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A Signature of Pre-Operative Biomarkers of Cellular Senescence to Predict Risk of Cardiac and Kidney Adverse Events after Cardiac Surgery

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Abstract

Importance: Improved pre-operative risk stratification methods are needed for targeted risk mitigation and optimization of care pathways for cardiac patients. This is the first report demonstrating pre-operative, aging-related biomarkers of cellular senescence and immune system function can predict risk of common and serious cardiac surgery-related adverse events.

Design: Multi-center 331-patient cohort study that enrolled patients undergoing coronary artery bypass grafing (CABG) surgery with 30-day follow-up. **Included a quaternary care center and two community-based hospitals.** Primary outcome was KDIGO-defined acute kidney injury (AKI). Secondary outcomes: decline in eGFR ≥25% at 30d and a composite of major adverse cardiac and kidney events at 30d (MACKE30). Biomarkers were assessed in blood samples collected prior to surgery.

Results: A multivariate regression model of six senescence biomarkers (p16, p14, LAG3, CD244, CD28 and suPAR) identified patients at risk for AKI (NPV 86.6%, accuracy 78.6%), decline in eGFR (NPV 93.5%, accuracy 85.2%), and MACKE30 (NPV 91.4%, accuracy 79.9%). Patients in the top risk tertile had 7.8 (3.3-18.4) higher odds of developing AKI, 4.5 (1.6-12.6) higher odds of developing renal decline at 30d follow-up, and 5.7 (2.1-15.6) higher odds of developing MACKE30 versus patients in the bottom tertile. All models remained significant when adjusted for clinical variables.

Conclusions: A network of senescence biomarkers, a fundamental mechanism of aging, can identify patients at risk for adverse kidney and cardiac events when measured pre-operatively. These findings lay the foundation to improve pre-surgical risk assessment with measures that capture heterogeneity of aging, thereby improving clinical outcomes and resource utilization in cardiac surgery.

Keywords: CABG; Acute kidney injury; Cellular senescence; Biological aging; Pre-operative risk assessment; P16

Introduction

Cellular senescence is a well-known aging mechanism linking deleterious subcellular changes with multi-system loss of organ function and physiologic decline. Senescent cells are permanently growth arrested but metabolically active, secreting pro-inflammatory and pro-fibrotic cytokines that contribute to chronic inflammation and impaired tissue regeneration. Senescent cells increase in abundance over time; however, senescent cell load varies dramatically between individuals and may be discordant with expectations

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based on chronological age and multi-morbidities [1,2]. The latter are routinely considered in medical decision making and form the foundation of pre-surgical risk assessment. Measures of senescence may provide a more accurate measure of age-related vulnerability, and pre-operative molecular biomarker measurement could allow for optimization of care pathways across the peri-operative period.

Cardiac surgery has been at the forefront of risk mitigation and outcomes reporting, including quality improvement initiatives such as the Society of Thoracic Surgeons (STS) Database and ERAS Cardiac [3]. However, post-operative morbidity and mortality after coronary artery bypass grafting (CABG), the most common form of cardiac surgery, remain high. Older, more co-morbid patients, increasing procedural costs, and value-based payments are driving strong interest in accurate, pre-operative patient stratification and targeted risk mitigation for cardiac patients. A better understanding of aging-related patient risk remains an unexplored area. In this study we report the use of a network of cellular senescence biomarkers to identify patients at-risk of cardiac surgery-associated adverse events.

