SUPPLEMENTARY APPENDIX

Performance analysis of AL amyloidosis cardiac biomarker staging systems with special focus on renal failure and atrial arrhythmia

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SUPPLEMENTAL INFORMATION

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SUPPLEMENTAL METHODS

Definition and application of the evaluated staging systems

The evaluated staging systems were applied based on the originally reported thresholds[13,17,21] and only modified with respect to the biomarker troponin. The risk stratification of AL patients is substantially improved by the application of high-sensitivity troponin (hsTnT)[18], which is used by many centers today. Some centers use cTnI instead. We aimed to take all available troponin assays into account by creating a combined binomial category “elevated troponin” (Table S1). The threshold for cTnI (0.1 µg/l) was derived from the original report of the MAYO2004 systems. For hsTnT, we used the recently suggested threshold 54 pg/ml[11], which we could validate in our own cohort (optimal cutpoint for survival prognostication = 54.0 pg/ml; maximally selected rank statistics M = 12.7). Moreover, each analysed troponin biomarker was at its respective threshold highly prognostic for overall survival (Table S2). We further justified our approach by illustrating the potential of prognostication according to the staging systems by KM-estimates (Fig S1) as well as performance analyses (Fig S2) for subsets of patients according to the availability of troponin assays (hsTnT 893 patients, cTnI 524 patients and cTnT 297 patients). The results and conclusions for each subset were well comparable to the entire cohort. To build the MAYO3b system, stage 1 and 2 were taken from the MAYO2004 system. The intermediate high risk “stage 3a” and the very high risk “stage 3b” were obtained by dividing the MAYO2004 stage 3 by an additional NT-proBNP cut-off of above 8.500 ng/l[23].

Definition of impaired renal function

In this study, we decided to define impaired renal function as eGFR<50 ml/min for the following reasons: (1) This threshold is already established in the amyloidosis community, as it was found to be prognostic for renal survival by the national Italian

amyloidosis center, which was further validated by our group[12]. (2) According to Fig S3, it can be expected that patients with eGFR of 50 ml/min and below are assigned approximately one higher stage in any of the staging systems compared to patients with normal renal function. Hence, it seems not necessary to take a lower cut-off such as eGFR below 30 or 20 ml/min to assess the impact of impaired renal function on the staging systems. (3) This threshold is close to the median of our cohort (62.5 ml/min). The criterion eGFR<50 ml/min was met by 415 patients (33.9%).

Definition of atrial arrhythmia

The diagnosis of atrial arrhythmia was based on patient history, clinical examination and ECG, which were performed on a routine basis at the time of laboratory measurement, usually the day of first presentation to our institution (4 patients without information about heart rhythm). Atrial fibrillation or atrial flutter was documented in 144 patients (11.8% of the evaluable patients). At the time of laboratory sampling, 89 of these patients had sinus rhythm ("intermittent AF"), 55 patients had atrial arrhythmia documented by ECG ("present AF") and another 39 patients had a pacemaker rhythm. The median NT-proBNP was markedly elevated not only in patients with present AF, but also in patients with intermittent AF and pacemaker rhythm when compared to patients with sinus rhythm (Table S4). A significant elevation in each AF subgroup could also be observed for median serum levels of hsTnT and cTNT, while median cTNI was elevated only with a trend to significance in patients with present AF and non-significantly in patients with pacemaker rhythm (Table S4). This was potentially due to low numbers of patients with this biomarker available in these subgroups. We decided to pool all three conditions into one category that is hereinafter referred to as "atrial arrhythmia" ("AF", 183 patients).

Supplemental statistical analysis

To visualize the distribution of heart and kidney organ involvement with respect to eGFR and albuminuria, conditional density estimates were computed by applying the Gaussian distribution as smoothing kernel and standard deviation as bandwidth. Four conditions were formed: heart involvement without kidney involvement, kidney involvement without heart involvement, involvement of both these organs and involvement of neither organ. The density was normalized by the fraction of the corresponding group size and stack positioned. The distribution of all patients was generated by normalization of the density to a maximum of one.

The optimal cutpoint for hsTnT was determined by maximally selected rank statistics. This is an outcome-oriented method providing a value of a cutpoint that corresponds to the most significant relation with a continuous predictor (survival).

SUPPLEMENTAL RESULTS

Distribution and organ involvement of subgroups with eGFR<50 ml/min and AF.

