Associations of temporal cardiometabolic patterns and incident SARS-CoV-2 infection among US blood donors with serologic evidence of vaccination

Elaine A. Yu, Mars Stone, Marjorie D. Bravo, Eduard Grebe, Roberta L. Bruhn, Marion C. Lanteri, Mary Townsend, Hany Kamel, Jefferson Jones, Michael P. Busch, Brian S. Custer

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Supplemental Table 1: Serologic definitions of incident SARS-CoV-2 infection among those with vaccination

Outcome of interest	Antibodies of SARS-CoV-2 protein antibodies		At risk population	Definitions for time at risk (person-days)	
	S *Total Ig*	**N** *Total Ig*		Among participants with SISV [a]	Among participants without SISV
SISV	+/-	+	Participants had serologic evidence of previous vaccination and no SARS-CoV-2 infection at baseline (anti-S + and anti-N -) [b]	Interval definition: Donation date $_{SISV}$ – donation date $_{penultimate\ donation\ prior\ to\ SISV}$	Donation date $_{last}$ – donation date $_{baseline}$

[a] The proportional hazards assumption was not met for two regressions. Multivariable Poisson regressions were used to evaluate these two associations; among people with SISV, the time at risk was defined at the difference between the dates of first donation and first SISV observation.
[b] This interpretation of antibody seropositivity is from the CDC's Interim Guidelines for COVID-19 Antibody Testing; specifically, this interpretation is applicable if vaccine status is not known.

Abbreviations: Centers for Disease Control and Prevention (CDC), coronavirus disease 2019 (COVID-19), nucleocapsid (N), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spike (S), SARS-CoV-2 infection among those with serologic evidence of vaccination (SISV)

Supplemental Table 2: Cardiometabolic indicators and definitions

Cardiometabolic Indicator	Units	Assay/instrument	Cardiometabolic Subgroups		Cut-off value(s)	Notes	Reference(s)
Body mass index	kg/m^2	---	Obese		≥ 30	Self-reported height and weight	World Health Organization [1]
			Overweight		≥ 25 and <30		
			Normal		≥ 18.5 and < 25		
			Underweight		<18.5		
Blood pressure (systolic, diastolic)	mm Hg	Validated automated vital sign instrument or manual sphygmomanometer [b]	Hypertensive	Yes	SBP ≥ 130 or DBP ≥ 80	---	American College of Cardiology / American Heart Association [2]
				No	SBP < 130 and DBP < 80		
Total cholesterol [a]	mg/dL	Beckman Coulter AU [c]	High		≥240 mg/dl	---	Adult Treatment Panel III (National Cholesterol Education Program) [3]
			Borderline high		≥200 to <240 mg/dl		
			Desirable		<200 mg/dl		

[a] Assayed from non-fasting serum sample. Data were missing if the blood donation was not successfully completed.
[b] Measured during blood donation visits
[c] Assayed by trained laboratory staff at Clinical Testing Solutions, Inc

Abbreviations: diastolic blood pressure (DBP), systolic blood pressure (SBP)

Supplemental Table 3: Modeling approach for associations between cardiometabolic health and humoral immune response against SARS-CoV-2

Does the risk of incident SARS-CoV-2 infections differ by temporal cardiometabolic health patterns?				
Definition of at-risk study population	***Dependent variable(s)***	***Independent variable(s)***	***Effect estimates***	***Regression(s)***
Serologic evidence of no previous SARS-CoV-2 infection and vaccination (Anti-N -, anti-S +) at first donation visit in study	Time (days) to incident SISV (anti-N +) or censoring	Baseline values of total cholesterol (mg/dL), BMI (kg/m2),[b] mean systolic and diastolic blood pressure (mm Hg) Temporal patterns of total cholesterol (mg/dL), systolic and diastolic blood pressure (mm Hg) during study follow-up period	aHR (95% CI)	<u>*Survival analysis*</u> [a] $Y\ (\text{time to event [incident SISV or censoring]})_i = \beta_{0\,i} + \beta_1\,(\text{CMD indicator})_i + \beta_2\,(\text{age})_i + \beta_3\,(\text{sex})_i + \beta_4\,(\text{race-ethnicity})_i + \beta_5\,(\text{geographic region})_i + \beta_n\,(\text{other covariates})_i$ where: $_i$ = participant CMD indicator = Baseline, temporal values (continuous [median, peak]) or categorical [temporal pattern subgroups]) of total cholesterol, BMI,[b] blood pressure (systolic, diastolic)

[a] PROC ICPHREG was utilized for proportional hazards regression analysis. In these regressions, covariates (age, sex, geographic region of residence) were based on self-report at the initial study visit.
[b] We only evaluated baseline BMI values since there was low variability across timepoints. During initial evaluation of 62,379 donors (with 344,166 donation), only 444 donors (0.7%) had mean and median BMI values that differed between baseline and other timepoints.

