

Leukocyte extracellular vesicles predict progression of systolic dysfunction in heart failure with mildly reduced ejection fraction (LYCHEE) – a prospective, multicentre cohort study

Short title: EVs predict systolic dysfunction in HFmrEF

Supplementary Materials

Supplementary Table 1. Results of univariable analysis to predict left ventricle systolic dysfunction using the concentration of leukocyte extracellular vesicles above the cut-off value and clinical variables.

	OR	95% CI		p-value
		Lower	Upper	
Leu EVs, $>1.35 \times 10^7 \text{ mL}^{-1}$	4.20	1.03	17.09	0.045
Age, years	1.00	0.95	1.05	0.854
Gender, male	2.02	0.53	7.77	0.306
BMI, kg/m^2	0.89	0.76	1.06	0.191
HF aetiology	1.49	0.39	5.59	0.400
Hypertension	1.08	0.21	5.63	0.929
Dyslipidemia	1.18	0.33	4.29	0.800
Diabetes mellitus	0.245	0.050	1.200	0.083
Metabolic syndrome	0.79	0.23	2.68	0.702
NYHA class	0.82	0.31	2.19	0.690
NT-proBNP, pg/mL	1.00	1.00	1.00	0.962
Creatinine, mg/dL	0.19	0.01	0.50	0.018
RBC, $\times 10^6/\mu\text{L}$	0.91	0.33	2.54	0.860
WBC, $\times 10^3/\mu\text{L}$	0.99	0.73	1.35	0.974
PLT, $\times 10^3/\mu\text{L}$	0.99	0.98	1.01	0.765
LVEF, %	1.12	0.93	1.36	1.120
IVSd, mm	0.96	0.79	1.15	0.631
PWd, mm	0.79	0.60	1.04	0.097
E/A	1.45	0.46	4.54	0.527
E/e' average	1.07	0.91	1.28	0.380

LAVI, ml/m ²	0.97	0.90	1.03	0.289
TRV, m/s	1.66	0.90	3.04	0.102
Beta-blockers	0.99	0.97	1.02	0.585
RAAS inhibitor	0.99	0.97	1.02	0.585
MRA	2.03	0.57	7.34	0.275
SGLT2-inhibitor	0.98	0.27	3.59	0.979
Diuretic	0.41	0.12	1.37	0.150
Statin	0.71	0.13	3.90	0.696

BMI – body mass index, CRP – C-reactive protein, CI - confidence interval, DecT – deceleration time, eGFR – estimated glomerular filtration rate, EVs - extracellular vesicles, E/A ratio - transmitral peak flow velocity in early diastole (E wave) to peak flow velocity in late diastole caused by atrial contraction (A wave), E/e' - transmitral peak flow velocity in early diastole (E wave) to average early peak wave velocity (e') of the mitral annulus, HF – heart failure, IVSd – interventricular septum diameter, LAVI - left atrium volume index, LVEF - left ventricle ejection fraction, MRA - mineralocorticoid receptor antagonist, NT-proBNP - N-terminal pro B natriuretic peptide, NYHA - New York Heart Association, OR – odds ratio, PLT – platelets, PWd – posterior wall diameter, RAAS – renin-angiotensin-aldosterone system, RBC - red blood cells, SGLT2i - sodium-glucose cotransporter-2 inhibitors, TRPG - tricuspid regurgitation peak gradient, WBC - white blood cells

Supplementary Table 2. Comparison of baseline characteristics between patients who experienced progression of diastolic dysfunction and those who did not during a median follow-up of 6.5 months.

