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ORIGINAL ARTICLE

Global coagulation assays in patients with diabetes mellitus

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Abstract

Background: There is significant heterogeneity in the incidence and severity of diabetes-associated vascular complications and there is no routine biomarker that accurately predicts these outcomes. This pilot study investigates the role of global coagulation assays in patients with diabetes mellitus.

Methods: In this cross-sectional study, patients with diabetes not on anticoagulation or dialysis and without active malignancy were recruited from endocrinology clinics. Blood samples were collected for global coagulation assays including thromboelastography (TEG), thrombin generation using calibrated automated thrombogram (CAT), and fibrin generation and fibrinolysis using the overall hemostatic potential (OHP) assay. The results were compared with healthy controls.

Results: A total of 147 adult patients including 19 with type 1 diabetes (T1DM), 120 with type 2 diabetes (T2DM), and eight with latent autoimmune diabetes were recruited. Compared with 153 healthy controls, patients with diabetes demonstrated higher maximum amplitude (68.6 vs 60.2 mm, $p < 0.001$) on TEG, and higher OHP (9.3 vs 6.4, $p < 0.001$) with comparable CAT parameters. Patients with T2DM were more hypercoagulable than those with T1DM on most biomarkers. Higher maximum amplitude, velocity index, and OHP were associated with increased risk of complications (C-stat 0.82). Patients with history of microvascular complications appear to have more hypercoagulable thrombin and fibrin generation than those without.

Conclusion: Patients with diabetes have more hypercoagulable profiles on global coagulation assays, particularly patients with T2DM and those with microvascular complications. Further studies with longitudinal follow-up are ongoing to evaluate the utility of global coagulation assays in predicting long-term patient outcomes.

KEYWORDS
diabetes mellitus, fibrin, fibrinolysis, thrombin, thromboelastography

Essentials

- There is no routine laboratory test that risk stratifies diabetic complications.
- This is a cross-sectional study involving patients with diabetes mellitus.
- Patients with diabetes have more hypercoagulable global coagulation assay parameters.
- Patients with microvascular complications appear more hypercoagulable than those without.

1 | INTRODUCTION

The global diabetes prevalence is estimated to be 9.3% (463 million people) in 2019 and rising to 10.9% (700 million) by 2045.[1] Diabetes also independently increases the risk of cardiovascular disease significantly with a mortality rate of 44% in patients with type 1 diabetes mellitus (T1DM) and 52% in patients with type 2 diabetes mellitus (T2DM).[2] Atherosclerosis tends to progress faster and extends more distally in patients with diabetes compared with those without diabetes.[3] Patients with diabetes may also have clinical events with less atherosclerotic burden and likely greater plaque instability.

One of the unmet needs in diabetes management is the ability to predict the development of complications so that active preventive measures can be introduced. Although glycated hemoglobin (HbA1c) is an established surrogate marker for diabetic complications, it may be inaccurate in some settings such as anemia, and studies have found a consistent optimal HbA1c for diabetes complications difficult to define given significant heterogeneity in cardiovascular complication presentations in patients with diabetes despite similar HbA1c levels.[4,5] While the vascular complications of diabetes may in part relate to Virchow's triad of endothelial dysfunction, hemodynamic changes, and hypercoagulability,[6,7] there is a lack of a reliable coagulation marker to evaluate its components. Current routine coagulation studies only measure time to clot formation (<5% of thrombin generation) and are poor predictors of thrombotic or bleeding risks.[8,9] Global coagulation assays, which measure the final components of the coagulation cascade, may provide a more complete assessment of an individual's hemostatic profile.[10,11]

The majority of data pertaining to global coagulation assays in diabetes have been in patients with T2DM. Previous studies comparing patients with T2DM to normal controls found that patients with diabetes demonstrated higher peak thrombin and endogenous thrombin potential (ETP)[12,13] as well as more hypercoagulable thromboelastography (TEG) parameters.[14] To the best of our knowledge, few studies have looked at the use of overall hemostatic potential (OHP) assay. OHP is derived from repeated spectrophotometric measurements of platelet-poor plasma and provides functional testing of fibrin generation and fibrinolysis. Antovic et al. found OHP to be higher in T1DM patients with complications compared to controls, although there were no significant differences in overall fibrinolytic potential (OFP).[15]

Hence, we designed a study to evaluate a combination of global coagulation assays in patients with diabetes, with the specific aim of determining whether a combination of these assays may assist in providing a better individualized cardiovascular disease risk assessment.

2 | METHODS

This was a cross-sectional study of patients recruited from the endocrinology clinic at Northern Health, a tertiary hospital in Melbourne, Australia, between February 2017 and August 2020. Inclusion criteria were adult patients with diabetes, as defined by the American Diabetes Association,[16] who were receiving medical therapy for their condition. Exclusion criteria included an inability to provide informed consent, active malignancy, and concurrent use of anticoagulants. Patients with diabetes on dialysis were also excluded to avoid the confounders associated with dialysis as dialysis is a known risk factor for significant morbidity and mortality independent of diabetes.[17] Basic characteristics such as age, weight, height, and cardiovascular risk factors were recorded. Written informed consent was obtained from every study participant. The data from the diabetes cohort were compared with healthy controls (n = 153) recruited between August 2013 and August 2019.[18,19] The healthy controls were recruited from staff and family members as well as through poster advertisement in the hospital and word of mouth. In addition to these exclusion criteria, the healthy controls were not known to have any modifiable cardiovascular risk factors nor were on any medications that may modify the coagulation parameters such as antiplatelet, oral contraceptive, or hormone replacement therapy. The project was approved by the Austin Health Human Research Ethics Committee (HREC/Austin/16/459).

Patients with diabetes were classified, based on clinical history, as having a history of microvascular (diabetic nephropathy, neuropathy, and/or retinopathy) or macrovascular (coronary artery disease, peripheral arterial disease, and/or cerebrovascular) disease. Although debate continues as to whether microvascular complications distinctly precede macrovascular complications or if both processes occur simultaneously as a continuum, macrovascular complications contribute significantly to diabetes-related deaths.[20,21] For this study, patients with microvascular-only complications were analyzed as a subgroup and those with macrovascular complications (with or without concurrent microvascular complications) as another subgroup.

