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The Stroke Riskometer™ App: Validation of a data collection tool and stroke risk predictor

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Background **The greatest potential to reduce the burden of stroke is by primary prevention of first-ever stroke, which constitutes three quarters of all stroke. In addition to population-wide prevention strategies (the 'mass' approach), the 'high risk' approach aims to identify individuals at risk of stroke and to modify their risk factors, and risk, accordingly. Current methods of assessing and modifying stroke risk are difficult to access and implement by the general population, amongst whom most future strokes will arise. To help reduce the burden of stroke on individuals and the population a new app, the Stroke Riskometer™, has been developed. We aim to explore the validity of the app for predicting the risk of stroke compared with current best methods.**

Methods **752 stroke outcomes from a sample of 9501 individuals across three countries (New Zealand, Russia and the Netherlands) were utilized to investigate the performance of a novel stroke risk prediction tool algorithm (Stroke Riskometer™) compared with two established stroke risk score prediction algorithms (Framingham Stroke Risk Score [FSRS] and QStroke). We calculated the receiver operating characteristics (ROC) curves and area under the ROC curve (AUROC) with 95% confidence intervals, Harrels C-statistic and D-statistics for measure of discrimination, R^2 statistics to indicate level of variability accounted for by each prediction algorithm, the Hosmer-Lemeshow statistic for calibration, and the sensitivity and specificity of each algorithm.**

Results **The Stroke Riskometer™ performed well against the FSRS five-year AUROC for both males (FSRS = 75·0% (95% CI 72·3%–77·6%), Stroke Riskometer™ = 74·0(95% CI 71·3%–76·7%) and females [FSRS = 70·3% (95% CI 67·9%–72·8%, Stroke Riskometer™ = 71·5% (95% CI 69·0%–73·9%)], and better than QStroke [males – 59·7% (95% CI 57·3%–62·0%) and comparable to females = 71·1% (95% CI 69·0%–73·1%)]. Discriminative ability of all algorithms was low (C-statistic ranging from 0·51–0·56, D-statistic ranging from 0·01–0·12). Hosmer-Lemeshow illustrated that all of the predicted risk scores were not well calibrated with the observed event data ($P < 0·006$).**

Conclusions **The Stroke Riskometer™ is comparable in performance for stroke prediction with FSRS and QStroke. All three algorithms performed equally poorly in predicting stroke events. The Stroke Riskometer™ will be continually developed and validated to address the need to improve the current stroke risk scoring systems to more accurately predict stroke, particularly by identifying robust ethnic/race ethnicity group and country specific risk factors.**

Key words: prevention, stroke prediction, Stroke Riskometer™ App, validation

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Introduction

Despite a steady decrease in stroke mortality over the last two decades (1), the global burden of stroke is increasing. Almost 17 million people are affected by stroke every year (68% increase from 1990) and there were 33 million stroke survivors in the world in 2010 (84% increase from 1990), many with disability (2). Unlike 30–40 years ago when most strokes occurred in people aged ≥75 years, now most (>60%) strokes affect people younger than 75 years (2). This, together with the global epidemic of major stroke risk factors (3,4), including diabetes (5) and overweight (6), suggests that the burden of stroke is likely to increase in the future, unless more effective prevention strategies are implemented.

As most (>70%) strokes are first-ever strokes, the prevention of first-ever stroke is a major priority. The two main approaches to the prevention of first-ever stroke are the population-wide

'mass' approach (reducing the level of exposure to stroke risk factors in all people in the region regardless of the individual's level of risk factors), and the individual-based 'high risk' approach. The 'high-risk' aims to identify individuals at risk of stroke (e.g. people with elevated blood pressure, dyslipidaemia, atrial fibrillation and carotid artery stenosis), and to modify their risk factors, and risk, accordingly [current methods of assessing stroke risk include two established stroke risk score prediction algorithms – the Framingham Stroke Risk Score (FSRS) (7) and QStroke (8)]. Although those with high-risk stroke benefit most from prevention strategies, the highest number of strokes and cardiovascular disease occur in people with only a mildly increased risk (9–11), mainly because there are greater numbers of people in this category of risk [according to Dalton *et al.* (12), about 90% of UK people aged 40–74 have low 10-year risk of stroke (<20%) as determined by QRisk2]. However, the general population, amongst whom most future strokes will arise, do not readily access and utilize these prediction models; the vast majority of people do not know their risk of having a stroke, do not know their risk factors, and do not know what to do about it (13–15).

Recent advances in mobile (smartphone) technologies and their worldwide use (about 1·4 billion users) offer unique opportunities to utilize these technologies for improving health and reducing burden from these disorders. Importantly, easily accessible and cost-effective risk-estimation systems are well suited to the developing world and other regions where access to medical facilities is limited (16), including elderly populations where smartphones are being increasingly used (17–19).

In recognition of the importance of e-research into noncommunicable disease (NCD) initiatives, the United Nations (UN) Economic and Social Council, the International Telecommunication Union (ITU) and the World Health Organization (WHO) have recently (June 2013) launched a new mHealth initiative for improving NCD prevention, treatment and policy enforcement (20). In order to inform and support these UN/ITU/WHO efforts, and to increase general awareness about stroke and its risk factors as well as to improve stroke and NCD prevention on an individual level, The National Institute for Stroke and Applied Neurosciences, AUT University recently developed an app called the Stroke Riskometer™. This app utilizes recent advances in risk presentation/communication (21,22), international guidelines on stroke and CVD prevention (23–28) and has the potential to significantly improve stroke and NCD prevention (29). The Stroke Riskometer™ algorithm was derived from the Framingham Stroke Risk Score (FSRS) prediction algorithm (7) and enhanced to improve accessibility and to include several additional major risk factors shown to be important for stroke, largely based on the INTERSTROKE study (4).

Endorsed by the World Stroke Organization, World Federation of Neurology and International Association on Neurology and Epidemiology, the app provides estimates of the absolute risk of stroke within the next 5 and 10 years for individuals aged ≥20 years. Importantly, the Stroke Riskometer™ provides not only their absolute risk of stroke development but also a baseline risk for comparison, thus allowing users to compare their risk of stroke with someone of the same age and gender who has no risk factors. The former represents a new paradigm for high-risk stroke prevention strategy (29), and enables a refined presentation of the traditional threshold-based approach in which people are categorized into low, moderate, and high-risk groups. This procedure enables not only those at high levels of risk, but also those at low- to moderate absolute risk, to reduce their risk of stroke. The app therefore allows a combination of both high-risk and population strategies, an approach shown to be the most effective for cardiovascular disease prevention (11).

The aim of this study was to compare the performance of the Stroke Riskometer™ prediction algorithm with two other commonly used stroke prediction algorithms – Framingham Heart Study Stroke Risk Score (FSRS) prediction algorithm (7) and QStroke (8).

Methods

Study design and data sources

Three study populations (80 308 person-years of observation in total) were used to validate the Stroke Riskometer™ algorithm: the Auckland Regional Community Stroke (ARCOS IV) 2011–2012 study (30), the Rotterdam Study (1990 – ongoing) (3,31), and Russian Cohort studies (1992 – ongoing; Dr M Kravchenko, unpublished data).

The ARCOS study is a population-based stroke register where all new stroke events (both hospitalized and nonhospitalized, fatal and nonfatal) in almost 1·2 million Auckland adult residents were prospectively ascertained using multiple overlapping sources of the information, including hospital admissions/referrals, community general practices and death certificates etc. (details of the study methodology have been described elsewhere) (30). For the purpose of the validation of the Stroke Riskometer™ we used a sub-set of ARCOS IV data on strokes in people aged 21–95 years ($n = 410$).

The Rotterdam Study has been described previously (3). It is an ongoing prospective population-based cohort study that focuses on the causes and consequences of chronic and disabling diseases in the elderly (31). The cohort started enrolment in 1990 and included 7983 participants aged ≥55 years living in Ommoord, a district of the city of Rotterdam in the Netherlands (participation rate 78%). Follow-up was complete until January 1, 2012, for 97·1% of potential person-years (32). The Rotterdam study contributed data from $n = 7713$ individuals who ranged in age from 55–90 years.

Russian cohort studies were conducted in Moscow ($n = 412$), Ulyanovsk ($n = 512$), Nal'chik ($n = 177$) and Minsk ($n = 277$) over various time periods starting from 1992. Study participants (men and women; age range 39–66 years) were followed up from 12 years (Moscow) to four-years (Ulyanovsk, Nal'chik and Minsk). The World Health Organization stroke diagnostic criteria (33) were used and a diagnosis of stroke was confirmed by a study neurologist across all these studies (over 90% of stroke patients had brain neuroimaging to establish a pathological type of stroke). All these studies have been approved by the local Ethics Committees.

Stroke risk factors and algorithm development

Risk scores from three stroke predictors were generated. Each scoring algorithm utilized a series of known or hypothesized stroke risk factors (Table 1), some of which are in addition to those used in the FSRS and are the central targets in the new WHO Global Action Plan for the NCD 2013–2020 (34). Distribution of each risk factor for each data set is listed in Table 2. The Stroke Riskometer™ algorithm was derived from the Framingham Stroke Risk Score (FSRS) prediction algorithm (7) but enhanced to include several additional major risk factors shown to be important for both ischaemic and haemorrhagic strokes, largely based on the INTERSTROKE study (4). The additional variables are listed in Table 1. Questions were based on recall such as 'Have you ever been told by a doctor that you have atrial fibrillation (irregular heartbeats)?' and 'Have you ever been told by a doctor that you have left ventricular hypertension?' such that no immediate medical test (e.g. an ECG is required) in order for users to provide an answer. These questions have been used and validated in cross-sectional studies (4). Beta-coefficients for each additional variable were derived from current literature and discussed amongst by a panel of stroke and health experts of the Stroke Riskometer™ Collaboration. Based on these discussions and available evidence, the following risk scores were added to the FSRS (7) risk score: 0·20 for being non-Caucasian (23,35), 0·20 for poor diet (i.e., consuming less than six servings of fruits and vegetables per day) (4), 0·10 for high alcohol consumption (i.e., consuming two or more standard drinks per day) (4,36,37), 0·10 for low physical activity (i.e., less than 2·5 hours per week) (15,23), 0·05 for family history of stroke or heart attack (23,38–41), 10 (for 5-year risk) and 15 (for 10-year risk) for previous stroke or transient ischaemic attack (TIA) (42), 1·80 for any cognitive problems and 1·40 for memory problems but no cognitive issues (43), 1·20 for previous traumatic brain injury (44), 0·20 plus 0·10 for any unit (0·01) increase in waist-to-hip ratio above 0·96 for males and 0·80 for females (45). In the absence of waist-to-hip ratio data we used BMI and scored 1·02 plus 0·10 for every unit (1 kg/m^2) above 24 kg/m^2 for Chinese, or above 23 kg/m^2 for South Asians or above 25 kg/m^2 for all other ethnicities (46) [different cut-off criteria for Chinese people were based on recommendations from the Chinese National Centre for Cardiovascular Disease (W. Wang, personal communication)]. In the absence of both waist-to-hip ratio and BMI data, waist circumference measures can be used adding 1·02 per unit (1 cm) above 103 cm for males and 89 cm for females (45). As each of the additional risk factors was added to the algorithm separately without taking into account interactions between the risk factors, we applied conservative beta-estimates to reduce the chance of overestimating the stroke risk (47,48). Algorithm testing prior to the app launch used a number of different methods. A very large number of hypothetical cases (many hundreds of different combination of risk factors) were entered into the tool to identify problems requiring resolution before clinical use. The tool then underwent clinical evaluation by stroke experts and general practitioners to compare the estimated 5-year and 10-year risk.

