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Relationship of coagulation and fibrinolytic variables with arterial structure and function in Africans

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ABSTRACT

Introduction: Although both coagulation and fibrinolysis are associated with cardiovascular disease (CVD) the underlying nature and pathways of many of these associations are still unclear. Our aim was to determine which of the current or 5-year prior levels of total fibrinogen, fibrinogen γ', plasminogen activator inhibitor-1 ($PAI\text{-}1_{act}$) and global fibrinolytic potential were the stronger determinant of arterial structure and function.
Materials and methods: This prospective study consisted of 2010 Africans over the age of 35 years with 5-year follow-up data available for 1288 participants. Cardiovascular measurements included arterial stiffness, blood pressure and carotid intima media thickness.
Results: Fibrinogen γ' showed stronger associations with blood pressure than total fibrinogen also in the presence of other CVD risk factors. $PAI\text{-}1_{act}$ was positively associated with blood pressure both cross-sectionally and prospectively, with the longitudinal association being the stronger determinant, also after adjustment for known CVD risk factors. Clot lysis time (CLT) was positively associated, both prospectively and cross-sectionally, with intima media thickness and negatively with markers of arterial stiffness but not after adjustment for known CVD risk factors.
Conclusions: Fibrinogen γ' was more strongly associated with CVD function than total fibrinogen. $PAI\text{-}1_{act}$ was significantly associated with blood pressure with changes in PAI-1 levels preceding changes in blood pressure. Different mechanisms may be at play determining arterial wall stiffness/thickening and blood pressure as observed from the opposing associations with $PAI\text{-}1_{act}$ and CLT. CLT was not independently related to cardiovascular measures as its associations were weakened in the presence of other known CVD risk factors.

Introduction

Haemostatic abnormalities such as hypercoagulability and hypofibrinolyis have been associated with thrombotic diseases such as cardiovascular diseases (CVD) [1]. The nature and pathways of these associations is, however, still under investigation. Some of the coagulation variables, such as fibrinogen, have been independently and consistently linked to the development of CVD in both cross-sectional and prospective data analysis [2]. Others, such as von Willebrand factor, FVII and FVIII have been associated with CVD only in cross-sectional analysis (e.g. case-control studies), or the association with future risk was significantly diminished after adjustment for other CVD risk factors, questioning its possible causal contribution to CVD development [2–5]. Also for fibrinogen there is discussion regarding its causality since Mendelian randomization studies have been inconclusive [6] and therapeutic lowering of fibrinogen has not shown benefit in studies of ischemic stroke [7].

Although hypofibrinolysis is considered to be associated with thrombosis, existing prospective studies have not consistently shown an independent association between fibrinolytic factors and CVD. While most studies have found significant associations between plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator antigen and/or activity (tPA_{ag}, tPA_{act}) and plasminogen with several types of CVD, these associations were significantly reduced in many of the studies after adjustment for other CVD risk factors [4,8–14]. One of the questions that are difficult to answer is whether changes in fibrinolysis play a causal role in thrombosis or whether they reflect changes induced by the ischemic insult. Some data is available investigating the relationship of fibrinolytic variables with CVD progression such as

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vascular stiffness, hypertension and intima media thickness (IMT) [15–19] but the topic remains as of yet inconclusive. The relationship between fibrinolysis and CVD can also be investigated through the use of assays reflecting global fibrinolytic potential (reported as clot lysis time, CLT, for example) instead of analyzing individual components of the fibrinolytic system. A limited number of studies exist that investigates the association between CLT and CVD events using different CLT assays [20–22] but no information is available regarding the association of CLT with markers of arterial structure and function, which would aid in elucidating the mechanisms behind the associations with CVD events.

Another haemostatic variable for which no prospective data exist, but which have been associated with CVD through case control studies, is fibrinogen γ'. Fibrinogen γ' arises from a splice variant of the γ chain mRNA resulting from an alternative polyadenylation signal in intron 9 [23]. Its levels have been associated with both venous and arterial thrombosis, but with opposite trends of lower levels in venous and higher levels in arterial thrombosis [23]. Data collected in prospective studies investigating the association between haemostatic and fibrinolytic variables and CVD have largely been obtained from white populations. Results from the ARIC study showed that, while fibrinolysis plays a role in the early stages of atherosclerosis in white participants, this was not the case in African-Americans [15]. Studies investigating these associations in other ethnicities such as Africans, who are considered an under-studied population in CVD etiology, are largely lacking.

The aim of our study was to investigate the association of selected haemostatic variables, including both coagulant and fibrinolytic proteins, with arterial structure and function in a prospective study including 2010 Africans. Total fibrinogen (the final substrate of coagulation), and its variant fibrinogen γ'(a novel, emerging risk factor) were selected as coagulation variables with PAI-1_{act} (the major inhibitor of clot lysis) and CLT (a global estimate of fibrinolytic potential) representing fibrinolytic variables. This was performed by investigating both the longitudinal (prospective) as well as cross-sectional associations of these variables with markers of arterial structure and function such as vascular stiffness (augmentation index (AI) and pulse wave velocity), blood pressure and carotid intima media thickness.