	Total population (N=74)	No progression of diastolic dysfunction (N=69)	Progression of diastolic dysfunction (N=5)	p-value
Baseline characteristics				
Age, years	70.0 (63.0-78.0)	69.5 (63.5-78.5)	73.0 (61.0-77.0)	1.000
Gender, male	59 (79.7%)	55 (80.0%)	9 (69.2%)	1.000
BMI, kg/m ²	27.8 (24.8-30.4)	27.7 (24.5-30.4)	29.0 (27.1-29.0)	0.494
HF ischemic etiology	56 (75.7%)	51 (73.9%)	5 (100%)	0.189
Co-morbidities				
Hypertension	62 (84.0%)	57 (83.6%)	5 (100%)	1.000
Dyslipidemia	49 (66.2%)	40 (65.2%)	4 (80%)	1.000
Diabetes	28 (37.8%)	26 (37.7%)	2 (40.0%)	0.918
Obesity (BMI>30 kg/m ²)	20 (27.0%)	18 (26.1%)	2 (40%)	1.000
Metabolic syndrome	32 (43.2%)	31 (44.9%)	1 (20%)	1.000
NYHA class	2 (2-2)	2 (2-2)	2 (2-2)	1.000
Laboratory data				
Cholesterol, mg/dL	141.5 (121.3-179.2)	140.0 (120.0-182.4)	144.4 (130.0-158.0)	0.587
HDL, mg/dL	48.0 (38.3-56.0)	47.0 (38.0-49.5)	54.1 (42.0-58.0)	0.790
LDL, mg/dL	74.0 (57.6-114.0)	74.0 (57.0-114.0)	81.0 (71.0-85.2)	0.504
TG, mg/dL	104.5 (80.0-152.0)	104.3 (79.7-152.0)	119.0 (102.8-147.0)	0.399
Creatinine, mg/dL	1.0 (0.9-1.2)	1.0 (0.9-1.2)	1.0 (1.0-1.2)	0.771
eGFR, mL/min/1.73 m ²	63.0±20.0	62.0 (58.0-82.0)	71.0 (51.0-82.0)	0.923
NT-proBNP, pg/mL	694 (308-1415)	685 (305-1327)	711 (327-1734)	0.721
CRP, mg/L	1.5 (1.0-6.2)	1.5 (1.0-5.5)	4.35 (1.05-7.9)	0.623
RBC, *10 ⁶ /μL	4.4±0.6	140.0 (120.0-182.4)	144.4 (130.0-158.0)	0.587
WBC, *10 ³ /μL	7.4 (6.1-8.5)	47.0 (38.0-49.5)	54.1 (42.0-58.0)	0.790
PLT, *10 ³ /μL	181 (161-230)	74.0 (57.0-114.0)	81.0 (71.0-85.2)	0.504
Baseline echocardiography				
LVEF, %	45.0 (43.0-47.0)	45.0 (43.0-48.0)	41.0 (41.0-45.0)	0.049
LAV, ml	93.0 (52.9-134.0)	82.0 (52.9-134.0)	94.8 (93.0-96.6)	0.890
LAVI, ml/m ²	50.1 (33.7-71.2)	51.5 (30.6-74.8)	46.0 (45-46.8)	0.530
E wave, m/s	0.8 (0.7-0.9)	0.8 (0.6-1.0)	0.7 (0.7-0.7)	0.460
A wave, m/s	0.8±0.3	0.8 (0.6-0.9)	0.7 (0.5-0.9)	0.820
E/A	0.9 (0.8-1.4)	0.9 (0.8-1.4)	0.9 (0.9-1.1)	1.000
DecT, ms	173.0 (146.0-222.0)	173.0 (151.0-222.0)	140.5 (124.0-229.0)	0.250
e' med, cm/s	6.6 (5.6-7.9)	6.6 (5.6-7.8)	6.3 (6.0-8.0)	0.868
e' lat, cm/s	9.3±2.9	8.5 (7.1-11.7)	10.0 (8.5-11.2)	0.517
E/e' average	10.1±4.6	10 (8.0-13.0)	30.5 (21.5-35.5)	0.531