Materials and Methods

Study design and participants

In a pilot study, patients (>50 years) undergoing primary elective or urgent, on-pump CABG (+/- valve) were prospectively enrolled at Johns Hopkins and Duke University Hospitals between September 2010 and March 2013 [4,5] and June 2015 and July 2017, respectively. Exlculsion criteria were emergency or salvage CABG, aortic aneurysm or congenital heart disease repair, primary ventricular assist device implantation, preoperative vasopressors or IABP, severe heart failure (LVEF <25%), end stage kidney disease (eGFR <15 mL/min/1.73m^2) or renal transplantation, chronic liver disease or cirrhosis. A total of 46 and 42 participants were enrolled from Duke and Johns Hopkins, respectively. In the GUARD-AKI study, patients (>40 years) undergoing non-emergency cardiac surgery using cardiopulmonary bypass (CABG +/- valve) were prospectively enrolled at WakeMed, Johns Hopkins, and Hoag Memorial Hospitals between October 2020 and July 2022. Exclusion criteria were emergency or salvage CABG, off-pump CABG, aortic aneurysm or congenital heart disease repair, primary ventricular assist device implantation, preoperative vasopressors or IABP, severe heart failure (LVEF <25%), end stage kidney disease (eGFR <30 mL/min/1.73m^2) or renal transplantation, chronic liver disease or cirrhosis. This study was registered with clinicaltrials.gov (NCT03635606) and approved by the IRB of Johns Hopkins University, Duke University, and a central IRB (WIRB/WCG). Power calculations for the GUARD-AKI study were based on the pilot study for a primary endpoint of AKI. The number was predetermined to achieve a 95% confidence interval (CI) width of 0.175% on the area under the curve (AUC) of the receiver operating curve (ROC) given that events to non-events occur in a 1:4 ratio such that: a random sample of 40 subjects from the AKI positive population and 158 subjects from the AKI negative population produce a two-sided 95.0% CI with a width of 0.175 when the sample AUC is 0.86. As such the lower limit of the 95% CI is 0.776 and the upper limit is 0.947. Upon study conduct, it was noted that the event to non-event ratio was 1:5 and hence enrollment was increased to achieve the minimum number of required events.

Outcome definitions

Patients were followed for the duration of their hospital stay and at a 30-day (30d) surgical follow-up. In all cohorts, development of stage 1 or higher postoperative AKI as defined by KDIGO was a primary endpoint (sCr increase of ≥0.3 mg/dL in the first 48h or ≥50% in peak sCr from baseline within 7 days post-surgery). Secondary outcomes: worsening renal function (≥25% eGFR reduction by 30d from baseline) and a composite of major adverse cardiac and kidney events (MACKE30; myocardial infarction, stroke, heart failure, cardiac-related death, and a ≥25% reduction in baseline eGFR). A creatinine-based, race-independent equation (CKD-EPI [6]) was used to calculate eGFR for all participants.

Sample collection and biomarker measurements

Peripheral blood samples were collected prior to surgery. 7.5ml of blood was used to isolate T cells [7] and the remaining sample was used to isolate plasma. Samples were stored at -80^{o}C until analysis. Gene expression of p14, p16, LAG3, CD28, and CD244 was analyzed by real-time qPCR and normalized to housekeeping genes. Positive and negative controls were included in each run, and Cts over 37 were considered below the limit of detection. Gene expression is reported as log2 of arbitrary units, as is standard for qPCR reporting. Expression of suPAR, sTNF-R1, and Activin A in plasma was measured by ELISA (R&D Systems) per manufacturer's instructions. In all analyses, lab personnel were blinded to clinical information and outcome measures.

Statistical analyses

Pilot Study—To build a predictive model for AKI, expression of p16, p14 (and their second- degree interaction), pre-operative sCr, as well as demographic and clinical variables such as gender, diabetes, and surgery type were tested in a multivariate logistic model, with factors chosen by backwards elimination. Akaike information criterion with correction for sample size (AICc) was used to find a model with both good fit to the truth and few parameters.

GUARD-AKI – Descriptive statistics were reported as mean (sd) for continuous variables, and as frequency (%) for categorical variables. Missing data for any reason was not imputed. If an outcome was missing, the subject was excluded from summary statistics and analyses.