Abbreviations: antibodies specific to SARS-CoV-2 nucleocapsid protein antibodies (anti-N), antibodies specific to SARS-CoV-2 spike protein antibodies (anti-S), body mass index (BMI), cardiometabolic disease (CMD), millimeter of mercury (mm Hg), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV-2 infection among those with serologic evidence of vaccination (SISV)

Supplemental Table 4: Comparison of blood pressure (mm Hg) and total cholesterol (mg/dL), stratified by incident SISV status [a]

Median (IQR)	**Overall** N_{donors} =13,930			**Incident SARS-CoV-2 infection**					
				Y N_{donors} =221			**N** N_{donors} =13,709		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Total cholesterol (mg/dL)	171.0 (148.0, 195.0)	180.0 (157.0, 204.0)	190.0 (166.0, 216.0)	171.0 (148.0, 189.0)	182.5 (160.0, 207.5)	198.0 (174.0, 219.0)	171.0 (148.0, 195.0)	180.0 (157.0, 204.5)	190.0 (165.0, 216.0)
Systolic blood pressure (mm Hg)	119.0 (110.0, 129.0)	126.0 (116.0, 136.0)	132.0 (122.0, 144.0)	121.0 (114.0, 128.0)	128.0 (120.0, 137.5)	136.0 (127.0, 148.0)	119.0 (110.0, 129.0)	125.5 (116.0, 136.0)	132.0 (122.0, 144.0)
Diastolic blood pressure (mm Hg)	72.0 (66.0, 78.0)	76.0 (70.0, 81.5)	80.0 (74.0, 86.0)	73.0 (69.0, 78.0)	78.5 (73.5, 83.0)	82.0 (78.0, 89.0)	72.0 (66.0, 78.0)	76.0 (70.0, 81.5)	80.0 (74.0, 86.0)

[a] All values are median (IQR) of donor summary values (e.g., minimum, median, maximum values of cholesterol or blood pressure across all donations of each individual). Data are reported among 13,930 donors with 39,736 donations.

Supplemental Table 5: Bivariable associations between baseline cardiometabolic indicators and probability of SISV

			Univariable regression [a]		
			HR	**95% CI**	**p** [b]
Total cholesterol	Categorical (%)	High at baseline	1.20	0.74, 1.94	0.47
	Continuous (per 10 units; mg/dL)	Baseline	1.00	0.97, 1.04	0.83
		Peak	1.04	1.01, 1.07	0.02
		Median	1.02	0.99, 1.06	0.20
Blood pressure	Categorical (%)	Hypertension at baseline	1.36	1.04, 1.78	0.02
	Continuous (per 10 units; mm Hg)	Peak systolic (mm Hg)	1.16	1.07, 1.26	<0.01
		Peak diastolic (mm Hg)	1.43	1.23, 1.67	<0.01
Adiposity	Categorical	Obesity at baseline	1.11	0.85, 1.47	0.43
	Continuous (per 10 units; kg/m^2)	BMI at baseline	1.27	1.03, 1.58	0.03
Age (years)			0.98	0.98, 0.99	<0.01
Male (%)			1.46	1.11, 1.91	0.01
Race-ethnicity (%)		White, non-Hispanic	Ref		
		Hispanic	1.08	0.77, 1.51	0.66
		Asian, non-Hispanic	0.28	0.10, 0.74	0.01
		Black, non-Hispanic	0.63	0.20, 1.97	0.43
		Other	0.63	0.20, 1.98	0.43

[a] Consistent was defined based on whether all study timepoints of a participant were categorized in the same subgroup.
[b] P-value was calculated from a chi-square test statistic.

Abbreviations: hazard ratio (HR), millimeters of mercury (mm Hg), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV-2 infection among those with serologic evidence of vaccination (SISV)

Supplemental Table 6: Baseline and temporal patterns of elevated total cholesterol and probability of SISV [a]

