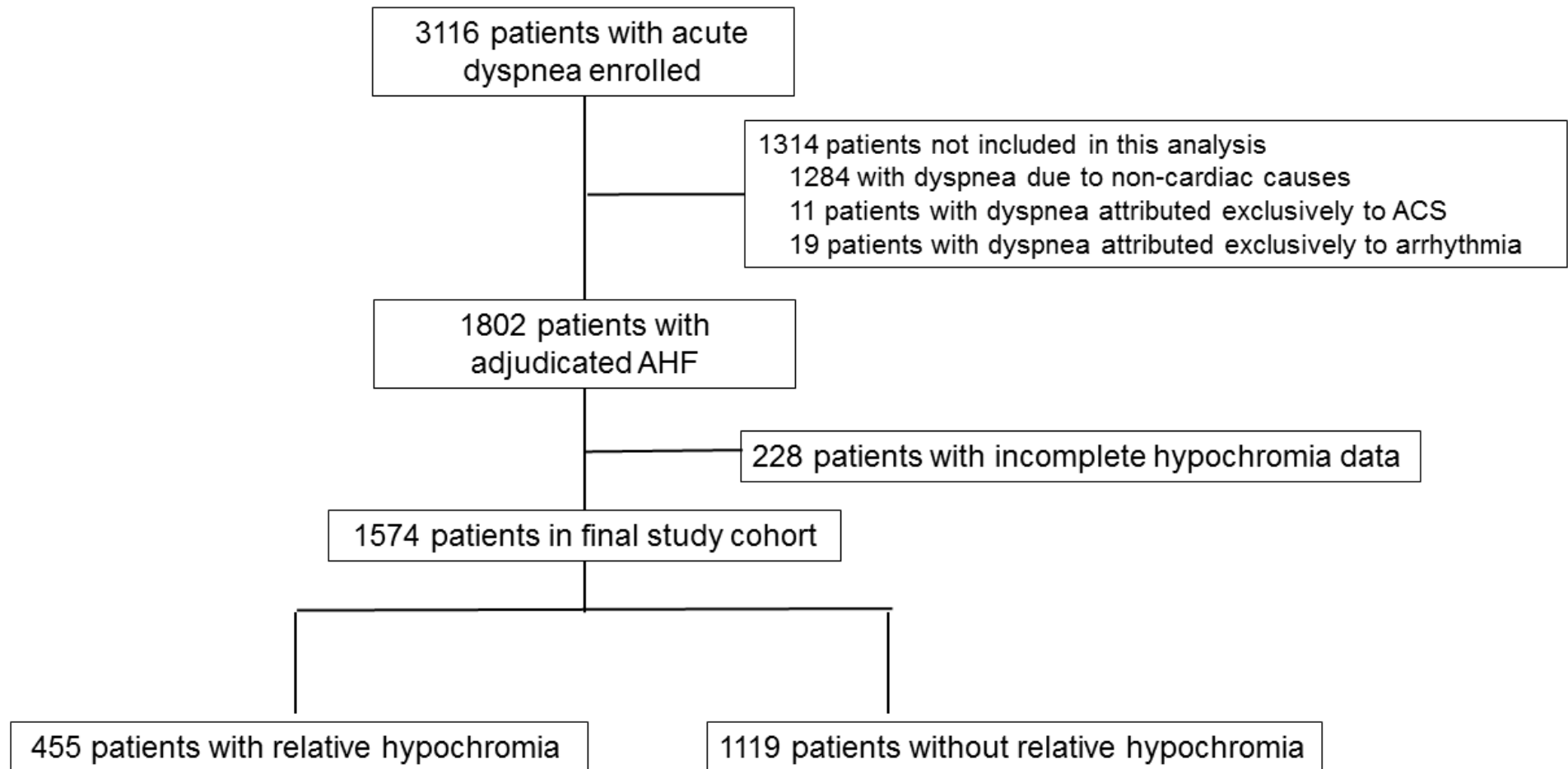


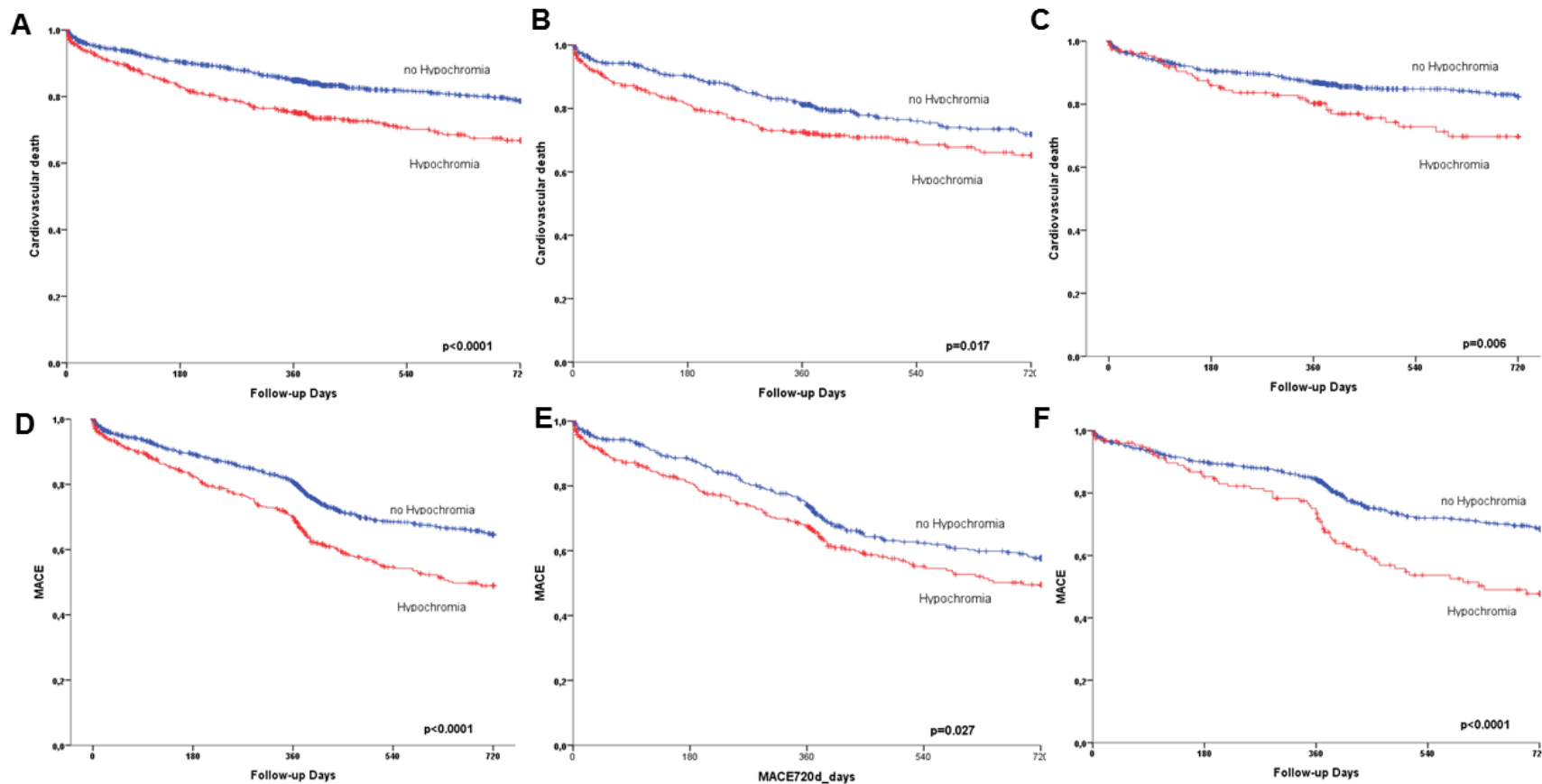
Supplemental Materials

Figures

Figure S1. Patient flow.



**Figure S2.** Cardiovascular death (A-C) and MACE\* (D-E) in patients with acute heart failure in the presence of relative hypochromia