Table 1 Stroke Riskometer™ variables

Variables	Definition
Age*	In years
Gender*	Males or Females
SBP*	Systolic blood pressure measured in mm/Hg
Antihypertensive treatment*	Any blood pressure lowering medications or antihypertensive medicines No = 0, Yes = 1
Diabetes*	Yes = 1, No = 0
CVD risk*	History of CVD (heart attack or peripheral artery disease) Yes = 1, No = 0
Smoking status*	Never, Ex-Smoker, Current
Atrial fibriliation*	Yes = 1, No = 0
Left ventricular hypertrophy by ecg*	Yes = 1, No = 0
Family history of stroke or heart attack*	Yes = 1, No = 0
Alcohol consumption	More than 2 standard drinks per day.
Stress	Significant stress as determined by the patient. Diagnosis of anxiety or depression.
Low physical activity	Less than 2·5 hours per week.
Waist to hip ratio (WHR)	In males, if WHR > 0·96 then add 0·20 + 0·10 for every unit (0·01) above this threshold In females, if WHR > 0·80 then add 0·20 + 0·10 for every unit (0·01) above this threshold
Non-Caucasian	Caucasian = 0, Non-Caucasian = 1
Poor diet	Less than six servings of fruit and vegetable per day = 1, More than or equal to six servings of fruit and vegetables per day = 0
Cognitive problems or dementia	Yes = 1, No = 0
Poor memory	No cognitive problems but has poor memory Yes = 1, No = 0
Previous TBI	Previous Traumatic Brain Injury Yes = 1, No = 0
BMI	If WHR not available. We added 0·10 for every unit (1) above 24 kg/m^2 for Chinese, or above 23 kg/m^2 for South Asians or above 25 kg/m^2 for all other ethnicities
Waist circumference	If WHR and BMI not available. We added 1·02 per unit (1 cm) above 103 cm waist circumference for males and 89 cm for females

Variables denoted with an asterix (*) comprise the existing Framingham Stroke Risk Score (FSRS) algorithm where the beta-coefficients differ for males and females. Variables in bold are new additions to the Stroke Riskometer™.

Table 2 Baseline characteristics of the validation cohorts (ARCOS, RUSSIA and ROTTERDAM) and which variables are required for each of the three risk score algorithms being assessed

			Data set					
			ARCOS ($n = 410$)		RUSSIA ($n = 1378$)		ROTTERDAM ($n = 7713$)	
Algorithm	Variables		Males	Females	Males	Females	Males	Females
F, R, Q	Age (years)		68·8 (13·2)	72·4 (15·7)	50·3 (6·2)	50·6 (6·4)	69·0 (8·7)	71·7 (10·2)
F, R, Q	SBP (mmHg)	Mean (SD)	156·8 (30·1)	157·3 (29·9)	135·8 (19·4)	130·8 (21·1)	138·7 (21·8)	140·0 (22·8)
R	Waist-to-hip ratio		0·9 (0·1)	0·9 (0·1)				
F, R, Q	BMI (kg/m^2)				27·8 (4·3)	27·5 (5·4)	25·6 (2·9)	26·7 (3·7)
R	Waist circumference (cm)		97·2 (15·9)	99·3 (14·5)				
		Categories	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
	Stroke Event		216 (100%)	194 (100%)	21 (4·4%)	24 (2·7%)	268 (8·6%)	407 (8·3%)
F, R, Q	Anti-hypertensive medication		172 (79·6%)	154 (79·4%)	162 (33·8%)	321 (35·8%)	915 (29·5%)	1728 (35·5%)
F, R	Diabetes		42 (19·8%)	39 (20·3%)	21 (4·4%)	43 (4·8%)	184 (6·1%)	331 (7·1%)
F, R, Q	Atrial Fibrillation		59 (27·3%)	87 (44·9%)	47 (9·8%)	69 (7·7%)	170 (5·5%)	208 (5·1%)
F, R, Q	Left ventricular hypertrophy		0 (0%)	0 (0%)	101 (26·8%)	130 (19·2%)	144 (4·8%)	194 (4·9%)
F, R, Q	History of CVD		100 (46·5%)	93 (47·9%)	57 (11·9%)	95 (10·6%)	752 (24·2%)	903 (18·5%)
R	Previous stroke or TIA		66 (30·6%)	62 (32·0%)	33 (6·9%)	33 (3·7%)	407 (13·1%)	704 (14·4%)
Q	Rheumatoid arthritis						26 (2·4%)	92 (4·7%)
Q	Chronic kidney disease						339 (16·8%)	661 (20·3%)
Q	Chronic coronary heart failure		22 (10·4%)	21 (11·0%)				
R	Cognitive problems or dementia						114 (3·9%)	368 (8·0%)
R	Poor memory						586 (19·2%)	1010 (21·6%)
R	Previous TBI						1071 (35·3%)	1270 (27·4%)
F, R, Q	Family history of stroke/heart attack		133 (61·6%)	112 (57·8%)	240 (49·9%)	492 (54·9%)	1548 (49·9%)	2523 (51·7%)
R, Q	Non-European		46 (21·3%)	46 (23·7%)	0 (0%)	0 (0%)	40 (1·4%)	56 (1·3%)
R, Q	Race-ethnicity	European	170 (78·7%)	148 (76·3%)	481 (100%)	897 (100%)	2846 (98·6%)	4281 (98·7%)
		Maori	5 (2·3%)	10 (5·2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		Pacific	9 (4·2%)	20 (10·3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		Chinese	7 (3·2%)	4 (2·1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		South Asian	8 (3·7%)	3 (1·6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		Other	17 (7·9%)	9 (4·6%)	0 (0%)	0 (0%)	40 (1·4%)	56 (1·3%)
R	Poor diet		102 (47·2%)	104 (53·6%)			1362 (62·2%)	1808 (56·7%)
R	Low physical activity		176 (81·5%)	154 (79·4%)	224 (46·7%)	430 (47·9%)		
R	High alcohol consumption		118 (54·6%)	104 (53·6%)	106 (22·0%)	14 (1·6%)		
R	Experienced stress		52 (24·1%)	39 (20·1%)	141 (29·5%)	470 (52·8%)		
F, R	Smoking	Yes	114 (52·8%)	88 (45·4%)	202 (42·0%)	137 (15·3%)	920 (29·63%)	805 (16·50%)
Q	Smoking status	Ex-Smoker/Light	77 (35·8%)	69 (36·1%)	117 (24·3%)	82 (9·1%)	2058 (66·28%)	1503 (30·81%)
		Current-Smoker/Moderate	34 (15·8%)	17 (8·9%)	18 (3·7%)	9 (1·0%)	294 (9·47%)	318 (6·52%)
		/Heavy			202 (42·0%)	137 (15·3%)	270 (8·69%)	216 (4·43%)

Important new variables required for the Stroke Riskometer™ algorithm but were not present in the data set assessed are represented by shaded grey boxes.
F, Framingham Stroke Risk Score (FSRS), R, Stroke Riskometer™, Q, Qstroke.

Algorithm validation

The performance of the Stroke Riskometer™ was tested across three data sets (ARCOS, Russian and Rotterdam) as greater precision is gained when assessing risk prediction models using multiple epidemiologic studies compared to single-studies (49). We also compared performance of the Stroke Riskometer™ with the FSRS (7) and QStroke (8) risk score equations. The five-year estimated risk of stroke for Russian and Rotterdam cohorts was calculated across the three different prediction algorithms. Estimates for 10-year stroke risk score were generated only for the Rotterdam study where data were available over a span of 10 years. Follow-up data for the ARCOS were limited to one-year and for Russian data sets – 4 to 12 years. We calculated Harrels C-statistic and Somer's D-statistic to measure discrimination (the ability of the algorithms to discriminate between stroke and nonstroke events). C-statistic values of 0·50 represent chance and 1 denotes the ability of the risk score to discriminate perfectly. D-statistics over 0·10 indicate that the risk score has a good ability to differentiate between an event and nonevent. Receiver operating characteristics (ROC) curve, Area Under the ROC Curve (AUROC) with 95% confidence intervals within each data set, sensitivity and specificity of each algorithm were also analyzed. R^2 statistic was calculated to indicate the level of variability accounted for by each prediction algorithm. Calibration was assessed using the H-L test (for goodness-of-fit statistics to examine differences between the observed and predicted risks from each algorithm) All analyses were performed in R (version 3·0·2) (50).

Results

Validation cohorts

A total of 752 new strokes that developed in a sample of 9501 individuals over the follow-up period (80 308 person-years of observation) across three studies (ARCOS, Russia and Rotterdam) were utilized to investigate the recently derived stroke risk prediction tool algorithm Stroke Riskometer™ against two established stroke risk score prediction algorithms [FSRS (7) and QStroke (8)]. The three data sets differed in their distribution of stroke outcomes and predictor variables required for each algorithm. The ARCOS data set was comprised of stroke only data whilst the Russian database was generated through a new cohort study, with 3·2% total strokes being observed. Of the Rotterdam study, 8·4% was comprised of strokes.

None of the three studies had all variables required for the Stroke Riskometer™ algorithm. The Russian data set was the most recent of the three data sets analyzed here so the average age was lowest (50 years for males and females; Table 2). Individuals in ARCOS and the Rotterdam study were similar in age (males 69 years and females 72 years). The Russian data set had the lowest average systolic blood pressure (SBP) while ARCOS had the highest. Both the Rotterdam and ARCOS studies had similar SBP values for males and females whilst the Russian data set had higher values in males. BMI was not recorded in ARCOS but was similar in males and females of the Russian data set (average for males = 27·8 kg/m^2 and females = 27·5 kg/m^2) and comparable with males and females in the Rotterdam study (average for males = 25·6 kg/m^2 and females = 26·7 kg/m^2). As BMI was not recorded in ARCOS waist circumference was used (for the Stroke Riskometer™ algorithm); women had greater waist circumference (99 cm) than men (97 cm). Due to inclusion of only patients with stroke, the ARCOS database had the highest percentage of individuals with diabetes (20%) compared to the Russian data set (range 4·4–4·8%) and Rotterdam (range 6·1–7·1%). A much higher proportion of the ARCOS database had individuals with a history of CVD, previous stroke/TIA event and were of non-European descent, compared to the Russian and Rotterdam cohorts (Table 2).