			High cholesterol at baseline as key independent variable			Total cholesterol at baseline as key independent variable [b]			Peak cholesterol as key independent variable [b]			Median cholesterol as key independent variable [b]			Temporal pattern subgroup of cholesterol as key independent variable [c]					
															Men			Women		
			Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
			aHR	95% CI	p [d]	aHR	95% CI	p [d]	aHR	95% CI	p [d]	aHR	95% CI	p [d]	aIRR	95% CI	p [d]	aIRR	95% CI	p [d]
Total cholesterol	Continuous (per 10 units; mg/dL)					1.02	0.99, 1.06	0.26	1.05	1.02, 1.08	<0.01	1.05	1.01, 1.09	0.02						
	Categorical	High at baseline	1.35	0.83, 2.18	0.23															
		Consistently desirable (normal)													Ref			Ref		
		Intermittently high [d]													1.90	1.32, 2.74	<0.01	0.73	0.43, 1.25	0.25
		Consistently borderline high													1.11	0.55, 2.21	0.78	1.09	0.58, 2.07	0.78
		Consistently high													1.33	0.42, 4.22	0.63	1.71	0.72, 4.04	0.22
Age (years)			0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01
Male (%)			1.56	1.18, 2.06	<0.01	1.59	1.20, 2.09	<0.01	1.63	1.24, 2.15	<0.01	1.64	1.24, 2.16	<0.01						
Race-ethnicity (%)		White, non-Hispanic	Ref			Ref			Ref			Ref								
		Hispanic	1.29	0.87, 1.92	0.20	1.29	0.87, 1.91	0.21	1.29	0.87, 1.91	0.21	1.28	0.87, 1.90	0.22						
		Asian, non-Hispanic	0.37	0.14, 1.02	0.05	0.37	0.14, 1.01	0.05	0.37	0.13, 1.00	<0.05	0.36	0.13, 0.99	<0.05						
		Black, non-Hispanic	0.49	0.16, 1.59	0.24	0.49	0.16, 1.57	0.23	0.49	0.15, 1.57	0.23	0.49	0.15, 1.55	0.22						
		Other	0.71	0.23, 2.25	0.56	0.71	0.23, 2.23	0.55	0.71	0.22, 2.22	0.55	0.71	0.22, 2.22	0.55						

[a] Models additionally adjusted for geographic region.
[b] Multivariable proportional hazards regression models (ICPHREG procedure in SAS) were fit to evaluate associations with time to event (incident SARS-CoV-2 infection or censoring) as the key outcome of interest among 13,930 donors with 39,736 donations.
[c] Given that the proportional hazard assumption was violated, multivariable Poisson regressions were used to evaluate these associations. Due to small sample cell sizes and model instability, race-ethnicity and geographic region were excluded as covariates in these models.
[d] P value was calculated from the chi-square test statistic.

Abbreviations: adjusted hazard ratio (aHR), adjusted incidence rate ratio (aIRR), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV-2 infection among those with serologic evidence of vaccination (SISV)

Supplemental Table 7: Baseline and temporal patterns of hypertension and probability of SISV [a]

			Hypertension at baseline as key independent variable [b]			Peak systolic blood pressure as key independent variable [b]			Peak diastolic blood pressure as key independent variable [b]			Temporal pattern subgroup of blood pressure as key independent variable [c]		
			Model 7			Model 8			Model 9			Model 10		
			aHR	95% CI	p [d]	aHR	95% CI	p [d]	aHR	95% CI	p [d]	aIRR	95% CI	p [d]
Blood pressure	Continuous (per 10 units; mm Hg)					1.18	1.08, 1.30	<0.01	1.31	1.12, 1.54	<0.01			
	Categorical (%)	Hypertension at baseline	1.23	0.93, 1.63	0.15									
		Consistent normotension										Ref		
		Intermittent hypertension										2.07	1.44, 2.96	<0.01
		Consistent hypertension										1.45	0.87, 2.42	0.16
Age (years)			0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01
Male (%)			1.50	1.14, 1.98	<0.01	1.39	1.05, 1.84	0.02	1.44	1.09, 1.90	0.01	1.43	1.08, 1.89	0.01
Race-ethnicity (%)	White, non-Hispanic		Ref			Ref			Ref			Ref		
	Hispanic		1.28	0.86, 1.89	0.22	1.26	0.85, 1.86	0.25	1.25	0.85, 1.85	0.26	1.27	0.85, 1.88	0.24
	Asian, non-Hispanic		0.37	0.14, 1.01	0.05	0.37	0.14, 1.01	0.05	0.36	0.13, 0.98	0.05	0.37	0.14, 1.02	0.06
	Black, non-Hispanic		0.49	0.15, 1.55	0.22	0.47	0.15, 1.48	0.20	0.46	0.14, 1.46	0.19	0.48	0.15, 1.53	0.22
	Other		0.71	0.22, 2.24	0.56	0.70	0.22, 2.21	0.54	0.69	0.22, 2.17	0.53	0.72	0.23, 2.27	0.58

[a] Models additionally adjusted for geographic region.
[b] Multivariable proportional hazards regression models (ICPHREG procedure in SAS) were fit to evaluate associations with time to event (incident SARS-CoV-2 infection or censoring) as the key outcome of interest among 13,930 donors with 39,736 donations.
[c] Given that the proportional hazard assumption was violated, a multivariable Poisson regression was used to evaluate this association.
[d] P value was calculated from the chi-square test statistic.