Entwistle A, et al., Cardiol Cardiovasc Med 2024
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To build a predictive model for each outcome, a subset of pre-selected factors was tested in a logistic model. These pre-identified factors were evaluated for inclusion using a randomly selected balanced dataset (50%) from GUARD-AKI. Expression of p14, p16, CD28, CD244, LAG3, suPAR (and their 2nd degree interactions), and sCr were evaluated. Using random sampling with replacement ≥200 iterations), logistic models were created using forward selection with model inclusion criteria of p-value ≤0.25 after forced inclusion of p14 and p16. The percentages of inclusion for each of the factors was calculated. Factors with percentage above 4% were considered for model retention and final model parameter estimates were derived using the entire sample. The performance of each model was assessed by ROC analysis. A composite factor was created using each risk model and a distribution of values was generated across the complete sample. Probabilities of predicting each event were also modeled categorically in tertiles, with the lowest tertile serving as a reference group to derive an odds ratio. The models were also adjusted for age, diabetes, CKD and CHF as potential effect modifiers. Optimal thresholds for each model were identified by examining a distribution of fitted probabilities versus classification of the outcome in question. Sensitivity, specificity, PPV, NPV, and accuracy were calculated at a threshold identified for each model. CI for sensitivity, specificity and accuracy are "exact" Clopper-Pearson CI and CI for PPV and NPV are standard logit Cs [8]. Two-tailed p values of less than 0.05 were considered statistically significant. Statistical analyses were performed in SAS version 9.4 and JMP 12.2.0 (SAS Institute, Cary, NC).

Results

Proof of concept of clinical relevance of biomarkers of senescence for risk prediction

To determine if measurements of senescence, measured pre-operatively, can identify patients at risk for adverse events after cardiac surgery, we measured expression of p16 mRNA, a well-established biomarker of cellular senescence [1,2], as well as the related transcript, p14, in peripheral blood T cells in a pilot study of 60 patients who underwent elective cardiac surgery at two centers. Study flow diagram and patient characteristics are shown in eFigure 1 and eTable 1 in the Supplement. Given the small sample size, we focused on the most common adverse event post cardiac surgery, AKI. In this cohort, 30% (18/60) of patients developed in-hospital AKI. A multivariate regression model that included p16, p14, the p16*p14 interaction, and pre-operative sCr could identify patients at risk for AKI with AUC of 0.76, 80% accuracy, and 86% NPV (eFigure 2).

Biomarker network used to characterize cellular senescence

While expression of p16 is a gold-standard measure and marker of established senescent cells [2,9], accumulation of senescent cells depends on both formation of senescent cells and their clearance by the immune system [10,12] (Figure 1A). With age and physiologic stress, there is an increase in the rate of formation of senescent cells, and a decline in immune surveillance capacity, leading to a progressive accumulation of senescent cells [10,11,13]. Thus, measuring biomarkers of immune function in addition to p16 may allow us to better capture potential age-related vulnerability to adverse events, challenges associated with managing inflammatory responses induced by cardiac surgery, and overall capacity to recover. Biomarkers of cellular senescence as well as age-dependent components of the adaptive and innate immune system (Figure 1) were selected for analysis based on studies of donors and patients in multiple clinical settings. Briefly, CD28 and LAG3 are established markers of T cell exhaustion [14,15]. CD244 was first described as an exhaustion marker, has been shown to correlate with age-dependent impairment of T cells [16], and more recently was shown to regulate autophagy [17], a process that may also involve p14 [18]. Finally, suPAR has been shown to be secreted by senescent cells [19,20]; it is an immune-derived pathogenic factor of kidney disease and potentially cardiovascular disease [21,22]. As expected, there is a large degree of association between these markers (Figure 1C) and a general, although weak, positive association with chronological age. CD28 expression declines with age consistent with a negative association with age and senescence. suPAR has the weakest association with senescent biomarkers consistent with the idea that total plasma levels reflect various sources of suPAR.

