CARDIOVASCULAR DISEASE AND ITS ASSOCIATED RISK FACTORS IN SOUTH AFRICAN CHRONIC KIDNEY DISEASE PATIENTS

DR HON-CHUN HSU

A thesis submitted to the Faculty of Health Sciences,

University of the Witwatersrand, Johannesburg,

for the degree Doctor of Philosophy

Johannesburg, South Africa

2022

Declaration

This is to certify that this thesis is my own work and has not been submitted before for any degree or examination in this or any other University.

I have worked closely with my supervisors Professor Patrick Dessein and Professor Gavin Norton. I recruited the patients in my practice and obtained their written informed consent. I conducted all clinical assessments and evaluated the blood results.

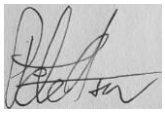
Ms Chanel Robinson who belongs to a research team at the School of Physiology assisted me with the applanation tonometry using Sphygmocor software and echocardiography.

Professor Patrick Dessein assisted me with the data analysis, interpretation of results and drafting of manuscripts. Professor Gavin Norton and Professor Angela Woodiwiss advised on the evaluation of diastolic function and gave valuable intellectual input.

I hereby certify that the studies contained in this thesis have been approved by the Committee for Research in Human Subjects, University of the Witwatersrand, Johannesburg. The ethics approval number is M15-08-43.

Signed on the 26th of February 2022.

Dr Hon-Chun Hsu (Student)



……

Prof Patrick H. Dessein (Supervisor 1)

……

Prof Gavin R. Norton (Supervisor 2)

……

Table of Contents

Page

Declaration…… 2

Publications arising from this work……4

Abstract……..5

Abbreviations……8

Acknowledgements……9

Introduction……...10

Study/Paper 1……22

Study/Paper 2……51

Study/Paper 3……85

Study/Paper 4……121

Summary of the results, implications and conclusions……146

Ethics approval……... 160

Publications and presentations arising from this work

1. Hsu H-C, Robinson C, Woodiwiss AJ, Norton GR, Dessein PH. Cardiovascular risk factors and disease in black compared to other Africans with chronic kidney disease. Int J Nephrol 2021 Feb19;2021:8876363. doi: 10.1155/2021/8876363. eCollection 2021.PMID: 33680512
2. Hsu H-C, Norton GR, Robinson C, Woodiwiss AJ, Dessein PH. Potential determinants of the E/e‘ ratio in non-dialysis compared to dialysis patients. Nephrology 2021;26:988-998.
3. Hsu H-C, Norton GR, Peters F, Robinson C, Dlongolo N, Solomon A, Teckie G, Woodiwiss AJ, Dessein PH. Association of post transplant anaemia and persistent hyperparathyroidism with diastolic function in stable kidney transplant recipients. Int J Nephrol Renovasc Dis 2021;14:1-13.
4. Hsu H-C, Robinson C, Norton GR, Woodiwiss AJ, Dessein PH. The optimal haemoglobin target in dialysis patients may be determined by its opposing effects on arterial stiffness and pressure pulsatility. Int J Nephrol Cardiovasc Dis 2020;13:385-395.

Potential determinants of diastolic function at different stages of chronic kidney disease. Joint paper delivered as a ***plenary talk*** at the **Second International Webinar on Nephrology, Urology and Kidney Failure** held on September 17, 2021 (Online Meeting).

Towards preventing heart failure with preserved ejection fraction in non-dialysis and dialysis patients, and stable kidney transplant recipients. Joint paper to be delivered as a ***Keynote Presentation*** at the **World Nephrology Congress** in Barcelona, Spain (recently postponed due to COVID-19).

Abstract

The markedly increased mortality in chronic kidney disease (CKD) patients is mostly due to an enhanced risk of cardiovascular disease (CVD). The most documented CKD induced cardiovascular abnormalities include impaired diastolic function that underlies heart failure with preserved ejection fraction and arteriosclerosis leading to impaired aortic function. CKD is highly prevalent in South Africa. The extent to which CKD enhances CVD risk among black Africans remains largely unknown.

This thesis comprises a series of investigations that evaluated CVD and its risk factors in black compared to other Africans (first study), examined potential determinants of diastolic function in predialysis and dialysis patients (second study) as well as stable kidney transplant recipients (third study), and tested the hypothesis that the optimal haemoglobin target in dialysis patients may be determined by its contrasting effects on arterial stiffness and pressure pulsatility (fourth study).

In the first study, we evaluated cardiovascular risk factors, aortic function by applanation tonometry using SphygmoCor software, cardiac function by echocardiography, atherosclerosis extent by carotid ultrasound and cardiovascular event rates in 115 consecutive predialysis (n=67) and dialysis patients (n=48) including 46 black and 69 other (32 Asian, 28 white and 9 mixed race) participants. Data were analysed in multivariable regression models. Overall, black compared to other African CKD patients had less frequent carotid artery plaque (30.4% versus 58.8%; OR (95% CI)=0.38 (0.16-0.91) despite an increased cardiovascular risk factor burden. In receiver operator characteristic curve analysis, the Framingham score performed well in identifying non-black but not black CKD patients with carotid plaque (area under the curve (AUC) (95% CI)=0.818 (0.714-0.921) and AUC (95% CI)=0.556 (0.375-0.921), respectively). Black compared to other African predialysis patients experienced larger Framingham scores and more adverse non-traditional cardiovascular risk factors, impaired arterial and diastolic function but similar cardiovascular event rates (OR (95% CI)=0.93 (0.22 to 3.87)). Among dialysis patients, black compared to other Africans had an overall similar traditional and non-traditional cardiovascular risk factor burden, similar arterial and diastolic function and reduced cardiovascular event rates (OR (95% CI)=0.22 (0.05 to 0.88)).

For the second study, we assessed cardiovascular risk factors, arterial function, N-terminal natriuretic peptide (NT-proBNP) levels as a marker of volume overload, and diastolic function in 103 (62 non-dialysis and 41 dialysis) patients. In established confounder adjusted analysis, dialysis status impacted the pulse wave velocity-E/e' relationship (interaction p=0.01) but not the NT-proBNP level-E/e' association (interaction p=0.1). Upon entering arterial function measures and NT-proBNP levels simultaneously in regression models, arterial function measures were associated with E/e' (p=0.008 to 0.04) in non-dialysis patients whereas NT-proBNP levels were related

to E/e' in dialysis patients (p=0.009 to 0.04). Bivariate associations were found between diabetes (p<0.0001) and E/e' in non-dialysis patients, and haemoglobin concentrations and E/e' (p=0.02) in those on dialysis. Upon adjustment for diabetes in non-dialysis patients, only central pulse pressure remained associated with E/e' (p=0.02); when haemoglobin concentrations were adjusted for in dialysis patients, NT-proBNP levels were no longer associated with E/e' (p=0.2). In separate models, haemoglobin levels were associated with E/e' independent of left ventricular mass index and preload and afterload measures (p=0.02 to 0.03).

For the third study, we evaluated traditional and non-traditional cardiovascular risk factors, carotid artery atherosclerosis, arterial function and diastolic function in 43 kidney transplant recipients with a transplant duration of $\geq$6 months, no acute rejection and a glomerular filtration rate of $\geq$15 ml/min/1.73m^2. The mean (SD; range) transplant duration was 12.3 (8.0; 0.5-33.8) years. Post transplantation anaemia and persistent secondary hyperparathyroidism were identified in 27.9% and 30.8% of the patients, respectively; 67.5% of participants were overweight or obese. In established confounder adjusted analysis, haemoglobin (partial R=-0.394, p=0.01) and parathyroid hormone concentrations (partial R=0.382, p=0.02) were associated with E/e'. In multivariable analysis, haemoglobin (partial R=-0.278, p=0.01) and parathyroid levels (partial R=0.324, p=0.04) were independently associated with E/e'. Waist-height ratio (partial R=-0.526, p=0.001 and partial R=-0.355, p=0.03), waist circumference (partial R=-0.433, p=0.008 and partial R=-0.393, p=0.02) and body mass index (partial R=-0.332, p=0.04 and partial R=-0.489, p=0.002) were associated with both e' and E/A, respectively, in established confounder adjusted analysis. The haemoglobin-E/e' (partial R=-0.422, p=0.02), parathyroid hormone-E/e' (partial R=0.434, p=0.03), waist-height ratio-e' (partial R=-0.497, p=0.007) and body mass index-E/A (partial R=-0.386, p=0.04) relationships remained consistent after additional adjustment for left ventricular mass index and cardiac preload and afterload measures.

For the fourth study, cardiovascular risk factors and arterial function was assessed in 48 dialysis patients. In established confounder and diabetes adjusted linear regression models, haemoglobin levels were directly associated with arterial stiffness (partial R=0.366, p=0.03) and inversely with central systolic pressure (partial R=-0.344, p=0.04), central pulse pressure (partial R=-0.403, p=0.01), peripheral pulse pressure (partial R=-0.521, p=0.001) and forward wave pressure (partial R=-0.544, p=0.001). The presence of heart failure and use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers and erythropoietin stimulating agents did not materially alter these relationships upon further adjustment for the respective characteristics in the models, and in sensitivity analyses. In receiver operator characteristic curve analysis, the optimal haemoglobin concentration cut-off values in predicting arterial stiffness and increased central pulse pressure were remarkably similar at 10.95 g/dl and 10.85 g/dl respectively, and with clinically useful sensitivities, specificities and positive and egative predictive values. In logistic regression

models, a haemoglobin value of >10.9 mg/dl was associated with both arterial stiffness (>10 m/sec; OR (95% CI) = 10.48 (1.57 – 70.08), p=0.02) and normal central pulse pressure (>50 mmHg; OR (95% CI) = 7.55 (1.58 – 36.03), p=0.01).

In conclusion, black compared to other African predialysis patients experience an increased traditional and non-traditional cardiovascular risk factor burden and more impaired arterial and diastolic function. Once on dialysis, black African patients sustain less frequent cardiovascular event rates, which may represent a survival bias. The atherosclerotic burden is smaller in black compared to other African CKD patients but the Framingham score is unreliable in identifying those with very high risk atherosclerosis, which is nevertheless present in ~30% of them. Cardiovascular risk factors that are independently associated with markers of diastolic function include impaired arterial function and diabetes in predialyis patients, volume overload and low haemoglobin concentrations in dialysis patients, and post transplantation anaemia and persistent secondary hyperparathyroidism in kidney disease transplant recipients. The optimal haemoglobin target in dialysis patients may be determined by its contrasting effects on aortic stiffness and pressure pulsatility. These findings have implications in our understanding of CVD risk and its management among CKD patients.

Abbreviations

AUC	Area under the curve
CI	Confidence interval
CKD	Chronic kidney disease
CVD	Cardiovascular disease
ESRD	End stage renal disease
HFpEF	Heart Failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
NT-proBNP	N-terminal natriuretic peptide
OR	Odds ratio
SD	Standard deviation
ROC	Receiver operator curve

Acknowledgements

Special thanks to Professor Patrick Dessein for inspiring me to pursue a PhD while in full time private practice. Your contagiousness, enthusiasm and guidance spurred enough confidence in me to complete this series of interesting studies. To Professor Gavin Norton, I am grateful that you accepted me into the School of Physiology and for your detailed guidance during the design and draft of the study. I would not have been able to complete this thesis without the patience and knowledge of my supervisors.

To my parents, thank you for always believing in me. I owe all my accomplishments to your love and kindness.

I am most grateful to all my patients who participated in this work. They gave up their precious time to help others benefit from their suffering. This selfless sacrifice inspired me to be a better doctor.

Lastly to my wife, thank you for taking such good care of our boys while I concentrated my efforts on this thesis. I could not have done it without you.

Introduction

Chronic kidney disease (CKD) contributes substantially to morbidity and mortality (Global Burden of Disease (GBD) Chronic Kidney Disease Collaboration, 2020). This is a worldwide phenomenon. The main risk factors for CKD are hypertension and diabetes. Increased mortality in CKD is mostly due to a markedly enhanced risk of cardiovascular disease (CVD). In this regard, a systematic analysis for the Global Burden of Disease, Injuries, and Risk Factors Study in 2017 revealed a global chronic kidney disease prevalence of 9.1% (95% confidence interval 8.5 to 9.8) in 2017 with an increase of 29.3% (26.4 to 32.6) since 1990. Globally, in 2017, 1.2 (1.2 to 1.3) million people died from CKD and the all-age mortality rate from CKD had increased by 41.4% (35.2 to 46.5) since 1990 (GBD Chronic Kidney Disease Collaboration, 2017). In addition, 1.4 (1.2-1.6) million cardiovascular disease related deaths were attributable to CKD.

CKD and CVD risk in black compared to other Africans

A recent sub-Saharan population study (George JA et al, 2019) reported an age-standardised population CKD prevalence of 10.7% (9.9 to 11.7). CKD prevalence were larger in South African sites (14.0% (11.9 to 16.4)) than in West African sites (9.5% (8.3 to 10.8)). Risk factors for CKD included older age, hypertension, diabetes and human immunodeficiency virus infection whereas male sex was protective. Regional differences in CKD and its risk factors were attributed to disparities in sociodemographic and epidemiological health transition stages.

Available data on CVD risk among CKD patients originates mostly in studies that were performed in high-income populations. Most patients with CKD reportedly die from CVD prior to developing kidney failure (Cozzolino M et al, 2018; Sarnak MJ et al, 2019). Both adverse traditional cardiovascular risk factor profiles and kidney disease specific factors including anaemia, chronic volume overload, calcium-phosphate imbalance and oxidative stress mediate CVD in CKD patients. CKD thereby causes cardiovascular changes that characteristically include advanced arteriosclerosis leading to impaired arterial function (Zanoli L et al, 2019) and left ventricular fibrosis that results in diastolic dysfunction and is often accompanied by hypertrophy (Wang X et al, 2019). CKD also

enhances atherosclerosis (Sarnak MJ et al, 2019). These changes result in an increased risk of heart failure, arrhythmias, sudden death, stroke and myocardial infarction. The risk of cardiovascular disease increases from 1.5 fold in patients with stage 2 CKD to 20 fold in those with end stage renal disease (ESRD) (Cozzolino M et al, 2018).

Sub-Saharan Africa comprises low and middle income countries (Sub-Saharan Africa Population 2020 (Demographics, Maps, Graphics); World Bank Country and Lending Groups: Country Classification). Due to the rapid current urbanization and consequent epidemiological health transition, cardiovascular risk factor profiles and their impact on CVD as well as cardiovascular event phenotypes differ in low or middle compared to high income populations (Solomon A et al, 2014). Black South African persons currently experience smaller age-standardized mortality rates from ischemic heart disease than their non-black counterparts (Pillay-Van Wijk V et al, 2016). By contrast, age-standardized mortality rates due to cerebrovascular and hypertensive heart disease are much larger in black compared to other South Africans (Pillay-Van Wijk V et al, 2016).

Compared to their white counterparts, black Americans with pre-dialysis CKD patients experience an enhanced risk of developing ESRD and CVD mortality (Choi AI et al, 2008; Carnethon MR et al 2017). However, survival is better in black compared to white Americans once they are on dialysis.

The extent to which CKD impacts CVD risk among black Africans remains largely unknown. In two retrospective cohort studies, sepsis was a more frequent cause of death than CVD among predominantly black African dialysis patients (Luyckx VA et al, 2009; Tamayo Isla RA et al, 2016). Black African dialysis patients may be less prone to coronary and aortic calcification (Freercks R et al, 2012). However, carotid artery plaque was identified in 38.1% of 58 black and 26 non-black African dialysis patients (Amira OC et al, 2012). A consistent shortcoming of these reported investigations is that the mean age of participants was as low as 36 to 42 years (Luyckx VA et al, 2009; Tamayo Isla RA et al, 2016; Freercks R et al, 2012; Amira OC et al, 2012).

Given these limited reported data on CVD and its risk factors among Africans with CKD, we compared cardiovascular risk factor profiles (Gunter S et al, 2018), large artery function including arterial stiffness, wave reflection and pressure pulsatility (Gunter S et al, 2018; Briet M et al, 2012; London GM, 2018), left ventricular systolic and diastolic function (Mokotedi L et al 2017), atherosclerosis extent and cardiovascular event rates between black and other African pre-dialysis and dialysis patients.

Potential determinants of the E/e' ratio in non-dialysis compared to dialysis patients

As alluded to above, impaired left ventricular diastolic function comprises a most characteristic cardiovascular abnormality induced by CKD (Wang X et al, 2019). Impaired diastolic function results from both reduced active and passive myocardial relaxation. Active relaxation is mostly estimated by the echocardiographically determined ratio of early (E) to late (A) diastolic filling or mitral inflow velocity (E/A) and the early diastolic mitral annulus motion (e'). Active relaxation involves high energy requiring rapid removal of cytosolic calcium into the sarcoplasmic reticulum, thin filament deactivation and cross-bridging kinetics. Passive relaxation is typically assessed by the E/e' ratio, which is a measure of left ventricular filling pressure. Left ventricular fibrosis impairs passive relaxation. E/A, e' and E/e' are indices that form part of currently recommended algorithms in the identification of patients with diastolic dysfunction (Nagueh SF, 2016).

Impaired diastolic function underlies the development of heart failure with preserved ejection fraction (HFpEF), the prevalence of which increases with decreasing kidney function. HFpEF is more prevalent and carries a worse prognosis than heart failure with reduced ejection fraction (HFrEF) in CKD patients (Kim MK et al, 2013; Ahmed A et al, 2007).

Impaired diastolic function in CKD patients is mediated by increased preload due to volume overload and anaemia as well as increased afterload caused by arteriosclerosis mediated arterial

stiffness. Arterial stiffness enhances the forward wave pressure and thereby wave reflection and pulse pressure (Wang X et al, 2019; Zanoli L et al, 2019).

Left ventricular hypertrophy comprises another highly prevalent cardiovascular abnormality in CKD (Wang X, 2019; Zanoli L, 2019). It is also due to increased preload and afterload and is a strong predictor of incident cardiovascular events. However, the reported mechanistic link between impaired diastolic function and left ventricular hypertrophy has been questioned (Wang X et al, 2019).

Circulating levels of N-terminal proBNP (NT-proBNP) are mostly used in the evaluation of patients with heart failure (Gutierrez MC et al, 2013). In CKD, NT-proBNP is a marker of volume overload (Kim J-S et al, 2017).

Previously reported cohort studies suggest that volume overload may be particularly important in the development of CVD among dialysis patients (Zoccalli C et al, 2017). By contrast, increased afterload may comprise a predominant cardiovascular risk mechanism among pre-dialysis patients (Chirinos JA et al, 2014). Additionally, anaemia is associated with volume overload (Hung S-C et al, 2015) as well as NT-proBNP concentrations in patients with HFpEF, whereas diabetes is strongly associated with impaired arterial function in CKD persons who are not on dialysis (Townsend RR, 2015).

We therefore hypothesized that, in established confounder adjusted analysis, impaired arterial function indices are more strongly associated with E/e' in non-dialysis compared to dialysis patients whereas NT-proBNP levels are more closely related to E/e' in dialysis compared to non-dialysis persons. We further examined whether traditional and non-traditional or renal cardiovascular risk factors including diabetes and haemoglobin levels, could explain the associations of arterial function measures and NT-proBNP levels with E/e' in CKD patients.

Association of post transplantation anaemia and persistent secondary hyperparathyroidism with diastolic function in stable kidney transplant recipients

Kidney transplant recipients experience a markedly improved quality of life and reduced CVD risk when compared to those on ongoing dialysis (Devine PA et al, 2019). Indeed, a functioning allograft can improve echocardiographically identified abnormalities. Nevertheless, kidney transplant recipients still experience a 3 to 5 fold increased risk of CVD and, in particular, non-atherosclerotic CVD (Lentine KL et al, 2005; Rigatto C et al, 2002). Approximately 18% of kidney transplant recipients still develop *de novo* heart failure within 3 years subsequent to transplantation (Lentine KL et al, 2005).

Reportedly, half of patients undergoing kidney transplantation have impaired diastolic function (Himelman RB et al, 1988). The determinants of diastolic function among stable kidney transplant recipients (transplant duration of $\geq$6 months, absence of acute rejection and glomerular filtration rate of $\geq$15 ml/min/1.37m^2) (Ducloux D et al, 2000) await further elucidation. In this regard, late post transplantation anaemia (Gafter-Gvili A et al, 2019) and persistent hyperparathyroidism (Santos RD et al, 2019) are present in 35% and 50% of kidney transplant recipients, respectively.

Anaemia can cause not only increased preload and left ventricular mass but also tissue hypoxia with consequent myocardial fibrosis (Mistry N et al, 2018; Caramelo C et al, 2007; D'Amario D et al, 2019; Lopez B et al, 2008). Secondary hyperparathyroidism can increase cardiac afterload by enhancing arteriosclerosis (Devine PA et al 2019) and induce cardiac hypertrophy and myocardial fibrosis directly (Fujii H et al, 2018).

In view of these reported findings, herein we hypothesized that post transplantation anaemia and persistent secondary hyperparathyroidism are potential determinants of diastolic function in stable kidney transplant recipients.

The optimal haemoglobin target in dialysis patients may be determined by its contrasting effects on arterial stiffness and pressure pulsatility

The prevalence of anaemia in ESRD patients is ~90% (Alemu B et al, 2021). It is caused by multiple factors including relative erythropoietin deficiency, iron deficiency, blood loss, reduced erythrocyte survival duration, inflammation, infection, underlying hematologic disease, hyperparathyroidism, hemolysis and nutritional factors (Fishbane S et al, 2018).

Upon treating severe anaemia with erythropoietin in dialysis patients, quality of life improves and the need for hospitalization and blood transfusion decreases when targeting a haemoglobin level of 11.0 g/dl (Jones M et al, 2004). However, upon targeting a haemoglobin level of ~13 g/dl with erythropoietin, the risk of all-cause mortality, stroke, hypertension and vascular access thrombosis increases (Promminitikul A et al, 2007; Palmer SC et al, 2010). Due to previously reported trial designs, the effects of a haemoglobin level of 11.5 to 13 g/dl on the vasculature remain currently unknown. The reasons why total correction of anaemia is associated with adverse CVD events in dialysis patients are unclear. Postulates include frequent underlying atherosclerotic disease, increased viscosity and platelet aggregation and increased peripheral vascular resistance. Notably in this regard, haemoglobin is a potent nitric oxide scavenger (Donadee C et al, 2011). Nitric oxide reduces arterial stiffness and peripheral vascular resistance (Wilkinson IB et al 2004). Given that CKD causes severe arteriosclerosis, it is therefore conceivable that increases in the haemoglobin level could enhance arterial stiffening further whereas a reductions in the haemoglobin level may further increase pressure pulsatility in the present context. Both arterial stiffness and pressure pulsatility increase CVD risk (Hashimoto J et al, 2010).

We therefore assessed the independent relationships of haemoglobin levels with arterial function measures including pulse wave velocity, wave reflection and pressure pulsatility in a cohort of dialysis patients.

References

Ahmed A, Rich MW, Sanders PW et al. Chronic kidney disease associated mortality in diastolic versus systolic heart failure: A propensity matched study. Am J Cardiol 2007;99:393-8.

Alemu B, Techane T, Dinegde NG, Tsige Y. Prevalence of anemia and its associated factors among chronic kidney disease patients attending selected public hospitals of Addis Ababa, Ethiopia: institutional-based cross-sectional study. Int J Nephrol Renovasc Dis 2021;14:67-75.

Amira OC, Naicker S, Manga P et al. Adiponectin and atherosclerosis risk factors in African hemodialysis patients: a population at low risk for atherosclerotic cardiovascular disease. Hemodialysis Int 2012;16:59-68.

Briet M, Boutouyrie P, Laurent S, London GM. Arterial stiffness and pulse pressure in CKD and ESRD. *Kidney Int 2012;*82:388-400.

Caramelo C, Justo S, Gil P. Anemia in heart failure: pathophysiology, pathogenesis, treatment, and incognitae. Rev Esp Cardiol 2007;60:848-860.

Carnethon MR, Pu J, Howard G et al. Cardiovascular health in African Americans: A scientific statement from the American Heart Association. Circulation 2017;136:e393-e423.

Chirinos JA, Khan A, Bansai N et al. Arterial stiffness, central pressures and incident hospitalized heart failure in the Chronic Renal Insufficiency Cohort (CRIC). Circ Heart Fail 2014;7(5):709-16.

Choi AI Rodriguez RA, Bacchetti P, Bertenthal D, Hernandez GT, O'Hare AM. White/black racial differences in risk of end-stage renal disease and death. Am J Med 2009;122:672-8.

Cozzolino M, Mangano M, Stucchi A et al. Cardiovascular disease in dialysis patients. Nephrol Dial Tranplant 2018;33 Suppl 3:iii28-iii34.

D'Amario D, Migliaro S, Borovac JA, Restivo A, Vergallo R, Galli M, et al. Microvascular dysfunction in heart failure with preserved ejection fraction. Front Physiol 2019;10:1347. doi: 10.3389/fphys.2019.01347. eCollection 2019.

Devine PA, Courtney AE, Maxwell AP. Cardiovascular risk in renal transplant recipients. J Nephrol 2019;32:389-399.

Ducloux D, Motte G, Challier R, Gibey R, Chalopin J-M. Serum homocysteine total and cardiovascular disease occurrence in chronic, stable renal transplant recipients: a prospective study. J Am Soc Nephrol 2000;11:134-137.

Freercks F, Swanepoel C, Carrara H, Moosa S, Lachman A, Rayner B. Vascular Calcification in South African dialysis patients: ethnic variation, prevalence, detection and haemodynamic correlates. *Nephrology 2012;*17:607-615.

Fujii H. Association between parathyroid hormone and cardiovascular disease. Ther Apher Dial 2018;22:236-241.

Gafter-Gvili A, Gafter U. Posttransplantation anemia in kidney transplant recipients. Acta Haematol 2019;142:37-43.

GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2020;395:709-33.

George JA, Brandenburg J-T, Fabian J. Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): a cross-sectional population study. Lancet 2019;7:e1632-43.

Gunter S, Robinson C, Woodiwiss AJ et al. Arterial wave reflection and subclinical atherosclerosis in rheumatoid arthritis. Clin Exp Rheumatol 2018;36:412-420.

Gutierrez MC, Moore PG, Liu H. Goal-directed therapy in intraoperative fluid and hemodynamic management. J Biomed Res 2013;27(5):357-65.

Hashimoto J, Ito S. Pulse pressure amplification, arterial stiffness, and peripheral wave reflection determine pulsatile flow waveform of the femoral artery. Hypertension 2010;56:926-33.

Kidney Disease: Improving. Global Outcomes (KDIGO) Anemia Work Group. KDIGO clinical practice guideline for anemia in chronic kidney disease. Kidney Int (Suppl) 2012;2:279-335.

Kim J-S, Yang J-W, Yoo JS, Choi S Ok, Han B-G. Association between E/e’ ratio and fluid overload in patients with predialysis chronic kidney disease. PLoS One 2017:13;12(9):e0184764. doi: 10.1371/journal.pone.0184764. eCollection 2017.

Kim MK , Kim B, Lee JY et al. Tissue Doppler-derived E/e' ratio as a parameter for assessing diastolic heart failure and as a predictor of mortality in patients with chronic kidney disease. Korean J Intern Med 2013;28:35-44.

Lentine KL, Schnitzler MA, Abbott KC et al. De novo congestive heart failure after kidney transplantation: a common concition with poor prognostic implications. Am J Kidney Dis 2005;46:720-733.

London GM. Arterial stiffness in chronic kidney disease and end-stage renal disease. Blood Purif 2018;45:154-158.

Lopez B, Gonzalez A, Hermida N, Laviades C, Diez J. Myocardial fibrosis in chronic kidney disease: potential benefits of torasemide. Kidney Int 2008;Suppl 111:S19-S23.

Luyckx VL, Yip A, Sofianou L, Jhangri GS, Mueller TF, Naicker S. Cardiac function in an African dialysis population with a low prevalence of pre-existing cardiovascular disease. Ren Fail 2009;31:211-220.

Mistry N, Mazer CD, Sled JG et al. Red blood cell antibody-induced anemia causes differential degrees of tissue hypoxia in kidney and brain. Am J Physiol Reg Integr Comp Physiol 2018;314:R611-R622.

Mokotedi L, Gunter S, Robinson C et al. The Impact of different classification criteria sets on the estimated prevalence and associated risk factors of diastolic dysfunction in rheumatoid arthritis. Int J Rheumatol 2017;2017:2323410.

Nagueh SF, Smiseth OA, Appleton CP et al. Recommendations for the evaluation of ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016;29:277-314.

Pillay-van Wyk V, Msemburi W, Laubscher R et al. Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. Lancet 2016;4:e642-e653.

Phrommintikul A, Haas SJ, Elsik M, Krum H. Mortality and target haemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: a meta-analysis. Lancet 2007:369:381-388.

Rigatto C, Parfrey P, Foley R, Negrijn C, Tribula C, Jeffrey J. Congestive heart failure in renal transplant recipients: risk factors, outcomes, and relationships with ischemic heart disease. J Am Soc Nephrol 2002;13:1084-1090.

Santos RD, Rossi A, Coyne D, Maw TT. Management of post-transplant hyperparathyroidism and bone disease. Drugs 2019;79:501-513.

Sarnak MJ, Amann K, Bangalore A et al. Chronic kidney disease and coronary artery disease. JACC State-of-the-Art Review. J Am Coll Cardiol 2019;74:1828-38.

Solomon A, Tsang L, Woodiwiss AJ, Millen AME, Norton GR, Dessein PH. Cardiovascular disease risk amongst African black patients with rheumatoid arthritis: the need for population specific stratification. Biomed Res Int 2014;2014, article ID 826095; http://dx.doi.org/10.1155/2014/826095.

Sub-Saharan Africa Population 2020 (demographics, maps, graphics): World Population Review. Http://worldpopulationreview.com/continents/sub-saharan-africa-population/ (Accessed 13 September 2020).

Tamayo Isla RA, Ameh OI, Mapiye D et al. Baseline predictors of mortality among predominantly rural-dwelling end-stage renal disease patients on chronic dialysis therapies in Limpopo, South Africa. PLoS One 2016;11:e0156642, 2016. doi: 10.1371/journal.pone.0156642. eCollection 2016.

Townsend RR. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. Curr Opin Nephrol Hypertens 2015;24(1):47-53.

Zoccali C, Moissl U, Chazot C et al. Chronic fluid overload and mortality in ESRD. J Am Soc Nephrol 2017;28(8):2491-7.

Zanoli L, Lentini P, Briet M et al. Arterial stiffness in the heart disease of CKD. J Am Soc Nephrol 2019;30:918-28.

Wang X, Shapiro JI. Evolving concepts in the pathogenesis of uraemic cardiomyopathy. Nat Rev 2019;15:159-74.

Wilkinson IB, Franklin SS, Cockcroft JR. Nitric oxide and the regulation of arterial stiffness. Hypertension 2004;44:112-116.

World Bank Country and Lending Groups: Country Classification, https://data-helpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending groups (Accessed 13 September 2020)

Study/Paper 1

Study/Paper 1 shows that black compared to other African predialysis patients experience an increased traditional and non-traditional cardiovascular risk factor burden and more impaired arterial and diastolic function. Once on dialysis, black African patients sustain less frequent cardiovascular event rates, which may represent a survival bias. The atherosclerotic burden is smaller in black compared to other African CKD patients but the Framingham score is unreliable in identifying those with very high risk atherosclerosis, which is nevertheless present in ~30% of them.

Research Article

Cardiovascular risk factor profiles and disease in black compared to other Africans with chronic kidney disease

Hon-Chun Hsu[1,2], Chanel Robinson[1], Angela J. Woodiwiss[1], Gavin R. Norton[1], Patrick H. Dessein[1,2,3,4]

[1]*Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[2]*Nephrology Unit, Milpark Hospital, Johannesburg, South Africa*
[3]*Internal Medicine Department, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[4]*Free University and University Hospital, Brussels, Belgium*

ORCID identifiers: Hon-Chun Hsu: https://orcid .org/0000-0003-4180-1552; Chanel Robinson: https://orcid .org/0000-0002-9385-2709; Angela J. Woodiwiss: https://orcid.org/0000-0001-2555-4125; Gavin R. Norton: https://orcid .org/0000-0001-6416-6104; Patrick H. Dessein: https://orcid .org/0000-0002-9357-4630

Correspondence should be addressed to Patrick H Dessein; patrick.dessein22@gmail.com

Abstract

Background and objectives. The extent to which chronic kidney disease (CKD) impacts cardiovascular disease (CVD) in black Africans is uncertain. We compared cardiovascular risk factors and CVD between black and other African CKD patients.

Methods. Cardiovascular risk factors, aortic and cardiac function, atherosclerosis extent and cardiovascular event rates were assessed in 115 consecutive pre-dialysis (n=67) and dialysis patients (n=48) including 46 black and 69 other (32 Asian, 28 white and 9 mixed race) participants. Data were analysed in multivariable regression models.

Results. Overall, black compared to other African CKD patients had less frequent carotid artery plaque (OR (95% CI)=0.38 (0.16-0.91) despite an increased cardiovascular risk factor burden. In receiver operator characteristic curve analysis, the Framingham score performed well in identifying non-black but not black CKD patients with carotid plaque (area under the curve (AUC) (95% CI)=0.818 (0.714-0.921) and AUC (95% CI)=0.556 (0.375-0.921), respectively). Black compared to other African pre-dialysis patients experienced larger Framingham scores and more adverse non-traditional cardiovascular risk factors, impaired arterial and diastolic function but similar cardiovascular event rates (OR (95% CI)=0.93 (0.22 to 3.87)). Among dialysis patients, black compared to other Africans had an overall similar traditional and non-traditional cardiovascular risk factor burden, similar arterial and diastolic function but increased systolic function (partial R=0.356, p=0.01 and partial R=0.315, p=0.03 for ejection fraction and systolic volume, respectively) and reduced cardiovascular event rates (OR (95% CI)=0.22 (0.05 to 0.88)).

Conclusion. Black compared to other African CKD patients have less frequent very high risk atherosclerosis and experience weaker cardiovascular risk factor-atherosclerotic CVD relationships. These disparities may be due to differences in epidemiological health transition stages. Among dialysis patients, black compared to other Africans have less cardiovascular events, which may represent a selection bias as previously documented in black American.

1. Introduction

The global prevalence of chronic kidney disease (CKD) was recently estimated at 9.1% [1], whereas that in sub-Saharan Africa was 10.7% and ranged from 6.6% to 14% in west compared to South African sites [2]. Most patients with CKD are more likely to die from cardiovascular disease (CVD) than develop kidney failure [3,4]. CVD in CKD is mediated by adverse traditional cardiovascular risk factor profiles and renal disease specific factors including calcium-phosphate imbalance, anaemia, chronic volume overload and oxidative stress [3,4]. The risk of cardiovascular disease increases from 1.5 fold in patients with stage 2 CKD to 20 fold in those with end stage renal disease (ESRD) [3].

