Citation: Clin Transl Sci (2021) 14, 354–361; doi:10.1111/cts.12886

ARTICLE

Performance of Plasma Adenosine as a Biomarker for Predicting Cardiovascular Risk

Trevor Simard[1,2,3,†], Richard G. Jung[1,2,†], Pietro Di Santo[1], F. Daniel Ramirez[1,4,5], Alisha Labinaz[1], Chantal Gaudet[1], Pouya Motazedian[1,6], Simon Parlow[1], Joanne Joseph[1], Robert Moreland[1], Jeffrey Marbach[1], Paul Boland[1], Steven Promislow[1], Juan J. Russo[1], Aun-Yeong Chong[1], Derek So[1], Michael Froeschl[1], Michel Le May[1] and Benjamin Hibbert[1,2,*]

Adenosine boasts promising preclinical and clinical data supporting a vital role in modulating vascular homeostasis. Its widespread use as a diagnostic and therapeutic agent have been limited by its short half-life and complex biology, though adenosine-modulators have shown promise in improving vascular healing. Moreover, circulating adenosine has shown promise in predicting cardiovascular (CV) events. We sought to delineate whether circulating plasma adenosine levels predict CV events in patients undergoing invasive assessment for coronary artery disease. Patients undergoing invasive angiography had clinical data prospectively recorded in the Cardiovascular and Percutaneous CIInical TriALs (CAPITAL) revascularization registry and blood samples collected in the CAPITAL Biobank from which adenosine levels were quantified. Tertile-based analysis was used to assess prediction of major adverse cardiovascular events (MACE; composite of death, myocardial infarction, unplanned revascularization, and cerebrovascular accident). Secondary analyses included MACE subgroups, clinical subgroups and adenosine levels. There were 1,815 patients undergoing angiography who had blood collected with adenosine quantified in 1,323. Of those quantified, 51.0% were revascularized and 7.3% experienced MACE in 12 months of follow-up. Tertile-based analysis failed to demonstrate any stratification of MACE rates (log rank, *P* = 0.83), when comparing low-to-middle (hazard ratio (HR) 1.10, 95% confidence interval (CI) 0.68–1.78, *P* = 0.70) or low-to-high adenosine tertiles (HR 0.95, 95% CI 0.56–1.57, *P* = 0.84). In adjusted analysis, adenosine similarly failed to predict MACE. Finally, adenosine did not predict outcomes in patients with acute coronary syndrome nor in those revascularized or treated medically. Plasma adenosine levels do not predict subsequent CV outcomes or aid in patient risk stratification.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Adenosine demonstrates promising preclinical and clinical data supporting its role as a vasculoprotective agent with limited data suggesting it may serve as a marker of clinical outcomes.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ We sought to definitively establish the role of circulating adenosine as a predictor of cardiovascular (CV) events in patients undergoing invasive assessment for coronary artery disease.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Systemic adenosine levels do not predict CV outcomes or aid in CV risk stratification.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Although risk stratification using systemic adenosine levels are not clinically useful, this does not preclude the potential of tissue-level adenosine differences nor the role of adenosine modulation for therapeutic benefit.

Adenosine serves as a crucial regulatory molecule in both intracellular and extracellular processes.[1] Adenosine homeostasis is closely regulated by an ongoing balance of production, degradation, and transport.[2,3] Adenosine signaling is complex with four primary adenosine receptors (1, 2A, 2B, and 3) facilitating extracellular signaling. These receptors are found on a multitude of vascular cells with differing responses based upon the cell and receptor

[†]Equally contributing authors.

[1]CAPITAL Research Group, Division of Cardiology, University of Ottawa Heart Institute, Ottawa, Ontario, Canada; [2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada; [3]Division of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota, USA; [4]Hôpital Cardiologique du Haut-Lévêque, CHU Bordeaux, Bordeaux-Pessac, France; [5]L'Institut de Rythmologie et Modélisation Cardiaque (LIRYC), Université de Bordeaux, Bordeaux-Pessac, France; [6]Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada. *Correspondence: Benjamin Hibbert (bhibbert@ottawaheart.ca)
Received: May 20, 2020; accepted: July 23, 2020. doi:10.1111/cts.12886

subtypes. Accordingly, adenosine signaling has been implicated in vascular homeostasis with considerable preclinical data supporting its role in vasculoprotection.[1]

Therapeutically, adenosine is limited by its relative instability and short half-life. As a hyperemic agent, it has found limited use in coronary flow assessment,[4] although dipyridamole is more commonly used to augment adenosine levels given its improved stability.[5] Dedicated molecules specifically targeting adenosine receptors of interest are also in use to further optimize the intended vs. unintended effects of adenosine modulation.[6] Indeed, adjunctive agents that augment adenosine levels have demonstrated promising results. Dipyridamole, augmenting adenosine through equilibrative nucleoside transporter-1 (ENT-1) inhibition, has demonstrated improved vascular patency and reduced restenosis in preclinical and clinical studies.[1] Similarly, cilostazol modestly augments adenosine levels in addition to cyclic adenosine monophosphate,[7] yielding reduced restenosis following coronary stenting.[8] Last, ticagrelor, a $P2Y_{12}$ receptor inhibitor, demonstrates improved clinical outcomes aside from its antiplatelet effects proposed to be related to pleiotropic effects via augmentation of adenosine[9] (via ENT-1 inhibition)[10] and possibly cyclic adenosine monophosphate.[11] However, the precise impact of ticagrelor on circulating adenosine levels in clinical trials remains the subject of debate.[12,13] Taken together, preclinical and clinical data supports that adenosine augmentation may portend vasculoprotective effects and thereby improve cardiovascular (CV) outcomes.