Validation and overall performance of the Stroke Riskometer™

As none of the three studies had all variables required for the Stroke Riskometer™ algorithm, we cannot fully validate this algorithm with the emphasis for the continuing development of the Stroke Riskometer™ algorithm. We present measures of overall performance, discrimination and calibration of the Stroke Riskometer™ algorithm based on available data. The FSRS and Stroke Riskometer™ algorithms gave comparable 5-year and 10-year risk scores for males and females within each data set (Fig. 1). Risk scores differed substantially by data set, reflecting the availability of predictors within each cohort. Each algorithm (FSRS, Stroke Riskometer™ and QStroke) explained 50% of the variation observed in the ARCOS data set (R^2 statistic, Table 3). With fewer stroke outcomes in the Russian and Rotterdam data sets, the reported R^2 was low across all cohorts for all algorithms, ranging from 0·31–5·22% (Table 3).

Discrimination

All three algorithms showed poor discriminative ability across each cohort (C-statistic range 0·50–0·53, D-statistic <0·05, Table 3). The ROC curves (Fig. 2) show that the FSRS and Stroke Riskometer™ algorithms behaved similarly for 5-year and 10-year risk scores for males and females, with area under the ROC curves ranging between 61% and 66% in the Rotterdam cohort (Fig. 2, Table 3). The QStroke algorithm outperformed the FSRS and Stroke Riskometer™ algorithms (Table 3).

When all three data sets (ARCOS, Russia and Rotterdam) were combined the Stroke Riskometer™ and FSRS algorithms had higher five-year AUROC values for males [FSRS AUROC = 75·0 (95% CI 72·5%–77·6%), Stroke Riskometer™ AUROC = 74·0% (95% CI 71·3%–76·7%)], for both FSRS and Stroke Riskometer™ C-statistic = 0·56 and D-statistic = 0·12) and females (FSRS AUROC = 70·3% (95% 67·9%–72·8%), Stroke Riskometer™ AUROC = 71·5% (95% CI 69·0%–73·9%), for both FSRS and Stroke Riskometer™ C-statistic = 0·54 and D-statistic = 0·08). There was no difference in the AUROC between the FSRS and Stroke Riskometer™ AUROC (DeLong's for correlated ROC curves; males $P = 0·013$, females $P = 0·140$). AUROC for QStroke were considerably lower (males AUROC = 59·7% (95% CI 57·3%–62·0 %), C-statistic = 0·52, D-statistic = 0·04 and for females AUROC = 71·1% (95% CI 69·0%–73·1%), C-statistic = 0·54, D-statistic = 0·08) (Fig. 2). A statistically significant difference in the AUROC between the QStroke and Stroke Riskometer™ was observed (DeLong's test for correlated ROC curves; males $P < 0·0001$, females $P = 0·779$).

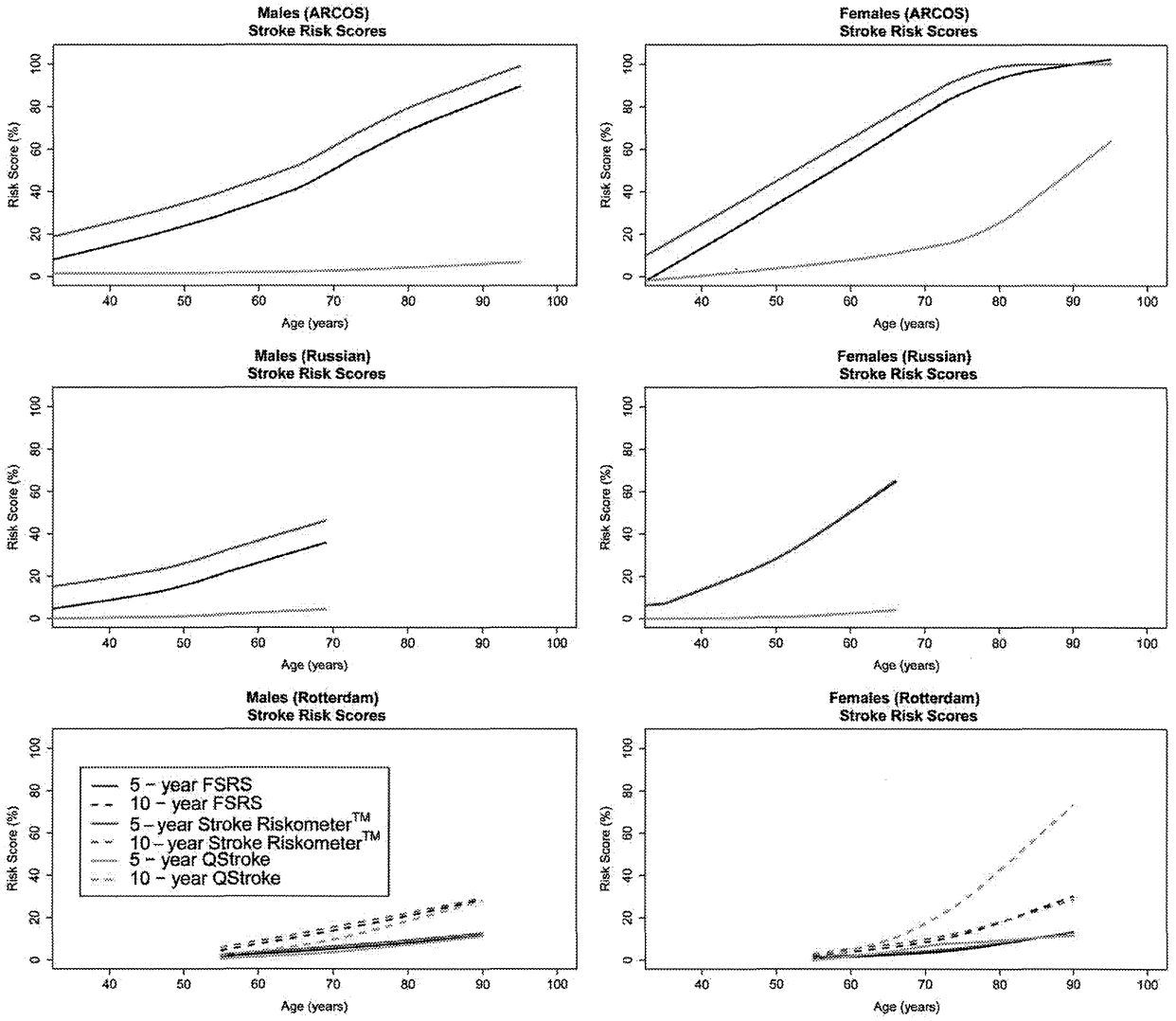

Fig. 1 Mean predicted risk score by age for Framingham Stroke Risk Score (FSRS) (black), Stroke Riskometer™ (red) and QStroke (green) for five-years for males and females.

Classification, sensitivity and specificity

Mean predicted stroke risk scores were on average higher in the group of observed stroke outcomes compared to individuals with no stroke outcome (Supplementary Fig. S1). Sensitivity and specificity was calculated for FSRS, Stroke Riskometer™ and QStroke predicted risk scores, which reached accuracy threshold of 50%, 70%, 80%, 85% and 90% (Table 4). The predicted risk scores were then categorized into 'High' risk (based on reaching 80% accuracy and >80% specificity, Table 4) and 'Low' otherwise, which were compared against each other (Table 5). Sensitivity for QStroke in males was low (10·6% for accuracy = 80%) compared to FSRS and Stroke Riskometer™ which had a sensitivity = 53·9% (FRSR) and 52·3% (Stroke Riskometer™) for accuracy = 80%.

In the Russian database we observed that both the Stroke Riskometer™ and FSRS algorithms classified most participants as high risk (63·6% five-year risk in males and 78·4% five-year risk in females). As ARCOS had all stroke events we would expect these to predominately to be categorized as 'High risk' this is observed for FSRS and Stroke Riskometer™ (males = 98·1% and females = 86·4%). A very high proportion of individuals in the ARCOS data set were classified as high risk for Stroke Riskometer™ but low risk on QStroke (males = 97·2% and females = 48·1% for five-year risk). A high proportion of females in the Rotterdam study were categorized as low-risk for Stroke Riskometer™ and high-risk for QStroke (13·2% for five-year risk in females) compared to 5·5% of males classified as low-risk for Stroke Riskometer™ and high-risk for QStroke (Table 5).

Calibration

Calibration plots of the predicted risk scores against the observed event for each tenth of predicted risk, separately for males and females, are shown in Fig. 3 (all data sources) and Supplementary Fig. S2 (Russian and Rotterdam cohorts). The Russian cohort illustrated that the QStroke algorithm was better calibrated for

Table 3 Validation statistics for Framingham Stroke Risk Score (FSRS), Stroke Riskometer™ and the Qstroke algorithm across all validation cohorts (ARCOS, RUSSIA and ROTTERDAM). Harrels C-statistic and Somer's D-statistic to measure discrimination (the ability of the algorithms to discriminate between stroke and nonstroke events). C-statistic values of 0·50 represent chance and 1 denotes the ability of the risk score to discriminate perfectly. D-statistics over 0·10 indicate that the risk score has a good ability to differentiate between an event and nonevent. AUROC = Area Under the Receiver operating characteristics Curve (AUROC) with 95% confidence intervals. R^2 statistic was calculated to indicate the level of variability accounted for by each prediction algorithm

			ARCOS		RUSSIA		ROTTERDAM	
			Mean (95% CI)		Mean (95% CI)		Mean (95% CI)	
Algorithm	Year	Statistic	Males	Females	Males	Females	Males	Females
FSRS	5	R^2 (%)	49·85 (49·73–50·08)	49·90 (49·88–49·92)	0·99 (0·08–3·14)	0·32 (0·001–1·54)	0·72 (0·21–1·81)	1·85 (0·89–3·40)
		C statistic	Stroke event data only		0·515 (0·514–0·516)	0·506 (0·505–0·506)	0·511 (0·511–0·511)	0·511 (0·511–0·512)
		D statistic			0·030 (0·029–0·031)	0·011 (0·011–0·011)	0·022 (0·021–0·022)	0·023 (0·023–0·023)
		AUROC			68·1 (58·5–77·7)	60·1 (49·1–72·0)	63·0 (57·9–68·0)	64·7 (60·1–69·4)
	10	R^2 (%)					0·91 (0·34–1·85)	2·05 (1·17–3·23)
		C statistic					0·518 (0·517–0·518)	0·521 (0·521–0·521)
		D statistic					0·035 (0·035–0·035)	0·042 (0·042–0·043)
		AUROC					61·2 (57·6–64·8)	64·2 (61·0–67·3)
Stroke Riskometer™	5	R^2 (%)	49·85 (49·73–50·08)	49·90 (49·88–49·92)	0·99 (0·08–3·14)	0·32 (0·001–1·54)	0·72 (0·21–1·81)	1·85 (0·89–3·40)
		C statistic	Stroke event data only		0·515 (0·514–0·516)	0·514 (0·513–0·514)	0·511 (0·511–0·511)	0·513 (0·512–0·513)
		D statistic			0·030 (0·029–0·031)	0·029 (0·028–0·029)	0·022 (0·022–0·023)	0·027 (0·026–0·027)
		AUROC			68·1 (58·5–77·7)	77·4 (69·2–85·6)	63·6 (58·5–68·5)	65·4 (61·0–69·7)
	10	R^2 (%)					0·91 (0·34–1·85)	0·91 (0·34–1·85)
		C statistic					0·517 (0·517–0·517)	0·522 (0·521–0·522)
		D statistic					0·033 (0·032–0·033)	0·045 (0·044–0·045)
		AUROC					60·4 (58·8–64·0)	64·6 (61·6–67·6)
QStroke	5	R^2 (%)	49·79 (49·73–50·3)	49·98 (49·88–50·04)	5·22 (1·54–14·22)	2·49 (0·24–9·77)	1·04 (0·43–2·13)	1·26 (0·60–2·36)
		C statistic	Stroke event data only		0·526 (0·524–0·527)	0·511 (0·511–0·512)	0·513 (0·513–0·513)	0·515 (0·515–0·515)
		D statistic			0·051 (0·050–0·052)	0·023 (0·022–0·023)	0·027 (0·027–0·027)	0·031 (0·030–0·031)
		AUROC			80·6 (72·3–88·9)	71·2 (59·2–83·8)	66·1 (61·9–70·2)	69·7 (66·3–73·1)
	10	R^2 (%)					0·97 (0·41–1·92)	0·97 (0·41–1·92)
		C statistic					0·520 (0·519–0·520)	0·526 (0·526–0·526)
		D statistic					0·039 (0·039–0·039)	0·053 (0·053–0·053)
		AUROC					62·5 (59·4–65·7)	67·6 (65·0–70·1)