TRPG, mmHg	26.0 (19.0-32.0)	25.0 (19.0-32.0)	30.5 (21.5-35.5)	0.673
LVEDd, mm	54.5 (50.0-57.0)	55.0 (50.0-57.0)	52.0 (51.0-55.0)	0.656
LVEDV, mL	135.9±34.0	132.0 (116.5-153.0)	137.0 (120.0-142.0)	0.981
LVESV, mL	71.5 (48.0-84.0)	67.0 (48.0-83.0)	96.0 (96.0-96.0)	1.000
IVSd, mm	11.0 (10.0-12.0)	11.0 (10.0-12.0)	12.0 (12.0-13.0)	0.222
PWd, mm	10.0 (9.0-11.0)	10.0 (9.0-11.0)	11.0 (11.0-12.0)	0.127
Pharmacotherapy at discharge				
Beta-blockers	68 (91.9%)	63 (91.3%)	5 (100.0%)	0.492
RAAS inhibitor	68 (91.9%)	63 (91.3%)	5 (100.0%)	0.492
MRA	41 (55.4%)	37 (53.6%)	4 (80.0%)	0.252
SGLT2-inhibitor	23 (31.1%)	21 (30.4%)	2 (40.0%)	0.655
Diuretic	42 (56.8%)	39 (56.5%)	3 (60.0%)	0.880
Statin	65 (87.8%)	60 (86.9%)	5 (100.0%)	0.389
Echocardiography at follow-up				
LVEF, %	46.5 (42.0-49.0)	47.0 (42.0-49.0)	45.0 (42.0-45.0)	0.049
LAVI, ml/m ²	46.1 (28.0-52.4)	46.1 (28.4-52.4)	35.1 (21.5-51.8)	0.697
E wave, m/s	0.7 (0.6-1.0)	0.7 (0.6-1.0)	0.7 (0.5-0.9)	0.729
A wave, m/s	0.8 (0.6-0.9)	0.8 (0.6-0.9)	0.7 (0.6-0.8)	0.330
E/A	0.9 (0.7-1.3)	0.9 (0.6-0.9)	1.0 (0.7-1.3)	0.757
DecT, ms	190.0 (114.5-230.0)	188.5 (121.5-235.5)	201.5 (135.5-212.5)	0.951
e' med, cm/s	6.0 (5.1-7.7)	6.0 (5.1-7.9)	6.0 (5.8-6.4)	0.934
e' lat, cm/s	9.1±3.4	8.9 (6.4-11.2)	10.0 (9.6-10.9)	0.521
E/e' average	9.3 (6.7-13.0)	9.4 (6.7-13.4)	9.3 (8.0-11.0)	0.782
TRPG, mmHg	22.0 (19.0-30.0)	22.0 (19.0-30.0)	20.0 (19.0-37.0)	1.000
LVEDd, mm	52.0 (47.0-58.0)	52.0 (47.0-58.0)	51.0 (48.0-60.0)	0.874
LVEDV, mL	141.7±45.9	145.0 (111.0-175.0)	68.9 (45.8-92.0)	0.900
LVESV, mL	74.9±27.7	77.0 (55.0-97.0)	68.9 (45.8-92.0)	0.698
IVSd, mm	10.0 (9.0-12.0)	10.0 (9.0-12.0)	11.0 (10.0-11.0)	1.000
PWd, mm	9.0 (8.0-11.0)	9.0 (8.0-10.5)	9.0 (8.0-11.0)	0.841