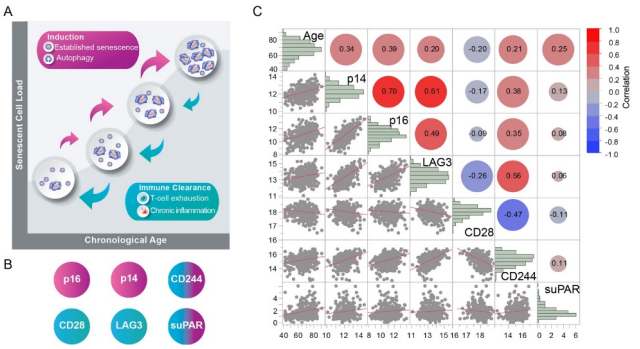

Figure 1: Cellular senescence network. A. Overall cellular senescence load results from two competing biological processes: induction of senescent cells due to cellular stress (magenta arrows) and clearance of senescent cells by the immune system (teal arrows). B. Network of cellular senescence biomarkers used in this study. Biomarkers with a primary known function in establishing senescence are shown in magenta, and those with immune system function are in shown in teal. C. Scatterplot correlation matrix of pre-operative levels of cellular senescence network biomarkers as well as chronological age. The color of each correlation circle represents the correlation between each pair of variables on a scale from red (+1) to blue (-1). The size of each circle represents the significance test between the variables. A larger circle indicates a more significant relationship, and the Pearson correlation coefficient is shown as a number and a line of linear fit on the corresponding scatterplot. The histograms (diagonal across the matrix) show the distribution of each biomarker in the entire cohort.

Entwistle A, et al., Cardiol Cardiovasc Med 2024
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GUARD-AKI study

Building on the pilot findings, a total of 331 participants who underwent cardiac surgery at three centers were enrolled consecutively and used in further analyses (eFigure 3). Baseline characteristics of the patients in the entire cohort and at each site are shown in Table 1 (and extended eTable 2). The average age was 67 ± 10 years, 79% of the participants were male and white. 52.3% of participants had elective surgery, and 87.0% surgeries were isolated CABG.

Table 1: Baseline characteristics and postoperative course for GUARD-AKI study participants.

	Entire Cohort	Site 1	Site 2	Site 3
	n=331	n=160	n=136	n=35
Demographics				
Age				
mean (sd)	67.2 (10)	66.2 (9.3)	69.1 (10.3)	64.8 (10.7)
range	43-91	43-91	43-87	45-84
Male gender, n (%)	262 (79.15)	121 (75.63)	114 (83.82)	27 (77.14)
Race, n (%)				
White	261 (78.85)	133 (83.13)	105 (77.21)	23 (65.71)
Black	28 (8.46)	22 (13.75)	1 (0.74)	5 (14.29)
BMI, mean (sd), kg/m^2	28.9 (5.7)	29.5 (6.3)	28.2 (4.9)	28.9 (4.9)
eGFR, mean (sd), mL/min/1.73m^2	82.9 (21.5)	80.8 (21.8)	86.1 (20.2)	80.4 (23.7)
LVEF, mean (sd), %	53.0 (10.0)	51.3 (9.7)	55.0 (10.1)	53.0 (9.9)
p14, log2, mean (sd)[a]	12.2 (0.7)	12.3 (0.7)	12.1 (0.7)	12.2 (0.6)
p16, log2, mean (sd)[a]	11.1 (1.1)	11.4 (1.0)	10.6 (1.1)	10.9 (1.0)
LAG3, log2, mean (sd)[a]	13.4 (0.8)	13.7 (0.8)	13.3 (0.8)	13.2 (0.7)
CD28, log2, mean (sd)[a]	18.0 (0.4)	17.9 (0.4)	18.0 (0.3)	18.0 (0.4)
CD244, log2, mean (sd)[a]	15.2 (0.8)	15.4 (0.8)	15.0 (0.6)	15.1 (0.8)
suPAR, mean (sd), ng/mL	1.8 (1.0)	1.9 (1.2)	1.5 (0.7)	1.9 (0.8)
Medical history				
Hypertension, n (%)	268 (80.97)	132 (82.5)	104 (76.47)	32 (91.43)
Diabetes, n (%)	140 (42.3)	75 (46.88)	46 (33.82)	19 (54.29)
Congestive heart failure, n (%)	95 (28.7)	67 (41.88)	26 (19.12)	2 (5.71)
Chronic kidney disease, n (%)	58 (17.5)	31 (19.4)	18 (13.2)	9 (25.7)
Atrial fibrillation, n (%)	46 (13.9)	20 (12.5)	23 (16.9)	3 (8.6)
Procedural characteristics				
Urgent surgery, n (%)	158 (47.73)	78 (48.75)	72 (52.94)	8 (22.86)
Isolated CABG procedure, n (%)	288 (87.01)	143 (89.38)	113 (83.09)	32 (91.43)
Post-operative course				
Time to extubation, h, mean (sd)	9.3 (13.1)	9.8 (3.9)	8.3 (19.6)	10.8 (8.5)
New onset Afib, n (%)	101 (30.51)	52 (32.5)	37 (27.21)	12 (34.29)
Acute kidney injury, n (%)				
All stages	66 (19.94)	31 (19.38)	30 (22.06)	5 (14.29)
Stage 2 or above	7 (2.1)	4 (2.5)	2 (1.47)	1 (2.86)
Need for RRT, n (%)	3 (0.91)	2 (1.25)	0 (0)	1 (2.86)
New onset ESRD, n (%)	2 (0.6)	0 (0)	2 (1.47)	0 (0)
LOS ICU, mean (sd), h	46.1 (46.9)	52.8 (39.1)	37.1 (54.7)	50.0 (42.1)
LOS hospital, mean (sd), d	6.9 (6.2)	7.4 (4.6)	6.4 (8.0)	7.0 (3.8)
Hospital death, n (%)	3 (0.91)	2 (1.25)	0 (0)	1 (2.86)
Discharge destination, n (%)				
Home	299 (91.16)	148 (93.67)	121 (88.97)	30 (88.24)
Skilled nursing facility	14 (4.27)	4 (2.53)	9 (6.62)	1 (2.94)
Inpatient rehabilitation	15 (4.57)	6 (3.8)	6 (4.41)	3 (8.82)

