

# Association of plasma soluble ST2 and galectin-3 with cardiovascular events and mortality following cardiac surgery

*(sST2 and gal-3 associate with post-op mortality)*

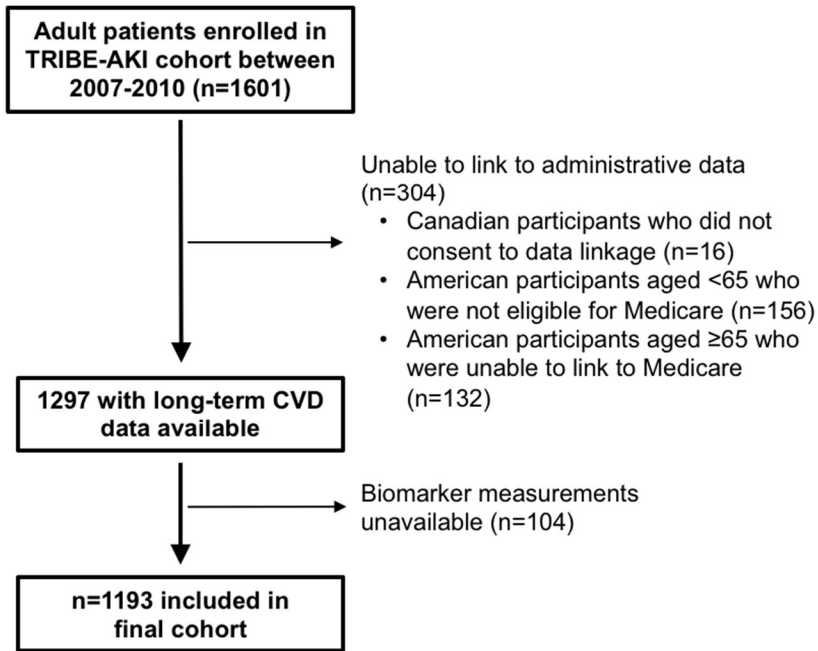
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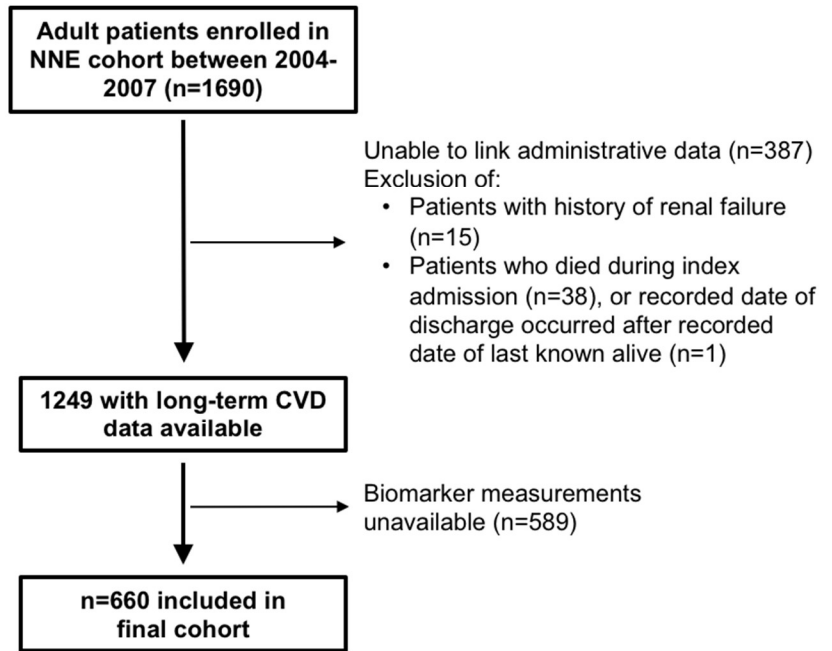
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### Supplemental Figure 1 a-b. Patient selection.

#### a) TRIBE-AKI



#### b) NNE



Supplemental Figure 1 a-b. Flow diagram of patient selection process for (a) TRIBE-AKI and (b) NNE cohorts.

