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ORIGINAL ARTICLE

Retinal Vasculature Reactivity During Flicker Light Provocation, Cardiac Stress and Stroke Risk in Africans: The SABPA Study

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Abstract

Structural and functional similarities exist between the retinal, cerebral and, as previously suggested, the coronary microvasculature. Retinal microvascular structure and functionality (in response to flicker-light-induced-provocation (FLIP)) may relate to coronary artery disease risk and possible stroke risk. We investigated associations between retinal vessel structure, functionality and cardiac stress markers (cardiac troponin T [cTnT], amino-terminal B-type natriuretic peptide [NT-proBNP]) to translate these retina–heart relationships to stroke risk. We included 317 African and Caucasian teachers' (aged 23–68 years), who participated in the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study. Fasting plasma and serum samples for cTnT and NT-proBNP were collected. Retinal vascular calibres were quantified from fundus images and dynamic retinal vessel calibre responses during FLIP. The University of California stroke risk score was applied to assess sub-clinical 10-year stroke risk. cTnT levels were similar in Africans and Caucasians, whereas NT-proBNP levels were lower in Africans. In Africans, a reduced arteriolar calibre and attenuated arteriolar dilation during FLIP was associated with higher cTnT ($p < 0.01$). Their larger retinal–venular calibre ($p < 0.02$) and attenuated arteriolar dilation during FLIP ($p < 0.05$) were associated with lower NT-proBNP. Again, exclusively in Africans, increased cardiac stress, wider venular calibres and retinal arteriovenous nicking predicted an increased 10-year stroke risk with odds ratios of 1.57 (95% CI, 1.34; 1.68, $p = 0.031$), 1.51 (95% CI, 1.26; 1.59, $p = 0.002$), 1.10 (95% CI, 0.94; 2.85, $p = 0.002$) and 1.06 (95% CI 0.83; 1.56, $p = 0.052$), respectively. None of these associations were evident in the Caucasian group. Investigating the retinal vasculature may serve as a tool to approximate sub-clinical coronary and cerebral microvasculature damage or dysfunction. These cardiac stress–retinal associations additionally predicted a greater stroke risk in the SABPA African cohort. Observable changes in the retinal vasculature may serve as markers for the identification and prediction of cardio-systemic and cerebral vascular morbidities and risks, thereby establishing a brain-heart link.

Keywords Retina · Dynamic retinal vessel responses · Flicker-light-induced-provocation (FLIP) · NT-proBNP · cTnT · Stroke · Ethnicity

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Abbreviations

AUC	Area under the curve
DVA	Dynamic vessel analyses
cTnT	Cardiac troponin T
MC	Maximum constriction
MD	Maximum dilation
NT-proBNP	Amino-terminal pro-B-type natriuretic peptide
OR	Odds ratio

Introduction

Structural and functional similarities exist between the retinal, cerebral and, as previously suggested, the coronary microvasculature [1]. Specifically, the retinal and cerebral vasculature

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show striking anatomical, functional and autoregulatory similarities—maintaining constant blood pressure (BP), despite changes in systemic BP [1]. The latter ensures adequate perfusion and the preservation of blood–ocular or blood–brain barriers [2]. However, the ability to maintain these autoregulatory capacities diminishes with increases in BP, such as during hypertension [3, 4]. The coronary arteries, possessing an elastic lamina, are tailored to accommodate frequent pulsatile changes, whereas the retinal and cerebral microvasculature's structure ensures adequate perfusion. Despite these anatomical differences, changes in retinal vessel calibres and functional responses to flicker-light-induced-provocation (FLIP) were present in patients suffering from coronary artery disease (CAD), compared to those without CAD [5].

A wider retinal venular calibre and attenuated dilation in response to FLIP have been associated with an increased risk for concurrent and future cerebrovascular events [5–8] and CAD [5, 9]. Yet, the relationship between the structure and functionality (in response to FLIP) of retinal vessels with cardiac stress markers in the risk stratification for stroke remains to be investigated. Furthermore, literature describing the overall retina–brain–heart link remains limited [10].

Until recently, cardiac troponin T (cTnT) was considered solely as a marker of cardiac apoptosis and necrosis [11, 12]. However, recent observations of detectable circulating cTnT levels in the general population have initiated a paradigm shift. Alternative mechanisms for cTnT release have been investigated, including increased myocardial stress (cardiac stress), due to increased pressure and/or volume load [11, 12]. In addition, small increases in cTnT levels were linked to endothelial dysfunction and small vessel disease (cerebral and coronary), rather than myocardial damage exclusively [13, 14]. This prompts the question whether cTnT can be linked to the cerebral microvasculature and subsequent stroke risk [15].