An overview of the subgroup frequencies and overlaps is provided with a Venn-diagram based on the frequencies of patients with heart and kidney organ involvement, impaired renal function and with AF (Fig S4A). Most patients with impaired renal function had both heart and kidney involved (59%, 246/415). However, among patients with impaired renal function there were subgroups of patients without kidney involvement (22%, 93/415) or without heart involvement (21%, 88/415). As expected, patients with AF were mostly found to have heart involvement (93%, 171/183).

To further illustrate the distribution of heart versus kidney organ involvement as a function of eGFR, kernel distribution evaluations were generated (Fig S4B). The estimated prevalence of both heart and kidney involvement increased almost linearly with decreasing renal function. However, while the heart was the most prevalent involved organ in patients with mild to moderate kidney injury, patients with severe or end stage kidney disease (eGFR <30 ml/min) had primarily kidney organ involvement (Fig S4B).

The survival of patients with decreased kidney function is mainly determined by heart involvement status and not negatively influenced by a higher degree of kidney organ involvement.

Patients with higher albuminuria, a hallmark of kidney organ involvement, showed a significantly longer median OS compared to patients with lower albuminuria based on a threshold of 1 g/day, which was close to the median of our population (67.5 vs. 22.4, months, p <0.0001, Fig S5A). Importantly, the same holds true for lower thresholds of albuminuria (e.g. 250 mg/day: 52.9 vs. 23.3, months, p <0.0001). This is explained by the fact, that patients with heart involvement have lower albuminuria (median of 470

mg/day) compared to patients without heart involvement (median of 3’413 mg/day, Fig S5B).