Presently available evidence on the effects of CKD on CVD originates in studies that were mostly performed in high income countries. Sub-Saharan Africa is a large continent that consists of low and middle income countries [5,6]. The sub-Saharan African black population is currently undergoing rapid urbanization and, consequently, an epidemiological health transition [7]. Cardiovascular risk factor profiles and their impact on CVD as well as cardiovascular event phenotypes differ in low or middle compared to high income populations [7]. Black South African persons currently experience smaller age-standardized mortality rates from ischemic heart disease than their non-black counterparts [8]. By contrast, age-standardized mortality rates due to cerebrovascular and hypertensive heart disease are much larger in black compared to other South Africans [8]. Compared to their white counterparts, black Americans with pre-dialysis chronic kidney disease experience an enhanced risk of CVD mortality [9]. This disparity further increases with CKD severity [9]. However, survival is better in black compared to white Americans once they are on dialysis [9].

The extent to which CKD impacts CVD risk among black Africans is less well established. The respective evidence derives from investigations that included only patients on dialysis [10-13]. In two retrospective cohort studies, sepsis was a more frequent cause of death than CVD among predominantly black African dialysis patients [10,12]. Another study revealed a markedly low prevalence of coronary and aortic calcification in black African dialysis patients [11]. Yet, Amira and colleagues [13] identified carotid artery plaque in 38.1% of 58 black and 26 non-black African dialysis patients. Notably, the mean age of participants in these studies was as low as 36 to 42 years [10-13]. In the present study, we compared cardiovascular risk factor profiles [14], large artery function including arterial stiffness, wave reflection and pressure pulsatility [14-16], left ventricular systolic and diastolic function [17], atherosclerosis extent and cardiovascular event rates between black and other African pre-dialysis and dialysis patients.

2. Patients and Methods

2.1. Patients. One hundred and fifteen consecutive pre-dialysis or dialysis patients that included 46 black and 69 other (32 Asian, 28 white and 9 mixed race) participants, were recruited at the Milpark Hospital in Johannesburg, South Africa. Pre-dialysis patients had a Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate (eGFR) [18] of <60 ml/min.1.73m^2 upon enrolment. Patients with infection or/and active cancer were excluded. The study was approved by the University of the Witwatersrand Human (Medical) Research Ethics Committee (protocol number: M15-08-43) in Johannesburg, South Africa and performed according to the 2013 revised Helsinki Declaration. Each patient gave written informed consent.

2.2. Methods. Baseline recorded characteristics comprised demographic features, CKD staging, lifestyle factors and anthropometric parameters. Dialysis patients were investigated on the day prior to undergoing the respective procedure. Each of these patients was dialysed thrice weekly.

2.2.1. Cardiovascular risk factors and their treatment. Traditional and non-traditional or kidney disease related cardiovascular risk factors and their treatment were recorded using previously reported methods [14] and as given in the Supplementary material (methods). The overall major traditional cardiovascular risk factor burden was estimated by calculating the Framingham score [19]. For this study, a high phosphate concentration was identified in patients with a phosphate level of >1.42 mmol/l or/and when a phosphate lowering agent (calcium carbonate or sevelamer in 48 and 1 (black dialysis patient) cases, respectively) was used.

2.2.2. Arterial function. Central hemodynamic characteristics were determined using a high-fidelity SPC-301 micromanometer (Millar instument, Inc., Houston, Texas), interfaced with a computer utilizing SphygmoCor software, version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). We evaluated arterial stiffness as estimated by aortic pulse wave velocity, wave reflection as represented by the augmentation index, reflected wave pressure and reflection magnitude, and pressure pulsatility measures including central systolic and pulse pressure, peripheral pulse pressure, pressure amplification and forward wave pressure as previously reported [14] and given in the Supplementary material (methods).

2.2.3. Left ventricular structure and function. Echocardiography was performed as recommended by the American Society of Echocardiography convention [17] and using a Philips CX50 POC Compact CompactXtreme Ultrasound System (Philips Medical Systems (Pty) Ltd, USA) equipped with a 1.8-4.2 MHz probe that allowed for M-mode, 2-D, pulsed and tissue Doppler measurements. We assessed left ventricular structure as represented by mass and hypertrophy, systolic function as estimated by ejection fraction and systolic volume, and diastolic function parameters

comprising the early (E)/late (atrial) diastolic wave (A) ratio, the peak mitral annulus motion during early diastole (e') and E/e' ratio, as previously described [17] and given in the Supplementary material (methods).

2.2.4. Carotid atherosclerosis. Carotid artery ultrasound was performed using a Philips CX50 POC Compact CompactXtreme Ultrasound System (Philips Medical Systems (Pty) Ltd, USA) attached to a linear array 4.0-12.0 MHz probe. The software provided for semi-automated border detection gives markedly reproducible data as previously described [14]. Images of at least 1cm length of the distal common carotid arteries were obtained. The optimal angle of incidence was used, defined as the longitudinal angle of approach where both branches of the internal and external carotid artery were visualized simultaneously. The carotid intima-media thickness (c-IMT) was defined as the mean of the left and right common carotid artery thickness. Plaque in the extracranial carotid tree was defined according to the Mannheim consensus criteria [20]. Carotid ultrasound measurements were made by the same observer that performed the arterial function and echocardiographic evaluations (CR). The intra-observer variability of ultrasound measurements is low in our setting [14].

2.2.5. Cardiovascular event rates. Cardiovascular event rates included ischemic heart disease (acute myocardial infarction, percutaneous transluminal coronary angioplasty and/or coronary artery bypass surgery), heart failure and/or cerebrovascular and/or peripheral arterial disease that was confirmed by a cardiologist, neurologist and vascular surgeon, respectively.

2.2.6. Data analysis. Data were analysed using the IBM SPSS statistics program (version 23.0 IBM, USA) and significance was set at $p \leq 0.05$. Significance was consistently analysed with two-sided tests. Results are expressed as mean (SD) or median (interquartile range, IQR) for continuous variables and percentages for categorical variables. Non-normally distributed characteristics were logarithmically transformed prior to entering them in multivariable regression models.

We compared traditional and non-traditional cardiovascular risk factor profiles, arterial function parameters, left ventricular structure and function variables, atherosclerosis markers and cardiovascular event rates between black and other African chronic kidney disease patients in age and sex adjusted multivariable regression models. Other established potential confounders or mediators of arterial function [14] were consistently adjusted for in additional models. We subsequently performed sensitivity analyses among pre-dialysis as well as dialysis patients. Differences among the 4 investigated groups that included black and other African pre-dialysis and black and other African dialysis patients were assessed by ANOVA, Kruskall-Wallis and chi-quare test for continuous normally distributed, continuous non-normally distributed and categorical variables, respectively. The performance of the Framingham score in identifying black and other

African chronic kidney disease patients with very high CVD risk as represented by carotid plaque presence was determined in receiver operator characteristic (ROC) curve analysis.

3. Results

3.1. Baseline patient characteristics. As given in Table 1, mean age was 5.6 years smaller in black compared to other CKD African patients (p=0.03). Sex and lifestyle factors did not differ in black compared to other African participants. In age and sex adjusted analysis, black patients were more frequently on dialysis (OR (95%CI)=3.18 (1.41 to 7.08)). Body weight and height were each smaller in black compared to other African patients. These difference reached significance for weight (p=0.02) only. Other anthropometric measures were similar in the two groups.

3.2. Traditional and non-traditional cardiovascular risk factors in black compared to other African patients with CKD. Cardiovascular risk factor profiles are presented in Table 2. In age and sex adjusted analysis, hypertension and diabetes were more prevalent in black compared to other African CKD patients. The use of insulin, diuretics and calcium channel blockers was more frequent whereas that of lipid lowering agents was less prevalent in black compared to other African patients. Systolic blood pressure, heart rate and the Framingham score were each larger in black compared to other African patients.

With regard to non-traditional cardiovascular risk factors, black patients had more frequently high phosphate concentrations, smaller calcium and haemoglobin levels and larger parathyroid and ferritin concentrations compared to other African participants. Black patients also more often received erythropoietin stimulating agent and intravenous iron therapy. In an additional logistic regression model in which diuretic agent use was adjusted for, black population origin remained associated with low calcium levels (partial R=-0.192, p=0.04). Black patients used erythropoietin stimulating agents and intravenous iron more often than their other African counterparts.

3.3. Arterial function in black compared to other African patients with CKD. As given in Table 3, in age and sex adjusted analysis, pressure pulsatility as represented by central systolic and pulse pressure, peripheral pulse pressure and forward wave pressure were each larger in black compared to other African CKD patients (model 1 in Table 3). Upon additional adjustment for other established confounders or mediators, these disparities persisted except for as relates to the forward wave pressure.

3.4. Left ventricular structure and function in black compared to other African patients with chronic kidney disease. Cardiac structure and function measures in black and other African CKD patients are shown in Table 4. In age and sex adjusted analysis, black African patients experienced a larger E/e' ratio and smaller e'.

3.5. Atherosclerosis and cardiovascular event rates in black compared to other African patients with chronic kidney disease. As given in Table 5, in age and sex adjusted analysis, carotid artery plaque prevalence was smaller in black compared to other African CKD patients. The frequency of cardiovascular events and carotid intima-media thickness were also smaller in black compared to other African patients but none of these differences reached significance.

3.6. Sensitivity analyses

3.6.1. Baseline characteristics in black compared to other African pre-dialysis and dialysis patients. Baseline demographic characteristics, lifestyle factors and anthropometric features did not differ significantly in black compared to other pre-dialysis as well as dialysis patients, as shown in Table 1.

3.6.2. Traditional and non-traditional cardiovascular risk factors in black compared to other African pre-dialysis and dialysis patients. Table 2 gives the traditional and non-traditional cardiovascular risk factors in black compared to other African pre-dialysis and dialysis patients. Among pre-dialysis patients, all black African patients were hypertensive as compared to 81.3% of their other African counterparts. Black patients also had a larger systolic blood pressure and used diuretics, calcium channel blockers and alpha blockers more frequently. Diabetes was more prevalent, haemoglobin A1c concentrations were larger and insulin was used more frequently in black compared to other African patients. The Framingham score was larger in black compared to other African patients. With regard to non-traditional cardiovascular risk factors, the prevalence of high phosphate levels and parathyroid concentrations were larger and vitamin D levels were smaller in black compared to other African patients.

Among dialysis patients, beta blockers were used less frequently and heart rate was larger in black compared to other African patients. With regard to non-traditional cardiovascular risk factors, the calcium x phosphate product was smaller in black compared to other African patients.

3.6.3. Arterial function in black compared to other African pre-dialysis and dialysis patients. Arterial function in black compared to other African pre-dialysis and dialysis patients are shown in Table 3. Among pre-dialysis patients, arterial wave reflection markers and, except for the forward wave pressure, pressure pulsatility parameters were larger in black compared to other African patients. Except for as relates to augmentation index and reflection magnitude, each of these disparities remained significant in regression models in which, besides age and sex, other established potential confounders or mediators were additionally adjusted for.

Among dialysis patients, arterial stiffness, wave reflection as well as pressure pulsatility measures did not differ significantly in black compared to other African patients.

3.6.4. Left ventricular structure and function in black compared to other African pre-dialysis and dialysis patients. Table 4 gives the left ventricular structure and function in black compared to other African pre-dialysis and dialysis patients. Among pre-dialysis patients, the E/e' ratio was larger and the e' was smaller in black compared to other African patients. The association of black population origin with E/e' ratio was unaltered upon additional adjustment for left ventricular hypertrophy (partial R=0.307, p=0.01) or hypertension (partial R=0.278, p=0.02) but was no longer present after diabetes was adjusted for (partial R=0.089, p=0.5).

Among dialysis patients, ejection fraction and stroke volume were larger in black compared to other African patients. These associations were materially unaltered upon additional adjustment for haemoglobin levels (partial R=0.356, p=0.01 and partial R=0.276, p=0.07).

3.6.5. Carotid atherosclerosis and cardiovascular event rates in black compared to other African pre-dialysis and dialysis patients. Among pre-dialysis patients, carotid atherosclerosis extent and cardiovascular event rates did not differ significantly in black compared to other African patients, as shown in Table 5.

Among dialysis patients, black compared to other African study participants were less likely to have experienced any cardiovascular event.

3.6.6. Differences among black and other African pre-dialysis and black and other African dialysis patients. Differences among the 4 investigated groups that included black and other African pre-dialysis and black and other African dialysis patients were confirmed upon inter-group comparisons. This was the case for body weight as a baseline characteristic (Table1), a substantial proportion of the traditional and non-traditional cardiovascular risk factors and their treatment (Table 2), pressure pulsatility measures (Table 3), E/e' (Table 4) and carotid artery plaque and intima-media thickness as well as ischemic heart disease (Table 5).

3.7. Association of cardiovascular risk factors with atherosclerosis in black compared with other African chronic kidney disease. The above mentioned results indicate that the cardiovascular disease risk factor burden was larger in black compared to other African chronic kidney disease patients. Despite this disparity, the atherosclerosis extent as estimated by carotid plaque prevalence was smaller in black compared to other African patients. In this regard, in interaction analysis, black population origin impacted the Framingham score – plaque prevalence relationship (OR (95% CI)=0.914 (0.862 to 0.970), interaction p=0.003). As given in Table 6, stratified analysis revealed that traditional cardiovascular risk factors were not related to carotid plaque in black African patients whereas age, sex, dyslipidemia and the Framingham score were associated with atherosclerosis among other Africans. The performance of the Framingham score in identifying black and other African chronic kidney disease patients with carotid plaque in ROC curve analysis

is shown in Figure 1. The area under the curve (AUC) (95% CI) for the association of the Framingham score with plaque was 0.556 (0.375 to 0.737) (p=0.6) in black compared to 0.818 (0.714 to 0.921) (p<0.0001) in other Africans.

Non-traditional cardiovascular risk factors were not associated with carotid plaque (data not shown).

4. Discussion

To our knowledge, this is the first study that compared the traditional and non-traditional cardiovascular risk factor burden and subclinical and established CVD between black and other pre-dialysis and dialysis African patients that were seen at the same centre. The main novel findings produced by our investigation were as follows: (1) overall, black compared to other African CKD patients experienced a larger traditional cardiovascular risk factor burden as estimated by the Framingham score, more adverse non-traditional cardiovascular risk factors, impaired arterial and diastolic function but less frequent very high risk atherosclerosis as represented by carotid artery plaque presence and numerically though not significantly smaller cardiovascular event rates; (2) black compared to other African pre-dialysis patients experienced a larger traditional cardiovascular risk factor burden, more adverse non-traditional cardiovascular risk factors, impaired arterial function and diastolic dysfunction but similar cardiovascular event rates, (3) among dialysis patients, black compared to other Africans had an overall similar traditional and non-traditional cardiovascular risk factor burden, similar arterial and diastolic function but increased systolic function and reduced cardiovascular event rates, and (4) in ROC curve analysis among all participants, the Framingham score was strongly associated with carotid artery plaque in non-black but not black African CKD patients.

We found that, as applies to American black pre-dialysis CKD patients [9], the prevalence of hypertension and diabetes were larger in black compared to other African study participants (100% versus 81.3% and 68.4% versus 16.7%, respectively). These differences translated into an overall increased major traditional cardiovascular risk factor burden. Moreover, hypertension was more severe in black pre-dialysis CKD patients in that despite the use of more intensive antihypertensive therapy, their systolic blood pressure was larger than in their non-black counterparts. Additionally, black African CKD patients had more frequent high phosphate levels, larger parathyroid hormone concentrations and lower vitamin D levels.

Severe arteriosclerosis is a characteristic vascular feature of CKD that results in impaired large artery function [21]. Increased arterial stiffness, wave reflection and pressure pulsatility in black persons were well documented in general population studies [22-24]. These central artery characteristics contribute to heart failure, arrhythmias, sudden death, stroke and myocardial infarction in CKD [15,16]. In this study, black African pre-dialysis CKD patients experienced increased

wave reflection and pressure pulsatility. Increased wave reflection contributes to enhanced pressure pulsatility, which is strongly associated with CVD and disease progression in CKD patients [21,25-27]. Effective management of impaired central artery function associates with improved survival in CKD [21]. Our results therefore argue for comprehensive cardiovascular risk factor management among black African pre-dialysis CKD patients.

We measured E/A ratio and e' as markers of left ventricular relaxation and E/e' ratio as an index of left ventricular filling pressure. The E/e' ratio and e' predict incident cardiac and cardiovascular events more strongly than the E/A ratio [28]. In this study, the E/e' ratio was larger and the e' smaller in black compared other African pre-dialysis CKD patients. Hypertension and diabetes are both important determinants of diastolic dysfunction in the general population [29]. In multivariate analysis, we found that diabetes but not hypertension explained the association of black population origin with impaired diastolic function among pre-dialysis CKD patients. In contrast to black African pre-dialysis patients, those on dialysis experienced similar cardiovascular risk factor profiles and arterial and left ventricular diastolic function to those recorded in other Africans. In this regard, Bellasi and colleagues [30] previously reported disparities in cardiovascular risk factors but similar arterial function in black compared to white American dialysis patients. More noticeably, we found that cardiovascular event rates were significantly reduced by 78% (OR (95% CI)=0.22 (0.05 to 0.88)) in black compared to other African dialysis patients. This is remarkably similar to what was reported among black American dialysis patients [9,30]. The likelihood of progressing from chronic kidney disease to end-stage renal disease is greater in African Americans than whites. However, once on dialysis, survival is better among African Americans compared with whites. In 2008, the mortality rate for patients on dialysis was 16% in African Americans compared with 24% in whites. This disparity may be due to black pre-dialysis CKD patients being sicker and therefore more likely to die, a phenomenon that would be expected to result in a selection of healthier persons that survive by the time they start dialysis [30]. This notion is supported by the DOPPS-1 report in which comorbidities, concurrent therapies and nutritional variables explained the reduced mortality rates in black compared to white dialysis patients [31]. The authors concluded that minority group dialysis patients should not be expected to survive longer than their white counterparts with similar characteristics. Interestingly, we also found that left ventricular function as estimated by the ejection fraction and stroke volume was more preserved in black compared to other African dialysis patients.

Besides the decreased cardiovascular event rates in black compared to other African dialysis patients, the most striking finding in this study was that, among all study participants, despite the more adverse cardiovascular risk factor profiles, impaired arterial function and left ventricular diastolic dysfunction, the atherosclerosis extent as represented by plaque presence was overall smaller in black compared to other African CKD patients (OR (95% CI)=0.38 (0.16 to 0.91)). Additionally, in black compared to other African pre-dialysis patients, despite more adverse cardiovascular risk factor profiles, cardiovascular event rates were not increased (OR (95% CI)=0.93

(0.0.22 to 3.87)). These results suggest that cardiovascular risk factors may be less strongly associated with atherosclerotic CVD in black compared to other African CKD patients. Indeed, black population origin impacted the Framingham score-plaque prevalence relationship (interaction p=0.003). In stratified analysis, the Framingham score was associated with carotid plaque in non-black but not black African CKD patients. ROC curve analysis confirmed that the Framingham score performed well in identifying very high risk atherosclerosis in non-black but not black African CKD patients (AUC (95% CI)= 0.818 (0.714 to 0.921) and AUC (95% CI)=0.556 (0.375 to 0.921)), respectively. We recently reported the same finding in black African patients with rheumatoid arthritis [32-34]. This may be attributable to a shorter lifetime exposure to cardiovascular risk factors as part of recent urbanization and an earlier epidemiological transition stage [32]. More importantly, this finding indicates that cardiovascular risk equations such as the Framingham score should not be relied upon in cardiovascular risk stratification among black African CKD patients [33,34].

The major limitations of the present study are the relatively small number of patients included, particularly in subgroups, its cross-sectional design and that all included participants were enrolled at a single centre. Strengths are that we performed a detailed assessment of not only cardiovascular risk factor profiles but also large artery and cardiac function as well as subclinical atherosclerosis and that recorded data were compared between black and other African CKD patients.

5. Conclusion

Overall, black compared to other African CKD patients currently experience less frequent severe atherosclerosis despite an increased cardiovascular risk factor burden. The Framingham score is useful in atherosclerotic CVD risk stratification among non-black but not black African CKD patients. Among pre-dialysis patients, black compared to other Africans have more adverse traditional and non-traditional cardiovascular risk factor profiles, impaired arterial function and diastolic dysfunction but similar cardiovascular event rates. These disparities may originate in differences in epidemiological transition stages. Black compared to other African dialysis patients have smaller cardiovascular event rates, which may represent a selection bias as previously documented in black Americans.

Supplementary material

Methods: Methods used in cardiovascular risk factor and arterial and left ventricular systolic and diastolic function recording.

Figure legend

FIGURE 1: Performance of the Framingham score in identifying non-black (A) and black (B) CKD patients with carotid artery plaque.

Data availability

The data used in this study can be obtained from the corresponding author.

Ethical approval

The study was approved by the University of the Witwatersrand Human (Medical) research Ethics Committee (protocol number: M15-08-43) in Johannesburg, South Africa.

Consent

Each patient gave written informed consent.

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

Conception and design of the study: H-C.H., P.H.D.

Arterial function evaluation and echocardiography: C.R.

Analysis of the data: H-C.H., P.H.D.

Interpretation of the data: H-C.H., P.H.D., C.R., G.R.N., A.J.W.

Drafting of the manuscript: H-C.H., P.H.D.

Revising the article: H-C.H., P.H.D., C.R., G.R.N., A.J.W.

Providing intellectual content of critical importance to the work described: H-C.H., P.H.D., C.R., G.R.N., A.J.W.

Final approval of the version to be published: H-C.H., P.H.D., C.R., G.R.N., A.J.W.

Acknowledgement

We thank Ms Noluntu Dlongolo for revising the manuscript. This study was supported by the South African National Research Foundation.

References

[1] GBD Chronic Kidney Disease Collaboration. “Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study: 2017,” *Lancet*, vol. 395, no. 10225, pp. 709-733, 2020.

[2] J.A. George, J-T. Brandenburg, J. Fabian et al., “Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): a cross-sectional population study,” *Lancet Glob Health,* vol. 7, no. 12, pp. e1632-1643, 2019.

[3] M. Cozzolino, M. Mangano, A. Stucchi et al., “Cardiovascular disease in dialysis patients,”*Nephrol Dial Tranplant,* vol. 33, suppl_3, pp. iii28-iii34, 2018.

[4] M.J. Sarnak, K. Amann, S Bangalore et al., “Chronic kidney disease and coronary artery disease: JACC State-of-the-Art Review,” *J Am Coll Cardiol,* vol. 74, no. 14, pp. 1823-1838, 2019.

[5] “Sub-Saharan Africa Population 2020 (demographics, maps, graphics): World Population Review,” http://worldpopulationreview.com/continents/sub-saharan-africa-population/ (Accessed 13 September 2020)

[6] “World Bank Country and Lending Groups: Country Classification,” https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups (Accessed 13 September 2020)

[7] A. Solomon, L. Tsang, A.J. Woodiwiss, A.M.E. Millen, G.R. Norton, P.H. Dessein, Cardiovascular disease risk amongst African black patients with rheumatoid arthritis: the need for population specific stratification. *Biomed Res Int* 2014;2014, article ID 826095; http://dx.doi.org/10.1155/2014/826095.

[8] V. Pillay-van Wyk, W. Msemburi, R. Laubscher et al., “Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study,” *Lancet,* vol. 4, no. 9, pp. e642-e653, 2016.

[9] M.R. Carnethon, J. Pu, G. Howard et al., “Cardiovascular health in African Americans: A scientific statement from the American Heart Association,” *Circulation*, vol. 136, no. 21, pp. e393-e423, 2017.

[10] V.L. Luyckx, A. Yip, L. Sofianou, G.S. Jhangri, T.F. Mueller, S. Naicker, “Cardiac function in an African dialysis population with a low prevalence of pre-existing cardiovascular disease,” *Ren Fail*, vol. 31, no. 3, pp. 211-220, 2009.

[11] R. Freercks, C. Swanepoel, H. Carrara, S. Moosa, A. Lachman, B. Rayner, “Vascular calcification in South African dialysis patients: ethnic variation, prevalence, detection and haemodynamic correlates,” *Nephrology*, vol. 17, no. 7, pp. 607-615, 2012.

[12] R.A. Tamayo Isla, O.I. Ameh, D. Mapiye et al. “Baseline predictors of mortality among predominantly rural-dwelling end-stage renal disease patients on chronic dialysis therapies in Limpopo, South Africa,” *PLoS One*, vol. 11, no. 6, pp. e0156642, 2016. doi: 10.1371/journal.pone.0156642. eCollection 2016.

[13] O.C. Amira, S. Naicker, P. Manga et al., “Adiponectin and atherosclerosis risk factors in African hemodialysis patients: a population at low risk for atherosclerotic cardiovascular disease,” *Hemodialysis Int*, vol. 16, no. 1, pp. 59-68, 2012.

[14] S. Gunter, C. Robinson, A.J. Woodiwiss et al., “Arterial wave reflection and subclinical atherosclerosis in rheumatoid arthritis,” *Clin Exp Rheumatol*, vol. 36, no. 3, pp. 412-420, 2018.

[15] M. Briet, P. Boutouyrie, S. Laurent, G.M. London, “Arterial stiffness and pulse pressure in CKD and ESRD,” *Kidney Int*, vol. 82, no. 4, pp. 388-400, 2012.

[16] G.M. London, “Arterial stiffness in chronic kidney disease and end-stage renal disease,” *Blood Purif*, vol. 45, no. 1-3, pp. 154-158, 2018.

[17] L. Mokotedi, S. Gunter, C. Robinson et al., “The Impact of different classification criteria sets on the estimated prevalence and associated risk factors of diastolic dysfunction in rheumatoid arthritis,” *Int J Rheumatol* 2017;2017:2323410.

[18] A.S. Levey, L.A. Stevens, C.H. Schmid et al., “A new equation to estimate glomerular filtration rate,” *Ann Intern Med*, vol. 150, no. 9, pp.604-612, 2009.

[19] R.B.Sr. D’Agostino, R.S. Vasan, M.J. Pencina et al., “General cardiovascular risk profile for use in primary care: the Framingham Heart Study,” *Circulation*, vol. 117, no. 6, pp. 743-753, 2008.

[20] P.J. Touboul, M.G. Hennerici, S. Meairs et al., “Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006,” *Cerebrovasc Dis*, vol. 23, no. 1, pp. 75-80, 2007.

[21] L. Zanoli, P. Lentini, M. Briet et al., “Arterial stiffness in the heart of CKD,” *J Am Soc Nephrol*, vol. 30, no. 6, pp. 918-928, 2019.

[22] M. Maritz, C.M.T. Fourie, J.M. van Rooyen, I.M. Kruger, A.E. Schutte, “A health profile associated with excessive alcohol use independently predicts aortic stiffness over 10 years in black South Africans,” *J Hypertens*, vol. 35, no. 11, pp. 2268-2275, 2017.

[23] J.A. Chirinos, J.G. Kips, M.J. Roman et al., “Ethnic differences in arterial wave reflections and normative equations for augmentation index,” *Hypertension* vol. 57, no. 6, pp. 1108-1116, 2011.

[24] T. Coutinho, S.T. Turner, T.H. Mosley, I.J. Kullo, “Biomarkers associated with pulse pressure in African-Americans and non-hispanic whites,” *Am J Hypertens*, vol. 25, no. 2, pp. 145-151, 2012.

[25] R.R. Townsend, J.A. Chirinos, A. Parsa A et al. “Central pulse pressure in chronic kidney disease: A CRIC ancillary study,” *Hypertension*, vol. 56, no. 3, pp. 518-524, 2010.

[26] A.A. House, C. Wanner, M.J. Sarnak et al., “Heart failure in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference,” *Kidney Int*, vol. 95, no. 6, pp. 1304-1317, 2019.

[27] L. Di Lullo, A. Gorini, D. Russo, A. Santoboni, C. Ronco, “Left ventricular hypertrophy in chronic kidney disease patients: from pathophysiology to treatment,” *Cardiorenal Med,* vol. 5, no. 4, pp. 254-266, 2015.

[28] T. Kuznetsova, L. Thijs, J. Knez, S.L. Herbot, Z Zhang, J.A. Staessen, “Prognostic value of left ventricular diastolic dysfunction in a general population,” *J Am Heart Assoc*, vol. 3, no. 3, pp. e000789, 2014. doi: 10.1161/JAHA.114.000789.

[29] C. Russo, Z. Jin, S. Homma et al., “Effect of diabetes and hypertension on left ventricular diastolic function in a high-risk population without evidence of heart disease,” *Eur J Heart Fail*, vol. 12, no 5, pp. 454-461, 2010.

[30] A. Bellasi A, Veledar E, Ferramosca E, Ratti C, Block G, Raggi P. “Markers of vascular disease do not differ in black and white hemodialysis patients despite different risk profile,” *Atherosclerosis*, vol. 197, no. 1, pp242-249, 2008.

[31] B.M. Robinson, M.M. Joffe, R.L. Pisoni, F.K. Port, H.I. Feldman, “Revisiting survival differences by race and ethnicity among hemodialysis patients: the dialysis outcomes and practice patterns study,” *J Am Soc Nephrol,* vol. 17, no. 10, pp. 2910-2918, 2006.

[32] A. Solomon, A.J. Woodiwiss, A.T. Abdool-Carrim, B.A. Stevens, G.R. Norton, P.H. Dessein, “The carotid artery atherosclerosis burden and its relation to cardiovascular risk factors in black and white Africans with established rheumatoid arthritis: a cross-sectional study,” *J Rheumatol*, vol. 39, no. 9, pp. 1798-1806, 2012.

[33] P.H. Dessein, A. Corrales, R. Lopez-Mejias et al., “The Framingham Score and the Systematic Coronary Risk Evaluation at Low Cutoff Values Are Useful Surrogate Markers of High-risk Subclinical Atherosclerosis in patients with Rheumatoid Arthritis,” *J Rheumatol*, vol. 43, no. 3, pp. 486-494, 2016.

[34] A. Solomon, Stanwix A.E., S. Castaneda et al., “Points to consider in cardiovascular disease risk management among patients with rheumatoid arthritis living in South Africa, an unequal middle income country,” *BMC Rheumatol*, vol. 16, no. 4, pp. 42. doi: 10.1186/s41927-020-00139-2. eCollection 2020.PMID: 32550295.

TABLE 1: Baseline characteristics in black compared to other African chronic kidney disease patients overall, and in sensitivity analysis among pre-dialysis and dialysis patients

	Chronic kidney disease patients									
	All patients			Pre-dialysis patients			Dialysis patients			Inter-group comparison[a]
Characteristic	Black African (n=46)	Other African (n=69)	p	Black African (n=19)	Other African (n=48)	p	Black African (n=27)	Other African (n=21)	p	p
Demographics										
Age (years)	**54.3 (14.6)**	**59.9 (13.3)**	**0.03**	55.7 (14.2)	60.3 (13.6)	0.2	53.3 (15.0)	59.1 (13.0)	0.2	0.1
Female sex	18 (39.1)	25 (36.2)	1.0	6 (31.6)	15 (31.3)	0.8	12 (44.4)	10 (47.6)	0.8	0.5
CKD stage										
Pre-dialysis	**19 (41.3)**	**48 (69.6)**	**0.005**	-	-	-	-	-	-	-
Dialysis	**27 (58.7)**	**21 (30.4)**	**0.005**	-	-	-	-	-	-	-
Lifestyle										
Current smoker	2 (4.4)	1 (1.5)	0.4	2 (10.5)	1 (2.1)	0.2	0 (0)	0 (0)	-	-
Ex-smoker	2 (4.4)	6 (8.7)	0.3	0 (0)	4 (8.3)	1.0	2 (7.4)	2 (9.5)	0.5	0.6
Alcohol	1 (2.2)	1 (1.5)	0.8	0 (0)	1 (2.1)	1.0	1 (3.7)	0 (0)	-	0.7
Exercise	19 (41.3)	23 (33.3)	0.4	6 (31.6)	11 (22.9)	0.5	13 (48.2)	12 (57.1)	0.4	0.06
Anthropometry										
Weight (kg)	**74.5 (13.0)**	**81.1 (16.8)**	**0.02**	76.3 (13.6)	83.1 (16.5)	0.07	73.1 (12.7)	76.7 (17.1)	0.5	0.04
Height (cm)	167.8 (9.8)	170.5 (10.4)	0.09	170.0 (7.9)	171.3 (9.7)	0.5	166.2 (10.7)	168.6 (11.3)	0.2	0.2
Waist (cm)	96.5 (13.1)	102.0 (15.3)	0.1	97.1 (10.4)	101.4 (14.8)	0.3	96.1 (14.9)	103.3 (16.7)	0.3	0.2

Neck (cm)	38.6 (3.8)	39.7 (4.5)	0.2	38.7 (4.7)	39.5 (5.5)	0.7	38.4 (3.1)	40.1 (5.1)	0.3	0.5
BMI (kg/m2)	26.6 (5.3)	27.9 (5.6)	0.2	26.5 (5.2)	28.3 (5.5)	0.2	26.7 (5.5)	27.0 (5.8)	0.8	0.4
Waist-hip ratio	0.98 (0.11)	0.97 (0.10)	0.2	0.97 (0.14)	0.97 (0.11)	0.6	0.99 (0.09)	0.97 (0.09)	0.3	0.8
Waist-height ratio	0.58 (0.09)	0.59 (0.09)	0.5	0.57 (0.07)	0.59 (0.09)	0.5	0.58 (0.11)	0.61 (0.11)	0.7	0.5

Data are expressed as mean (SD) or number (percent) and were analysed in age and sex adjusted linear of logistic regression models as appropriate. [a]For differences among black and other African pre-dialysis patients and black and other African dialysis patients. Significant differences are shown in bold. Abbreviations: CKD: chronic kidney disease; BMI: body mass index.