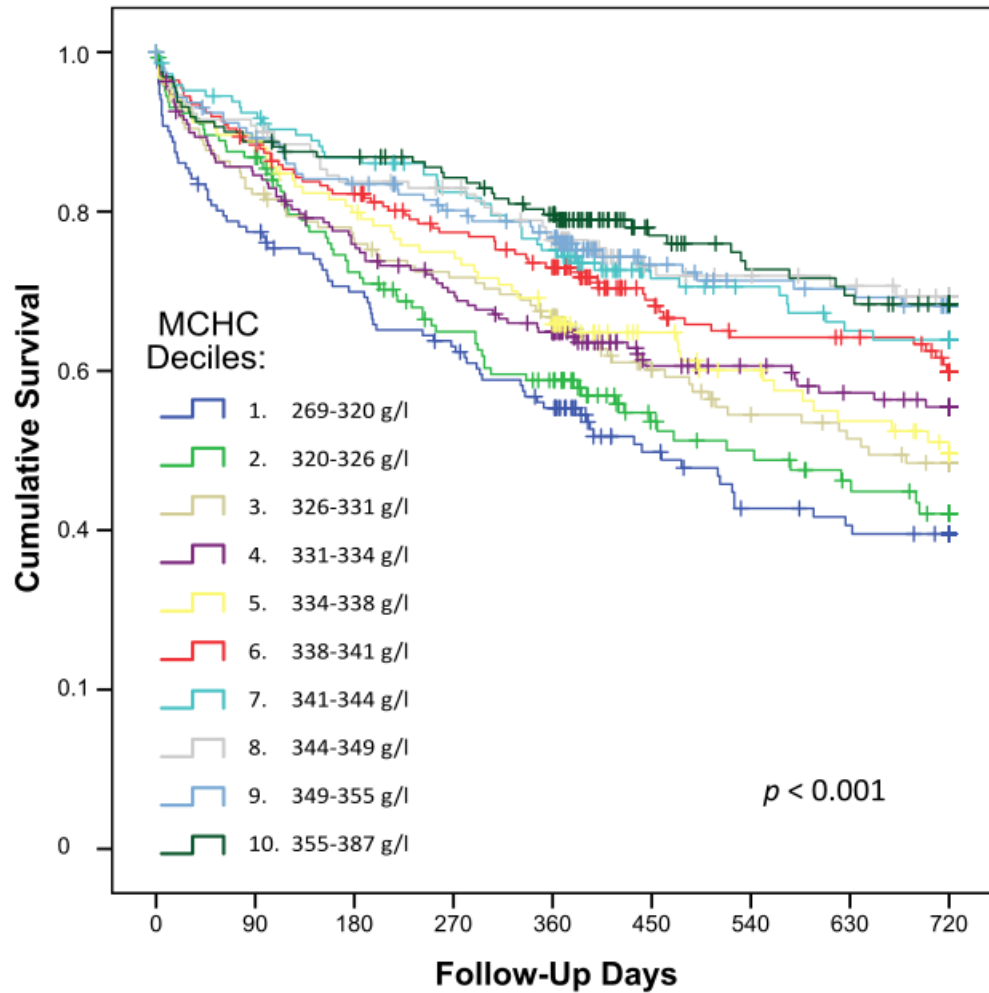


720-day cardiovascular death in patients stratified according to hypochromia (MCHC $\leq$ 330g/l) vs. non-hypochromia in the overall AHF cohort (A), in anemic AHF patients according to hypochromia (B), and in non-anemic AHF patients according to hypochromia (C). 720-day mortality due to MACE in patients stratified according to hypochromia (MCHC $\leq$ 330g/l) vs. non-hypochromia in the overall AHF cohort (D), in anemic AHF patients (E) and in non-anemic AHF patients (F) according to hypochromia.

\* the composite of cardiovascular mortality, admission for AHF or other cardiovascular events