Materials and Methods

Study Cohort

The Prospective Urban and Rural Epidemiological (PURE) study is a large-scale cohort study that tracks changing lifestyles, risk factors and chronic disease in rural and urban areas of 21 countries from all major continents over 12 years. For further details see Teo et al. [24]. In South Africa the baseline data was collected in 2005 and the first follow-up data in 2010. Two thousand and ten randomly selected African participants (1260 women and 750 men) living the North West Province of South Africa participated in the PURE study in 2005. These participants were recruited from 6000 randomly selected households from two communities based on representativeness and feasibility for long-term follow-up, according to the guidelines stipulated in the overarching PURE study [24,25]. Apparently healthy black South Africans, older than 35, were eligible to participate. Exclusion criteria were use of chronic medication for non-communicable diseases and/or any self-reported acute illness. In 2010, 1288 of the original 2010 participants were available for follow-up. Reasons for the smaller number of participants were: refusal to participate again or withdrawal from the study, relocation, not being available on days of sample collection or acute illness or death (n = 230). Efforts to retain participants and limit loss during the five year period was made through three monthly follow-up visits by trained field workers as well as completion of annual follow-up reports. The study complied with the Helsinki Declaration and was approved by the ethics committee of the North-West University. Subjects signed informed consent before taking part in the study. All data were treated confidentially and all analyses were performed with coded data with the operator performing laboratory assays blind to patient status.

Blood Processing

Fasting blood samples were collected with minimum stasis from the antecubital veins of participants between 07:00 and 11:00 am. For the analysis of fibrinogen γ', total fibrinogen, PAI-1_{act} and turbidimetric measurement of clot formation and lysis, blood was collected into citrate tubes (1 part of 0.1 M Na citrate to 9 parts of blood). Samples were centrifuged at 2000 x g for 15 minutes at 10 °C within 30 minutes of collection. Aliquots were frozen on dry ice, stored in the field at -18 °C and then after 2-4 days at -82 °C until analysis.

Analysis of Coagulation and Fibrinolysis Parameters

Coagulation and fibrinolytic variables were analysed in 2005 and 2010. Total fibrinogen concentrations were measured using a modified Clauss method on an automated coagulation analyser (Multifibrin U-test, BCS analyser, Dade Behring, Deerfield, IL, USA for the 2005 samples and ACL-200, Instrumentation Laboratories, Milan, Italy for 2010). One hundred and forty of the 2005 samples were re-run with the 2010 samples to exclude the possibility that batch differences were artefacts resulting from the use of the different analysers. Fibrinogen γ' was measured by ELISA using the 2.G.H9 mouse monoclonal coating antibody against the human γ' sequence from Santa Cruz Biotechnology (Santa Cruz, USA) for antigen capture and a goat polyclonal HRP-conjugated antibody against human fibrinogen from Abcam for development (Cambridge, USA) [26,27]. PAI-1_{act} was measured using an indirect enzymatic method (Spectrolyze PAI-1, Trinity Biotech, Bray, Ireland). Plasma fibrinolytic potential of tissue factor induced clots, lysed by exogenous tPA was analyzed using turbidimetric analysis (A405nm) according to the method of Lisman et al. [28], with slightly modified tissue factor and tPA concentrations in order to obtain clot lysis times of about 60 min (intra-assay CV = 3.6%, between plate CV = 4.5%). Final concentrations were tissue factor (125 x diluted – an estimated final concentration of 59 pM according to Duckers et al. [29]; Dade Innovin, Siemens, Marburg, Germany), $CaCl_2$ (17 mmol/l), tPA (100 ng/ml; Actilyse, Boehringer Ingelheim, Ingelheim, Germany) and phospholipid vesicles (10 μmol/l; Rossix, Mölndal, Sweden). CLT was defined as the time from the midpoint in the transition from the initial baseline to maximum turbidity, which is representative of clot formation, to the midpoint in the transition from maximum turbidity to the final baseline turbidity, which represents the lysis of the clot [28]. The coefficient of variance for all assays was < 10%.

Cardiovascular Measurements

The following cardiovascular measurements were performed at the follow-up in 2010. Estimated central (cSBP) and brachial (bSBP) systolic blood pressure (also measured in 2005) and augmentation index (AI) were measured with participants seated upright with the right arm supported at heart level, using the Omron HEM 9000AI (Omron HealthCare, Kyoto, Japan). A SonoSite Micromax Ultrasound system (SonoSite Inc., Bothell, WA) and 6-13 MHz linear array transducer were used to determine carotid intima-media thickness (cIMT). Images from at least two optimal angles of the left and right common carotid artery were obtained. Measurements were conducted using the semi-automated program; Artery Measurement Systems II v1.139 (Chalmers University of Technology, Gothenburg, Sweden). Far wall measurements were used. Pulse wave velocity was measured using noninvasively accessible superficial pulses and the Complior SP device (Artech-Medical, Pantin, France) in an upper limb muscular artery, over the carotid radial segment (carotid-radial PWV [crPWV]), reflecting vascular stiffness in muscular arteries and in an elastic muscular mixed arterial segment