

Supplemental information

Methemoglobin as a marker of acute anemic stress in cardiac surgery

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Figure S1: Patient flow chart, related to Table 1. Numbers include patients enrolled in the pilot TRICS II trial.

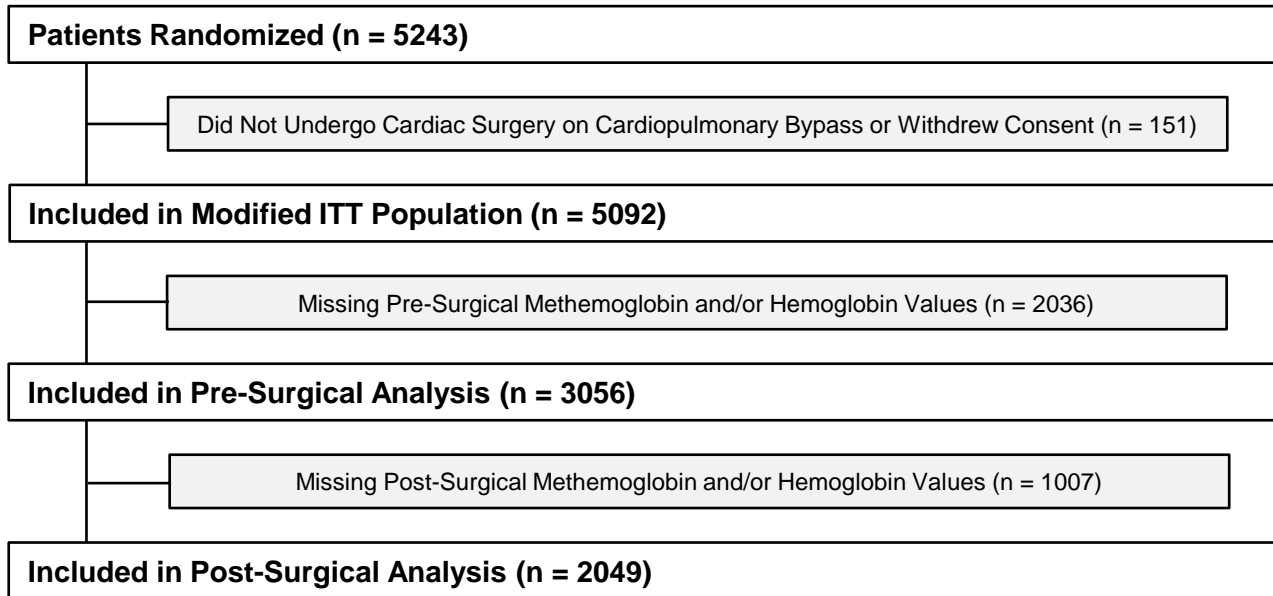


Figure S2: Distribution of methemoglobin concentrations at the pre-surgical and post-surgical timepoints, related to Table 1

