Supplementary material

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Supplementary Figure 1: Midkine stability is ascertained by storage at -80°C and preclusion of repeated freeze-thaw cycles. (A) Serum samples from 3 different patients were stored at 4°C, -20°C, or -80°C. Quantification of midkine was carried out after 4 days following immediate thawing before performance of the ELISA. **(B)** Serum samples from the same patients were stored at 4°C, -20°C, or -80°C for four days. Before quantification by midkine ELISA samples were thawed and subjected to another freezing/thawing cycle (1st thawing) and two more freezing/thawing cycles (2nd thawing). **(C)** Long term stability was tested with serum samples from two patients that were included for ELISA quantification after 2 and 3 years storage at -80°C.

Supplementary Figure 2: Correlation of applied heparin with delta midkine levels. (A) The doses of applied non-fractionated heparin were correlated with changes in serum midkine levels after a short (r^2=0.06, p=0.03) and long dialysis-free interval (r^2=0.17, p<0.001). **(B)** For non-fractionated heparin similar correlations of delta midkine levels after a short (r^2=0.002, p=0.88) and long dialysis-free interval (r^2=0.01, p=0.76) were performed in 12 patients (average 2,958 ± 1,389 IU). The increase of midkine levels was similar after both intervals, 17.6 ± 17 ng/ml and 14.5 ± 11.9 ng/ml, respectively, and did not positively correlate with the dose of fractionated heparin.

Supplementary Figure 3: Serum midkine levels before and after dialysis treatment. (A) Δmidkine values (midkine post – pre-hemodialysis) were calculated for the short and long interval, yielding no significant difference. Furthermore the ΔΔmidkine levels (Δmidkine short interval - Δmidkine long interval) were calculated to assess the variability of midkine changes. **(B)** For the subgroups of diabetic (n=33) versus non-diabetic (n=50) patients analyses were performed. Δmidkine (midkine levels after dialysis - before dialysis) were calculated for the short and long dialysis-free interval. **(C)** For the subgroups of patients diagnosed with hypervolemia (n=51) versus euvolemia (n=32), Δmidkine (serum midkine levels after dialysis - before dialysis) were calculated for the short and long dialysis-free interval. Here, significant differences for the subgroup comparisons were confirmed. **(D)** ROC curve analyses showing the prognostic power of absolute midkine values or ∆midkine values to predict diabetes (cohort with n=70 diagnosed with diabetes; n=101 without diabetes),

hypervolemia (cohort with n=83 diagnosed with hypervolemia; n=88 without hypervolemia). In the last panels prediction of both conditions combined is tested (cohort with n=32 diagnosed with diabetes and hypervolemia; n=51 without diabetes andhypervolemia). AUC, area under the curve. **(E)** ROC curve analyses showing the prognostic power of ∆midkine values with binary logistic regression performed following short and long dialysis-free intervals to diagnose hypervolemia. Selected cut-off points for ∆midkine of the short and long intervals are indicated.

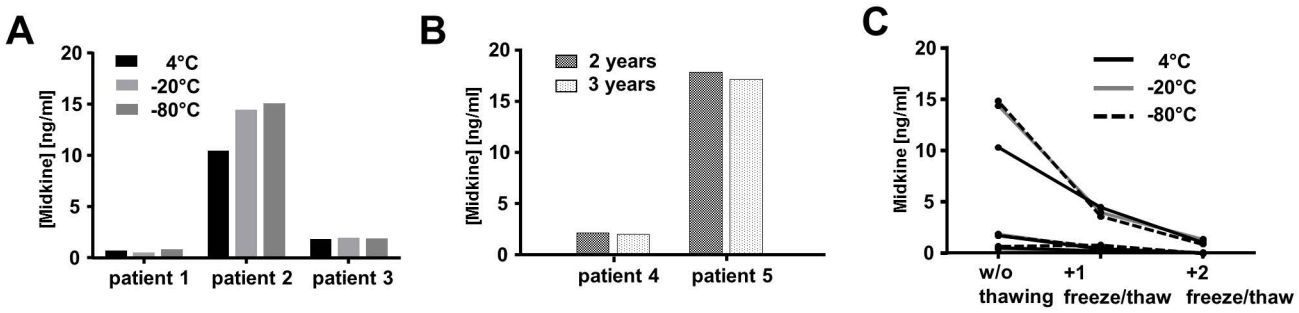
Supplementary Figure 4: Kaplan-Meier survival curves for tenascin-C and galectin. Serum concentrations of tenascin-C **(A, B)** and galectin **(C,D)** were determined. Kaplan-Meier survival curves indicate that both parameters are not predictive for overall survival.