SUPPLEMENTAL FIGURES

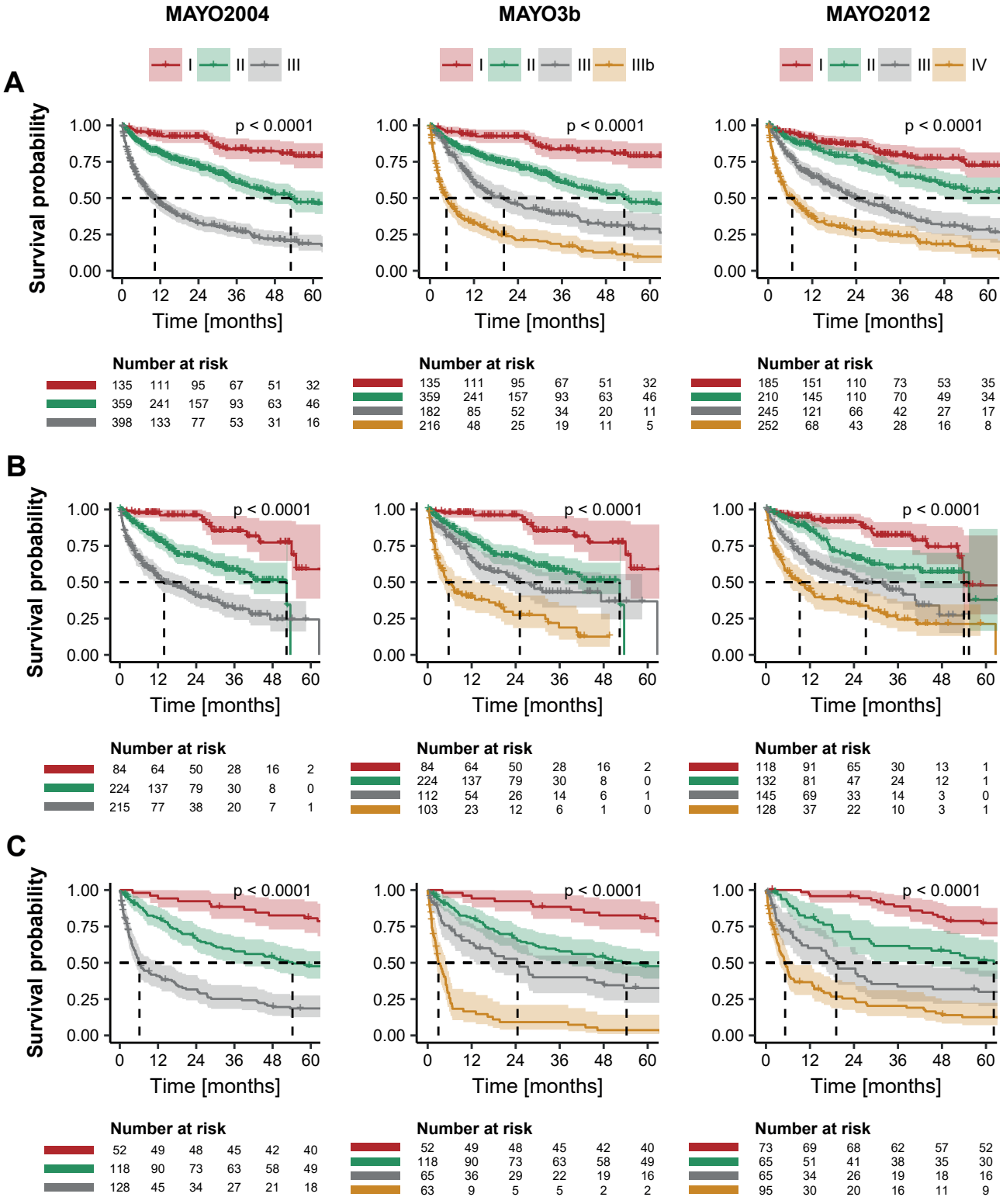

Figure S1. Overall survival according to the staging systems in subsets of patients with hsTnT, cTNI and cTnT.

Kaplan-Meier-Plots. Shaded areas indicate the 95% CI. **(A)** Subset with available hsTnT. **(B)** Subset with available cTNI. **(C)** Subset with available cTnT.