Blood sample collection was done by peripheral venipuncture using a 21G needle. Routine investigations such as full blood count, coagulation studies, D-dimer, von Willebrand factor studies, HbA1c, lipid levels, and renal function tests were performed. The Alinity c HbA1c enzymatic assay was certified to the National Glycohemoglobin Standardization Program, standardized to International Federation of Clinical Chemistry and Laboratory Medicine and traceable to the Diabetes Control and Complications Trial. The STA fibrinogen kit was used to quantitatively determine fibrinogen levels by the Clauss method, whereas D-dimer was measured using the immunoturbidimetric method with the STA-LIATEST D-Di Plus kit. The laboratory cutoff for D-dimer elevation

is >500 ng/ml FEU. The von Willebrand factor antigen was measured with the immunoturbidimetric method using the STA-vWF Liatest kit. Factor VIII was measured using the one-stage activated partial thromboplastin time (APTT) based assay with STA-Immunodeficient plasma in which commercial lyophilized plasma lacking factor VIII was mixed with varying dilutions of the calibrator plasma with normal factor activity and tested with the APTT reagent TriniCLOT aPTT S to derive a standard curve. The patient plasma was then mixed with the factor-deficient plasma and an APTT derived and plotted on the curve. Antithrombin and Protein C were measured by the chromogenic method using the STA-STACHROM-Antithrombin III kit and STA-STACHROM Protein C respectively, whereas free Protein S was quantified using a latex particle-based agglutination assay with the STA-Liatest Free Protein S kit. The coagulation assays were performed on the STA-Max analyzers as per the manufacturer's recommendations.

Thromboelastography was performed on whole citrate blood within 4 h of collection, and the remaining tubes were double centrifuged at 2500 g for 10 minutes to obtain platelet-poor plasma and stored at −80°C within 2 h of collection. These samples were used for batch testing for calibrated automated thrombogram (CAT) and OHP. TEG was performed in real time (with manufacturer's quality controls run periodically), whereas CAT was performed in batches during the respective recruitment period of the different cohorts. OHP was a relatively newer assay and the samples of both cohorts were performed in batches during the same period. Both CAT and OHP assays were also performed alongside reference controls of known values. All samples from both cohorts were subjected to the same collection, processing, and storage protocols.

2.1 | Thromboelastography

This assay was performed as per the manufacturer's recommendations. A total of 1000 µl of the citrated blood was pipetted into a tube containing 40 µl of kaolin; 340 µl of this mix was then added to a cup preheated to 37°C, containing 20 µl of 0.2 M calcium chloride. The cups then oscillate around a suspended pin, attached to a detector via a torsion wire. Fibrin strands begin to form and create torsion around the wire. When fibrinolysis occurs, the clot degrades and reduces the torque on the wire. The TEG analyzer (TEG 5000 Hemonetics; Braintree) assesses these torque changes and generates a graph. Routine TEG parameters of R-time (min, clotting time), K-time (min, clot kinetics), maximum amplitude (MA, mm, maximum clot strength), a-angle (°, clot strengthening), and Ly30 (%, clot lysis) are recorded.

2.2 | Calibrated automated thrombogram

This assay measures the rate and extent of thrombin generation following tissue factor stimulus by continuously comparing the cleavage of the fluorogenic substrate to a calibrator.[10] A total of 80 µl of platelet-poor plasma was added to either 20 µl of platelet-poor plasma reagent (5 pM tissue factor; 4 µM phospholipids) or 20 µl of thrombin calibrator (to correct for differences in sample color, inner filter fluorescence, and substrate consumption).[22] All samples were run in triplicate. Coagulation was triggered with the addition of calcium chloride in a buffer with fluorogenic substrate (FluCa-kit). Readings from the automated fluorometer (>60 min) were analyzed by the software, Thrombinoscope BV (Diagnostica Stago, France), and used to generate a thrombin curve. Parameters calculated include lag time, thrombin peak height, ETP (amount of thrombin), and velocity index.

2.3 | Overall hemostatic potential assay

Overall hemostatic potential is derived from a fibrin aggregation curve formed from repeated spectrophotometric measurements of platelet-poor plasma.[23] A total of 75 µl of thawed platelet poor plasma was added to wells with 75 µl of buffer containing either (1) Tris, NaCl, $CaCl_2$ (final concentration 66 nM Tris, 130 mM NaCl, 35 ml $CaCl_2$; pH 7.0) and thrombin (0.006 IU/ml) to generate the overall coagulation potential (OCP) or (2) Tris, NaCl, $CaCl_2$, thrombin and tissue plasminogen activator (600 ng/ml) to generate the OHP. The two fibrin-aggregation curves (OCP and OHP) are calculated from the FLUOstar Optima (BMG Labtech) plate reader at 405 nM. The difference between the area underneath the two curves gives the OFP.

2.4 | Statistical analysis

Descriptive analysis was conducted to compare healthy controls and patients with diabetes using Stata version 15.1 (StataCorp). For continuous variables, normality was determined through Shapiro-Wilk test, with normally distributed variables reported as mean and standard deviation and differences between groups tested using the Student *t*-test. Non-normally distributed variables were reported as median and interquartile range (IQR), with Mann-Whitney (rank-sum) test used to test for differences across groups. Spearman's correlation coefficients were calculated for an assessment of correlation between continuous variables because of the non-normal distribution of the majority of the continuous variables. Propensity score matching was conducted with age and gender included as the two matching variables to balance the characteristics across the healthy controls and diabetes cohorts. Nearest neighbor and 1:1 matching using the Stata "psmatch2" program with no replacement was considered, with percentage standardized differences used to confirm the matching was appropriate.[24] Univariate analysis was conducted to identify variables associated with complications within the diabetes subgroup. Receiver operating curve analysis and the Youden Index was used to identify thresholds maximizing these associations for continuous variables. Variables with p values < 0.2 were considered in multivariable analysis with the collinearity between variables also assessed to ensure that highly collinear variables were not considered in the same multivariable analysis. These preliminary analyses found that fibrinogen was highly correlated with a number of variables, including MA and OHP and

TABLE 1 Patient demographics and investigations of the study groups compared to normal controls (values reported as median [IQR] unless otherwise stated)