Supplementary Figure 5: Correlative analyses of midkine, NTproANP and uPAR. NTproANP and uPAR serum concentrations were correlated (Pearson) with absolute midkine serum values in the whole dialysis cohort **(A,C)** or selected patients diagnosed with diabetes **(B,D)**.

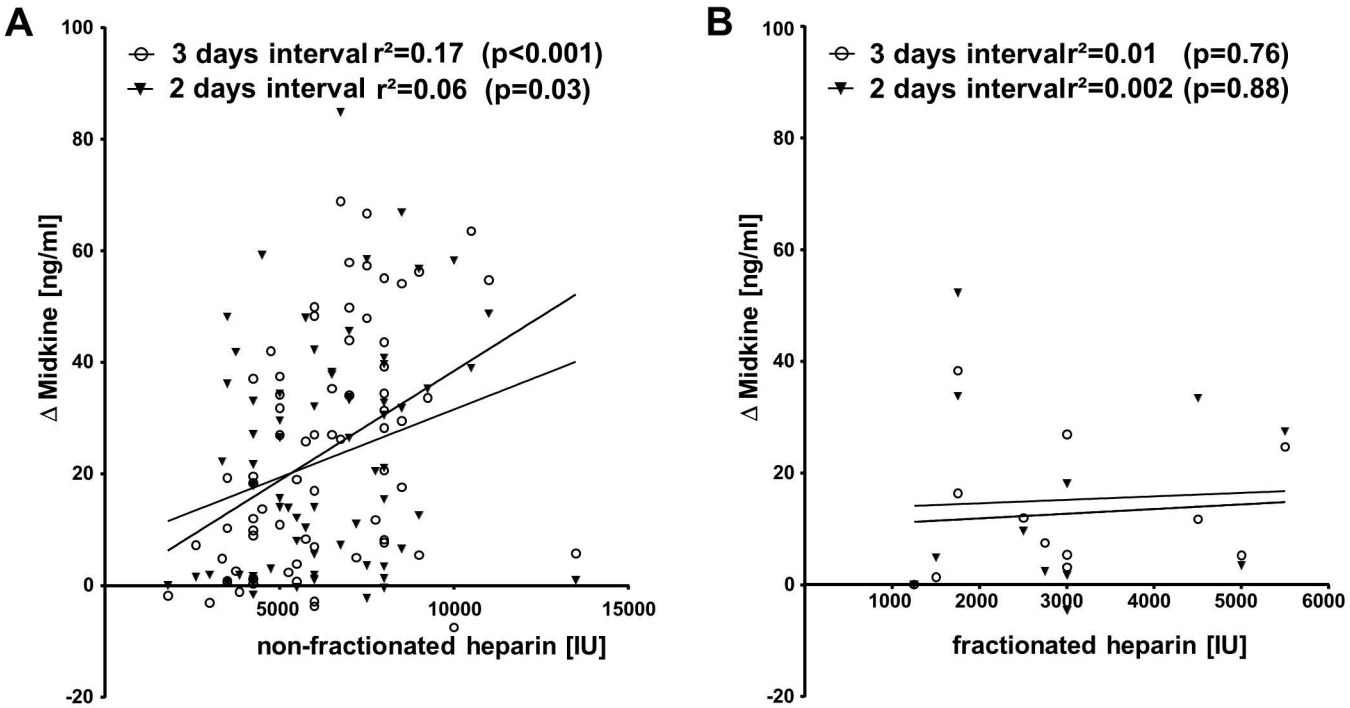
Supplementary Figure 6: Kaplan-Meier survival curves for ADMA. (A) Patients with less-than-average ADMA serum levels before the long dialysis-free interval (group 1) were compared with those having above-average ADMA levels (group 2). Over a 36 months observation period, censoring was performed for overall mortality and **(B)** cardiovascular mortality.