Diagnostically, circulating adenosine levels have not been widely adopted owing in part to the technical challenges with its quantification and notable biological variability.[14] Despite this, small series have suggested a role for adenosine levels in risk stratifying CV outcomes. Subjects with coronary artery disease (CAD) and genetically reduced adenosine deaminase (AMPD1) activity, theoretically increasing adenosine levels, have demonstrated improved CV survival.[15] Similarly, we previously reported diminished adenosine levels in those with advanced age and obstructive CAD, although no association with established risk factors was noted.[14] However, following revascularization, patients with AMPD1 did not demonstrate improved CV outcomes.[16] This study was limited by its small size, nonconventional revascularization strategies, and lack of difference in adenosine levels between the cohorts—supporting the challenges of utilizing adenosine as a biomarker.[16] Patients post-revascularization are at elevated risk of recurrent CV events,[17] and exogenous adenosine has shown promise in improving vascular healing in preclinical models.[18] Given the uncertainty of the utility of adenosine as a biomarker, we sought to determine whether circulating adenosine levels are predictive of future CV events in patients undergoing invasive assessment for CAD.

METHODS

Adenosine sample collection and quantification

Adenosine samples were collected at the time of invasive angiography as previously described.[14] Briefly, blood samples were collected via the plastic arterial access sheath (Terumo Medical, Somerset, NJ) or, rarely, if required by peripheral venipuncture. Samples were collected in prefilled Greiner BioOne Vacuette tubes containing 2 mL of ice-cold stop solution as reported.[14] Hemolyzed samples were excluded.[19] Samples were centrifuged and stored at −80°C to await processing. Samples were then processed via the Waters Oasis MCX (Mixed-mode, strong Cation-eXchange) columns and transferred for high-performance liquid chromatography analysis on a Waters Alliance E2695 separating module system with sample quantification by Waters 2489 UV/visible detector at 260 nM as previously reported.[14] This methodology underwent thorough development and validation with ongoing quality-of-care metrics to ensure robust adenosine quantification as described.[14]

Population and patient outcomes

The University of Ottawa Heart Institute provides sole revascularization services to over 1.2 million people.[20,21] The Cardiovascular and Percutaneous CIInical TriALs (CAPITAL) revascularization registry is a web-based registry that prospectively indexes data on patients undergoing coronary angiography. Baseline demographics, CV risk factors and definitions have been previously described.[14] Patients undergo follow-up assessment in the year following their angiography where clinical outcomes in the year following revascularization are recorded in the registry. Outcomes were prospectively recorded by individuals blinded to adenosine levels. Myocardial infarction (MI) was recorded according to the universal definition of MI or clinical diagnosis of MI by treating physicians.[22] Repeat unplanned revascularization included patients undergoing repeat coronary intervention not planned following their index procedure and included both target vessel and non-target vessel revascularization. Death included both cardiac and noncardiac death. Stroke included both ischemic or hemorrhagic events as diagnosed by the treating neurologist or on cross-sectional imaging.

The primary outcome of interest was major adverse cardiovascular events (MACE) composed of death, MI, stroke, or unplanned revascularization at 1-year follow-up. Secondary outcomes of interest included individual MACE components and clinical subgroups.

The collection and use of blood samples in the CAPITAL Biobank was approved by the Ottawa Health Science Network Research Ethics Board (Protocol #20160516-01H) and informed consent was obtained from all patients for collection. The outcomes study assessment was approved by Ottawa Health Science Network Research Ethics Board (#20190224-01H and #20180562-01H) to evaluate clinical outcomes following revascularization.

Statistical analysis

Data are reported as mean ± SD, median ± interquartile range, or number and percentage (%) where appropriate. Plasma adenosine levels were compared using Mann–Whitney *U* tests. Plasma adenosine levels were categorized into tertiles (33rd and 67th percentiles) and Kaplan–Meier plots generated to assess event distributions with comparison by log rank tests. Subsequent Cox proportional-hazard models were then used to determine hazard ratios (HRs) and 95% confidence intervals (CIs). Multivariable Cox

proportional-hazards model was also performed incorporating variables known to impact adenosine levels and post-revascularization MACE, including age (years), sex, diabetes mellitus type 2, hypertension, dyslipidemia, CAD, acute coronary syndrome, and revascularization, as documented previously and highlighted in univariate analysis (**Table S1**).[14,23] We estimated an incidence rate of 9.5% and 15.5% (effect size of 60%) between the lowest and highest adenosine tertiles with an $\alpha = 0.05$ and $\beta = 0.20$ which yielded a sample size of 476 per tertile for a total sample of 1,428 subjects. All statistical analyses were completed using SAS version 9.4 (SAS Institute, Cary, NC). All figures were created using GraphPad Prism version 8 (GraphPad Software, La Jolla, CA). The $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

During the study period, 1,815 patients underwent angiography and had blood collected in the CAPITAL Biobank for further analysis with 1-year follow-up completed. Within the collected samples, 1,323 had adenosine levels quantified from the time of index angiography and were included in our analysis. Of the 1,323 patients included, 675 (51.0%) underwent revascularization by either percutaneous coronary intervention (PCI; 70.8%) or coronary artery bypass grafting (CABG; 28.7%) or both (0.4%). Medical therapy was pursued in 648 patients (49.0%) with 64.5% having obstructive CAD and 35.5% have no obstructive CAD at the time of angiography (**Figure 1**). Mean age was 66.8 ± 11.6 years, 29.3% were women, risk factors included 65.2% with hypertension, 61.1% with dyslipidemia, 30.5% with diabetes, 18.1% active smokers, and 14.9% with a family history of CAD. Indications for angiography included 59.7% with acute coronary syndrome (ACS) and 37.7% with stable CAD. Patients had a history of PCI (27.7%), MI (23.3%), CABG (6.5%), peripheral artery disease (6.7%), and cerebrovascular accident (CVA; 6.5%). Medications at the time of angiography included aminosalicylic acid 90.9%), P2Y12 (89.4%), angiotensin-converting enzyme/angiotensin receptor blocker; 53.5%), beta-blocker (59.2%), and statin (80.9%; **Table 1**). Adenosine values were stratified according tertile (i) 0–33rd (low adenosine, $n = 436$) with a cutoff of 693.2 nM; (ii) 34–67th (middle adenosine, $n = 451$) with a cutoff level of 902.9 nM; and (iii) 68–100th (high adenosine, $n = 436$) with a cutoff level of 1141.9 nM with baseline characteristics stratified by tertiles (**Table 1**).