	Unmatched Cohorts						Propensity Score Matched Cohorts		
Factor	Healthy Controls	Diabetes Mellitus	*p* Value	T1 DM or LADA	T2 DM	*p* Value[a]	Healthy Controls	Diabetes Mellitus	*p* Value
N	153	147		27	120		74	74	
Sex									
Female	98 (64.1%)	67 (45.6%)	0.002	14 (51.9%)	53 (44.2%)	0.004	50 (68%)	47 (64%)	0.73
Male	55 (35.9%)	80 (54.4%)		13 (48.1%)	67 (55.8%)		24 (32%)	27 (36%)	
Age, mean (SD)	42 (17)	60 (15)	<0.001	50 (15)	62 (14)	<0.001	52 (14)	50 (11)	0.22
Glycated hemoglobin (%)	5.4 (5.1, 5.6)	7.6 (6.7, 8.6)	<0.001	7.9 (7.3, 9.7)	7.3 (6.6, 8.5)	<0.001	5.4 (5.2, 5.7)	7.9 (6.7, 8.9)	<0.001
Creatinine (μmol/L)	68.5 (59.5, 81.0)	83.0 (65.0, 118.0)	<0.001	71.0 (61.0, 86.0)	88.0 (66.5, 122.5)	<0.001	67.0 (60.0, 80.0)	69.0 (58.0, 89.0)	0.30
eGFR (ml/min/1.73 m^2)	100.5 (89.5, 115.0)	76.0 (53.0, 94.0)	<0.001	93.0 (83.0, 103.0)	70.5 (49.0, 90.1)	<0.001	96.0 (84.0, 105.0)	91.0 (70.0, 104.0)	0.26
Albumin (g/L)	42.5 (40.0, 44.0)	39.0 (36.0, 42.0)	<0.001	40.0 (37.0, 42.0)	39.0 (35.0, 42.0)	<0.001	42.0 (40.0, 43.0)	39.0 (35.0, 42.0)	<0.001
Total cholesterol (mmol/L)	5.1 (4.4, 6.0)	4.2 (3.6, 5.1)	**<0.001**	4.4 (3.6, 5.7)	4.1 (3.5, 5.0)	<0.001	5.2 (4.5, 6.1)	4.6 (3.7, 5.4)	<0.001
Triglycerides (mmol/L)	1.0 (0.7, 1.4)	1.9 (1.2, 2.6)	<0.001	1.3 (0.9, 1.9)	2.1 (1.3, 2.7)	<0.001	1.1 (0.8, 1.6)	1.7 (1.2, 2.6)	<0.001
Low-density lipoprotein cholesterol (mmol/L)	3.0 (2.3, 3.6)	2.1 (1.6, 2.9)	<0.001	2.6 (1.7, 3.8)	2.1 (1.5, 2.7)	<0.001	3.0 (2.3, 3.7)	2.4 (1.8, 3.2)	0.001
High-density lipoprotein cholesterol (mmol/L)	1.6 (1.3, 1.9)	1.1 (0.9, 1.4)	<0.001	1.4 (1.1, 1.7)	1.1 (0.9, 1.3)	<0.001	1.5 (1.3, 1.9)	1.2 (1.0, 1.4)	<0.001
Prothrombin time (s)	11.0 (10.3, 12.4)	11.7 (10.9, 12.5)	<0.001	11.2 (10.5, 12.3)	11.8 (10.9, 12.6)	<0.001	11.0 (10.3, 12.4)	11.3 (10.7, 12.1)	0.32
Activated partial thromboplastin time (s)	28.1 (26.1, 31.0)	27.3 (26.0, 29.0)	0.04	26.8 (25.9, 28.3)	27.4 (26.0, 29.4)	0.04	28.0 (26.1, 31.0)	26.9 (25.9, 28.6)	0.02
Fibrinogen (g/L)	2.9 (2.5, 3.5)	3.8 (3.2, 4.6)	<0.001	3.3 (2.9, 4.3)	3.9 (3.3, 4.7)	<0.001	3.2 (2.7, 3.5)	3.6 (3.0, 4.5)	<0.001
D-dimer									
Negative	135 (88.2%)	85 (57.8%)	<0.001	22 (81.5%)	63 (52.5%)	<0.001	63 (85%)	51 (69%)	0.006
Positive	14 (9.2%)	61 (41.5%)		5 (18.5%)	56 (46.7%)		9 (12%)	23 (31%)	
Unknown	4 (2.6%)	1 (0.7%)		0 (0.0%)	1 (0.8%)		2 (3%)	0 (0%)	
Von Willebrand factor antigen (%)	102.0 (86.0, 140.0)	154.0 (115.0, 191.0)	<0.001	150.0 (103.0, 193.0)	154.5 (117.0, 188.0)	<0.001	104.0 (85.5, 146.5)	143.0 (103.0, 171.0)	0.001
Factor VIII (%)	105.0 (86.0, 145.0)	163.0 (125.0, 200.0)	<0.001	164.0 (125.0, 185.0)	163.0 (126.0, 200.5)	<0.001	108.0 (86.0, 148.5)	164.0 (120.0, 186.0)	<0.001
Antithrombin (%)	102.0 (96.0, 110.0)	100.0 (93.0, 108.0)	0.26	104.5 (98.0, 111.0)	100.0 (92.0, 107.5)	0.20	101.5 (90.5, 106.0)	104.5 (95.0, 111.0)	0.08
Protein C (%)	104.5 (93.5, 127.5)	125.5 (107.0, 144.0)	<0.001	115.0 (97.0, 135.0)	127.0 (110.0, 144.5)	<0.001	99.5 (90.0, 112.0)	127.0 (109.0, 145.0)	<0.001

TABLE 1 (Continued)