**Supplemental Table 1. Characteristics of assays used for sST2 and gal-3 quantification.**

Assay characteristics		sST2 (n=40)	gal-3 (n=38)
<b>Meso Scale range of detection</b>		0.69 to 8300 pg/ml	2951 to 28790 pg/ml
<b>Correlation coefficient (r)<sup>a</sup></b>		0.98	0.78 <sup>b</sup>
<b>Coefficient of variation (CV)</b>		16.63%	15.76%
<b>Linearity of Dilution (% recovery)</b>	2-fold	103%	105%
	4-fold	101%	113%
	8-fold	104%	104%
	16-fold	95%	94%
<b>Spike Recovery (% recovery)</b>	Low spike	95%	92%
	Medium spike	105%	92%
	High spike	96%	87%

**Supplemental Table 1. Characteristics of assays used for sST2 and gal-3 quantification.** <sup>a</sup>Correlation coefficients are provided in comparison to biomarker levels obtained using FDA-approved assays. <sup>b</sup>Two samples were excluded from this calculation due to handling issues.

**Supplemental Table 2 a. Characteristics of patient cohorts by pre-operative sST2.**

Patient characteristics	Pre-operative sST2 (TRIBE-AKI)					Pre-operative sST2 (NNE)				
	All (N=1193)	T1 (n=397)	T2 (n=398)	T3 (n=398)	p-value	All (N=660)	T1 (n=212)	T2 (n=234)	T3 (n=214)	p-value
Age at surgery (SD)	73 (8.4)	73 (8.6)	73 (8.4)	73 (8.4)	0.6	71 (7.9)	71 (8.1)	72 (6.8)	71 (8.8)	0.17
Male	837 (70%)	250 (63%)	281 (71%)	306 (77%)	<0.001	501 (76%)	145 (68%)	180 (77%)	176 (82%)	0.003
<b>Pre-operative comorbidities</b>										
Diabetes	444 (37%)	123 (31%)	161 (40%)	160 (40%)	0.007	273 (41%)	74 (35%)	91 (39%)	108 (50%)	0.003
Hypertension	955 (80%)	308 (78%)	325 (82%)	322 (81%)	0.3	555 (84%)	173 (82%)	204 (87%)	178 (84%)	0.3
Myocardial infarction	302 (26%)	97 (25%)	103 (26%)	102 (26%)	0.9	309 (47%)	77 (36%)	109 (47%)	123 (57%)	<0.001
Congestive heart failure	244 (20%)	78 (20%)	65 (16%)	101 (25%)	0.006	86 (13%)	16 (8%)	26 (11%)	44 (21%)	<0.001
<b>Pre-operative renal function</b>										
Serum creatinine	1.1 (0.3)	1.0 (0.3)	1.1 (0.3)	1.1 (0.4)	0.003	1.2 (0.7)	1.1 (0.3)	1.1 (0.4)	1.4 (1.2)	<0.001
eGFR (ml/min per 1.73 m <sup>2</sup> )										
≥ 90	129 (11%)	38 (10%)	48 (12%)	43 (11%)		95 (14%)	34 (16%)	31 (13%)	30 (14%)	
60-90	661 (55%)	235 (59%)	220 (55%)	206 (52%)	0.07	358 (54%)	126 (59%)	130 (56%)	102 (48%)	0.004
≤ 60	403 (34%)	124 (31%)	130 (33%)	149 (37%)		207 (31%)	52 (25%)	73 (31%)	82 (38%)	
<b>Characteristics of surgery and hospitalization</b>										
Elective surgery	1023 (86%)	341 (86%)	347 (87%)	335 (84%)	0.5	183 (28%)	60 (28%)	69 (29%)	54 (25%)	0.6
CABG with valve repair	285 (24%)	91 (23%)	91 (23%)	104 (26%)	0.5	34 (5%)	**	**	**	**
CABG	575 (48%)	186 (47%)	209 (52%)	180 (45%)		577 (93%)	183 (93%)	219 (95%)	117 (93%)	
Valve	332 (28%)	121 (30%)	99 (25%)	112 (28%)		**	**	**	**	**