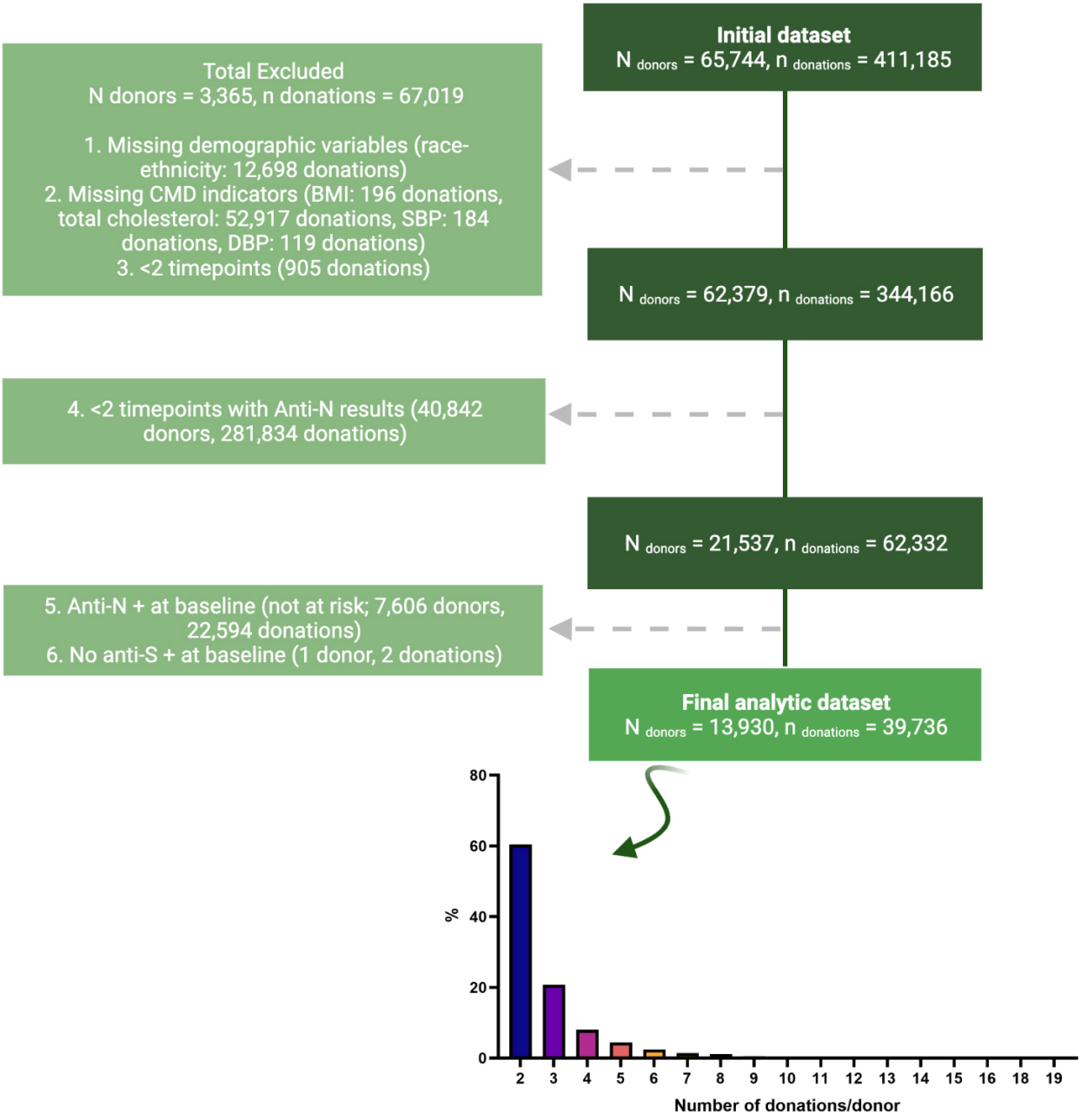
Abbreviations: adjusted hazard ratio (aHR), adjusted incidence rate ratio (aIRR), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV-2 infection among those with serologic evidence of vaccination (SISV)

Supplemental Table 8: Elevated baseline BMI and risk of SISV [a, b]

		Men			Women [c]			Overall		
		Model 11			Model 12			Model 13		
		aHR	95% CI	p [d]	aHR	95% CI	p [d]	aHR	95% CI	p [d]
BMI (per 10 units; kg/m)^2		1.44	1.07, 1.93	0.01	0.97	0.68, 1.37	0.84			
Obesity								1.01	0.76, 1.33	0.95
Age (years)		0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01	0.98	0.97, 0.99	<0.01
Male (%)								1.55	1.18, 2.04	<0.01
Race-ethnicity (%)	White, non-Hispanic	Ref			Ref			Ref		
	Hispanic	1.29	0.79, 2.11	0.31	0.66	0.35, 1.22	0.18	1.29	0.87, 1.92	0.20
	Asian, non-Hispanic	[e]			0.59	0.21, 1.62	0.31	0.38	0.14, 1.03	0.06
	Black, non-Hispanic	0.63	0.19, 2.05	0.44	[e]			0.49	0.15, 1.57	0.23
	Other	0.36	0.05, 2.57	0.31	0.96	0.23, 3.93	0.95	0.71	0.23, 2.24	0.56