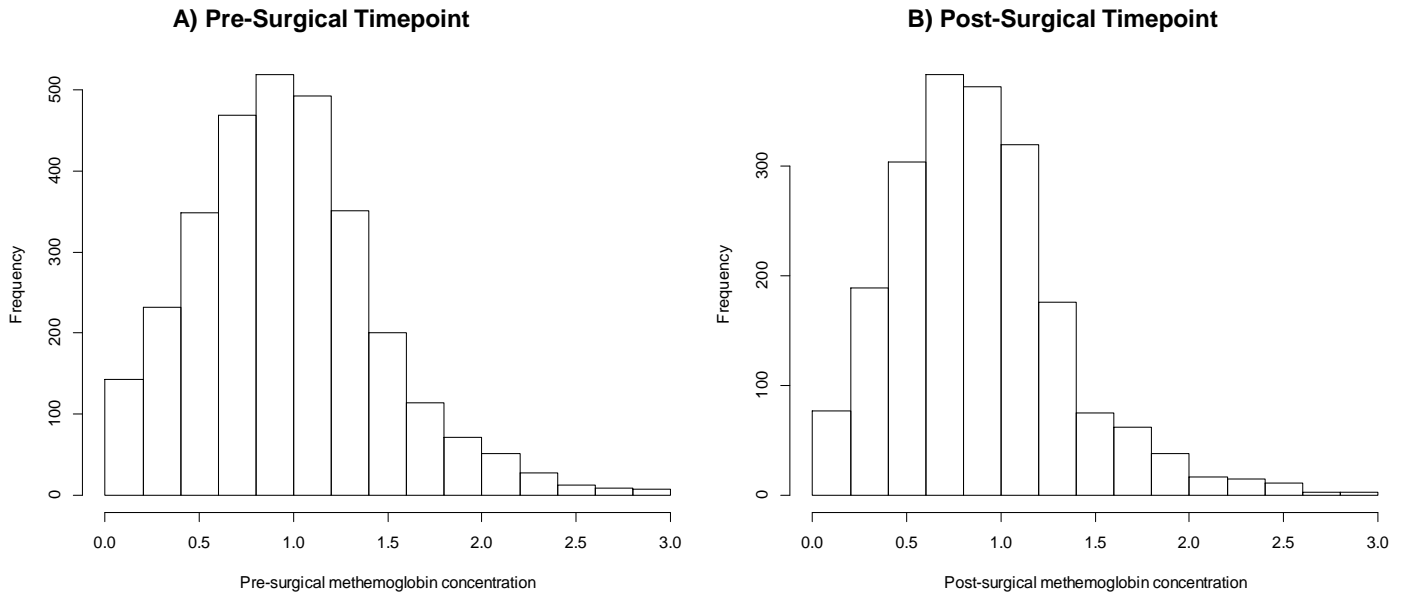


Figure S3: Scatterplots and correlations between hemoglobin versus methemoglobin before and after surgery, related to Figure 1. [Panel A]; post-surgical hemoglobin versus post-surgical methemoglobin [Panel B]; and Delta (difference between pre-surgical and post-surgical) hemoglobin versus post-surgical methemoglobin [Panel C] in the whole cohort. The plotted lines of best fit and 95% confidence intervals were estimated using restricted cubic splines.

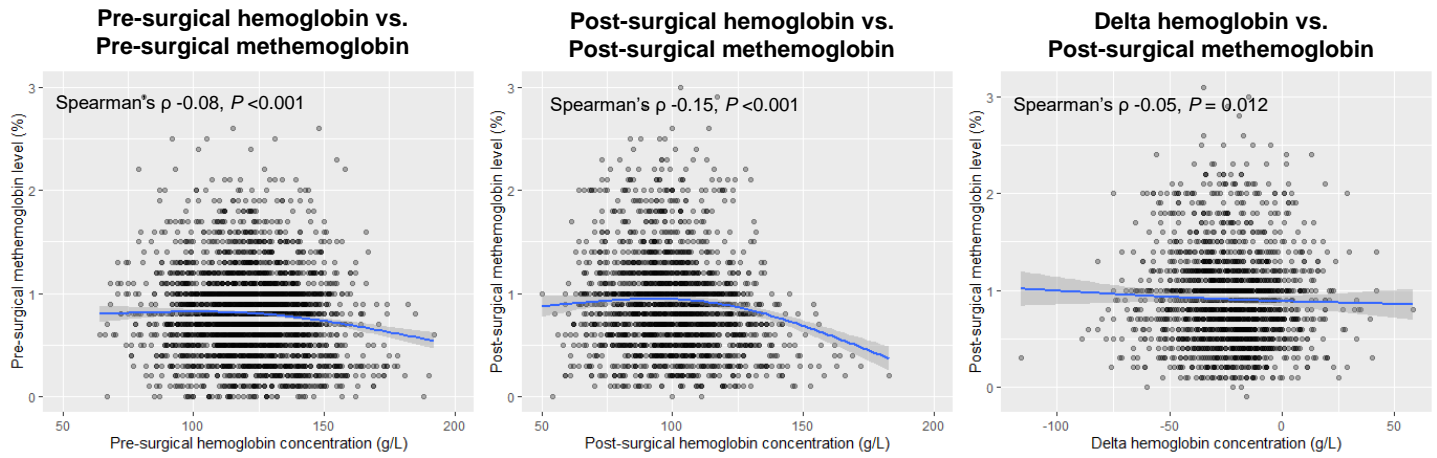


Figure S4: Adjusted association between hemoglobin and methemoglobin before and after surgery, subgroup analysis, related to Figure 1. The adjusted association between: pre-surgical hemoglobin versus pre-surgical methemoglobin [Panels A]; post-surgical hemoglobin versus post-surgical methemoglobin [Panel B]; and Delta hemoglobin (difference between pre-surgical and post-surgical) versus post-surgical methemoglobin [Panel C] stratified by transfusion. The respective sex subgroup analyses are presented in Panels D, E, and F; and the respective ethnicity subgroup analyses are presented in Panels G, H, and I. The plotted lines of best fit and 95% confidence intervals were estimated using linear regression models that included a methemoglobin-by-subgroup interaction term and adjustment for age, sex, diabetes status, preoperative renal function, preoperative left ventricular function, baseline chronic pulmonary disease, planned surgery, and red blood cell transfusion. There was no evidence of non-linear associations for the delta analysis; therefore, linear associations are presented. P values were obtained from a chi-squared tests adjusted for all interactions in the model. Ethnicity data were not collected in the TRICS-II pilot study.