Patient characteristics	Pre-operative sST2 (TRIBE-AKI)					Pre-operative sST2 (NNE)				
	All (N=1193)	T1 (n=397)	T2 (n=398)	T3 (n=398)	p-value	All (N=660)	T1 (n=212)	T2 (n=234)	T3 (n=214)	p-value
On-Pump	1075 (90%)	355 (89%)	356 (89%)	364 (91%)	0.48	585 (89%)	189 (89%)	223 (95%)	173 (80%)	0.54
Perfusion time (min)	104 (80, 140) <sup>a</sup>	102 (77, 133)	100 (79, 140)	111 (87, 144)	0.004	104 (87, 122)	100 (81, 116)	105 (90, 122)	107 (91, 130)	0.006
Days hospitalized	6 (5, 9)	6 (5, 8)	6 (5, 8)	7 (5, 9)	<0.001	8 (6, 12)	8 (6, 11)	8 (6, 12)	10 (7, 14)	0.02
Acute Kidney Injury	420 (35%)	114 (29%)	137 (34%)	169 (42%)	<.001	273 (41%)	62 (29%)	97(41%)	114 (53%)	0.006
<b>Post-operative complications</b>										
Stroke	7 (1%)	3 (1%)	2 (1%)	2 (1%)	0.87	**	**	**	**	**
Re-operation	57 (5%)	16 (4%)	17 (4%)	24 (6%)	0.35	26 (4%)	**	**	**	**
Prolonged Ventilation	39 (3%)	7 (2%)	7 (2%)	25 (6%)	<.001	38 (6%)	**	12 (2%)	16 (2%)	**
DSWI	9 (1%)	4 (1%)	4 (1%)	1 (0%)	0.36	**	**	**	**	**

**Supplemental Table 2 b. Characteristics of patient cohorts by pre-operative gal-3.**

Patient characteristics	Pre-operative gal-3 (TRIBE-AKI)					Pre-operative gal-3 (NNE)				
	All (N=1193)	T1 (n=397)	T2 (n=398)	T3 (n=398)	p-value	All (N=660)	T1 (n=220)	T2 (n=230)	T3 (n=210)	p-value
Age at surgery (SD)	73 (8.4)	73 (8.7)	73 (8.7)	74 (7.8)	0.08	71 (7.9)	70 (7.6)	71 (7.6)	72 (8.5)	0.04
Male	837 (70%)	302 (76%)	286 (72%)	249 (63%)	<0.001	501 (76%)	179 (81%)	176 (77%)	146 (70%)	0.02
<b>Pre-operative comorbidities</b>										
Diabetes	444 (37%)	143 (36%)	152 (38%)	149 (37%)	0.8	273 (41%)	83 (38%)	92 (40%)	98 (47%)	0.2
Hypertension	955 (80%)	307 (77%)	317 (80%)	331 (83%)	0.1	555 (84%)	181 (82%)	195 (85%)	179 (85%)	0.6
Myocardial infarction	302 (26%)	97 (25%)	115 (29%)	90 (23%)	0.09	309 (47%)	109 (50%)	100 (43%)	100 (48%)	0.4
Congestive heart failure	244 (20%)	57 (14%)	89 (22%)	98 (25%)	<0.001	86 (13%)	20 (9%)	27 (12%)	39 (19%)	0.01
<b>Pre-operative renal function</b>										
Serum creatinine	1.1 (0.3)	1.0 (0.3)	1.1 (0.3)	1.1 (0.4)	0.003	1.2 (0.7)	1.1 (0.4)	1.1 (0.5)	1.4 (1.1)	<0.001
≥ 90	129 (11%)	56 (14%)	44 (11%)	29 (7%)		95 (14%)	38 (17%)	36 (16%)	21 (10%)	
eGFR (ml/min per 1.73 m <sup>2</sup> )										
60-90	661 (55%)	226 (57%)	233 (59%)	202 (51%)	<0.001	358 (54%)	141 (64%)	117 (51%)	100 (48%)	<0.001
≤ 60	403 (34%)	115 (29%)	121 (30%)	167 (42%)		207 (31%)	41 (19%)	77 (33%)	89 (42%)	
<b>Characteristics of surgery and hospitalization</b>										
Elective surgery	1023 (86%)	356 (90%)	329 (83%)	338 (85%)	0.02	183 (28%)	63 (29%)	60 (26%)	60 (29%)	0.79
CABG with valve repair	285 (24%)	95 (24%)	82 (21%)	109 (27%)	0.09	34 (5%)	**	**	**	**
CABG	575 (48%)	199 (50%)	209 (53%)	167 (42%)		577 (93%)	189 (86%)	204 (88%)	184 (87%)	
Valve	332 (28%)	103 (26%)	107 (27%)	122 (31%)		**	**	**	**	**

