.4 - 8.8]	0.2
Lateral MV E/e’	6.6 [5.7 - 8.2]	5.7 [4.9 - 7.1]	0.6
Septal e’ (cm/s)	8 [7.7 - 11]	10 [9 - 11]	0.2
Lateral e’ (cm/s)	12 [11 - 17]	13 [12 - 15]	0.6
TAPSE (mm)	24.5 [22.3 - 27.3]	24.0 [22.3 - 25.8]	0.8
Mitral E to A ratio	1.20 [1.00 - 1.50]	1.30 [0.825 - 1.65]	0.9
IVC diameter (mm)	17.0 [11.8 - 20.3]	16.0 [12.0 - 18.0]	0.9

Data presented as median (IQR), mean (SD) or n (%).
* p values show a comparison among the groups using either Student’s *t*-test or the Mann-Whitney *U* test for continuous variables and corrected χ^2 test or a 2-sided Fisher’s exact test for categorical variables.

Abbreviations: L/min- Litres per minute; L/min/m^2-Litres per minute per square metre; mm- millimetre; LV-Left ventricle; mL-millilitre; mL/m^2-millilitre per square metre; m/s-metre per second; gm- gram; gm/m^2 -gram per square metre; RVSP-right ventricular systolic pressure; TRV-tricuspid regurgitation velocity; mmHg- millimetres of mercury; LA-left atrial; RA- right atrial; MV E/e’-early mitral inflow velocity (E) over the early diastolic mitral annular velocity (e′); TAPSE-tricuspid annular plane systolic excursion; IVC – inferior vena cava.

Supplementary Table 3
Factors associated with RVSP in patients with portal hypertension

Variables	**Correlation*, r**	**P value**

Age	0.29	0.1
Oxygen saturation (%)	-0.32	0.08
Cardiac output (L/min)	0.27	0.1
Cardiac index (L/min/m^2)	0.30	0.1
LV mass index (gm/m^2)	0.38	0.03
LA volume (ml)	0.52	<0.01
LA volume Index (mL/m^2)	0.54	<0.01
RA volume (mL)	0.48	<0.01
RA volume index (mL/m^2)	0.57	<0.01
Septal MV E/e'	0.25	0.2
Lateral MV E/e'	0.38	0.03
TAPSE (mm)	0.35	0.08
IVC diameter (mm)	0.21	0.3
AST/ALT ratio	0.27	0.1
Liver stiffness measured using VCTE (kPa)	-0.07	0.7
Wedged hepatic venous pressure (mmHg) †	0.08	0.7
Free hepatic vein pressure (mmHg) †	0.46	0.02
Hepatic venous pressure gradient (mmHg) †	-0.14	0.5

*Spearman correlation. † 30 patients underwent transjugular portal pressure measurement.
Abbreviations: RVSP- right ventricular systolic pressure; L/min- Litres per minute; L/min/m^2-Litres per minute per square metre; LV-Left ventricle; gm/m^2-gram per square metre; mm-millimetre; mL-millilitre; mL/m^2-millilitre per square metre; MV E/e'-early mitral inflow velocity (E) over the early diastolic mitral annular velocity (e′); TAPSE-tricuspid annular plane systolic excursion; mmHg- millimetres of mercury; IVC-inferior vena cava; AST/ALT-aspartate aminotransferase to alanine aminotransferase; kPa-kilopascals; LA-left atrial; RA- right atrial.