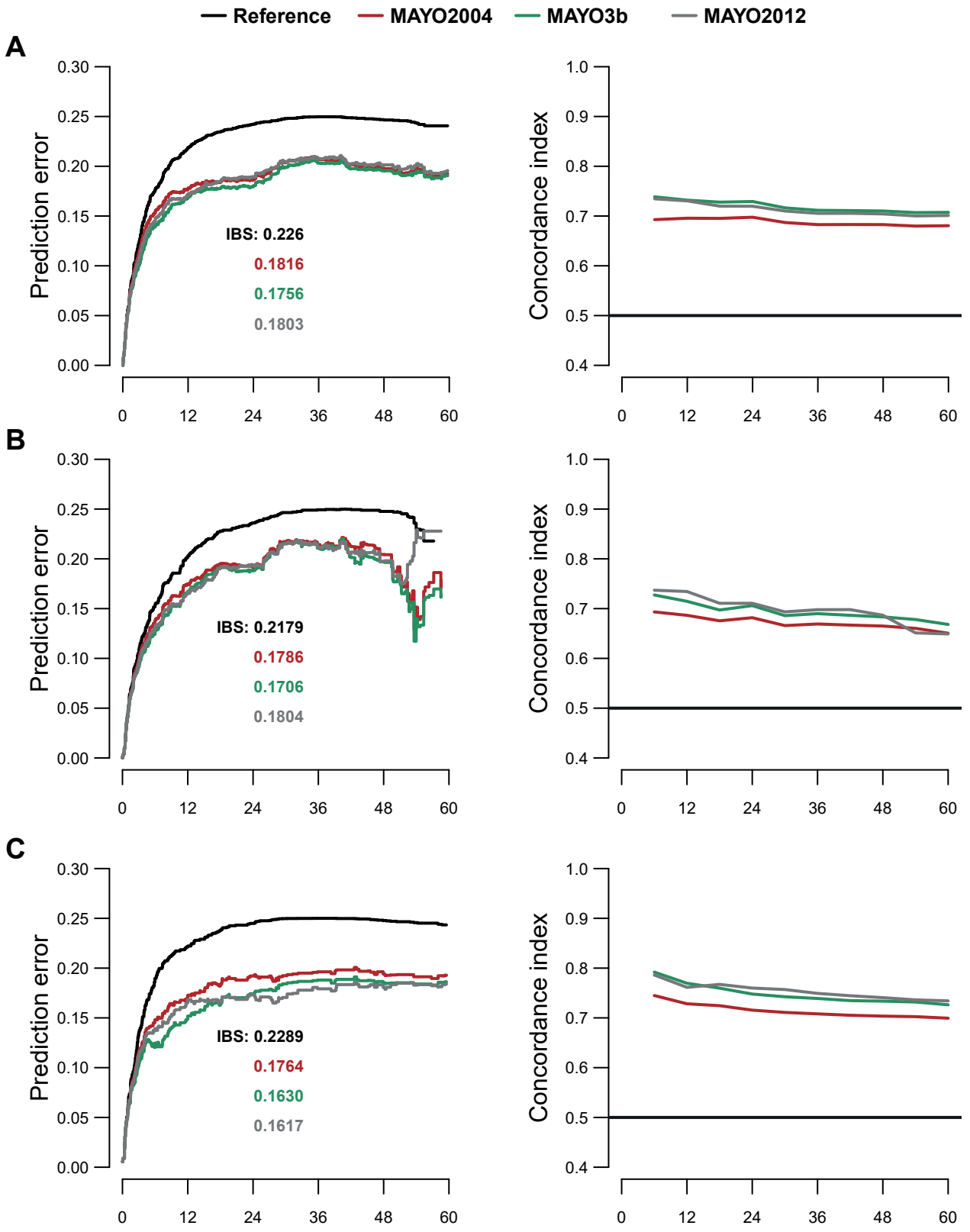

Figure S2. Performance of the staging systems in subsets of patients with hsTnT, cTNI and cTnT.

Curves of time-dependent prediction errors as well as time-dependent concordance indices of the scoring systems. The "IBS" (Integrated Brier Score) for each staging system is given with the respective color, as indicated by the legend. Reference is the marginal Kaplan-Meier estimator, ignoring the predictors. A concordance index of 0.5 indicates random chance. **(A)** Subset with available hsTnT. **(B)** Subset with available cTNI. **(C)** Subset with available cTnT.