Patient characteristics	Pre-operative gal-3 (TRIBE-AKI)					Pre-operative gal-3 (NNE)				
	All (N=1193)	T1 (n=397)	T2 (n=398)	T3 (n=398)	p-value	All (N=660)	T1 (n=220)	T2 (n=230)	T3 (n=210)	p-value
On-Pump	1075 (90%)	353 (89%)	352 (88%)	370 (93%)		585 (95%)	191 (94%)	208 (94%)	186 (93%)	
Perfusion time (min)	104 (80, 140) <sup>a</sup>	100 (77, 131)	102 (77, 137)	111 (85, 145)	0.01	104 (87, 122)	103 (83, 122)	103 (86, 123)	106 (90, 122)	0.5
Days hospitalized	6 (5, 9)	6 (5, 8)	6 (5, 8)	7 (5, 10)	<0.001	8 (6, 12)	8 (6, 11)	8 (6, 12)	10 (7, 14)	0.02
Acute Kidney Injury	420 (35%)	122 (31%)	138 (35%)	160 (40%)	0.02	273 (41%)	64 (29%)	87 (38%)	94 (45%)	0.003
<b>Post-operative complications</b>										
Stroke	7 (1%)	2 (1%)	2 (1%)	3 (1%)	0.87	**	**	**	**	**
Re-operation	57 (5%)	20 (5%)	15 (4%)	22 (6%)	0.47	26 (4%)	**	**	**	**
Prolonged Ventilation	39 (3%)	14 (4%)	8 (2%)	17 (4%)	0.19	38 (6%)	**	17 (2%)	12(2%)	**
DSWI	9 (1%)	3 (1%)	3 (1%)	3 (1%)	0.9	**	**	**	**	**

**Supplemental Table 2 a-b. Characteristics of patient cohorts by pre-operative sST2 and gal-3.** Numbers are provided as n (percentage) or median (interquartile range). **a)** Tertiles are defined for sST2 as follows. For TRIBE-AKI: tertile 1 (T1) 0.08 – 3.0, tertile 2 (T2) 3.0 – 4.4, and tertile 3 (T3) 4.4 – 89.5 in ng/ml. For NNE: tertile 1 (T1) 1.1 - 3.3, tertile 2 (T2) 3.3 – 4.9, tertile 3 (T3) 4.9 – 75.0 in ng/ml. **b)** Tertiles are defined for gal-3 as follows. For TRIBE-AKI: tertile 1 (T1) 1.1 – 6.6, tertile 2 (T2) 6.6 – 10.3, tertile 3 (T3) 10.3 – 97.8 in ng/ml. For NNE: tertile 1 (T1) 1.6 – 8.7, tertile 2 (T2) 8.7 – 13.8, tertile 3 (T3) 13.8 – 102.4 in ng/ml. <sup>a</sup>Information was not available for a small number of patients (less than 50). \*\*n < 11 with inability to pull data from Medicare data server. SD = standard deviation; eGFR = estimated glomerular filtration rate; ICU = intensive care unit; DSWI = deep sternal wound infection.

**Supplemental Table 3a. Events included in the primary composite outcome.**

	# (%) Events	
	TRIBE-AKI (n = 1193)	NNE (n = 660)
Primary composite outcome	220 (18%)	212 (32%)
Cardiovascular event	122 (10%)	100 (15%)
Death	98 (8%)	112 (17%)

**Supplemental Table 3a. Primary outcome events.** Events within each cohort are separated by component of primary outcome (CV event or death).