Supplementary Table 4 Echocardiographic features among NCPH patients

Characteristics	NCPH (n=32)	Preclinical NCPH (n=10)	p value*
Left ventricular ejection fraction (%)	62 [59 - 65]	62 [61 - 65]	0.7
Cardiac output (L/min)	4.9 [3.6 - 5.8]	5.1 [4.3 - 5.8]	0.9
Cardiac index (L/min/m^2)	2.6 [2.0 - 3.2]	2.8 [2.7 - 2.9]	0.8
LV diastolic volume (mL)	124 [101 - 144]	97 [90 - 121]	0.01
LV diastolic volume index (mL/m^2)	66.0 [57.7 - 71.8]	56.2 [47.4 - 58.2]	<0.01
LV mass index (gm/m^2)	78.8 [20.9]	79.3 [24.1]	0.9
LV global longitudinal strain (%)	-21.6 [-24.2 - -20.1]	-21.4 [-22.3 - -19.5]	0.3
RV free wall strain (%)	-26.7 [-28.6 - -24.6]	-25.6 [-28.0 - -24.7]	0.7
TRV (m/s)	2.3 [2.0 - 2.6]	2.3 [2.1 - 2.5]	0.9
RVSP (mmHg)	26 [22 - 33]	25 [23 - 29]	0.5
LA volume (ml)	62.0 [48.8 - 81.0]	47.5 [34.5 - 58.3]	<0.01
LA volume Index (mL/m^2)	34.9 [25.8 - 40.7]	23.2 [21.7 - 28.0]	<0.01
RA volume (mL)	46.0 [32.5 - 56.3]	28.5 [26.3 - 43.0]	0.3
RA volume index (mL/m^2)	25.5 [16.7 - 29.9]	16.8 [13.2 - 24.0]	0.2
Septal MV E/e’	8.9 [7.5 - 11.2]	7.3 [6.1 - 8.5]	<0.01
Lateral MV E/e’	6.5 [5.7 - 8.5]	5.2 [4.6 - 6.6]	0.1
Septal e’ (cm/s)	8 [8 - 11]	11 [10 - 11]	0.03
Lateral e’ (cm/s)	12 [11 - 14]	14 [11 - 15]	0.4
TAPSE (mm)	25 [22 - 28]	24 [23 - 25]	0.6
Mitral E to A ratio	1.2 [0.9 - 1.5]	1.4 [1.2 - 1.5]	0.8
IVC diameter (mm)	16.0 [12.5 - 18.5]	19.5 [12.0 - 21.0]	0.8
TAPSE/RVSP (mm/mmHg)	0.88 [0.75-1.02]	0.89 [0.79-1.02]	0.6

Data presented as median (IQR), mean (SD) or n (%).

* p values show a comparison among the groups using either Student’s *t*-test or the Mann-Whitney *U* test for continuous variables and corrected χ^2 test or a 2-sided Fisher’s exact test for categorical variables.

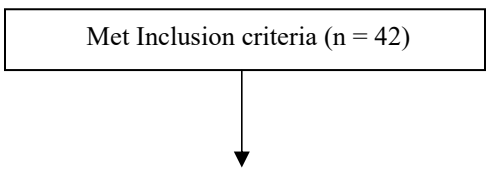
Abbreviations: L/min- Litres per minute; L/min/m^2-Litres per minute per square metre; mm- millimetre; LV-Left ventricle; mL-millilitre; mL/m^2-millilitre per square metre; m/s-metre per second; gm- gram; gm/m^2 -gram per square metre; RVSP-right ventricular systolic pressure; TRV-tricuspid regurgitation velocity; mmHg- millimetres of mercury; LA-left atrial; RA- right atrial; MV E/e’-early mitral inflow velocity (E) over the early diastolic mitral annular velocity (e′); TAPSE-tricuspid annular plane systolic excursion; IVC – inferior vena cava.

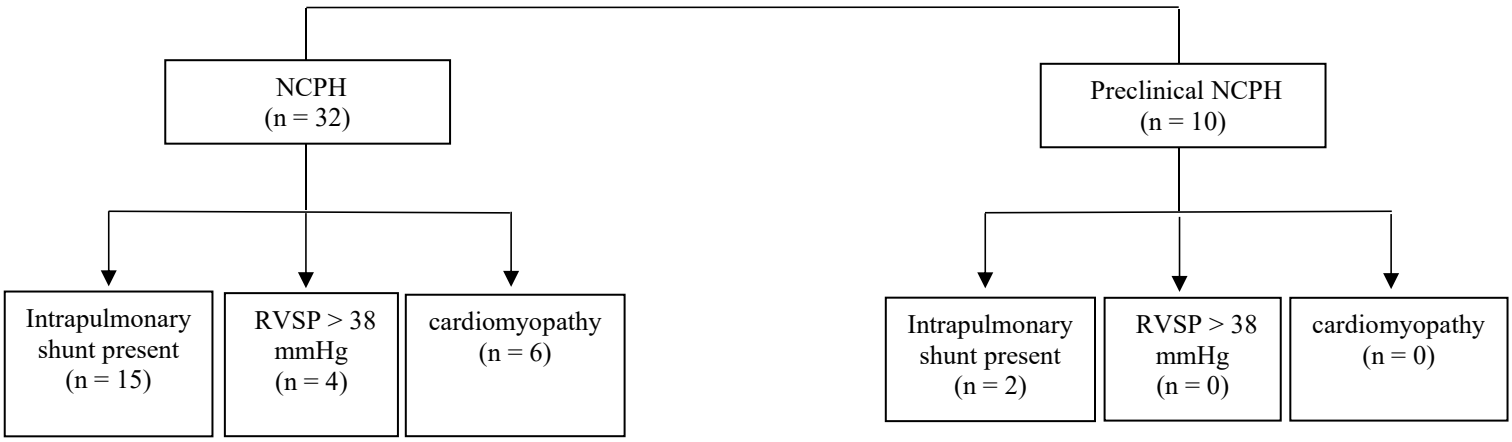
Supplementary Table 5 Echocardiographic factors associated with Bile acids