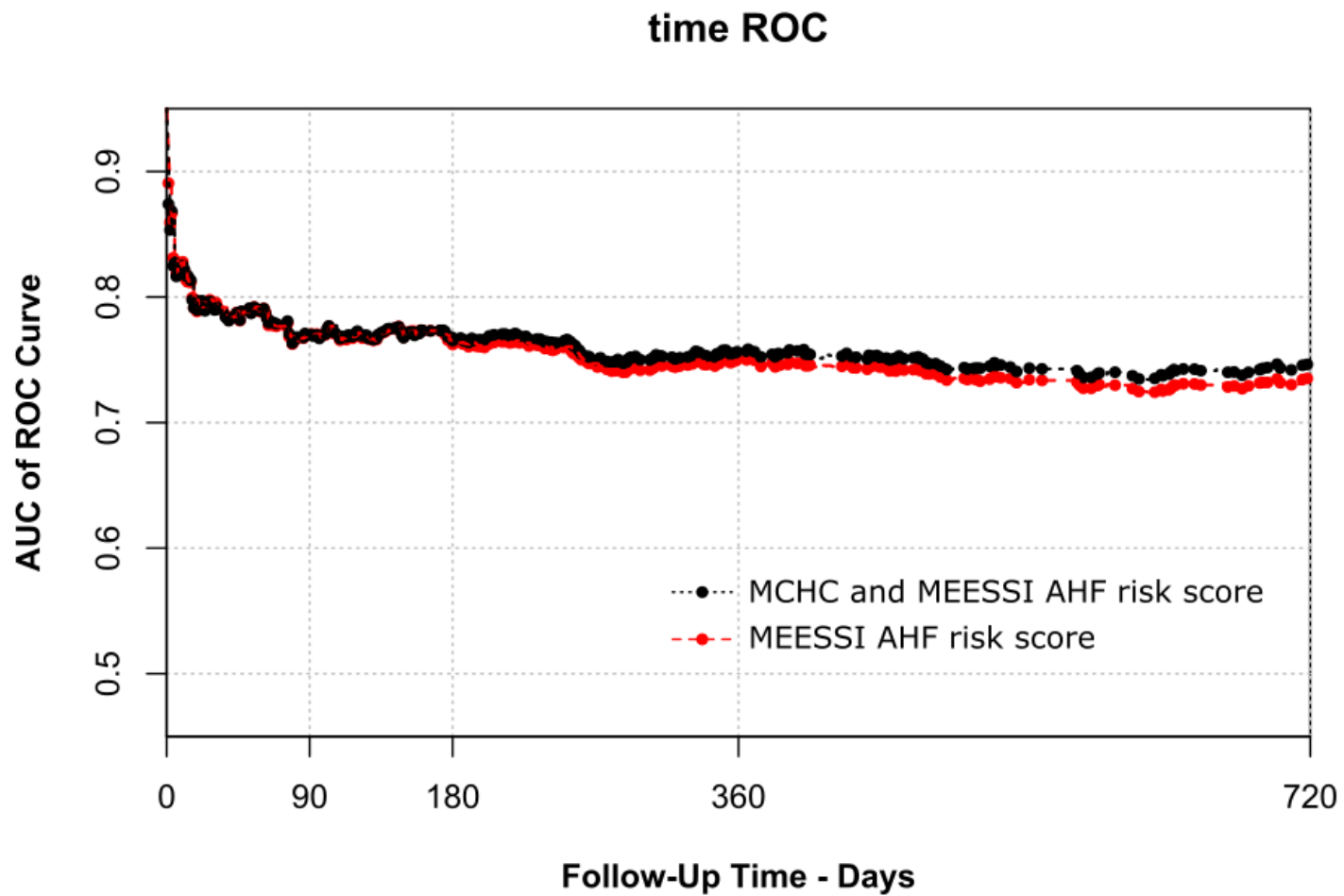
AHF: Acute heart failure. MACE: Major adverse cardiovascular events.

**Figure S4.** Survival of AHF patients according to MCHC deciles.



AHF: Acute heart failure. MCHC: mean corpuscular hemoglobin concentration.

**Figure S3.** Time dependent ROC curves describing the prognostic performance of the MEESSI AHF risk score and its combination with MCHC to predict survival during 720 days follow-up.



AHF: Acute heart failure; AUC of ROC curve: area under the time dependent receiver operating characteristic curve; MCHC: mean corpuscular hemoglobin.

## Tables

**Table S1A:** Characteristics of anemic patients (n=712) with acute heart failure (AHF) according to the presence of relative hypochromia