**Supplemental Table 3b. Hazard ratios for components of the primary outcome.**

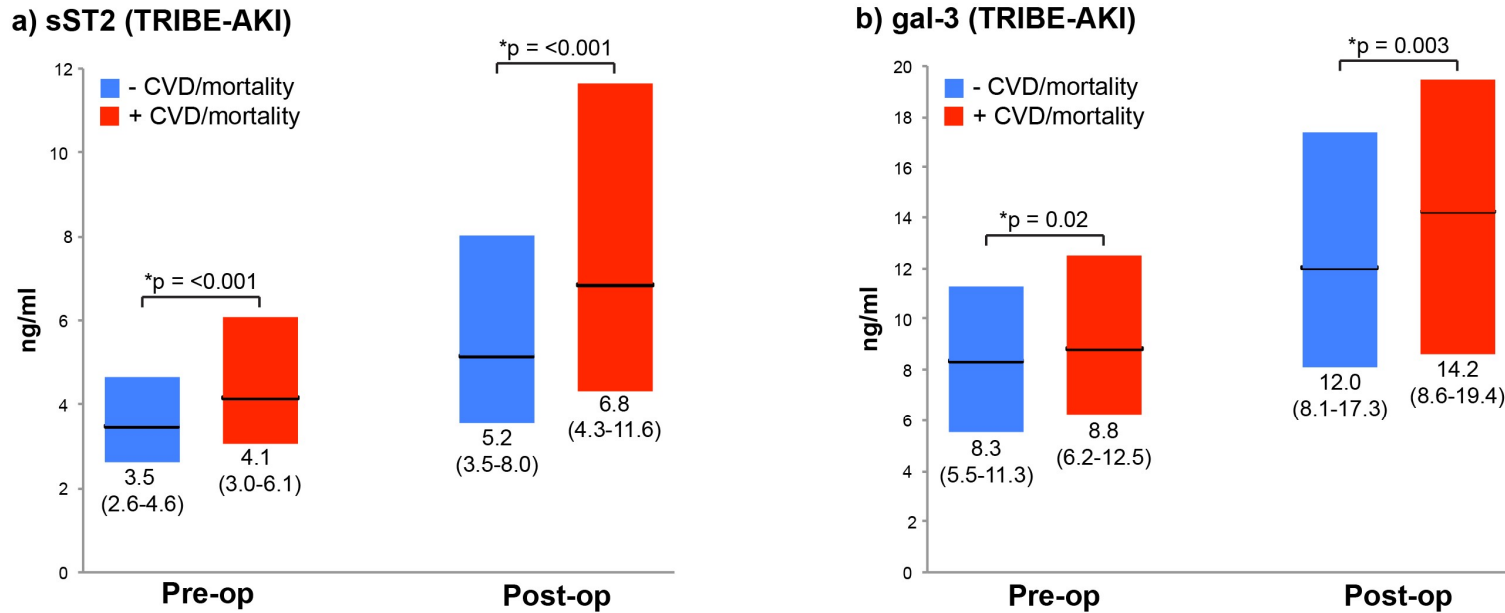
Post-op Biomarker (Log-transformed)	TRIBE-AKI (n=1193). Hazard Ratios (95% CI)					
	Primary composite outcome: CV events or Death (222 Events)		CV Events only (122 Events)		Heart Failure Events (74 events)	Non-Heart Failure CV events (48 events)
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Unadjusted
sST2	1.39 (1.23, 1.56)	1.31 (1.14, 1.50)	1.32 (1.12, 1.56)	1.21 (1.00, 1.46)	1.47 (1.18, 1.83)	0.91 (0.63, 1.32)
Gal-3	1.37 (1.11, 1.68)	1.22 (0.99, 1.51)	1.28 (0.98, 1.68)	1.13 (0.86, 1.49)	2.27 (1.48, 3.48)	0.76 (0.50, 1.15)

Due to low event rates we did not complete analyses on each type of CV events. CV events were categorized as Heart Failure events or Non-Heart Failure events. Non-Heart Failure CV events include: myocardial infarction (26 events), revascularization or coronary syndrome (22 events). Adjusted analyses were not completed due to low event rates.