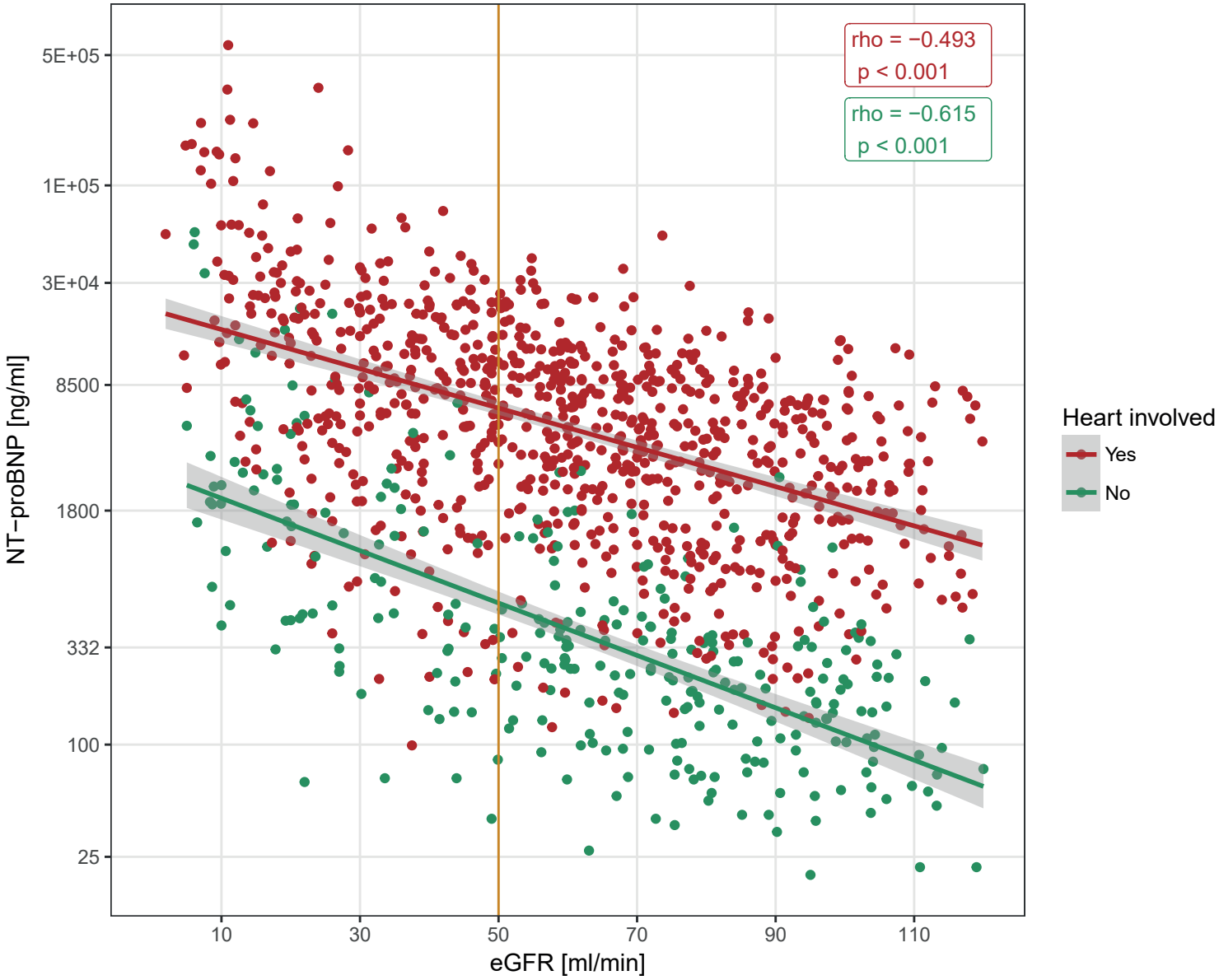

Figure S3. Influence of heart involvement and eGFR on NT-proBNP serum levels.

Scatter plot of NT-proBNP serum levels as a function of eGFR, colored by heart involvement status. The y-axis is log10 transformed. Smoothing lines are added with shadows indicating 95% confidence intervals. Spearman rho and respective p-values are indicated in matching colours. 54 Patients with eGFR >120 ml/min are not shown.

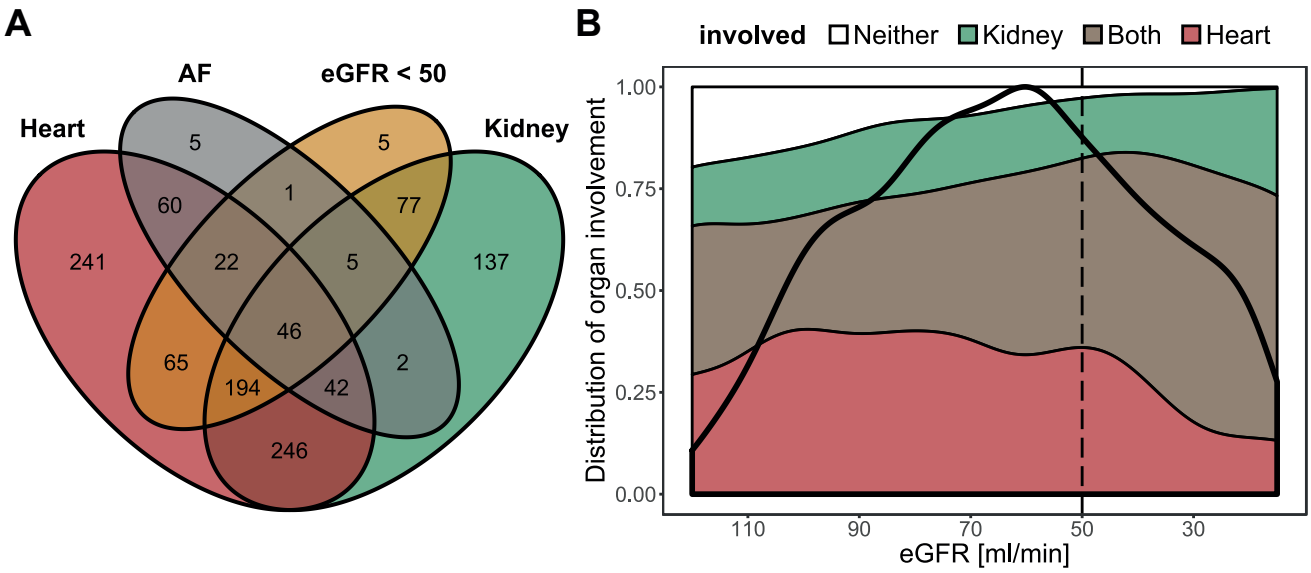

Figure S4. Distribution and organ involvement of subgroups with eGFR<50 ml/min and AF.

(A) Venn-Diagram. Seventy-six patients had neither heart or kidney involvement, nor AF or pacemaker nor eGFR<50, resulting in a total frequency of patients in any of the subgroups of 1148. Frequencies of subgroups are as follows: Heart (916), Kidney (749), AF or pacemaker (183), EGFR<50 (415). **(B)** The distribution of heart and kidney organ involvement with respect to eGFR, generated by conditional kernel density estimates of 1108 patients. The thick overlaid line represents the distribution of all patients. Patients with eGFR >120 ml/min (n =54) and eGFR <15 ml/min (n =62) are not shown. The dashed line indicates eGFR 50 ml/min.