A recent investigation established a positive relationship between cTnT and amino-terminal pro-B type natriuretic peptide (NT-proBNP) [16]. NT-proBNP is a recognised dynamic marker of cardiac stress [17], which is also functionally linked to retinal epithelial cells, glial cell regulation [17, 18] and, recently, with retinal microvascular damage [15]. Lower levels of NT-proBNP are associated with early microvasculature changes, including loss of endothelial integrity [20], haemodynamic modifications and reduced densities of both coronary and cerebral microvasculature, signifying an increased risk for cerebrovascular events [21].

Therefore, we aimed to examine associations between retinal vessel structure and functionality with cardiac stress markers, NT-proBNP and cTnT, in a bi-ethnic cohort from South Africa. Furthermore, as the urban-dwelling Africans presented a greater hypertensive prevalence profile than Caucasians [22], we hypothesised that smaller arteriolar calibres, wider venular calibres, arteriovenous nicking (AV nicking) and diminished arteriolar and venular dilation during FLIP will be associated with increased cardiac stress and an accompanying higher stroke risk in Africans.

Materials and Methods

Study Design and Participants

The participants were initially recruited as part of phase 1 of the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study. All participants included in phase 1 (2008–2009) were invited to take part in the follow-up or phase two (2011–2012). Of the initial 409 participants, 359 reported for the second phase of the study. We only included participants who took part in the second phase of the study, as no retinal measurements were taken during the first phase. The study sample comprised of urban male and female African and Caucasian teachers ($N = 359$) from the North-West Province, South Africa, aged between 23 and 68 years. The motivation behind the exclusive selection of teachers was to obtain a socio-economic equated sample from a similar working environment; cultural differences could however not be excluded. Additional exclusion criteria for this study included epilepsy ($n = 1$) and unsuccessful retinal vessel recordings ($n = 41$). Regarding retinal vessel reactivity measurements in response to FLIP, only individuals with a quality score of equal or greater than 2.5 were included (Supplementary Information). The majority of the unsuccessful retinal measurements were found in the African group. Finally, a total of 139 Africans and 178 Caucasians were included in the current study (sub-population $N = 317$).

Ethical Considerations

The SABPA study obtained ethical approval from the Health Research Ethics Committee (HREC) of the NWU and extended approval was granted for the second phase. Written informed consent was obtained from all volunteers prior to participation. All procedures and objectives were explained to the participants prior to their recruitment and adhered to the applicable institutional guidelines and terms, as stated by the Declaration of Helsinki (2004).

General Procedure of Investigation

Clinical assessments were done over a 2-day period during the second phase or follow-up—similar to baseline. Before 08:00 of the first clinical assessment day, participants were each fitted with an ambulatory blood pressure (ABPM) and two-lead ECG monitor device (Cardiotens CE120®; Meditech CE120; Meditech, Budapest, Hungary). This device also obtained event BP prior and after retinal vessel assessments. A

24-h standardised diet commenced and participants subsequently continued with their normal daily activities, reporting any peculiarities such as nausea, headaches, visual disturbances, palpitations, fainting, stress and physical activity, on the issued 24-h diary cards. At 15:00, participants were transported to the NWU Metabolic Unit Research Facility for clinical measurements including the retinal vessel imaging.

Retinal Vessel Analyses

Participants abstained from caffeinated and alcoholic beverages, smoking, strenuous physical activity and food consumption for at least 1 h prior to the measurements. They were familiarised with the experimental setup and were examined for acute angle anterior chamber glaucoma risk by a registered nurse. Diastolic ocular perfusion pressure (DOPP) was calculated (Supplementary Information). The retinal vessel analyser (Imedos Systems UG, Jena, Germany) was used for digital fundus imaging with a Carl Zeiss FF450^{Plus} camera (Carl Zeiss, Meditech Jena, Germany) to perform dynamic and static retinal vessel analyses. Fifteen minutes prior to the measurement, a drop of tropicamide (1% Alcon, 1% tropicamide and 0.01% benzalkonium chloride (*m*/*v*)) was used to induce mydriasis in the right eye. If the right eye was not suitable, the left eye was used. Dynamic vessel analysis with flicker stimulation was performed first, followed by image capturing for structural (static) vessel analysis. In static vessel analysis we calculated the central retinal artery and vein equivalent (CRAE and CRVE respectively) and subsequently determined the arteriovenous ratio (AVR) as previously described in the SABPA study [23]. The presence of retinopathy and AV nicking was determined by a registered ophthalmologist from a colour retinal image (see Supplementary Methods).

For functional (dynamic) vessel analysis a standard flicker protocol by IMEDOS Systems was used. During FLIP, the duration of the baseline was 50 s, followed by a 20-s flicker period and an 80-s recovery (also referred to as second baseline) period. There were three flicker cycles in total, lasting an added total of 350 s for the entire measurement. The camera was set at a 30° angle with the participant focusing on the tip of a fixation rod, and an artery and vein segment (as long as possible) were primarily selected in the upper or lower temporal quadrant of the fundus image. The quality of the FLIP measurements for each participant were assessed subjectively using a newly developed, previously described, scoring method and extensively described in the Supplementary Methods (Smith W, Vilsert W, Kotliar K, 2018).