Variables	Anemia with hypochromia (n=297)	Anemia without hypochromia (n=415)	p-value
<b>Demographics</b>			
Age (years)	80 (72-85)	80 (73-85)	0.509
Female (%)	44	35	<b>0.015</b>
Weight (kg)	73 (62-87)	74 (64-83)	0.933
BMI (kg/m <sup>2</sup> )	26 (23-30)	26 (23-29)	0.352
<b>Medical history</b>			
Coronary Artery Disease (%)	66	61	0.179
Hypertension (%)	86	83	0.301
Peripheral artery disease (%)	23	21	0.496
Stroke (%)	19	21	0.413
Dyslipidemia (%)	62	61	0.965
Diabetes (%)	39	37	0.661
Current or ex-smoker (%)	66	59	0.058
Chronic kidney disease (%)	64	59	0.180
COPD (%)	32	21	0.002
Active malignancies (%)	2	1	0.593
<b>AHF precipitating conditions</b>			
Worsening Heart Failure	66	66	0.972
Infection	17	25	0.012
Cardiogenic shock	2	1	0.593
<b>Laboratory data</b>			
Hemoglobin (g/l)	108 (94-116)	113 (105-120)	<b>&lt;0.001</b>
Hematocrit (%)	34 (29-36)	33 (31-35)	0.300
MCHC (g/l)	322 (316-326)	341 (335-347)	<b>&lt;0.001</b>
MCH (pg)	28 (26-30)	31 (29-32)	<b>&lt;0.001</b>
Creatinine (μmol/l)	131 (92-179)	119 (89-172)	0.239
Urea (mmol/l)	13 (9-19)	11 (7-17)	<b>0.002</b>
eGFR (ml/min/1.73m <sup>2</sup> )	40 (27-60)	45 (29-64)	0.093
Albumin (g/l)	33 (31-36)	34 (31-36)	0.627
CRP (mg/l)	16 (6-35)	16 (5-43)	0.952
Sodium (mmol/l)	139 (137-142)	138 (135-141)	<b>&lt;0.001</b>
Potassium (mmol/l)	4.3 (3.9-4.7)	4.2 (3.9-4.6)	0.114
<b>Clinical parameters</b>			
sBP (mmHg)	131 (117-148)	130 (117-157)	0.053
dBP (mmHg)	73 (62-83)	75 (63-89)	0.069
HR (beats/min)	82 (69-102)	83 (70-99)	0.069
LV ejection fraction (%)	45 (30-55)	45 (32-57)	0.400
Atrial fibrillation (%)	48	42	0.135
<b>Cardiac biomarker</b>			
NT-proBNP (ng/l)	6351 (3449-12429)	5849 (2521-12309)	0.091
hs-TnT(ng/l)	47 (30-61)	40 (25-81)	0.085
<b>Medication at admission</b>			
ACE inhibitors (%)	45	45	0.948
ARBs (%)	27	26	0.744
CCB (%)	25	31	0.085
Beta blockers (%)	59	63	0.317
Diuretics (%)	82	74	0.017
Aldosterone-receptor Antagonists (%)	14	14	0.977
ASS (%)	43	47	0.320
Marcumar (%)	46	35	0.011

### Abbreviations:

ARBs: Angiotensin II Receptor Blockers; ASS: acetylsalicylic acid; AT: Aldosterone-receptor antagonists; BMI: Body mass index; CKD-Epi: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate calculated according to CKD-EPI; CCB: Calcium Channel Blocker; BNP: B-type brain natriuretic peptide; COPD: Chronic obstructive pulmonary disease; CRP: C-reactive protein, dBP: diastolic blood pressure; HR: heart rate; LV: left ventricular; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin; NT-proBNP: N-terminal pro-B-type natriuretic peptide; sBP: systolic blood pressure; hs-TnT: high sensitive Troponin T.

**Table S1B:** Characteristics of non-anemic acute heart failure (AHF) patients according to the presence of relative hypochromia (n=861)