Factor	Unmatched Cohorts						Propensity Score Matched Cohorts		
	Healthy Controls	Diabetes Mellitus	*p* Value	T1 DM or LADA	T2 DM	*p* Value[a]	Healthy Controls	Diabetes Mellitus	*p* Value
Protein S (%)	93.0 (80.0, 105.0)	110.5 (95.0, 129.0)	<0.001	105.0 (93.0, 118.0)	112.5 (95.0, 129.5)	<0.001	89.5 (78.5, 106.0)	111.0 (94.0, 130.0)	<0.001
Body mass index, (kg/m^2)		30.9 (25.8, 37.0)		27.4 (24.2, 31.4)	32.3 (27.1, 39.1)	<0.001		33.2 (27.1, 39.1)	
Systolic blood pressure (mm Hg)		131.0 (120.0, 145.0)		120.0 (115.0, 138.0)	134.5 (125.0, 145.0)	0.02		130.0 (120.0, 140.0)	
Framingham Risk Score									
High		94 (63.9%)		12 (44.4%)	82 (68.3%)	<0.001		28 (38%)	
Low		24 (16.3%)		12 (44.4%)	12 (10.0%)			23 (31%)	
Moderate		28 (19.0%)		3 (11.1%)	25 (20.8%)			23 (31%)	
Unknown		1 (0.7%)		0 (0.0%)	1 (0.8%)				
Hypertension		106 (72.1%)		12 (44.4%)	94 (78.3%)	<0.001		44 (59%)	
Hyperlipidemia		108 (73.5%)		16 (59.3%)	92 (76.7%)	0.09		51 (69%)	
Obesity		83 (56.5%)		8 (29.6%)	75 (62.5%)	0.002		46 (62%)	
Current smoker		32 (21.8%)		10 (37.0%)	22 (18.3%)	0.04		21 (28%)	
Any complications		81 (55.1%)		10 (37.0%)	71 (59.2%)	0.05		31 (42%)	
History of peripheral neuropathy		26 (17.7%)		2 (7.4%)	24 (20.0%)	0.17		13 (17.6%)	
History of diabetic nephropathy (nondialysis)		42 (28.6%)		2 (7.4%)	40 (33.3%)	0.008		13 (17.6%)	
History of diabetic retinopathy		35 (23.8%)		7 (25.9%)	28 (23.3%)	0.80		17 (23.0%)	
History of coronary artery disease		53 (29.4%)		2 (7.4%)	30 (25.0%)	0.04		11 (15.0%)	
History of cerebrovascular accidents		16 (8.6%)		0	12 (10.0%)	0.12		5 (7.0%)	
History of peripheral artery disease		20 (10.7%)		0	11 (9.2%)	0.22		4 (5.0%)	

Abbreviations: eGFR, estimated glomerular filtration rate; IQR, interquartile range; LADA, latent autoimmune diabetes of adult onset; SD, standard deviation; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

[a]*p* values of patients with type 1 patients compared with patients with type 2 diabetes.

therefore was not included in the multivariable modelling. Sensitivity analysis was conducted to assess the effect of including these collinear variables in the final multivariable model. C-statistics (area under the receiver operating curve), Schwarz Bayesian Information Criterion, and the Hosmer-Lemeshow test were used to compare model fit and select the preferred model. Statistical significance was set at a p value < 0.05.

3 | RESULTS

A total of 147 patients with diabetes consisting of 19 with T1DM, 8 with latent autoimmune disease in adults (LADA), and 120 with T2DM were recruited. For the purposes of data analysis, patients with LADA were collectively analyzed with T1DM. Table 1 shows the characteristics of the patients with comparisons to the healthy control cohort.

3.1 | Patient demographics and investigations

The mean age of the diabetes cohort was 60 (±15) years. Sixty-seven (45.6%) of the patients were female and the median duration of diagnosis was 14 years for T1DM (6.5, 25.0) and 10 years for T2DM (5.0, 17.0; $p = 0.05$). A total of 63.9% (n = 94) were classified as high Framingham risk score (≥20%) including 12 with T1DM (44.4%) and 82 with T2DM (68.3%). Sixty-two patients (42.2%) were on antiplatelet therapy. A total of 42.5% (n = 54) of the patients with T2DM were

TABLE 2 Global coagulation assay parameters for controls compared with patients with diabetes (values reported as median [IQR] unless otherwise stated)

	Unmatched Cohorts		
Factor	**Healthy Controls**	**Diabetes Mellitus**	***p* Value**
N	153	147	
R-time (min)	6.3 (5.2, 7.5)	6.5 (5.7, 7.8)	0.26
K-time (min)	2.2 (1.8, 2.6)	1.8 (1.4, 2.2)	<0.001
a-angle (°)	58.1 (50.3, 64.3)	52.8 (45.7, 63.3)	0.06
Maximum amplitude (mm), mean (SD)	60.2 (6.3)	68.6 (5.9)	<0.001
Lysis 30 (%)	0.5 (0.0, 1.3)	0.0 (0.0, 0.5)	<0.001
Lag time (min)	3.2 (2.7, 3.7)	4.0 (3.3, 4.7)	<0.001
Endogenous thrombin potential (nM.min), mean (SD)	1335.1 (258.1)	1282.9 (265.9)	0.09
Peak thrombin (nM), mean (SD)	219.7 (67.1)	214.5 (68.2)	0.50
Velocity index (nM/min)	64.3 (43.3, 93.5)	60.2 (41.0, 82.4)	0.31
Overall coagulation potential (unit), mean (SD)	35.5 (9.7)	40.5 (10.1)	<0.001
Overall hemostatic potential (unit)	6.4 (4.8, 9.4)	9.3 (6.6, 13.7)	<0.001
Overall fibrinolytic potential (%)	81.1 (77.4, 84.1)	75.1 (69.2, 81.8)	<0.001

Abbreviations: DM, diabetes mellitus; IQR, interquartile range; SD, standard deviation.

insulin-dependent, whereas 28.3% (n = 36) were on a sodium-glucose co-transporter-2 inhibitor and 60.5% (n = 89) were on metformin. Fifty-seven patients (38.8%) were on aspirin and 99 patients (67.3%) were on statin therapy. Patients with T1DM had higher HbA1c levels than those with T2DM (7.9% vs 7.3%, $p < 0.001$).

Propensity score matching provided 74 patients in both cohorts, with percentage standardized differences of −15.7% (reduced from 119.4%) for age and 8.3% (reduced from 40.4%) for sex, indicating a closer match in the propensity score matched cohort when compared with the overall cohort (Table 1). Von Willebrand factor antigen, factor VIII, fibrinogen, and proteins C and S were all significantly higher in patients with diabetes. There was also a higher proportion of patients with positive D-dimer in the diabetes group (31% vs 12%, $p = 0.006$).

3.2 | Comparison of global coagulation assays between patients with diabetes and healthy controls

3.2.1 | Thromboelastography

Overall, patients with diabetes were more hypercoagulable with higher MA (69.5 vs 60.8 mm, $p < 0.001$) and reduced Ly30 (0.0% vs 0.4%, $p = 0.003$) compared with healthy controls (Table 2, Figure 1). Patients with T2DM also demonstrated more hypercoagulable TEG parameters compared with those with T1DM. There was no correlation between HbA1c level and MA (rho = −0.026, $p = 0.76$).