**Supplemental Table 3b. Hazard ratios for components of the primary outcome.** Associations of post-operative biomarkers with separate components of the primary outcome (CV event or death).



**Supplemental Figure 2 a-b Biomarker trends for pre and post-operative sST2 and Gal-3**



**Supplemental Figure 2 a-b. Biomarker trends for pre- and post-operative (a) sST2 and (b) gal-3 for the TRIBE-AKI cohort.** Data demonstrates median values with 25<sup>th</sup> – 75<sup>th</sup> percentiles. **(a)** Higher sST2 levels were associated with incidence of the primary outcome in pre-operative ( $p < 0.001$ ) and post-operative ( $p < 0.001$ ) stages. **(b)** Higher gal-3 levels were associated with incidence of the primary outcome in pre-operative ( $p = 0.020$ ) and post-operative ( $p = 0.003$ ) stages. N = 1193.

**Supplemental Table 4. Hazard ratios for the primary outcome after adjustment for NT-proBNP and troponin T.**

Biomarker			Hazard Ratio (95% CI) of cardiovascular event or mortality				
			TRIBE-AKI				
			Event rate per 1000py	Unadjusted	Adjusted <sup>a</sup> + NT-proBNP	Adjusted <sup>a</sup> + troponin T	Adjusted <sup>a</sup> + NT-proBNP and troponin T
sST2	Pre-op	Log		1.85 (1.52, 2.25)	1.40 (1.12, 1.76)	1.53 (1.22, 1.92)	1.35 (1.07, 1.71)
		T1	48.5	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )
		T2	64.8	1.31 (0.95, 1.82)	1.46 (1.03, 2.09)	1.42 (0.99, 2.02)	1.40 (0.98, 2.01)
		T3	100.2	2.00 (1.48, 2.70)	1.71 (1.22, 2.41)	1.72 (1.22, 2.43)	1.57 (1.11, 2.22)
	Post-op	Log		1.39 (1.23, 1.56)	1.14 (0.98, 1.33)	1.24 (1.07, 1.43)	1.14 (0.97, 1.33)
		T1	47.0	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )
		T2	62.0	1.31 (0.94, 1.83)	1.32 (0.93, 1.88)	1.39 (0.97, 1.98)	1.34 (0.94, 1.91)
		T3	104.2	2.18 (1.61, 2.95)	1.74 (1.22, 2.49)	1.93 (1.36, 2.76)	1.74 (1.22, 2.49)
Gal-3	Pre-op	Log		1.24 (1.01, 1.53)	1.14 (0.92, 1.42)	1.07 (0.87, 1.33)	1.12 (0.90, 1.39)
		T1	59.4	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )
		T2	69.1	1.17 (0.87, 1.59)	1.09 (0.79, 1.52)	1.13 (0.82, 1.57)	1.11 (0.80, 1.55)
		T3	82.7	1.39 (1.03, 1.87)	1.21 (0.87, 1.67)	1.07 (0.77, 1.49)	1.13 (0.81, 1.56)
	Post-op	Log		1.37 (1.11, 1.68)	1.21 (0.96, 1.52)	1.22 (0.97, 1.54)	1.20 (0.95, 1.51)
		T1	55.7	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )
		T2	66.2	1.19 (0.87, 1.63)	1.10 (0.78, 1.55)	1.11 (0.79, 1.55)	1.09 (0.78, 1.54)
		T3	90.2	1.61 (1.20, 2.16)	1.42 (1.02, 1.99)	1.45 (1.04, 2.02)	1.38 (0.99, 1.93)

**Supplemental Table 4. Hazard ratios for the primary outcome after adjustment for NT-proBNP and troponin T.** NT-proBNP and troponin T values were not available for the NNE cohort. Data was adjusted for clinical covariates, covariates with NT-proBNP or troponin T, or covariates with both NT-proBNP and troponin T. Definitions of tertiles with n for each tertile are as listed in Table 1 and Table 2. <sup>a</sup>Adjusted model included Society of Thoracic Surgery (STS) score, sex, cardiopulmonary bypass time, non-elective surgery, hypertension, and congestive heart failure.