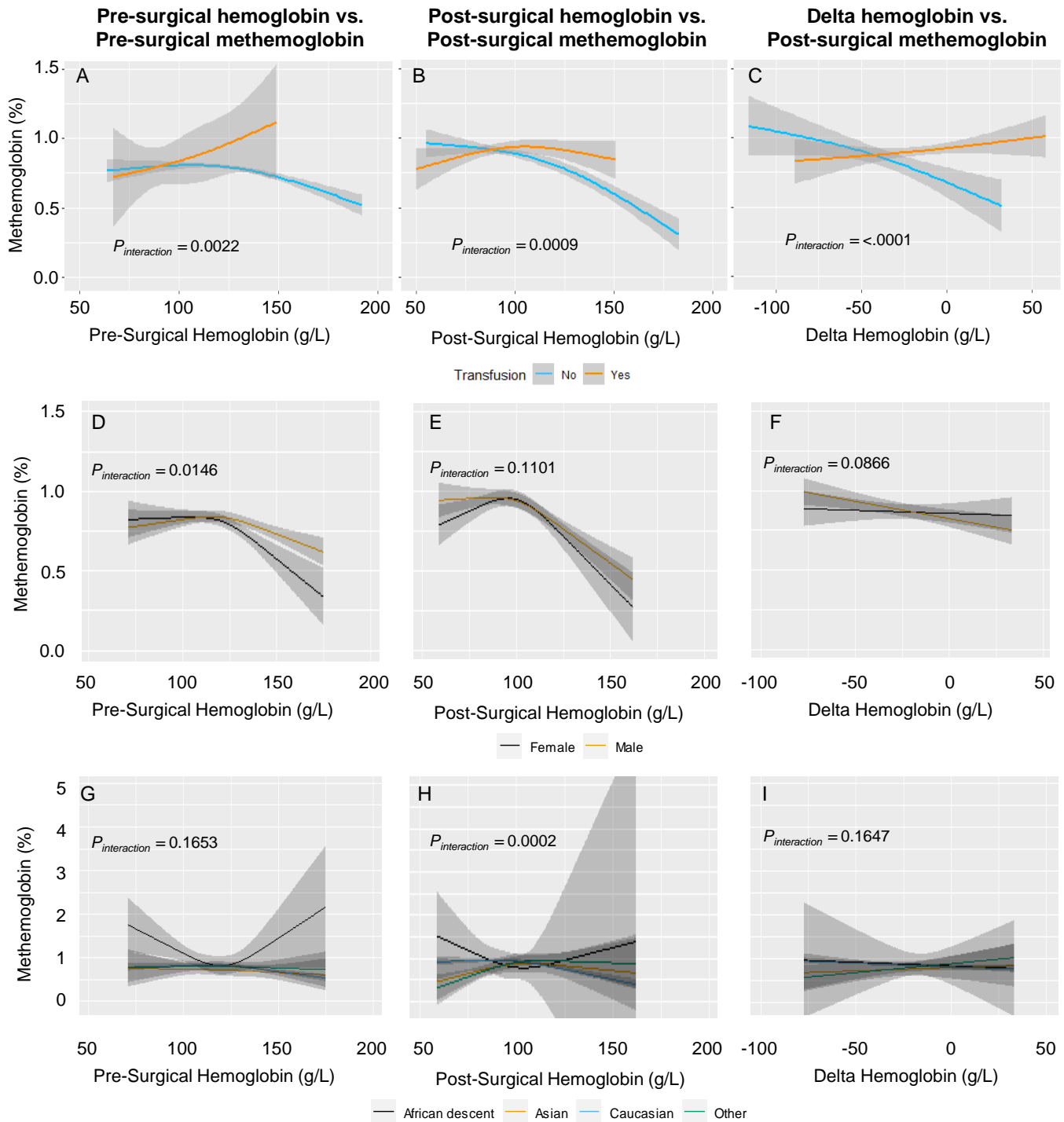


Figure S5: Subgroup analysis of association between post-surgical methemoglobin and the primary composite outcome, related to Figure 2. CI, Confidence interval. Each separate model included a methemoglobin-by-subgroup interaction term and adjustment for age, sex, diabetes status, preoperative renal function, preoperative left ventricular function, baseline chronic pulmonary disease, planned surgery, red blood cell transfusion, and hemoglobin concentration. Ethnicity data were not collected in the TRICS-II pilot study.