[a]arbitrary units, see Methods

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Entwistle A, et al., Cardiol Cardiovasc Med 2024
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Preoperative biomarkers of cellular senescence predict AKI

Stage 1 or higher AKI occurred in 20% of patients postoperatively. To determine if pre-operative biomarkers of cellular senescence can predict the incidence of AKI, we performed regression analyses that included biomarkers of cellular senescence, their pairwise interactions, and serum creatinine (Figure 2A). The resulting multivariate model had an AUC of 0.76. The cut-off for determining patients at risk for AKI was chosen to balance false positives and false negatives (eFigure 4). At a cut-off of 30% probability, our model could identify patients at risk for AKI with 78.6% accuracy, 86.7% specificity, and 86.6% NPV.

Preoperative biomarkers of senescence predict decline in renal function at 30 days

Patient characteristics at a 30d post-surgical follow-up are shown in Table S3. Eleven percent of patients had a decline in kidney function (decline in eGFR ≥25% from baseline). A regression model predicting the incidence of eGFR decline at 30 days had an AUC of 0.73 (Figure 2B). For this model, the cut-off was established to minimize false negatives (eFigure 5). At a cut-off of 18% probability, our model could identify patients at risk for renal function decline with 85.2% accuracy, 89.6% specificity, and 93.5% NPV.

Incidence of AKI is largely non-overlapping with incidence of eGFR decline at 30 days

While these models demonstrated that biomarkers of senescence can predict patients at risk for both AKI and eGFR decline at 30d, model differences included elimination of sCr as a variable in the eGFR decline model. When examined closely, we found that only 21% of patients who had

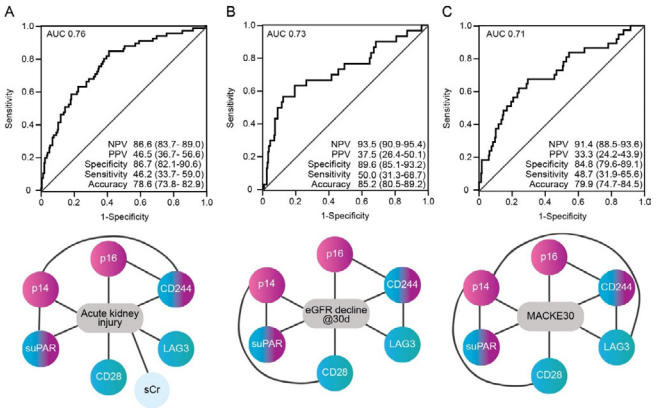

Figure 2: Cellular senescence-based models predict cardiac surgery-associated adverse events. (A) AKI, (B) decline in eGFR between baseline and a 30d post-operative visit, and (C) a composite of major adverse cardiac and kidney events at the 30d post-op (MACKE30). Biomarkers comprising each predictive model are shown. Interactions between biomarkers that are included in the predictive models are shown by connecting lines. The performance of each model was assessed by ROC analysis and performance metrics for each model at a selected threshold are shown (value and 95% CI).