TABLE 2: Traditional and non-traditional cardiovascular risk factors and their treatment in black compared to other African chronic kidney disease patients overall, and in sensitivity analysis among pre-dialysis and dialysis patients

	Chronic kidney disease patients									
	All patients			Pre-dialysis patients			Dialysis patients			Inter-group comparison[b]
Characteristic	Black African (n=46)	Other African (n=69)	Model[a]	Black African (n=19)	Other African (n=48)	Model[a]	Black African (n=27)	Other African (n=21)	Model[a]	p
Traditional CV risk factors and their treatment										
Categorical variables			OR (95% CI)			OR (95% CI)			OR (95% CI)	
Hypertension	**45 (97.8)**	**59 (85.5)**	**9.05 (1.08-75.59)**	19 (100)	39 (81.3)	-	26 (96.3)	20 (95.2)	1.51 (0.08-28.03)	**0.04**
Dyslipidemia	30 (71.4)	53 (85.5)	0.41 (0.15-1.12)	12 (70.6)	41 (91.1)	0.25 (0.06-1.10)	18 (72.0)	12 (70.6)	0.89 (0.21-3.72)	0.1
Diabetes	**21 (45.7)**	**19 (27.5)**	**3.10 (1.30-7.40)**	**13 (68.4)**	**8 (16.7)**	**13.28 (5.51-50.27)**	8 (29.6)	11 (52.4)	0.51 (0.13-2.01)	**<0.0001**
Antihypertensive treatment	**45 (97.8)**	**59 (85.5)**	**9.05 (1.08-75.59)**	19 (100)	39 (81.3)	-	26 (96.3)	20 (95.2)	1.51 (0.08-28.03)	**0.04**
ACEI/ARB	36 (81.8)	54 (79.4)	1.07 (0.40-2.87)	15 (83.3)	38 (79.2)	1.41 (0.33-6.03)	21 (80.1)	16 (80.0)	0.70 (0.14-3.47)	1.0
Beta blocker	21 (47.7)	30 (44.4)	1.22 (0.51-2.45)	9 (50.0)	16 (33.3)	2.31 (0.72-7.26)	**12 (46.2)**	**14 (70.0)**	**0.20 (0.05-0.88)**	**0.04**
Diuretic	**20 (44.4)**	**19 (27.5)**	**2.56 (1.11-5.94)**	**11 (61.1)**	**13 (27.1)**	**5.52 (1.59-19.17)**	9 (33.3)	6 (28.6)	1.34 (0.36-5.05)	0.07
Calcium channel blocker	**26 (57.8)**	**23 (33.8)**	**2.70 (1.22-6.00)**	**10 (52.6)**	**11 (22.9)**	**3.69 (1.18-11.50)**	16 (61.5)	12 (60.0)	1.14 (0.33-3.91)	**0.002**
Alpha blocker	11 (25.6)	13 (19.1)	1.73 (0.67-4.48)	**7 (38.9)**	**8 (16.7)**	**5.48 (1.31-22.87)**	4 (16.0)	5 (25.0)	0.53 (0.11-2.45)	0.2
Lipid lowering therapy	**21 (47.7)**	**51 (75.0)**	**0.33 (0.14-0.75)**	9 (50.0)	37 (77.1)	0.32 (0.09-1.08)	12 (46.2)	14 (70.0)	0.31 (0.08-1.12)	**0.03**
Statin	**21 (47.7)**	**51 (75.0)**	**0.33 (0.14-0.75)**	9 (50.0)	37 (77.1)	0.32 (0.09-1.08)	12 (46.2)	14 (70.0)	0.31 (0.08-1.12)	**0.03**
Ezetimibe	4 (9.1)	7 (10.4)	0.96 (0.26-3.61)	2 (11.1)	5 (10.4)	1.26 (0.21-7.59)	2 (7.7)	2 (10.5)	0.72 (0.09-5.95)	1.0
Insulin	**14 (31.1)**	**12 (17.4)**	**2.60 (1.03-6.61)**	**9 (47.4)**	**5 (10.4)**	**7.67 (2.07-28.41)**	5 (19.2)	7 (33.3)	0.67 (0.16-2.89)	**0.007**
OGLA	8 (17.8)	6 (8.7)	3.37 (0.98-11.61)	4 (21.1)	4 (8.3)	3.26 (0.64-16.56)	4 (15.4)	2 (9.5)	3.24 (0.40-26.49)	0.5
Continuous variables			Partial R p			Partial R p			Partial R p	
Systolic blood pressure	**148 (24)**	**137 (18)**	**0.271 0.004**	**145 (25)**	**135 (16)**	**0.265 0.03**	150 (24)	141 (21)	0.185 0.2	**0.02**
Diastolic blood pressure (mmHg)	84 (13)	82 (10)	0.032 0.7	80 (10)	82 (8)	-0.150 0.2	86 (13)	82 (15)	0.083 0.6	0.2
Mean blood pressure (mmHg)	105 (14)	100 (11)	0.175 0.06	101 (13)	100 (9)	0.093 0.5	107 (14)	102 (14)	0.155 0.3	0.07
Total cholesterol (mmol/l)	4.3 (1.2)	4.2 (1.2)	0.005 1.0	4.2 (1.2)	4.2 (1.1)	-0.006 1.0	4.3 (1.2)	4.3 (1.3)	-0.001 1.0	1.0
LDL-cholesterol (mmol/l)	2.3 (0.9)	2.4 (1.0)	-0.047 0.6	2.2 (0.7)	2.3 (0.9)	-0.094 0.5	2.4 (1.0)	2.5 (1.1)	-0.038 0.8	0.8

HDL cholesterol (mmol/l	1.14 (0.43)	1.14 (0.42)	-0.007	0.9	1.03 (0.37)	1.12 (0.42)	-0.087	0.5	1.22 (0.45)	1.18 (0.41)	0.058	0.7	0.5
Non-HDL cholesterol (mmol/l)	3.1 (1.1)	3.1 (1.0)	-0.017	0.8	3.2 (1.3)	3.1 (1.0)	0.025	0.9	3.1 (1.1)	3.3 (1.1)	-0.086	0.6	0.9
Triglycerides (mmol/l)	1.3 (0.9-1.8)	1.5 (1.1-2.1)	-0.080	0.4	1.5 (1.1-1.9)	1.4 (1.1-2.2)	0.033	0.8	1.4 (0.8-1.8)	1.6 (1.2-1.8)	-0.127	0.4	0.4
Cholesterol-HDL cholesterol ratio	3.8 (2.7-5.2)	3.8 (3.1-4.8)	-0.022	0.6	3.7 (3.1-5.4)	3.8 (3.1-4.9)	0.073	0.6	3.8 (2.6-5.1)	3.9 (3.1-4.8)	-0.122	0.4	0.9
Triglycerides-HDL cholesterol ratio	1.2 (0.7-2.0)	1.4 (0.9-2.1)	-0.050	0.8	1.7 (1.2-2.3)	1.5 (1.0-2.1)	0.102	0.4	1.0 (0.6-2.0)	1.3 (0.9-2.4)	-0.111	0.5	0.2
Haemoglobin A1c (%)	5.6 (5.1-7.0)	5.5 (5.2-6.3)	0.106	0.3	**6.9 (5.7-8.3)**	**5.6 (5.3-6.2)**	**0.429**	**<0.0001**	5.4 (4.9-5.7)	5.4 (4.9-7.3)	-0.094	0.5	**0.004**
Framingham score	**23.4 (21.7)**	**22.6 (16.5)**	**0.223**	**0.01**	**27.9 (23.3)**	**21.8 (16.3)**	**0.327**	**0.008**	20.2 (20.2)	24.3 (14.0)	0.046	0.8	0.5
Heart rate (beats per minute)	**79 (14)**	**72 (14)**	**0.196**	**0.04**	76 (15)	72 (15)	0.067	0.6	**81 (13)**	**72 (12)**	**0.350**	**0.02**	**0.05**
Antihypertensives (n)	**2.5 (1.1)**	**2.0 (1.2)**	**0.215**	**0.02**	**2.8 (1.2)**	**2.0 (1.2)**	**0.388**	**0.001**	2.3 (1.1)	2.5 (1.1)	-0.149	0.3	**0.008**
Non-traditional CV risk factors and their treatment													
Categorical variables			OR (95% CI)				OR (95% CI)				OR (95% CI)		
High phosphate	**34 (77.3)**	**31 (44.9)**	**3.99 (1.67-9.54)**		**9 (52.9)**	**12 (25.0)**	**3.31 (1.01-10.89)**		25 (92.6)	19 (90.5)	1.20 (0.15-9.92)		**<0.0001**
Phosphate binder	22 (52.4)	25 (37.3)	1.71 (0.77-3.81)		4 (23.5)	8 (16.7)	1.55 (0.39-6.10)		18 (72.0)	17 (89.5)	0.27 (0.05-1.52)		**<0.0001**
Vitamin D replacement	27 (67.5)	31 (47.7)	2.27 (0.98-5.28)		9 (50.0)	14 (31.1)	1.96 (0.62-6.17)		18 (81.8)	17 (85.0)	1.06 (0.19-6.03)		**<0.0001**
Erythropoietin stimulating agent	**29 (63.0)**	**25 (36.2)**	**3.00 (1.36-6.62)**		6 (31.6)	5 (10.4)	3.69 (0.95-14.35)		23 (85.2)	20 (95.2)	0.34 (0.03-3.81)		**<0.0001**
Intravenous iron	**26 (56.5)**	**24 (34.8)**	**2.50 (1.14-5.48)**		5 (26.3)	5 (10.4)	3.07 (0.75-12.59)		21 (77.8)	19 (90.5)	0.44 (0.08-2.61)		**<0.0001**
Sevelamer	1 (2.0)	0 (0)	-		0 (0)	0 (0)	-		1 (4.0)	0 (0)	-		
Continuous variables			Partial R	p			Partial R	p			Partial R	p	
Dialysis duration (months)	30 (12-48)	36 (12-48)	-0.090	0.6	-	-	-	-	30 (12-36)	36 (12-42)	-0.138	0.4	-
Phosphate (mmol/l)	1.2 (0.8-1.6)	1.2 (1.0-1.6)	-0.022	0.8	1.3 (0.9-1.6)	1.2 (0.9-1.4)	0.076	0.6	1.3 (0.8-1.7)	1.6 (1.1-2.1)	-0.268	0.07	**0.04**
Calcium (mmol/l)	**2.3 (2.1-2.4)**	**2.3 (2.2-2.4)**	**-0.205**	**0.03**	2.2 (2.1-2.4)	2.3 (2.2-2.4)	-0.120	0.4	2.2 (2.0-2.4)	2.3 (2.2-2.4)	-0.254	0.09	0.2
Calcium x phosphate	2.5 (1.8-3.8)	2.8 (2.2-3.7)	-0.100	0.3	3.0 (2.0-3.6)	2.9 (2.2-3.1)	0.010	0.9	**2.9 (1.8-3.8)**	**3.7 (2.6-4.6)**	**-0.364**	**0.01**	**0.02**
Intact PTH (pg/ml)	**287 (141-531)**	**84 (64-334)**	**0.270**	**0.007**	**232 (92-370)**	**70.0 (50-111)**	**0.426**	**0.001**	369 (172-686)	621 (197-801)	-0.149	0.4	**<0.0001**
Haemoglobin (g/dl)	**10.7 (9.6 -12.5)**	**12.6 (10.7-14.2)**	**-0.202**	**0.03**	11.5 (10.0-14.9)	13.5 (10.6-15.1)	-0.072	0.6	10.6 (9.5-11.9)	11.0 (10.7-12.5)	-0.199	0.2	**0.001**
Transferrin saturation (%)	22.0 (17.4-29.0)	22.9 (17.5-30.2)	-0.037	0.7	24.1 (13.7-30.6)	21.0 (17.0-29.0)	-0.144	0.3	22.6 (20.0-30.0)	25.0 (18.5-28.9)	-0.013	0.9	0.3
Ferritin (ng/ml)	**325 (113-573)**	**166 (51-365)**	**0.203**	**0.03**	191 (113-490)	115 (38-224)	0.198	0.1	364 (124-725)	361 (168-609)	0.029	0.8	**0.001**
Albumin (g/l)	36.8 (8.0)	38.6 (5.5)	-0.157	0.1	35.3 (9.2)	38.9 (5.4)	-0.235	0.06	37.8 (7.0)	38.0 (5.9)	-0.025	0.9	0.2
Vitamin D (nmol/l)	19.0 (8.8)	19.8 (9.3)	-0.050	0.6	**15.5 (5.4)**	**20.9 (9.9)**	**-0.269**	**0.03**	21.4 (9.9)	17.4 (7.2)	0.204	0.2	0.07
Uric acid (mmol/l)	0.31 (0.24-0.40)	0.37 (0.28-0.46)	-0.184	0.06	0.38 (0.32-0.48)	0.42 (0.35-50.8)	-0.157	0.2	0.28 (0.21-0.32)	0.28 (0.17-0.34)	0.029	0.9	**<0.0001**
Hs-C-reactive protein (mg/l)	8.2 (2.6-28.1)	6.0 (2.0-15.5)	0.073	0.5	4.7 (1.3-22.3)	4.1 (2.0-15.1)	-0.050	0.7	12.3 (4.1-32.1)	7.7 (1.9-25.4)	0.181	0.2	0.4

Data are expressed as mean (SD), median (interquartile range) or number (percentage). [a]Adjusted for age and sex; [b]for differences among black and other African pre-dialysis and black and other African dialysis patients.

Significant associations are shown in bold. Abbreviations: CV: cardiovascular; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; OGLA: oral glucose lowering agents; LDL: low density lipoprotein; HDL: high density lipoprotein; PTH: intact parathyroid hormone; hs: high sensitivity.

TABLE 3: Arterial function in black compared to other African chronic kidney disease patients overall, and in sensitivity analysis among pre-dialysis and dialysis patients

	Chronic kidney disease patients																
	All patients						Pre-dialysis patients						Dialysis patients					Inter-group comparison[a]
Arterial function	Black African (n=46)	Other African (n=69)	Model 1[a]		Model 2[b]		Black African (n=19)	Other African (n=48)	Model 1[a]		Model 2[b]		Black African (n=27)	Other African (n=21)	Model 1[a]		Model 2[b]		p
			Partial R	p	Partial R	p			Partial R	p	Partial R	p			Partial R	p	Partial R	p	
PWV (m/s)	12.0 (3.8)	11.4 (4.4)	0.126	0.2	0.031	0.8	12.2 (3.5)	10.9 (3.7)	0.230	0.09	0.210	0.1	11.9 (4.1)	12.2 (5.5)	0.016	0.9	-0.143	0.4	0.6
Aix (%)	66.6 (16.4)	66.8 (16.7)	0.089	0.4	0.030	0.8	**73.3 (18.0)**	**64.5 (15.7)**	**0.299**	**0.03**	0.229	0.1	62.2 (14.0)	71.8 (18.2)	-0.170	0.3	-0.223	0.2	0.08
RWP (mmHg)	25.0 (13.8-29.0)	20.5 (15.0-25.0)	0.100	0.2	0.152	0.1	**24.0 (14.0-29.0)**	**19.5 (13.5-23.0)**	**0.280**	**0.04**	**0.307**	**0.03**	25.0 (13.0-29.0)	21.5 (15.8-30.5)	0.126	0.4	0.037	0.8	0.1
Rm (%)	67.4 (16.8)	67.2 (16.6)	0.099	0.3	0.047	0.7	**74.3 (18.4)**	**64.9 (15.6)**	**0.309**	**0.02**	0.250	0.09	62.9 (14.4)	72.3 (18.2)	-0.159	0.3	-0.213	0.2	0.08
CSBP (mmHg)	**136 (22)**	**127 (17)**	**0.265**	**0.006**	**0.225**	**0.02**	**132 (21)**	**125 (15)**	**0.251**	**0.04**	**0.361**	**0.005**	139 (22)	132 (22)	0.208	0.2	0.074	0.7	**0.03**
CPP (mmHg)	**50 (19)**	**43 (13)**	**0.292**	**0.002**	**0.247**	**0.01**	**51 (20)**	**41 (11)**	**0.367**	**0.003**	**0.390**	**0.002**	50 (18)	48 (17)	0.142	0.4	0.056	0.7	**0.04**
PPP (mmHg)	**64 (22)**	**54 (16)**	**0.283**	**0.002**	**0.240**	**0.01**	**65 (23)**	**53 (13)**	**0.358**	**0.003**	**0.405**	**0.001**	64 (22)	59 (21)	0.148	0.3	0.113	0.5	**0.03**
FWP (mmHg)	**35.2 (12.1)**	**31.1 (9.5)**	**0.204**	**0.04**	0.159	0.1	32.5 (12.9)	30.4 (19.2)	0.097	0.5	0.151	0.3	37.0 (11.5)	32.6 (10.2)	0.251	0.1	0.209	0.2	0.1

Descriptive results are expressed as mean (SD) or median (interquartile range). Significant associations are show in bold. [a]Adjusted for age and sex; [b]additionally adjusted for height, weight, heart rate and mean arterial pressure; [c]for differences among black and other African pre-dialysis and black and other African dialysis patients. Abbreviations: PWV: pulse wave velocity; Aix: augmentation index; RWP: reflected wave pressure; Rm: reflection magnitude; CSBP: centralsystolic blood pressure; CPP: central pulse pressure; PPP: peripheral pulse pressure; FWP: forward wave pressure.

TABLE 4: Left ventricular structure and function in black compared to other African chronic kidney disease patients overall, and in sensitivity analysis among pre-dialysis and dialysis patients

	Chronic kidney disease patients											
	All patients				Pre-dialysis patients				Dialysis patients				Inter-group comparison[b]
Characteristic	Black African (n=46)	Other African (n=69)	Model[a]		Black African (n=19)	Other African (n=48)	Model[a]		Black African (n=27)	Other African (n=21)	Model[a]		p
Categorical variables			OR (95% CI)				OR (95% CI)				OR (95% CI)		
LV hypertrophy	21 (45.7)	27 (39.1)	1.50 (0.65 to 3.48)		6 (31.6)	18 (37.5)	0.77 (0.22 to 2.75)		15 (55.6)	9(42.9)	1.78 (0.55 to 6.27)		0.3
LV concentric remodelling	15 (33.3)	18 (26.9)	1.36 (0.59 to 3.15)		7 (36.8)	13 (27.7)	1.53 (0.49 to 4.78)		8 (30.8)	5 (25.0)	1.35 (0.35 to 5.14)		0.9
Reduced ejection fraction	5 (11.1)	17 (25.3)	0.41 (0.14 to 1.26)		2 (10.5)	11 (23.9)	0.46 (0.07 to 2.62)		3 (11.5)	6 (30.0)	0.32 (0.07 to 1.52)		0.3
Continuous variables			Partial R	p			Partial R	p			Partial R	p	
LV mass index (g/m^2)	104.1 (50.9)	91.4 (38.6)	0.147	0.1	99.2 (38.2)	88.6 (38.2)	0.132	0.3	107.6 (58.8)	97.9 (39.6)	0.084	0.06	0.3
LV relative wall thickness	0.39 (0.12)	0.38 (0.12)	0.023	0.8	0.42 (0.13)	0.38 (0.10)	0.132	0.3	0.37 (0.10)	0.39 (0.15)	-0.058	0.7	0.6
Ejection fraction (%)	65.9 (13.7)	61.2 (14.6)	0.159	0.1	65.7 (15.7)	63.3 (13.8)	0.071	0.6	**66.1 (12.3)**	**56.4 (15.8)**	**0.356**	**0.01**	0.1
Stroke volume (ml/beat)	74.1 (24.7)	66.9 (24.2)	0.150	0.1	67.6 (22.3)	69.2 (24.3)	-0.025	0.9	**78.9 (25.7)**	**61.6 (23.6)**	**0.315**	**0.03**	0.1
E/A	1.10 (0.48)	1.01 (0.36)	0.053	0.6	1.19 (0.56)	1.06 (0.38)	0.093	0.5	1.04 (0.41)	0.91 (0.27)	0.135	0.4	0.2
E/e’	**11.5 (5.1)**	**9.7 (4.0)**	**0.254**	**0.007**	**11.3 (5.4)**	**8.8 (3.6)**	**0.307**	**0.01**	11.7 (4.1)	11.7 (5.0)	0.070	0.7	**0.02**
Average e’ (cm/s)	**8.2 (2.6)**	**8.7 (2.7)**	**-0.201**	**0.03**	**8.4 (2.4)**	**9.1 (2.7)**	**-0.246**	**0.04**	8.0 (2.8)	7.8 (2.6)	-0.049	0.8	0.2

Data are expressed as mean (SD), median (interquartile range) or number (percentage). [a]Adjusted for age and sex; [b]for differences among black and other African pre-dialysis and black and other African dialysis patients. Significant associations are shown in bold. Abbreviations: LV: left ventricular; E: early diastolic wave; A: late or atrial diastolic wave; e’: peak velocity during early diastole.

TABLE 5: Carotid atherosclerosis and cardiovascular event rates in black compared to other African chronic kidney disease patients overall, and in sensitivity analysis among pre-dialysis and dialysis patients

	Chronic kidney disease patients									
	All patients			Pre-dialysis patients			Dialysis patients			Inter-group comparison[b]
Characteristic	Black African (n=46)	Other African (n=69)	Model[a]	Black African (n=19)	Other African (n=48)	Model[a]	Black African (n=27)	Other African (n=21)	Model[a]	p
Categorical variables			OR (95% CI)			OR (95% CI)			OR (95% CI)	
Carotid atherosclerosis										
Plaque	**14 (30.4)**	**40 (58.8)**	**0.38 (0.16-0.91)**	7 (36.8)	29 (61.7)	0.43 (0.12-1.51)	7 (25.9)	11 (52.4)	0.35 (0.10-1.29)	**0.01**
Cardiovascular events										
Heart failure	4 (8.7)	8 (11.6)	0.79 (0.22-2.86)	1 (5.3)	4 (8.3)	0.68 (0.07-6.72)	3 (11.1)	4 (19.1)	0.59 (0.11-3.08)	0.5
Ischemic heart disease	4 (8.7)	18 (26.1)	0.33 (0.10-1.15)	4 (21.1)	11 (22.9)	1.26 (0.31-5.23)	0 (0)	7 (33.3)	-	**0.02**
Cerebrovascular event	1 (2.2)	4 (5.8)	0.44 (0.05-4.17)	0 (0)	2 (4.2)	-	1 (3.7)	2 (9.5)	0.39 (0.09-4.87)	0.5
Peripheral arterial disease	0 (0)	2 (2.9)	-	0 (0)	2 (4.2)	-	0(0)	0(0)	-	-
Any cardiovascular event	8 (17.4)	24 (34.8)	0.49 (0.19-1.30)	4 (21.1)	14 (29.2)	0.93 (0.22-3.87)	**4 (14.8)**	**10 (47.6)**	**0.22 (0.05-0.88)**	0.07
Continuous variable			Partial R p			Partial R p			Partial R p	
Carotid atherosclerosis										
Intima-media thickness (mm)	0.610 (0.580-0.725)	0.690 (0.560-0.875)	-0.116 0.2	0.650 (0.580-0.725)	0.690 0.560-0.875)	-0.058 0.6	0.580 (0.503-0.691)	0.645 (0.545-0.763)	-0.095 0.5	**0.04**

Data are expressed as number (percentage) or median (interquartile range) as appropriate. [a]Adjusted for age and sex; [b]for differences among black and other African pre-dialysis and black and other African dialysis patients. Significant associations are shown in bold.

TABLE 6: Cardiovascular risk factors in black and other African chronic kidney disease patients with and without carotid plaque.

	Chronic kidney disease patients					
	Black African patients			Other African patient		
CVD risk factor	Plaque (n=14)	No plaque (n=32)	OR (95% CI)	Plaque (n=40)	No plaque (n=28)	OR (95% CI)
Age (years)	57.6 (15.9)	52.8 (14.0)	1.02 (0.98 to 1.07)	**66.1 (8.3)**	**52.1 (14.0)**	**1.13 (1.06 to 1.20)**
Female sex	7 (50)	11 (34.4)	1.90 (0.53 to 6.84)	**7 (17.5)**	**18 (64.3)**	**0.12 (0.04 to 0.36)**
Hypertension	14 (100)	31 (96.8)	-	34 (85.0)	25 (85.7)	0.94 (0.24 to 3.71)
Dyslipidemia	11 (78.6)	19 (59.4)	2.90 (0.53 to 15.69)	**33 (82.5)**	**19 (67.9)**	**13.90 (1.61 to 119.78)**
Diabetes	8 (57.1)	13 (40.6)	1.95 (0.55 to 6.95)	12 (30.0)	7 (25.0)	1.29 (0.43 to 3.83)
Smoking	0 (0)	2 (6.3)	-	1 (2.5)	0 (0)	-
Framingham score	25.0 (20.5)	22.7 (22.4)	1.01 (0.98 to 1.03)	**29.8 (15.8)**	**13.2 (11.7)**	**1.10 (1.04 to 1.16)**

Data are expressed as mean (SD) or number (percent) and were analysed in logistic regression models. Significant associations are shown in bold. Abbreviations: CVD: cardiovascular disease.

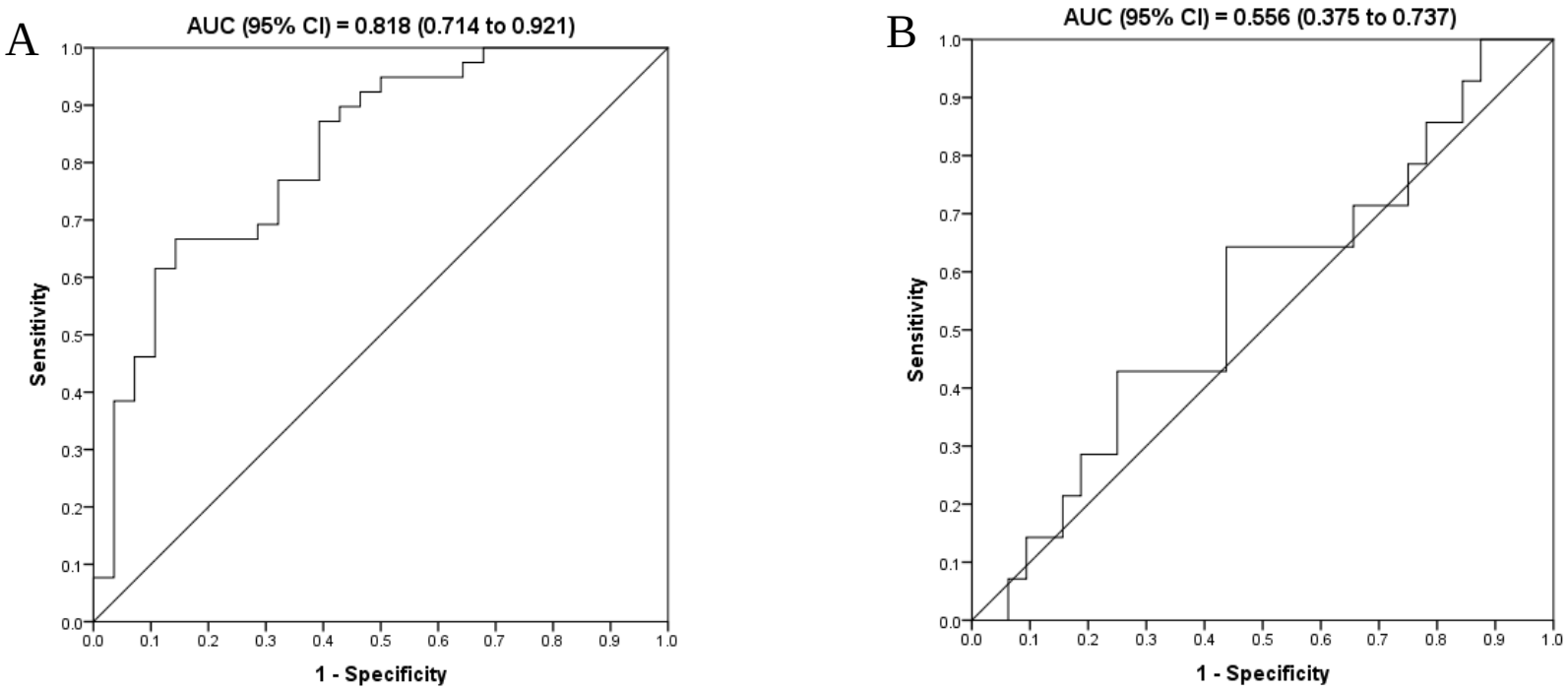


FIGURE 1: Performance of the Framingham score in identifying non-black (A) and black (B) CKD patients with carotid artery plaque.

Supplementary material

Methods

Cardiovascular risk factors. Hypertension was identified when the systolic or/and diastolic blood pressure were ≥ 140mmHg and 90mmHg, respectively, or/and antihypertensive agents were used. Analyses were performed on fasting blood samples using routine laboratory tests for the determination of lipid, glucose, phosphate, calcium, haemoglobin, albumin, uric acid and ferritin concentrations, transferrin saturation percentages. Intact parathyroid hormone levels were measured by an electrochemiluminescent immunoassay on Roche Cobas. High-sensitive C-reactive protein and vitamin D concentrations were both estimated on Abbott Architect using an immunoturbidimetric assay and chemoluminescent microparticle immunoassay, respectively. Dyslipidemia was diagnosed when the total cholesterol: HDL cholesterol ratio was >4 or/and lipid lowering drugs were employed. Diabetes was identified in patients with a fasting glucose level of >7 mmol/l and in those that used glucose lowering medications.

Arterial function. Subsequent to resting for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded for a time period of ten consecutive waveforms (heart beats). Calibration of the pulse wave was done by manual measurement (auscultation) of brachial BP taken immediately prior to recordings. A validated generalized transfer function incorporated in the SphygmoCor software was used to convert the peripheral pressure wave form into a central aortic waveform. The results were discarded when the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less that 80 mV. The aortic pulse wave velocity was calculated as distance in meters divided by transit time in seconds. The time delay in pulse waves between the carotid and femoral sites was assessed by employing an ECG-derived R wave as a fudicial point. Pulse transit time was calculated as the mean of 10 consecutive beats. The difference between the distance from the femoral sampling site to the suprasternal notch and the distance from the carotid sampling site to the suprasternal notch represented the distance the pulse wave travels. Magnitude of the forward and reflected wave components of the aortic pressure waveform was determined by the SphygmoCor software, which separates the aortic waveform by using a modified triangular waveform. Augmentation index was calculated as (second systolic peak/first systolic peak) x 100, reflection magnitude as (reflected wave amplitude/forward wave amplitude) x 100 and pulse pressure amplification as (radial pulse pressure/aortic pulse pressure) x 100. All measurements were made by a single experienced observer (CR) who was unaware of the cardiovascular risk factor profiles of the patients. Brachial blood pressure was recorded in all patients. Technically sound measurements of the central pressure wave and pulse wave velocity were obtained in 151 and 140 patients, respectively.

Left ventricular structure and function. Echocardiography was performed with the patient in the partial left decubitus position. Left ventricular dimensions were determined by measuring the left ventricular internal end diastolic and end systolic diameters and wall thickness (inter-ventricular septal and posterior wall thickness) in the parasternal long axis view by two-dimensional directed M-mode echocardiography. Left ventricular end diastolic and systolic volumes were assessed using the Teichholz method [1]. Stroke volume was determined from the difference between LV end diastolic and systolic volumes as evaluated upon employing the Z-derived method [2]. Left ventricular ejection fraction was calculated as [(left ventricular end diastolic volume – left ventricular end systolic volume) / left ventricular end diastolic volume] x 100. Left ventricular mass was determined using a standard formula [3] and indexed to body surface area (left ventricular mass index). Left ventricular relative wall thickness was calculated as (left ventricular diastolic posterior wall thickness x 2) / left ventricular end diastolic diameter [4]. Left ventricular hypertrophy was identified when left ventricular mass index was >95 g/m^2 for women and >115 g/m^2 for men [3].

Left ventricular diastolic function was assessed using pulsed Doppler and tissue Doppler imaging [5]. Transmitral flow patterns were recorded at the mitral valve leaflet tips using pulsed Doppler in the apical four chamber view. From the mitral valve inflow velocity curve, the early (E) and late (atrial-A) diastolic wave were measured and the ratio of early and late diastolic filling (E/A) was calculated. To perform tissue Doppler imaging, the velocity of myocardial tissue lengthening was measured by placing the curser at the septal and lateral corners of the mitral annulus in the apical four chamber view. To determine diastolic function using tissue Doppler imaging, the early diastolic mitral annulus motion (e') was recorded. Because mitral E is dependent on ventricular relaxation as well as left atrial driving forces (pressures), while e' is dependent on relaxation alone, E/e' ratio is considered to be an index of left ventricular filling pressures. The E/e' ratio was calculated as mitral E/the average of septal and lateral e'.

Echocardiographic measurements were made by the same observer that performed the arterial function evaluation (CR). Intra-observer variation for echocardiographic measurements is low in our setting [6].

References

[1] L.E. Teichholz, T. Kreulen, M.V. Herman, R. Gorlin, “Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy,” *Am J Cardiol*, vol. 37, no. 7, pp. 7-11, 1976.

[2] G. De Simone, R.B. Devereux, A. Ganau et al., “Estimation of the left ventricular chamber and stroke volume by limited M-Mode echocardiography and validation by two-dimensional and Doppler echocardiography,” *Am J Cardiol*, vol. 78, no. 7, pp. 801-807, 1996.

[3] R.M. Lang, L.P. Badano, V. Mor-Avi et al., “Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging,” *Eur Heart J Cardiovasc Imaging*, vol. 16, no. 3, pp. 233-271, 2015.

[4] A. Ganau, R.B. Devereux, M.J. Roman et al., “Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension,” *J Am Coll Cardiol*, vol. 19, no. 7, pp. 1550-1558, 1992.

[5] S.F. Nagueh, O.A. Smiseth, C.P. Appleton et al., “Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update form the American Society of Echocardiography and the European Association of Cardiovascular Imaging,” *J Am Soc Echocardiogr,* vol. 29, no. 4, pp. 277-314, 2016.

[6] L. Molokotedi, S. Gunter, C. Robinson et al. “The impact of different classification criteria on the estimated prevalence and associated risk factors of diastolic dysfunction in rheumatoid arthritis. Int J Rheumatol 2017;2017:2323410.

Study/Paper 2:

Study/Paper 2 shows that cardiovascular risk factors that are independently associated with E/e' include impaired arterial function and diabetes in predialyis patients, and volume overload and low haemoglobin concentrations in dialysis patients.

Potential determinants of the E/e' ratio in non-dialysis compared to dialysis patients

Hon-Chun Hsu[1,2], Gavin R. Norton[1], Chanel Robinson[1], Angela J. Woodiwiss[1], Patrick H. Dessein[1,3,4]
[1]Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[2]Nephrology Unit, Milpark Hospital, Johannesburg, South Africa
[3]Internal Medicine Department, University of the Witwatersrand, Johannesburg, South Africa
[4]Free University and University Hospital, Brussels, Belgium

Corresponding author:

Patrick H Dessein
Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology and Internal Medicine Department, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; Free University and University Hospital, Brussels, Belgium
Postal address: 80 Scholtz Road, Norwood 2117, Johannesburg, South Africa
E-mail: patrick.dessein22@gmail.com
Telephone number: +27(0)66 2491-468

Short running title: E/e' ratio in non-dialysis and dialysis patients

Abstract

Aim: We hypothesized that arterial function and N-terminal natriuretic peptide (NT-proBNP) levels as a marker of volume overload, relate differently to E/e' as an index of diastolic function in dialysis compared to non-dialysis patients with chronic kidney disease. We further examined whether cardiovascular risk factors attenuated these relationships.

Methods: We assessed cardiovascular risk factors and determined arterial function indices by applanation tonometry using SphygmoCor software and E/e' by echocardiography in 103 (62 non-dialysis and 41 dialysis) patients.