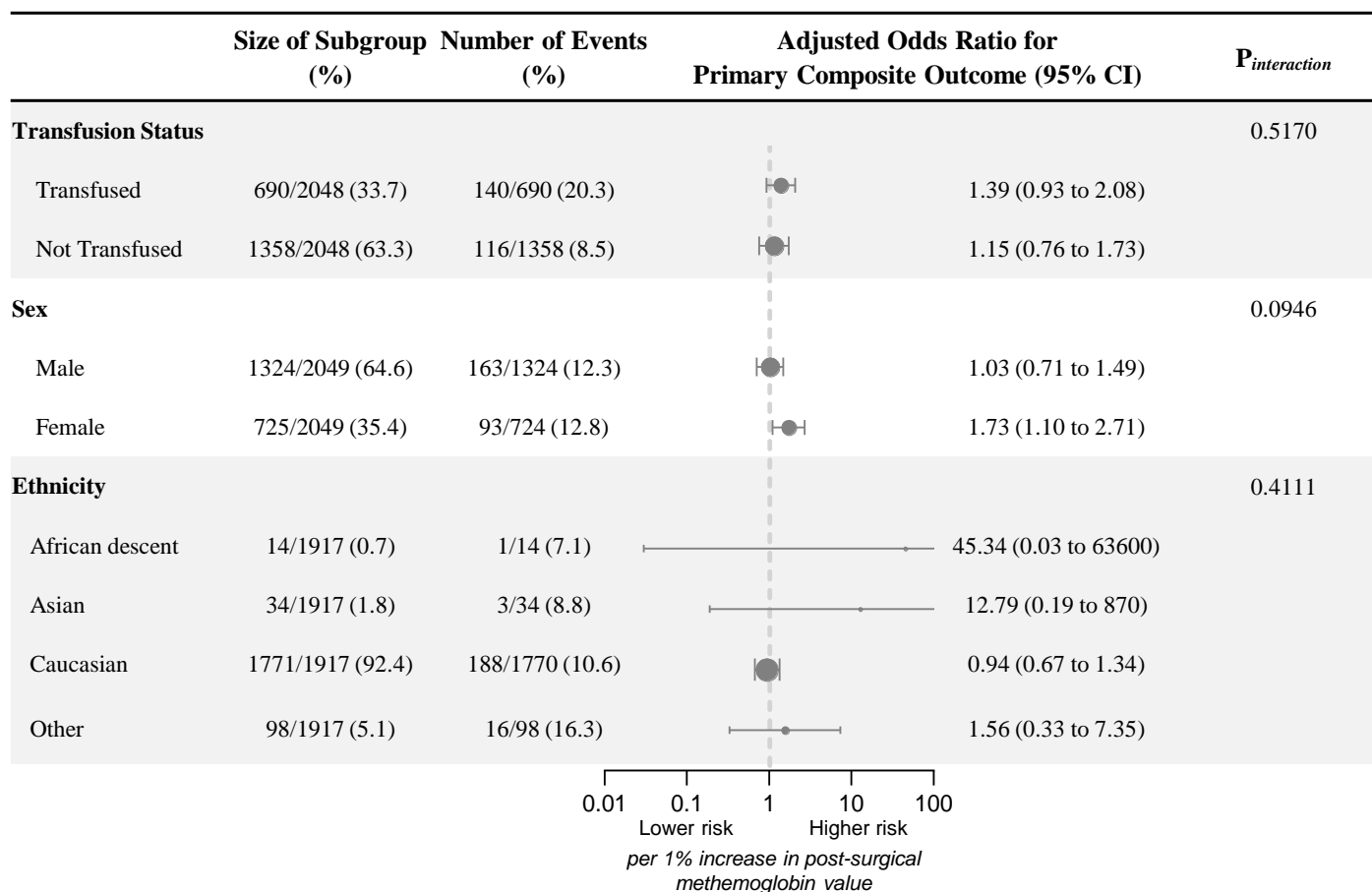
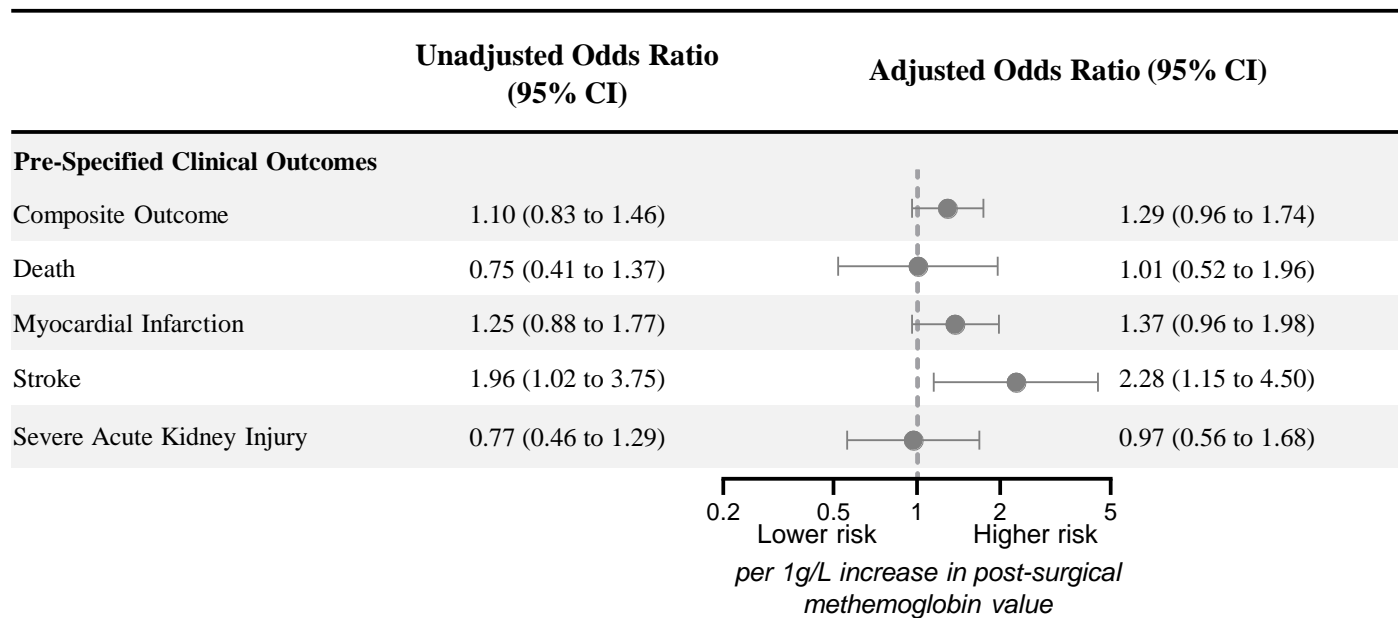