[a] Multivariable proportional hazards regression models (ICPHREG procedure in SAS) were fit to evaluate associations with time to event (incident SARS-CoV-2 infection or censoring) as the key outcome of interest among 13,930 donors with 39,736 donations.
[b] Models additionally adjusted for geographic region.
[c] This model initially did not convergence; the reported values were in a model without geographic region as a covariate.
[d] P value was calculated from the chi-square test statistic.
[e] No estimate due to small sample cell size.
Abbreviations*:* adjusted hazard ratio (aHR), body mass index (BMI), hazard ratio (HR), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV-2 infection among those with serologic evidence of vaccination (SISV)

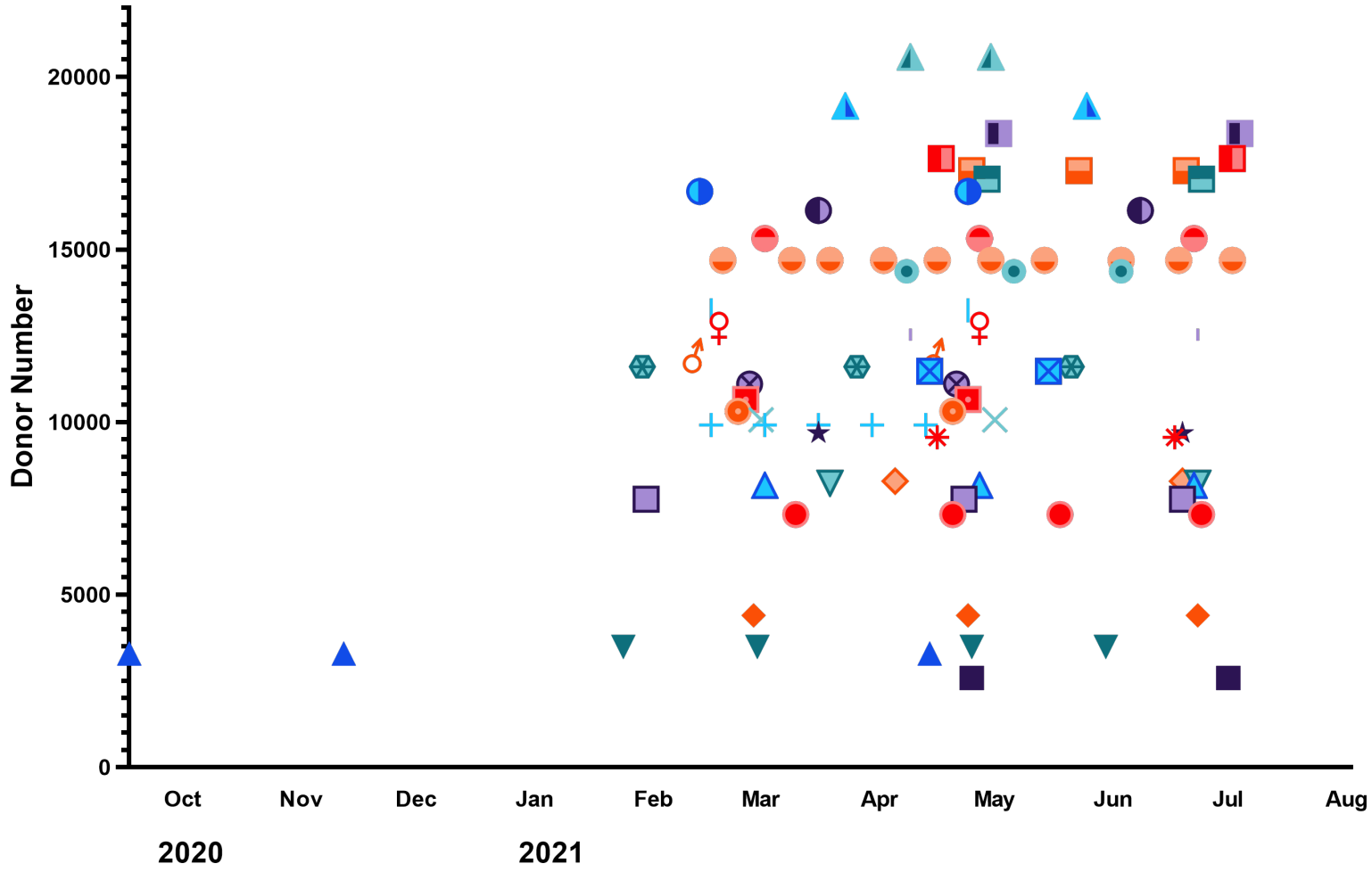
Supplemental Figure 1: Study participant flowchart per inclusion and exclusion criteria [a, b]