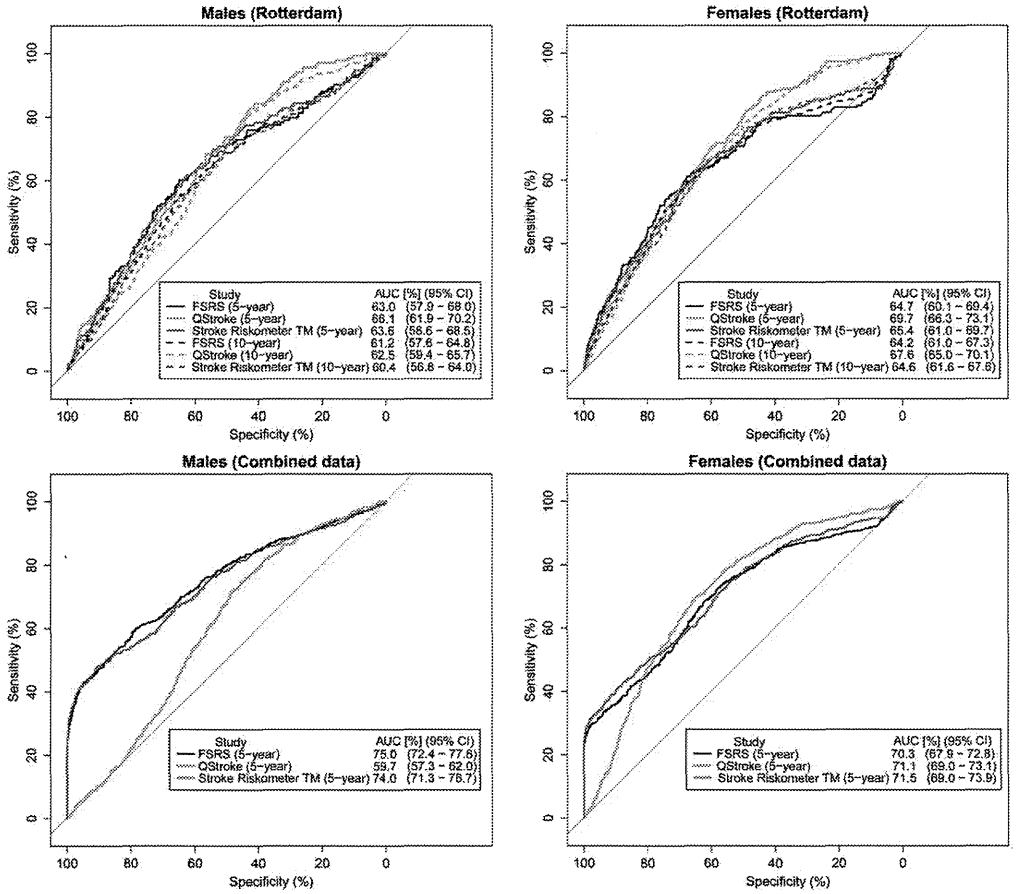

Fig. 2 Receiver-operating characteristic (ROC) curves for Framingham Stroke Risk Score (FSRS) (black), Stroke Riskometer™ (red) and QStroke (green) algorithms for 5 and 10-year risks.

the data set of all three algorithms, for a database with few strokes (Supplementary Fig. S2). An improved calibration for the FSRS and Stroke Riskometer™ algorithms compared to QStroke was observed for the Rotterdam data set, particularly for females. The QStroke algorithm was shown to over-estimate stroke risk in females whilst FSRS and Stroke Riskometer™ over-estimated stroke risk in males (Supplementary Fig. S2). Visual assessment of five-year risk scores from the combined data (ARCOS, Russia and Rotterdam) highlighted that the Stroke Riskometer™ algorithm was better calibrated compared to QStroke, especially for females (Fig. 3). All predicted risk scores were not well calibrated to our data sets (Table 6, H-L tests $P < 0{\cdot}006$).

Discussion

The Stroke Riskometer™ is comparable in performance to two widely used stroke risk scoring systems. The variation found in our study may be due to several factors. The higher R^2 values for ARCOS are explained by the high number of stroke outcome data available. Many variables required for the QStroke algorithm were not available within the ARCOS data set (such as rheumatoid arthritis, chronic kidney disease, Table 2) therefore it is likely that the QStroke risk scores we observed under-estimate stroke risk, particularly amongst males in ARCOS. A large proportion of females in ARCOS were classified as high risk in both Stroke Riskometer™ and QStroke scoring (>50%, Table 5). Conversely, the Rotterdam study had a more complete data set of variables required for the QStroke algorithm calculation (Table 2), however this appears to have led to over-estimation of the stroke risk amongst females (Fig. 1, Supplementary Fig. S2C) and an inconsistency across predicted risk scores with 13% categorized as low-risk for Stroke Riskometer™ and high-risk for QStroke (Table 5). Sensitivity was low for the QStroke risk scores generated for males (10·6% for 5-year and 8·9% for 10-year risk scores) and females (35·9% for 5-year and 36·7% for 10-year risk scores, Table 4), when specificity was high (= 80%, Table 4) compared to the sensitivity for FSRS and Stroke Riskometer™ for males (53% for 5-year and 10-year risk scores) and females (42% for 5-year and 10-year risk scores, Table 4) for FSRS, and (45% for 5-year and 10-year risk scores, Table 4 for Stroke Riskometer™, when specificity was high (= 80%, Table 4). The developers of QStroke have previously highlighted that their algorithm over-predicts stroke risk in females (8). It should also be noted that QStroke was developed for predicting ischaemic stroke specifically, and not for predicting any type of stroke as developed for Stroke Riskometer™ and FSRS.

Table 4 Performance of risk score algorithms (Framingham Stroke Risk Score (FSRS), Stroke Riskometer™ and QStroke) across three validation cohorts (ARCOS, RUSSIA and ROTTERDAM) combined across different thresholds meeting 50%, 70%, 80%, 85% and 90% accuracy

		5-year risk				10-year risk			
Algorithm	Subset	Threshold [Accuracy (%)]	Number classified as high risk (%)	Sensitivity (%)	Specificity (%)	Threshold [Accuracy (%)]	Number classified as high risk (%)	Sensitivity (%)	Specificity (%)
FSRS	Males	4·25 (50)	2202 (58·6)	82·49	45·02	11·3 (50)	2200 (58·51)	82·49	45·08
		8·6 (70)	1273 (33·9)	63·38	70·61	22 (70)	1270 (33·78)	63·38	70·70
		13 (80)	807 (21·5)	53·92	83·45	32 (80)	798 (21·22)	53·72	83·73
		18 (85)	538 (14·3)	46·28	90·56	42 (85)	542 (14·41)	46·48	90·47
		30 (90)	245 (6·5)	35·21	97·85	65 (90)	227 (6·04)	33·80	98·19
	Females	3·2 (50)	3190 (55·6)	78·89	47·04	8 (50)	3185 (55·52)	78·89	47·16
		9·5 (70)	1801 (31·4)	55·28	71·30	23 (70)	1770 (30·85)	54·44	71·84
		19·5 (80)	1055 (18·4)	42·04	84·33	42 (80)	1064 (18·55)	42·38	84·19
		28 (85)	677 (11·8)	35·51	90·92	57 (85)	672 (11·71)	35·51	91·02
		42 (90)	361 (6·3)	30·15	96·44	79 (90)	304 (5·30)	29·65	97·49
Stroke Riskometer™	Males	5·7 (50)	2184 (58·1)	81·49	45·42	13 (50)	2223 (59·12)	82·90	44·50
		14·5 (70)	1218 (32·4)	59·56	71·71	30 (70)	1225 (32·58)	61·37	71·81
		21·5 (80)	770 (20·5)	52·31	84·37	43 (80)	789 (20·98)	52·52	83·82
		27 (85)	515 (13·7)	46·48	91·30	55 (85)	514 (13·67)	46·08	91·27
		45 (90)	188 (5·0)	31·79	99·08	72 (90)	279 (7·42)	38·43	97·30
	Females	4·5 (50)	3212 (56·0)	80·07	46·75	10 (50)	3219 (56·12)	80·40	46·67
		13·5 (70)	1803 (31·4)	55·61	71·33	27 (70)	1787 (31·15)	56·28	71·70
		22 (80)	1069 (18·6)	44·89	84·34	45 (80)	1080 (18·83)	45·06	84·17
		29 (85)	734 (12·8)	39·03	90·20	57 (85)	745 (12·99)	39·20	90·00
		45 (90)	336 (5·9)	31·66	97·08	77 (90)	373 (6·50)	33·00	96·52
QStroke	Males	2·5 (50)	2130 (56·6)	73·44	45·91	6·7 (50)	2090 (55·59)	72·43	46·98
		5·3 (70)	910 (24·2)	26·76	76·19	13·5 (70)	912 (24·25)	26·96	76·16
		8·6 (80)	357 (9·5)	10·06	90·59	22 (80)	325 (8·64)	8·85	91·39
		14 (85)	77 (2·0)	2·21	97·98	33 (85)	78 (2·07)	2·21	97·95
	Females	2·4 (50)	3270 (57·0)	84·25	46·13	6·3 (50)	3269 (56·99)	84·25	46·15
		7·7 (70)	1806 (31·5)	59·13	71·68	19 (70)	1822 (31·76)	59·63	71·43
		23 (80)	978 (17·1)	35·85	85·08	48 (80)	1012 (17·64)	36·68	84·52
		70 (85)	391 (6·8)	12·56	93·80	95 (85)	422 (7·36)	13·74	93·33