Results: In established confounder adjusted analysis, dialysis status impacted the pulse wave velocity-E/e' relationship (interaction p=0.01) but not the NT-proBNP level-E/e' association (interaction p=0.1). Upon entering arterial function measures and NT-proBNP levels simultaneously in regression models, arterial function measures were associated with E/e' (p=0.008 to 0.04) in non-dialysis patients whereas NT-proBNP levels were related to E/e' in dialysis patients (p=0.009 to 0.04). Bivariate associations were found between diabetes (p<0.0001) and E/e' in non-dialysis patients, and haemoglobin concentrations and E/e' (p=0.02) in those on dialysis. Upon adjustment for diabetes in non-dialysis patients, only central pulse pressure remained associated with E/e' (p=0.02); when haemoglobin concentrations were adjusted for in dialysis patients, NT-proBNP levels were no longer associated with E/e' (p=0.2). In separate models, haemoglobin levels were associated with E/e' independent of left ventricular mass index and preload and afterload measures (p=0.02 to 0.03).

Conclusions: The main determinants of E/e' may differ in non-dialysis compared to dialysis patients. These include arterial function and diabetes in non-dialysis patients, and volume overload and anaemia in dialysis patients.

KEYWORDS:

chronic kidney disease, E/e', arterial function indices, NT-proBNP levels, haemoglobin

Introduction

Impaired diastolic function and consequent heart failure with preserved ejection fraction (HFpEF) are highly prevalent in patients with chronic kidney disease (CKD).[1,2] The frequency of diastolic dysfunction increases in relation to CKD severity.[2] Accordingly, up to 85% of dialysis patients have some degree of diastolic dysfunction.[2] The prevalence of HFpEF is larger than that of heart failure with reduced ejection fraction (HFrEF) in CKD.[1] Moreover, mortality rates associated with HFpEF are larger than those related to HFrEF in CKD patients.[3]

Hemodynamic factors that mediate impaired diastolic function in CKD comprise increased afterload and preload.[4] With regard to increased afterload, CKD is characterized by marked arteriosclerosis, which increases arterial stiffness as is mostly estimated by carotid-femoral pulse wave velocity.[5] Increased arterial stiffness results in a larger forward wave pressure, which enhances wave reflection. These changes translate into an increased pulse pressure and reduced coronary perfusion.

Increased preload in CKD is mostly due to volume overload and anaemia.[4] In this regard, anaemia may lead to diastolic dysfunction via three pathways: (a) hyperdynamic circulation and increased preload, (b) myocardial ischemia and (c) myocardial fibrosis.[4,6-8] Both increased afterload and preload in CKD are associated with left ventricular hypertrophy, which is the most frequently identified cardiovascular abnormality in CKD.[4] Left ventricular hypertrophy is traditionally considered to be involved in the development of diastolic dysfunction among patients with CKD.[4] However, in an experimental model of chronic kidney disease, diastolic dysfunction occurred prior to changes in left ventricular geometry.[9]

N-terminal proB-type natriuretic peptide (NT-proBNP) is secreted by cardiomyocytes in response to stretch caused by increased left ventricular volume and pressure as well as other factors including particularly hypoxia.[10,11] In CKD, increased NT-proBNP concentrations is a marker of fluid overload.[12] NT-pro-BNP levels predict cardiovascular events and all-cause mortality in haemodialysis patients.[13] Kim and colleagues recently reported an association of NT-proBNP concentrations with volume overload as well as diastolic dysfunction in non-dialysis CKD stage 5 patients.[14]

The impact of fluid overload on cardiovascular and all-cause mortality is well documented in dialysis patients.[15,16] By contrast, increased afterload may be a more important cardiovascular risk determinant in non-dialysis patients. Indeed, whereas pulse wave velocity independently predicted incident heart failure and mortality in non-dialysis patients that participated in the Chronic Renal Insufficiency Cohort,[17,18] it did not meaningfully improve cardiovascular and all-cause mortality risk discrimination and reclassification beyond clinical risk scores in 2 large dialysis cohorts.[19] We therefore hypothesized that, in established confounder adjusted analysis, impaired arterial function indices are more strongly associated with E/e' in non-dialysis compared to dialysis patients whereas NT-proBNP levels are more closely related to E/e' in dialysis compared to non-dialysis persons. E/e' is calculated as the ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity. It represents left ventricular filling pressure and is one of the echocardiographic indices of diastolic function.[1,14] Importantly in the present context, diabetes is related to impaired arterial function in CKD[20] whereas haemoglobin concentrations are inversely associated with natriuretic peptides in patients with HFpEF.[21,22] In view of these reported findings, we further examined whether traditional and non-traditional or renal cardiovascular risk factors including diabetes and haemoglobin levels, could explain the associations of arterial function measures and NT-proBNP levels with E/e' in CKD patients.

1 METHODS

1.1 Patients

One hundred and three patients including 62 non-dialysis and 41 dialysis participants were enrolled at the Milpark Hospital in Johannesburg, South Africa. Patients with previously diagnosed heart failure, infection or/and active cancer were excluded. Only one male non-dialysis patient had paroxysmal atrial fibrillation and he experienced sinus rhythm at the time he was investigated. Non-dialysis patients had a Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate (eGFR) of <60 ml/min/1.73m^2 upon enrolment. The mean (SD) eGFR in non-dialysis patients was 33.4 (17.9) ml/min/1.73m^2; 34 (54.8%), 16 (25.8%)

and 12 (19.4%) of them had stage 3, stage 4 and stage 5 CKD, respectively. Dialysis was performed thrice weekly for 4 hours per session at the haemodialysis unit at Milpark Hospital. The study was carried out in accordance with the Helsinki Declaration as revised in 2013. Study approval was obtained from the University of the Witwatersrand Human (Medical) research Ethics Committee (protocol number: M15-08-43) in Johannesburg, South Africa. Each patient provided written informed consent prior to participation.

1.2 Methods

Patient characteristics that were recorded included demographic variables, lifestyle factors, anthropometric features, traditional and non-traditional cardiovascular risk factors, established cardiovascular disease, arterial function indices, echocardiographic features and systemic vascular resistance. All investigations were performed on a single day. In dialysis patients, the data were recorded on the day prior to undergoing the respective procedure.

Traditional and non-traditional or renal cardiovascular risk factors were recorded as previously reported and provided in the online Supporting Information (Methods). For the present study, high phosphate was considered present when the phosphate concentration was >1.42 mmol/l or/and phosphate lowering drugs including calcium carbonate or sevelamer therapy in 41 and 1 patients, respectively, was used. Mean arterial blood pressure for the peripheral waveform was determined electronically by the SphygmoCor device (see below) and using the formula

$$MP = \frac{\sum_{i=T_0}^{T_F} P_i}{n}$$

where T_0=start of the waveform; T_F=end of waveform; P_i=pressure points and n=number of pressure points. The mean blood pressure was recorded during 8 consecutive heartbeats once the pulse wave form was consistent with less than 5% variation in pulse height and diastolic pressure.

Established cardiovascular disease comprised ischemic heart disease and cerebrovascular and peripheral arterial disease, the presence of which was confirmed by a cardiologist, neurologist and vascular surgeon, respectively.

Applanation tonometry and SphygmoCor software were used to determine central hemodynamic features as previously reported and provided in the online Supporting Information (Methods). We determined aortic pulse wave velocity, augmentation index, reflected wave pressure and reflection magnitude, central systolic and pulse pressure, peripheral pulse pressure and forward wave pressure.

NT-proBNP concentrations were determined by an electrochemiluminescence immunoassay on Cobas (Roche Diagnostics, Mannheim, Germany). The measurement range was 10 to 35000 pg/ml. The intra-assay and inter-assay coefficients of variation were 3.0% and 4.8%, respectively.

Echocardiography was performed in accordance with recent recommendations[23,24] and using a Philips CX50 POC Compact CompactXtreme Ultrasound System (Philips Medical Systems (Pty) Ltd, USA) equipped with a 1.8-4.2 MHz probe that allowed for M-mode, 2-D, pulsed and tissue Doppler measurements. We assessed left ventricular mass index by the linear method using 2D-guided M-mode echocardiography and indexed to body surface area, left ventricular end diastolic volume and diastolic function variables including the early (E)/late (atrial) diastolic wave (A) ratio, the peak mitral annulus motion during early diastole (averaged septal and lateral e') and E/e' ratio, as previously described and provided in the online Supporting Information (Methods).

Systemic vascular resistance was calculated from mean arterial pressure, right atrial pressure and cardiac output according to the equation (mean arterial pressure – right atrial pressure)=systemic vascular resistance x cardiac output assuming that right atrial pressure=10 mmHg. Right atrial pressure was taken as a fixed value, as in previous studies among CKD patients.[25.] Further details are given in the online Supporting Information.

1.3 Data analysis

Results are given as mean (SD), median (interquartile range) or percentages as appropriate. Non-normally distributed variables were logarithmically transformed prior to entering them in linear multivariate regression models.

We compared the recorded characteristics between non-dialysis and dialysis patients in age, sex and race adjusted linear or logistic regression models as appropriate, with additional adjustment for waist-height ratio, mean blood pressure and heart rate upon comparing arterial function parameters and for left ventricular mass index upon comparing diastolic function markers.

The subsequent analyses aimed at identifying factors that were associated with E/e'. For this, potential established confounders were considered when they were related to E/e' with a p value of <0.2. These included age (p=0.03), waist-height ratio (p=0.13), mean blood pressure (p=0.17) and heart rate (p=0.1). In this regard, sex (p=0.6), race (p=0.9), body mass index (p=0.47) and waist-hip ratio (p=0.6) did not show a tendency to relate to E/e'. Sex was nevertheless forced into the models.

Given the above, the associations of arterial function markers and NT-proBNP with E/e' were first assessed in age and sex and subsequently in established confounder (age, sex, waist-height ratio, mean blood pressure and heart rate) adjusted models; additional adjustment for left ventricular mass index and left ventricular end diastolic volume was made when deemed indicated. To assess the impact of CKD status (non-dialysis versus dialysis) on arterial function-diastolic function and NT-pro-BNP-diastolic function relationships, we added interaction terms together with their components to the models. This was followed by stratified analysis, i.e. analysis in non-dialysis and dialysis patients separately. Subsequently, we re-assessed the arterial function-diastolic function and NT-pro-BNP-diastolic function relationships after additional adjustment for patient characteristics that were associated with diastolic function in bivariate analysis.

Statistical analysis was performed on IBM SPSS statistics program (version 23.0 IBM, USA). Significance was set at $p < 0.05$.

2 RESULTS

2.1 Recorded characteristics in non-dialysis and dialysis patients

The recorded characteristics in non-dialysis and dialysis patients are given in Table 1. Black patients were more often on dialysis whereas the reverse applied to white participants. In age, sex and race adjusted analysis, systolic and mean blood pressure were significantly larger in dialysis compared to non-dialysis patients.

With regard to non-traditional/renal cardiovascular risk factors, dialysis patients experienced significantly more frequent high phosphate concentrations, larger intact parathyroid hormone and ferritin concentrations and smaller haemoglobin and uric acid levels and were more often treated with intravenous iron and erythropoietin stimulating agents compared to non-dialysis participants.

NT-proBNP concentrations were larger in dialysis compared to non-dialysis patients. As given in Table 2, among arterial function parameters, central systolic blood pressure was significantly larger in dialysis compared to non-dialysis patients.

As also shown in Table 2, on echocardiography, the left ventricular mass index tended to be larger in dialysis compared to non-dialysis patients (p=0.07). E' was significantly smaller and E/e' significantly larger in dialysis compared to non-dialysis patients; upon additional adjustment for left ventricular mass index, e' remained significantly smaller in dialysis patients whereas E/e' no longer differed between dialysis and non-dialysis participants.

2.2 Established confounder adjusted associations of arterial function and NT-proBNP concentrations with diastolic function in all, non-dialysis and dialysis patients

In all patients, central systolic blood pressure (β (SE)=0.133 (0.050), p=0.009, model R^2=0.172), central pulse pressure (β (SE)=0.110 (0.035), p=0.002, model R^2=0.194), peripheral pulse pressure (β (SE)=0.062 (0.025), p=0.01, model R^2=0.160), forward wave pressure (β (SE)=0.108 (0.046), p=0.02, model R^2=0.184) and NT-proBNP (β (SE)=1.870 (0.540), p=0.001, model R^2=0.220) were associated with E/e'. Arterial function parameters and NT-proBNP concentrations were not associated with e' and E/A (data not shown).

As shown in Supplementary Table 1, CKD status (non-dialysis versus dialysis) impacted the pulse wave velocity-E/e' relationship significantly (interaction *p*=0.01). As given in Supplementary Table 2, CKD stage did not influence the NT-proBNP-E/e' association (interaction *p*=0.1).

Table 3 shows the age and sex adjusted and established confounder adjusted associations of arterial function and NT-proBNP levels with E/e' in stratified analysis. In non-dialysis but not dialysis patients, pulse wave velocity, reflected wave pressure, central systolic and pulse pressure, peripheral pulse pressure and forward wave pressure were each significantly associated with E/e'. NT-proBNP was significantly associated with E/e' in both non-dialysis and dialysis patients. Further adjustment for left ventricular mass or left ventricular end diastolic volume did not materially alter any of the significant relationships as also given in Table 3.

Arterial function parameters and NT-pro-BNP levels were not associated with E/A and e' in stratified analysis (data not shown).

Among non-dialysis patients, the estimated glomerular filtration rate was not associated with E/e' (β (SE)=-0.031 (0.034); partial R=-0.123; *p*=0.4).

2.3 Independent established confounder adjusted associations of arterial function and NT-proBNP concentrations with diastolic function in non-dialysis and dialysis patients

Table 4 gives the established confounder adjusted associations of arterial function measures and NT-proBNP concentrations with E/e' in non-dialysis and dialysis patients when both potential determinants of E/e' were entered simultaneously in the models. In non-dialysis patients, pulse wave velocity, central systolic and pulse pressure and peripheral pulse pressure but not NT-proBNP were significantly associated with E/e'. In dialysis patients, NT-proBNP was significantly associated (when entered together with pulse wave velocity or peripheral pulse pressure) or tended to be associated (when entered together with augmentation index (p=0.05), reflected wave pressure (p=0.06), reflection magnitude (p=0.05), central systolic pressure (p=0.05) or forward wave pressure (p=0.05)) with E/e' whereas arterial function parameters were not related to E/e'.

2.4 Arterial function-E/e' relationships after additional adjustment for diabetes in non-dialysis patients and the NT-proBNP levels-E/e' relationship after additional adjustment for haemoglobin concentrations in dialysis patients

Among the baseline recorded patient characteristics as given in Table 1, bivariate associations were found between diabetes (R=0.530, p<0.0001), erythropoietin stimulating agent use (R=0.252, p=0.04) and high phosphate concentrations (R=0.246, p=0.05) with E/e' in non-dialysis patients whereas haemoglobin (R=-0.359, p=0.02) concentrations were related to E/e' in those on dialysis. Table 5 shows that upon additional adjustment for diabetes in established confounder adjusted models in non-dialysis patients, only the central pulse pressure-E/e' relationship (partial R=0.301, p=0.02) remained significant; in each of the respective models, diabetes was independently associated with E/e' (partial R=0.497, p=0.002, partial R=0.501, p<0.0001, partial R=0.458, p=0.002, partial R=0.501, p<0.0001, partial R=0.476, p<0.0001, partial R=0.457, p=0.001, partial R=0.444, p=0.001, and partial R=0.461, p=0.0001 for pulse wave velocity, augmentation index log reflected wave pressure, reflection magnitude, central systolic blood pressure, central pulse pressure and forward wave pressure, respectively). Upon additional adjustment for haemoglobin levels in dialysis patients, NT-proBNP was no longer associated with E/e'. The attenuation of arterial function indices-E/e' relations upon additional adjustment for diabetes in non-dialysis patients and the NT-proBNP-E/e' association upon additional adjustment for haemoglobin concentrations in dialysis patients is further illustrated in Figure 1.

As shown in the online Supporting Information (Supplementary Table 1), additional adjustment for erythropoietin concentrations or high phosphate levels in non-dialysis patients did not materially alter the arterial function-E/e' associations as were given above and in Table 3.

2.5 Associations of haemoglobin concentrations with E/e' in dialysis patients

Given the above mentioned finding that haemoglobin concentrations weakened the NT-proBNP-E/e' relationship in dialysis patients, we assessed the independent association of

haemoglobin concentrations with E/e'. Table 6 shows that haemoglobin concentrations were associated with E/e' when adjusted for age, sex, waist-height ratio, mean blood pressure and heart rate (first model). The second model shows that the haemoglobin-E/e' relationship was independent of left ventricular mass index. In the third model, left ventricular end diastolic volume and central systolic blood pressure were additionally adjusted for to account for pre-load and afterload, respectively. In the fourth model, mean blood pressure was replaced by systemic vascular resistance to account for arteriolar dilatation. In the fifth model, left ventricular end diastolic volume was omitted to avoid collinearity (Pearson R=-0.500, p<0.0001) between arteriolar dilatation and preload. Across the models, haemoglobin was similarly associated with E/e'.

3 DISCUSSION

To our knowledge, this is the first study that compared the associations of arterial function indices and NT-proBNP concentrations as a reported marker of volume overload,[12] with diastolic function between non-dialysis and dialysis patients with CKD. Our main findings were as follows: (1) CKD status (dialysis versus non-dialysis) influenced the relationship of pulse wave velocity with E/e' independent of established confounders (interaction *p*=0.01); accordingly, in stratified analysis, arterial stiffness, wave reflection and central pressures were significantly associated with E/e' in non-dialysis but not dialysis patients; (2) although NT-pro-BNP concentrations were associated with E/e' in both non-dialysis and dialysis patients, upon entering arterial function measures and NT-proBNP levels simultaneously in regression models, only arterial function indices were associated with E/e' in non-dialysis patients and only NT-proBNP concentrations was related to E/e' in dialysis participants; (3) diabetes attenuated the arterial function-E/e' associations in non-dialysis patients whereas haemoglobin levels attenuated the NT-pro-BNP-E/e' relations in dialysis patients; (4) in separate models, haemoglobin concentrations were associated with E/e' independent of preload and afterload measures among dialysis patients. Taken together, these findings suggest that potential determinants of diastolic function may, at least to an extent, differ in non-dialysis compared to dialysis patients.

It is notable that diabetes prevalence and arterial function measures were overall similar among non-dialysis and dialysis patients in the present study. However, as expected, NT-proBNP concentrations were larger and haemoglobin concentrations were smaller in dialysis compared to non-dialysis participants. We believe that volume overload and anaemia may therefore, at least to some extent, override potential effects of impaired arterial function and diabetes on E/e' in CKD patients once they require dialysis. By contrast, the effects of impaired arterial function and diabetes on E/e' may outweigh those of volume overload and anaemia among those with milder CKD, i.e. non-dialysis patients.

NT-proBNP levels are associated with diastolic function in the general population.[10,26] Increased NT-proBNP production in CKD is reportedly due to volume overload and left ventricular hypertrophy.[12] It is therefore of interest that in the present study, NT-proBNP concentrations were associated with E/e' ratio independent of established confounders that included left ventricular mass index and end diastolic volume as a marker of cardiac preload,[27] in both non-dialysis and dialysis patients. Strikingly, haemoglobin concentrations that were associated with E/e' among dialysis patients in bivariate analysis, weakened the NT-proBNP-E/e' relationship in the respective group. In additional models among dialysis patients, haemoglobin concentrations were associated with E/e' independent of not only left ventricular mass index but also arterial function, systemic vascular resistance and left ventricular end diastolic volume in dialysis patients.

NT-proBNP levels are most valuable in the management of heart failure patients.[10,27] Increased NT-proBNP production is generally considered to result from an increased ventricular mechanical load.[10,28] However, the mechanisms that regulate natriuretic peptide gene expression are not fully elucidated.[10] In this regard, systemic hypoxia and particularly hypoxia at the cardiomyocyte level comprise stimuli that can directly stimulate natriuretic peptide production.[10,11,28] With regard to haemoglobin concentrations, besides their effects on tissue hypoxia and myocardial fibrosis,[6-8] anaemia is associated with increased NT-pro-BNP concentrations in persons without heart failure and kidney disease.[29] Furthermore, in patients with HFpEF,[21]

haemoglobin concentrations are associated with those of NT-pro-BNP and diastolic dysfunction severity. In another study,[22] haemoglobin levels were related to natriuretic peptide concentrations in patients with HFpEF but not those with HFrEF. Intravenous iron therapy reduces NT-proBNP levels in patients with heart failure and chronic kidney disease.[30] These reported data and our current findings suggest that low haemoglobin concentrations may mediate reduced diastolic function through impaired cardiomyocyte oxygenation rather than its hemodynamic effects[4] in dialysis patients.

In this study, diabetes was associated with E/e' in bivariate analysis among non-dialysis patients. Upon additional adjustment for diabetes, we found that the arterial function-E/e' relationships were consistently attenuated among non-dialysis patients and only the association between central pulse pressure and diastolic function remained significant (p=0.02). In a previous study among non-dialysis patients, those with diabetic nephropathy experienced more impaired diastolic function compared to chronic glomerulonephritis participants.[31] Also, in the Chronic Renal Insufficiency Cohort study, carotid-femoral pulse wave velocity was 2 m/s faster in diabetic compared to non-diabetic participants, this within any decade.[20] Our current results suggest that diabetes can impact diastolic function directly as well as through its effects on central pulse pressure in non-dialysis CKD patients.

In non-CKD persons, pulse wave velocity and wave reflection mediate cardiac afterload in early and systole, respectively.[32, 33] Both increased pulse wave velocity and wave reflection contribute to an enhanced central pulse pressure and thereby E/e'. [32, 33] In line with these reported findings in the non-CKD population, in the present study, each of the respective arterial function measures was associated with E/e' in non-dialysis patients. Furthermore, the central pulse pressure-E/e' remained significantly associated with E/e' even after additional adjustment for diabetes.

Although increased left ventricular mass is generally perceived as an essential intermediate step in the pathophysiology of CKD as well as hypertension induced diastolic dysfunction,[4] impaired diastolic function may antedate left ventricular hypertrophy.[4,9] We found that E/e' was larger in dialysis compared to non-dialysis patients and this difference was no longer

significant after adjustment for left ventricular mass index. Nevertheless, arterial function measure-E/e', NT-pro-BNP-E/e' and haemoglobin-E/e' relationships were consistently unaltered upon adjustment for left ventricular mass index.

Arterial function and NT-proBNP concentrations were not associated with E/A in this study. This is not surprising as E/A cannot reliably distinguish between diastolic function and altered loading conditions.[34] E/e' is strongly associated with incident cardiovascular events in both CKD and non-CKD patients.[1,35]

The present study has limitations. The study design was cross-sectional, which precludes determining cause-effect relationships. The number of enrolled patients was small, particularly in the dialysis group. Haemoglobin and NT-pro-BNP concentrations were assessed on one occasion only, i.e. on the same day that other measurements were made. Conceptually, anaemia may need to be present for prolonged time periods in order for myocardial fibrosis to develop. We may therefore have underestimated the potential impact of low haemoglobin levels on diastolic function in dialysis patients. With regard to NT-pro-BNP concentrations, a single measurement at the time of arterial and cardiac function evaluation is likely most appropriate as CKD patients often experience major changes in volume status over time. All participants were enrolled at a single centre. We did not assess volume status by bioimpedance spectroscopy,[12,14] which may have been useful in the present context. We also did not consistently measure troponin concentrations, which reportedly comprise a useful biomarker of diastolic function in both non-CKD and CKD persons.[36,37] Finally, in non-dialysis patients, addressing proteinuria may have provided further insights. A strength of this investigation is that our conclusions originate in multivariable regression models in which we consistently adjusted for potential established confounders as well as those that were identified in bivariate analysis.

In conclusion, the main determinants of diastolic function may differ in non-dialysis compared to dialysis patients. These include arterial function and diabetes in non-dialysis patients and volume overload and anaemia in dialysis patients.

Acknowledgements

The study was supported in part by the South African National Research Foundation. We thank Ms Nolontu Dlongolo for assisting with data management and Dr Gloria Teckie and Dr Ferande Peters for providing intellectual input.

Conflict of Interest Statement

The authors have not conflicts of interest to declare.

REFERENCES

1. Kim MK , Kim B, Lee JY, Kim JS, Han B-G, Choi SO, et al. Tissue Doppler-derived E/e' ratio as a parameter for assessing diastolic heart failure and as a predictor of mortality in patients with chronic kidney disease. *Korean J Intern Med* 2013;28(1):35-44.
2. Farshid A, Pathak R, Shadbolt B, Arnolda L, Talaulikar G. Diastolic function is a strong predictor of mortality in patients with chronic kidney disease. *BMC Nephrology* 2013;14:280.
3. Ahmed A, Rich MW, Sanders PW, Perry GJ, Bakris GL, Zile MR, et al. Chronic kidney disease associated mortality in diastolic versus systolic heart failure: A propensity matched study. *Am J Cardiol* 2007;99(3):393-8.
4. Wang X, Shapiro JI. Evolving concepts in the pathogenesis of uraemic cardiomyopathy. *Nat Rev Nephrol* 2019;15(3):159-175.
5. Zanoli L, Lentini P, Briet M, Castellino P, House AA, London GM, et al. Arterial stiffness in the heart of CKD. *J Am Soc Nephrol* 2019;30(6):918-928.
6. Mistry N, Mazer CD, Sled JG, Lazarus AH, Cahill LS, et al. Red blood cell antibody-induced anemia causes differential degrees of tissue hypoxia in kidney and brain. *Am J Physiol Reg Integr Comp Physiol* 2018;314:R611-R622.
7. Caramelo C, Justo S, Gil P. Anemia in heart failure: pathophysiology, pathogenesis, treatment, and incognitae. *Rev Esp Cardiol* 2007;60:848-860.
8. D'Amario D, Migliaro S, Borovac JA, Restivo A, Vergallo R, Galli M, et al. Microvascular dysfunction in heart failure with preserved ejection fraction. *Front Physiol* 2019;10:1347. doi: 10.3389/fphys.2019.01347. eCollection 2019.
9. Winterberg PD, Jiang R, Maxwell JT, Wang B, Wagner MB. Myocardial dysfunction occurs prior to changes in ventricular geometry in mice with chronic kidney disease (CKD). *Physiol Rep* 2016;4(5):e12732.
10. Sergeeva IA, Christoffels VM. Regulation of expression of atrial and brain natriuretic peptide, biomarkers for heart development and disease. *Biochim Biophys Acta* 2013;1832(12):2403-13.

11. Arjamaa O, Nikinmaa M. Hypoxia regulates the natriuretic peptide system. *In J Physiol Patophysiol Pharmacol* 2011;3(3):191-201.
12. Ekinci C, Karabork M, Siriopol D, Dincer N, Covic A, Kanbay M. Effects of volume overload and current techniques for the assessment of fluid status in patients with renal disease. *Blood Purif* 2018;46(1):34-47.
13. Kawagoe C, Sato Y, Toida T, Nakagawa H, Yamashita Y, Fukuda A, et al. N-terminal-pro-B-type-natriuretic peptide associated with 2-year mortality from both cardiovascular and non-cardiovascular origins in prevalent hemodialysis patients. *Ren Fail* 2018;40(1):127-34.
14. Kim J-S, Yang J-W, Yoo JS, Choi S Ok, Han B-G. Association between E/e' ratio and fluid overload in patients with predialysis chronic kidney disease. *PLoS One* 2017:13;12(9):e0184764. doi: 10.1371/journal.pone.0184764. eCollection 2017.
15. Kalantar-Zadeh K, Redigor DL, Kovesdy CP, Van Wiyck D, Bunnapradist S, Horwich TB, et al. Fluid retention is associated with cardiovascular mortality in patients undergoing long-term hemodialysis. *Circulation* 2009;119(5):671-9.
16. Zoccali C, Moissl U, Chazot C, Mallamaci F, Tripepi G, Arkossy A, et al. Chronic fluid overload and mortality in ESRD. *J Am Soc Nephrol* 2017;28(8):2491-7.
17. Chirinos JA, Khan A, Bansai N, Dies DL, Feldman HI, Ford V et al. Arterial stiffness, central pressures and incident hospitalized heart failure in the Chronic Renal Insufficiency Cohort (CRIC). *Circ Heart Fail* 2014;7(5):709-16.
18. Townsend RR, Anderson AH, Chirinos JA, Feldman HI, Grunwald JE, Nessel L, et al. Association of pulse wave velocity with chronic disease progression and mortality. Findings from the CRIC Study (Chronic Renal Insufficiency Cohort). *Hypertension* 2018;71(6):1101-7.
19. Tripepi G, Agharazii M, Pannier B, D'Arrigo G, Mallamaci F, Zoccali C, et al. Pulse wave velocity and prognosis in end-stage kidney disease. *Hypertension* 2018;71(6):1126-32.
20. Townsend RR. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. *Curr Opin Nephrol Hypertens* 2015;24(1):47-53.

21. Brucks S, Little WC, Chao T, Rideman RL, Upadhya B, Wesley-Farrington D, et al. Relation of anemia to diastolic heart failure and the effect on outcome. *Am J Cardiol* 2004;93(8):1055-7.

22. Wu AHB, Omland T, Knudsen CW, McCord J, Nowak RM, Hollander JE, et al. Relationship of B-type natriuretic peptide and anemia in patients with and without heart failure: A substudy from the Breathing Not Properly (BNP) Multinational Study. *Am J Hematol* 2005;80(3):174-80.

23. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2015;28:1-39.

24. Nagueh SF, Smiseth OA, Appleton CP, Byrd 3rd BF, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2016;29:277-314.

25. Santosh S, Chu C, Mwangi J, Marayan M, Mosman A, Nayak R, et al. Changes in pulmonary artery systolic pressure and right ventricular function in patients with end-stage renal disease on maintenance dialysis. *Nephrology* 2019;24:74-80.

26. McGrady M, Reid CM, Shiel L, Wolfe R, Boffa U, Liew D, et al. N-terminal B-type natriuretic peptide and the association with left ventricular diastolic function in a population at high risk of incident heart failure: results of the SCReening Evaluation of the Evolution of New-Heart Failure Study (SCREEN-HF). *Eur J Heart Fail* 2013;15 (5):573-80.

27. Gutierrez MC, Moore PG, Liu H. Goal-directed therapy in intraoperative fluid and hemodynamic management. *J Biomed Res* 2013;27(5):357-65.

28. Cao Z, Jia Y, Zhu B. BNP and NT-proBNP as diagnostic biomarkers for cardiac dysfunction in both clinical and forensic medicine. *Int J Mol Sci* 2019;20(8):1820. doi: 10.3390/ijms20081820.

29. Willis MS, Lee ES, Grenache DG. Effect of anemia on plasma concentrations of NT-proBNP. *Clin Chim Acta* 2005;358(1-2):175-81.
30. Toblli JE, Lombrana A, Duarte P, Gennaro FD. Intravenous iron reduces NT-Pro-Brain natriuretic peptide in anemic patients with chronic heart failure and renal insufficiency. *J Am Coll Cardiol* 2007;50(17):1657-65.
31. Miyazato J, Horio T, Takiuchi S, Kamide K, Sasaki O, Nakamura S, et al. Left ventricular diastolic dysfunction in patients with chronic renal failure: impact of diabetes mellitus. *Diabet Med* 2005;22(6):730-6.
32. Chirinos JA, Segers P, Hughes T, Townsend R. Large-artery stiffness in health and disease. *J Am Coll Cardiol* 2019;9:1237-63.
33. Peterson VR, Woodiwiss AJ, Libhaber CD, Raymond A, Sareli P, Norton GR. Cardiac diastolic dysfunction is associated with aortic wave reflection, but not stiffness in a predominantly young-to-middle-aged community sample. *Am J Hypertens* 2016;29:1148-57.
34. von Bibra H, Sutton MStJ. Diastolic dysfunction in diabetes and the metabolic syndrome: promising potential for diagnosis and prognosis. *Diabetologia* 2010;53(6):1033-45.
35. Kutznetsova T, Thijs L, Knez J, Herbots L, Zhang Z, Staessens JA. Prognostic value of left ventricular diastolic dysfunction in a general population. *J Am Heart Assoc* 2014;3(3): e000789. doi: 10.1161/JAHA.114.000789.
36. Myhre PL, Claggett B, Ballantyne C, Selvin E, Rosjo H, Omland T, et al. Association between circulating troponin concentrations, left ventricular systolic and diastolic functions, and incident heart failure in older adults. *JAMA Cardiol* 2019;4:997-1006.
37. Kitagawa M, Sugiyama H, Morinaga H, Inoue T, Takiue K, Kikumoto Y, ety al. Serum high-sensitivity cardiac troponin is a significant biomarker of left-ventricular diastolic dysfunction in subjects with non-diabetic chronic kidney disease. *Nephron Extra* 2011;1:166-177.

Supporting Information:

Additional supporting information on methods (cardiovascular risk factors, arterial function and echocardiography) and results (Supplementary Table 1, 2 and 3) may be found online in the Supporting Information section at the end of this article.

Figure legend:

FIGURE 1 Partial correlations (95% CI) for the associations of arterial function measures with E/e' before (open circles) and after (closed circles) additional adjustment for diabetes in non-dialysis patients and haemoglobin concentrations in dialysis patients. Age, sex, waist-height ratio, mean blood pressure and heart rate were adjusted for in all models. CI, confidence interval; PWV, pulse wave velocity; adj., adjusted; DM, diabetes mellitus; log, logarithmically transformed; Pb, backward pressure or reflected wave pressure; SBP, systolic blood pressure; PP, pulse pressure; NT-pro-BNP, N-terminal B-type natriuretic peptide.