Variables	Total bile acids		Primary bile acid		Secondary bile acids	
	Correlation (r)	p value	Correlation (r)	p value	Correlation (r)	p value
LV diastolic volume (mL)	0.50	<0.01	0.50	<0.01	0.26	0.1
LV diastolic volume index (mL/m^2)	0.51	<0.01	0.52	<0.01	0.21	0.2
LA volume (ml)	0.36	0.02	0.36	0.02	0.24	0.1
LA volume Index (mL/m^2)	0.41	<0.01	0.41	<0.01	0.26	0.1

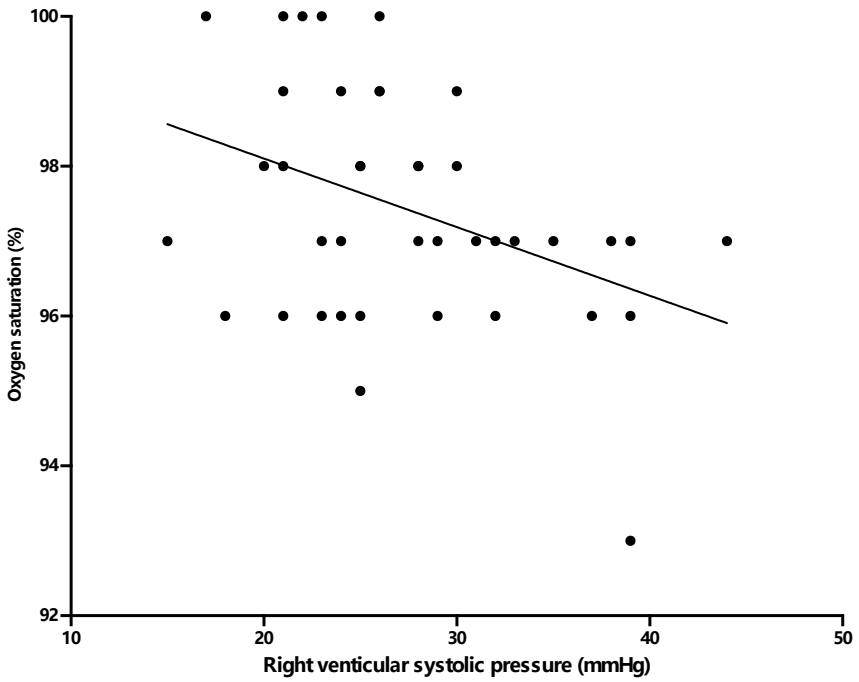
p values for correlation calculated using Spearman test.

Abbreviations: LV-Left ventricle; mL-millilitre; mL/m^2-millilitre per square metre; LA-left atrial