in-hospital AKI also had a decline in eGFR at 30 days. And vice versa, the majority (63%) of patients who had decline in eGFR did not have AKI during their hospital stay. To better understand this phenomenon, we performed a post-hoc analysis comparing patient characteristics, multi-morbidities, and surgical factors across the AKI and eGFR decline endpoints. As expected, AKI was associated with known AKI risk factors such as age, CKD, PVD, need for blood products post-operatively, as well as biomarkers of kidney inflammation (Activin A, sTNF-R1, and suPAR) (Table 2). Surprisingly, none of these risk factors for AKI were associated with the incidence of eGFR decline. Moreover, while pre-operative CKD was associated with AKI, it was not associated with post-operative eGFR decline and 80% of patients showing this eGFR decline did not have pre-existing CKD. Thus, for most patients with eGFR decline at 30 days after surgery, this adverse event may represent a new loss of kidney function, and not AKI-to-CKD progression. Incidence of new onset post-surgical atrial fibrillation (NOAF) was associated with the incidence of eGFR decline at 30 days, and not incidence of AKI. And while not statistically significant, patients with pre-operative congestive heart failure were enriched in the group with eGFR decline as compared to the group with no eGFR decline (37% vs. 28%). Together, these results suggest different etiologies for AKI versus eGFR decline at 30d.

Preoperative biomarkers of senescence predict a composite of cardiac and kidney events at 30 days

Finally, a composite of cardiac and kidney adverse events at 30 days post-surgery (MACKE30) was tested as an outcome. A MACKE30 event occurred in 13.4% of patients. A multivariate regression model to predict MACKE30 had AUC of 0.71 (Figure 2C). Similar to the model for eGFR decline, the cut point was established to minimize false negatives (eFigure 6). At a cut-off of 19% probability, our model could identify patients at risk for MACKE30 with 79.9% accuracy, 84.8% specificity, and 91.4% NPV.

Models based on senescence biomarkers are not improved by patient characteristics

To further define the value of a biomarker-based model for risk prediction, we calculated the odds ratio of developing an outcome based on the biomarker model alone versus the biomarker model adjusted for demographics and multi-morbidities that are commonly used in patient care. Patients in the top tertile of the senescence biomarker-based model of AKI had 7.8 (95%CI 3.3-18.4, p=0.0001) higher odds of developing AKI than patients in the bottom tertile (Figure 3). Adjustment for clinical variables decreased the odds to 5.5 (95%CI 2.2-13.7), but remained highly statistically significant (p=0.0003). Similarly, odds of decline in eGFR and MACKE30 remained statistically significant after the adjustment. These data suggest that biomarkers of cellular senescence are identifying patients at risk for cardiac surgery-associated adverse events independently of clinical factors.

Entwistle A, et al., Cardiol Cardiovasc Med 2024
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Table 2: Patient and surgical characteristics that are associated with risk of AKI are not associated with risk of eGFR decline at 30d. Only patients with ascertained outcomes for both AKI and 30d eGFR decline were included in the analysis.