[a] Donations were collected between April 2, 2020 and October 31, 2021 in the initial dataset, and August 29, 2020 and September 30, 2021 in the final analytic dataset. A large proportion of donors were excluded based on the eligibility criterion of having two anti-N measurements. This is a secondary analysis, and this large number of excluded blood samples reflects the two phases of laboratory testing algorithms in the parent study. The two phases were approximately the periods prior to and after vaccine approval and availability in the US. The respective reflex testing excluded blood samples with serologic evidence of previous infection (2020) and no vaccination (2021) from further anti-N assays; we note that both were also exclusion criteria in our final analytic dataset.
[b] Figure created with BioRender.com.

Abbreviations*:* antibodies specific to SARS-CoV-2 nucleocapsid protein antibodies (anti-N), antibodies specific to SARS-CoV-2 spike protein antibodies (anti-S), body mass index (BMI), cardiometabolic disease (CMD), diastolic blood pressure (DBP), systolic blood pressure (SBP)

Supplemental Figure 2: Donations throughout study period among donors included in final analytic dataset [a]

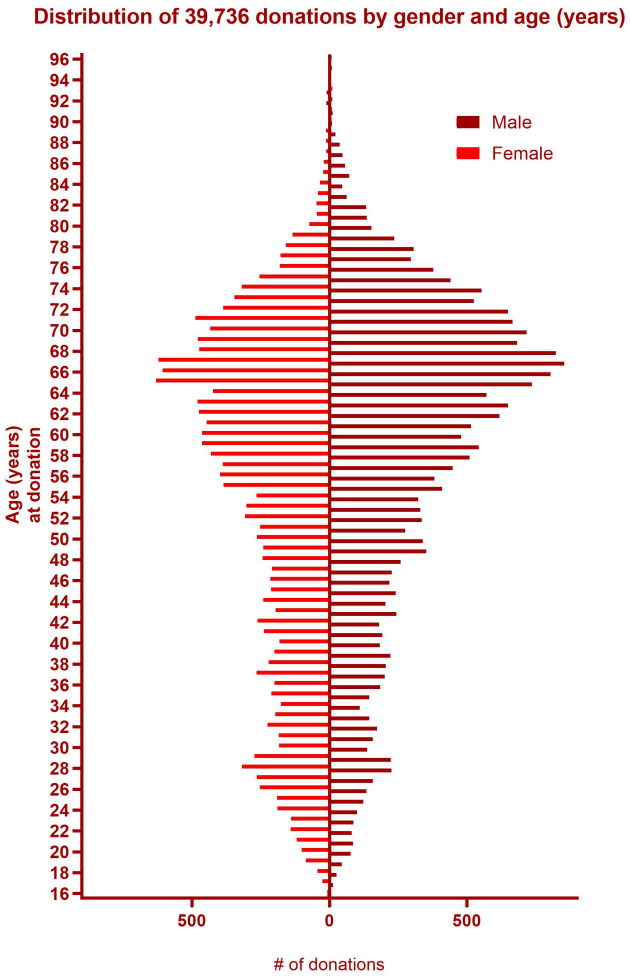


[a] Each unique symbol represents donations from one individual study participant (donor). In this figure, a selected subset of 33 donors is included. This visualization illustrates the heterogeneity of inter-donation intervals.

Supplemental Figure 3: Number of study donations stratified by gender and age

A: Among the total number of blood samples ($N_{donations}$ = 39,736) in this study, the respective numbers in gender-[a] and age-stratified subgroups are visually represented in a study pyramid.