TABLE 1 Baseline recorded characteristics in non-dialysis and dialysis patients

Characteristic	Chronic kidney disease patients		
	Non-dialysis (n=62)	Dialysis (n=41)	*p*
Demographics			
Age (years)	58.6 (14.1)	55.3 (15.1)	0.4
Female sex (%)	20 (32.2)	18 (43.9)	0.1
Black (%)	**18 (29.0)**	**24 (58.5)**	**0.004**
Asian (%)	21 (33.9)	9 (21.9)	0.1
White (%)	**21 (33.9)**	**3 (7.3)**	**0.006**
Mixed (%)	2 (3.2)	5 (12.2)	0.2
Anthropometry			
Body mass index (kg/m^2)	27.8 (5.6)	27.2 (5.9)	0.5
Waist-hip ratio	0.96 (0.12)	0.98 (0.09)	0.09
Waist-height ratio	0.59 (0.09)	060 (0.11)	0.5
Major traditional risk factors			
Hypertension (%)	53 (85.5)	39 (95.1)	0.1
Systolic blood pressure (mmHg)	**138 (20)**	**146 (21)**	**0.02**
Diastolic blood pressure (mmHg)	81 (9)	85 (15)	0.1
Mean blood pressure (mmHg)	**100 (11)**	**105 (14)**	**0.02**
Heart rate (beats/min)	74 (15)	76 (12)	0.4
Dyslipidemia (%)	49 (86.0)	25 (69.4)	0.06
Diabetes (%)	21 (33.9)	14 (34.2)	0.9
Non-traditional/renal risk factors			
Dialysis duration, months	-	36 (12-48)	-
Estimated GFR	33.4 (17.9)	-	-
Phosphate (mmol/l)	1.2 (0.9-1.4)	1.4 (0.9-1.7)	0.1
Calcium (mmol/l)	2.3 (2.2-2.4)	2.3 (2.1-2.4)	0.2
High phosphate	**12 (19.7)**	**30 (81.1)**	**<0.0001**
Intact PTH (pg/ml)	**83.0 (56.0-195.6)**	**520.3 (193.0-790.0)**	**<0.0001**
Haemoglobin (g/dl)	**12.9 (10.2-15.1)**	**10.8 (9.8-12.0)**	**<0.0001**

Transferrin saturation (%)	20.5 (16.7-27.2)	22.6 (18.0-26.1)	0.4
Ferritin (ng/ml)	**124 (49-247)**	**360 (146-603)**	**<0.0001**
Uric acid (mmol/l)	**0.42 (0.12)**	**0.25 (0.09)**	**<0.0001**
Intravenous iron therapy (%)	**9 (14.5)**	**34 (82.9)**	**<0.0001**
ESA therapy (%)	**10 (16.1)**	**38 (92.7)**	**<0.0001**
Cardiovascular disease	13 (20.9)	7 (17.1)	0.8
NT-proBNP (pg/ml)	**527 (78-1901)**	**4060 (1100-13022)**	**<0.0001**
SVR (mmHg/l/min)	21.1 (15.9-27.1)	20.4 (16.1-24.1)	0.6

Data are expressed as mean (SD) or median (interquartile range) unless indicated otherwise, and were analysed in age, sex and race adjusted linear or logistic regression models as appropriate. Significant differences are shown in bold. Abbreviations: GFR, glomerular filtration rate; PTH, intact parathyroid hormone; ESA, erythropoietin stimulating agent; NT-proBNP, N-terminal B-type natriuretic peptide; SVR, systemic vascular resistance.

TABLE 2 Arterial function indices and echocardiographic characteristics in non-dialysis and dialysis patients

Characteristic	Expected values	Chronic kidney disease patients		
		Non-dialysis (n=62)	Dialysis (n=41)	*p*
Arterial function				
Pulse wave velocity (mm/sec)		11.1 (2.3)	11.4 (4.3)	0.5/0.6*
Augmentation index (%)		66.9 (16.9)	66.3 (16.7)	0.9/0.9*
Reflected wave pressure (mmHg)		20.8 (14.3-25.0)	25.0 (15.0-29.0)	0.1/0.1*
Reflection magnitude (%)		67.5 (17.1)	66.9 (16.7)	0.9/0.9*
Central systolic BP (mmHg)		127 (17.2)	135 (20.9)	**0.01/0.02***
Central pulse pressure (mmHg)		47 (15)	48 (16)	0.09/0.1*
Peripheral pulse pressure (mmHg)		57 (18)	61 (21)	0.1/0.2*
Forward wave pressure (mmHg)		31 (10.4)	35 (11)	0.1/0.1*
Echocardiographic characteristics				
Left ventricular mass index (g/m^2)	men: 49-115; women: 43-95	88.7 (37.3)	101.9 (54.1)	0.07
Left ventricular EDV (ml)	men: 62-150; women: 46-106	125 (59)	140 (70)	0.2
Ejection fraction (%)	men: 52-72; women: 54-74	65.3 (13.9)	62.3 (13.9)	0.1
E/A	>0.8 to <2.0	1.05 (0.38)	0.97 (0.35)	0.1/0.1*,*
E' (cm/sec)	≥8.5	**9.0 (2.6)**	**8.1 (2.8)**	**0.01/0.02***,*
E/e'	≤14	**9.3 (4.3)**	**11.0 (4.6)**	**0.02**/0.05*,*

Data are expressed as mean (SD) or median (interquartile range) and were analysed in age, sex and race adjusted linear regression models as appropriate. Significant differences are shown in bold. Abbreviations: EDV, end diastolic volume; BP, blood pressure.
*Additionally adjusted for waist-height ratio, mean blood pressure and heart rate.
*,*Additionally adjusted for left ventricular mass index.

TABLE 3 Established confounder adjusted associations of arterial function and NT-proBNP levels with E/e' in non-dialysis and dialysis patients

	Chronic kidney disease patients					
	Non-dialysis			Dialysis		
	β (SE)	*p*	Model R^2	β (SE)	*p*	Model R^2
Pulse wave velocity	**0.599 (0.188)**	**0.002†**	**0.206**	-0.113 (0.169)	0.5†	0.080
	0.661 (0.203)	**0.002‡**	**0.268**	-0.098 (0.192)	0.6‡	0.111
	0.626 (0.206)	**0.004§**	**0.287**	-0.127 (0.196)	0.5§	0.133
	0.692 (0.201)	**0.001¶**	**0.323**	-0.045 (0.198)	0.8¶	0.145
Augmentation index	0.013 (0.032)	0.7†	0.160	-0.038 (0.061)	0.5†	0.107
	0.004 (0.037)	0.9‡	0.220	-0.074 (0.069)	0.3‡	0.155
	0.003 (0.037)	0.9§	0230	-0.075 (0.072)	0.3§	0.155
	-0.008 (0.037)	0.8¶	0.294	-0.081 (0.065)	0.2¶	0.187
Log reflected wave pressure	6.551 (3.280)	0.05†	0.222	4.100 (5.062)	0.4†	0.114
	8.568 (4.131)	**0.04‡**	**0.289**	2.380 (6.202)	0.7‡	0.126
	8.262 (4.296)	0.06§	0.291	2.569 (6.351)	0.7§	0.127
	9.694 (3.996)	**0.02¶**	**0.382**	0.129 (6.119)	0.9¶	0.140
Reflection magnitude	0.013 (0.031)	0.7†	0.160	-0.044 (0.061)	0.5†	0.111
	0.003 (0.036)	0.9‡	0.220	-0.079 (0.068)	0.2‡	0.161
	0.003 (0.036)	0.9§	0.230	-0.081 (0.071)	0.2§	0.161
	-0.009 (0.036)	0.8¶	0.295	-0.085 (0.064)	0.2¶	0.193
Central systolic BP	0.059 (0.033)	0.08†	0.125	0.041 (0.037)	0.3†	0.129
	0.229 (0.071)	**0.002‡**	**0.264**	0.058 (0.078)	0.5‡	0.135
	0.213 (0.073)	**0.005§**	**0.275**	0.062 (0.080)	0.5§	0.137
	0.261 (0.069)	**<0.0001¶**	**0.349**	0.016 (0.077)	0.8¶	0.141
Central pulse pressure	**0.122 (0.035)**	**0.001†**	**0.235**	0.060 (0.051)	0.2†	0.133
	0.158 (0.043)	**0.001‡**	**0.295**	0.051 (0.064)	0.9‡	0.137
	0.149 (0.044)	**0.002§**	**0.306**	0.051 (0.065)	0.5§	0.138
	0.176 (0.042)	**<0.0001¶**	**0.379**	0.026 (0.064)	0.7¶	0.145
Peripheral pulse pressure	**0.088 (0.029)**	**0.004†**	**0.203**	0.033 (0.036)	0.4†	0.100
	0.125 (0.036)	**0.001‡**	**0.284**	0.027 (0.039)	0.5‡	0.131
	0.118 (0.037)	**0.002§**	**0.296**	0.027 (0.040)	0.5§	0.131
	0.139 (0.035)	**<0.0001¶**	**0.364**	0.013 (0.038)	0.7¶	0.147
Forward wave pressure	0.085 (0.046)	0.06†	0.215	0.108 (0.077)	0.2†	0.149
	0.117 (0.054)	**0.03‡**	**0.296**	0.102 (0.088)	0.2‡	0.160
	0.113 (0.055)	**0.04§**	**0.300**	0.101 (0.089)	0.3§	0.161
	0.136 (0.051)	**0.01¶**	**0.399**	0.076 (0.087)	0.4¶	0.163
Log NT-proBNP level	**1.495 (0.665)**	**0.03†**	**0.155**	**2.374 (1.056)**	**0.03†**	**0.202**
	1.650 (0.714)	**0.03‡**	**0.206**	2.427 (1.215)	0.05‡	0.229
	1.414 (0.770)	0.07§	0.211	2.476 (1.253)	0.05§	0.230
	1.638 (0.693)	**0.02¶**	**0.291**	**3.106 (1.106)**	**0.008¶**	**0.341**

NT-pro-BNP, N-terminal pro B-type natriuretic peptide; Log, logarithmically transformed; BP, blood pressure; SE, standard error. Significant associations are shown in bold.

† Adjusted for age, sex.

‡Adjusted for age, sex, waist-height ratio, mean blood pressure and heart rate.

§Adjusted for age, sex, waist-height ratio, mean blood pressure, heart rate and left ventricular mass index.

¶Adjusted for age, sex, waist-height ratio, mean blood pressure, heart rate and left ventricular end diastolic volume

TABLE 4 Independent established confounder adjusted associations of arterial function measures and NT-proBNP levels with E/e' in non-dialysis and dialysis patients

	Chronic kidney disease patients					
	Non-dialysis			Dialysis		
	β (SE)	*p*	Model R^2	β (SE)	*p*	Model R^2
Pulse wave velocity	**0.533 (0.244)**	**0.03**		-0.045 (0.181)	0.8	
Log NT-proBNP level	1.236 (0.750)	0.1	**0.300**	**3.180 (1.129)**	**0.009**	**0.314**
Augmentation index	-0.010 (0.048)	0.8		-0.071 (0.069)	0.3	
Log NT-proBNP level	0.627 (0.732)	0.4	0.243	2.465 (1.248)	0.05	0.263
Log reflected wave pressure	7.677 (5.073)	0.1		2.576 (5.997)	0.7	
Log NT-proBNP level	0.276 (0.710)	0.7	0.290	2.457 (1.267)	0.06	0.239
Reflection magnitude	-0.011 (0.047)	0.8		-0.075 (0.068)	0.3	
Log NT-proBNP level	0.629 (0.733)	0.4	0.243	2.449 (1.244)	0.05	0.267
Central systolic BP	**0.207 (0.082)**	**0.01**		0.064 (0.079)	0.4	
Log NT-proBNP level	1.027 (0.704)	0.1	**0.311**	2.504 (1.241)	0.05	0.248
Central pulse pressure	**0.136 (0.049)**	**0.008**		0.050 (0.062)	0.4	
Log NT-proBNP level	1.061 (0.688)	0.1	**0.328**	2.430 (1.237)	0.05	0.247
Peripheral pulse pressure	**0.107 (0.040)**	**0.01**		0.042 (0.045)	0.4	
Log NT-proBNP level	1.141 (0.687)	0.1	**0.320**	**2.662 (1.243)**	**0.04**	**0.252**
Forward wave pressure	**0.128 (0.061)**	**0.04**		0.100 (0.086)	0.3	
Log NT-proBNP level	0.533 (0.662)	0.4	**0.329**	2.474 (1.241)	0.05	0.270

Arterial function parameters and NT-pro-BNP levels were entered together in multivariable regression models adjusted for age, sex, race, waist-height ratio, mean arterial pressure and heart rate. NT-proBNP, N-terminal pro B-type natriuretic peptide; Log, logarithmically transformed; BP, blood pressure;
SE, standard error. Significant associations are shown in bold.

TABLE 5 Arterial function-E/e' relationships after additional adjustment for diabetes in non-dialysis patients and NT-proBNP levels-E/e' relationship after additional adjustment for haemoglobin concentrations in dialysis patients

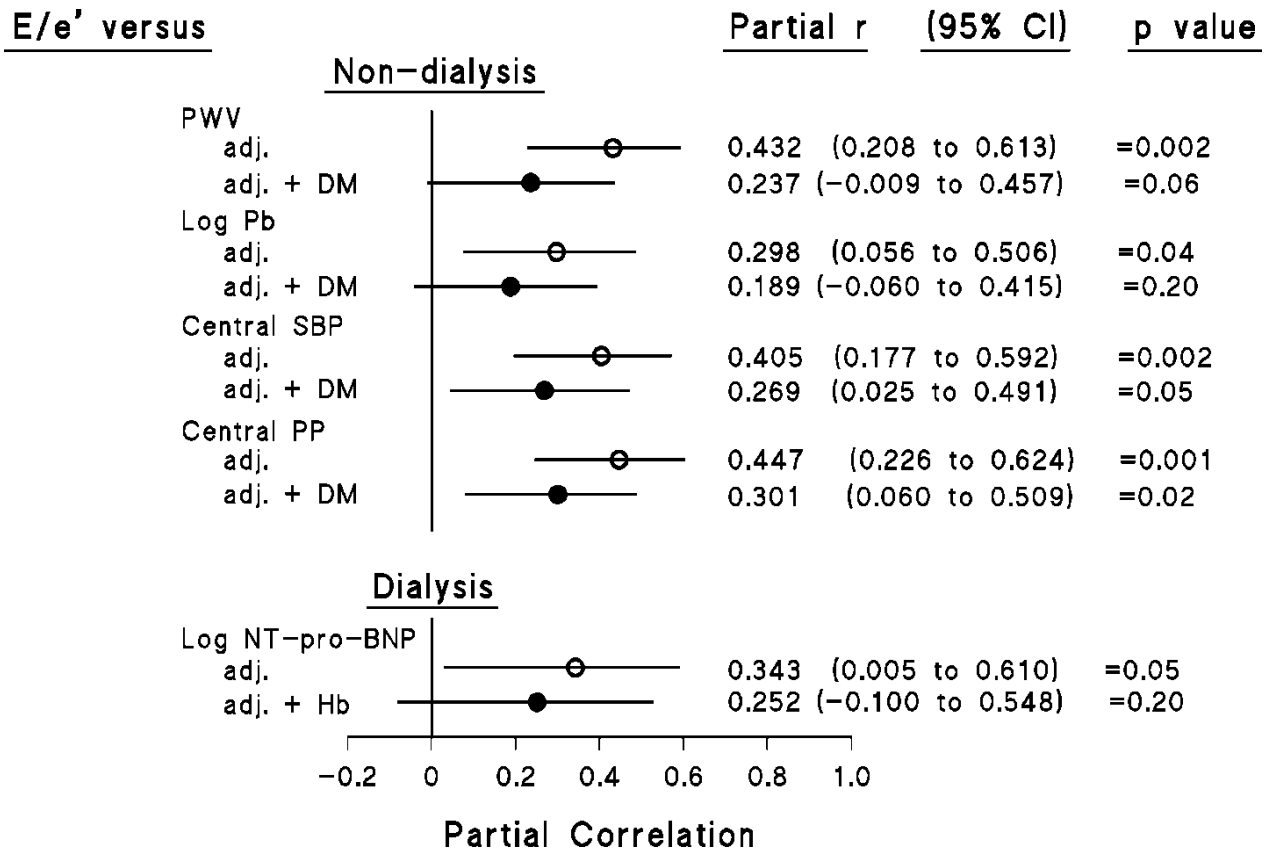
	Chronic kidney disease patients					
	Non-dialysis			Dialysis		
	β (SE)	p	Model R^2	β (SE)	p	Model R^2
Pulse wave velocity	0.384 (0.202)	0.06	0.415			
Augmentation index	-0.004 (0.032)	0.9	0.416			
Log reflected wave pressure	5.112 (3.854)	0.2	0.438			
Reflection magnitude	-0.005 (0.031)	0.9	0.321			
Central systolic BP	0.135 (0.067)	0.05	0.355			
Central pulse pressure	**0.096 (0.042)**	**0.02**	**0.442**			
Peripheral pulse pressure	0.068 (0.036)	0.06	0.425			
Forward wave pressure	0.077 (0.050)	0.1	0.446			
Log NT-pro-BNP level				1.760 (1.257)	0.2	0.291

Associations were assessed in age, sex, waist-height ratio, mean blood pressure and heart rate adjusted models. NT-proBNP, N-terminal pro B-type natriuretic peptide; Log, logarithmically transformed; BP, blood pressure; SE, standard error. Significant associations are shown in bold.

TABLE 6 Association of haemoglobin concentrations with E/e' in dialysis patients

	β (SE)	*p*	Model R^2
Haemoglobin concentration	**-0.877 (0.401)**	**0.03†**	**0.233**
	-0.896 (0.411)	**0.03‡**	**0.236**
	-1.061(0.432)	**0.02§**	**0.298**
	-1.011 (0.441)	**0.03¶**	**0.312**
	-0.990 (0.454)	**0.03Φ**	**0.241**

SE, standard error. Significant associations are shown in bold.
†Adjusted for age, sex, waist-height ratio, mean arterial pressure and heart rate.
‡Adjusted for age, sex, waist-height ratio, mean arterial pressure, heart rate and left ventricular mass index.
§Adjusted for age, sex, waist-height ratio, mean arterial pressure, heart rate, left ventricular end diastolic volume and central systolic blood pressure.
¶Adjusted for age, sex, waist-height ratio, heart rate, left ventricular end diastolic volume, central systolic blood pressure and log systemic vascular resistance.
ΦAdjusted for age, sex, waist-height ratio, heart rate, central systolic blood pressure and log systemic vascular resistance.


E/e' versus
Partial r
(95% CI)
p value
Non−dialysis
PWV
adj.
0.432 (0.208 to 0.613)
=0.002
adj. + DM
0.237 (−0.009 to 0.457)
=0.06
Log Pb
adj.
0.298 (0.056 to 0.506)
=0.04
adj. + DM
0.189 (−0.060 to 0.415)
=0.20
Central SBP
adj.
0.405 (0.177 to 0.592)
=0.002
adj. + DM
0.269 (0.025 to 0.491)
=0.05
Central PP
adj.
0.447 (0.226 to 0.624)
=0.001
adj. + DM
0.301 (0.060 to 0.509)
=0.02
Dialysis
Log NT−pro−BNP
adj.
0.343 (0.005 to 0.610)
=0.05
adj. + Hb
0.252 (−0.100 to 0.548)
=0.20
−0.2
0
0.2
0.4
0.6
0.8
1.0
Partial Correlation

SUPPORTING INFORMATION

METHODS

Cardiovascular risk factors

Cardiovascular risk factors were recorded using previously described methods.[1] Hypertension was identified when the systolic or/and diastolic blood pressure were ≥ 140mmHg and 90mmHg, respectively, or/and antihypertensive agents were used. Analyses were performed on fasting blood samples using routine laboratory tests for the determination of lipid, glucose, phosphate, calcium, haemoglobin, albumin, uric acid and ferritin concentrations, transferrin saturation percentages. Intact parathyroid hormone levels were measured by an electrochem-iluminescent immunoassay on Roche Cobas. High-sensitive C-reactive protein and vitamin D concentrations were both estimated on Abott Architect using an immunoturbidimetric assay and chemoluminescent microparticle immunoassay, respectively. Dyslipidemia was diag-nosed when the total cholesterol: HDL cholesterol ratio was >4 or/and lipid lowering drugs were employed. Diabetes was identified in patients with a fasting glucose level of $\geq$7 mmol/l and in those that used glucose lowering medications.

Arterial function

Arterial function was evaluated using previously described methods.[1] Pulse wave velocity, central aortic blood pressure and reflected and forward wave pressures were evaluated using a high-fidelity SPC-301 micromanometer (Millar instument, Inc., Houston, Texas), interfaced with a computer utilizing SphygmoCor software, version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Subsequent to resting for 15 minutes in the supine posi-tion, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded for a time period of ten consecutive waveforms (heart beats). Calibration of the pulse wave was done by manual measurement (auscultation) of brachial BP taken immediately prior to recordings. An extensively validated generalized transfer function incorporated in the SphygmoCor software was used to convert the peripheral pressure wave form into a central

aortic waveform.[2-4] The results were discarded when the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less that 80 mV. The aortic pulse wave velocity was calculated as distance in meters divided by transit time in seconds. The time delay in pulse waves between the carotid and femoral sites was assessed by employing an ECG-derived R wave as a fudicial point. Pulse transit time was calculated as the mean of 10 consecutive beats. The difference between the distance from the femoral sampling site to the suprasternal notch and the distance from the carotid sampling site to the suprasternal notch represented the distance the pulse wave travels. Magnitude of the forward and reflected wave components of the aortic pressure waveform was determined by the SphygmoCor software, which separates the aortic waveform by using a modified triangular waveform. Augmentation index was calculated as (second systolic peak/first systolic peak) x 100, reflection magnitude as (reflected wave amplitude/forward wave amplitude). All measurements were made by a single experienced observer who was unaware of the cardiovascular risk factor profiles of the patients.

Echocardiography

Echocardiography was performed as previously described.[5] Left ventricular dimensions were determined using two-dimensional directed M-mode echocardiography in the parasternal long axis view. Left ventricular dimensions were determined using two-dimensional directed M-mode echocardiography in the parasternal long axis view. Left ventricular end diastolic and systolic volumes were determined using the Teichholz method.[6] Stroke volume was determined from the difference between left ventricular end diastolic and systolic volumes as evaluated upon employing the Z-derived method. Cardiac output was determined as stroke volume x heart rate. Left ventricular mass was determined using a standard formula (left ventricular mass=0.8 x (1.04 (left ventricular end diastolic diameter + left ventricular septal wall thickness + left ventricular end diastolic posterior wall thickness)^3 – (left ventricular end diastolic diameter)^3 + 0.6) and indexed to body surface area.

Left ventricular diastolic function was assessed using pulsed Doppler and tissue Doppler imaging.[7] Transmitral flow patterns were recorded at the mitral valve leaflet tips using pulsed Doppler in the apical four chamber view. From the mitral valve inflow velocity curve, the early (E) and late (atrial-A) diastolic wave were measured and the ratio of early and late diastolic filling (E/A) was calculated. To perform tissue Doppler imaging, the velocity of myocardial tissue lengthening was measured by placing the curser at the septal and lateral corners of the mitral annulus in the apical four chamber view. To determine diastolic function using tissue Doppler imaging, the early diastolic mitral annulus motion (e') was recorded. Because mitral E is dependent on ventricular relaxation as well as left atrial driving forces (pressures), while e' is dependent on relaxation alone, E/e' ratio is considered to be an index of left ventricular filling pressures. The E/e' ratio was calculated as mitral E/the average of septal and lateral e'. Echocardiographic measurements were made by the same experienced observer that performed the arterial function measurements. The intra-observer variation for echocardiographic measurements is low in our setting.[5]

REFERENCES

1. Gunter S, Robinson C, Woodiwiss AJ, Norton GR, Hsu H-C, Solomon A, et al. Arterial wave reflection and subclinical atherosclerosis in rheumatoid arthritis. Clin Exp Rheumatol 2018;36(3):412-420.
2. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. Eur Heart J 1993 Feb;14(2):160-7.
3. Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure wave from. Hypertension 2001 Oct;38(4):932-937.
4. Gallagher D, Adji A, O'Rourke MF. Validation of the transfer function technique for generating central from peripheral upper limb pressure waveform. Am J Hypertens 2004 Nov;17(11 Pt 1):1059-67.
5. Mokotedi L, Gunter S, Robinson C, Norton GR, Woodiwiss AJ, Tsang L, et al. The impact of different criteria sets on the estimated prevalence and associated risk factors of diastolic dysfunction in rheumatoid arthritis. Int J Rheumatol 2017;2017:2323410. doi: 10.1155/2017/2323410.
6. Techholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. Am J Cardiol 1976 Jan;37(1):7-11.
7. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Evardsen T et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update form the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016 Dec;17(12):1321-60.

SUPPLEMENTARY TABLE 1 Impact of chronic kidney disease status (non-dialysis versus dialysis) on the pulse wave velocity-E/e' relationship in a multivariable regression model

Variable	β (SE)	t	Partial R	*p*
Constant	-8.425 (7.103)	-1.186		0.2
Age	0.037 (0.035)	1.062	0.117	0.3
Female sex	1.086 (0.966)	1.125	0.124	0.3
Waist-height ratio	0.951 (5.024)	0.189	0.021	0.9
Mean blood pressure	0.021 (0.037)	0.569	0.063	0.6
Heart rate	-0.048 (0.032)	-0.154	-0.164	0.1
Dialysis status	**9.323 (2.984)**	**3.124**	**0.328**	**0.002**
Pulse wave velocity	**1.266 (0.430)**	**2.943**	**0.311**	**0.004**
Dialysis status*pulse wave velocity	**-0.675 (0.256)**	**-2.631**	**-0.281**	**0.01**

Significant associations are shown in bold.

Study/Paper 3:

Study/Paper 3 shows that post transplantation anaemia and persistent secondary hyperparathyroidism are associated with E/e', and adiposity measures with e' and E/A in kidney disease transplant recipients.

Association of post transplantation anaemia and persistent secondary hyperparathyroidism with diastolic function in stable kidney transplant recipients

Subtitle: Diastolic function in kidney transplant recipients

Hon-Chun Hsu[1,2], Gavin R. Norton[1], Ferande Peters[1], Chanel Robinson[1], Noluntu Dlongolo[3], Ahmed Solomon[4], Gloria Teckie[5], Angela J. Woodiwiss[1], Patrick H. Dessein[1,6,7]

[1]Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa,

[2]Nephrology Unit, Milpark Hospital, Johannesburg, South Africa,

[3]Rheumatology Unit, Rosebank Hospital, Johannesburg, South Africa,

[4]Division of Rheumatology, Charlotte Maxeke Johannesburg Academic Hospital and Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa,

[5]Division of Nephrology, Department of Medicine, Chris Hani Baragwanath Hospital and Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa,

[6]Internal Medicine Department, University of the Witwatersrand, Johannesburg, South Africa,

[7]Internal Medicine Department, Free University and University Hospital, Brussels, Belgium

ORCID identifiers: Hon-Chun Hsu: https://orcid .org/0000-0003-4180-1552; Angela J. Woodiwiss: https://orcid.org/0000-0001-2555-4125; Chanel Robinson: https://orcid .org/0000-0002-9385-2709; Gavin R. Norton: https://orcid .org/0000-0001-6416-6104; Patrick H. Dessein: https://orcid .org/0000-0002-9357-4630

Correspondence: Patrick H Dessein

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; Internal Medicine Department, University of the Witwatersrand, Johannesburg, South Africa;

Internal Medicine Department, Free University and University Hospital, Brussels, Belgium

Tel +27(0)662491468

Postal address: 80 Scholtz Road, Norwood 2117, Johannesburg, South Africa

E-mail: patrick.dessein22@gmail.com

Abstract

Introduction: We hypothesized that post transplantation anaemia and persistent secondary hyperparathyroidism are potential determinants of diastolic function in stable kidney transplant recipients.

Methods: We assessed traditional and non-traditional cardiovascular risk factors and determined carotid artery intima-media thickness and plaque by ultrasound, arterial function by applanation tonometry using SphygmoCor software and diastolic function by echocardiography in 43 kidney transplant recipients with a transplant duration of $\geq$6 months, no acute rejection and a glomerular filtration rate of $\geq$15 ml/min/1.73m^2.

Results: Mean (SD; range) transplant duration was 12.3 (8.0; 0.5-33.8) years. Post transplantation anaemia and persistent secondary hyperparathyroidism were identified in 27.9% and 30.8% of the patients, respectively; 67.5% of participants were overweight or obese. In established confounder adjusted analysis, haemoglobin (partial R=-0.394, p=0.01) and parathyroid hormone concentrations (partial R=0.382, p=0.02) were associated with E/e'. In multivariable analysis, haemoglobin (partial R=-0.278, p=0.01) and parathyroid levels (partial R=0.324, p=0.04) were independently associated with E/e'. Waist-height ratio (partial R=-0.526, p=0.001 and partial R=-0.355, p=0.03), waist circumference (partial R=-0.433, p=0.008 and partial R=-0.393, p=0.02) and body mass index (partial R=-0.332, p=0.04 and partial R=-0.489, p=0.002) were associated with both e' and E/A, respectively, in established confounder adjusted analysis. The haemoglobin-E/e' (partial R=-0.422, p=0.02), parathyroid hormone-E/e' (partial R=0.434, p=0.03), waist-height ratio-e' (partial R=-0.497, p=0.007) and body mass index-E/A (partial R=-0.386, p=0.04) relationships remained consistent after additional adjustment for left ventricular mass index and cardiac preload and afterload measures.

Conclusions: Haemoglobin and parathyroid hormone concentrations as well as adiposity measures are independently associated with diastolic function in kidney transplant recipients. Whether adequate management of post transplantation anaemia, persistent secondary hyperparathyroidism and excess adiposity can prevent the development of heart failure with preserved ejection fraction in kidney transplant recipients merits further investigation.

Keywords: haemoglobin, parathyroid hormone, obesity, diastolic function, kidney transplantation

Introduction

Among patients with end-stage kidney disease (ESRD), kidney transplant recipients experience a markedly better quality of life and reduced mortality rates compared to those treated with ongoing dialysis.[1,2] Also, cardiovascular disease risk is increased 10 to 20 fold in dialysis patients as compared to 3 to 5 fold in kidney transplant recipients.[1]

Impaired diastolic function together with left ventricular hypertrophy and pronounced fibrosis comprise the most characteristic cardiac abnormalities in chronic kidney disease (CKD) patients.[3] Impaired diastolic function generally results from reduced left ventricular active relaxation and increased left ventricular stiffness.[3,4] Left ventricular stiffening is mediated by fibrosis and causes increased cardiac filling pressures through impaired passive relaxation. [3,4]

A functioning allograft in kidney transplant recipients can improve echocardiographically identified cardiac abnormalities[5] and stabilize those detected by cardiac magnetic resonance imaging.[6] Despite these potentially favourable effects of kidney transplantation on cardiac function and structure, ~18% of kidney transplant recipients develop *de novo* heart failure within 3 years subsequent to transplantation.[7] This is 2 to 5 times higher than that in population-based cohorts.[7] By contrast, de novo ischemic heart disease reportedly occurs at a similar frequency in kidney transplant recipients and the general population.[8] Kidney transplant recipients may be particularly prone to non-atherosclerotic cardiac disease.

Impaired diastolic function underlies the development of heart failure with preserved ejection fraction (HFpEF).[3,4] HFpEF is more prevalent and associated with larger mortality rates than heart failure with reduced ejection fraction (HFrEF) in patients with CKD.[9,10]

Hemodynamic factors that cause impaired diastolic function in CKD comprise increased preload due to volume overload and anaemia, and increased afterload mediated by heightened peripheral resistance and marked arteriosclerosis that results in arterial stiffness and enhanced wave reflection and pressure pulsatility.[3,11] Other reported determinants of impaired diastolic function in CKD patients encompass aberrant bone mineral metabolism including secondary hyperparathyroidism, inflammation and oxidative stress.[3,11]

Impaired diastolic function may be present in more than half of patients undergoing kidney

transplantation.[12] However, the determinants of diastolic function in particularly stable kidney transplant recipients (transplant duration of ≥6 months, absence of acute rejection and glomerular filtration rate of ≥15 ml/min/1.73m^2)[13] await elucidation. Importantly in the present context, late post-transplantation anaemia[14] and persistent secondary hyperparathyroidism[15] have been reported in up to 35% and 50% of kidney transplant recipients, respectively. Conceptually, anaemia and hyperparathyroidism could mediate impaired diastolic function in kidney transplant recipients. In this regard, anaemia not only increases cardiac preload and left ventricular mass but can also cause tissue hypoxia with consequent myocardial fibrosis.[16-20] Secondary hyperparathyroidism can increase cardiac afterload through enhancing arteriosclerosis[1,11] and directly induce cardiac hypertrophy and myocardial fibrosis.[21]

In this study, we hypothesized that post transplantation anaemia and persistent secondary hyperparathyroidism are potential determinants of diastolic function in stable kidney transplant recipients.

Patients and Methods

Patients

The study was performed in line with the Helsinki Declaration as revised in 2013. All organs were donated voluntarily with written informed consent, which was conducted in accordance with the Declaration of Istanbul.[23] The University of the Witwatersrand Human (Medical) Research Ethics Committee (protocol number: M15-08-43) in Johannesburg, South Africa approved the study protocol. Written informed consent was obtained in each patient prior to participation. Forty three consecutive stable kidney transplant recipients were enrolled as outpatients at the Milpark Hospital in Johannesburg, South Africa. Mean (SD; range) transplant duration was 12.3 (8.0; 0.5 to 33.8) years. Median (IQR) duration of dialysis prior to kidney transplantation was 2.0 (0.5-4.6) years and 4 (9.3%) patients were not dialysed prior to receiving a kidney transplant. Only 2 (4.7%) patients had a patent arteriovenous fistula. Patients with a transplant duration of <6 months, acute rejection, an estimated glomerular filtration rate[22] of ≤15 ml/min/1.73m^2, previously diagnosed heart failure, myocardial infarction,

active infection or/and cancer were excluded. The criteria selected for stable kidney transplant status were based on a previously reported study by Ducloux and colleagues.[13]

Methods

Patient characteristics that were recorded included demographic variables, traditional and non-traditional cardiovascular risk factors, carotid atherosclerosis measures, arterial function, echocardiographic parameters and systemic vascular resistance. All investigations were carried out on a single day at the time of the study.

Traditional and non-traditional or renal cardiovascular risk factors were recorded as previously reported[24] and provided in the online Supplementary Methods. Intact parathyroid hormone levels were determined by an electrochemiluminescence immunoassay "ECLIA" on Cobas (Roche Diagnostics, Mannheim, Germany). This assay employs a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1-37) and a monoclonal antibody labelled with a ruthenium complex[a)] reacts with the C-terminal fragment (38-84). The antibodies used in this assay are reactive with epitopes in the amino acid region 26-32 and 37-42. The measurement range is 1.2 to 5000 pg/ml. The intra-assay and inter-assay coefficients of variation are each <3.5%. Vitamin D concentrations were measured using the Alinity i 25-OH Vitamin D assay (Abbott Ireland Diagnostics Division Lisnamuck, Longford, Ireland), which is a chemiluminescent microparticle immunoassay that quantifies 25-hydroxyvitamin D on the Alinity I Analyser. The measurement range is 3.5 to 154.2 ng/ml. The intra-assay and inter-assay coefficients of variation are each <4.6%. Mean or distending arterial blood pressure for the peripheral waveform was determined electronically by the SphygmoCor device (see below) and using the formula

$$MP = \frac{\sum_{i=T_0}^{T_F} P_i}{n}$$

where T_0=start of the waveform; T_F=end of waveform; P_i=pressure points and n=number of pressure points.

Atherosclerosis was assessed by carotid artery ultrasound employing a Philips CX50 POC Compact CompactXtreme Ultrasound System (Philips Medical Systems (Pty) Ltd, USA) attached to a linear array 4.0-12.0 MHz probe. The software provided for semi-automated border detection gives markedly reproducible data as previously described[21]. Images of at least 1cm length of the distal common carotid arteries were obtained. The optimal angle of incidence was used, defined as the longitudinal angle of approach where both branches of the internal and external carotid artery were visualized simultaneously. The carotid intima-media thickness (c-IMT) was defined as the mean of the left and right common carotid artery thickness. Plaque in the extracranial carotid tree was defined according to the Mannheim consensus criteria[25]. Carotid ultrasound measurements were made by the same observer that performed the arterial function and echocardiographic evaluations (CR). The intra-observer variability of ultrasound measurements is low in our setting[24].