Table 5 Comparing the scoring of the three risk score algorithms as 'High' or 'Low' risk for Framingham Stroke Risk Score (FSRS), Stroke Riskometer™ and the Qstroke algorithm across all validation cohorts (ARCOS, RUSSIA and ROTTERDAM). Thresholds for 'High' risk in each algorithm for males and females was selected for 80% accuracy and >80% specificity (Table 4)

			Number of patients (%)					
Algorithm	Comparison	Subset	RUSSIA		ARCOS		ROTTERDAM	
Stroke Riskometer™ vs. FSRS			5-year risk	10-year risk	5-year risk	10-year risk	5-year risk	10-year risk
Low risk on Stroke Riskometer™	Low risk on FSRS	Males	20 (4·16%)		0 (0·00%)		2410 (78·63%)	2522 (82·28%)
High risk on Stroke Riskometer™	Low risk on FSRS		155 (32·22%)		3 (1·40%)		275 (8·97%)	163 (5·32%)
Low risk on Stroke Riskometer™	High risk on FSRS		0 (0·00%)		1 (0·47%)		17 (0·55%)	6 (0·20%)
High risk on Stroke Riskometer™	High risk on FSRS		306 (63·62%)		210 (98·13%)		363 (11·84%)	374 (12·20%)
Low risk on Stroke Riskometer™	Low risk on FSRS	Females	190 (21·18%)		3 (1·40%)		4114 (88·51%)	4188 (90·10%)
High risk on Stroke Riskometer™	Low risk on FSRS		1 (0·11%)		6 (2·80%)		194 (4·17%)	119 (2·56%)
Low risk on Stroke Riskometer™	High risk on FSRS		0 (0·00%)		0 (0·00%)		3 (0·00%)	0 (0·00%)
High risk on Stroke Riskometer™	High risk on FSRS		703 (78·37%)		185 (86·45%)		337 (7·25%)	341 (7·34%)
Stroke Riskometer™ vs. QStroke			RUSSIA		ARCOS		ROTTERDAM	
Low risk on Stroke Riskometer™	Low risk on QStroke	Males	20 (4·16%)		0 (0·00%)		2258 (73·67%)	2346 (76·54%)
High risk on Stroke Riskometer™	Low risk on QStroke		439 (91·27%)		208 (97·20%)		376 (12·27%)	279 (9·10%)
Low risk on Stroke Riskometer™	High risk on QStroke		0 (0·00%)		1 (0·47%)		47 (5·51%)	182 (5·94%)
High risk on Stroke Riskometer™	High risk on QStroke		22 (4·57%)		5 (2·34%)		262 (8·55%)	258 (8·42%)
Low risk on Stroke Riskometer™	Low risk on QStroke	Females	190 (21·18%)		3 (1·40%)		3505 (75·41%)	3557 (76·53%)
High risk on Stroke Riskometer™	Low risk on QStroke		686 (76·48%)		103 (48·13%)		168 (3·61%)	116 (2·50%)
Low risk on Stroke Riskometer™	High risk on QStroke		0 (0·00%)		0 (0·00%)		612 (13·17%)	631 (13·58%)
High risk on Stroke Riskometer™	High risk on QStroke		18 (2·01%)		88 (41·12%)		363 (7·81%)	344 (7·40%)

For FSRS: Male 5-year = 13·0%, Male 10-year = 32·0%, Female 5-year = 19·5%, Female 10-year = 42·0%. For Stroke Riskometer™: Male 5-year = 21·5%, Male 10-year = 43·0%, Female 5-year = 22·0%, Female 10-year = 45·0%. For QStroke: Male 5-year = 8·6%, Male 10-year = 22·0%, Female 5-year = 23·0%, Female 10-year = 48·0%.

Whilst the discriminative abilities of all three algorithms across all data sets appeared to be comparable, they were also very low (C-statistic ranging from 0·51–0·56, D-statistic ranging from 0·01–0·12). H-L calibration statistics illustrated that all of the predicted risk scores did not align well to observed event data, $P < 0·006$. This may be due to the QStroke risk score algorithms being developed from UK-based data and while the data sets being utilized here are predominately European, they were not UK-based individuals. The FSRS has been externally validated in several different European cohorts but with inconsistent result, some studies attaining appropriate levels of discrimination but over-estimation of risk of stroke (51), however other studies have shown the FSRS has poor discrimination and under-estimates stroke risk (52). QStroke was recently created and validated in a subset of the British cohort data used to develop their algorithm and showed good levels of discrimination; however the authors did acknowledge a tendency to overestimate female stroke risk (8). In a large cohort of black and white adults the FSRS overestimated observed stroke rates, particularly in certain ethnic subgroups where the FSRS suggested there should be approximately twice as many strokes occurring than was detected (53).

This indicates that there is still a need to improve current stroke risk scoring systems to more accurately predict stroke risk across different populations/countries. We have shown that there is a level of overlap in the variables considered in these algorithms, however it may be that the weights assigned to each risk factor need to be generated to be country/or ethnic-specific as some risk factors may hold more importance in some groups compared to

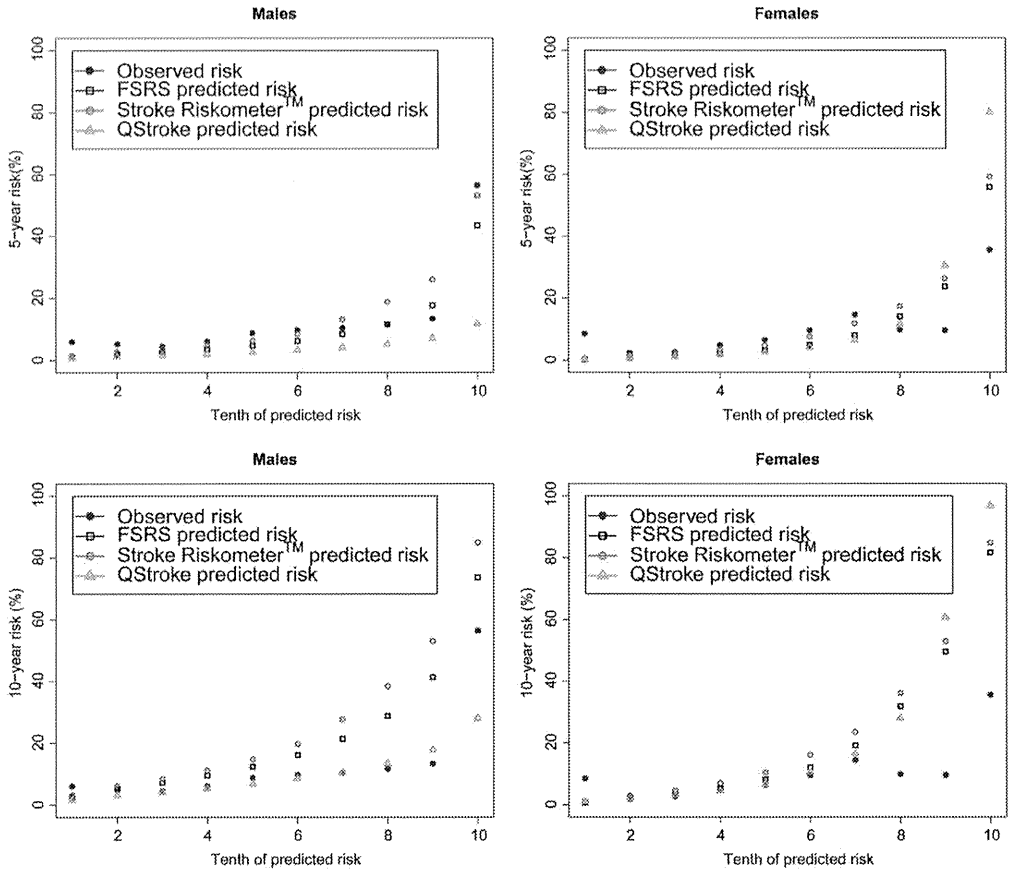

Fig. 3 Mean predicted risk (%) vs. observed stroke events in deciles of predicted risk for Framingham Stroke Risk Score (FSRS) (black), Stroke Riskometer™ (red) and QStroke (green) algorithms.

Table 6 Performance of the goodness-of-fit of each algorithm reported as the Hosmer-Lemeshow calibration statistic for Framingham Stroke Risk Score (FSRS), Stroke Riskometer™ and QStroke against observed stroke events at 5-years for the Russian and 5-years and 10-years for the Rotterdam and combined (ARCOS, Russia and Rotterdam data set)

			Hosmer-Lemeshow Test	
Data	Risk score	Subset	5-year risk	10-year risk
RUSSIA	FSRS	Females	$\chi2 = 58{\cdot}12$, $P \leq 0{\cdot}0001$	
		Males	$\chi2 = 133{\cdot}65$, $P \leq 0{\cdot}0001$	
	Stroke Riskometer™	Females	$\chi2 = 321{\cdot}92$, $P \leq 0{\cdot}0001$	
		Males	$\chi2 = 36{\cdot}84$, $P \leq 0{\cdot}0001$	
	QStroke	Females	$\chi2 = 3{\cdot}33$, $P = 0{\cdot}912$	
		Males	$\chi2 = 318{\cdot}81$, $P \leq 0{\cdot}0001$	
Rotterdam	FSRS	Females	$\chi2 = 69{\cdot}95$, $P \leq 0{\cdot}0001$	$\chi2 = 222{\cdot}02$, $P \leq 0{\cdot}0001$
		Males	$\chi2 = 100{\cdot}58$, $P \leq 0{\cdot}0001$	$\chi2 = 356{\cdot}01$, $P \leq 0{\cdot}0001$
	Stroke Riskometer™	Females	$\chi2 = 298{\cdot}95$, $P \leq 0{\cdot}0001$	$\chi2 = 588{\cdot}20$, $P \leq 0{\cdot}0001$
		Males	$\chi2 = 2247{\cdot}03$, $P \leq 0{\cdot}0001$	$\chi2 = 20\,297{\cdot}53$, $P \leq 0{\cdot}0001$
	QStroke	Females	$\chi2 = 21{\cdot}68$, $P = 0{\cdot}006$	$\chi2 = 70{\cdot}10$, $P \leq 0{\cdot}0001$
		Males	$\chi2 = 796{\cdot}93$, $P \leq 0{\cdot}0001$	$\chi2 = 949{\cdot}04$, $P \leq 0{\cdot}0001$
Combined	FSRS	Females	$\chi2 = 196{\cdot}70$, $P \leq 0{\cdot}0001$	$\chi2 = 304{\cdot}91$, $P \leq 0{\cdot}0001$
		Males	$\chi2 = 153{\cdot}78$, $P \leq 0{\cdot}0001$	$\chi2 = 726{\cdot}04$, $P \leq 0{\cdot}0001$
	Stroke Riskometer™	Females	$\chi2 = 547{\cdot}29$, $P \leq 0{\cdot}0001$	$\chi2 = 1\,811{\cdot}14$, $P \leq 0{\cdot}0001$
		Males	$\chi2 = 1699{\cdot}96$, $P \leq 0{\cdot}0001$	$\chi2 = 11\,552{\cdot}55$, $P \leq 0{\cdot}0001$
	QStroke	Females	$\chi2 = 1441{\cdot}52$, $P \leq 0{\cdot}0001$	$\chi2 = 270{\cdot}42$, $P \leq 0{\cdot}0001$
		Males	$\chi2 = 1587{\cdot}38$, $P \leq 0{\cdot}0001$	$\chi2 = 1\,822{\cdot}10$, $P \leq 0{\cdot}0001$