Absolute vessel diameters (measured in standardised measuring units (MU)) of the vessel segment were selected for each measurement. Each was calculated individually as the median value over the last 30 s of the first baseline phase prior to FLIP. Parameters derived from the smoothed averaged curve during FLIP, used in the current study, included the following: (1) the percentage maximal dilation in response to FLIP ($MD_{arteriole}$). (2) The area under the reaction curve during FLIP (AUC) was determined for arteries and veins and calculated as the percentage change per second (% s). The latter provided information on the curve form during FLIP (0–20 s) and theoretically describes the longevity, time and intensity of the vessel's response. For the values under the 100% line, the area was negative. (3) The percentage absolute maximal constriction after FLIP (MC) was the minimum value occurring after maximum FLIP-induced dilation and expressed as a percentage from baseline.

Biochemical Analyses

After an overnight stay, fasting blood samples were obtained from the antebrachial vein branches of each participant's dominant arm with a sterile winged infusion set by a registered nurse. Blood samples were handled according to the standardised protocol, and serum and plasma samples were frozen at − 80 °C until analysed in duplicate. Cotinine values were determined by means of a homogeneous immunoassay with a Modular Roche automized (Switzerland). γGT and ultra-high sensitivity C-reactive protein (hs-CRP) were analysed with the Konelab™20i (ThermoScientific, Vantaa, Finland). Tumour necrosis factor-alpha (TNF-α) was measured via a Quantikine HS Elisa Human serum TNF-alpha Immuno-assay (R&D Systems, Minneapolis, MN USA), with inter- (15%) and intra (17.8%)-assay variabilities. Glycated haemoglobin (HbA_{1c}) EDTA whole blood HbA_{1c} and serum cholesterol and high-density lipoprotein (HDL) were determined with a turbidimetric inhibition immunoassay and a homogeneous enzymatic colorimetric assay respectively (Integra 400 plus, Roche, Switzerland). NT-proBNP and cTnT were measured via an electrochemiluminescence method on the Roche® e411 (Roche®, Basel, Switzerland), with inter-batch variability 4.6% and intra-batch variability 4.2%. Below detectable limit, cTnT values (31.3% of all cTnT analyses, $N = 104$) were logarithmically calculated according to the method developed by Croghan and Egeghy [24].

Cardiovascular Risk Indicators

The 10-year UCLA risk composite score included gender, SBP, hypertensive drugs, diabetes, smoking habit, perfusion deficits, atrial fibrillation, and electrocardiography (ECG) left ventricular hypertrophy (American Heart and Stroke certified UCLA Medical Centre, Primary Stroke Centre, Santa Monica, Los Angeles, USA). Medium to high stroke probability was termed as scores of 5.2 and greater. Additionally, we applied recently defined cTnT cut-points predictive of clinical 24H hypertension. These cut-points were defined as cTnT ≥

4.2 pg/mL for the total African group and NT-proBNP levels below the age-and-gender-specific reference values [25–27].

Statistical Analyses

Statistica version 13.3 (TIBCO Software Inc., Palo Alto, USA, 2018) was used for data analyses. Normality was tested for all variables. Logarithmically transformed γGT, CRP, cotinine and HbA_{1c} levels were used in correlation models. Characteristics between ethnic groups were calculated with *t*-tests. Chi-square (X^2) statistics were used to determine proportions and prevalence data. A priori covariates included age, body surface area (BSA), cotinine, γGT, HbA_{1c}, total cholesterol:HDL cholesterol ratio, TNF-α, hypertensive/diabetic retinopathy and 24-h pulse pressure [23]. Single two-way ANCOVAs determined ethnic × gender differences for all cardiac stress and retinal vessel markers, independent of a priori selected co-variates. One-way ANCOVA was used to determine the least square mean difference in risk markers between ethnic groups, independent of a priori selected covariates. Multivariate linear regression analyses were used to determine associations between retinal vessel analyses (RVA) parameters and cardiac stress markers in several models. The dependent variables included (1) structure: CRAE, CRVE, AVR and (2) reactivity/functionality during FLIP: arteriole maximum dilation (MD), maximum constriction (MC), area under the curve $(AUC)_{arteriole}$ and venular (AUC_{venule}), both during FLIP. Independent variables included NT-proBNP, cTnT, along with the a priori selected covariates in all models. For all dynamic retinal analyses, vessel segment diameter was included as a covariate. Additional adjustments were made for CRVE in the CRAE models and vice versa. For all the aforementioned analyses, significance was set at $p < 0.05$ (two-tailed) and the *F* to enter was fixed at 2.5 in regression models. Odds ratios (OR) (95% confidence intervals (CI)) were computed in several models to determine the probability of a brain–heart link to predict stroke risk. Therefore, the following was calculated: (1) the probability of cardiac stress (cTnT ≥ 4.2 pg/mL; NT-proBNP below the age and gender specific reference value [25–27]) and (2) wider retinal venular calibres (CRVE ≥ 248 MU [22, 23]) and AV nicking (Henderson et al., 2011) to predict medium–high UCLA 10-year stroke risk.