Variables	No anemia with hypochromia (n=158)	No anemia without hypochromia (n=703)	p-value
<b>Demographics</b>			
Age (years)	77 (69-84)	78 (68-85)	0.486
Female, (%)	47	45	0.715
Weight (kg)	74 (63-85)	75 (64-88)	0.217
BMI (kg/m <sup>2</sup> )	26 (23-29)	26 (23-30)	0.190
<b>Medical history</b>			
Coronary Artery Disease (%)	54	44	<b>0.027</b>
Hypertension (%)	65	77	<b>0.002</b>
Peripheral artery disease (%)	14	15	0.605
Stroke (%)	15	15	0.849
Dyslipidemia (%)	51	48	0.537
Diabetes (%)	28	23	0.131
Current or ex-smoker (%)	61	64	0.504
Chronic kidney disease (%)	41	32	0.030
COPD (%)	32	24	0.042
Malignancies (%)			
<b>Laboratory data</b>			
Hemoglobin (g/l)	139 (130-150)	140 (132-151)	0.252
Hematocrit (%)	43 (40-47)	40 (38-44)	<b>&lt;0.001</b>
MCHC (g/l)	325 (320-328)	343 (338-351)	<b>&lt;0.001</b>
MCH (pg)	30 (28-31)	31 (30-32)	<b>&lt;0.001</b>
Creatinine (μmol/l)	106 (81-142)	94 (75-124)	<b>0.002</b>
Urea (mmol/l)	10 (7-15)	8 (6-11)	<b>&lt;0.001</b>
eGFR (ml/min/1.73m <sup>2</sup> )	51 (36-74)	59 (42-78)	<b>0.004</b>
Albumin (g/l)	35 (32-38)	36 (33-38)	0.443
CRP (mg/l)	9 (4-24)	8 (3-27)	0.602
Sodium (mmol/l)	139 (137-141)	139 (136-141)	0.102
Potassium (mmol/l)	4.3 (3.9-4.8)	4.1 (3.8-4.3)	<b>&lt;0.001</b>
<b>Clinical parameters</b>			
sBP (mmHg)	135 (119-159)	140 (122-159)	0.159
dBp (mmHg)	86 (76-95)	83 (71-97)	0.289
HR (beats/min)	100 (85-114)	90 (76-110)	0.006
LV ejection fraction (%)	40 (25- 56)	45 (30- 57)	0.117
Atrial fibrillation (%)	47	44	0.508
<b>Cardiac biomarker</b>			
NT-proBNP (ng/l)	5354 (2781-10128)	3943 (1812-7766)	<b>0.001</b>
hs-TnT(ng/l)	33 (20-65)	33 (18-58)	0.209
<b>Medication at admission</b>			
ACE inhibitors (%)	40	40	0.883
ARBs (%)	16	23	0.070
CCB (%)	9	19	<b>0.003</b>
Beta blockers (%)	59	57	0.592
Diuretics (%)	67	63	0.356
Aldosterone-receptor Antagonists (%)	15	11	0.196

**Abbreviations:**

ARBs: Angiotensin II Receptor Blockers; BMI: Body mass index; CKD-Epi: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate calculated according to CKD-EPI; CCB: Calcium Channel Blocker; BNP: B-type brain natriuretic peptide; CRP: C-reactive protein, dBp: diastolic blood pressure; HR: heart rate; LV: left ventricular; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin, NT-proBNP: N-terminal pro-B-type natriuretic peptide; sBP: systolic blood pressure; hs-TnT: high sensitive Troponin T.

**Table S2:** Multivariable Cox proportional hazards model for all-cause mortality, cardiovascular death and combined endpoint of all-cause mortality or AHF-related hospitalizations with MCHC≤335

	Hazard ratio	p-value	95% CI
<b>All-cause mortality</b>			
Age (years)	1.033	<0.001	1.019-1.048
Sex	0.682	0.001	0.539-0.862
BMI	0.956	<0.001	0.932-0.980
eGFR (ml/min/1.73m <sup>2</sup> )	0.987	<0.001	0.982-0.993
Hemoglobin at admission (g/l)	1.000	0.884	0.995-1.006
MCHC≤335 (g/l)	1.571	<0.001	1.251-1.974
Ig NT-proBNP (ng/l)	2.073	<0.001	1.489-2.884
LV Ejection Fraction (%)	1.003	0.431	0.955-1.011
<b>Cardiovascular death</b>			
Age (years)	1.020	0.001	1.011-1.046
Sex	0.757	0.063	0.564-1.015
BMI	0.955	0.004	0.925-0.985
eGFR (ml/min/1.73m <sup>2</sup> )	0.983	<0.001	0.976-0.991
Hemoglobin at admission (g/l)	1.000	0.937	0.993-1.008
MCHC≤335 (g/l)	1.562	0.002	1.173-2.078
Ig NT-proBNP (ng/l)	1.986	0.001	1.304-3.025
LV Ejection Fraction (%)	0.993	0.191	0.983-1.003
<b>All-cause mortality &amp; AHF rehospitalization</b>			
Age (years)	1.017	0.006	1.005-1.029
Sex	0.793	0.023	0.642-0.981
BMI	0.975	0.022	0.954-0.996
eGFR (ml/min/1.73m <sup>2</sup> )	0.990	<0.001	0.985-0.995
Hemoglobin at admission (g/l)	1.001	0.746	0.996-1.006
MCHC≤335 (g/l)	1.538	<0.001	1.251-1.892
Ig NT-proBNP (ng/l)	1.902	<0.001	1.413-2.561
LV Ejection Fraction (%)	1.001	0.864	0.993-1.008

**Abbreviations:** AHF: acute heart failure, BMI: Body mass index; NT-proBNP: N-terminal pro-B-type natriuretic peptide, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate calculated according to CKD-EPI, LV = left ventricle; MCHC: mean corpuscular hemoglobin concentration.