Figure S6: Sensitivity analysis of the association between post-surgical absolute methemoglobin concentration and pre-specified clinical outcomes, related to Figure 2. CI; Confidence interval. Absolute methemoglobin was calculated by taking the product of methemoglobin fraction and hemoglobin concentration. There was no evidence of a non-linear associations for any outcomes; therefore, linear associations are presented. Models adjusted for age, sex, diabetes status, preoperative renal function, preoperative left ventricular function, baseline chronic pulmonary disease, planned surgery, red blood cell transfusion, and hemoglobin concentration.



Data S1: List TRICS investigators, related to STAR-Methods.

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Data S2: Standard definitions used for adjudicating clinical outcomes, related to STAR-Methods.

Death (all causes)

Myocardial infarction as defined according to the task force for the European Society of Cardiology, the American College of Cardiology Foundation, the American Heart Association, and the World Heart Federation as follows:

a) Early perioperative MI (intraoperatively or within 72 hours after surgery): Detection of cardiac troponin (preferred) or CK-MB values more than 10 times the 99th percentile of the upper limit from a normal baseline (i.e. the 99th percentile or less of the upper limit of normal (ULN)) during the first 72 hours following surgery, and at least one of the following: a) new left bundle branch block; b) development of new pathological Q waves on the ECG; c) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; d) identification of a new intracoronary thrombus of the new graft or native coronary artery(ies) by angiography or autopsy. if preoperative cardiac enzymes are elevated, the postoperative enzymes should be increased by more than 10 times the ULN from the baseline value (i.e. greater than baseline plus 10 times the ULN).

b) Late perioperative MI (more than 72 hours after surgery): Detection of a rise and/or fall of cardiac troponin (preferred) or CK-MB values, with at least one value above the ULN, and with at least one of the following: a) symptoms of ischemia, as long as the symptoms/signs are not explained by another proven clinical condition (pulmonary embolism, myocarditis, etc.); b) new, or presumed new, significant ST-T wave changes or new left bundle branch block; c) development of new Q waves on the ECG; d) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; e) identification of a new intracoronary thrombus of the new graft or native coronary artery (ies) by angiography or autopsy.