3.2.2 | Calibrated automated thrombogram

The lag time was more prolonged in patients with diabetes (3.8 vs 3.3 min, $p < 0.001$) compared with healthy controls, with lower velocity index (57.6 vs 68.4, $p = 0.05$, Table 2). However, there were no significant differences in ETP and peak height. Patients with T1DM had comparable TEG results compared with those with T2DM. There were no correlations between HbA1c levels and CAT parameters (ETP: rho = −0.059, $p = 0.48$; peak: rho = −0.120, $p = 0.15$; velocity index: rho = −0.149, $p = 0.07$).

3.2.3 | Overall hemostatic potential

Overall hemostatic potential (8.8 vs 7.3 units, $p = 0.003$) was significantly higher in patients with diabetes, particularly in those with T2DM, whereas the OFP was lower (75.8% vs 79.2%, $p = 0.001$) when compared with healthy controls (Table 2). OHP and OFP demonstrated only minimal correlation with D-dimer (rho = 0.299, $p < 0.001$ and rho = −0.195, $p = 0.02$, respectively), and no correlation to LY30 (rho = −0.153, $p = 0.07$ and rho = 0.169, $p = 0.05$, respectively) and HbA1c (rho = −0.104, $p = 0.22$ and rho = 0.107, $p = 0.21$, respectively).

3.3 | Associations between global coagulation assays with microvascular and macrovascular complications in patients with diabetes

Eighty-one patients with diabetes had a previous history of vascular complications attributable to diabetes: 35 had microvascular complications only and 46 had macrovascular (with or without microvascular) complications. Patients with T1DM were more likely to have microvascular complications, whereas patients with T2DM were more likely to have macrovascular complications ($p = 0.02$). The use of aspirin overall did not appear to influence the global coagulation assay

Propensity Score Matched Cohorts					
Healthy Controls	**Diabetes Mellitus**	***p* Value**	**T1 DM or LADA**	**T2 DM**	***p* Value**
74	74		21	53	
6.3 (5.2, 7.3)	6.6 (5.6, 7.8)	0.11	6.0 (5.4, 7.6)	6.8 (6.2, 7.8)	0.19
2.2 (1.7, 2.4)	1.9 (1.5, 2.2)	0.067	2.0 (1.8, 2.2)	1.8 (1.4, 2.2)	0.08
59.4 (48.1, 66.0)	51.9 (44.7, 62.0)	0.030	47.1 (45.1, 53.2)	54.8 (44.7, 65.6)	0.01
60.8 (6.6)	69.5 (5.9)	<0.001	67.0 (5.3)	70.5 (5.9)	<0.001
0.4 (0.0, 1.1)	0.0 (0.0, 0.6)	0.003	0.4 (0.0, 1.7)	0.0 (0.0, 0.2)	<0.001
3.3 (3.0, 3.7)	3.8 (3.3, 4.5)	<0.001	3.4 (3.1, 4.0)	4.0 (3.6, 4.6)	<0.001
1352.6 (268.8)	1319.7 (288.4)	0.47	1264.4 (273.7)	1341.7 (293.7)	0.44
226.9 (67.3)	213.2 (75.7)	0.25	201.7 (71.4)	217.8 (77.5)	0.35
68.4 (47.3, 93.5)	57.6 (33.8, 80.4)	0.049	52.6 (29.4, 80.6)	57.7 (40.3, 74.9)	0.13
36.9 (9.6)	40.0 (10.9)	0.075	38.7 (8.9)	40.5 (11.7)	0.16
7.3 (5.6, 10.3)	8.8 (6.8, 13.6)	0.003	7.8 (6.1, 10.7)	9.3 (7.4, 14.8)	0.002
79.2 (76.2, 82.9)	75.8 (68.0, 82.2)	0.001	79.3 (70.7, 82.8)	74.8 (64.3, 79.7)	<0.001