Patient outcomes

Patient outcomes were assessed at 1 year from their revascularization procedure, as previously described.[14] MACE occurred in 7.3% of patients at 1 year. When stratifying outcomes by adenosine tertiles we noted no significant differences in MACE rates between the tertiles (log rank $P = 0.83$). Similarly, no differences in HRs were noted when comparing the low to middle (HR 1.10, 95% CI 0.68–1.78, $P = 0.70$) or low to high adenosine tertiles (HR 0.95, 95% CI 0.56–1.57, $P = 0.84$; **Figure 2a**).

Secondary outcome analysis of the individual MACE components similarly revealed no changes among the subgroups (**Figure 2b–e**): death (log rank $P = 0.95$) and low vs middle (HR 1.02, 95% CI 0.55–1.92, $P = 0.94$) or low to high adenosine tertiles (HR 0.93, 95% CI 0.49–1.77, $P = 0.82$); MI (log rank $P = 0.95$), low vs. middle (HR 1.17, 95% CI 0.36–3.83, $P = 0.80$) or low to high adenosine tertiles (HR 0.98, 95% CI 0.28–3.38, $P = 0.98$); or unplanned revascularization (log rank $P = 0.49$); and low vs. middle (HR 1.01, 95% CI 0.53–1.90, $P = 0.98$) or low vs. high adenosine tertiles (HR 0.69, 95% CI 0.34–1.39, $P = 0.29$). When assessing CVA individually there was a noted difference with CVA stratified by adenosine tertiles (log rank $P = 0.03$), with no differences observed between low vs. middle (HR 4.37, 95% CI 0.95–20.23, $P = 0.059$) or low vs. high adenosine tertiles (HR 0.97, 95% CI 0.14–6.89, $P = 0.98$; **Figure 2c**). Adjusted analyses for age, ACS, diabetes mellitus type 2, sex, CAD, revascularization, hypertension, and dyslipidemia did not change the observed trends with no difference seen in MACE between low vs. middle (HR 1.11, 95% CI 0.68–1.80, $P = 0.68$) or low vs. high adenosine tertiles (HR 1.05, 95% CI 0.63–1.74, $P = 0.85$), nor in unplanned revascularization between low vs. middle (HR 1.06, 95% CI 0.56–2.01, $P = 0.86$) or low vs. high adenosine tertiles (HR 0.70, 95% CI, 0.34–1.42, $P = 0.32$; **Table 2**).

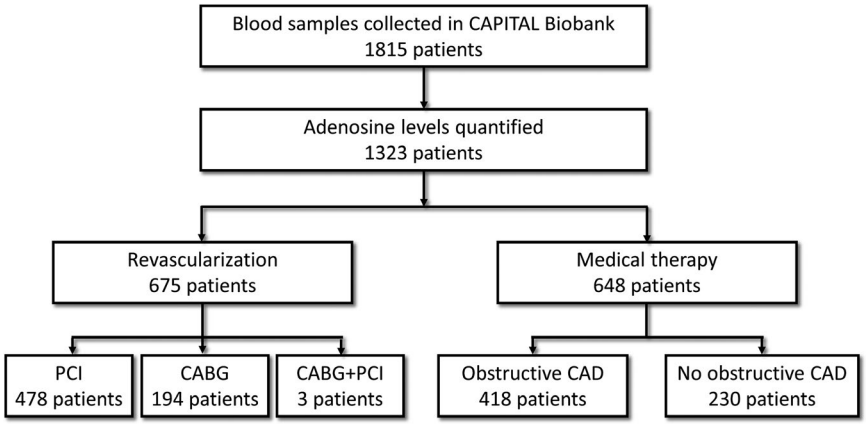

Figure 1 Patient flow diagram. CABG, coronary artery bypass grafting; CAD, coronary artery disease; CAPITAL, Cardiovascular and Percutaneous Intervention Trials; PCI, percutaneous coronary intervention.