**Supplemental Table 5. Interaction values of the primary composite outcome with incidence of CHF.**

Biomarker			Unadjusted Hazard Ratio (95% CI) of cardiovascular event or mortality					
			TRIBE-AKI			NNE		
			- CHF	+ CHF	Interaction p-value	- CHF	+ CHF	Interaction p-value
sST2	Pre-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.9	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.2
		T2	1.30 (0.88, 1.92)	1.51 (0.83, 2.73)		1.34 (0.95, 1.89)	1.32 (0.89, 1.94)	
		T3	1.84 (1.27, 2.66)	2.15 (1.27, 3.63)		1.49 (1.06, 2.10)	1.46 (0.98, 2.17)	
	Post-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.5	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.4
		T2	1.40 (0.95, 2.05)	1.11 (0.56, 2.20)		1.01 (0.70, 1.45)	1.05 (0.70, 1.58)	
		T3	1.84 (1.26, 2.67)	2.18 (1.26, 3.80)		1.74 (1.26, 2.41)	1.75 (1.20, 2.56)	
Gal-3	Pre-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.04	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.01
		T2	0.89 (0.61, 1.30)	1.62 (0.92, 2.86)		1.62 (1.14, 2.30)	1.77 (1.18, 2.67)	
		T3	1.36 (0.96, 1.92)	1.19 (0.66, 2.13)		1.79 (1.26, 2.54)	2.10 (1.39, 3.16)	
	Post-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.9	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.05
		T2	1.21 (0.83, 1.75)	1.09 (0.61, 1.95)		1.22 (0.85, 1.74)	1.22 (0.80, 1.85)	
		T3	1.51 (1.05, 2.18)	1.38 (0.82, 2.32)		1.87 (1.34, 2.62)	2.03 (1.38, 2.99)	

**Supplemental Table 5. Interaction values of the primary composite outcome with incidence of CHF.** Hazard ratios for risk of the primary composite outcome with 95% confidence intervals are shown.

**Supplemental Table 6. Interaction values of the primary composite outcome with incidence of AKI.**

Biomarker			Unadjusted Hazard Ratio (95% CI) of cardiovascular event or mortality					
			TRIBE-AKI			NNE		
			- AKI	+ AKI	Interaction p-value	- AKI	+ AKI	Interaction p-value
sST2	Pre-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.8	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.8
		T2	1.35 (0.92, 1.98)	1.22 (0.65, 2.28)		1.32 (0.93, 1.87)	1.13 (0.72, 1.79)	
		T3	2.01 (1.40, 2.88)	1.65 (0.94, 2.89)		1.50 (1.06, 2.12)	1.32 (0.83, 2.10)	
	Post-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.5	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.5
		T2	1.35 (0.93, 1.96)	0.95 (0.46, 1.93)		1.00 (0.70, 1.44)	0.80 (0.51, 1.27)	
		T3	2.09 (1.46, 2.98)	1.35 (0.73, 2.50)		1.69 (1.21, 2.34)	1.31 (0.84, 2.05)	
Gal-3	Pre-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.03	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.1
		T2	1.41 (0.99, 2.01)	0.57 (0.31, 1.04)		1.56 (1.10, 2.23)	1.97 (1.22, 3.17)	
		T3	1.38 (0.96, 1.98)	1.06 (0.63, 1.80)		1.80 (1.26, 2.55)	2.14 (1.30, 3.51)	
	Post-op	T1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.1	1.0 ( <i>referent</i> )	1.0 ( <i>referent</i> )	0.3
		T2	1.29 (0.91, 1.84)	0.71 (0.36, 1.40)		1.22 (0.85, 1.76)	1.47 (0.92, 2.34)	
		T3	1.37 (0.96, 1.96)	1.47 (0.83, 2.61)		1.90 (1.35, 2.67)	1.97 (1.22, 3.18)	

**Supplemental Table 6. Interaction values of the primary composite outcome with incidence of AKI.** Hazard ratios for risk of the primary composite outcome with 95% confidence intervals are shown.