Sensitivity Analyses

Forward stepwise regression analyses with the same set of covariates were repeated in several models in both ethnic groups. Analyses were computed by excluding participants with diabetes ($n = 36$) and those using angiotensin converting enzyme inhibitors ($n = 52$). None of these variables statistically significantly ($p < 0.05$) influenced the outcome.

Results

Interaction testing revealed that significant differences existed between Africans and Caucasians, but not between men and women, independent of a priori selected covariates for NT-proBNP ($F_{1,\ 317} = 4.0$, $p = 0.046$), cTnT ($F_{1,\ 317} = 27.1$, $p < 0.001$), arteriole MD (%) ($F_{1,\ 308} = 19.27$, $p < 0.001$) and venular MD (%) ($F_{1,\ 308} = 20.65$, $p = 0.024$).

Baseline Characteristics

Table 1 displays a comparison between the characteristics of ethnic groups. The Caucasian group were slightly older. Africans displayed lower BSA, but a poorer cardiometabolic profile with higher CRP, HbA_{1c} and total cholesterol:HDL cholesterol ratio, compared to their Caucasian counterparts ($p < 0.05$). Caucasians revealed higher NT-proBNP ($p < 0.001$) levels, but similar cTnT values were observed between ethnic groups. The African group had higher 24H BP and PP; pre-and-post FLIP BP ($p < 0.001$), intra-ocular pressure (IOP) ($p < 0.001$) and DOPP ($p = 0.010$) values; hypertensive/diabetic retinopathy prevalence of 86% compared to 39% in Caucasians; and prevalence of AV nicking of 64% vs 21% in Caucasians.

Unadjusted and Adjusted Retinal Calibres

In Table 2, the African group revealed a higher CRVE ($p < 0.001$) and smaller ARV ($p < 0.001$). A comparison between dynamic retinal vessel parameters revealed that both arteriolar and venular maximal dilation in response to FLIP were greater in Africans ($p < 0.05$). The $AUC_{arteriole}$ confirmed the significantly higher arteriolar vessel response during FLIP ($p < 0.05$) in Africans. NT-proBNP and cTnT trends remained similar to the unadjusted analyses in both ethnicities.

Structure: Static Retinal Calibres and Cardiac Stress Markers

Table 3 reveals that in Africans CRAE was inversely associated with cTnT, whereas CRVE was positively and ARV inversely associated with NT-proBNP ($p = 0.019$). None of these associations were significant in the Caucasian group.

Functionality: Retinal Vessel Calibres in Response to FLIP and Cardiac Stress Markers

Concerning vessel responses to FLIP (Table 3), only arteriolar maximum dilation was inversely associated with cTnT ($p = 0.026$) in the African group. In addition, $AUC_{arteriole}$ during FLIP, depicting the longevity, length and duration of vessel reactivity, was inversely related to cTnT and NT-proBNP ($p = 0.049$).