Table 1 Baseline characteristics

			Adenosine tertiles					
	Total cohort		**Low**		**Middle**		**High**	
Number	1323		436		451		436	
Age, mean ± SD	66.8	11.6	67.0	11.4	67.3	11.8	66.2	11.6
Sex, female, *n* %	387	29.3	112	25.7	144	31.9	131	30.0
Hypertension, *n* %	863	65.2	287	65.8	281	62.3	295	67.7
Dyslipidemia, *n* %	808	61.1	266	61.0	277	61.4	265	60.8
Diabetes, *n* %								
Type I	8	0.6	4	0.9	1	0.2	3	0.7
Type II	396	29.9	132	30.3	116	25.7	148	33.9
Diet	24	1.8	8	1.8	7	1.6	9	2.1
Oral hypoglycemic agents	266	20.1	85	19.5	82	18.2	99	22.7
Insulin	106	8.0	39	8.9	27	6.0	40	9.2
Smoking, *n* %								
Never	765	57.8	261	59.9	253	56.1	251	57.6
Remote	319	24.1	95	21.8	113	25.1	111	25.5
Active	239	18.1	80	18.3	85	18.8	74	17.0
Family history of CAD, *n* %	197	14.9	71	16.3	56	12.4	70	16.1
Indication for angiography, *n* %								
ACS	790	59.7	176	40.4	176	39.0	181	41.5
Staged PCI	150	11.3	54	12.4	52	11.5	44	10.1
Stable CAD	499	37.7	174	39.9	173	38.4	152	34.9
Atrial fibrillation, *n* %	137	10.4	38	8.7	47	10.4	52	11.9
Medical history, *n* %								
PCI	366	27.7	124	28.4	119	26.4	123	28.2
MI	308	23.3	107	24.5	92	20.4	109	25.0
CABG	86	6.5	26	6.0	28	6.2	32	7.3
PAD	89	6.7	25	5.7	30	6.7	34	7.8
CVA	86	6.5	24	5.5	23	5.1	39	8.9
Bleed	23	1.7	9	2.1	4	0.9	10	2.3
Heart failure	100	7.6	33	7.6	25	5.5	42	9.6
Medications, *n* %								
ASA	1203	90.9	404	92.7	404	89.6	395	90.6
P2Y12	1183	89.4	401	92.0	395	87.6	387	88.8
ACEi/ARB	708	53.5	224	51.4	242	53.7	242	55.5
Beta-blocker	783	59.2	255	58.5	273	60.5	255	58.5
Calcium channel blocker	185	14.0	62	14.2	62	13.7	61	14.0
Statin	1070	80.9	364	83.5	360	79.8	346	79.4
Revascularized, *n* %	675	51.0	238	54.6	214	47.5	223	51.1

ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; ACS, acute coronary syndrome; ASA, acetylsalicylic acid; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CVA, cardiovascular accident; MI, myocardial infarction; PAD, peripheral artery disease; PCI, percutaneous coronary intervention.

Subgroup analyses were performed to further assess three cohorts of patients—demonstrating no significant differences between adenosine tertiles in MACE or unplanned revascularization: (i) medical therapy for obstructive CAD (**Figure S1**)—MACE (log rank $P = 0.16$) and low vs. middle (HR 0.68, 95% CI 0.36–1.26, $P = 0.22$) or low vs. high adenosine tertiles (HR 0.54, 95% CI 0.27–1.06, $P = 0.07$), and unplanned revascularization (log rank $P = 0.77$) and low vs. middle (HR 1.02, 95% CI 0.41–2.58, $P = 0.96$) or low vs. high adenosine tertiles (HR 0.72, 95% CI 0.26–2.03, $P = 0.54$); (ii) revascularization cohort (**Figure S2**)—MACE (log rank $P = 0.16$) and low vs. middle (HR 1.89, 95% CI 0.75–4.74, $P = 0.17$) or low vs. high adenosine tertiles (HR 2.34, 95% CI 0.96–5.68, $P = 0.06$) and unplanned revascularization (log rank $P = 0.86$) and low vs. middle (HR 0.92, 95% CI 0.32–2.08, $P = 0.86$) or low vs. high adenosine tertiles (HR 0.79, 95% CI 0.31–2.00, $P = 0.62$); and (iii) ACS cohort (**Figure S3**)—MACE (log rank $P = 0.32$) and low vs. middle (HR 0.57, 95% CI 0.27–1.21, $P = 0.14$) or low vs. high adenosine tertiles (HR 0.74, 95% CI 0.37–1.50, $P = 0.41$)