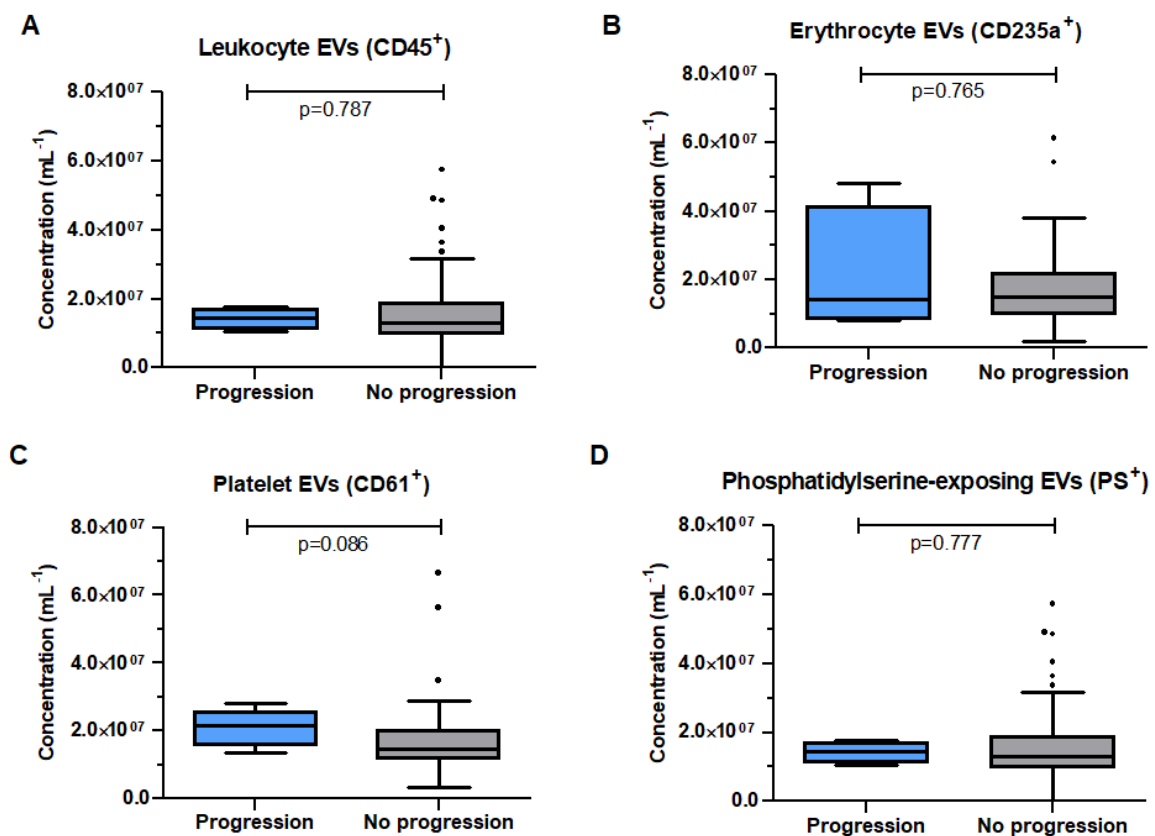
Bold p-values indicates significantly different (< 0.05). Data are shown as number (percentage), median (interquartile range) or mean +/- standard deviation. BMI – body mass index, CRP – C-reactive protein, DecT – deceleration time, eGFR – estimated glomerular filtration rate, E/A ratio - transmitral peak flow velocity in early diastole (E wave) to peak flow velocity in late diastole caused by atrial contraction (A wave), E/e' - transmitral peak flow velocity in early diastole (E wave) to average early peak wave velocity (e') of the mitral annulus, HF – heart failure, HDL – high-density lipoproteins, IVSd – interventricular septum diameter, LAVI - left atrium volume index, LDL – low-density lipoproteins, LVEF - left ventricle ejection fraction, LVEDV – left ventricle end-diastolic volume, LVESV – left ventricle end-systole volume, MRA - mineralocorticoid receptor antagonist, NT-proBNP - N-terminal pro B natriuretic peptide, NYHA - New York Heart Association, PLT – platelets, PWd – posterior wall diameter, RAAS – renin-angiotensin-aldosterone system, RBC - red blood cells, SGLT2i - sodium-glucose cotransporter-2 inhibitors, TG – triglycerides, TRPG - tricuspid regurgitation peak gradient, WBC - white blood cells

Supplementary Table 3. Correlations between baseline plasma concentrations of extracellular vesicles (EVs) and AGE skin accumulation and echocardiographic parameters of systolic and diastolic dysfunction. Significant correlations are made bold and marked with a star. * p<0.05, **p<0.01, ***p<0.001.

Marker	LVEF	E/A ratio	E/e' ratio	LAVI	TRPG
Leukocyte EVs	0.011	-0.038	0.081	-0.035	0.149
Erythrocyte EVs	0.019	-0.062	-0.163	-0.048	0.021
Platelet EVs	-0.047	-0.158	-0.213	-0.80	-0.015
PS-exposing EVs	-0.121	-0.025	-0.109	-0.061	-0.121
Skin AGE	0.004	0.378	0.124	0.018	0.469**

E/A ratio - transmitral peak flow velocity in early diastole (E wave) to peak flow velocity in late diastole caused by atrial contraction (A wave), E/e' - transmitral peak flow velocity in early diastole (E wave) to average early peak wave velocity (e') of the mitral annulus, LAVI - left atrium volume index, LVEF - left ventricle ejection fraction, TRPG - tricuspid regurgitation peak gradient

Supplementary Figure 1. Baseline plasma concentrations of extracellular vesicles (EVs) in patients who did and did not experience the progression of left ventricle diastolic dysfunction at follow-up (panels A-D). The flow cytometer detection range was 80-10,000 nm and >50 MESF PE for erythrocyte EVs; 150-1,000 nm and >50 MESF PE for leukocyte EVs; 50-10,000 nm and >50 MESF APC for platelet EVs and 500-100,000 nm and >50 MESF APC for phosphatidylserine (PS)-exposing EV. **Number of patients: 74**



Supplementary Figure 2. Correlations between blood counts and blood count-derived extracellular vesicles (EVs). **Number of patients: 74**