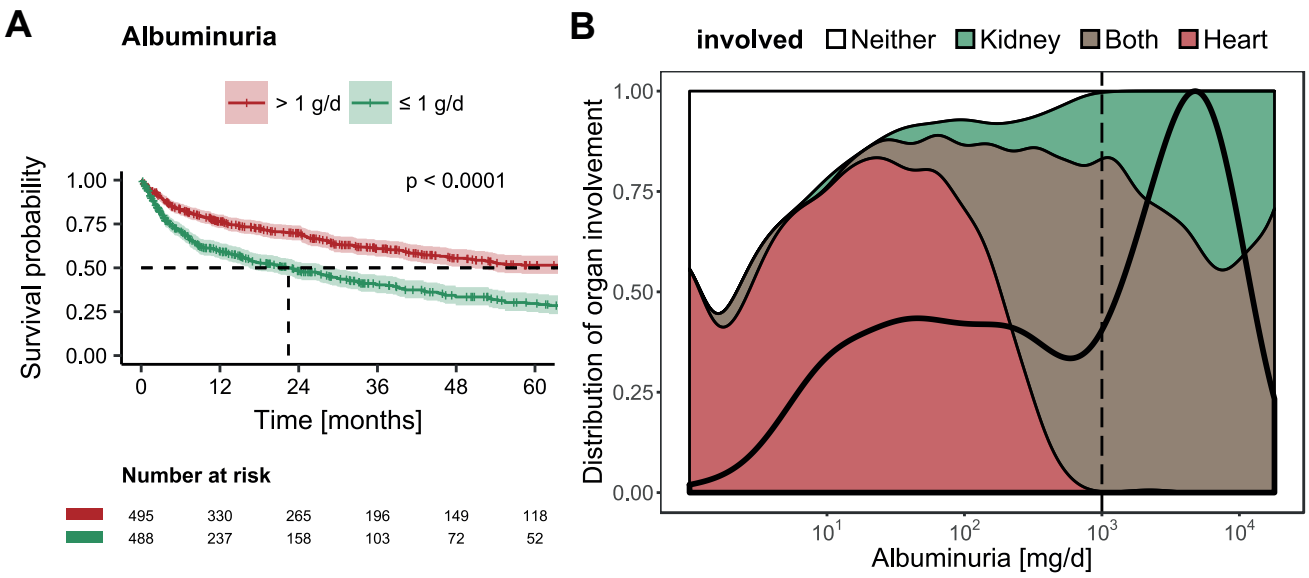

Figure S5. Survival and distribution of patients with heart and kidney involvement according to albuminuria >1g/day.

(A) Kaplan-Meier-Plot depicting overall survival. Median OS in months (with corresponding 95% confidence intervals): ">1 g/d" 67.5 (50.3 - 86.1), "≤1 g/d" 29.1 (16.4 - 29.1). **(B)** The distribution of heart and kidney organ involvement with respect to albuminuria, generated by conditional kernel density estimates of 983 patients with available data. The thick overlaid line represents the distribution of all patients. The dashed lines indicates albuminuria 1 g/day.