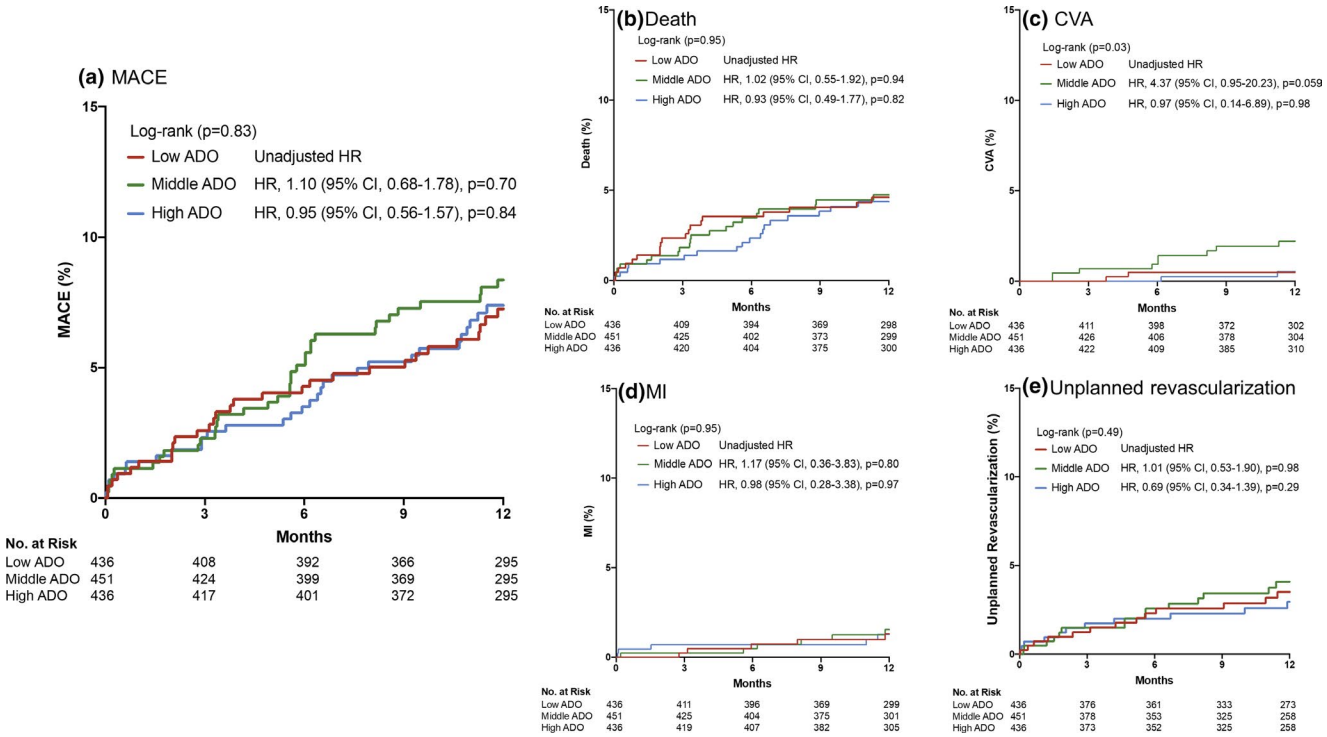

Figure 2 Adenosine levels and cardiovascular outcomes. (**a**) Major adverse cardiac events (MACE) including death, cerebrovascular accident (CVA), myocardial infarction (MI), and unplanned revascularization did not demonstrate any difference between middle or high adenosine tertiles. Similar trends were observed when analyzing by subgroups with no differences between tertiles noted for (**b**) death, (**d**) MI, and (**e**) unplanned revascularization. (**c**) CVA demonstrated higher incidence of stroke in the middle adenosine tertile. Adenosine levels stratified into tertiles (0–33rd, 34–66th, and 67–100th) for analysis. Kaplan–Meier curves generated and compared via log rank with subsequent unadjusted hazard ratios (HRs) compared by Cox proportional hazards model. $P < 0.05$ was defined as statistically significant. ADO, adenosine levels; CI, confidence interval.

and unplanned revascularization (log rank $P = 0.96$) and low vs. middle (HR 1.12, 95% CI 0.45–2.75, $P = 0.81$) or low vs. high adenosine tertiles (HR 1.01, 95% CI 0.40–2.55, $P = 0.98$). Moreover, from a clinical perspective, those who experienced MACE were more likely to be older (72.4 ± 11.5 vs. 66.1 ± 11.4 years, $P < 0.0001$) and have hypertension (73.4% vs. 64.1%, $P = 0.02$), dyslipidemia (72.2% vs. 59.6%, $P = 0.002$), diabetes (46.2% vs 27.7%, $P < 0.0001$), and a history of MI (30.4% vs. 22.3%, $P = 0.02$), CABG (10.8% vs. 5.9%, $P = 0.02$), and peripheral artery disease (11.4% vs. 6.1%, $P = 0.01$). Patients with MACE were less likely to present as stable CAD (29.1% vs. 38.9%, $P = 0.02$) and less likely to undergo revascularization (39.2% vs. 52.6%, $P = 0.002$; **Table S1**).

Finally, we evaluated adenosine values within each outcome cohort with no significant differences noted in any comparison (**Figure 3**): (i) MACE—absent (n = 1,227) vs. present (n = 96, 902.2 nM (621.4–1,292.3 nM) vs. 914.2 585.0–1,324.5 nM), $P = 0.86$); (ii) death—absent (n = 1,266) vs. present (n = 57, 904.1 nM (621.4–1,285.2 nM] vs. 857.0 nM (540.8–1,418.7 nM), $P = 0.68$); (iii) MI—absent (n = 1307) vs. present (n = 16, 902.8 nM (619.9–1,292.3 nM) vs. 838.7 nM (562.8–1,294.9 nM), $P = 0.77$); (iv) CVA—absent (n = 1,310) vs. present (n = 13; 902.2 nM (619.1–1,295.7 nM)

Table 2 Clinical outcomes predicted by plasma adenosine levels

	MACE			Unplanned revascularization		
	Event rates (%)	HR (95% CI)	*P* value	Event rates (%)	HR (95% CI)	*P* value
Low adenosine	7.1	–		4.4	–	
Unadjusted analysis (relative to low adenosine)						
Middle adenosine	7.8	1.10 (0.68–1.78)	0.70	4.2	1.01 (0.53–1.90)	0.98
High adenosine	6.9	0.95 (0.56–1.57)	0.84	3.0	0.69 (0.34–1.39)	0.29
Adjusted analysis (relative to low adenosine)[a]						
Middle adenosine	7.8	1.11 (0.68–1.80)	0.68	4.2	1.06 (0.56–2.01)	0.86
High adenosine	6.9	1.05 (0.63–1.74)	0.85	3.0	0.70 (0.34–1.42)	0.32

CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiac event.
[a]Adjusted for age (years), sex, type 2 diabetes, hypertension, dyslipidemia, coronary artery disease, acute coronary syndrome, and revascularization.