Supplementary Figure 7. Effect of fluid removal on midkine release in patients with hypervolemia. In patients diagnosed with hypervolemia (n=21) appropriate fluid management was planned by increasing net fluid removal during dialysis sessions. Serum midkine levels after long dialysis-free intervals were quantified. 8/21 patients successfully removed additional 0.5-1.0 kg (n=3) or >1.0 kg (n=5). **(A)** ∆Midkine levels were calculated as maximal difference to baseline in the 3 week intervention period with subgrouping according to weight loss: >0.5 to 1.0 kg and >1.0 kg. **(B)** Change of midkine values calculated as [%] change to baseline values again with subgrouping according to weight loss: >0.5 to 1.0 kg and >1.0 kg.

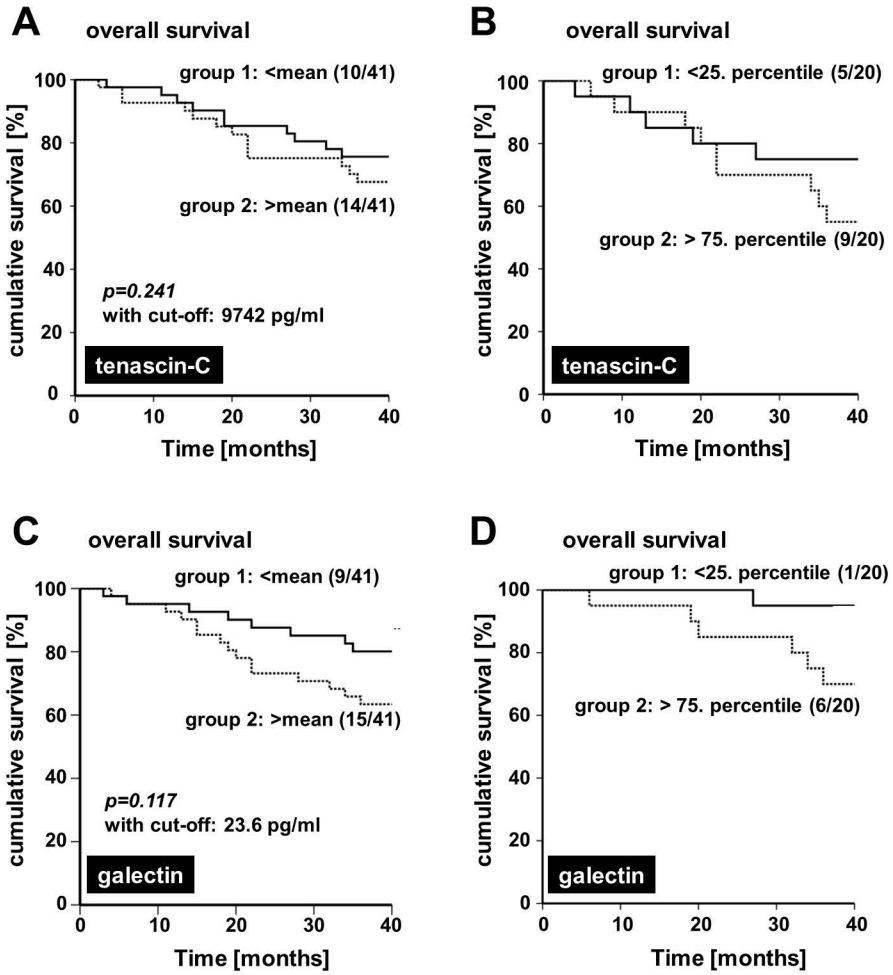
Supplementary Figure 1



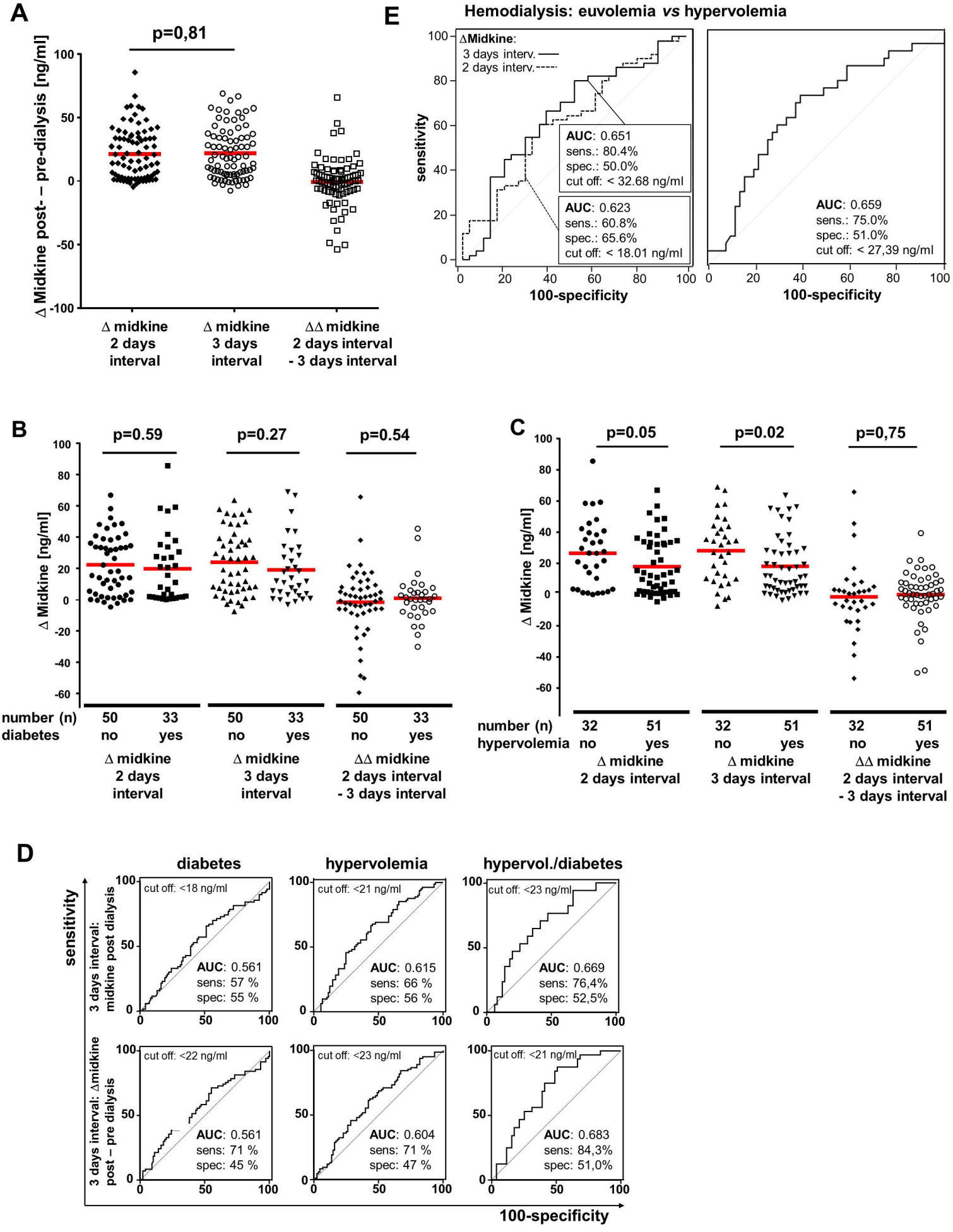
Supplementary Figure 2



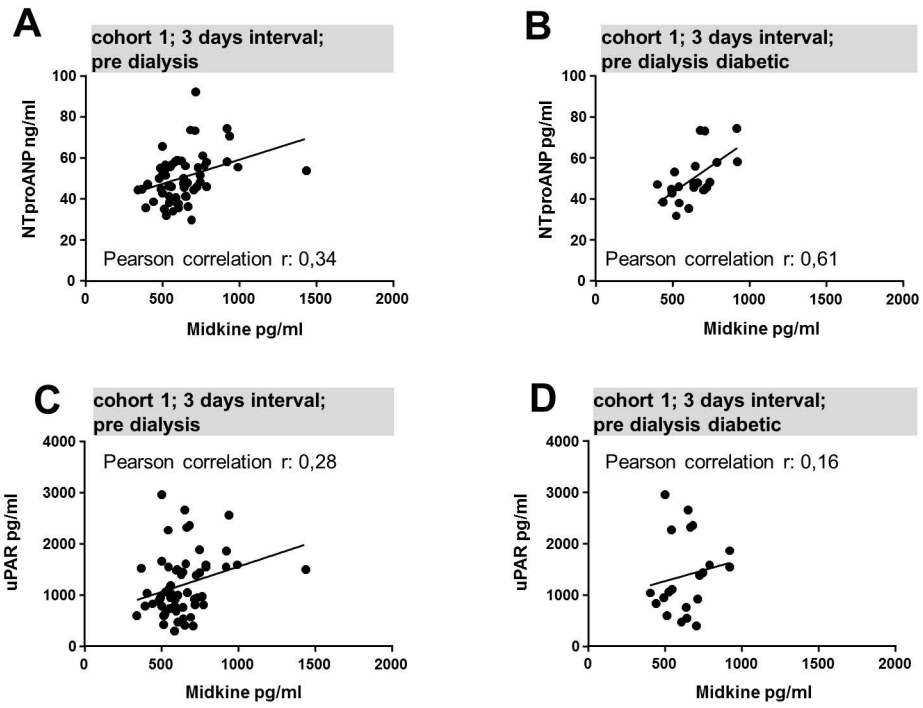
Supplementary Figure 4



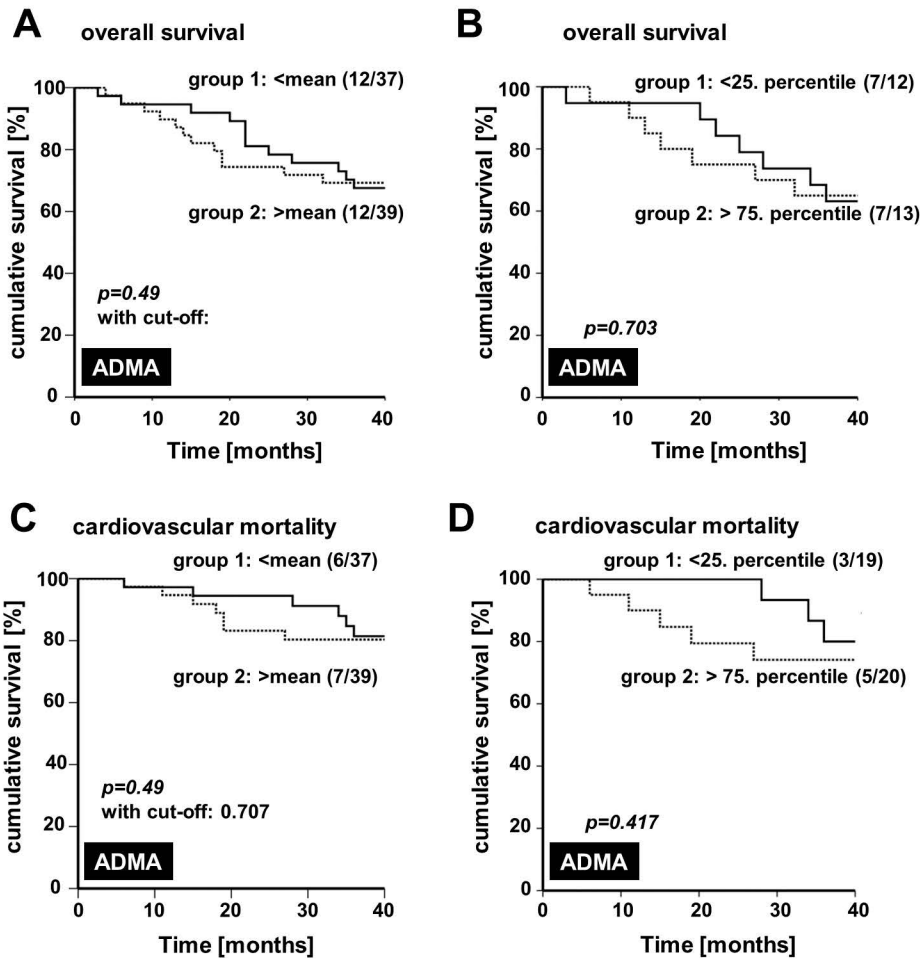
Supplementary Figure 3



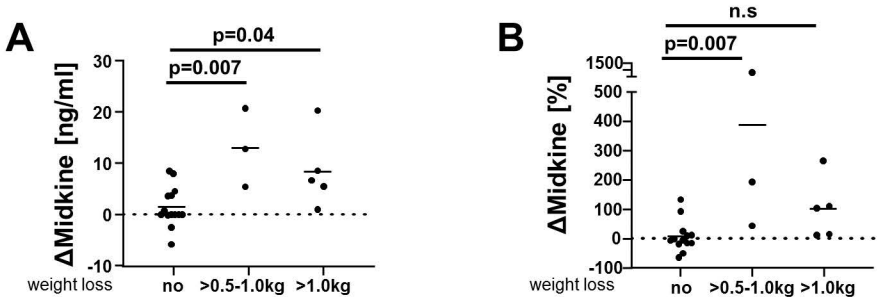
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Table 1

Fluid homeostasis assessment

	hypervolemia defined by		
	comet tail sign lungs	elevated weight (> +0.5 kg)	V. cava diameter > 20mm