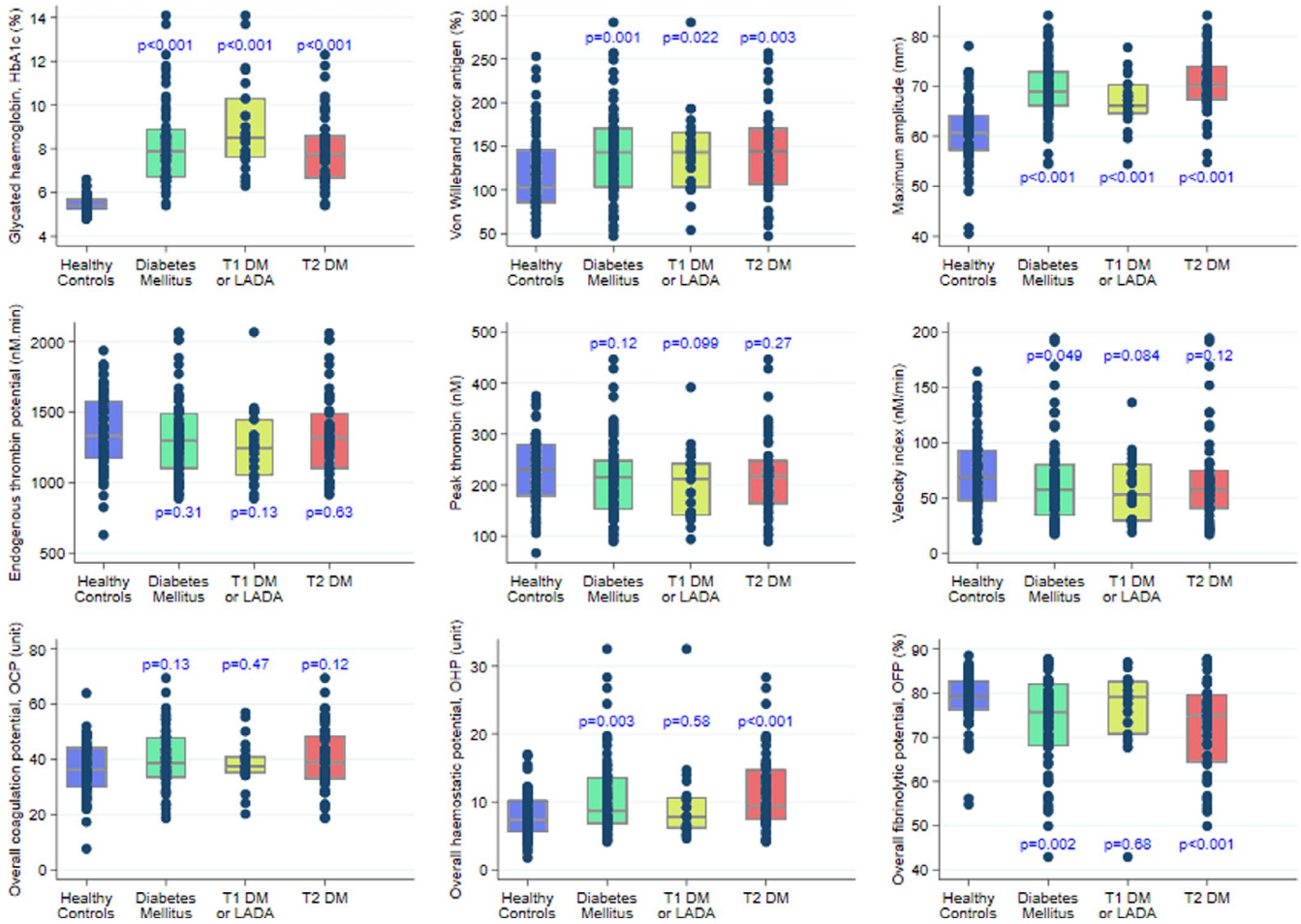

FIGURE 1 Comparison of global coagulation assay variables between healthy controls (blue bar) and patients with diabetes mellitus (green bar) following propensity score matching. Abbreviations: LADA, latent autoimmune diabetes in adults (yellow bar); T1DM, type 1 diabetes mellitus; T2DM type 2 diabetes mellitus (red bar)

TABLE 3 Laboratory investigations and global coagulation assay parameters for patients with diabetes divided into groups according to complications (values reported as median [IQR] unless otherwise stated)

Factor	No Complication	Any Complications	*p* Value[a]	Microvascular Complications	*p* Value[b]	Macrovascular Complications[e]	*p* Value[c]	*p* Value[d]
N	66	81		35		46		
Sex								
Female	36 (55%)	31 (38%)	0.07	10 (29%)	0.02	21 (46%)	0.34	0.25
Male	30 (45%)	50 (62%)		25 (71%)		25 (54%)		
Age (years), mean (SD)	56 (17)	64 (12)	0.001	60 (14)	0.18	67 (9)	<0.001	0.02
Age (years)								
≤57.5	36 (55%)	22 (27%)	0.001	14 (40%)	0.15	8 (17%)	<0.001	0.05
>57.5	30 (45%)	59 (73%)		21 (60%)		38 (83%)		
Diabetes mellitus type								
Type 1 or latent autoimmune diabetes	17 (26%)	10 (12%)	0.05	8 (23%)	0.81	2 (4%)	0.004	0.02
Type 2 diabetes	49 (74%)	71 (88%)		27 (77%)		44 (96%)		
No. of cardiovascular risk factors	4 (3, 4)	5 (4, 6)	<0.001	4 (3, 5)	0.05	5 (5, 6)	<0.001	<0.001
HbA1c (%), median (IQR)	7.4 (6.6, 8.4)	7.6 (6.8, 8.8)	0.22	7.6 (6.7, 8.6)	0.42	7.7 (6.8, 8.9)	0.22	0.84
HbA1c (%)								
≤7.75	43 (65%)	40 (49%)	0.08	17 (49%)	0.13	23 (50%)	0.10	1.00
>7.75	23 (35%)	39 (48%)		17 (49%)		22 (48%)		
Unknown	0	2 (2%)		1 (2%)		1 (2%)		
Fibrinogen (g/L)	3.5 (3.0, 4.3)	4.3 (3.4, 5.4)	<0.001	4.3 (3.4, 5.3)	0.001	4.0 (3.3, 4.7)	0.07	0.19
Factor VIII (%), median (IQR)	159 (119, 200)	166 (130, 200)	0.35	168 (146, 218)	0.13	159 (116, 194)	0.91	0.20
Von Willebrand factor antigen (%), median (IQR)	144.5 (105.0, 172.0)	159.0 (127.0, 211.0)	0.07	168.0 (134.0, 219.0)	0.02	144.5 (101.0, 207.5)	0.54	0.12
Estimated glomerular filtration rate (ml/min/1.73 m^2)	89.0 (70.0, 106.0)	58.0 (39.0, 88.3)	<0.001	55.5 (30.0, 91.0)	<0.001	62.0 (44.0, 86.8)	<0.001	0.45
Maximum amplitude, (mm) mean (SD)	68.0 (5.5)	69.1 (6.2)	0.27	69.8 (6.6)	0.14	68.5 (5.9)	0.66	0.34
Maximum amplitude								
≤68.15	38 (58%)	32 (40%)	0.04	14 (40%)	0.15	18 (39%)	0.09	0.90
>68.15	26 (39%)	48 (59%)		21 (60%)		27 (59%)		
Unknown	2 (3%)	1 (1%)		0		1 (2%)		
Endogenous thrombin potential (nM.min), mean (SD)	1269.5 (286.6)	1293.8 (249.1)	0.58	1367.2 (268.4)	0.10	1235.0 (218.1)	0.50	0.02
Peak thrombin (nM), mean (SD)	199.9 (64.3)	226.4 (69.4)	0.02	247.9 (73.7)	<0.001	209.2 (61.3)	0.45	0.01
Velocity index (nM/min), median (IQR)	57.9 (30.3, 73.3)	64.8 (42.6, 94.9)	0.02	70.1 (48.8, 113.7)	0.002	54.4 (41.0, 86.7)	0.33	0.04
Velocity index (nM/min)								
≤86.4	59 (89%)	54 (67%)	0.001	22 (63%)	0.002	32 (70%)	0.02	0.36
>86.4	7 (11%)	27 (33%)		13 (37%)		14 (30%)		
Overall coagulation potential (unit), mean (SD)	38.7 (10.2)	41.9 (9.9)	0.06	43.7 (11.3)	0.03	40.5 (8.5)	0.34	0.15

TABLE 3 (Continued)