Central hemodynamic characteristics were determined by applanation tonometry and SphygmoCor software as previously reported[24] and provided in the online Supplementary Material. Aortic pulsewave velocity, augmentation index, reflected wave pressure and reflection magnitude, central systolic and pulse pressure, peripheral pulse pressure, pulse pressure amplification and forward wave pressure were measured.

NT-proBNP was determined by an electrochemiluminescence immunoassay on Cobas (Roche Diagnostics, Mannheim, Germany). The measurement range is 10 to 35000 pg/ml. The intra-assay and inter-assay coefficients of variation are 3.0% and 4.8%, respectively.

Echocardiography was performed in accordance with the American Society of Echocardiography convention[26] and employing a Philips CX50 POC Compact CompactXtreme Ultrasound System (Philips Medical Systems (Pty) Ltd, USA) equipped with a 1.8-4.2 MHz probe that allowed for M-mode, 2-D, pulsed and tissue Doppler measurements. We evaluated geometric characteristics, left ventricular systolic function and diastolic function variables including the

early (E)/late (atrial) diastolic wave (A) ratio, the peak mitral annulus motion during early diastole (e') and E/e' ratio. E/A and e' are markers of active left ventricular relaxation, and E/e' is an index of left ventricular filling pressure.[4,27] Our methodology was previously described[27] and is provided in the online Supplementary Material.

Systemic vascular resistance was calculated from mean arterial pressure, right atrial pressure and cardiac output according to the equation (mean arterial pressure – right atrial pressure)=systemic vascular resistance x cardiac output assuming that right atrial pressure=0 mmHg.

Data analysis

Results are given as mean (SD), median (interquartile range) or number (percentages) as appropriate. Non-normally distributed variables were logarithmically transformed prior to entering them in multivariate linear regression models.

The associations of cardiovascular risk factors with E/e', e' and E/A were first assessed in age, sex and race adjusted models. This was followed by established confounder (age, sex, race, weight, height, mean blood pressure and heart rate)[24] adjusted analysis; when anthropometric characteristic-diastolic function relationships were evaluated, weight and height were not included as potential established confounders in the models. Associations were then reassessed in multivariate models in which potential determinants as identified in the previous models were entered simultaneously.

Subsequently, we assessed whether cardiovascular risk factor-diastolic function relationships were explained by left ventricular mass, cardiac preload (left ventricular end diastolic volume)[28] or/and cardiac afterload (central systolic blood pressure[29] and systemic vascular resistance[30]) by adding the respective variables to the models.

Statistical analysis was performed on IBM SPSS statistics program (version 23.0 IBM, USA). Significance was set at $p<0.05$.

Results

Patient characteristics

The main recorded patient characteristics are given in Table 1. Mean (SD) age was 53.5 (10.8) years, approximately one third were women and black patients comprised the largest proportion (46.52%) of enrolled study subjects. Mean body mass index (BMI) was in the overweight range (26.9%); overweight (BMI=25-29.9 kg/m^2) and obesity (BMI >30 kg/m^2) were present in 19 (44.2%) and 10 (23.3%) patients, respectively. Hypertension, dyslipidemia and diabetes were present in 86.0%, 83.7% and 25.6% of participants, respectively. Antihypertensive agents, lipid lowering drugs (statins with or without ezetimibe), insulin therapy and oral glucose lowering medications were employed in 37 (86.0%), 33 (76.7%), 6 (14.0%) and 5 (11.6%) of patients, respectively.

Mean (SD) estimated glomerular filtration rate[22] was 63 (22) ml/min/1.73m^2; glomerular filtration rate ranged from 22 to 104 ml/min/1.73m^2. Median (IQR) intact parathyroid hormone levels were 68.0 (49.0-155.6) pg/ml; 21 (48.8%) patients had raised intact parathyroid concentrations (>65 pg/ml), and among those with a time since transplantation of more than 1 year (n=39), the respective levels were >130 pg/ml in 12 (30.8%), which is in keeping with persistent secondary hyperparathyroidism[15]. Vitamin D, calcium and phosphate supplementation was used in 15 (34.8%), 3 (7%) and 1 (2.3%) of the participants.

While mean haemoglobin concentrations (13.9 g/dl) were in the normal range, 12 (27.9 %) patients had anaemia (haemoglobin <12 g/dl in women and <13 g/dl in men)[14], which was severe (haemoglobin <11g/dl)[14] in 4 (9.4%) of study participants. None of the patients were treated with intravenous or oral iron, or erythropoietin stimulating agents.

Mean (IQR) carotid intima-media thickness was 0.583 (0.495-0.706) and plaque was found in less than one third of the participants (n=13 (30.2%)).

Mean (SD) pulse wave velocity was 9.9 (3.0) m/sec; mean (SD) augmentation index, reflection magnitude and pulse pressure amplification were 62.1 (17.0)%, 63.0 (17.4)%, 135 (19), respectively; median (IQR) central pulse pressure was 40 (33-45) mmHg.

Median (IQR) NT-proBNP concentrations were 188 (33-400) pg/ml.

Mean (SD) E/e' was 9.4 (3.9) whereas an E/e' of >14 that is in keeping with diastolic dysfunction,[4,9] was present in 3 (7%) of participants. Ejection fraction was <50% in 4 (9.3) patients of which only one (2.3%) had an ejection fraction of <40%.

Employed immunosuppressive therapy comprised glucocorticoids (n=41 (95.3%)), mycophenolate mofetil (n=31 (72.1%)), calcineurin (n=30 (69.8%)) and mTOR (n=14 (32.6%)) inhibitors and azathioprine (n=8 (18.6%)).

Associations of cardiovascular risk factors with E/e', e' and E/A

Table 2 shows the associations of cardiovascular risk factors with E/e'. In age, sex and race adjusted analysis, estimated GFR and haemoglobin and intact parathyroid hormone concentrations were associated with E/e' (model 1). Upon further adjustment for established confounders in the present context, only haemoglobin and intact parathyroid hormone levels remained related to E/e' (model 2). Subsequently, EGFR and haemoglobin concentrations were entered together with intact parathyroid hormone levels in separate multivariate models (models 3 and 4) as the former two variables were strongly collinear (Spearman's rho=0.525). Only haemoglobin and intact parathyroid hormone concentrations were significantly or with borderline significance (p=0.08) associated with E/e'. When all three cardiovascular risk factors were entered simultaneously in a stepwise regression model (model 5), haemoglobin and intact parathyroid hormone levels were associated with E/e' whereas estimated glomerular filtration rate was excluded from the model. Sex was also retained with a *P* value of 0.02 by SPSS in Model 5.

Table 3 gives the associations of cardiovascular risk factors with e' and E/A. Three anthropometric characteristics including waist-height ratio, waist circumference and body mass index were associated with both e' and E/A in age, sex and race (model 1) as well as in established confounder (model 2) adjusted analysis. Waist-hip ratio was not associated with e' and E/A (partial R=-0.178, *P*=0.3 and partial R=-0.197, *P*=0.2, respectively, in age, sex and race adjusted models).

Measures of atherosclerosis and arterial function, NT-proBNP concentrations and cardiovascular drug use, vitamin D, calcium and phosphate supplementation as well as immunosuppressive agent use were not related to E/e', e' and E/A (data not shown).

Associations of haemoglobin and intact parathyroid levels and anthropometric measures with diastolic function parameters after additional adjustment for left ventricular mass index and preload and afterload measures

Table 4 shows the associations of haemoglobin and intact parathyroid hormone concentrations with E/e' as well as those of anthropometric measures with e' and E/A after additional adjustment (to established confounders) for left ventricular mass index (Model 1), cardiac preload as estimated by left ventricular end diastolic volume (Model 2), cardiac afterload as estimated by central systolic blood pressure and systemic vascular resistance (Model 3) and left ventricular mass index together with left ventricular end diastolic volume, central systolic blood pressure and systemic vascular resistance (Model 4). The cardiovascular risk factor-diastolic function relationships were consistent in each of the respective models.

Impact of time since transplantation on the associations of haemoglobin and intact parathyroid levels and anthropometric measures with diastolic function parameters

In this study, there was heterogeneity in terms of transplant duration as it ranged from 0.5 to as much as 33.8 years. The median transplant duration was 13.28 years. To determine whether this can affect the generalizability of our results, we first compared the relevant recorded characteristics in patients with a transplant duration of <13.3 years compared to $\geq$13.3 years. We then assessed the impact of transplant duration on the associations of haemoglobin and intact parathyroid levels and anthropometric measures with diastolic function parameters by adding interaction terms (together with their individual components) to the age, sex and race adjusted models in Tables 2 and 3 and performing stratified analysis.

As given in Table 5, in age, sex and race adjusted analysis, compared to patients with a transplant duration of <13.3 years, those with a transplant duration of $\geq$13.3 years were more

frequently female and less often Asian, and had less abdominal adiposity, lower heart rates and larger haemoglobin levels and central systolic blood pressures. Intact parathyroid hormone concentrations also tended to be lower (p=0.07) in patients with a transplant duration of ≥13.3 years compared to <13.3 years. The estimated glomerular filtration rate was 61.1 ml/min/1.73m^2 and 65.1ml/min/1.73m^2 (p=0.5) in patients with a transplant duration of ≥13.3 years compared to <13.3 years.

Transplant duration did not impact the estimated glomerular filtration rate-E/e' (interaction p=0.6), haemoglobin-E/e' (interaction p=0.2) and intact parathyroid hormone-E/e' (interaction p=0.5) relationships. However, transplantation duration did impact the waist-height-e' association (interaction p=0.03). Transplant duration did not significantly impact other anthropometric measure-e' and anthropometric-E/A relationships (interaction p=0.1 to 0.8).

As shown in Supplementary Table 1, in line with the interaction analysis results and in age, sex and race adjusted models, the associations of estimated glomerular filtration rate and haemoglobin and intact parathyroid hormone levels with E/e' appeared similar in patients with a transplant duration of <13.3 years compared to ≥13.3 years. None of these associations were significant, this presumably because of the small number of patients in both groups. Further adjustment for other potential confounders (see Table 2) did not alter these results (data not shown).

As given in Tables 6 and 7 and also in keeping with the interaction analysis results, significant anthropometric measure-e' and, to a lesser extent, anthropometric-E/A relationships were found in patients with a transplant duration of ≥13.3 years but not <13.3 years.

Discussion

To our knowledge, this is the first study that evaluated potential determinants of diastolic function in stable kidney transplant participants. Our main findings were as follows: (1) haemoglobin concentrations were inversely associated with E/e' whereas intact parathyroid hormone levels were directly related to E/e'; (2) anthropometric measures including waist-height ratio, waist circumference and body mass index were each associated with both e' and E/A; (3) the

haemoglobin-E/e', intact parathyroid hormone-E/e', anthropometric measure-e' and anthropometric measure-E/A relationships were each independent of left ventricular mass index and cardiac preload and afterload measures and (4) estimated glomerular filtration rate was associated with E/e' but this relationship was no longer significant after adjustment for established confounders as well as haemoglobin and intact parathyroid hormone levels.

Post transplantation anaemia was previously reported to be associated with all-cause mortality, graft loss and heart failure.[14,31] Our current findings suggest that post transplantation anaemia may contribute to heart failure through increasing left ventricular stiffness and pressures due to impaired left ventricular passive relaxation.[3,4,27] Anaemia is associated with left ventricular hypertrophy, which is a strong independent predictor of cardiovascular mortality in CKD patients.[3] However, impaired diastolic function predates left ventricular hypertrophy in CKD.[3,32] This argues against an important role of left ventricular hypertrophy in impaired diastolic function. Our finding that post transplantation anaemia was associated with E/e' independent of left ventricular mass supports this notion. Moreover, abnormal left ventricular geometry as represented by hypertrophy and concentric remodelling was present in only 15 (34.9%) patients. This may have been consequential to the selection of patients with favourable cardiovascular disease risk profiles for transplantation, the consistent use of antihypertensive agents with overall adequate blood pressure control and post transplantation regression of left ventricular hypertrophy.[5] Anaemia also remained significantly associated with E/e' when we adjusted for cardiac preload and afterload measures. Our results therefore indicate that post transplantation anaemia may mediate impaired diastolic function through cardiomyocyte hypoxia and consequent cardiac fibrosis.[16-20]

Our finding that 27.9% of our patients had post transplantation anaemia is in keeping with previously reported findings.[14] The causes of anaemia differ somewhat in kidney transplant recipients compared to dialysis patients and include graft rejection and immunosuppressant therapy.[14] Based on a randomized trial and a large observational study, Gafter-Gvili and colleagues[14] recently recommended targeting a haemoglobin of 12.5 to 13g/dl in kidney transplant recipients with appropriate erythropoietin stimulating agent and iron therapy.

None of our patients received iron or erythropoietin stimulating agent therapy. In this regard, in the STRESAM study,[33] only 5 % of patients received treatment with an erythropoietin stimulating agent. Taken together, post transplantation anaemia comprises a comorbidity that may be in need of more systematic management[14] than is currently practised. Whether this can improve cardiovascular outcomes in kidney transplant recipients requires further study.

Increased parathyroid hormone concentrations are associated with cardiovascular events and mortality in CKD and non-CKD patients.[21] However, the role of high parathyroid hormone levels as a cardiovascular risk factor in stable kidney transplant recipients has not been reported thus far. In this study, 30.8% of our patients had persistent secondary hyperparathyroidism, which is also in line with previous reports.[15] We found that, as applied to haemoglobin concentrations, parathyroid levels were associated with E/e' independent of established confounders, left ventricular hypertrophy and cardiac preload and afterload measures in kidney transplant recipients. In this regard, experimental studies using rat cardiomyocytes have shown that parathyroid hormone can cause mitochondrial Ca2+ excess that results in oxidative stress, necrotic cell death and consequent fibrosis.[21] In another study, parathyroidectomy prevented the development of myocardial hypertrophy, fibrosis and apoptosis in 5/6 nephrectomised rats.[34] Also, the use of the calcimimetic agent cinacalcet in haemodialysis patients for 20 weeks decreased not only parathyroid hormone, calcium and phosphate levels but also the left ventricular mass index and E/e' ratio.[35] These reported data together with our current findings indicate that the impact of persistent hyperparathyroidism and its adequate management[15] on cardiovascular outcomes in kidney transplant recipients also deserves further study.

Even a fully functional kidney allograft does not entirely restore the glomerular filtration rate.[1] A reduction in glomerular filtration rate is reportedly an independent risk factor for cardiovascular disease and mortality in kidney transplant recipients.[1] In the present study, an age, sex and race adjusted association was found between the estimated glomerular filtration rate and E/e' but this relationship was explained by established confounders and parathyroid hormone and haemoglobin concentrations.

Excess adiposity is prevalent and its association with heart failure was previously identified in kidney transplant recipients.[1, 31] Overweight or obesity was present in 67.5% of our patients. Except for waist-hip ratio, adiposity measures were independently associated with e' and E/A. Excess adiposity is also associated with impaired left ventricular active relaxation in non-CKD persons[4,36,37] and can be reversed by weight loss.[38] Dialysis duration prior to kidney transplantation comprises another reported risk factor for heart failure in kidney transplant recipients.[31] In this study, dialysis duration prior to kidney transplantation was not associated with E/e', e' or E/A (data not shown).

In the present investigation, the heterogeneity in relation to transplant duration called for performing a stratified analysis. In patients with a prolonged transplant duration (≥13.3 years), haemoglobin concentrations were larger, intact parathyroid hormone concentrations tended to be smaller and abdominal adiposity was reduced compared to those with a shorter transplant duration. Conceptually, this may represent an improvement of the respective cardiovascular risk factors over time after transplantation. Alternatively, our respective results may originate in a selection bias due to an overall better survival among patients with a prolonged compared to shorter transplant duration. Indeed, anaemia, bone mineral disorder and excess adiposity can increase cardiovascular disease and mortality in kidney transplant patients.[39,40] More importantly, our interaction analysis results indicated that transplant duration did not influence the potential impact of haemoglobin and parathyroid hormone levels on E/e'. By contrast, abdominal adiposity was inversely associated with measures of left ventricular relaxation in patients with a prolonged but not shorter transplant duration. This may suggest that exposure to excess adiposity may need to be prolonged for it to reduce left ventricular relaxation in kidney transplant recipients. Taken together, the role of kidney transplant duration on cardiovascular risk requires further study in future larger and longitudinal investigations.

Our study has limitations. Firstly, the study design was cross-sectional, which precludes determining cause-effect relationships. Secondly, as applies to previously reported investigations by us,[37,41,42] we assessed the associations of cardiovascular risk factors with E/e', e' and E/A as continuous variables, which requires inclusion of both high and low values. As only 3

(7%) patients had an E/e' of >14 that is in keeping with diastolic dysfunction,[4,9] the number of included patients was too small to reliably assess associations of cardiovascular risk factors with an increased E/e' in adequately adjusted logistic regression models. Thirdly, as we did not determine atrial volume index and tricuspid regurgitation velocity, we could not apply the currently suggested algorithm for the detection of diastolic dysfunction[4] in this patient population. However, given that at a mean age of 53.5 years, 86% of our patients were hypertensive and 25.6% had diabetes, it is highly likely that a substantial proportion of them had diastolic dysfunction. Fourthly, haemoglobin concentrations were assessed on one occasion only, i.e. on the same day that other measurements were made. Anaemia may need to be present for prolonged time periods in order for myocardial fibrosis to develop. We may therefore have underestimated the effect of low haemoglobin concentrations on diastolic function. Fifthly, all participants were enrolled at a single centre. Sixthly, the number of patients included was relatively small, particularly in the analysis stratified by transplantation duration. A strength of this investigation is that our conclusions originate in multivariable regression models in which we consistently adjusted for potential established confounders.

In conclusion, haemoglobin and parathyroid hormone concentrations as well as adiposity measures are independently associated with diastolic function in kidney transplant recipients. Whether adequate management of post transplantation anaemia,[14] persistent secondary hyperparathyroidism[15] and excess adiposity[1] can prevent the development of heart failure with preserved ejection fraction in kidney transplant recipients merits further investigation.

Ethics statement

This study was performed according to the Helsinki Declaration of 1975 as revised in 2013. All organs were donated voluntarily with written informed consent, which was conducted in accordance with the Declaration of Istanbul. The study was approved by the University of the Witwatersrand Human (Medical) Research Ethics Committee (protocol number: M15-08-43) in Johannesburg, South Africa. Written informed consent was obtained in each patient prior to participation.

Author contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

The study was supported by the South African National Research Foundation.

Disclosure

The authors declare no conflict of interest.

References

1. Devine PA, Courtney AE, Maxwell AP. Cardiovascular risk in renal transplant recipients. J Nephrol 2019;32(3):389-399.
2. Sarier M, Ozen NS, Guler H, Duman I, Yuksel Y, Tekin S, et al. Prevalence of sexually transmitted diseases in asymptomatic renal transplant recipients. Exp Clin Transplant. 2018. PMID: 29619908.
3. Wang X, Shapiro JI. Evolving concepts in the pathogenesis of uraemic cardiomyopathy. Nat Rev Nephrol 2019;15(3):159-175.
4. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016;29(4):277-310.
5. Hawwa N, Shrestha K, Hammadah M, Yeo PSD, Fatica R, Tang WHW. Reverse remodelling and prognosis in contemporary patients with cardiac dysfunction. J Am Coll Cardiol 2015;66(16):1779-1787.
6. Patel RK, Mark PB, Johnston N, McGregor E, Dargie HJ, Jardine AG. Renal transplantation is not associated with regression of left ventricular hypertrophy: a magnetic resonance study. Clin J Am Soc Nephrol 2008;3(6):1807-1811.
7. Lentine KL, Schnitzler MA, Abbott KC, Li L, Burroughs TE, Irish W, et al. De novo congestive heart failure after kidney transplantation: a common condition with poor prognostic implications. Am J Kidney Dis 2005;46(4):720-733.
8. Rigatto C, Parfrey P, Foley R, Negrijn C, Tribula C, Jeffrey J. Congestive heart failure in renal transplant recipients: risk factors, outcomes, and relationships with ischemic heart disease. J Am Soc Nephrol 2002;13(4):1084-1090.
9. Kim MK , Kim B, Lee JY, Kim JS, Han B-G, Choi SO, et al. Tissue Doppler-derived E/e' ratio as a parameter for assessing diastolic heart failure and as a predictor of mortality in patients with chronic kidney disease. Korean J Intern Med 2013;28(1):35-44.

10. Ahmed A, Rich MW, Sanders PW, Perry GJ, Bakris GL, Zile MR, et al. Chronic kidney disease associated mortality in diastolic versus systolic heart failure: a propensity matched study. Am J Cardiol 2007;99(3):393-8.
11. Escoli R, Carvalho MJ, Cabrita A, Rodrigues A. Diastolic dysfunction, an underestimated new challenge in dialysis. Ther Apher Dial 2019;23(2):108-117.
12. Himelman RB, Landzberg JS, Simonson JS, Amend W, Bouchard A, Merz R, et al. Cardiovascular consequences of renal transplantation: changes in left ventricular morphology and function. J Am Coll Cardiol 1988:12(4):915-923.
13. Ducloux D, Motte G, Challier R, Gibey R, Chalopin J-M. Serum homocysteine total and cardiovascular disease occurrence in chronic, stable renal transplant recipients: a prospective study. J Am Soc Nephrol 2000;11:134-137.
14. Gafter-Gvili A, Gafter U. Posttransplantation anemia in kidney transplant recipients. Acta Haematol 2019;142(1):37-43.
15. Santos RD, Rossi A, Coyne D, Maw TT. Management of post-transplant hyperparathyroidism and bone disease. Drugs 2019;79(5):501-513.
16. Mistry N, Mazer CD, Sled JG, Lazarus AH, Cahill LS, et al. Red blood cell antibody-induced anemia causes differential degrees of tissue hypoxia in kidney and brain. Am J Physiol Reg Integr Comp Physiol 2018;314:R611-R622.
17. Caramelo C, Justo S, Gil P. Anemia in heart failure: pathophysiology, pathogenesis, treatment, and incognitae. Rev Esp Cardiol 2007;60:848-860.
18. D'Amario D, Migliaro S, Borovac JA, Restivo A, Vergallo R, Galli M, et al. Microvascular dysfunction in heart failure with preserved ejection fraction. Front Physiol 2019;10:1347. doi: 10.3389/fphys.2019.01347. eCollection 2019.
19. Lopez B, Gonzalez A, Hermida N, Laviades C, Diez J. Myocardial fibrosis in chronic kidney disease: potential benefits of torasemide. Kidney Int 2008;Suppl 111:S19-S23.
20. Losi MA, Memoli B, Contaldi C, Barbati G, Del Prete M, Betocchi S, et al. Myocardial fibrosis and diastolic dysfunction in patients on chronic haemodialysis. Nephrol Dial Transplant 2010;25:1950-1954.

21. Fujii H. Association between parathyroid hormone and cardiovascular disease. Ther Apher Dial 2018;22(3):236-241.
22. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro 3rd AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150(9):604-12.
23. International Summit on Transplant Tourism and Organ Trafficking. The Declaration of Istanbul on Organ Trafficking and Transplant Tourism. Clin J Am Soc Nephrol 2008;3:1227-1231.
24. Gunter S, Robinson C, Woodiwiss AJ, Norton GR, Hsu H-C, Solomon A, et al. Arterial wave reflection and subclinical atherosclerosis in rheumatoid arthritis. Clin Exp Rheumatol 2018;36(3):412-20.
25. Touboul PJ, Hennerici MJ, Meairs S, et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis 2007;23(1):75-80.
26. Sahn DJ, De Maria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58(6):1072-83.
27. Mokotedi L, Gunter S, Robinson C, Norton GR, Woodiwiss AJ, Tsang L, et al. The impact of different criteria sets on the estimated prevalence and associated risk factors of diastolic dysfunction in rheumatoid arthritis. Int J Rheumatol 2017;2017:2323410. doi: 10.1155/2017/2323410.
28. Kapoor KPM, Bhardwaj V, Sharma A, Kiran U. Global end-diastolic volume an emerging preload marker vis-à-vis other markers – have we reached our goal? Ann Card Anaesth 2016;19:699-704.
29. Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, et al. Central blood pressure measurements and antihypertensive therapy. Hypertension 2007;50:154-160.

30. Collins S, Martindale J. Optimizing hypertensive acute heart failure management with afterload reduction. Curr Hypertens Rep 2018;20:9.
31. House AA, Wanner C, Sarnak MJ, Pina IL, McIntyre W, Komenda P, et al. Heart failure in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int 2019;95(6):1304-1317.
32. Winterberg PD, Jiang R, Maxwell JT, Wang B, Wagner MB. Myocardial dysfunction occurs prior to changes in ventricular geometry in mice with chronic kidney disease (CKD). Physiol Rep 2016;4(5):e12732.
33. Vanrenterghem Y, Ponticelli C, Morales JM, et al. Prevalence and magement of anemia in renal transplant recipients: a European survey. Am J Transplant 2003;3(7):835-845.
34. Rodriguez-Ayala E, Avila-Diaz M, Foyo-Niembro E, Amato D, Ramirez-San-Juan E, Paniagua R. Effect of parathyroidectomy on cardiac fibrosis and apoptosis: possible role of aldosterone. Nephron Physiol 2006;103(3):112-118.
35. Choi SR, Lim JH, Kim MY, Hong Y-A, Chung BH, Chung S, et al. Cinacalcet improves endothelial dysfunction and cardiac hypertrophy in patients on hemodialysis with secondary hyperparathyroidism. Nephron Clin Pract 2012;122(1-2):1-8.
36. Russo C, Jin Z, Homma S, Rundek T, Elkind MSV, Sacco RL et al. Effect of obesity and overweight on left ventricular diastolic function: a community-based study in an elderly cohort. J Am Coll Cardiol 2011;57(12):1368-1374.
37. Libhaber CD, Norton GR, Majane OHI, Libhaber E, Essop MR, Brooksbank R, et al. Contribution of central and general adiposity to abnormal left ventricular diastolic function in a community sample with high prevalence of obesity. Am J Cardiol 2009;104(11):1527-1233.
38. Leichman J, Wilson EB, Scarborough T, Agullar D, Miller III CC, Yu S, et al. Dramatic reversal of derangements in muscle metabolism and diastolic left ventricular function after bariatric surgery. Am J Med 2008;121(11):966-973.

39. Carminatti M, Tedesco-Silva H, Fernandes NMS, Sanders-Pinheiro H. Chronic kidney disease progression in kidney transplant recipients: a focus on traditional risk factors. Nephrology 2019;24:141-147.
40. Erturk T, Berber I, Cakir U. Effect of obesity on clinical outcomes of kidney transplant patients. Transplant Proc 2019;51:1093-1095.
41. Bamaiyi AJ, Norton GR, Peterson V, Libhaber CD, Sarelli P, Woodiwiss AJ. Limited contribution of left ventricular mass and remodelling to the impact of blood pressure on diastolic function in a community sample. J Hypertens 2019;37(6):1191-9.
42. Bello H, Norton GR, Peterson VR, Mmopi KN, Mthembu N, Libhaber CD, et al. Hemodynamic determinants of age versus left ventricular diastolic function relations across the full adult age range. Hypertension 2020;75:1574-1583.

Table 1 Main recorded patient characteristics in 43 kidney transplant recipients

Demographics		High-sensitivity CRP (mg/l)	3.4 (1.7-6.9)
Age (years)	53.5 (10.8)	Phosphate (mmol/l)	1.0 (0.8-1.2)
Female sex	14 (32.6)	Calcium (mmol/l)	2.3 (2.2-2.4)
Black	20 (46.52)	IPTH (pg/ml)	68.0 (49.0-155.6)
Asian	11 (25.58)	Haemoglobin (g/dl)	13.9 (2.4)
Mixed	6 (13.95)	Transferrin saturation (%)	22.7 (10.1)
White	6 (13.63)	Ferritin (ng/ml)	181 (59-302)
Traditional CV risk factors		Albumin (g/l)	38.5 (5.1)
Lifestyle factors		Vitamin D (ng/l)	23.0 (9.4)
Current smoker	0 (0)	Uric acid (mmol/l)	0.33 (0.29-0.45)
Ex-smoker	1 (2.3)	**Arterial function**	
Anthropometry		Pulse wave velocity (m/sec)	9.9 (3.0)
Body mass index (kg/m^2)	26.9 (4.4)	Reflected wave pressure (mmHg)	18.7 (14.0-22.5)
Waist (cm)	98 (13)	Central systolic BP (mmHg)	128 (117-134)
Waist-hip ratio	0.97 (0.13)	**NT-proBNP (pg/ml)**	188 (33-400)
Weight (kg)	78.7 (17.0)	**Echocardiographic characteristics**	
Height (m)	1.70 (0.11)	E/e’	9.4 (3.9)
Waist-height ratio	0.57 (0.07)	E’ (cm/sec)	8.8 (2.7)
Major traditional CV risk factors		E/A	1.01 (0.38)
Hypertension	37 (86.0)	E wave velocity (cm/sec)	77 (23)
Systolic BP (mmHg)	139 (130-144)	A wave velocity (cm/sec)	83 (29)
Diastolic BP (mmHg)	86 (9)	LV mass index (g/m^2)	82.4 (66.2-109.3)
Mean BP	102 (97-107)	LV hypertrophy	7 (16.6)
Heart rate (beats/min)	71 (11)	Concentric remodelling	8 (18.6)
Dyslipidemia	36 (83.7)	LV concentric hypertrophy	1 (2.3)
Total cholesterol (mmol/l)	4.4 (1.0)	LV septal wall thickness (mm)	9.3 (1.5)
LDL cholesterol (mmol/l)	2.3 (0.6)	LV posterior wall thickness (mm)	9.6 (8.6-10.7)
HDL cholesterol (mmol/l)	1.10 (0.90-1.40)	LV relative wall thickness	0.39 (0.34-0.45)
Non-HDL cholesterol (mmol/l)	3.1 (0.9)	LV EDV indexed to BSA (ml/m^2)	62 (25)
Cholesterol-HDL cholesterol ratio	3.8 (1.2)	LV ESV indexed to BSA (ml/m^2)	19 (12-28)

Triglycerides (mmol/l)	1.60 (1.29-2.25)	LV ejection fraction (%)	74 (65-79)
Diabetes	11 (25.6)	Stroke volume (ml/beat)	73 (29)
Non-traditional/renal risk CV factors		Cardiac output (l/min)	5.3 (2.9)
Estimated GFR (ml/min/1.73m^2)	63 (22)	**SVR (mmHg/l/min)**	**20.8 (15.5-28.6)**

Data are expressed as mean (SD), median (interquartile range) or number (%). Subheadings are shown in bold.

Abbreviations: CV, cardiovascular; BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; GFR, glomerular filtration rate; CRP, C-reactive protein; iPTH, intact parathyroid hormone; NT-proBNP, N-terminal B-type natriuretic peptide; LV, left ventricular; EDV, end diastolic volume; ESV, end systolic volume; BSA, body surface area; SVR, systemic vascular resistance.

Table 2 Associations of cardiovascular risk factors with E/e' in 43 kidney transplant recipients

	Model 1[a]				Model 2[b]				Model 3[c]				Model 4[d]				Model 5[e]			
CV risk factor	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2
EGFR	**-0.061 (0.026)**	**-0.351**	**0.02**	**0.241**	**-0.052 (0.032)**	**-0.270**	**0.1**	**0.282**	**-0.049 (0.031)**	**-0.273**	**0.1**	**0.390**				**0.441**				**0.243**
Haemoglobin	**-0.589 (0.251)**	**-0.356**	**0.02**	**0.244**	**-0.700 (0.284)**	**-0.394**	**0.01**	**0.346**					**-0.696 (0.300)**	**-0.390**	**0.02**		**-0.691 (0.278)**	**-0.278**	**0.01**	
Log IPTH	**3.767 (1.596)**	**0.366**	**0.02**	**0.254**	**4.178 (1.818)**	**0.382**	**0.02**	**0.341**	**3.972 (1.783)**	**0.377**	**0.03**		3.132 (1.761)	0.309	0.08		**3.079 (1.480)**	**0.324**	**0.04**	

Significant associations are shown in bold. Abbreviations: SE: standard error; β: regression coefficient; EGFR, estimated glomerular filtration rate; log: logarithmically transformed; other abbreviations: see Table 1.

[a]Adjusted for age, sex and race.

[b]Adjusted for age, sex, race, height, weight, log mean arterial pressure and heart rate.

[c]Adjusted for age, sex, race, height, weight, log mean arterial pressure and heart rate with entering of EGFR and log iPTH in a multivariate model; haemoglobin was not entered due to collinearity with EGFR.

[d]Adjusted for age, sex, race, height, weight, log mean arterial pressure and heart rate with entering of haemoglobin and log iPTH in a multivariate model; EGFR was not entered due to collinearity with haemoglobin.

[e]Adjusted for age, sex, race, height, weight, log mean arterial pressure and heart rate with entering of EGFR, haemoglobin and log PTH in a stepwise regression model; female sex comprised the only other variable that was associated with E/e' (β (SD)=2.775 (1.161); partial R=0.370; *P*=0.02) in model 5.

Table 3 Associations of cardiovascular risk factors with e' and E/A in 43 kidney transplant recipients

	E'								E/A							
	Model 1[a]				Model 2[b]				Model 1[a]				Model 2[b]			
Cardiovascular risk factor	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2
Waist-height ratio	**-15.474 (4.857)**	**-0.464**	**0.003**	**0.465**	**-16.069 (4.450)**	**-0.526**	**0.001**	**0.624**	**-1.516 (0.721)**	**-0.331**	**0.04**	**0.480**	**-1.559 (0.715)**	**-0.355**	**0.03**	**0.509**
Waist	**-0.064 (0.027)**	**-0.365**	**0.02**	**0.467**	**-0.070 (0.025)**	**-0.433**	**0.008**	**0.578**	**-0.009 (0.004)**	**-0.387**	**0.01**	**0.504**	**-0.009 (0.004)**	**-0.393**	**0.02**	**0.439**
BMI	**-0.164 (0.074)**	**-0.339**	**0.03**	**0.420**	**-0.148 (0.070)**	**-0.332**	**0.04**	**0.551**	**-0.033 (0.009)**	**-0.498**	**0.001**	**0.566**	**-0.031 (0.009)**	**-0.489**	**0.002**	**0.507**

Significant associations are shown in bold. Abbreviations: BMI, body mass index; other abbreviations: see Table 2.

[a]Adjusted for age, sex and race.

[b]Adjusted for age, sex, race, log mean arterial pressure and heart rate.