Table 1 Baseline characteristics between ethnicities

Variable	Africans ($N = 139$)	Caucasians ($N = 178$)	p value
Lifestyle and biochemical measurements			
Age (years)	48 ± 8	50 ± 10	0.037
BSA (m^2)	1.94 ± 0.24	2.04 ± 0.29	0.001
Physical activity (kcal/day)	3318.47 ± 1257.10	3462.78 ± 1633.80	0.356
Serum cotinine* (ng/mL)	32.61 (18.60, 36.00)	21.63 (10.45, 31.00)	0.242
γGT* (U/L)	37.55 (22.30, 67.30)	18.00 (11.90, 29.60)	< 0.001
CRP* (mg/L)	4.61 (2.00, 9.60)	2.59 (0.99, 3.98)	< 0.001
TNF-α	2.48 ± 2.18	2.74 ± 1.68	0.198
$HbA1_c$ (%)	6.19 ± 1.33	5.59 ± 0.67	< 0.001
Total cholesterol:HDL cholesterol	4.91 ± 1.67	4.43 ± 1.48	0.005
cTnT (pg/mL)	4.68 ± 3.71	5.04 ± 1.15	0.349
NT-proBNP (pg/mL)	61.85 ± 68.44	80.98 ± 72.17	< 0.001
Cardiovascular measurements			
24H ABPM SBP (mmHg)	139 ± 18	128 ± 11	< 0.001
24H ABPM DBP (mmHg)	88 ± 11	79 ± 8	< 0.001
24H ABPM PP (mmHg)	52 ± 10	48 ± 7	< 0.001
DVA pre-FLIP SBP (mmHg)	141 ± 21	133 ± 15	< 0.001
DVA pre-FLIP DBP (mmHg)	88 ± 13	83 ± 11	< 0.001
DVA post-FLIP SBP (mmHg)	139 ± 19	132 ± 13	< 0.001
DVA post-FLIP DBP (mmHg)	88 ± 13	83 ± 10	< 0.001
IOP (R) (mmHg)	16.51 ± 4.14	14.95 ± 3.42	< 0.001
DOPP (R) (mmHg)	71.68 ± 13.31	68.12 ± 10.77	0.007
Static retinal parameters			
CRAE (MU)	149.68 ± 1.20	151.01 ± 1.00	0.317
CRVE (MU)	249.19 ± 1.80	236.68 ± 1.60	< 0.001
ARV	0.60 ± 0.06	0.64 ± 0.04	< 0.001
Dynamic retinal parameters			
Arteriole MD (%)	4.11 ± 2.46	3.51 ± 1.94	0.015
Arteriole MC (%)	− 1.54 ± 1.17	− 1.70 ± 1.33	0.272
Venule MD (%)	4.72 ± 2.35	4.10 ± 1.85	0.008
AUC during FLIP			
$AUC\ FLIP_{arteriole}$ (% s)	57.88 ± 39.15	50.28 ± 33.08	0.061
$AUC\ FLIP_{venule}$ (% s)	58.51 ± 35.66	49.54 ± 26.59	0.010
History			
MI events, *N* (%)	1 (0.58)	1 (0.54)	0.959
Hypertensive, *N* (%)	112 (80.58)	63 (35.39)	< 0.001
Diabetic, *N* (%)	27 (19.42)	10 (5.62)	0.003
Atrial fibrillation, *N* (%)	3 (2.16)	3 (1.69)	0.959
NT-proBNP below reference value, N (%)	85 (61.15)	104 (58.42)	0.052
Hypertensive/diabetic retinopathy, *N* (%)	120 (86.33)	69 (38.76)	< 0.001
Arteriovenous nicking, *N* (%)	89 (64.03)	38 (21.35)	< 0.001

Data expressed as arrhythmic mean ± SD

N number of participants, % percentage change, *CRP* C-reactive protein, *TNF-α* tumour necrosis factor alpha, HbA_{1c} glycated haemoglobin, *γGT* gamma glutamyl transferase, *NT-proBNP* N-terminal pro-brain natriuretic peptide, *cTnT* cardiac troponin T, *ABPM* ambulatory blood pressure measurement, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *IOP* intra-ocular pressure, *DOPP* diastolic ocular perfusion pressure, *FLIP* flicker-light-induced-provocation, *CRAE* central retinal artery equivalent, *CRVE* central retinal vein equivalent, *MD* maximum dilation during FLIP, *MC* maximum constriction during FLIP, *AUC* area under the curve during FLIP, *MI* myocardial infarction

*Data presented as median and interquartile ranges

Table 2 Comparison of static and dynamic retinal vessel calibres and cardiac stress markers between ethnicities, independent of a priori covariates

Variable	Africans ($N = 139$)	Caucasians (N = 178)	p value
Static vessel analyses			
CRAE (MU)	149.78 ± 1.09	150.94 ± 0.95	0.287
CRVE (MU)	248.39 ± 1.71	237.49 ± 1.50	< 0.001
AVR	0.61 ± 0.004	0.64 ± 0.004	< 0.001
Dynamic vessel analyses			
Arteriolar MD (%)	3.98 ± 0.19	3.58 ± 0.16	0.030
Arteriolar MC (%)	− 1.47 ± 0.11	− 1.72 ± 0.09	0.190
Venular MD (%)	4.73 ± 0.17	4.05 ± 0.16	0.014
AUC data			
FLIP $AUC_{arteriole}$	55.49 ± 3.10	51.47 ± 2.70	0.111
FLIP AUC_{venule}	58.02 ± 2.53	49.42 ± 2.28	0.027
Biochemical markers			
cTnT (pg/mL)	4.80 ± 0.27	4.98 ± 0.25	0.344
NT-proBNP (pg/mL)	64.86 ± 5.64	79.56 ± 5.17	0.009

Data expressed as an arithmetic mean (± SE). A priori covariates included age, body surface area, cotinine, gamma glutamyl transferase, $HbA1_c$, total cholesterol:HDL ratio, hypertensive/diabetic retinopathy, and 24H pulse pressure. For NT-proBNP models, thyroxin and triiodothyronine were added

FLIP flicker-light-induced-provocation, *AVR* artery-to-vein ratio, *CRAE* central retinal artery equivalent, *CRVE* central retinal vein equivalent, *MD* maximum dilation, *MC* maximum constriction, *AUC* area under the curve, *cTnT* cardiac troponin T, *NT-proBNP* N-terminal pro-brain natriuretic peptide

In Fig. 1a, the 10-year stroke risk probability in Africans is illustrated in individuals with cTnT levels of ≥ 4.2 pg/mL, NT-proBNP levels below the normal reference. In Africans, a medium–high 10-year stroke risk was associated with an OR of 1.51 (95% CI, 1.04; 1.44, $p = 0.002$) and 1.57 (95% CI, 1.26; 1.59, $p = 0.032$), for increased cTnT levels and for NT-proBNP below reference values respectively. In Fig. 1b, retinal stroke markers, CRVE ≥ 248 MU (OR of 1.10 [95% CI, 0.94; 2.85], $p < 0.001$) and AV nicking (OR 1.06 [95% CI, 0.83; 1.56] $p = 0.052$), predicted a medium–high 10-year stroke risk. Excluding participants with diabetes and those using angiotensin converting enzymes inhibitors did not influence the outcome.