number of patients (n)	11	38	22

Supplementary Table 2

ADMA, SDMA, DGMV, L-arginine, delta midkine serum levels

Delta Midkine	3 days interval above mean [24,9 ng/ml] n=42	3 days interval below mean [24,9 ng/ml] n=41	p-value
ADMA [µmol/l]	0.49 ± 0.99	0.49 ± 0.09	0.88
SDMA [µmol/l]	1.28 ± 0.36	1.33 ± 0.1	0.65
DGMV [mmol/l]	334.96 ± 218.34	356.07 ± 385.85	0.77
L-arginine [µmol/l]	22.78 ± 9.4	24.11 ± 20.7	0.72

Supplementary Table 3. HR for ΔMidkine, uPAR, and ANP adjusted for age, gender, fluid status, and diabetes. (*Abbreviations: CI, confidence intervall; HR, hazard ration.)*

	HR	95% CI
ΔMidkine		
HR_{Age}	**1.059**	**1.026 - 1.094**
HR_{Gender}	0.905	0.447 - 1.830
$HR_{Hypervolemia}$	1.367	0.652 - 2.863
$HR_{Diabetes}$	0.898	0.457 - 1.762
$HR_{\Delta Midkine}$	**2.204**	**1.244 - 3.641**
uPAR		
HR_{Age}	**1.052**	**1.017 - 1.088**
HR_{Gender}	1.083	0.533 - 2.199
$HR_{Hypervolemia}$	1.720	0.824 - 3.592
$HR_{Diabetes}$	0.987	0.501 - 1.943
HR_{uPAR}	**2.421**	**1.153 - 5.086**
ANP		
HR_{Age}	**1.055**	**1.022 - 1.090**
HR_{Gender}	0.951	0.468 - 1.934
$HR_{Hypervolemia}$	1.729	0.833 - 3.590
$HR_{Diabetes}$	1.036	0.529 - 2.029
HR_{ANP}	1.352	0.678 - 2.697