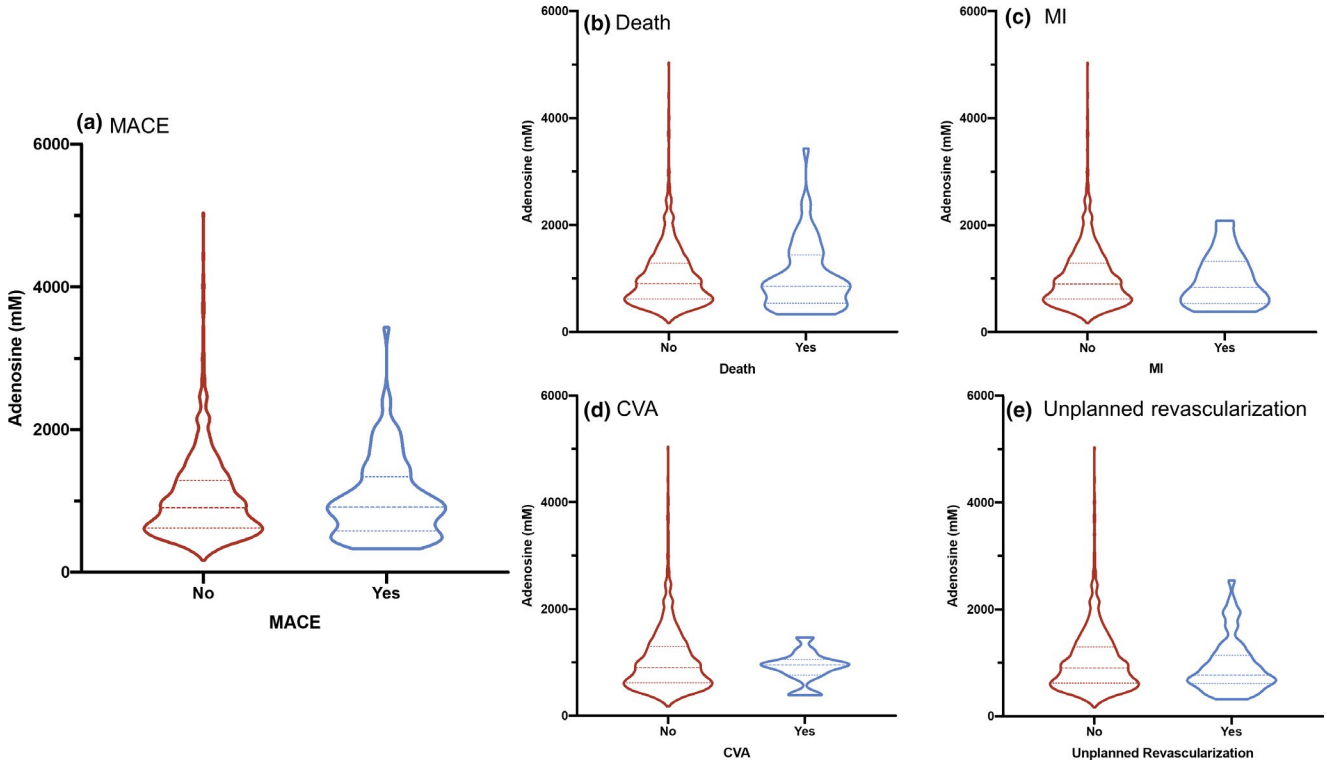

Figure 3 Comparison of adenosine levels across cardiovascular outcome groups. Circulating adenosine values presented according to each cohort with no significant differences noted among: (**a**) MACE—absent (n = 1,165) vs. present (n = 158, 902.8 nM (619.9–1,285.2 nM) vs. 904.0 (616.7–1,295.7 nM), P = 0.99). (**b**) Death—absent (n = 1,255) vs. present (n = 68, 903.3 nM (621.4–1,283.1 nM) vs. 897.8 nM (543.9–1,458.1 nM), P = 0.99). (**c**) MI—absent (n = 1,293) vs. present (n = 30, 902.8 nM (619.9–1,292.3 nM) vs. 907.0 nM (709.2–1,341.0 nM), P = 0.63). (**d**) CVA—absent (n = 1,294) vs. present (n = 29, 897.6 nM (618.3–1,292.3 nM] vs. 970.0 nM (837.0–1,256.7 nM), P = 0.25). (**e**) Unplanned revascularization—absent (n = 1,263) vs. present (n = 60, 905.7 nM (619.6–1,302.2 nM) vs. 789.8 nM (616.0–1,127.2), P = 0.15). Data was presented as median (interquartile range (IQR)) and compared using Mann–Whitney U test. $P < 0.05$ was defined as statistically significant. CVA, cerebrovascular accident; MACE, major adverse cardiac events; MI, myocardial infarction.

vs. 953.2 nM (802.0–1,007.3 nM), P = 0.82); (v) unplanned revascularization—absent (n = 1,272) vs. present (n = 51, 904.3 nM (619.9–1,297.9 nM) vs. 770.7 nM (615.4–1,144.8), P = 0.26).

DISCUSSION

In the current study, we sought to evaluate the ability of plasma adenosine levels to risk stratify patients and predict MACE. Contrary to our hypothesis, adenosine levels were not found to predict MACE rates at 1 year of follow-up, nor did it predict any of the individual components. Multivariable adjustment for factors known to influence outcomes and adenosine levels similarly did not demonstrate a clear role of adenosine for risk stratifying patients at the time of invasive angiography. Hence, this study does not support the use of plasma adenosine in risk stratification of cardiac patients for 1 year events.

Prior to our analysis, adenosine's role as a predictor of CV outcomes was inconclusive. Individuals with genetic mutations (AMPD1) leading to reduced adenosine deaminase activity provide a unique perspective on the role of adenosine in CV outcomes. Elevated adenosine levels have been noted in patients with heart failure (HF) previously, supporting a compensatory role as a counter-regulatory molecule in response to rising catecholamine levels endemic to patients with HF.[24] Therapeutically, dipyridamole-induced adenosine augmentation was suggested to improve HF severity.[25] Moreover, less-adverse outcomes have been noted in patients with HF with AMPD1 mutations; although adenosine levels were not assessed, limiting discernment as to whether circulating or tissue adenosine differences led to the observed changes.[26,27] Indeed, AMPD1 is most active in the skeletal muscle, suggesting tissue levels may be more directly affected.[28] Moreover, when 367 patients with obstructive CAD were stratified by AMPD1 status, they demonstrated improved CV outcomes, although adenosine levels were not quantified.[15] However, 161 patients undergoing revascularization (PCI and CABG) did not demonstrate differential circulating adenosine levels by AMPD1 status, nor did AMPD1 status predict CV outcomes, suggesting alternative pathways for its observed effects.[16] Indeed, these discrepant results reflect an incomplete understanding of adenosine biology, in that the observed outcome effects may actually be driven by modification of local cardiac/vascular adenosine levels with systemic adenosine levels providing a relatively minor role as supported by work in patients with HF.[16,29] Similarly, our findings may suggest that local adenosine levels may be more relevant and predictive of vascular healing. While

direct coronary sampling has demonstrated reduced local adenosine levels post-PCI,[30] the technical barriers of this approach would preclude meaningful adoption as a clinical tool.