others (54). It is also likely that there are further unknown stroke risk factors that still need to be identified and included in a stroke prediction assessment tool. For one such example we refer to the recent evidence from Yusuf *et al.* (55) that populations from low to middle-income countries are at highest risk of cardiovascular events have the lowest risk factor burden (55), suggesting that the major 'missing piece in the equation' of the effective CVD prevention is the impaired ability of resource-limited health systems to effectively identify and modify cardiovascular risk. It is our expectation that the Stroke Riskometer™ will be further developed to account for these factors (we are currently collecting data on country) such that in future iterations of the Stroke Riskometer™ we hope to refine the algorithm to be able to provide country and ethnic specific-stroke risk prediction estimates, using both current research such as Yusuf *et al.* (55) and data collected from the current Stroke Riskometer™ App to improve overall predictability and applicability of our algorithm across all populations. Furthermore, an algorithm for all major noncommunicable disease, such as stroke, ischaemic heart disease (IHD), dementia and diabetes mellitus that share common risk factors, should be developed and validated in different populations. The main weakness of this validation study was that analyses were restricted due to the lack of currently available data on the variables shown to be important determinants of stroke.

The Stroke Riskometer™ availability on a portable device (smartphone) with constant proximity to the user, enables individuals to assess their own risk of stroke in the privacy and comfort anytime, anywhere. Unlike web-based versions, no internet connection required to use the app or access its information. In addition, the app offers a higher level of interactivity via sending direct reminders to the smartphone that is always on hand when needed. Moreover, the availability of the app on the smartphone app stores that has global reach, and vast consumer base of various age groups allows wide range of consumers to benefit from the stroke risk assessment tool and allows the crowdsourcing of large research database. Finally, users who are at increased (even slightly increased) risk are provided with ways to reduce their risk of stroke according to their individual risk profile and recommended to seek medical attention. This could rapidly transform epidemiologic research and monitoring of health status of individuals, especially in the area of chronic NCD (17).

Current risk scores will inevitably become outdated with improvements in clinical outcomes and data recording and changes in population demographics (56). With the Lite version of the Stroke Riskometer™ being made freely available globally on both iOS and Android smartphones and users invited to partake in a large-scale study we will have the potential to amass a large database. Ethical approval for the study has been received. Anonymous data from individuals who consent to participate in the study will be collected and securely stored at study coordinating centre (AUT University, NZ). The aim of these planned epidemiological studies based on the Stroke Riskometer™ will be to generate a global, population-specific stroke and NCD risk scoring system. We will further assess the Stroke Riskometer™ in a cohort study to establish the efficiency of the algorithm and assess if the new collections of recommendations are useful for motivating users to actively reduce their risk of stroke.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Bar plots of mean predicted risk scores for observed stroke and nonstroke events at (A) 5-years and (B) 10-years for FSRS (black), Stroke Riskometer™ (red) and QStroke (green) algorithms across the (1A) Rotterdam and (1B) combined [ARCOS, Russia and Rotterdam] data sets for males (left) and females (right).

Fig. S2. Mean predicted risk (%) vs. observed stroke events in deciles of predicted risk for Framingham Stroke Risk Score (FSRS) (black), Stroke Riskometer™ (red) and QStroke (green) algorithms for males (left) and females (right) for (A) 5-year predicted risks for the Russian data set, (B) 5-year predicted risks for the Rotterdam data set and (C) 10-year predicted risks for the Rotterdam data set.

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Salivary inflammatory cytokines may be novel markers of carotid atherosclerosis in a Japanese general population: The Suita study☆

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ABSTRACT

Objective: Salivary biomarkers have been recently useful of periodontal disease, which is also risk factor of atherosclerosis. However, there are few studies of the association between salivary inflammatory cytokines and carotid atherosclerosis. We aimed to clarify the association between salivary inflammatory cytokines and periodontal disease and carotid atherosclerosis in a general urban population.
Methods: We studied 608 Japanese men and women (mean age: 65.4 years) in the Suita study. Carotid atherosclerosis was evaluated by high-resolution ultrasonography with atherosclerotic indexes of intima-media thickness (IMT). Periodontal status was evaluated by the Community Periodontal Index (CPI). Salivary levels of interleukin-1β, interleukin-6, tumor necrosis factor-α (TNF-α), and prostaglandin E2 (PGE2) were measured by enzyme linked immunosorbent assay. The risks of carotid atherosclerosis (≥75th percentiles of mean- [0.88 mm] and Max-IMT [1.50 mm]) according to the quartiles of salivary inflammatory cytokines were compared using of adjusted-logistic regression models.
Results and conclusion: All salivary inflammatory cytokines were positively associated with CPI. The adjusted odds ratios for carotid atherosclerosis of mean-IMT in the highest quartile of interleukin-6 and TNF-α were higher than those in the lowest quartiles (OR = 2.32 and 2.88; 95% confidence intervals = 1.19–4.51 and 1.51–5.49, respectively). The adjusted odds ratio for carotid atherosclerosis of mean-IMT in the highest quartile of PGE2 was greater than those in the lowest quartile in women (OR = 2.78; 95% confidence intervals = 1.11–6.95). In conclusion, higher levels of salivary inflammatory cytokines were associated with both periodontal disease and carotid atherosclerosis. Selected salivary inflammatory cytokines may be useful screening markers for periodontal disease and carotid atherosclerosis.

1. Introduction

Inflammatory cytokines in plasma have associated with carotid atherosclerosis. Recent investigations have shown that plasma levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) were associated to the severity and progression of carotid artery plaques [1–8]. Prostaglandin E2 (PGE2) in plasma was related to sub-clinical atherosclerosis in apparently healthy subjects exposed to cardiovascular risk factors [9].

On the other hand, previous studies have suggested that periodontal disease has associated with cardiovascular disease (CVD) [10,11]. The findings from cross-sectional and longitudinal epidemiologic studies were supported by in vitro and animal studies describing plausible mechanisms linking periodontal infection to development of atherosclerotic diseases, to the triggering of clinical coronary events or to both [12]. One recent investigation has reported that carotid IMT regressed with moderate to severe periodontal disease after periodontal therapy in Aboriginal Australian adults, suggesting periodontal disease and atherosclerosis are positively associated [13].

☆ This article encompasses the doctoral dissertation of Takayuki Kosaka.

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Until now, periodontal pocket examination has been used typically for diagnosis of periodontal disease. However, recently it has been suggested that select salivary biomarkers could have utility for monitoring periodontal health [14–17]. Saliva contains locally and systemically derived biomarkers of periodontal disease [18]. We focused on attention to saliva as a useful biological sample which reflects the oral health condition because saliva samples can be collected easily and non-invasively differently from blood samples. However, there is no study on the association between salivary inflammatory cytokines and carotid atherosclerosis, in Asia. The purpose of this study was therefore to investigate the association between not only salivary inflammatory cytokines and periodontal status but also those cytokines and carotid atherosclerosis. In this study, we assessed the hypothesis that selected inflammatory cytokines in saliva are related to periodontal disease and carotid atherosclerosis in a general urban Japanese population.

2. Methods

2.1. Study participants

We studied 608 Japanese (271 men and 337 women, mean age: 65.4 years) who underwent a medical check-up, dental examination, and carotid ultrasonography between June 2008 and March 2012 in the Suita study [19]. Before the study started, the study protocol was approved by the Ethics Committee of the National Cerebral and Cardiovascular Center (M19-62), and only individuals who provided informed consent after receiving a full explanation of the study purpose and methods both in writing and orally were including among subjects.

2.2. Carotid ultrasonography

Carotid atherosclerosis was evaluated by high-resolution ultrasonography with a 7.5-MHz transducer that produced an axial resolution of 0.1 mm (SSA-780A, TOSHIBA, Tokyo, Japan). We measured the carotid arteries from the superior border of the collarbone to the inferior margin of the mandible. Details of the methods used for the carotid ultrasonic examination have been described elsewhere [20]. Each index of carotid atherosclerosis was defined as follows. Mean-IMT was defined as the mean of the proximal and distal walls for both sides of the common carotid arteries at a point 10 mm proximal to the beginning of each carotid artery bulb. Max-IMT was defined as the maximum IMT in the entire scanned area. The risks of carotid atherosclerosis were defined by the cut-off values: 75th percentile for mean- [0.88 mm] and Max-IMT [1.50 mm].

2.3. Periodontal tissue examination

Periodontal status was evaluated on the basis of the Community Periodontal Index (CPI) by means of partial 10 index teeth recording [21]. This examination was performed by five dentists who had undergone calibration in advance. A total of 10 teeth were examined, comprising the maxillary and mandibular left and right first and second molars, the maxillary right central incisor, and the mandibular left central incisor, and if this test could not be performed because of loss of one or both of the central incisors concerned, the same tooth on the opposite side was examined. No evaluation was performed if all the relevant teeth were missing. Periodontal status was examined using a CPI probe (YDM, Tokyo, Japan) to evaluate each tooth with respect to six periodontal pockets according to the following criteria, and the highest-value code was recorded. The CPI codes were as follows: no signs of inflammation of the gingiva (Code 0), evident bleeding after probing (Code 1), dental calculus deposits (including those detected by probing up to 4 mm beneath the gingival margin, Code 2), periodontal pocket of depth $\geq$4 mm but <6 mm (Code 3), periodontal pocket of depth $\geq$6 mm (Code 4). Cohen's κ value for the consistency between the periodontal tissue examinations of the five dentists was 0.78.

2.4. Assessment of salivary inflammatory cytokines

Participants were conducted to chew a piece of paraffin gum for 2 min to stimulate salivary flow. Subsequently, subjects were asked to spit the saliva into a test tube. Samples, which were stored at −80 °C until use, were delivered to the laboratory for analyses.

Salivary inflammatory cytokines were determined by enzyme-linked immunosorbent assay (ELISA). IL-1β (Quantikine HS Human IL-1β, R&D Systems, Minneapolis, MN USA), IL-6 (Quantikine Human IL-6, R&D Systems, Minneapolis, MN USA), TNF-α (Quantikine HS Human TNF-α, R&D Systems, Minneapolis, MN USA) were determined using conventional sandwich ELISA [22]. PGE2 was determined using competitive ELISA (prostaglandin E2 EIA Kit monoclonal, Cayman Chemical, Ann Arbor, MI USA). These analyses were performed according to the manufacture's recommended protocols. The average absorbance readings of the samples were then compared with concentrations of the standard curve, and the salivary concentration of each cytokine was calculated. Limits of detection of IL-1β, IL-6, TNF-α and PGE2 were 0.125 pg/mL, 3.12 pg/mL, 0.5 pg/mL and 15 pg/mL, respectively. The coefficient of variations of IL-1β, IL-6, TNF-α and PGE2 were 2.1%, 5.8%, 4.2%, and 7.6%, respectively.