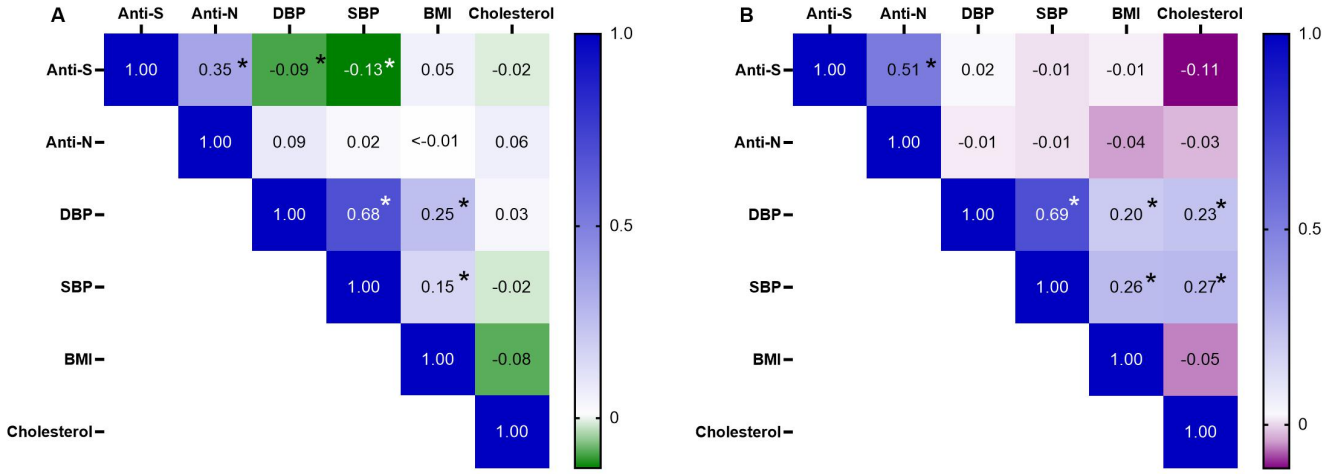
A



[a] Each timepoint is categorized based on self-reported gender for the study visit.

Abbreviations: body mass index (BMI), millimeters of mercury (mm Hg)

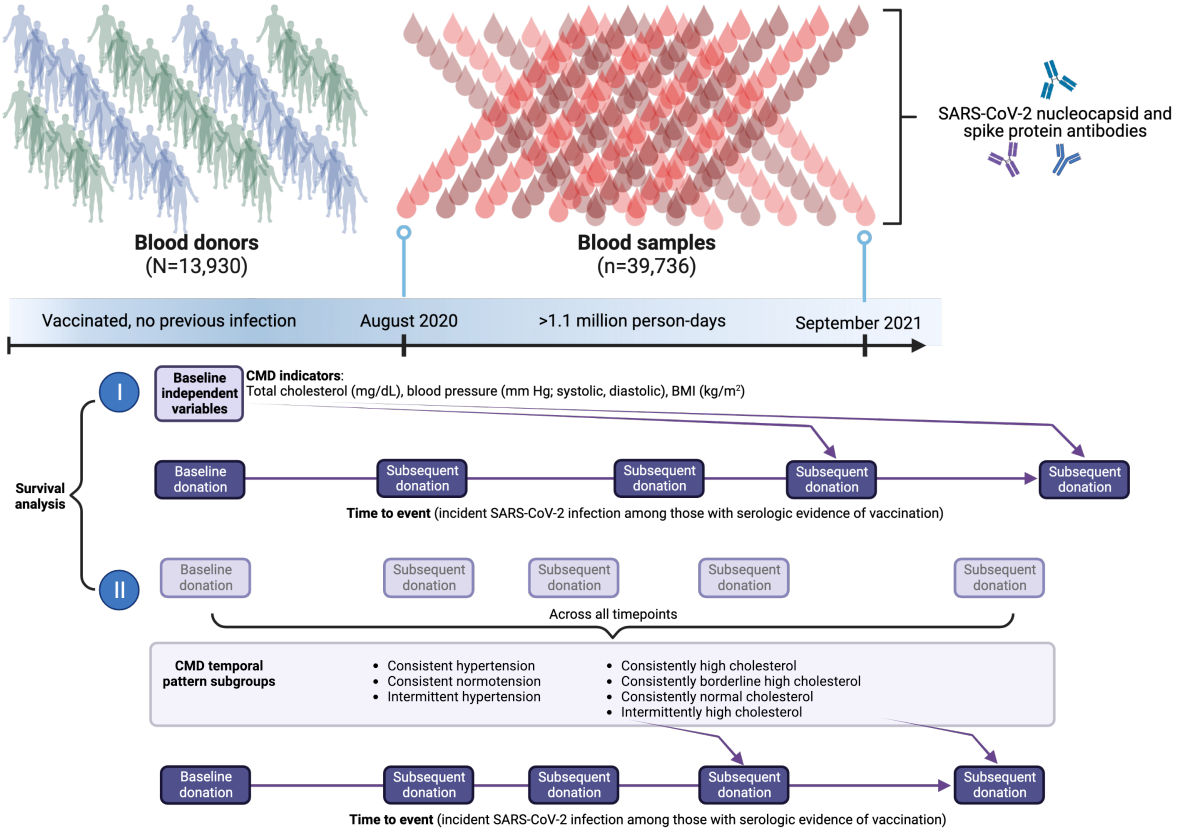
Supplemental Figure 4: Spearman rank correlation coefficients between anti-SARS-CoV-2 nucleocapsid and spike protein antibodies and cardiometabolic health indicators in those with incident SISV. All timepoints were considered in correlations stratified by gender (A men, B women).