Table 4 Established confounder adjusted associations of cardiovascular risk factors with E/e', e' and E/A after additional adjustment for left ventricular mass and cardiac preload and afterload measures in 43 kidney transplant recipients

	β (SE)	Partial R	*P*	Model R^2
Haemoglobin versus E/e'				
Model 1[a]	**-0.675 (0.297)**	**-0.378**	**0.03**	**0.345**
Model 2[b]	**-0.649 (0.308)**	**-0.349**	**0.04**	**0.350**
Model 3[c]	**-0.945 (0.333)**	**-0.472**	**0.008**	**0.433**
Model 4[d]	**-0.907 (0.390)**	**-0.422**	**0.02**	**0.447**
Log intact PTH versus E/e'				
Model 1[a]	**4.113 (1.879)**	**0.377**	**0.03**	**0.347**
Model 2[b]	**4.026 (1.819)**	**0.375**	**0.03**	**0.366**
Model 3[c]	**4.880 (1.927)**	**0.445**	**0.01**	**0.418**
Model 4[d]	**4.594 (1.988)**	**0.434**	**0.03**	**0.458**
Waist-height ratio versus e'				
Model 1[e]	**-16.214 (4.564)**	**-0.532**	**0.001**	**0.663**
Model 2[f]	**-19.603 (4.638)**	**-0.593**	**<0.0001**	**0.664**
Model 3[g]	**-15.332 (5.700)**	**-0.478**	**0.006**	**0.638**
Model 4[h]	**-16.649 (6.042)**	**-0.497**	**0.007**	**0.658**
BMI versus E/A				
Model 1[e]	**-0.029 (0.009)**	**-0.491**	**0.003**	**0.623**
Model 2[f]	**-0.027 (0.010)**	**-0.419**	**0.01**	**0.602**
Model 3[g]	**-0.022 (0.010)**	**-0.372**	**0.03**	**0.678**
Model 4[h]	**-0.026 (0.012)**	**-0.386**	**0.04**	**0.697**

Significant associations and subheadings are shown in bold. Abbreviations: see Tables 2 and 3.

[a]Adjusted for age, race, sex, weight, height, log mean arterial blood pressure, heart rate and log left ventricular mass index.

[b]Adjusted for age, race, sex, weight, height, log mean arterial blood pressure, heart rate and cardiac preload as estimated by left ventricular end diastolic volume.

[c]Adjusted for age, race, sex, weight, height, log mean arterial blood pressure, heart rate and cardiac afterload as estimated by log central systolic blood pressure and log systemic vascular resistance.

[d]Adjusted for age, race, sex, weight, height, log mean arterial blood pressure, heart rate left ventricular mass index and end diastolic volume, log central systolic blood pressure and log systemic vascular resistance.

[e]Adjusted for age, race, sex, log mean arterial blood pressure, heart rate and log left ventricular mass index.

[f]Adjusted for age, race, sex, log mean arterial blood pressure, heart rate and cardiac preload as estimated by left ventricular end diastolic volume.

[g]Adjusted for age, race, sex, log mean arterial blood pressure, heart rate and cardiac afterload as estimated by log central systolic blood pressure and log systemic vascular resistance.

[h]Adjusted for age, race, sex, log mean arterial blood pressure, heart rate left ventricular mass index and end diastolic volume, log central systolic blood pressure and log systemic vascular resistance.

Table 5 Recorded characteristics in 43 kidney transplant recipients upon stratification by median transplant duration

	Transplant duration <13.3 years (n=22)	Transplant duration ≥13.3 years (n=21)	*P*
Demographics			
Age (years)	52.4 (12.0)	54.6 (9.6)	0.6
Female sex	**4 (18.1)**	**10 (47.6)**	**0.04**
Black	8 (36.4)	12 (57.1)	0.3
Asian	**10 (45.5)**	**1 (4.8)**	**0.01**
Mixed	2 (9.1)	4 (19.0)	0.2
White	2 (9.1)	4 (19.0)	0.4
Traditional CV risk factors			
Anthropometry			
Body mass index (kg/m^2)	27.3 (3.8)	26.5 (5.1)	0.5
Waist (cm)	**103 (12)**	**92 (13)**	**0.007**
Waist-height ratio	**0.60 (0.06)**	**0.55 (0.08)**	**0.009**
Major traditional CV risk factors			
Mean blood pressure (mmHg)	101 (95-103)	105 (97-111)	0.06
Heart rate (beats/min)	**75 (10)**	**68 (11)**	**0.02**
Non-traditional/renal CV risk factors			
Estimated GFR (ml/min/1.73m^2)	65 (23)	61 (20)	0.5
IPTH (pg/ml)	125.7 (53.6-261.0)	54.7 (42.2-82.0)	0.07
Haemoglobin (g/dl)	**13.1 (2.5)**	**14.7 (1.5)**	**0.02**
Arterial function			
Central systolic blood pressure (mmHg)	**121 (116-126)**	**135 (123-146)**	**0.04**
Echocardiographic characteristics			
E/e'	9.8 (4.4)	9.0 (3.5)	0.3
E' (cm/sec)	8.5 (2.8)	9.1 (2.7)	0.06
E/A	0.96 (0.39)	1.07 (0.38)	0.2
LV mass index (g/m^2)	82.5 (62.8-112.4)	82.4 (68.0-105.9)	0.4
LV EDV indexed to BSA (ml/m^2)	67 (28)	57 (20.4)	0.1
SVR (mmHg/min)	20.4 (14.7-28.3)	22.7 (16.7-33.7)	0.2

Data are expressed as mean (SD), median (interquartile range) or number (%), and were analysed in age, sex and race adjusted linear or logistic regression models as appropriate. Subheadings and significant associations are shown in bold. Abbreviations: see Table 1.

Table 6 Associations of cardiovascular risk factors with e' in 43 kidney transplant recipients upon stratification by median transplant duration

	Transplant duration <13.3 years (n=22)								Transplant duration >13.3 years (n=21)							
	Model 1[a]				Model 2[b]				Model 1[a]				Model 2[b]			
Cardiovascular risk factor	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2
Waist-height ratio	-1.541 (11.166)	-0.034	0.9	0.338	-13.364 (13.347)	-0.268	0.3	0.553	**-23.275 (6.775)**	**-0.652**	**0.003**	**0.666**	**-18.930 (6.384)**	**-0.621**	**0.01**	**0.762**
Waist	0.012 (0.054)	0.056	0.8	0.471	-0.020 (0.065)	-0.085	0.8	0.522	**-0.09 (0.040)**	**-0.486**	**0.04**	**0.556**	**-0.077 (0.036)**	**-0.501**	**0.04**	**0.710**
BMI	0.017 (0.144)	0.029	0.9	0.499	-0.051 (0.174)	-0.077	0.8	0.551	**-0.265 (0.097)**	**-0.566**	**0.014**	**0.605**	-0.197 (0.105)	-0.448	0.08	0.691

Significant associations are shown in bold. Abbreviations: see Table 2.

[a]Adjusted for age, sex and race.

[b]Adjusted for age, sex, race, log mean arterial pressure and heart rate.

Table 7 Associations of cardiovascular risk factors with E/A in 43 kidney transplant recipients upon stratification by median transplantation duration

	Transplant duration <13.3 years (n=22)								Transplant duration >13.3 years (n=21)							
	Model 1[a]				Model 2[b]				Model 1[a]				Model 2[b]			
Cardiovascular risk factor	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2
Waist-height ratio	-0.461 (1.519)	-0.078	0.8	0.537	-1.439 (1.964)	-0.207	0.5	0.551	-1.222 (1.178)	-0.251	0.3	0.472	-0.737 (1.218)	-0.160	0.6	0.548
Waist	-0.005 (0.008)	-0.167	0.5	0.547	-0.009 (0.009)	-0.256	0.4	0.562	-0.009 (0.006)	-0.354	0.1	0.507	-0.007 (0.006)	-0.298	0.2	0.578
BMI	-0.020 (0.019)	-0.259	0.3	0.564	-0.027 (0.023)	-0.311	0.2	0.579	**-0.035 (0.013)**	**-0.547**	**0.01**	**0.605**	-0.029 (0.016)	-0.432	0.09	0.623

Significant associations are shown in bold. Abbreviations: see Table 3.

[a]Adjusted for age, sex and race.

[b]Adjusted for age, sex, race, log mean arterial pressure and heart rate.

Supplementary Material

Methods

Cardiovascular risk factors

Hypertension was identified when the systolic or/and diastolic blood pressure were ≥ 140mmHg and 90mmHg, respectively, or/and antihypertensive agents were used. Analyses were performed on fasting blood samples using routine laboratory tests for the determination of lipid, glucose, phosphate, calcium, haemoglobin, albumin, uric acid and ferritin concentrations, transferrin saturation percentages. High-sensitive C-reactive protein levels were estimated on Abbott Architect using an immunoturbidimetric assay. Dyslipidemia was diagnosed when the total cholesterol: HDL cholesterol ratio was >4 or/and lipid lowering drugs were employed. Diabetes was identified in patients with a fasting glucose level of ≥7 mmol/l and in those that used glucose lowering medications.

Arterial function

Subsequent to resting for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded for a time period of ten consecutive waveforms (heart beats). Calibration of the pulse wave was done by manual measurement (auscultation) of brachial BP taken immediately prior to recordings. A validated generalized transfer function incorporated in the SphygmoCor software was used to convert the peripheral pressure wave form into a central aortic waveform. The results were discarded when the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less that 80 mV. The aortic pulse wave velocity was calculated as distance in meters divided by transit time in seconds. The time delay in pulse waves between the carotid and femoral sites was assessed by employing an ECG-derived R wave as a fudicial point. Pulse transit time was calculated as the mean of 10 consecutive beats. The difference between the distance from the femoral sampling site to the suprasternal notch and the distance from the carotid sampling site to the suprasternal notch represented the distance the pulse wave travels. Magnitude of the forward and reflected wave components of

the aortic pressure waveform was determined by the SphygmoCor software, which separates the aortic waveform by using a modified triangular waveform. Augmentation index was calculated as (second systolic peak/first systolic peak) x 100, reflection magnitude as (reflected wave amplitude/forward wave amplitude) x 100 and pulse pressure amplification as (radial pulse pressure/aortic pulse pressure) x 100. All measurements were made by a single experienced observer (CR) who was unaware of the cardiovascular risk factor profiles of the patients. Brachial blood pressure was recorded in all patients. Technically sound measurements of the central pressure wave and pulse wave velocity were obtained in 40 and 38 patients, respectively.

Left ventricular structure and function

Echocardiography was performed with the patient in the partial left decubitus position. Left ventricular dimensions were determined by measuring the left ventricular internal end diastolic and end systolic diameters and wall thickness (left ventricular septal and posterior wall thickness) in the parasternal long axis view by two-dimensional directed M-mode echocardiography. Left ventricular end diastolic and systolic volumes were assessed using the Teichholz method and indexed to body surface area[1]. Stroke volume was determined from the difference between LV end diastolic and systolic volumes as evaluated upon employing the Z-derived method[2]. Cardiac output was determined as stroke volume x heart rate. Left ventricular ejection fraction was calculated as [(left ventricular end diastolic volume – left ventricular end systolic volume) / left ventricular end diastolic volume] x 100. Left ventricular mass was determined using a standard formula[3] and indexed to body surface area (left ventricular mass index). Left ventricular relative wall thickness was calculated as (left ventricular diastolic posterior wall thickness x 2) / left ventricular end diastolic diameter[4]. Left ventricular hypertrophy was identified when left ventricular mass index was >95 g/m^2 for women and >115 g/m^2 for men[3]. Concentric remodelling was defined as the presence of relative wall thickness of >0.45. Concentric left ventricular hypertrophy was considered present when the relative wall thickness was >0.45 and left ventricular hypertrophy was absent.

Left ventricular diastolic function was assessed using pulsed Doppler and tissue Doppler imaging[5]. Transmitral flow patterns were recorded at the mitral valve leaflet tips using ulsed Doppler in the apical four chamber view. From the mitral valve inflow velocity curve, the early (E) and late (atrial-A) diastolic wave were measured and the ratio of early and late diastolic filling (E/A) was calculated. To perform tissue Doppler imaging, the velocity of myocardial tissue lengthening was measured by placing the curser at the septal and lateral corners of the mitral annulus in the apical four chamber view. To determine diastolic function using tissue Doppler imaging, the early diastolic mitral annulus motion (e') was recorded. Because mitral E is dependent on ventricular relaxation as well as left atrial driving forces (pressures), while e' is dependent on relaxation alone, E/e' ratio is considered to be an index of left ventricular filling pressures. The E/e' ratio was calculated as mitral E/the average of septal and lateral e'. Echocardiographic measurements were made by the same observer that performed the arterial function evaluation (CR). Intra-observer variation for echocardiographic measurements is low in our setting[6].

References

1. Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. Am J Cardiol 1976;37:7-11.
2. de Simone G, Devereux RB, Ganau A, et al. Estimation of the left ventricular chamber and stroke volume by limited M-Mode echocardiography and validation by two-dimensional and Doppler echocardiography. Am J Cardiol 1996;78(1):801-807.
3. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015;16(7):233-271.
4. Ganau A, Devereux RB, Roman MJ, et al. Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. J Am Coll Cardiol 1992;19(7):1550-1558.
5. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update form the American Society of
   Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016;29(4):277-314.
6. Mokotedi L, Gunter S, Robinson C, et al. The Impact of different classification criteria sets on the estimated prevalence and associated risk factors of diastolic dysfunction in rheumatoid arthritis. Int J Rheumatol. 2017;2017:2323410.

Supplementary Table 1 Associations of cardiovascular risk factors with E/e' in 43 kidney transplant recipients upon stratification by median transplant duration

	Transplant duration <13.3 years (n=22)				Transplant duration >13.3 years (n=21)			
CV risk factor	β (SE)	Partial R	*P*	Model R^2	β (SE)	Partial R	*P*	Model R^2
EGFR	-0.060 (0.042)	-0.326	0.1	0.239	-0.064 (0.034)	-0.421	0.08	0.454
Haemoglobin	-0.597 (0.359)	-0.375	0.1	0.268	-0.350 (0.512)	-0.169	0.5	0.355
Log IPTH	3.988 (2.209)	0.422	0.09	0.304	2.027 (3.242)	0.154	0.5	0.352

Data were analysed in age, sex and race adjusted linear regression models. Abbreviations: CV, cardiovascular; β: regression coefficient; EGFR, estimated glomerular filtration rate; log: logarithmically transformed; iPTH intact parathyroid hormone.

Study/Paper 4:

Study/paper 4 shows that the optimal haemoglobin target in dialysis patients may be determined by its contrasting effects on aortic stiffness and pressure pulsatility.

The optimal haemoglobin target in dialysis patients may be determined by its contrasting effects on arterial stiffness and pressure pulsatility

Subtitle: Haemoglobin and arterial function in dialysis

Hon-Chun Hsu[1,2], Chanel Robinson[1], Gavin R. Norton[1], Angela J. Woodiwiss[1], Patrick H. Dessein[1,3,4]

[1]Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa,

[2]Nephrology Unit, Milpark Hospital, Johannesburg, South Africa,

[3]Internal Medicine Department, University of the Witwatersrand, Johannesburg, South Africa,

[4]Free University and University Hospital, Brussels, Belgium ORCID identifiers: Hon-Chun Hsu: https://orcid .org/0000-0003-4180-1552; Chanel Robinson: https://orcid .org/0000-0002-9385-2709; Angela J. Woodiwiss: https://orcid.org/0000-0001-2555-4125; Gavin R. Norton: https://orcid .org/0000-0001-6416-6104; Patrick H. Dessein: https://orcid .org/0000-0002-9357-4630

Correspondence: Patrick H Dessein; E-mail: patrick.dessein22@gmail.com; Postal address: 80 Scholtz Road, Norwood 2117, Johannesburg, South Africa

Abstract

Introduction: It remains unclear why the optimal haemoglobin target is lower in patients with chronic kidney disease (CKD) than in non-CKD persons. Arteriosclerosis and consequent impaired arterial function comprise a central cardiovascular risk mechanism in CKD. We hypothesized that the optimal haemoglobin target depends on its opposing effects on arterial stiffness and pressure pulsatility in CKD.

Methods: Arterial stiffness (aortic pulse wave velocity), wave reflection (augmentation index, reflected wave pressure and reflection magnitude), and pressure pulsatility (central systolic and pulse pressure, peripheral pulse pressure, pressure amplification and forward wave pressure) were assessed in 48 dialysis patients.

Results: In established confounder and diabetes adjusted linear regression models, haemoglobin levels were directly associated with arterial stiffness (partial R=0.366, p=0.03) and inversely with central systolic pressure (partial R=-0.344, p=0.04), central pulse pressure (partial R=-0.403, p=0.01), peripheral pulse pressure (partial R=-0.521, p=0.001) and forward wave pressure (partial R=-0.544, p=0.001). The presence of heart failure and use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers and erythropoietin stimulating agents did not materially alter these relationships upon further adjustment for the respective characteristics in the models, and in sensitivity analyses. In receiver operator characteristic curve analysis, the optimal haemoglobin concentration cut-off values in predicting arterial stiffness and increased central pulse pressure were remarkably similar at 10.95 g/dl and 10.85 g/dl respectively, and with clinically useful sensitivities, specificities and positive and negative predictive values. In logistic regression models, a haemoglobin value of > 10.9 mg/dl was associated with both arterial stiffness (>10 m/sec; OR (95% CI) = 10.48 (1.57 – 70.08), p=0.02) and normal central pulse pressure (>50 mmHg; OR (95% CI) = 7.55 (1.58 – 36.03), p=0.01).

Conclusions: This study suggests that the optimal haemoglobin target in dialysis patients is ~11g/dl and determined by its differential and contrasting effects on arterial stiffness and pressure pulsatility.

Keywords: haemoglobin target, dialysis, arterial stiffness, pressure pulsatility

Introduction

Arteriosclerosis encompasses hypertrophy and fibrosis of the medial layer in large arteries[1]. Chronic kidney disease (CKD) is characterized by marked premature arteriosclerosis that causes impaired arterial function[2-4]. Arterial stiffness results in an accelerated forward wave, which increases central systolic blood pressure and enhances wave reflection that occurs at bifurcations and due to changes in composition and progressive narrowing of the vessels along the arterial tree. The increased reflected wave arrives earlier at the heart, i.e. in systole rather than diastole, and thereby reduces diastolic blood pressure and coronary perfusion. The increased forward and reflected waves enhance pressure pulsatility and decrease pulse pressure amplification, which increases transmission of pulsatile pressure into the microcirculation leading to CKD progression. Increased arterial stiffness, wave reflection and pressure pulsatility thereby each contribute to cardiovascular event rates including heart failure, arrhythmias, stroke and myocardial infarction in CKD[5-7].

Accelerated arteriosclerosis in CKD is mediated by adverse traditional as well as non-traditional or renal impairment specific cardiovascular risk factors[2-8].

In observational studies, anaemia is associated with CVD in CKD[9]. However, the optimal haemoglobin target in CKD patients is reportedly substantially lower in CKD compared to non-CKD individuals[10-11]. Indeed, treatment with erythropoietin stimulating agents in CKD patients is associated increased CVD events when a haemoglobin level of ~13 g/dl is targeted[12,13]. In this regard, nitric oxide reduces arterial stiffness and peripheral vascular resistance[14] whereas haemoglobin is a potent nitric oxide scavenger[15]. High haemoglobin levels are associated with arterial stiffness in non-CKD persons[16-18]. Other investigations in persons with and without CKD indicate that anaemia increases pressure pulsatility[19,20]. In the present study, we therefore hypothesized that the optimal haemoglobin target in CKD may depend on its contrasting effects on arterial stiffness and pressure pulsatility in CKD. We assessed the independent relationships of haemoglobin levels with arterial function measures including pulse wave velocity, wave reflection and pressure pulsatility in a cohort of dialysis patients.

Patients and Methods

Patients

Forty-eight dialysis patients were enrolled at the Milpark Hospital in Johannesburg, South Africa. Patients with infection or/and active cancer were excluded. This study was performed according to the Helsinki Declaration of 1975 as revised in 2013 and was approved by the University of the Witwatersrand Human (Medical) research Ethics Committee (protocol number: M15-08-43) in Johannesburg, South Africa. Written informed consent was obtained in each patient prior to participation.

Methods

The recorded characteristics included demographic features, lifestyle factors, anthropometric measures, traditional and non-traditional cardiovascular risk factors, the presence of established cardiovascular disease including heart failure, arterial function and other hemodynamic characteristics that included systemic vascular resistance, stroke volume and work, cardiac output and left ventricular mass index. Data recording was performed on the day prior to undergoing dialysis, which was applied thrice weekly in each of these patients.

Cardiovascular risk factors

We recorded traditional and non-traditional or renal cardiovascular risk factors as previously reported[21] and given in the online Supplementary Materials. For the present study, high phosphate was considered present when the phosphate concentration was >1.42 mmol/l or/and phosphate lowering drugs including calcium carbonate or sevelamer therapy in 44 and 1 patients, respectively, was employed. Mean arterial blood pressure for the peripheral waveform was determined electronically by the SphygmoCor device (see below) and using the formula

$$MP = \frac{\sum_{i=T_0}^{T_F} P_i}{n}$$

where T_0=start of the waveform; T_F=end of waveform; P_i=pressure points and n=number of pressure points.

Established cardiovascular disease

Ischemic heart disease, heart failure and cerebrovascular and peripheral arterial disease that was confirmed by a cardiologist, neurologist and vascular surgeon, respectively, comprised recorded established cardiovascular disease.

Arterial function

Applanation tonometry and SphygmoCor software were used to determine central hemodynamic features as previously reported[21] and given in the online Supplementary Materials. We assessed aortic pulse wave velocity, augmentation index, reflected wave pressure and reflection magnitude, central systolic and pulse pressure, peripheral pulse pressure, pressure amplification and forward wave pressure.

Other hemodynamic parameters

Echocardiography was performed as recommended by the American Society of Echocardiography convention and using a Sonosite M-Turbo ultrasound (SonoSite® Inc., Bothell, WA, USA)[22]. We assessed stroke volume, cardiac output and left ventricular mass index. Further details are given in the online Supplementary Materials. Systemic vascular resistance was calculated from mean arterial pressure and cardiac output according to the equation mean arterial pressure=systemic vascular resistance x cardiac output assuming that right atrial pressure=0 mmHg. Stroke work was calculated as stroke volume x systolic blood pressure x 0.0144, and expressed in gram-meters/beat.

Data analysis

Statistical analysis was performed on IBM SPSS statistics program (version 23.0 IBM, USA) and significance was set at $p \leq 0.05$. Results are expressed as mean (SD) or median (interquartile range, IQR) for continuous variables and percentages for categorical variables. Logarithmic transformation was applied when non-normally distributed data were analysed in multivariable regression models.

The associations of lifestyle factors, anthropometric measures and major traditional and non-traditional/renal cardiovascular risk factors with arterial function parameters were first assessed in established confounder[21,23] including age, sex, race, weight, height, heart rate and mean arterial blood pressure adjusted linear regression models.

Subsequently, the independent associations of recorded cardiovascular risk factors with arterial function parameters were assessed by entering established confounders together with those that were related to arterial function in the previous analysis into single models.

As heart failure[24] and treatment with angiotensin converting enzyme inhibitors or angiotensin blockers[25] as well erythropoietin stimulating agents[26] can also impact arterial function, the potential influence of the respective factors was assessed in separate models and sensitivity analyses.

The above mentioned analyses revealed that haemoglobin concentrations were directly associated with arterial stiffness and inversely related to pressure pulsatility. We therefore investigated the respective relationships further by performing receiver operator characteristic (ROC) curve analysis.

Finally, we assessed bivariate associations among haemoglobin concentrations and other hemodynamic characteristics by determining Pearson correlation coefficients.

Results

Patient characteristics

The recorded characteristics are given in Table 1. The mean (SD) age was 55.8 (14.3) years and 22 (45.8%) were women. More than half of the study participants (56.3%) were of black population origin. Hypertension, dyslipidemia and diabetes were present in 44 (91.7%), 30 (71.4%) and 19 (39.6%), respectively. Angiotensin converting enzyme inhibitors or angiotensin receptor blockers and erythropoietin stimulating agents were used in 37 (77.1%) and 43 (89.6%) of the patients, respectively. Among those with cardiovascular disease (n=14 (29.6%)), 7 (14.6%) had heart failure. The pulse wave velocity and left ventricular mass index were both large with a median (interquartile range) and mean (SD) value of 11.2 (7.8-14.9) and 103.4 (51.0), respectively.

Associations of haemoglobin concentrations with arterial function

As given in Table 2, in age, sex, race, weight, height, mean blood pressure and heart rate adjusted linear regression models, haemoglobin levels were directly associated with pulse wave velocity (p=0.05) and inversely with central systolic blood pressure (p=0.03), central pulse pressure (p=0.01), peripheral pulse pressure (p=0.001) and forward wave pressure (p=0.001) (model 1 in Table 2).

Apart from diabetes that was associated with pulse wave velocity (p=0.01), augmentation index (p=0.01), reflected wave pressure (p=0.02), reflection magnitude (p=0.02) and central pulse pressure (p=0.02), and heart rate and mean blood pressure for which data are not shown as these characteristics were included as established confounders in the models, none of the other traditional and non-traditional/renal risk factors as given in Table 1 were associated with arterial function parameters. Model 2 in Table 2 shows that upon additional adjustment for diabetes, haemoglobin concentrations remained associated with pulse wave velocity (p=0.03), central systolic blood pressure (p=0.04), central pulse pressure (p=0.01), peripheral pulse pressure (p=0.001) and forward wave pressure (p=0.001).

When we additionally adjusted for the presence of heart failure (model 3 in Table 2), the associations of haemoglobin concentrations with arterial function remained consistent (p=0.03, p=0.04, p=0.01, p=0.001 and p=0.002 for pulse wave velocity, central systolic blood pressure, central pulse pressure, peripheral pulse pressure and forward wave pressure, respectively.

As shown in model 4 in Table 2, upon additional adjustment for the use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers, haemoglobin concentrations remained associated with pulse wave velocity (p=0.03), peripheral pulse pressure (p=0.002) and forward wave pressure (p=0.005) but their relationships with central systolic blood pressure and central pulse pressure no longer reached significance (p=0.1 and p=0.06, respectively.

As given in model 5 in Table 2, when we additionally adjusted for treatment with erythropoietin stimulating agents, haemoglobin levels remained associated with pulse wave velocity (p=0.03), central systolic blood pressure (p=0.04), central pulse pressure (p=0.01), peripheral pulse pressure (p=0.001) and forward wave pressure (p=0.001).

Associations of haemoglobin concentrations with arterial function in sensitivity analyses

To further assess whether the presence of heart failure and angiotensin converting enzyme inhibitor or angiotensin receptor blocker and erythropoietin stimulating agent therapy could influence the impact of haemoglobin concentrations on arterial function, we performed sensitivity analyses. These results are shown in Table 3.

In patients without heart failure (n=41), haemoglobin levels were associated with pulse wave velocity (p=0.01), central pulse pressure (p=0.02), peripheral pulse pressure (p=0.004) and forward wave pressure (p=0.004) whereas their relationship with central systolic blood pressure did not reach significance (p=0.07). Haemoglobin concentrations were further directly associated with reflection magnitude (p=0.04). When the mean arterial pressure was replaced by systemic vascular resistance in the respective model, the association of haemoglobin levels with reflection magnitude was not materially altered (partial R=0.334, p=0.08).

Among patients on angiotensin converting enzyme inhibitor or angiotensin receptor blocker therapy, haemoglobin levels were associated with central pulse pressure (p=0.03), peripheral pulse pressure (0.002) and forward wave pressure (p=0.003) but their relationship with pulse wave velocity and central systolic blood pressure did not reach significance (p=0.07 and p=0.09, respectively).

In patients treated with erythropoietin stimulating agents, haemoglobin concentrations were associated with pulse wave velocity (p=0.03), central pulse pressure (p=0.02), peripheral pulse pressure (p=0.004) and forward wave pressure (p=0.002) whereas their relationship with central systolic blood pressure did not reach significance (p=0.1).

Haemoglobin levels were further associated with augmentation index (p=0.05) and reflection magnitude (p=0.03). When the mean arterial pressure was replaced by systemic vascular resistance in the respective model, the association of haemoglobin levels with reflection magnitude was not materially altered (partial R=0.317, p=0.1).

Haemoglobin concentrations, arterial stiffness and increased central pulse pressure

Arterial stiffness (pulse wave velocity >10 m/sec)[27,28] and increased pulse pressure (>50 mmHg)[29] were recorded in 46.3% and 28.7% of the patients, respectively. Given the independent associations of haemoglobin concentrations with arterial stiffening and low pressure pulsatility among these patients (Table 2), we determined the accuracy of haemoglobin concentrations in predicting arterial stiffness and a normal central pulse pressure in ROC curve analysis. This is shown in Figure 1. The area under the curve (AUC) of the ROC curve was associated with arterial stiffness (Figure 1A; AUC=0.670) and normal pulse pressure (Figure 1B; AUC=0.717). To estimate the optimal cut-off values for haemoglobin concentrations in determining arterial function, we calculated the Youden index. The optimal haemoglobin concentration cut-off value in predicting the presence of arterial stiffness was 10.95 g/dl with a corresponding sensitivity, specificity, and positive and negative predictive value as determined by applying Bayes' theorem of 45.7%, 77.8%, 84.8% and 60.9%, respectively; the optimal haemoglobin concentration cut-off value in predicting the presence of a normal pulse pressure was 10.85 g/dl with a corresponding sensitivity, specificity, and positive and negative predictive value of 72.7%, 81.8%, 77.1% and 78.0%, respectively.

A haemoglobin value of >10.9 mg/dl was associated with arterial stiffness (OR (95% CI) = 4.77 (1.23 – 18.53), p=0.02) and normal central pulse pressure (OR (95% CI) = 7.88 (1.96 – 31.57), p=0.004). In established confounder adjusted logistic regression models, a haemoglobin value of >10.9 mg/dl remained associated with arterial stiffness (OR (95% CI) = 10.48 (1.57 – 70.08), p=0.02) and normal central pulse pressure (OR (95% CI) = 7.55 (1.58 – 36.03), p=0.01).

Associations among haemoglobin levels and other haemodynamic characteristics

Associations among haemoglobin concentrations and other hemodynamic characteristics are given in Table 4. Haemoglobin levels were directly associated with systemic vascular resistance (p=0.05) and inversely related to stroke volume (p=0.05), cardiac output (p=0.03) and stroke work (p=0.01). Systemic vascular resistance was inversely associated with stroke volume (p<0.0001), cardiac output (p<0.0001), stroke work and left ventricular mass index (p=0.01). Stroke volume was directly associated with cardiac output (p<0.0001), stroke work

(p=0.01) and left ventricular mass index. Cardiac output was directly associated with stroke work (p<0.0001) and left ventricular mass index (p=0.409). Stroke work was directly associated with left ventricular mass index (p=0.02). Additionally, cardiac output was directly associated with pressure pulsatility measures including peripheral pulse pressure (R=0.340, p=0.02), central pulse pressure (R=0.299, p=0.05) and forward wave pressure (R=0.377, p=0.01), and tended to be directly associated with central systolic pressure (R=0.292, p=0.06).

Discussion

To our knowledge, this is the first study that simultaneously assessed the potential impact of haemoglobin concentrations on pulse wave velocity, wave reflection and pressure pulsatility as arterial function markers, as well as other hemodynamic characteristics in dialysis patients. The most striking and novel finding in this study was that haemoglobin concentrations were associated not only with arterial stiffness but also simultaneously with low pressure pulsatility measures including central systolic blood pressure, central and peripheral pulse pressure as well as forward wave pressure. In ROC curve analysis, the optimal haemoglobin concentration cut-off values in predicting arterial stiffness and increased central pulse pressure were remarkably similar at 10.95 g/dl and 10.85 g/dl respectively, and with clinically useful sensitivities, specificities and positive and negative predictive values. A haemoglobin value of >10.9 mg/dl was independently and strongly associated with both arterial stiffness (OR (95% CI) = 10.48 (1.57 – 70.08), p=0.02) and normal central pulse pressure (OR (95% CI) = 7.55 (1.58 – 36.03), p=0.01).

Anaemia is a highly prevalent comorbidity in dialysis patients[30]. Anaemia in CKD is mediated by multiple factors including reduced erythropoietin synthesis and release, iron deficiency and chronic inflammation[31]. Anaemia causes reduced viscosity and increased nitric oxide production and activation mediated vasodilatation and decreased peripheral vascular resistance[19,32,33]. Nitric oxide additionally reduces arterial stiffness, this to a larger extent than peripheral vascular resistance[14]. Haemoglobin is a potent nitric oxide scavenger[15]. The use of nitric oxide donors comprises a potential therapeutic intervention for arterial stiffness[34]. The

vascular effects of anaemia or low haemoglobin result in reduced cardiac afterload, increased venous return, preload, left ventricular filling pressure and end-diastolic volume with consequent enhanced stroke volume and work. Increased stroke volume translates into enhanced cardiac output that is associated with enhanced pressure pulsatility[35,36]. Anaemia together with arterial stiffness thereby ultimately engender left ventricular hypertrophy and heart failure in CKD[19,37]. Our findings of a direct and concurrent inverse relationship of haemoglobin concentrations with arterial stiffness and pressure pulsatility measures, respectively, as well as the associations among haemoglobin levels and hemodynamic measures including systemic vascular resistance, stroke volume, stroke work, left ventricular mass index, cardiac output and pressure pulsatility parameters are each in keeping with these reported hemodynamic effects of anaemia in persons with and without CKD.

Our results are also in line with reported data in population and non-dialysis patient studies. The Korea National Health and Nutrition Examination Survey 2010-2012 revealed a relationship of anaemia with pulse pressure[38]. In the Chronic Renal Insufficiency Cohort Ancillary Study[39], haemoglobin concentrations were inversely associated with peripheral pulse pressure. A direct association of haemoglobin concentrations with arterial stiffness has also been reported in two population studies[16,17] and patients with hypertension[18].

In contrast to our findings, Schwartz and colleagues[40] previously reported in an inverse association between haemoglobin levels and aortic pulse wave velocity. The mean age (SD) age was 64.4 (15.8) years in the Schwarz study compared to 56.4 (13.3) years in ours. Notably, a high aortic pulse wave velocity strongly predicts overall and cardiovascular mortality in end-stage renal disease among patients <60 years but loses its prognostic value in older patients[41]. The potential impact of age on haemoglobin-arterial function relationships in CKD merits further study. Wave reflection and pressure pulsatility were not explored in the Schwartz study.