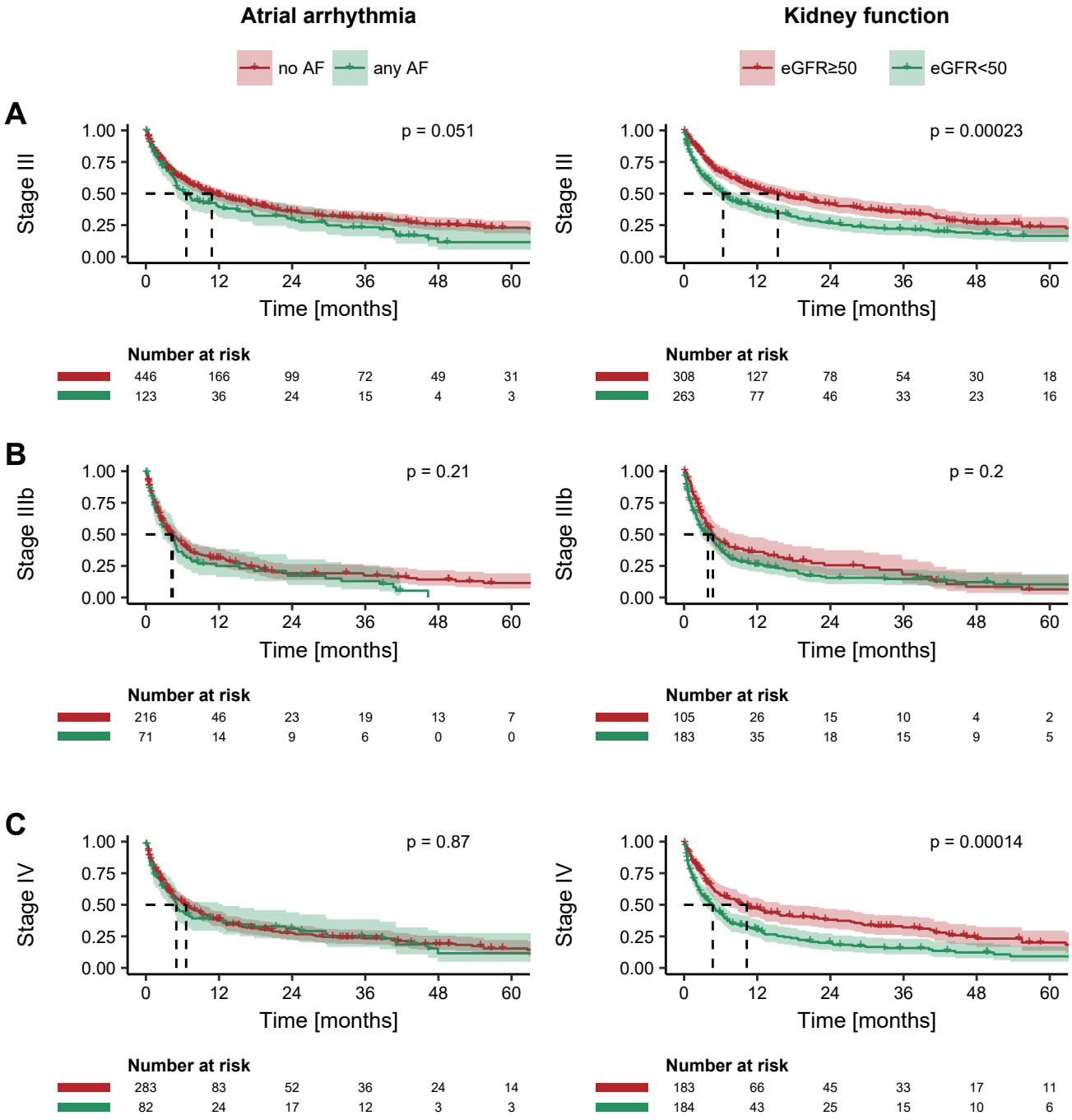

Figure S6. OS of patients in the most unfavourable stages of each staging system according to eGFR<50 and AA.

Kaplan-Meier-Plots depicting overall survival (OS) from diagnosis, stratified by the respective highest stages of the MAYO2004 system **(A)**, MAYO3b system **(B)** and MAYO2012 system **(C)**. Shaded areas indicate the 95% CI estimates. Median OS in months (with corresponding 95% confidence intervals) were as follows. MAYO2004 Stage III: Atrial arrhythmia "no" 10.7 (8.7 - 14.6), "yes" 6.6 (5.0 - 12.6). EGFR ≥50 ml/min 14.9 (10.7 - 21.4), <50 ml/min 6.4 (5.0 - 8.7). Stage IIIb: Atrial arrhythmia "no" 4.4 (3.3 - 5.8), "yes" 4.2 (2.6 - 6.0). EGFR ≥50 ml/min 4.7 (3.5 - 8.2), <50 ml/min 3.9 (2.6 - 5.3). MAYO2012 Stage IV: Atrial arrhythmia "no" 6.6 (5.0 - 8.8), "yes" 5.0 (3.9 - 12.6). EGFR ≥50 ml/min 9.6 (6.5 - 15.8), <50 ml/min 4.7 (3.3 - 6.4).

SUPPLEMENTAL TABLES

Table S1. Biomarker thresholds and application of MAYO staging systems.

System	MAYO2004	MAYO3b	MAYO2012
NTproBNP [ng/l]	332	8500	1800
cTnT [µg/l]	0.035	-	0.025
cTnI [µg/l]	0.1	-	0.1
hsTnT [pg/ml]	54	-	54
dFLC [mg/l]	-	-	180

Biomarkers were counted as elevated when the biomarker value is equal to or greater than the given threshold. Patients without any elevated biomarker score "1" in every system. One point is added to the score for each elevated biomarker, whereby any elevated troponin (cTnT, cTnI, hsTnT) is cumulated in only one point for elevated troponin. For example: A patient with cTnI and hsTnT available at baseline, will be assigned one point for elevated troponin if any of these two biomarkers is elevated. The MAYO3b score is built as follows: Stages 1-2 are taken from the MAYO2004 score and stage 3 of the MAYO2004 score is further divided by the given threshold of NT-proBNP to generate stages 3a and 3b.