	no AKI	AKI	p	no eGFR decline	eGFR decline	p
	n=220	n=53		n=243	n=30	
Demographic and clinical variables						
Age, mean (sd)	67 (10)	71 (9)	**0.004**	67 (10)	70 (9)	0.13
Male, n (%)	174 (79)	40 (75)	0.57	193 (79)	21 (70)	0.24
Pre-op eGFR, mean (sd)	85 (20)	72 (24)	**<0.0001**	83 (21)	83 (20)	0.9
CKD, n (%)	28 (13)	20 (38)	**<0.0001**	42 (17)	6 (20)	0.71
Hypertension, n (%)	171 (78)	47 (89)	0.07	194 (80)	24 (80)	0.98
CHF, n (%)	64 (29)	16 (30)	0.87	69 (28)	11 (37)	0.35
Atrial fibrillation	33 (12)	13 (20)	0.13	36 (15)	4 (13)	NA[a]
MI, n (%)	71 (32)	23 (43)	0.13	83 (34)	11 (37)	0.78
PVD, n (%)	8 (94)	7 (83)	**0.02**	13 (5)	2 (7)	NA[a]
Diabetes, n (%)	89 (40)	25 (47)	0.37	106 (44)	8 (27)	0.08
Insulin-depend diabetes, n (%)	33 (15)	12 (23)	0.18	40 (16)	5 (17)	0.98
CABG + valve surgery, n (%)	25 (11)	11 (21)	0.07	31 (13)	5 (17)	0.55
Use of post-op blood products	39 (18)	19 (36)	**0.004**	51 (21)	7 (23)	0.77
Post-op NOAF, n (%)	59 (27)	21 (40)	0.07	60 (25)	20 (67)	**<0.0001**
Pre-op kidney biomarkers						
Activin A, pg/ml, mean (sd)[b]	185 (138)	252.9 (210.6)	**0.02**	205 (174)	182 (80)	0.69
sTNF-R1, pg/ml, mean (sd)[c]	1360 (775)	1849(1019)	**0.004**	1462 (832)	1547 (642)	0.14
suPAR, ng/ml, mean (sd)[d]	1.7 (0.9)	2.4 (1.3)	**<0.0001**	1.8 (1.0)	2.0 (1.1)	0.51

[a] less than 5 events per group
[b] 193 samples with an AKI endpoint and 155 samples with a 30d eGFR decline endpoint were used for Activin A testing
[c] 165 samples with an AKI endpoint and 128 samples with a 30d eGFR decline endpoint were used for TNFR1 testing
[d] 329 samples with an AKI endpoint and 271 samples with a 30d eGFR decline endpoint were used for suPAR testing

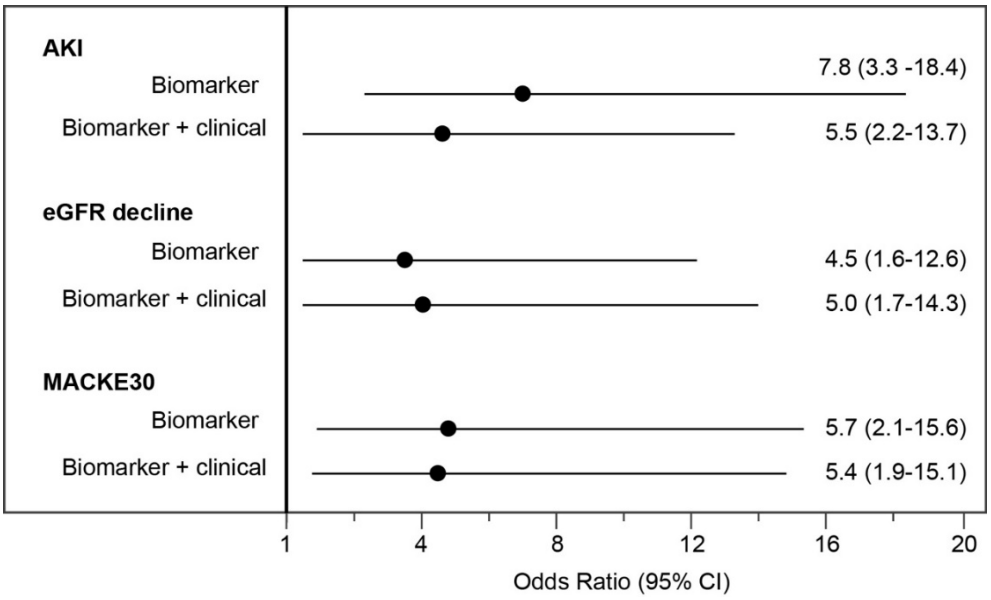

Figure 3: Predictive ability of senescence-based models is unchanged after adjustment for clinical variables. Odds ratios, with 95% CI, are plotted for cellular senescence-based models (Biomarker) for AKI, 30d decline in eGFR, and MACKE30. Each model was also adjusted for age, CKD, diabetes, and CHF (Biomarker + clinical).