2.5. Cardiovascular risk factors

Systolic (SBP) and diastolic blood pressure (DBP) were taken as the average classified into one of four blood pressure (BP) categories (optimal, normal, and high-normal blood pressure, and hypertension) based on BP values according to the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH) 2014 criteria [23] : optimal (SBP <120 mmHg and DBP <80 mmHg), normal (SBP = 120~129 mmHg and DBP = 80~84 mmHg), high-normal BP (SBP = 130~139 mmHg and DBP = 85~89 mmHg) and hypertensive (SBP $\geq$140 mmHg, DBP $\geq$90 mmHg, and/or the use of antihypertensive drugs). If the SBP and DBP reading for a subject were in different categories, the subjects were categorized into the higher of the two BP categories. We performed routine blood tests that included serum total cholesterol, HDL cholesterol and glucose levels. Fasting serum glucose categories were defined as follows: diabetes mellitus (DM, fasting serum glucose $\geq$7.0 mmol/L [126 mg/dL)] or the use of medications for DM), impaired fasting glucose (fasting serum glucose levels from 5.6 to 6.9 mmol/L [100~125 mg/dL]), and normoglycemia (fasting serum glucose levels <5.6 mmol/L [<100 mg/dL]). Physicians or nurses administered questionnaires covering personal habits and present illness. Smoking, drinking and using antilipidemic drug status were divided into current, former and never. Body mass index (BMI) was calculated as weight (kg) divided by height (m) [2].

2.6. Statistical analysis

Associations between salivary inflammatory cytokines and CPI were estimated by the Spearman's rank correlation coefficients. The associations of salivary inflammatory cytokines and mean- and Max-IMT were examined using analysis of covariance (ANCOVA) adjusting for cardiovascular risk factors. The risks of carotid atherosclerosis ($\geq$75th percentiles of mean- [0.88 mm] and Max-IMT [1.50 mm]) according to the quartiles of salivary inflammatory cytokines were compared using of logistic regression models,

Table 1
Characteristics of study subjects according to CPI category.

	CPI 0–2	CPI 3	CPI 4
Number, *n*	295	230	83
Age	65.4 ± 8.2	66.1 ± 8.3	64.8 ± 8.0
Interleukin-1β, pg/mL	338 ± 343	459 ± 401	530 ± 419
Interleukin-6, ng/mL	6.7 ± 8.0	8.6 ± 8.9	12.6 ± 12.7
Tumor necrosis factor-α, pg/mL	0.63 ± 0.71	1.06 ± 1.25	1.23 ± 1.50
ProstaglandinE2, pg/mL	51.6 ± 48.2	61.3 ± 52.1	87.6 ± 80.8
Mean-IMT, mm	0.79 ± 0.12	0.82 ± 0.12	0.85 ± 0.14
Maximum-IMT, mm	1.33 ± 0.56	1.47 ± 0.62	1.46 ± 0.59
Hypertension, %	27.8	31.7	38.6
Total cholesterol, mg/dL	213 ± 33	206 ± 29	210 ± 34
HDL-cholesterol, mg/dL	65 ± 16	61 ± 16	62 ± 18
Diabetes, %	7.5	7.0	8.4
Body mass index, kg/m^2	22.5 ± 3.1	22.6 ± 3.0	23.3 ± 2.8
Antihyperlipidemic drug using, %	27.1	25.7	33.7
Current smoking, %	10.5	10.4	13.3
Current drinking, %	43.4	53.9	59.0

Values are means ± standard deviations or frequencies (%).
IMT, intima-medial thickness; and CPI, Community Periodontal Index.

adjusting for cardiovascular risk factors. *P* values <0.05 were considered significant for all comparisons. All analyses were performed with PASW Statistics 21 (SPSS Inc., IBM, Tokyo, Japan).

3. Results

Detailed demographic data between 3 groups according to the periodontal status are summarized in Table 1. In the different CPI groups, salivary inflammatory cytokines and mean-IMT increased accordance with progression of periodontal disease. All salivary inflammatory cytokines were positively associated with CPI (IL-1β: $r = 0.17$, $P < 0.001$; IL-6: $r = 0.15$, $P < 0.001$; TNF-α: $r = 0.17$, $P < 0.001$; PGE2: $r = 0.16$, $P < 0.001$).

The multivariable-adjusted mean-IMT (Table 2) in subjects with the highest quartile of TNF-α was greater than that in subjects with the lowest quartile (0.831 ± 0.009 mm vs 0.779 ± 0.010 mm, $P < 0.001$). The multivariable-adjusted Max-IMT in subjects with the highest quartile of PGE2 was greater than that in subjects with the lowest quartile in women (1.319 ± 0.048 mm vs 1.183 ± 0.050 mm, $P = 0.04$).

The multivariable-adjusted odds ratios for carotid atherosclerosis of mean-IMT (Table 3) in subjects with the highest quartiles of IL-6 and TNF-α were greater than those in subjects with the lowest quartiles (OR 2.32; 95% CI 1.19–4.51 for IL-6: OR 2.88; 95% CI 1.51–5.49 for TNF-α). The multivariable-adjusted odds ratio for carotid atherosclerosis of mean-IMT in subjects the highest quartile of PGE2 was greater than that in subjects with the lowest quartile in women (OR 2.78; 95%CI 1.11–6.93).

Table 2
Multivariable-adjusted mean and maximum IMT according to the quartiles of salivary inflammatory cytokines.

	Q1 (Low)	Q2	Q3	Q4 (High)	*P* for trend
IL-1β					
Men and women ($n = 590$)					
Mean-IMT	0.809 ± 0.009	0.805 ± 0.009	0.808 ± 0.009	0.805 ± 0.009	0.827
Maximum-IMT	1.382 ± 0.044	1.372 ± 0.044	1.388 ± 0.044	1.410 ± 0.044	0.609
Men ($n = 262$)					
Mean-IMT	0.830 ± 0.015	0.826 ± 0.015	0.844 ± 0.017	0.807 ± 0.016	0.469
Maximum-IMT	1.589 ± 0.081	1.547 ± 0.079	1.511 ± 0.088	1.518 ± 0.083	0.510
Women ($n = 328$)					
Mean-IMT	0.792 ± 0.012	0.793 ± 0.012	0.781 ± 0.011	0.801 ± 0.012	0.807
Maximum-IMT	1.207 ± 0.045	1.241 ± 0.047	1.283 ± 0.043	1.318 ± 0.045	0.065
IL-6					
Men and women ($n = 539$)					
Mean-IMT	0.800 ± 0.010	0.808 ± 0.009	0.808 ± 0.010	0.812 ± 0.010	0.402
Maximum-IMT	1.416 ± 0.050	1.406 ± 0.046	1.391 ± 0.048	1.415 ± 0.048	0.939
Men ($n = 248$)					
Mean-IMT	0.830 ± 0.017	0.826 ± 0.015	0.809 ± 0.017	0.842 ± 0.015	0.786
Maximum-IMT	1.598 ± 0.092	1.617 ± 0.081	1.448 ± 0.095	1.593 ± 0.081	0.644
Women ($n = 291$)					
Mean-IMT	0.775 ± 0.012	0.794 ± 0.011	0.802 ± 0.011	0.784 ± 0.012	0.522
Maximum-IMT	1.256 ± 0.051	1.228 ± 0.048	1.330 ± 0.045	1.248 ± 0.051	0.741
TNF-α					
Men and women ($n = 541$)					
Mean-IMT	0.779 ± 0.010	0.798 ± 0.009	0.819 ± 0.010	0.831 ± 0.009[a]	<0.001
Maximum-IMT	1.410 ± 0.049	1.309 ± 0.046	1.401 ± 0.049	1.487 ± 0.046	0.130
Men ($n = 253$)					
Mean-IMT	0.801 ± 0.014	0.832 ± 0.016	0.824 ± 0.017	0.860 ± 0.016[a]	0.014
Maximum-IMT	1.591 ± 0.078	1.462 ± 0.086	1.553 ± 0.095	1.649 ± 0.086	0.485
Women ($n = 288$)					
Mean-IMT	0.760 ± 0.014	0.770 ± 0.011	0.809 ± 0.011	0.806 ± 0.011	0.002
Maximum-IMT	1.223 ± 0.056	1.168 ± 0.047	1.279 ± 0.047	1.350 ± 0.046	0.034
PGE2					
Men and women ($n = 535$)					
Mean-IMT	0.823 ± 0.010	0.796 ± 0.010	0.808 ± 0.010	0.822 ± 0.010	0.810
Maximum-IMT	1.431 ± 0.048	1.378 ± 0.047	1.400 ± 0.047	1.419 ± 0.048	0.947
Men ($n = 243$)					
Mean-IMT	0.862 ± 0.015	0.809 ± 0.016	0.826 ± 0.017	0.826 ± 0.016	0.202
Maximum-IMT	1.714 ± 0.083	1.578 ± 0.087	1.464 ± 0.092	1.532 ± 0.088	0.096
Women ($n = 292$)					
Mean-IMT	0.789 ± 0.013	0.785 ± 0.012	0.793 ± 0.011	0.819 ± 0.012	0.080
Maximum-IMT	1.183 ± 0.050	1.213 ± 0.047	1.330 ± 0.045	1.319 ± 0.048	0.020

Data are expressed multivariable-adjusted means of mean-IMT and standard errors adjusting for age, (sex), drinking and smoking habits, body mass index, total cholesterol, HDL cholesterol, hypertension, diabetes, and antihyperlipidemic drug uses.
[a] $p < 0.05$, compared with Q1.

Table 3
Adjusted odds ratios (95% CI) for stenosis defined by mean-IMT according to the quartiles of salivary inflammatory cytokines.