Whereas circulating adenosine levels may not predict future events, this does not exclude adenosine as a viable therapeutic target, particularly when considering its tissue-level characteristics. As discussed, numerous agents known to modulate adenosine have shown favorable impacts on vascular healing and cardiovascular outcomes, including dipyridamole,[1] cilostazol,[7,8] and ticagrelor.[9] Although all of these agents have demonstrated favorable impact on clinical outcomes, the mechanism of action may be on local rather than circulating adenosine levels. Dipyridamole is commonly used in i.v. formulation leading to transient peaks in central adenosine levels[31] lessening with repeated stimuli,[32] whereas sustained oral administration up to 5 days demonstrated early changes in circulating adenosine, lessening after 48 hours.[33] However, dipyridamole is known to more potently inhibit ENT-1 than ticagrelor, prolonging detectable levels of adenosine following adenosine administration in the presence of both agents.[34] Although ticagrelor has been shown to augment circulating adenosine in the setting of ACS, other studies have failed to consistently demonstrate this effect.[12,13] Taken together, these findings suggest that although therapeutic modulation of adenosine may represent a viable target, the effect on adenosine levels may occur transiently at a systemic level, with more sustained augmentation locally. Moreover, adenosine receptor levels have been shown to dynamically respond to changes in adenosine levels, reflecting yet another pathway by which vasculoprotective effects are mediated, while not appreciably impacting systemic adenosine levels.[35] Indeed, using systemic adenosine levels as a target diagnostically or therapeutically is challenged by its innate properties, variations between local and systemic levels and the complex homeostatic mechanisms regulating adenosine levels. Hence, although monitoring adenosine levels may not provide prognostication abilities, modulating adenosine biology still carries significant promise as a therapeutic approach and remains the focus of ongoing investigation.

Our study is not without limitations. First, our population is heterogenous with a variety of indications for invasive angiography and differential revascularization strategies, although assessment of these various subgroups similarly failed to demonstrate prognostic effects. Second, our follow-up period was limited to 1 year and we cannot rule out the possibility that adenosine may predict longer term outcomes; however, we are unable to determine this within the confines of the current study. Finally, systemic quantification of adenosine levels is subject to biological and analytical variation, which may impact levels quantified.[14]

CONCLUSION

Systemic adenosine levels are not predictive of CV events at one year in patients undergoing angiography. Although therapeutic targeting of adenosine and associated pathways may yet be beneficial, risk stratification using this molecule is not clinically useful.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Funding. This work was supported by the UOHIAMO AFP Innovations Funding Competition for Innovative Clinical Projects and CFI (Canadian Foundation for Innovation). The Vered-Beanlands Endowed Fellowship (T.S.). The Canadian Institutes of Health Research (Vanier Research Graduate Scholarship (R.G.J.) and Banting Postdoctoral Fellowship (F.D.R.)), the Royal College of Physicians and Surgeons of Canada (Detweiler Travelling Fellowship (F.D.R.)).

Conflicts of Interest. The authors declared no competing interests for this work.

Author Contributions. T.S., R.G.J., and B.H. designed the research and wrote the manuscript. T.S., R.G.J., A.L., C.G., P.M., S.P., J.J., R.M., J.M., P.B., S.P., J.J.R., A.Y.C., D.S., M.F., M.L.M., and B.H. performed the research. T.S., R.G.J., F.D.R., and P.D.S. analyzed the data. T.S. and C.G. contributed new reagents/analytical tools.