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Discussion

This is the first report of using pre-operative, aging-related biomarkers of cellular senescence and immune system function to predict risk of common and serious cardiac surgery-related adverse events. Biomarkers of aging represent a significant conceptual departure from other measures of patient risk that, historically, have been disease or organ-specific, and thereby fail to capture the multi-system vulnerability associated with aging. While senescent cells accumulate over time, senescence measures vary drastically between same-aged individuals. The data presented here support the assertion that senescent cell load is a key, multi-system risk factor for adverse events after cardiac surgery. Senescence-based risk alone appears sufficient to stratify patients by risk of AKI, eGFR decline at 30 days, and MACKE30 and remains a significant predictor after adjustement for clinical and demographic variables. Given the interdependent and perpetuating nature of kidney and cardiac function, there has been considerable effort in the past to predict risk of AKI. The only available molecular diagnostic, NephroCheck, uses kidney-specific biomarkers to identify injury after it occurs, eliminating the opportunity for prevention and the earliest intervention [23]. Predictive algorithms based on clinical variables alone have focused on only the most severe outcomes [24], or utilize intra-operative and post-operative variables that preclude use in everyday practice. In contrast, the biomarkers used in this study are measured pre-operatively and results can be integrated into clinical decision making throughout the peri-operative period. Further, cellular senescence biomarkers are not kidney-specific, but rather capture overall organismal aging as well as immune system status. The multi-system nature of aging vulnerability captured here underlies the success of this study.

We also observed that there is only 21% overlap between the patients who experienced AKI and those with a ≥25% decline in eGFR at 30 days, suggesting different etiologies. New post-operative eGFR decline, in the absence of prior renal impairment (AKI or CKD), may instead associate more closely with cardiac dysfunction, since the population is enriched with those experiencing NOAF, and potentially congestive heart failure [25,26]. Early, pre-operative identification of risk through senescence biomarker testing both reveals unexpected risk and allows for careful targeting of peri-operative interventions, such as the recently proposed order set for preventing AKI [27]. These interventions include precise goal-directed fluid management, adjusted hemodynamic monitoring and nursing parameters, reduced nephrotoxin exposure, and tailored metabolic management. In addition, patients with low-risk biomarker status could be identified as candidates for an accelerated recovery plan, with potential for earlier removal of monitors, intravascular lines, and bladder catheters, earlier transfer to sub-acute care, more opioid-sparing analgesic approaches, and earlier hospital discharge. The predictive models in this study were designed with high specificity and negative predictive values to prioritize true negatives, i.e., higher confidence in "fast track" recovery status, at the cost of more false positives, i.e., utilization of a prevention focused order set in some patients who are not at-risk. This approach is well-aligned with the rapidly expanding ERAS movement which has demonstrated that multi-modal, peri-operative interventions can improve patient outcomes, reduce costs, meet quality goals, and improve patient and staff satisfaction [3, 28]. Beyond the discovery nature of this study, other limitations include enrollment during the pandemic and limited number clinical sites, although these represent a geographically and socio-economically diverse "real-world" patient cohort. Future studies to validate these predictive models and demonstrate clinical utility in everyday surgical practice need to be conducted.

Conclusion

In summary, this is the first report utilizing senescence biomarkers for risk prediction to pre-operatively identify cardiac surgery patients at-risk of adverse events. These findings lay the foundation for future studies that can bring molecular aging to pre-surgical risk assessment, improving both clinical outcomes and resource utilization in cardiac surgery.

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Conflict of Interest

NM is a co-founder of Sapere Bio. AE, NM, SLS and AK hold equity in the company and are inventors on intellectual property applications.

Data Availability

Due to the informed consent and data privacy policies, the clinical data are not publicly available, i.e., accessible for anyone, for any purpose without a review by the Central IRB on a project-by-project basis. Requests for raw data can be made to the corresponding author.

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