Percutaneous coronary intervention (PCI) related MI is included in the late MI group, and is arbitrarily defined by elevation of troponin values more than 5 times the 99th percentile of the ULN from a normal baseline, or a rise of troponin values more than 20% if the baseline values are elevated and are stable or falling. In addition, either 1) symptoms suggestive of myocardial ischemia; or 2) new ischemic ECG changes; or 3) angiographic findings consistent with a procedural complication; or 4) imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality are required.

New focal neurological deficit (stroke) lasting more than 24 hours confirmed by clinical assessment and brain imaging.

Severe acute kidney injury (excluding dialysis during CPB), excluding patients who were on dialysis preoperatively.

Seizure: defined as generalized or focal tonic-clonic movements consistent with seizure; or EEG demonstrating epileptiform discharges; or diagnosis of seizures by neurologist or neurosurgeon consultation

Delirium based on one of the following criteria: Confusion Assessment Method (CAM) for the ICU / CAM positive (even on 1 occasion); or Intensive Care Delirium Screening Checklist score > 3; or more than one dose of haloperidol or similar antipsychotic drug; or documented delirium by neurologist or neurosurgeon or psychiatrist consultation

Encephalopathy: defined as unexpected delayed awakening or severely altered mental status (unconscious despite no sedative medication for more than 5 days), or encephalopathy documented by neurologist or neurosurgeon or psychiatrist consultation

Table S1: Characteristics of patients excluded due to missing data, related to Table 1

Characteristic	Patients included in the pre-surgical analysis (N=3056)	Patients excluded in the pre-surgical analysis (N=2036)	SMD	Patients included in the post-surgical analysis (N=2049)	Patients excluded in the post-surgical analysis (N=3043)	SMD
Preoperative Characteristics						
Allocated to Restrictive Strategy	1527/3056 (50.0)	1028/2036 (50.5)	0.01	1028/2049 (50.2)	1527/3043 (50.2)	0.01
Age, years	72.6±9.8	72.0±10.7	0.06	72.4±9.8	72.3±10.4	0.01
Male Sex	1958/3056 (64.1)	1312/2036 (64.4)	0.01	1324/2049 (64.6)	1946/3043 (64.0)	0.01
Ethnicity ^a			0.31			0.28
African descent	33/2902 (1.1)	3/1985 (0.2)		14/1917 (0.7)	22/2970 (0.7)	
Asian	74/2902 (2.5)	183/1985 (9.2)		34/1917 (1.8)	223/2970 (7.5)	
Caucasian	2679/2902 (92.3)	1715/1985 (86.4)		1771/1917 (92.4)	2623/2970 (88.3)	
Other	116/2902 (4.0)	84/1985 (4.2)		98/1917 (5.1)	102/2970 (3.4)	
Body Mass Index, kg/m ²	27.9±5.1	28.1±5.3	0.03	27.9±5.1	28.0±5.3	0.03
EuroSCORE I	7.9±1.9	7.9±1.9	0.01	7.9±1.9	7.9±1.9	0
Previous Cardiac Surgery	364/3056 (11.9)	260/2036 (12.8)	0.03	234/2049 (11.4)	390/3043 (12.8)	0.04
Recent Myocardial Infarction (≤90 days)	708/3056 (23.2)	500/2036 (24.6)	0.03	499/2049 (24.4)	709/3043 (23.3)	0.03
Emergency Surgery	38/3056 (1.2)	36/2036 (1.8)	0.04	29/2049 (1.4)	45/3043 (1.5)	0.01
Left Ventricular Function			0.13			0.06
Good	1944/3053 (63.7)	1200/2036 (58.9)		1266/2048 (61.8)	1878/3041 (61.8)	
Moderate	884/3053 (29.0)	636/2036 (31.2)		622/2048 (30.4)	898/3041 (29.5)	
Poor	187/3053 (6.1)	150/2036 (7.4)		134/2048 (6.5)	203/3041 (6.7)	
Very Poor	38/3053 (1.2)	50/2036 (2.5)		26/2048 (1.3)	62/3041 (2.0)	
Diabetes	823/3056 (26.9)	573/2036 (28.1)	0.03	540/2049 (26.4)	856/3043 (28.1)	0.04
Pulmonary Hypertension	247/3052 (8.1)	189/2036 (9.3)	0.04	170/2045 (8.3)	266/3043 (8.7)	0.02
Renal Function			0.09			0.09
Normal (CC >85ml/min)	1054/2901 (36.3)	725/1983 (36.6)		697/1916 (36.4)	1082/2968 (36.5)	
Moderate (CC >50 and <85)	1338/2901 (46.1)	907/1983 (45.7)		856/1916 (44.7)	1389/2968 (46.8)	
Severe (CC <50)	482/2901 (16.6)	313/1983 (15.8)		344/1916 (18.0)	451/2968 (15.2)	
Dialysis (regardless of CC)	27/2901 (0.9)	38/1983 (1.9)		19/1916 (1.0)	46/2968 (1.5)	
Treated Hypertension	2286/3056 (74.8)	1497/2036 (73.5)	0.03	1545/2049 (75.4)	2238/3043 (73.5)	0.04
Preoperative Aspirin Use	1624/3052 (53.2)	1064/2030 (52.4)	0.02	1085/2049 (53.0)	1603/3033 (52.9)	0.01
Preoperative Anticoagulant Use	732/3056 (24.0)	406/2034 (20.0)	0.10	518/2049 (25.3)	620/3041 (20.4)	0.12
Preoperative Hemoglobin, g/L	131.3±17.2	129.8±18.3	0.08	131.4±17.2	130.2±18.0	0.07
Preoperative RBC Transfusion	61/3056 (2.0)	40/2036 (2.0)	0.01	48/2049 (2.3)	53/3043 (1.7)	0.04
Operative Characteristics						
Planned Surgery			0.12			0.09
CABG Only	752/3054 (24.6)	558/2035 (27.4)		534/2047 (26.1)	776/3042 (25.5)	
CABG and Valve	594/3054 (19.4)	402/2035 (19.8)		369/2047 (18.0)	627/3042 (20.6)	
Other Surgery and CABG	240/3054 (7.9)	196/2035 (9.6)		166/2047 (8.1)	270/3042 (8.9)	
Valve Only	905/3054 (29.6)	569/2035 (28.0)		595/2047 (29.1)	879/3042 (28.9)	
Other Surgery	563/3054 (18.4)	310/2035 (15.2)		383/2047 (18.7)	490/3042 (16.1)	
Duration of cardiopulmonary bypass	122.8±61.0	120.4±58.7	0.04	116.5±54.4	125.4±63.4	0.15
Intraoperative vasoactive medication for ≥1h	2142/3008 (71.2)	1188/2012 (59.0)	0.26	1374/2010 (68.4)	1956/3010 (65.0)	0.07
Clinical Outcomes						
Composite Outcome	375/3054 (12.3)	265/2035 (13.0)	0.022	256/2049 (12.5)	384/3040 (12.6)	0.004
Death	96/3053 (3.1)	96/2035 (4.7)	0.081	56/2048 (2.7)	136/3040 (4.5)	0.093