**Table S3:** Patients with AHF (n=1574) stratified by heart failure phenotypes, precipitating and predisposing conditions and corresponding MCHC levels.

<b>AHF phenotypes</b>		<b>Yes</b>	<b>No</b>	<b>p-value</b>
Hypertensive heart failure	n (%)	141 (9)	1433 (91)	0.004
	MCHC (g/l)	341 (332-349)	337 (328-346)	
Worsening heart failure	n (%)	1008 (64)	566 (36)	0.366
	MCHC (g/l)	338 (329-346)	338 (328-346)	
Pulmonary edema	n (%)	39 (2)	1535 (98)	0.014
	MCHC (g/l)	334 (324-340)	338 (329-346)	
Right heart failure	n (%)	33 (2)	1541(98)	0.559
	MCHC (g/l)	337 (324-347)	338 (329-346)	
Cardiogenic shock	n (%)	16 (19)	2826 (99)	0.192
	MCHC (g/l)	335 (321-346)	341 (332-350)	
<b>AHF precipitating conditions</b>				
ACS	n (%)	86 (5)	1488 (95)	0.027
	MCHC (g/l)	341 (330-349)	337 (329-346)	
Arrhythmia	n (%)	392 (25)	1282 (75)	0.288
	MCHC (g/l)	339 (329-347)	337 (329-346)	
Infection	n (%)	324 (21)	1250 (79)	0.019
	MCHC (g/l)	340 (330-348)	337 (328-340)	
<b>Predisposing factors for AHF</b>				
Post-MI	n (%)	125 (9)	1449 (92)	0.357
	MCHC (g/l)	336 (324-346)	338 (329-346)	
Rhythmogenic AHF (chronic arrhythmia)	n (%)	179 (11)	1395 (89)	0.036
	MCHC (g/l)	340 (331-348)	338 (328-346)	
Rhythmogenic AHF (chronic Afib only)	n (%)	160 (10)	1414 (90)	0.089
	MCHC (g/l)	339 (331-348)	338 (328-346)	
Cardiomyopathy	n (%)	75 (5)	1499 (95)	0.418
	MCHC (g/l)	338 (330-348)	338 (329-346)	
Pulmonary hypertension	n (%)	122 (8)	1452 (92)	0.046
	MCHC (g/l)	335 (324-344)	338 (329-346)	
<b>Vital Signs at Presentation</b>				
SpO2	n (%)	312 (22)	1197 (78)	0.058
	MCHC (g/l)	336 (326-346)	338 (330-347)	
RR<90syst.	n (%)	31 (2)	1509 (98)	0.332
	MCHC (g/l)	337 (328-342)	338 (329-346)	

**Abbreviations:** AHF: acute heart failure; MI: myocardial infarction; MCHC: mean corpuscular hemoglobin concentration.



**Table S4:** Multivariable Cox proportional hazards model for all-cause mortality in AHF patients with arrhythmias and ACS.

	Hazard ratio	p-value	95% CI
<b>All-cause mortality</b>			
Arrhythmia*	0.637	0.001	0.486- 0.834
ACS*	1.193	0.400	0.791- 1.798
Age (years)	1.034	<0.001	1.020- 1.048
Sex	0.725	0.007	0.573- 0.918
BMI	0.958	0.001	0.934- 0.982
eGFR (ml/min/1.73m <sup>2</sup> )	0.987	<0.001	0.981-0.993
Hemoglobin at admission (g/l)	1.002	0.586	0.996-1.008
Hypochromia (yes/no)*	1.528	<0.001	1.207-1.935
Ig NT-proBNP (ng/l)	2.028	<0.001	1.461-2.815
LV Ejection Fraction (%)	1.002	0.709	0.993-1.010

\* Interaction *p*-values between Hypochromia and AHF precipitating conditions: 0.281 for arrhythmia and AHF; 0.151 for ACS and AHF.