Factor	No Complication	Any Complications	*p* Value[a]	Microvascular Complications	*p* Value[b]	Macrovascular Complications[e]	*p* Value[c]	*p* Value[d]
Overall hemostatic potential (unit), median (IQR)	7.8 (6.0, 12.7)	10.8 (7.4, 14.0)	0.02	12.6 (8.4, 19.3)	0.001	9.2 (6.8, 12.2)	0.36	0.01
Overall hemostatic potential (unit)								
≤7.965	34 (52%)	22 (27%)	0.005	8 (23%)	0.01	14 (31%)	0.03	0.25
>7.965	30 (45%)	57 (70%)		26 (74%)		31 (67%)		
Unknown	2 (3%)	2 (2%)		1 (3%)		1 (2%)		
Overall fibrinolytic potential (%), median (IQR)	77.6 (70.2, 82.3)	74.5 (68.0, 79.7)	0.13	70.9 (60.8, 77.3)	0.007	75.1 (70.5, 81.8)	0.95	0.01

Abbreviations: IQR, interquartile range; SD, standard deviation.

[a]*p* value comparing patient without any known vascular complications with those with known vascular complications.

[b]*p* value comparing patients with diabetes with microvascular complications with patients without complications.

[c]*p* value comparing patients with diabetes with macrovascular complications with patients without complications.

[d]*p* value of patients with macrovascular complications (with/without microvascular complications) compared with those with microvascular complications only.

[e]Any macrovascular complication, with or without microvascular complications.

TABLE 4 Final prediction model for variables significantly associated with complications

	Final Model			
Variable	Odds Ratio	95% CI	*p* Value	Assigned Weighted Score
Maximum amplitude (mm)				
≤68.15	1			
>68.15	4.24	1.66–10.88	0.003	4
Unknown	9.80	0.29–336.31	0.21	
Velocity index (nM/min)				
≤86.4	1			
>86.4	5.50	1.78–16.98	0.003	5
Overall hemostatic potential (unit)				
≤7.965	1			
>7.965	2.11	0.92–4.87	0.08	2
Unknown	0.96	0.18–5.04	0.97	
Sex				
Female	1			
Male	3.08	1.29–7.36	0.01	3
Age (years)				
≤57.5	1			
>57.5	4.64	1.85–11.66	0.001	4
HbA1c (%)				
≤7.75	1			
>7.75	4.35	1.72–10.97	0.002	4

Abbreviations: CI, confidence interval; HbA1c, glycated hemoglobin.

parameters (MA 68.7 vs 68.0 mm, $p = 0.74$; ETP 1245.1 vs 1257.5 nM.min, $p = 0.54$; velocity index 64.3 vs 59.4 nM/min, $p = 0.13$; OHP 10.4 vs 8.7 μ, $p = 0.83$). The choice of antidiabetic medications (insulin, sodium-glucose co-transporter-2 inhibitors, or metformin) and statins also did not significantly influence the global coagulation assay parameters in this study. The duration of diabetes diagnosis demonstrated no correlation with the main global coagulation parameters (MA: rho = −0.021, $p = 0.81$; ETP: rho = −0.007, $p = 0.93$; OHP: rho = 0.067, $p = 0.44$; Table 2).

Patients with diabetes with any complications were older (64 vs 56 years old, $p = 0.001$). These patients demonstrated more hypercoagulable global coagulation parameters with elevated thrombin peak (226.4 vs 199.9 nM, $p = 0.02$) and velocity index (64.8 vs 57.9 nM/min, $p = 0.02$) as well as higher OHP (10.8 vs 7.8, $p = 0.02$; Table 3). Those with microvascular complications only unexpectedly had more hypercoagulable thrombin generation with higher OHP and reduced OFP compared with those with macrovascular (with or without microvascular) complications. When patients with diabetes with microvascular only complications were excluded, the remaining patients with diabetes had lower ETP (1257.3 vs 1335.1 n.min, $p = 0.02$) compared with healthy controls.

3.4 | Prediction model based on global coagulation assay parameters

We evaluated if global coagulation assays could predict the presence of existing diabetic complications, with all variables included in Table 3 considered in the development of the multivariable model. The variables found to be significantly associated with complications included male gender (OR 3.08, 95% CI: 1.29–7.36), aged over 57.5 (OR 4.64, 95% CI: 1.85–11.66), MA >68.15 mm (OR 4.24, 95% CI: 1.66–10.88), velocity index >86.4 nM/min (OR 5.50, 95% CI: 1.78–16.98), OHP >7.965 units (OR 2.11, 95% CI: 0.92–4.87), and HbA1c >7.75% (OR 4.35, 95% CI: 1.72–10.97) (Table 4). The OHP variable

was not statistically significant ($p = 0.08$), but was included in the model because it slightly improved the overall performance of the risk prediction and was identified as a clinically important variable that warranted inclusion. This model provided a C-statistic of 0.82, with the assigned weighted scores for each category of variable as indicated in Table 4.

4 | DISCUSSION

T1DM and T2DM have different etiologies, although both diseases are characterized by altered insulin metabolism, dyslipidemia, endothelial dysfunction, oxidative stress, and inflammation.[25] In this study, patients with diabetes were more hypercoagulable with higher MA on TEG, increased OHP using fibrin generation, but comparable thrombin generation parameters to healthy controls. Factor VIII and von Willebrand factor antigen were significantly higher in the patients with diabetes compared with healthy controls, indicating possible underlying inflammation,[26] which can contribute to an overall prothrombotic state.[27,28]

Although both diseases are associated with dysregulation of coagulation via distinct mechanisms, few studies have looked at whether the subtypes have different influences on prothrombotic factors or global coagulation assays.[25] The TEG results showed higher MA in patients with diabetes compared with healthy controls in line with previous viscoelastic studies including one study of pediatric patients with T1DM[14] and one of patients with T2DM.[29] LY30, a marker of clot breakdown in the viscoelastic setting, was also significantly lower in patients with diabetes compared with healthy controls, with this effect more pronounced in patients with T2DM compared with patients with T1DM, suggesting hypofibrinolysis in the diabetes population consistent with previous studies.[30] The duration of diabetes therapy did not appear to influence the key global coagulation parameters in contrast to the findings by Konieczynska et al.[31]