Myocardial Infarction	200/3054 (6.5)	107/2034 (5.3)	0.055	144/2049 (7.0)	163/3039 (5.4)	0.069
Stroke	46/3054 (1.5)	50/2035 (2.5)	0.068	31/2049 (1.5)	65/3040 (2.1)	0.047
Severe Acute Kidney Injury	104/3054 (3.4)	61/2035 (3.0)	0.023	77/2049 (3.8)	88/3040 (2.9)	0.048
Encephalopathy	38/3054 (1.2)	15/2035 (0.7)	0.051	31/2049 (1.5)	22/3040 (0.7)	0.075
Seizure	51/3054 (1.7)	45/2035 (2.2)	0.039	32/2049 (1.6)	64/3040 (2.1)	0.07
Delirium	367/3054 (12.0)	243/2035 (11.9)	0.002	218/2049 (10.6)	392/3040 (12.9)	0.041
Hospital length of stay, days	10.6±6.1	10.7±6.5	0.007	10.4±6.0	10.8±6.5	0.060

SMD, standard mean difference; CC, creatinine clearance; RBC, red blood cell; CABG, coronary artery bypass graft surgery. Values presented as n (%) or mean ± standard deviation. The median pre-surgical methemoglobin value was 0.8%. ^aEthnicity data were not collected in the TRICS-II pilot study.

Table S2: Sensitivity analysis of the association between hemoglobin and methemoglobin before and after surgery: TRICS-III patients with additional adjustment for ethnicity, related to Figure 1.