[a] P <0.05 indicated by *. Correlations between the same variable (1.00) visualized but p values not included.
[b] Units: S/CO (anti-N, anti-S), mm Hg (systolic and diastolic blood pressure), kg/m^2 (BMI), mg/dL (total cholesterol)

Abbreviations*:* antibodies specific to SARS-CoV-2 nucleocapsid protein antibodies (anti-N), antibodies specific to SARS-CoV-2 spike protein antibodies (anti-S), body mass index (BMI), millimeter of mercury (mm Hg), signal-to-cutoff ratio (S/CO), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV-2 infection among those with serologic evidence of vaccination (SISV)

Supplemental Figure 5: Graphical abstract



Supplemental Methods

Additional data collection details and laboratory assays

Self-reported information included demographics (e.g., age, gender, race, and ethnicity) and anthropometry (height [in], weight [lb]). Prior to every blood donation, diastolic and systolic blood pressure (millimeters of mercury [mm Hg]) was measured by automated digital sphygmomanometer. If the first blood pressure measurement was within prespecified ranges (90-180 mm Hg for systolic, 50-100 for diastolic), this measurement was recorded. If the donor had blood pressure outside of these ranges, there was a second measurement, which was included in this analysis. Per blood collection organization donation eligibility criteria, donors with blood pressure outside of the prespecified range were deferred from donating. As exceptions, a medical director can approve allogeneic donors based on evaluations of the donor in person and the completed health questionnaire, with determination that donors are healthy and at low risk for adverse consequences caused by blood donation.

Laboratory assays for SARS-CoV-2 anti-N and anti-S responses

Binding antibody assays targeted the S1 subunit of spike glycoprotein (VITROS total immunoglobulin [Ig]; Ortho Clinical Diagnostics; Rochester, NY, USA) and complete nucleocapsid protein (total Ig) of SARS-CoV-2. Two anti-N assays were used during this study period (Roche Elecsys [Indianapolis, IN, USA] prior to and on July 7, 2021; Ortho Clinical Diagnostics [Rochester, NY, USA] beginning July 8, 2021), which were previously validated (34). Before August 31, 2021, only specimens that were anti-S seropositive were subsequently tested for anti-N. From September 2021, all donations were tested in parallel for anti-S and anti-N (VITROS chemiluminescent total Ig assay; Ortho Clinical Diagnostics). Testing of Vitalant donation specimens was performed at five laboratories operated by Creative Testing Solutions. Seropositivity was defined per manufacturers' instructions for use.

Cardiometabolic indicator temporal patterns

We evaluated cardiometabolic patterns with four approaches. First, we evaluated baseline values of BMI, total cholesterol, blood pressure. Baseline was defined as the first donation (study) visit that was included in the final analytic dataset. Second, we defined the peak cholesterol and blood pressure (systolic, diastolic) as the maximum values among all available timepoints of each donor. Third, median cholesterol and blood pressure was calculated based on all timepoints of the donor. Lastly, temporal pattern subgroups of total cholesterol and blood pressure were defined based on the following categories. We categorized each participant as having consistently high (≥240 mg/dL), borderline high (<240 and ≥200 mg/dL), and normal (<200 mg/dL; desirable) circulating total cholesterol concentrations if all available timepoints of the participant were in one of these ranges; otherwise, the participant was categorized as intermittently elevated. For hypertension patterns, we sequentially defined three subgroups: 1) consistently hypertensive (systolic blood pressure ≥130 or diastolic blood pressure ≥ 80); 2) consistently normotensive (systolic blood pressure <130 or diastolic blood pressure <80); and 3) intermittent hypertension status.

Geographic region

Geographic region of blood donation was included as a covariate in multivariable models. A complete list of the 18 geographic region categories, specifically airport codes, is below.

Albuquerque, NM (ALB)
Billings, MT (BIL)
Cheyenne, WY (CYS)
Denver, CO (DEN)
El Paso, TX (ELP)
Fargo, ND (FAR)
Lafayette, LA (LAF)
Las Vegas, NV (LAS)
Lubbock, TX (LBB)
McAllen, TX (MCA)
Memphis, TN (MEM)
Phoenix, AZ (PHX)
Rapid City, SD (RAP)
Reno, NV (RNO)

Sacramento, CA (SAC)
San Francisco, CA (SFO)
Spokane, WA (SPK)
Ventura, CA (VTA)

Online Supplemental Materials References

1. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894. Geneva: 2000.
2. Whelton PK, Carey RM, Aronow WS, Casey DE, Jr., Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension (Dallas, Tex : 1979)*. 2018;71:e13-e115. doi: 10.1161/hyp.0000000000000065
3. Expert Panel on Detection E, Adults ToHBCi. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486-2497. doi: 10.1001/jama.285.19.2486