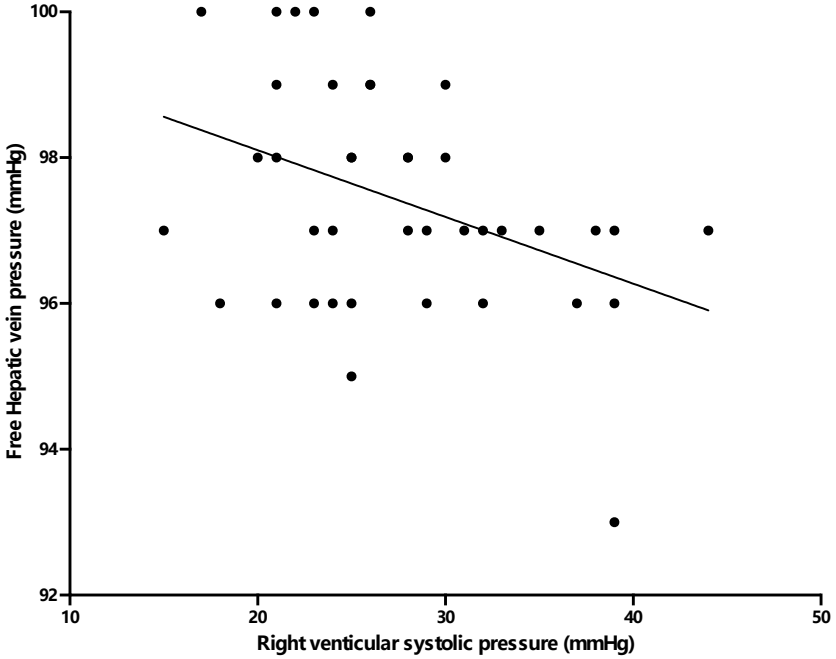




Supplementary Figure 1. Consort diagram of the cohort. PH-portal hypertension; RVSP-right ventricular systolic pressure; LVDD-left ventricular diastolic volume; LVSD- left ventricular systolic volume. Portal hypertension was defined by presence of gastric, esophageal, or ectopic varices, portal hypertensive gastropathy, or the presence of ascites or portosystemic shunting in imaging.

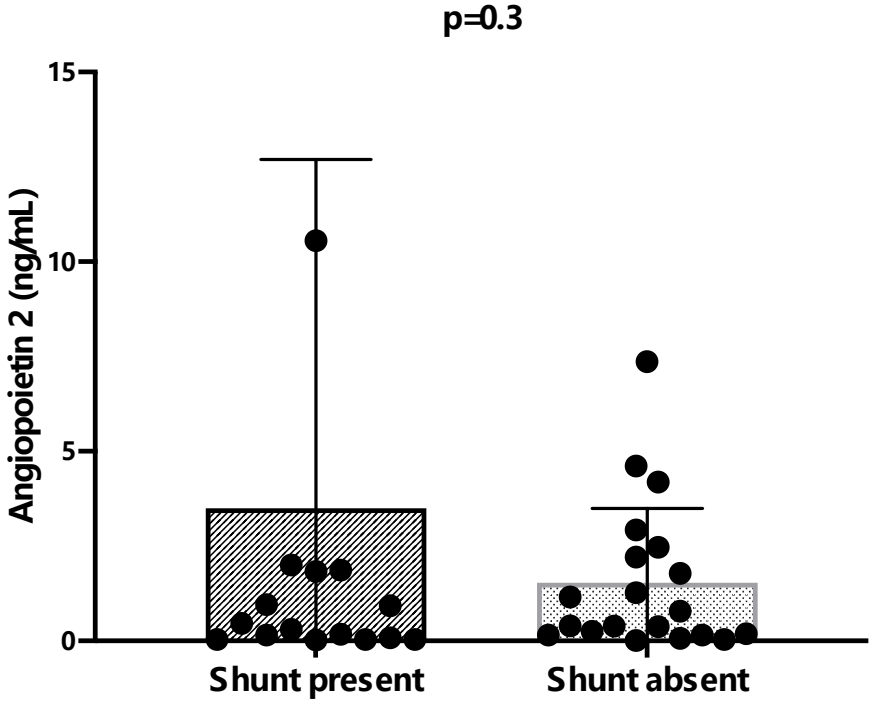


i)