	Adjusted Association Per 1% Absolute Increase in Methemoglobin (95% CI)
Pre-Surgical Hb vs Pre-Surgical MetHb	
No anemia (150g/L vs 125g/L)	-0.12 (-0.16 to -0.08)*
Mild anemia (125g/L vs 100g/L)	-0.01 (-0.05 to 0.02)
Moderate anemia (100g/L vs 75g/L)	0.02 (-0.02 to 0.07)
Severe anemia (75g/L vs 50g/L)	0.02 (-0.02 to 0.07)
Post-Surgical Hb vs Post-Surgical MetHb	
No anemia (150g/L vs 125g/L)	-0.23 (-0.28 to -0.17)*
Mild anemia (125g/L vs 100g/L)	-0.18 (-0.23 to -0.14)*
Moderate anemia (100g/L vs 75g/L)	0.02 (-0.03 to 0.07)
Severe anemia (75g/L vs 50g/L)	0.06 (0.00 to 0.12)
Delta Hb vs Post-Surgical MetHb	
per 25g/L increase in Hb (linear)	-0.03 (-0.06 to 0.00)*

*P <0.05. CI, Confidence interval; Hb, hemoglobin concentration; MetHb, methemoglobin. Ethnicity data were not collected in the TRICS-II pilot study. There was no evidence of a non-linear association between delta (difference between pre-surgical and post-surgical) hemoglobin versus post-surgical methemoglobin; therefore, the linear association is presented. Models adjusted for age, sex, diabetes status, preoperative renal function, preoperative left ventricular function, baseline chronic pulmonary disease, planned surgery, and red blood cell transfusion.

Table S3: Sensitivity analysis of the association between post-surgical methemoglobin concentration and pre-specified clinical outcomes: additional adjustment for ethnicity, related to Figure 2.

	Adjusted Odds Ratio Per 1% Absolute Increase in Methemoglobin (95% CI)
Composite Outcome	1.00 (0.72 to 1.40)
Death	0.92 (0.48 to 1.79)
Myocardial Infarction	0.81 (0.50 to 1.29)
Stroke	2.21 (1.09 to 4.51)
Severe Acute Kidney Injury	1.12 (0.66 to 1.90)

CI; Confidence interval. Ethnicity data were not collected in the TRICS-II pilot study. There was no evidence of a non-linear associations for any outcomes; therefore, linear associations are presented. Models adjusted for age, sex, diabetes status, preoperative renal function, preoperative left ventricular function, baseline chronic pulmonary disease, planned surgery, red blood cell transfusion, and hemoglobin concentration.

Table S4: Predictive performance of post-surgical methemoglobin on clinical outcomes, related to Figure 2.

	AUC	<u>Continuous Net Reclassification Index: % (95% CI)</u>		
		Events	Non-events	Overall
<u>Pre-specified Clinical Outcomes</u>				
Composite Outcome				
Baseline model	0.71			
Plus post-surgical methemoglobin	0.71	4.69 (-7.55 to 16.9)	13.58 (8.99 to 18.2)	18.27 (5.2 to 31.3)
Death				
Baseline model	0.84			
Plus post-surgical methemoglobin	0.84	17.86 (-7.91 to 43.63)	-9.05 (-13.43 to -4.68)	8.8 (-17.34 to 34.94)
Myocardial Infarction				
Baseline model	0.65			
Plus post-surgical methemoglobin	0.66	9.72 (-6.53 to 26)	14.47 (10.02 to 18.9)	24.19 (7.34 to 41)
Stroke				
Baseline model	0.71			
Plus post-surgical methemoglobin	0.71	-9.68 (-44.7 to 25.4)	28.7 (24.5 to 32.9)	19.02 (-16.3 to 54.3)
Severe acute kidney injury				
Baseline model	0.79			
Plus post-surgical methemoglobin	0.79	-6.49 (-28.78 to 15.8)	10.98 (6.58 to 15.4)	4.48 (-18.24 to 27.2)
<u>Exploratory Neurological Outcomes</u>				
Encephalopathy				
Baseline model	0.82			
Plus post-surgical methemoglobin	0.86	22.6 (-11.7 to 56.9)	48.5 (44.6 to 52.3)	71 (36.5 to 105.5)
Seizure				
Baseline model	0.79			
Plus post-surgical methemoglobin	0.79	-6.25 (-40.83 to 28.3)	13.76 (9.43 to 18.1)	7.51 (-27.34 to 42.4)
Delirium				
Baseline model	0.64			
Plus post-surgical methemoglobin	0.65	-16.1 (-29.26 to -3)	30.9 (26.493 to 35.2)	14.7 (0.888 to 28.6)

CI, Confidence interval; AUC, Area under the receiver operating characteristic curve. For each outcome, the baseline mode included hemoglobin, age, sex, diabetes status, transfusion status, renal impairment, chronic pulmonary disorder, left ventricular function, and planned surgery.