	Q1 (Low)	Q2	Q3	Q4 (High)	*P* for trend
IL-1β					
Men and women (n = 590)					
Case, n	37	39	42	46	
Age-adjusted	1	1.16 (0.67–2.03)	1.26 (0.73–2.19)	1.28 (0.74–2.20)	0.354
Multivariable-adjusted	1	1.08 (0.61–1.92)	1.24 (0.70–2.18)	1.05 (0.60–1.86)	0.755
Men (n = 262)					
Case, n	20	20	25	21	
Age-adjusted	1	1.00 (0.46–2.18)	2.05 (0.92–4.54)	1.01 (0.46–2.19)	0.597
Multivariable-adjusted	1	0.95 (0.41–2.22)	2.17 (0.92–5.14)	0.69 (0.30–1.62)	0.780
Women (n = 328)					
Case, n	17	19	17	25	
Age-adjusted	1	1.33 (0.60–2.96)	0.88 (0.40–1.94)	1.60 (0.75–3.43)	0.394
Multivariable-adjusted	1	1.26 (0.55–2.85)	0.84 (0.37–1.91)	1.48 (0.67–3.27)	0.521
IL-6					
Men and women (n = 539)					
Case, n	19	45	37	51	
Age-adjusted	1	2.03 (1.08–3.83)	1.61 (0.84–3.08)	2.42 (1.28–4.55)	0.024
Multivariable-adjusted	1	2.09 (1.08–4.05)	1.73 (0.88–3.39)	2.32 (1.19–4.51)	0.041
Men (n = 248)					
Case, n	12	25	15	33	
Age-adjusted	1	1.67 (0.72–3.90)	1.16 (0.46–2.90)	2.36 (1.03–5.44)	0.083
Multivariable-adjusted	1	1.56 (0.62–3.89)	0.96 (0.36–2.58)	2.02 (0.81–5.03)	0.233
Women (n = 291)					
Case, n	7	20	22	18	
Age-adjusted	1	2.64 (0.99–7.01)	2.42 (0.92–6.34)	2.43 (0.86–6.61)	0.157
Multivariable-adjusted	1	3.10 (1.13–8.55)	2.57 (0.95–6.96)	2.75 (0.96–7.83)	0.127
TNF-α					
Men and women (n = 541)					
Case, n	23	41	38	50	
Age-adjusted	1	2.18 (1.18–4.05)	2.43 (1.29–4.56)	2.80 (1.53–5.14)	0.002
Multivariable-adjusted	1	2.40 (1.25–4.61)	2.51 (1.29–4.89)	2.88 (1.51–5.49)	0.001
Men (n = 253)					
Case, n	15	23	18	31	
Age-adjusted	1	2.56 (1.14–5.78)	2.87 (1.20–6.84)	4.25 (1.90–9.50)	<0.001
Multivariable-adjusted	1	2.98 (1.23–7.21)	2.80 (1.08–7.27)	3.94 (1.63–9.53)	0.005
Women (n = 288)					
Case, n	8	18	20	19	
Age-adjusted	1	1.99 (0.75–5.27)	2.33 (0.89–6.13)	2.01 (0.76–5.29)	0.204
Multivariable-adjusted	1	2.24 (0.81–6.21)	2.83 (1.02–7.85)	2.80 (1.00–7.83)	0.060
PGE2					
Men and women (n = 535)					
Case, n	33	25	46	52	
Age-adjusted	1	0.54 (0.29–1.01)	1.19 (0.67–2.11)	1.26 (0.71–2.24)	0.094
Multivariable-adjusted	1	0.54 (0.28–1.04)	1.12 (0.61–2.04)	1.13 (0.62–2.05)	0.254
Men (n = 243)					
Case, n	22	13	25	24	
Age-adjusted	1	0.44 (0.19–1.04)	1.44 (0.65–3.21)	0.76 (0.35–1.68)	0.864
Multivariable-adjusted	1	0.42 (0.16–0.97)	1.09 (0.45–2.63)	0.50 (0.21–1.18)	0.422
Women (n = 292)					
Case, n	11	12	21	28	
Age-adjusted	1	0.74 (0.28–1.91)	1.26 (0.53–3.01)	2.36 (0.99–5.60)	0.014
Multivariable-adjusted	1	0.90 (0.33–2.40)	1.20 (0.48–3.01)	2.78 (1.11–6.93)	0.014

Stenosis was defined as ≥75th percentiles of mean-IMT [0.88 mm].
Odds ratios are estimated by the use of multivariable-adjusted logistic regression models adjusting for age, (sex), drinking and smoking habits, body mass index, total cholesterol, HDL cholesterol, hypertension, diabetes, and antihyperlipidemic drug uses. CI, confidence intervals.

Conducting similar analyses, the multivariable-adjusted odds ratio for carotid atherosclerosis of Max-IMT in subjects with the highest quartile of any salivary inflammatory cytokine was not significantly higher than those in subjects with the lowest quartile (Table 4). However, the multivariable-adjusted odds ratios for carotid atherosclerosis of Max-IMT was significantly increased according with quartiles in IL6 and TNFα in women (P = 0.035, 0.047, respectively).

4. Discussion

In this study, higher salivary IL-6 and TNF-α were associated with the periodontal status and carotid atherosclerosis. To our knowledge, this is the first population study to show the relationship between salivary inflammatory cytokines and periodontal disease and carotid atherosclerosis. Salivary IL-6 and TNF-α may indicate intensity of carotid IMT. Salivary inflammatory cytokines may be novel markers of intensities of carotid atherosclerosis, which may be useful screening markers for CVDs as a preventive medical point of view.

Salivary inflammatory cytokines were positively associated with CPI, which is widely used for assessing the periodontal disease, where periodontal disease is an inflammatory disorder in the oral tissue, and therefore the result means that salivary inflammatory cytokines were elevated by progression of periodontal disease. Thus, it appears that salivary inflammatory cytokines are useful markers reflecting periodontal statuses.

Atherosclerotic extension is caused by vascular endothelial dysfunction and strongly correlated to carotid atherosclerosis [24].

Table 4
Adjusted odds ratios (95% CI) for stenosis defined by Max-IMT according to the quartiles of salivary inflammatory cytokines.

	Q1 (Low)	Q2	Q3	Q4 (High)	*P* for trend
IL-1β					
Men and women (n = 590)					
Case, n	34	41	38	44	
Age-adjusted	1	1.38 (0.80–2.38)	1.20 (0.69–2.09)	1.35 (0.79–2.31)	0.385
Multivariable-adjusted	1	1.35 (0.77–2.37)	1.28 (0.72–2.26)	1.34 (0.76–2.35)	0.386
Men (n = 262)					
Case, n	21	27	21	26	
Age-adjusted	1	1.49 (0.71–3.14)	1.33 (0.61–2.94)	1.34 (0.63–2.83)	0.545
Multivariable-adjusted	1	1.56 (0.73–3.33)	1.35 (0.60–3.03)	1.29 (0.60–2.79)	0.717
Women (n = 328)					
Case, n	13	14	17	18	
Age-adjusted	1	1.21 (0.52–2.81)	1.24 (0.55–2.79)	1.41 (0.63–3.16)	0.416
Multivariable-adjusted	1	1.17 (0.50–2.77)	1.29 (0.56–2.93)	1.45 (0.63–3.33)	0.371
IL-6					
Men and women (n = 539)					
Case, n	23	35	38	49	
Age-adjusted	1	1.13 (0.61–2.09)	1.38 (0.75–2.55)	1.88 (1.03–3.41)	0.023
Multivariable-adjusted	1	1.09 (0.58–2.06)	1.55 (0.82–2.92)	1.77 (0.95–3.30)	0.035
Men (n = 248)					
Case, n	13	25	19	35	
Age-adjusted	1	1.50 (0.65–3.45)	1.53 (0.64–3.70)	2.42 (1.07–5.48)	0.037
Multivariable-adjusted	1	1.09 (0.58–2.06)	1.55 (0.82–2.92)	1.77 (0.95–3.30)	0.084
Women (n = 291)					
Case, n	10	10	19	14	
Age-adjusted	1	0.75 (0.29–1.97)	1.44 (0.60–3.41)	1.28 (0.51–3.21)	0.307
Multivariable-adjusted	1	0.73 (0.27–1.97)	1.57 (0.64–3.83)	1.44 (0.55–3.71)	0.204
TNF-α					
Men and women (n = 541)					
Case, n	35	32	28	49	
Age-adjusted	1	0.81 (0.46–1.44)	0.81 (0.45–1.46)	1.43 (0.83–2.47)	0.171
Multivariable-adjusted	1	0.92 (0.50–1.68)	0.95 (0.51–1.77)	1.65 (0.93–2.95)	0.080
Men (n = 253)					
Case, n	28	21	15	30	
Age-adjusted	1	0.82 (0.39–1.73)	0.78 (0.35–1.76)	1.48 (0.71–3.06)	0.339
Multivariable-adjusted	1	0.91 (0.42–1.96)	0.77 (0.33–1.81)	1.49 (0.68–3.22)	0.402
Women (n = 288)					
Case, n	7	11	13	19	
Age-adjusted	1	1.16 (0.41–3.26)	1.41 (0.51–3.86)	2.17 (0.83–5.69)	0.075
Multivariable-adjusted	1	1.24 (0.43–3.59)	1.63 (0.57–4.62)	2.49 (0.91–6.84)	0.047
PGE2					
Men and Women (n = 535)					
Case, n	31	29	37	47	
Age-adjusted	1	0.77 (0.42–1.40)	0.97 (0.54–1.73)	1.25 (0.70–2.20)	0.298
Multivariable-adjusted	1	0.83 (0.44–1.54)	1.09 (0.59–2.01)	1.30 (0.71–2.36)	0.267
Men (n = 243)					
Case, n	24	20	18	30	
Age-adjusted	1	0.75 (0.34–1.64)	0.65 (0.29–1.48)	1.03 (0.48–2.23)	0.992
Multivariable-adjusted	1	0.73 (0.33–1.64)	0.61 (0.26–1.43)	0.94 (0.42–2.10)	0.812
Women (n = 292)					
Case, n	7	9	19	17	
Age-adjusted	1	1.02 (0.35–2.96)	2.15 (0.83–5.61)	2.07 (0.78–5.51)	0.053
Multivariable-adjusted	1	1.02 (0.35–3.02)	2.10 (0.79–5.62)	2.13 (0.78–5.85)	0.058

Stenosis was defined as ≥75th percentiles of Max-IMT [1.50 mm].
Odds ratios are estimated by the use of multivariable-adjusted logistic regression models adjusting for age, (sex), drinking and smoking habits, body mass index, total cholesterol, HDL cholesterol, hypertension, diabetes, and antilipidemic drug uses.
CI, confidence intervals.

Recently, it has been suggested that periodontitis is associated with endothelial dysfunction in subjects without cardiovascular risk factors, as well as hypertensive patients [25]. By producing persistent subclinical infectious causing a chronic inflammatory state such as chronic periodontitis, infectious agents might contribute to gradual plaque enlargement [26]. In our study, salivary inflammatory cytokines were positively associated with periodontal disease. We assume that selected inflammatory cytokines have some impact on vascular endothelial dysfunction, and then cause atherosclerotic extension.

In our study, selected salivary inflammatory cytokines were positively associated with mean-IMT, nevertheless we found weak associations between those and Max-IMT. Nakashima et al. showed that risk factors for the maximum IMT and mean-IMT are somewhat different although the study was performed limited in hemodialysis patients [27]. Some other risk factors which were not used in our study may affect increasing IMT. The reason why associations only between salivary inflammatory cytokines and mean-IMT were clear may need further consideration.

Only one study has reported the association between pulse pressure of the common carotid artery and salivary PGE2, whereas IMT was not [28], which differs from the current study, due to different background of the subjects and methods for sampling saliva and using confounding factors in analyses. We observed the association in larger subjects. Interestingly, the absence of significant association between salivary PGE2 levels and carotid