Table S2. Median OS according to different troponins/thresholds.

Biomarker	Threshold	elevated	not elevated	p-value
cTnT	0.025 µg/l	7.6 (5 - 17)	92 (70 - 130)	<0.001
cTnT	0.035 µg/l	6.2 (5 - 13)	74.7 (62 - 98)	<0.001
cTnI	0.1 µg/l	15.7 (11 - 24)	52.9 (44 - NR)	<0.001
hsTnT	54 pg/ml	10.3 (8 - 13)	74.5 (62 - NR)	<0.001

Overall survival (OS) is presented as median (95% CI). NR: not reached.

Table S3. Correlation of eGFR with biomarkers.

	rho	n	p-value
cTnT	-0.43	298	<0.001
cTnI	-0.23	523	<0.001
hsTnT	-0.40	894	<0.001
NTproBNP	-0.46	1224	<0.001
dFLC	-0.04	1224	0.1699

Spearman's rank correlation of each cardiac biomarker with eGFR. The combined influence of heart involvement and eGFR on NT-proBNP serum levels is shown in Figure S3.

Table S4. Median serum levels of biomarkers in patients with atrial arrhythmia.

	Sinus rhythm	Intermittent AF		Present AF		Pacemaker	
	Median	Median	p-value	Median	p-value	Median	p-value
cTnT [µg/l]	0.02	0.055	0.037	0.08	0.028	0.10	0.008
cTnI [µg/l]	0.06	0.125	<0.001	0.09	0.087	0.11	0.136
hsTnT [pg/ml]	43	66	<0.001	87	<0.001	58	0.011
NTproBNP [ng/l]	2652	4756	<0.001	10247	<0.001	7626	<0.001
dFLC [mg/l]	198.6	205.1	0.310	427.3	0.013	278.9	0.209

P-values are from Wilcoxon rank sum test with continuity correction, each individual AF-subgroup compared to the respective group with sinus rhythm.

Table S5. Kaplan-Meier estimation and logrank tests of Mayo scores with respect to eGFR and AF subgroups (Figure 2).

Stage		All Patients		EGFR <50 ml/min		AF	
		Median OS	p	Median OS	p	Median OS	p
M2004	I	129.8 (97.9 - NR)	<0.001	NR (28.9 - NR)	0.390	NR (28.7 - NR)	0.311
	II	53.6 (45.5 - 71.9)	<0.001	52.4 (45.1 - 74.2)	<0.001	31.9 (15.1 - NR)	0.003
	III	10.3 (7.7 - 13.1)	-	6.4 (5 - 8.7)	-	6.6 (5 - 12.6)	-
M3b	I	129.8 (97.9 - NR)	<0.001	NR (28.9 - NR)	0.390	na (28.7 - NR)	0.374
	II	53.6 (45.5 - 71.9)	<0.001	52.4 (45.1 - 74.2)	0.009	31.9 (15.1 - NR)	0.515
	III	23.8 (17.1 - 31.2)	<0.001	22.1 (13.1 - 37.4)	<0.001	17.5 (11.6 - NR)	<0.001
	IIIB	4.4 (3.4 - 5.3)	-	3.9 (2.6 - 5.3)	-	4.2 (2.6 - 6)	-
M2012	I	129.8 (97.5 - NR)	<0.001	71.9 (52.4 - NR)	0.136	39.3 (28.7 - NR)	0.921
	II	72.1 (53 - 95.2)	<0.001	50.3 (34.4 - 96.9)	0.014	31.9 (24.4 - NR)	0.004
	III	23.8 (18.5 - 29.9)	<0.001	19.6 (11.1 - 37.4)	<0.001	11.1 (7.4 - 17.5)	0.418
	IV	6.2 (5 - 8.7)	-	4.7 (3.3 - 6.4)	-	5 (3.9 - 12.6)	-

Overall survival in months is given as median (95% confidence interval). P-values are calculated based on logrank test and correspond to the comparison with the respective subsequent stage. M2004: MAYO2004 stage; M3b: MAYO3b stage; M2012: MAYO2012 stage. NR: not reached.