Discussion

The present study examined the relationship between retinal vessel structure and functionality (in response to FLIP), cardiac stress markers and stroke risk in a bi-ethnic population from South Africa. Our findings in Africans revealed that reduced retinal arteriolar calibres were associated with higher cTnT levels, whereas an attenuated arteriolar dilatory response to FLIP (MD and AUC_{artery}) was associated with increased cardiac stress (both cTnT and NT-proBNP). Venular calibre was positively associated with NT-proBNP. Cardiac stress markers, wider venules and AV nicking predicted an increased, medium–high sub-clinical stroke risk in Africans. Observable changes in the retinal vasculature and cardiac stress markers support the notion that these markers may serve as investigative tools for the prediction of cardio-and cerebral vascular risks and thereby establishing a more defined brain-heart link.

Structure: Static Retinal Calibres, Cardiac Stress Markers, Stroke Risk and Ethnicity

The African group exhibited higher ambulatory, pre-and post-FLIP BP, diastolic ocular perfusion pressure (DOPP) levels and 64% of participants with AV nicking. Elevated resting BP levels have previously been associated with structural microvascular changes, directly relating to cardiac stress, stroke and hypertension [8, 23, 28]. Hypertension may manifest as retinopathy, which is in its own right, predictive of stroke and CAD risk [8, 23, 28]. Complementary to the former, AV nicking is a recognised phenomenon related to arteriosclerotic thickening of the small vessel walls and subsequent stroke risk [29]. Sustained high systemic BP, such as observed in the African group, alters perfusion pressures and adequate blood flow to peripheral tissues [23]. The modified haemodynamic responses observed during such high-pressure conditions directly contribute to faulty retinal autoregulation, as the autoregulatory capabilities tend to vanish with increased BP and ocular pressures [1]. This sustained high-pressure system leads to a decrease in and eventual diminishing of myogenic control mechanisms (Bayliss effect), ultimately responsible for autoregulation [3, 4]. Here, we were able to link the retinal microvasculature (a surrogate for the brain microcirculation) to markers of heart function and structure, via cTnT and NT-

Table 3 Independent associations between static and dynamic retinal vessel parameters and cardiac stress markers in Africans

Africans ($N = 139$)			
	CRAE (MU) β (95% CI)	CRVE (MU) β (95% CI)	AVR β (95% CI)
Adjusted R^2	0.37	0.24	0.38
cTnT (pg/mL)	− 0.21 (− 0.38, − 0.04) $p = 0.012$	NS	NS
NT-proBNP (pg/mL)	NS	0.20 (0.039, 0.36) $p = 0.019$	− 0.25 (− 0.41, − 0.09) $p = 0.003$
Dynamic retinal parameters in response to FLIP:			
	Arteriole MD (%) β (95% CI)	$AUC_{arteriole}$ β (95% CI)	Venular MD (%) β (95% CI)
Adjusted R^2	0.15	0.34	0.03
Age (years)	− 0.30 (− 0.37, − 0.21) $p = 0.001$	− 0.33 (− 0.41, − 0.25) $p < 0.001$	–
cTnT (pg/mL)	− 0.19 (− 0.35, 0.03) $p = 0.026$	− 0.12 (− 0.29, 0.05) $p = 0.081$	–
NT-proBNP (pg/mL)	–	− 0.12 (− 0.30, − 0.06) $p = 0.049$	NS

No significant associations were observed for vein dilation or AUC_{vein}, during FLIP, with any of the cardiac stress markers. All analyses were adjusted for age, cotinine, TNF-α, total cholesterol:HDL–cholesterol ratio, glycated haemoglobin (HbA_{1c}), 24H PP, hypertensive/ diabetic retinopathy. Additional adjustments were made for CRVE in the CRAE model and vice versa. For all dynamic retinal analyses, vessel segment diameter was included as a covariate. For NT-proBNP models, thyroxin and triiodothyronine were added as covariates. No statistical significant associations existed between MC of the artery and the cardiac stress markers

ns not significant, *CI* confidence interval, *AVR* artery-to-vein ratio, *CRAE* central retinal artery equivalent, *CRVE* central retinal vein equivalent, *MD* maximum dilation during FLIP, *MC* maximum constriction during FLIP, *AUC* during FLIP, *cTnT* cardiac troponin T, *NT-proBNP* N-terminal pro-brain natriuretic peptide