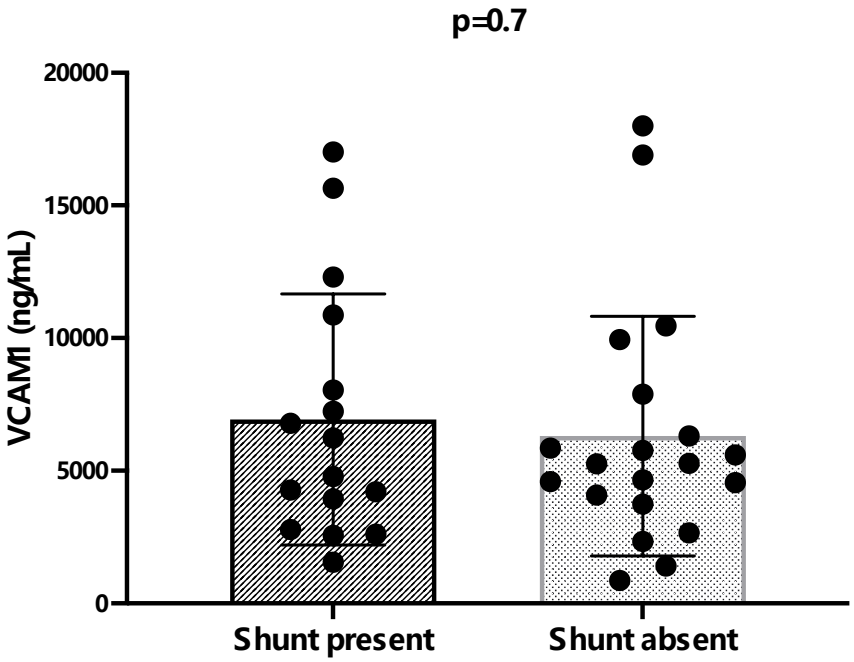


ii)

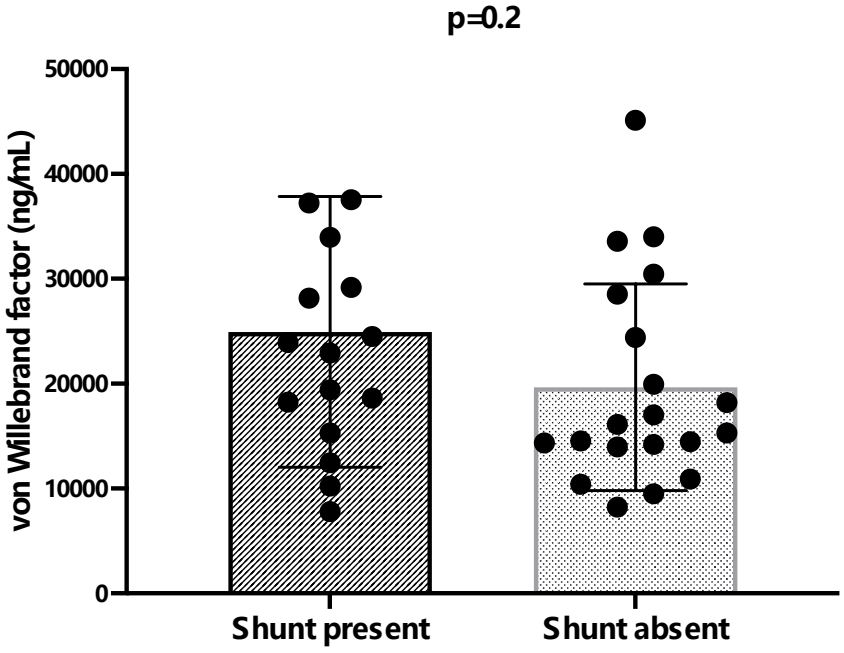
Supplementary Figure 2 Correlation between right ventricular systolic pressure with (i) oxygen saturation (ii) free right hepatic vein pressure



a)


p=0.7
VCAM1 (ng/mL)
20000
15000
10000
5000
0
Shunt present
Shunt absent

b)


p=0.2
von Willebrand factor (ng/mL)
50000
40000
30000
20000
10000
0
Shunt present
Shunt absent

c)

Supplementary Figure 3 Comparing angiogenesis factors among those with and without shunting a) angiopoietin-2 b) vascular cell adhesion molecule 1 (VCAM1), and c) von Willebrand factor. Mean and standard deviation values provided in the graph.

Reference

1. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. Jan 2015;28(1):1-39 e14. doi:10.1016/j.echo.2014.10.003
2. Soliman OI, Geleijnse ML, Meijboom FJ, et al. The use of contrast echocardiography for the detection of cardiac shunts. *Eur J Echocardiogr*. Jun 2007;8(3):S2-12. doi:10.1016/j.euje.2007.03.006
3. Guzzetti E, Capoulade R, Tastet L, et al. Estimation of Stroke Volume and Aortic Valve Area in Patients with Aortic Stenosis: A Comparison of Echocardiography versus Cardiovascular Magnetic Resonance. *J Am Soc Echocardiogr*. Aug 2020;33(8):953-963 e5. doi:10.1016/j.echo.2020.03.020