1. Simard, T. *et al.* Adenosine as a marker and mediator of cardiovascular homeostasis: a translational perspective. *Cardiovasc. Hematol. Disord. Drug Targets* **19**, 109–131 (2018).
2. Loffler, M., Morote-Garcia, J.C., Eltzschig, S.A., Coe, I.R. & Eltzschig, H.K. Physiological roles of vascular nucleoside transporters. *Arterioscler. Thromb. Vasc. Biol.* **27**, 1004–1013 (2007).
3. Yegutkin, G.G. Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. *Biochim. Biophys. Acta* **1783**, 673–694 (2008).
4. Chen, J.-F., Eltzschig, H.K. & Fredholm, B.B. Adenosine receptors as drug targets—what are the challenges? *Nat. Rev. Drug Discov.* **12**, 265–286 (2013).
5. Kim, H.-H. & Liao, J.K. Translational therapeutics of dipyridamole. *Arterioscler. Thromb. Vasc. Biol.* **28**, s39–s42 (2008).
6. Al Jaroudi, W. & Iskandrian, A.E. Regadenoson: a new myocardial stress agent. *J. Am. Coll. Cardiol.* **54**, 1123–1130 (2009).
7. Liu, Y. *et al.* Inhibition of adenosine uptake and augmentation of ischemia-induced increase of interstitial adenosine by cilostazol, an agent to treat intermittent claudication. *J. Cardiovasc. Pharmacol.* **36**, 351–360 (2000).
8. Douglas, J.S. *et al.* Coronary stent restenosis in patients treated with cilostazol. *Circulation* **112**, 2826–2832 (2005).
9. Nylander, S. & Schulz, R. Effects of P2Y12 receptor antagonists beyond platelet inhibition–comparison of ticagrelor with thienopyridines. *Br. J. Pharmacol.* **173**, 1163–1178 (2016).
10. Van Giezen, J.J.J., Sidaway, J., Glaves, P., Kirk, I. & Björkman, J.A. Ticagrelor inhibits adenosine uptake in vitro and enhances adenosine-mediated hyperemia responses in a canine model. *J. Cardiovasc. Pharmacol. Ther.* **17**, 164–172 (2012).
11. Li, X., Wang, Q., Xue, Y., Chen, J. & Lv, Q. Ticagrelor compared with clopidogrel increased adenosine and cyclic adenosine monophosphate plasma concentration in acute coronary syndrome patients. *Basic Clin. Pharmacol. Toxicol.* **120**, 610–614 (2017).
12. Ariotti, S. *et al.* Effects of ticagrelor, prasugrel, or clopidogrel on endothelial function and other vascular biomarkers: a randomized crossover study. *JACC Cardiovasc. Interv.* **11**, 1576–1586 (2018).
13. Bonello, L. *et al.* Ticagrelor increases adenosine plasma concentration in patients with an acute coronary syndrome. *J. Am. Coll. Cardiol.* **63**, 872–877 (2014).
14. Simard, T. *et al.* Evaluation of plasma adenosine as a marker of cardiovascular risk: analytical and biological considerations. *J. Am. Heart Assoc.* **8**, e012228 (2019).
15. Anderson, J.L. *et al.* A common variant of the AMPD1 gene predicts improved cardiovascular survival in patients with coronary artery disease. *J. Am. Coll. Cardiol.* **36**, 1248–1252 (2000).
16. Andreassi, M.G. *et al.* AMPD1 (C34T) polymorphism and clinical outcomes in patients undergoing myocardial revascularization. *Int. J. Cardiol.* **101**, 191–195 (2005).
17. Madhavan, M.V. *et al.* Stent-related adverse events >1 year after percutaneous coronary intervention. *J. Am. Coll. Cardiol.* **75**, 590–604 (2020).
18. Albayrak, G. *et al.* Inhibitory effect of adenosine on intimal hyperplasia and proliferation of smooth muscle cells in a carotid arterial anastomosis animal model. *Vascular* **23**, 124–131 (2015).

19. Huszar, E.B., Barát, E. & Kollai, M. Isocratic high-performance liquid chromatographic determination of plasma adenosine. *Chromatographia* **42**, 318–322 (1996).
20. Simard, T. *et al.* Impact of center experience on patient radiation exposure during transradial coronary angiography and percutaneous intervention: a patient-level, international, collaborative, multi-center analysis. *J. Am. Heart Assoc.* **5**, e003333 (2016).
21. Le May, M.R. *et al.* A citywide protocol for primary PCI in ST-segment elevation myocardial infarction. *N. Engl. J. Med.* **358**, 231–240 (2008).
22. Thygesen, K. *et al.* Fourth universal definition of myocardial infarction (2018). *Circulation* **138**, e618–e651 (2018).
23. Jung, R.G. *et al.* Evaluation of plasminogen activator inhibitor-1 as a biomarker of unplanned revascularization and major adverse cardiac events in coronary angiography and percutaneous coronary intervention. *Thromb. Res.* **191**, 125–133 (2020).
24. Funaya, H. *et al.* Plasma adenosine levels increase in patients with chronic heart failure. *Circulation* **95**, 1363–1365 (1997).
25. Kitakaze, M. *et al.* Elevation of plasma adenosine levels may attenuate the severity of chronic heart failure. *Cardiovasc. Drugs Ther.* **12**, 307–309 (1998).
26. Feldman, A.M., Wagner, D.R. & McNamara, D.M. AMPD1 gene mutation in congestive heart failure. *New Insights Pathobiol. Dis. Progr.* **99**, 1397–1399 (1999).
27. Loh, E. *et al.* Common variant in AMPD1 gene predicts improved clinical outcome in patients with heart failure. *Circulation* **99**, 1422–1425 (1999).
28. Morisaki, T. *et al.* Molecular basis of AMP deaminase deficiency in skeletal muscle. *Proc. Natl. Acad. Sci.* **89**, 6457–6461 (1992).
29. Kalsi, K. Decreased cardiac activity of AMP deaminase in subjects with the AMPD1 mutation—a potential mechanism of protection in heart failure. *Cardiovasc. Res.* **59**, 678–684 (2003).
30. Guieu, R. *et al.* The use of HPLC to evaluate the variations of blood coronary adenosine levels during percutaneous transluminal angioplasty. *Clin. Chim. Acta* **230**, 63–68 (1994).
31. Hegedus, K., Keresztes, T., Fekete, I. & Molnar, L. Effect of i.v. dipyridamole on cerebral blood flow, blood pressure, plasma adenosine and cAMP levels in rabbits. *J. Neurol. Sci.* **148**, 153–161 (1997).
32. Pasini, F.L. *et al.* Pharmacological preconditioning of ischemic heart disease by low-dose dipyridamole. *Int. J. Cardiol.* **56**, 17–27 (1996).
33. German, D.C., Kredich, N.M. & Bjornsson, T.D. Oral dipyridamole increases plasma adenosine levels in human beings. *Clin. Pharmacol. Ther.* **45**, 80–84 (1989).
34. Nylander, S. *et al.* Ticagrelor inhibits human platelet aggregation via adenosine in addition to P2Y12 antagonism. *J. Thromb. Haemost.* **11**, 1867–1876 (2013).
35. Fenouillet, E. *et al.* Adenosine receptor profiling reveals an association between the presence of spare receptors and cardiovascular disorders. *Int. J. Mol. Sci.* **20**, 5964 (2019).