proBNP, in Africans, although not in Caucasians. Smaller estimated size of the central retinal artery (CRAE) was associated with higher cTnT. A smaller central retinal artery diameter and wider vein diameter (CRVE) links to a greater CVD and stroke risk profile and reflects long-term exposure to high BP [30, 31]. Higher cTnT levels have also been identified as an independent stroke risk marker and predictor of cerebral micro-bleeds [32, 33]. This prediction of cerebral micro-bleeds may imply a role of cTnT in other sub-clinical microvessel diseases. Indeed, our findings support this notion, as the relative risk of developing stroke in the subsequent 10 years was about 50% more increased in Africans when cTnT levels were above 4.2 ng/mL (OR = 1.51). Overall, higher systemic levels of cTnT predicted AV nicking and CRVE ≥ 248 MU, in Africans (OR 1.26 and 0.98), respectively (Supplementary Fig. S1).

Yet another association that existed solely in the African group is that of NT-proBNP with a wider venular calibre. This might indicate that an increased volume load, due to pressure build-up in the venous system and sub-clinical cardiac stress. This possibly contributes to wider venular diameters, eventual diminishing of myogenic control mechanisms and autoregulation, in an attempt to expand the accommodation capacity [34–36]. The latter modified haemodynamic response may also contribute to stroke risk, as its end-result lies in disrupting the autoregulatory competence of both the cerebral and retinal microvasculature. Our previous work supports this finding, in that increased diastolic ocular perfusion pressure, or hypoperfusion, was associated with an increased vein diameter [22], a recognised risk predictor for stroke [8]. Currently, we showed that a mean hypertensive state, higher cTnT, lower NT-proBNP levels and structural changes in retinal vasculature were all independently related to stroke risk.

Lower levels of NT-proBNP were directly linked to increased stroke risk in a North American bi-ethnic population [20, 21]. Our findings, therefore, support this observation with an increased 10-year stroke probability in Africans (OR = 1.57) existing when NT-proBNP levels were below the age- and gender specific reference ranges. Additionally, a greater CRVE (≥ 248 MU) and AV nicking predicted a slightly higher UCLA stroke risk score in Africans (ORs = 1.10 and 1.06).

Functionality: Dynamic Retinal Vasculature Responses' to FLIP, Cardiac Stress Markers and Stroke

The current study revealed contrasting ethnic associations of cTnT and the retinal arteriole's ability to maximally dilate in response to FLIP, possibly due to sustained high BP and the accompanying endothelial impairment. cTnT is associated with coronary microvascular-and endothelial function [37]. This introduces the possibility that a compromised haemodynamic state (sustained hypertension, volume load, increased DOPP and increased ischemia) and accompanying endothelial dysfunction additionally contribute to alterations in retinal

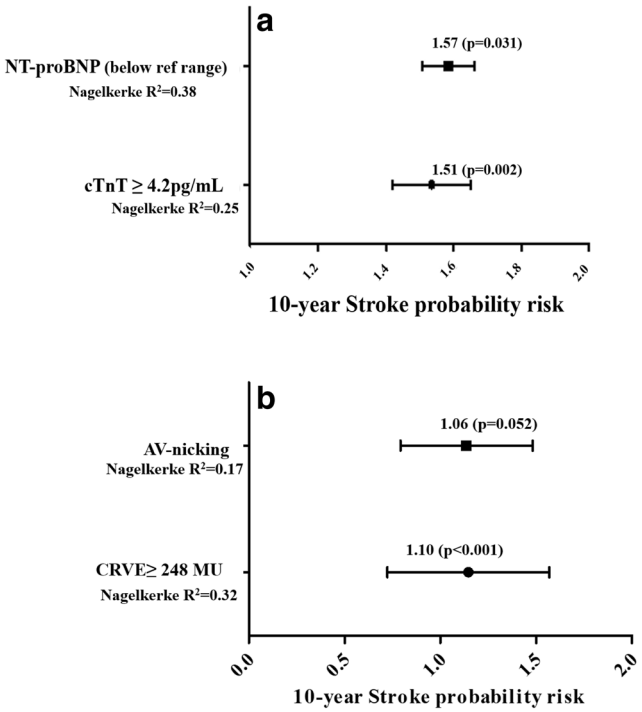

Fig. 1 Odds ratios (95% CI) predicting **a** probability of medium–high 10-year stroke risk with a cTnT level of ≥ 4.2 pg/mL and NT-proBNP levels below the age-and-gender specific reference values for Africans and **b** probability of medium–high 10-year stroke risk with AV nicking and CRVE > 248 MU in Africans. Strength of modelled relationships depicted as Nagelkerke^2 values (R^2). These odds ratios were exclusively present in Africans ($N = 107$)