The fibrin generation parameters, OCP and OHP, were significantly higher in patients with diabetes compared with healthy controls, with a lower OFP indicating hypercoagulability and impaired fibrinolysis in patients with diabetes. There have been limited studies looking at OHP assays in patients with diabetes, with one previous study in patients with T1DM and no known studies in patients with T2DM. Antovic et al. found increased OCP and OHP in patients with T1DM with complications, with no significant differences reported in OFP,[15] in contrast to this study. Furthermore, in this study, patients with diabetes with known complications had significantly higher OHP compared with those without. In addition, OHP did not appear to correlate well with other fibrinolytic markers such as Ly30 (rho = −0.153, $p = 0.07$) and D-dimer (rho = 0.299, $p < 0.001$), suggesting that the fibrinolytic pathway may be more complex than currently understood.[30] Other coagulation proteins involved in fibrinolysis which may be useful to investigate further include tissue plasminogen activator, plasminogen activator inhibitor-1, and plasmin generation. Another reason for the discrepancy may be due to the different methodologies between assays including Ly30 being a whole blood assay measuring viscoelastic properties, whereas OHP is performed on platelet poor plasma and directly measures fibrin formation. Furthermore, OHP is fluorogenic based, whereas D-dimer is an immunoturbidimetric assay.

Despite the changes seen in the fibrin pathways, there were no significant differences in CAT parameters between the patients and healthy controls, consistent with Beijers et al., who found only modest and nonsignificant changes in thrombin peak and ETP in their cohort of patients with T2DM.[32] These findings contrast other studies that had reported higher peak thrombin[12,13] and ETP[13] in patients with diabetes. This may be due to differences in the method of running CAT because Tripodi et al. used 1pM tissue factor, which may be more sensitive than 5pM in detecting hypercoagulability.[33] However, Kim et al., who used 5pM tissue factor, also found enhanced thrombin generation in patients with diabetes and attributed this to increased levels of coagulation factors (II, V, VII, VIII, X) and low levels of anticoagulant (protein C).[13] However, within the diabetes cohort, we found that patients with complications had higher thrombin peak and velocity index.

Similarly, we observed significant differences in global coagulations assays in macrovascular and microvascular diseases, with these markers being paradoxically lower in patients with macrovascular complications (Table 3) compared with those microvascular only complications. This includes key parameters such as a lower ETP and OHP, despite being older, increased cardiovascular risk factors, and having similar HbA1c and renal function. Similarly, aspirin and statins did not appear to impact the global coagulation assays in this study. This contradictory phenomenon is interesting given that macrovascular disease is typically associated with increased mortality.[20,21] The cause of these unexpected findings is difficult to explain. The pathophysiology behind microvascular and macrovascular disease is distinctly different and extremely complex,[34] There is a paucity of data on global coagulation assays in distinguishing between microvascular and macrovascular complications in diabetes and hence these findings require further validation. However, we postulate that this “paradox” may be due to the microvascular bed providing a larger endothelial surface area, resulting in potentially a greater volume of vasculopathy and activation of coagulation when diseased. Another potential reason for the paradoxically low global coagulation parameters, as we have previously hypothesized, could be a compensatory response of the coagulation system in response to endothelial dysfunction. We have previously described that in the healthy control population that patients with “flattened” thrombin generation curves had poorer lipid profile and were males.[18,19] Of note, the LURIC study has also previously reported that ETP was inversely associated with cardiovascular death and endothelial dysfunction.[35] This is consistent with the findings in this study in which ETP is lower in patients with diabetes compared with healthy controls when those with microvascular complications were excluded.

This study also evaluated how these parameters may best reflect the presence of existing diabetic complications, which is a novel

approach to the best of our knowledge. The parameters most associated with the presence of diabetic complications were MA, velocity index, OHP, and HbA1c in combination with age and sex (Table 4), with a c-stat of 0.82. We acknowledge that the prediction model is limited by the small sample size of this pilot study cohort. Previous studies have also reported that a consistent optimal HbA1c for diabetes complications is difficult to define because HbA1c alone is not sufficient in the risk stratification,[4,5] whereas the Framingham Heart Score was developed to predict first cardiovascular events in primary prevention setting, rather than to predict complications in patients with established risk factors.[36] Nevertheless, the receiver operating curve of 0.82 suggests that the model may improve the prediction of the presence of complications in patients with diabetes. In addition, the model is reproducible assuming other studies adopt similar preanalytical and analytical methods.

We acknowledge that this study has limitations, such as the heterogeneous sampling of patients with varying cardiovascular risk factors as well as recording bias in patient records. Furthermore, the patients were not actively investigated for previously unreported complications, and we acknowledge that it possible that patients categorized with microvascular-only complications may have silent macrovascular complications. Additionally, because blood sampling was only performed at a single time point, we were unable to evaluate any changes in coagulation profile over time. Although there were significant differences in age and gender between the healthy and diabetes population, propensity score matching was used to minimize this effect. It was not possible to have sufficient power to detect statistical significance for all variables considered in the modelling process due to the limited sample size. A larger sample size would be required to confirm the variables selected in the model and provide a validation cohort to test the predictive performance of the risk prediction model. An advantage of this study, however, is the evaluation of all three global coagulation assays in both patients with T1DM and T2DM.

5 | CONCLUSION

This study suggests that global coagulation assays may have a role in furthering our understanding of diabetes and diabetic complications. Patients with diabetes have a more hypercoagulable profile as demonstrated through TEG and OHP assays, with patients with T2DM appearing to be more hypercoagulable than in those with T1DM. Patients with known diabetic complications were also found to have more hypercoagulable global coagulation assay parameters, though those with macrovascular complications appear to have fewer hypercoagulable features. This study is ongoing to further investigate whether these tests have a clinical benefit in predicting risk of future cardiovascular events.

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RELATIONSHIP DISCLOSURE

No conflict of interest to declare. The manuscript has been read and approved by all the listed authors.

AUTHOR CONTRIBUTIONS

Hui Yin Lim was involved in the planning, recruitment, data collection and analysis and writing up the draft. Brandon Lui and Anna Kwok were involved in the recruitment, data analysis, and review of the draft. Mark Tacey was involved in the data analysis and review of the draft. Suresh Varadarajan was involved in the recruitment and review of paper. Geoffrey Donnan, Harshal Nandurkar, and Prahlad Ho were involved in the planning, data analysis and review of the draft.

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SUPPORTING INFORMATION

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