**Abbreviations:** ACS: acute coronary syndrome; AHF: acute heart failure; MI: myocardial infarction, NT-proBNP: N-terminal pro-B-type natriuretic peptide

**Table S5:** Interaction of Hypochromia with different AHF phenotypes and precipitating factors for predicting all-cause mortality at 720 days.

	interaction <i>p</i> -value
<b>AHF phenotypes</b>	
Hypertensive Heart Failure	0.529
Worsening Heart Failure	0.837
Pulmonary Edema	0.269
Right Heart Failure	0.015
Cardiogenic shock	0.469
<b>AHF precipitating conditions</b>	
ACS	0.397
Arrhythmia	
Infection	0.794

**Table S6:** Interaction of Hypochromia with different AHF phenotypes and precipitating factors for predicting all-cause mortality or AHF rehospitalizations at 720 days.

	interaction <i>p</i> -value
<b>AHF phenotypes</b>	
Hypertensive Heart Failure	0.319
Worsening Heart Failure	0.701
Pulmonary Edema	0.147
Right Heart Failure	0.013
Cardiogenic shock	0.718
<b>AHF precipitating conditions</b>	
ACS	0.500
Arrhythmia	0.494
Infection	0.331

**Abbreviations:** ACS: acute coronary syndrome; AHF: acute heart failure.

**Table S7.** Risk factors for the MEESSI AHF risk score.

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<b>Risk factors</b>
Systolic blood pressure
Age
NT-proBNP
Potassium level
Elevated troponin level
NYHA class IV
Respiratory rate
Low-output symptoms
Oxygen saturation
Episode associated with ACS
Hypertrophy on ECG
Creatinine plasma concentration

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**Abbreviations:** ACS: acute coronary syndrome; AHF: acute heart failure; NT-proBNP: N-terminal pro-B-type natriuretic peptide



### Combined data

MEESSI AHF risk score	MEESSI AHF risk score										% reclassified
	0-0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0	
0-0.1	0	0	0	0	0	0	0	0	0	0	0
0.1-0.2	76	17	0	0	0	0	0	0	0	0	82
0.2-0.3	0	253	76	0	0	0	0	0	0	0	77
0.3-0.4	0	0	0	268	115	0	0	0	0	0	30
0.4-0.5	0	0	0	0	0	0	0	0	0	0	-
0.5-0.6	0	0	0	0	0	149	73	0	0	0	33
0.6-0.7	0	0	0	0	0	0	0	0	0	0	-
0.7-0.8	0	0	0	0	0	0	51	39	0	0	57
0.8-0.9	0	0	0	0	0	0	0	0	18	0	0
0.9-1.0	0	0	0	0	0	0	0	0	0	3	0

NRI (categorical) (95% CI): 0.321 (0.242-0.400); p<0.001

NRI (continuous) (95% CI): 0.279 (0.169-0.390); p<0.001

IDI (95% CI): 0.011 (0.004-0.017); p<0.001

IDI: Integrated discrimination index. NRI: Net reclassification improvement.

**Supplementary Table S9.** Spearman`s rank correlation analysis between Hypochromia and demographics, biomarkers and echo parameters. Correlation was calculated with measurements obtained on admission.

		MCHC
Albumin	Spearman`s rank (rs)	0.136
	p-value	<0.001
GFR	Spearman`s rank (rs)	-0.206
	p-value	<0.001
Potassium	Spearman`s rank (rs)	-0.207
	p-value	<0.001
Sodium	Spearman`s rank (rs)	-0.118
	p-value	<0.001
LA diameter (mm)	Spearman`s rank (rs)	0.134
	p-value	<0.001
LVEF (%)	Spearman`s rank (rs)	0.079
	p-value	0.008
LVEDD (%)	Spearman`s rank (rs)	-0.043
	p-value	0.183
LVESD (%)	Spearman`s rank (rs)	-0.026
	p-value	0.468
Septum (mm)	Spearman`s rank (rs)	0.029
	p-value	0.416
Posterior wall (mm)	Spearman`s rank (rs)	-0.021
	p-value	0.559

LA: Left atrium. LVEDD: Left ventricular end diastolic diameter. LVEF: Left ventricular ejection fraction. LVESD: Left ventricular end-systolic diameter. MCHC: mean corpuscular hemoglobin concentration.