vessel autoregulation. This might possibly be by way of pressure-alterations and/or desensitised responses to endothelial-linked messengers, such as nitric oxide [22]. Schneider et al. [13] showed that cTnT does not only associate with general endothelial function, but specifically with dysfunction related to cerebrovascular pathology. Higher levels of cTnT was associated with increased risk for vascular dementia. cTnT may possibly also contribute to sub-clinical small vessel disease affecting the retina (retinopathy), heart (CAD) [9] and the brain (stroke and dementia) [13]. To further link cTnT's role to stroke risk, we refer to the inverse association with retinal arteriolar dilation in the current study. A recent study conducted by Conzen [38] reported diminished retinal arteriolar dilation in patients with sub-arachnoid haemorrhage. This might not only support the attenuated arteriolar dilation response to FLIP with increased cTnT levels in Africans but indirectly links a diminished arterial dilatory response to an increased risk for stroke. Additional cardiac stress markers (NT-proBNP) may relate to this dynamic profile, as literature regarding cTnT and the microvasculature is sparse.

The lower levels of NT-proBNP in Africans, accompanied by higher BP, may indicate and relate to early endothelial dysfunction and subsequent desensitisation to the vaso-reactive, cardio-protective effect of BNP. This scenario indicates that a pre-existent increase in cardiac stress might influence the dilatory ability of the arteriole during FLIP. This, in turn, may affect the general autoregulatory capacity of the retinal microvasculature. We carefully suggest that the arterioles may subsequently take a longer time to autoregulate during FLIP and may possibly affect the recovery of typical vessel diameter after FLIP. Retinal autoregulation is controlled by glial cell activity [28]. Glial cells not only regulate the release of BNP, but certain actions of glial cells are also controlled by the binding of BNP [18]. Expression of BNP has also been detected in astroglial cells, which cohesively function with the microvasculature [19]. Neurons, glial cells and the cerebral endothelium exhibit a unique relationship as they function as a cohesive unit, similar to that of the retinal microvasculature [38]. A diminished vessel response capacity and possibly impaired autoregulatory ability may additionally support the increased stroke risk observed at lower NT-proBNP levels. However, whether alterations in NT-proBNP levels precede and/or occur during ischemic stroke or perfusion deficits remains unknown. Therefore, we carefully suggest that in an already compromised high pressure system, lower bioavailability of NT-proBNP may contribute to the impaired autoregulatory ability of the retinal and cerebral microvasculature. This may either be due to pressure-induced endothelial dysfunction or by way of the previously described glial cell interaction. The latter not only notably links the retinal vasculature to the cerebral circulation but may also provide additional support for NT-proBNP as an independent indicator of increased stroke risk probability.

Limitations and Recommendations

Limitations of the current study may include the specific population (only including African and Caucasians teachers from one demographic area) as well as the cross-sectional design, which prevents identification of physiological mechanistic cause-and-effect relationships. Furthermore, as the risk profile differs distinctly between our African and Caucasian teachers, we are unable to evaluate whether the differences are ethnic specific, or whether the results are due to the observed higher risk in the African cohort. Unfortunately, the sample size of pair-matched risk profiles was too small to perform meaningful statistical analyses. Several investigations have identified increased psychosocial stress experienced by this vulnerable African cohort, as one of the main contributors to this observed higher risk profile [22, 23, 25, 26]. However, the exploration of psychosocial stress influences is not within the scope of the current investigation and we suggest assessing the effect of psychosocial stress on the structure and function of the retinal vasculature, cardiac stress markers and stroke risk. We further recommend future retinal vessel investigations to include beat-to-beat BP monitoring, as cardio-metabolic

demands increase during the FLIP, and blood pressure variations will occur in order to maintain homeostasis.

Conclusion

Our findings in Africans revealed that reduced retinal arteriolar calibres were associated with cTnT, whereas an attenuated dilatory response to FLIP was inversely associated with both cTnT and NT-proBNP. A wider venular calibre was positively associated with NT-proBNP. These cardiac stress–retinal associations additionally predicted, albeit sub-clinically, an increased stroke risk in the SABPA Africans. These results support the notion that structural and functional similarities exist between retinal, cerebral and coronary circulations. Observable changes in both the retinal vasculature and cardiac stress markers may serve as investigative tools for the identification, discovery and prediction of cerebrovascular risk.

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Compliance with Ethical Standards

The SABPA study obtained ethical approval from the Health Research Ethics Committee (HREC) of the NWU and extended approval was granted for the second phase (ethics number: NWU-00036-07-S6). Written informed consent was obtained from all volunteers prior to participation. All procedures and objectives were explained to the participants prior to their recruitment and adhered to the applicable institutional guidelines and terms, as stated by the Declaration of Helsinki (2004). Please also refer to uploaded SABPA Protocol article.

Conflict of Interest The authors declare that they have no conflict of interest.

Disclaimers None.

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