Partial correction of severe anaemia targeting a haemoglobin level of 11.0 g/dl with erythropoietin stimulating agents in dialysis patients improves quality of life and reduces hospitaliza-

tion and the need for transfusion[42]. However, recent meta-analyses have documented an increased risk of all-cause mortality, stroke, hypertension and vascular access thrombosis[12,13] when a normal haemoglobin level of ~13 g/dl is targeted with erythropoietin stimulating agents in CKD patients. The reason(s) why the optimal haemoglobin target as relates to CVD risk is lower in CKD compared to non-CKD individuals and the potential involved mechanisms remain largely unknown. Postulates include highly prevalent underlying atherosclerotic disease in CKD, increased viscosity and platelet aggregation, endothelial damage, vasoconstriction and increased peripheral vascular resistance[31]. Moreover, given the design of recent trials, the effect of a haemoglobin level of 11.5 to 13 g/dl on the vasculature in CKD patients is currently unknown[10,11]. Accordingly, the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guideline and a commentary on a recent meta-analysis state that targeting a haemoglobin level of 11.5 to 13 g/dl may still need to be considered in some CKD patients[10,11]. It is in this context that our findings in ROC curve analysis are particularly relevant as they indicate that a haemoglobin target of 10.9 g/dl in dialysis patients is associated with optimal arterial function comprising of a lower frequency of both arterial stiffness and increased pressure pulsatility. Taken together, the optimal haemoglobin target in CKD may be determined by its effects on arteriosclerosis, which is a key mechanism of increased CVD in CKD[2,6]. Notably, our results also suggest that the haemoglobin level reached is more important than the intervention used to reach the respective level.

Heart failure is associated with reduced pulse wave velocity and wave reflection[24]. Additionally, angiotensin converting enzyme inhibitors and angiotensin receptor blockers can improve arterial function[25], and erythropoietin stimulating agents can impair nitric oxide production[26]. When we adjusted for these characteristics in separate models and performed sensitivity analyses, the partial R values for the haemoglobin-pulse wave velocity and haemoglobin-pressure pulsatility relations were materially unaltered. However, in sensitivity analyses, haemoglobin concentrations were additionally associated with reflection magnitude among patients without heart failure as well as in those using erythropoietin stimulating agents. Reduced nitric oxide activity is also implicated in increased wave reflection[43].

Interestingly, when we replaced mean arterial pressure by systemic vascular resistance in the respective models, the results were also materially unaltered. This suggests that potential effect of haemoglobin concentrations on reflection magnitude in dialysis patients is located centrally rather than at the peripheral or arteriolar level and is therefore unlikely to improve upon treatment with vasodilators.

Our study has limitations. Its design was cross-sectional, which precludes determining cause-effect relations. The numbers of patients, particularly in sensitivity analyses, were small. However, our main conclusions originated in comprehensively adjusted multivariable regression models. The strength of our study is that we performed a detailed evaluation of aortic function using SphygmoCor and other hemodynamic characteristics.

In conclusion, this study suggests that the optimal haemoglobin target in dialysis patients is ~11g/dl and determined by its differential and contrasting effects on arterial stiffness and pressure pulsatility.

Ethics statement

This study was performed according to the Helsinki Declaration of 1975 as revised in 2013 and was approved by the University of the Witwatersrand Human (Medical) research Ethics Committee (protocol number: M15-08-43) in Johannesburg, South Africa. Written informed consent was obtained in each patient prior to participation.

Acknowledgement

We thank Ms Noluntu Dlongolo for revising the manuscript.

Author contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

The study was supported by the South African National Research Foundation.

Disclosure

The authors declare no conflict of interest.

References

1. Moody WE, Edwards NC, Chue CD, Ferro CJ, Townsend JN. Arterial disease in chronic kidney disease. Heart 2013;99:365-372.
2. Zanoli L, Lentini P, Briet M, et al. Arterial stiffness in the heart of CKD. J Am Soc Nephrol 2019;30:918-928.
3. London GM. Arterial stiffness in chronic kidney disease and end-stage renal disease. Blood Purif 2018;45:154-8.
4. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. Circulation 2001;103:987-992.
5. Briet M, Boutouyrie P, Laurent S, London GM. Arterial stiffness and pulse pressure in CKD and ESRD. Kidney Int 2012;82:388-400.
6. London GM. Arterial stiffness in chronic kidney disease and end-stage renal disease. Blood Purif 2018;45:154-158.
7. Fernandez-Fresnedo G, Rodrigo E, de Francisco ALM, de Castro SS, Castaneda O, Arias M. Role of pulse pressure on cardiovascular risk in chronic kidney disease patients. J Am Soc Nephrol 2006;17:S246-S249.
8. McIntyre NJ, Fluck RJ, McIntyre CW, Fakis A, Taal MW. Determinants of arterial stiffness in chronic kidney diseased stage 3. PLoS One 2013;8:e55444.
9. Major RW, Cheng MRI, Grant RA, et al. Cardiovascular disease risk factors in chronic kidney disease: a systematic review and meta-analysis. PLoS One 2018;13:e0192895.
10. Wyatt CM, Drueke TB. Higher hemoglobin levels and quality of life in patients with advanced chronic kidney disease: no longer a moving target? Kidney Int 2016;89:971-975.
11. Kidney Disease: Improving. Global Outcomes (KDIGO) Anemia Work Group. KDIGO clinical practice guideline for anemia in chronic kidney disease. Kidney Int (Suppl) 2012;2:279-335.

12. Phrommintikul A, Haas SJ, Elsik M, Krum H. Mortality and target haemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: a meta-analysis. Lancet 2007:369:381-388.
13. Palmer SC, Navaneethan SD, Craig JC, et al. Meta-analysis: erythropoiesis-stimulating agents in patients with chronic kidney disease. Ann Int Med 2010;153:23-33.
14. Wilkinson IB, Franklin SS, Cockcroft JR. Nitric oxide and the regulation of arterial stiffness. Hypertension 2004;44:112-116.
15. Donadee C, Raat NJH, Kanias T, Tejero J, Lee JS, Kelly EE. Nitric oxide scavenging by red cell microparticles and cell-free haemoglobin as a mechanism for the red cell storage lesion. Circulation 2011;124:465-476.
16. Zhang Z-Z, Wang P, Kong X-L, Mao W-L, Cui M-Y. Association of haemoglobin with arterial stiffness evaluated by carotid-femoral pulse wave velocity among Chinese adults. Chronic Dis Transl Med. 2018 Jul 13;5(2):122-128.
17. Kawamoto R, Tabara Y, Kohara K, Miki T, Kusunoki T, Tatoh T. A slightly low haemoglobin level is beneficially associated with arterial stiffness in Japanese community-dwelling women. Clin Exp Hypertension 2012;34:92-98.
18. Chen H, Hua Q, Hou H. Association of haemoglobin with ambulatory arterial stiffness index in untreated essential hypertensive patients without anemia. Intern Med 2011;50:2759-2765.
19. Lullo LD, Gorini A, Russo D, Santoboni A, Ronco C. Left ventricular hypertrophy in chronic kidney disease patients: from pathophysiology to treatment. Cardiorenal Med 2015;5:254-266.
20. London GM, Gyerin AP, Marchais SJ, et al. Cardiac and arterial interactions in end-stage renal disease. Kidney Int 1996;50:600-608.
21. Gunter S, Robinson C, Woodiwiss AJ, et al. Arterial wave reflection and subclinical atherosclerosis in rheumatoid arthritis. Clin Exp Rheumatol 2018;36:412-420.

22. American Society of echocardiography: Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072-1083.
23. Townsend RR. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. Curr Opin Nephrol Hypertens 2015;24:47-53.
24. Feola M, Testa M, Ferreri C, Rosso GL, Rossi A, Ruocco G. The analysis of arterial stiffness in heart failure patients in comparison with healthy subjects with cardiovascular risk factors. J Clin Med 2019;8:1721; doi:10.3390/jcm8101721.
25. Liu M, Li G-L, Li Y, Wang J-G. Effects of various antihypertensive drugs on arterial stiffness and wave reflections. Pulse 2013;1:97-107.
26. Scalera F, Kielstein JT, Martens-Lobenhoffer J, Postel SC, Tager M, Bode-Borger SM. Erythropoietin increases asymmetric dimethylarginine in endothelial cells: role of dimethylarginine dimethylaminohydrolase. Am J Soc Nephrol 2005;16:892-898.
27. 2018 ESC/ESH guidelines for the management of hypertension. Eur Heart J 2018;39:3021-3104.
28. Van Bortel LM, Laurent S, Boutouyrie P, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens 2012;30:445-448.
29. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2013;31:1281-1357.
30. Fishbane S, Spinowitz B. Update on anemia in ESRD and earlier stages of CKD: core curriculum 2018. Am J Kidney Dis 2018;71:423-35.
31. Fishbane S, Besarab A. Mechanisms of increased mortality risk with erythropoietin treatment to higher haemoglobin targets. Clin J Am Soc Nephrol 2007;2:1274-82.
32. Ni Z, Morcos S, Vaziri ND. Up-regulation of renal and vascular nitric oxide synthase in iron-deficiency anaemia. Kidney Int 1997;52:195-201.

33. Metivier F, Marchais SJ, Guerin AP, Pannier B, London GM. Pathophysiology of anaemia: focus on the heart and blood vessels. Nephrol Dial Transplant 2000;15:14-18.
34. Chirinos JA, Segers P, Hughes T, Townsend R. Large-artery stiffness in health and disease. State-of-the-art review. J Am Coll Cardiol 2019;9:1237-1263.
35. Fazelli N, Hahan J-O. Estimation of cardiac output and peripheral resistance using square-wave-approximated aortic flow signal. Front Physiol 2012;3:298. doi: 10.3389/fphys.2012.00298. eCollection 2012.
36. Papaioannou TG, Vardoulis O, Stergiopulos N. The systolic volume balance method for noninvasive estimation of cardiac output based on pressure wave analysis. Am J Physiol Heart Circ Physiol 2012;302:H2064-H2073.
37. London GM, Gyerin AP, Marchais SJ, et al. Cardiac and arterial interactions in end-stage renal disease. Kidney Int 1996;50:600-608.
38. Yoon H, Lee JH, Kim GS, et al. The relationship between anaemia and pulse pressure and hypertension: The Korea National Health and Nutrition Examination Survey 2010-2012. Clin Exp Hypertension 2018:40:650-655.
39. Townsend RR, Chirinos JA, Parsa A, et al. Central pulse pressure in chronic kidney disease: A CRIC ancillary study. Hypertension 2010;56:518-24.
40. Schwartz CP, Koppelstaetter C, Amann E, Mayer G. Impact of anemia on aortic pulse wave velocity in hemodialysis patients. Kidney Blood Press Res 2009;32:210-216.
41. Ferreira JP, Girerd N, Pannier B, Rossignol P, London GM. High pulse wave velocity defines a very high risk cohort of dialysis patients under age 60. Am J Nephrol 2017;45:72-81.
42. Jones M, Ibels L, Schenkel B, Zagari M. Impact of epoetin alfa on clinical end points in patients with chronic renal failure. Kidney Int 2004;65:757-767.
43. Weber T, Maas R, Auer J, et al. Arterial wave reflections and determinants of endothelial function. A hypothesis based on peripheral mode of action. Am J Hypertens 2007;20:256-262.

Table 1 Recorded patient characteristics in 48 dialysis patients

Demographics			
Age (years)	55.8 (14.3)	Dialysis duration (months)	24 (12-36)
Female sex	22 (45.8)	Phosphate (mmol/l)	1.4 (0.9-1.8)
Black	27 (56.25)	Calcium (mmol/l)	2.3 (2.1-2.4)
Asian	11 (22.92)	Calcium x phosphate	3.0 (2.2-4.0)
Mixed	6 (12.5)	Phosphate binder use	35 (79.5)
White	4 (8.33)	High phosphate	44 (83.3)
Lifestyle		IPTH	470.3 (182.0-785.8)
Alcohol use (units per week)	1 (2.1)	High sensitivity CRP (mg/l)	8.9 (2.5-29.9)
Current smoker	0 (0)	Haemoglobin (g/dl)	10.8 (1.7)
Ex-smoker	4 (8.3)	Transferrin saturation (%)	22.9 (19.1-28.9)
Anthropometry		Ferritin (ng/ml)	361 (128-654)
Body mass index (kg/m^2)	26.8 (5.6)	Albumin (g/l)	37.9 (6.5)
Waist (cm)	99 (16)	Vitamin D (nmol/l)	19.7 (9.0)
Waist-hip ratio	0.98 (0.09)	Uric acid (mmol/l)	0.28 (0.12)
Weight (kg)	74.7 (14.7)	ESA use	43 (89.6)
Height (m)	1.67 (0.11)	Cardiovascular disease	14 (29.2)
Waist-height ratio	0.60 (0.11)	Heart Failure	7 (14.6)
Major traditional risk factors		Arterial function	
Hypertension	44 (91.7)	Pulse wave velocity (m/sec)	11.2 (7.8-14.9)
Systolic BP (mmHg)	146 (22)	Augmentation index (%)	27.1 (16.2)
Diastolic BP (mmHg)	85 (14)	Reflected wave pressure (mmHg)	25.0 (15.0-29.5)
Mean BP	105 (14)	Reflection magnitude (%)	67.0 (16.6)
Heart rate (beats/min)	75 (17)	Central systolic BP (mmHg)	136 (22)
Dyslipidemia	30 (71.4)	Central pulse pressure (mmHg)	49 (17)
Total cholesterol (mmol/l)	4.3 (1.1)	Pulse pressure amplification	132 (29)
LDL cholesterol (mmol/l)	2.4 (1.0)	Forward wave pressure (mmHg)	35 (11)
HDL cholesterol (mmol/l)	1.13 (0.90-1.55)	Other hemodynamics	
Non-HDL cholesterol (mmol/l)	3.1 (1.0)	Stroke volume (ml/beat)	71.4 (26.0)
Triglycerides (mmol/l)	1.33 (0.88-1.80)	Cardiac output (l/min)	5.5 (2.2)
Diabetes	19 (39.6)	Stroke work (gram-meters/beat)	153.2 (66.8)
ACEI/ARB therapy	37 (77.1)	Left ventricular mass index (g/m2)	103.4 (51.0)
Non-traditional/renal risk factors		SVR (mmHg/l/min)	20.4 (15.2-24.3)

Data are expressed as mean (SD), median (interquartile range) or number (%). Abbreviations: BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; iPTH, intact parathyroid hormone; CRP, C-reactive protein; ESA, erythropoietin stimulating agent; SVR, systemic vascular resistance.

Table 2 Associations of haemoglobin concentrations with arterial function in 48 dialysis patients

	Model 1[a]				Model 2[b]				Model 3[c]				Model 4[d]				Model 5[e]			
Arterial function parameter	β (SD)	Partial R	*P*	Model R^2	β (SD)	Partial R	*P*	Model R^2	β (SD)	Partial R	*P*	Model R^2	β (SD)	Partial R	*P*	Model R^2	β (SD)	Partial R	*P*	Model R^2
Log PWV	**0.030 (0.015)**	**0.329**	**0.05**	**0.314**	**0.031 (0.014)**	**0.366**	**0.03**	**0.450**	**0.031 (0.014)**	**0.378**	**0.03**	**0.476**	**0.031 (0.014)**	**0.375**	**0.03**	**0.468**	**0.031 (0.014)**	**0.374**	**0.03**	**0.461**
Aix	1.835 (1.215)	0.232	0.1	0.552	1.920 (1.183)	0.284	0.1	0.589	1.954 (1.192)	0.291	0.1	0.589	1.946 (1.220)	0.293	0.1	0.588	1.920 (1.192)	0.238	0.1	0.597
Log RWP	-0.027 (0.014)	-0.322	0.07	0.552	-0.026 (0.014)	-0.323	0.07	0.586	-0.026 (0.014)	-0.321	0.08	0.587	-0.020 (0.014)	-0.262	0.2	0.613	-0.026 (0.014)	-0.324	0.08	0.588
Rm	1.951 (1.226)	0.275	0.1	0.430	2.044 (1.184)	0.301	0.1	0.592	2.076 (1.194)	0.307	0.09	0.599	1.995 (1.224)	0.299	0.1	0.582	2.052 (1.190)	0.305	0.1	0.601
CSBP	**-2.231 (1.035)**	**-0.347**	**0.03**	**0.798**	**-2.150 (1.022)**	**-0.344**	**0.04**	**0.809**	**-2.151 (1.038)**	**-0.344**	**0.04**	**0.809**	-1.514 (1.091)	-0.246	0.1	0.816	**-2.153 (1.039)**	**-0.344**	**0.04**	**0.810**
CPP	**-3.126 (1.227)**	**-0.400**	**0.01**	**0.541**	**-3.005 (1.189)**	**-0.403**	**0.01**	**0.583**	**-3.004 (1.208)**	**-0.402**	**0.01**	**0.583**	-2.357 (1.208)	-0.336	0.06	0.480	**-3.003 (1.208)**	**-0.402**	**0.01**	**0583**
PPP	**-5.828 (1.616)**	**-0.510**	**0.001**	**0.390**	**-5.831 (1.589)**	**-0.521**	**0.001**	**0.426**	**-5.807 (1.612)**	**-0.520**	**0.001**	**0.426**	**-5.570 (1.670)**	**-0.502**	**0.002**	**0.424**	**-5.599 (1.611)**	**-0.506**	**0.001**	**0.43**
PPamp	-2.838 (2.138)	-0.222	0.2	0.502	-2.856 (2.173)	-0.223	0.2	0.502	-2.851 (2.206)	-0.223	0.2	0.503	-3.531 (2.280)	-0.272	0.1	-0.530	-2.939 (2.160)	-0.234	0.1	0.524
FWP	**-3.111 (0.862)**	**-0.544**	**0.001**	**0.480**	**-3.087 (0.870)**	**-0.544**	**0.001**	**0.489**	**-3.080 (0.884)**	**-0.543**	**0.002**	**0.490**	**-2.658 (0.858)**	**-0.512**	**0.005**	**0.546**	**-3.091 (0.880)**	**-0.546**	**0.001**	**0.49**

Significant associations are shown in bold. Abbreviations: β: regression coefficient; Log: logarithmically transformed; PWV: pulse wave velocity; Aix: augmentation index; RWP: reflected wave pressure; Rm: reflection magnitude; CSBP: central systolic blood pressure; CPP: central pulse pressure; PPP: peripheral pulse pressure; PPamp: pulse pressure amplification; FWP: forward wave pressure.

[a]Adjusted for age, sex, race, height, weight, mean arterial pressure and heart rate.

[b]Adjusted for age, sex, race, height, weight, mean arterial pressure, heart rate and diabetes.

[c]Adjusted for age, sex, race, height, weight, mean arterial pressure, heart rate, diabetes and heart failure.

[d]Adjusted for age, sex, race, height, weight, mean arterial pressure, heart rate, diabetes and angiotensin converting enzyme inhibitor or angiotensin receptor blocker therapy.

[e]Adjusted for age, sex, race, height, weight, mean arterial pressure, heart rate, diabetes and erythropoietin stimulating agent therapy.

Table 3 Associations of haemoglobin concentrations with arterial function in sensitivity analyses among patients without heart failure and on ACEI or ARB and ESA agent therapy

	Patients without heart failure (n= 41)				Patients on ACEI or ARB therapy (n=37)				Patients on ESA therapy (n=43)			
Arterial function	β (SD)	Partial R	*P*	Model R^2	β (SD)	Partial R	*P*	Model R^2	β (SD)	Partial R	*P*	Model R^2
Log PWV	**0.037 (0.015)**	**0.435**	**0.01**	**0.390**	0.031 (0.017)	0.357	0.07	0.440	**0.031 (0.014)**	**0.376**	**0.03**	**0.409**
Aix	2.347 (1.212)	0.355	0.06	0.621	1.792 (1.399)	0.269	0.2	0.519	**2.097 (1.030)**	**0.365**	**0.05**	**0.677**
Log RWP	-0.020 (0.014)	-0.264	0.2	0.550	-0.027 (0.016)	-0.350	0.1	0.643	-0.026 (0.014)	-0.327	0.08	0.537
Rm	**2.509 (1.203)**	**0.378**	**0.04**	**0.626**	1.861 (1.405)	0.278	0.2	0.512	**2.225 (1.015)**	**0.389**	**0.03**	**0.687**
CSBP	1.971 (1.017)	-0.334	0.07	0.796	-1.994 (1.159)	-0.338	0.09	0.824	-2.149 (1.051)	0.239	0.1	0.773
CPP	**-2.809 (1.212)**	**-0.407**	**0.02**	**0.555**	**-2.697 (1.230)**	**-0.416**	**0.03**	**0.644**	**-3.011 (1.244**	**0.404**	**0.02**	**0.535**
PPP	**-5.476 (1.723)**	**-0.508**	**0.004**	**0.382**	**-6.344 (1.807)**	**-0.567**	**0.002**	**0.447**	**-5.406 (1.763)**	**-0.482**	**0.004**	**0.336**
PPamp	-2.862 (2.484)	-0.216	0.3	0.496	-4.199 (2.764)	-0.302	0.1	0.525	-3.149 (2.091)	-0.265	0.1	0.557
FWP	**-2.888 (0.922)**	**-0.524**	**0.004**	**0.437**	**-2.190 (2.008)**	**-0.588**	**0.003**	**0.587**	**-3.125 (0.903)**	**-0.554**	**0.002**	**0.445**

Associations were assessed in age, sex, race, weight, height, heart rate, mean arterial pressure and diabetes adjusted linear regression models. Significant associations are shown in bold. Abbreviations: β: regression coefficient; Log: logarithmically transformed; PWV: pulse wave velocity; Aix: augmentation index; RWP: reflected wave pressure; Rm: reflection magnitude; CSBP: central systolic blood pressure; CPP: central pulse pressure; PPP: peripheral pulse pressure; PPamp: pulse pressure amplification; FWP: forward wave pressure.

Table 4 Bivariate associations among haemoglobin concentrations and hemodynamic variables

	Haemoglobin	Log SVR	Stroke volume	Cardiac output	Stroke work	LVMI
Haemoglobin	-					
Log SVR	**0.285 (0.05)**	-				
Stroke volume	**-0.290 (0.05)**	**-0.869 (<0.0001)**	-			
Cardiac output	**-0.319 (0.03)**	**-0.926 (<0.0001)**	**0.910 (<0.0001)**	-		
Stroke work	**-0.367 (0.01)**	**-0.732 (<0.0001)**	**0.925 (<0.0001)**	**0.867 (<0.0001)**		
LVMI	-0.154 (0.3)	**-0.359 (0.01)**	**0.346 (0.01)**	**0.409 (0.005)**	**0.337 (0.02)**	**-**

Results are expressed as Pearson correlation (p). Significant associations are shown in bold. Abbreviations: Log: Logarithmically transformed; SVR: systemic vascular resistance; LVMI: left ventricular mass index.

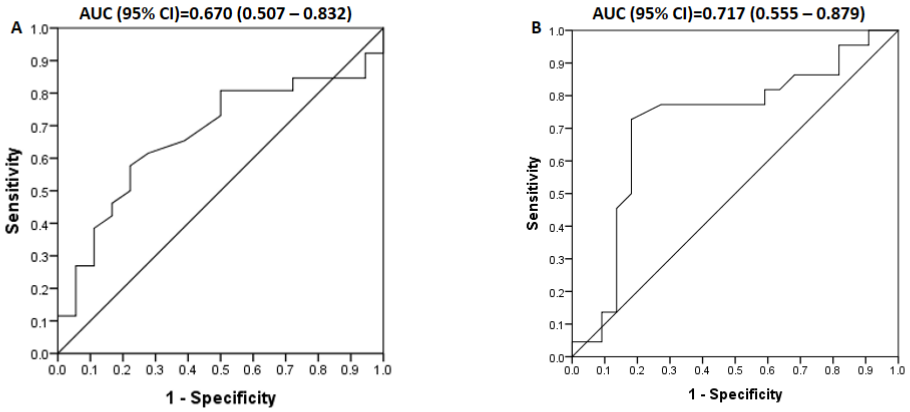


Figure 1: Receiver operator characteristic curves showing the accuracy of haemoglobin concentrations in predicting the presence of arterial stiffness (pulse wave velocity >10 m/sec) (A) and a normal central pulse pressure (<50 mmHg) (B). AUC: area under the curve.

Supplementary data

Methods

Cardiovascular risk factors

Hypertension was identified when the systolic or/and diastolic blood pressure were ≥ 140mmHg and 90mmHg, respectively, or/and antihypertensive agents were used. Analyses were performed on fasting blood samples using routine laboratory tests for the determination of lipid, glucose, phosphate, calcium, haemoglobin, albumin, uric acid and ferritin concentrations, transferrin saturation percentages. Intact parathyroid hormone levels were measured by an electrochemiluminescent immunoassay on Roche Cobas. High-sensitive C-reactive protein and vitamin D concentrations were both estimated on Abott Architect using an immunoturbidimetric assay and chemoluminescent microparticle immunoassay, respectively. Dyslipidemia was diagnosed when the total cholesterol: HDL cholesterol ratio was >4 or/and lipid lowering drugs were employed. Diabetes was identified in patients with a fasting glucose level of ≥7 mmol/l and in those that used glucose lowering medications.

Arterial function

Pulse wave velocity, central aortic blood pressure and reflected and forward wave pressures were evaluated using a high-fidelity SPC-301 micromanometer (Millar instument, Inc., Houston, Texas), interfaced with a computer utilizing SphygmoCor software, version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Subsequent to resting for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded for a time period of ten consecutive waveforms (heart beats). Calibration of the pulse wave was done by manual measurement (auscultation) of brachial BP taken immediately prior to recordings. An extensively validated generalized transfer function incorporated in the SphygmoCor software was used to convert the peripheral pressure wave form into a central aortic waveform[1-3]. The results were discarded when the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less that 80 mV. The aortic pulse wave velocity was calculated as distance in meters divided by transit time in seconds. The time delay in pulse waves between the carotid

and femoral sites was assessed by employing an ECG-derived R wave as a fudicial point. Pulse transit time was calculated as the mean of 10 consecutive beats. The difference between the distance from the femoral sampling site to the suprasternal notch and the distance from the carotid sampling site to the suprasternal notch represented the distance the pulse wave travels. Magnitude of the forward and reflected wave components of the aortic pressure waveform was determined by the SphygmoCor software, which separates the aortic waveform by using a modified triangular waveform. Augmentation index was calculated as (second systolic peak/first systolic peak) x 100, reflection magnitude as (reflected wave amplitude/forward wave amplitude) x 100 and pulse pressure amplification as (radial pulse pressure/aortic pulse pressure) x 100. All measurements were made by a single experienced observer who was unaware of the cardiovascular risk factor profiles of the patients. Brachial blood pressure was recorded in all patients. Technically sound measurements of the central pressure wave and pulse wave velocity were obtained in 44 patients each.

Other hemodynamic characteristics

Left ventricular dimensions were determined using two-dimensional directed M-mode echocardiography in the parasternal long axis view. Left ventricular dimensions were determined using two-dimensional directed M-mode echocardiography in the parasternal long axis view. Left ventricular end diastolic and systolic volumes were determined using the Teichholz method[4]. Stroke volume was determined from the difference between left ventricular end diastolic and systolic volumes as evaluated upon employing the Z-derived method[1]. Cardiac output was determined as stroke volume x heart rate. Left ventricular mass was determined using a standard formula[1] (left ventricular mass=0.8 x (1.04 (left ventricular end diastolic diameter + left ventricular septal wall thickness + left ventricular end diastolic posterior wall thickness)^3 – (left ventricular end diastolic diameter)^3 + 0.6) and indexed to body surface area. Echocardiographic measurements were made by the same experienced observer that performed the arterial function measurements. The intra-observer variation for echocardiographic measurements is low in our setting[5].

References

1. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. Eur Heart J 1993;14:160-167.
2. Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure wave from. Hypertension 2001;38:932-937.
3. Gallagher D, Adji A, O'Rourke MF. Validation of the transfer function technique for generating central from peripheral upper limb pressure waveform. Am J Hypertens 2004;17:1059-1067.
4. Techholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. Am J Cardiol 1976;37:7-11.
5. Mokotedi L, Gunter S, Robinson C, Norton GR, Woodiwiss AJ, Tsang L, Dessein PH, Millen AME. The impact of different classification criteria on the estimated prevalence and associated risk factors of diastolic dysfunction. Int J Rheumatol; 2017;2017:2323410, Article ID 2323410.

Summary of the findings, potential implications and conclusions

This thesis comprises 4 original studies on subclinical and/or established CVD and its risk factors among South African patients with CKD that were recruited at Milpark Hospital in Johannesburg, South Africa. Enrolled participants included predialysis and dialysis patients as well as kidney transplant recipients.

In the **first investigation**, we compared cardiovascular risk factor profiles and disease between black compared and other African predialysis and dialysis patients. Black American CKD patients experience more rapid CKD progression and a mostly similar or larger overall

mortality rate compared to their white counterparts (Choi AI et al, 2009; Hounkpatin HO et al, 2020). Increased overall mortality is particularly noticeable in black Americans with an eGFR of 45 to 59 ml/min/1.73m^2 (Choi AI et al, 2009). Hypertension and diabetes are also more prevalent in black American CKD patients. Cardiovascular mortality is increased in black predialysis patients but decreased once they are on dialysis, which is likely due to a survival bias (Carnethon MR et al, 2017).

It is interesting and important to consider how these reported findings compare to those in the first study of the present thesis. In this regard, we assessed cardiovascular risk factor profiles, aortic function including pulse wave velocity, wave reflection and central systolic and pulse pressure, left ventricular diastolic and systolic function, atherosclerosis extent as well as cardiovascular event rates. We found that in adjusted analysis, among predialysis patients, black compared to other Africans have more adverse traditional and non-traditional cardiovascular risk factor profiles, enhanced wave reflection and central pressures, and impaired diastolic function but similar cardiovascular event rates. By contrast, black compared to other African dialysis patients have similar traditional and non-traditional cardiovascular risk factor profiles and aortic and diastolic function but smaller cardiovascular event rates. The latter finding suggests the presence of a selection bias as was previously documented in black American dialysis patients. With regard to African predialysis patients, our findings may be due to an earlier epidemiological transition stage. However, given the considerably increased cardiovascular risk and subclinical cardiovascular disease burden in black African predialysis patients, it is also feasible if not likely that cardiovascular mortality is actually increased in this group. We could not address this likely possibility in black compared to other African CKD patients as our study design was cross-sectional. The same applies to disease progression. Future longitudinal studies are clearly needed to address these pertinent issues.

The other major finding of the first study of this thesis is that, overall, black compared to other African CKD patients currently experience significantly less frequent severe atherosclerosis

despite an increased cardiovascular risk factor burden. It should be noted in this regard that, while prevalent atherosclerosis is enhanced by CKD (Drueke TB et al, 2010), arteriosclerosis with consequent impaired arterial function (Zanoli L et al, 2009) and myocardial fibrosis that leads to impaired diastolic function that is often accompanied by left ventricular hypertrophy (Wang X et al, 2019), rather than atherosclerosis *per se* (Drueke TB et al, 2010) comprise the core CKD induced cardiovascular changes. In other words, CKD patients may be particularly predisposed to non-artherosclerotic CVD, which in itself is strikingly more prevalent even in the black African population at large (Pillay-van Wyk V et al, 2016). More importantly, the first study of this thesis revealed that the Framingham score is not reliable (area under the curve (AUC) of the receiver operator characteristic (ROC) curve=0.556 (0.375-0.737), p=0.6) in identifying black African CKD patients with very high risk atherosclerosis as represented by plaque presence; by contrast, the Framingham score performed remarkably well (AUC of the ROC curve=0.818 (0.714-0.921), p <0.0001) in determining whether other African CKD patients were at very high risk of atherosclerotic CVD.

The findings of the first study of this thesis therefore have important clinical implications. Firstly, consistent and adequate CVD risk management is strongly indicated in black African predialysis patients. Secondly, carotid and femoral artery ultrasound may need to be consistently performed in black African CKD patients in order to identify those with very high risk atherosclerosis, which was still present in 30.4% of them. The same phenomenon is well documented in black African patients with rheumatoid arthritis (Solomon A et al, 2020). These issues may need to be addressed in order to reduce racial disparities in disease outcomes among African CKD patients.

In the **second study** of this thesis, we compared potential determinants of the E/e' ratio between non-dialysis and dialysis patients. As dealt with in the introduction of this thesis, E/e' comprises one of the important parameters that are used in currently recommended algorithms

in the identification of patients with diastolic dysfunction. Based on previously reported investigations, we assessed the potential influence of volume overload and impaired arterial function on the E/e' ratio in predialysis compared to dialysis patients. As also based on previously reported data, we further explored whether cardiovascular risk factors including diabetes and haemoglobin concentrations could attenuate the respective relationships.

We found that CKD status (dialysis versus non-dialysis) influenced the relationship of pulse wave velocity with E/e' independent of established confounders (interaction p=0.01). Consequently, in stratified analysis, arterial function measures were significantly associated with E/e' in non-dialysis but not dialysis patients. Although NT-pro-BNP concentrations were associated with E/e' in both non-dialysis and dialysis patients, upon entering arterial function measures and NT-proBNP levels simultaneously in regression models, only arterial function indices were associated with E/e' in non-dialysis patients and only NT-proBNP concentrations was related to E/e' in dialysis participants. Diabetes attenuated the arterial function-E/e' associations in non-dialysis patients whereas haemoglobin levels attenuated the NT-pro-BNP-E/e' relations in dialysis patients. In additional separate models, haemoglobin concentrations were associated with E/e' independent of preload and afterload measures and left ventricular mass among dialysis patients.

Given these results, potential determinants of diastolic function may indeed, at least to an extent, differ in non-dialysis compared to dialysis patients. In this regard, NT-proBNP concentrations were markedly larger and haemoglobin levels substantially smaller in dialysis compared to predialysis patients. Arterial function was largely similar among both groups. We therefore believe that volume overload and anaemia may override the effects of impaired arterial function and diabetes on E/e' once CKD patients are on dialysis.

In relation to impaired arterial function, pulse wave velocity and wave reflection mediate cardiac afterload in early and late systole, respectively (Chirinos J et al, 2019; Peterson VR et al, 2016). It
is therefore noticeable that both pulse wave velocity and wave reflection were associated with E/e' in predialysis patients.

Anaemia may impair diastolic dysfunction by causing a hyperdynamic circulation and volume overload and myocardial ischemia and fibrosis (Wang X, 2019; Mistry N et al, 2018; Caramelo C et al, 2007; D'Amario D et al, 2019). Anaemia can also cause left ventricular hypertrophy. It is therefore of interest that, in separate models among dialysis patients, haemoglobin concentrations were associated with E/e' independent of measures of cardiac preload and afterload as well as left ventricular mass. Anaemia may therefore impair diastolic function through reduced cardiomyocyte oxygenation in CKD patients. This hypothesis requires further study.

What are the main potential implications of the present findings? It is conceivable that volume overload, anaemia, impaired arterial function as well as diabetes are involved in the development of impaired diastolic function in at least some patients with CKD, irrespective of their predialysis, dialysis and even renal transplant status (Wang X et al, 2019; Zanoli L et al, 2019). Indeed, renal transplant recipients do not experience a fully restored glomerular filtration rate (Devine PA et al, 2019). In the present context, our cross-sectional study design and relatively small number of particularly dialysis patients (n=41) are important limitations. Nevertheless, our results do suggest that, overall, impaired arterial function and diabetes are predominant potential risk factors for impaired diastolic function in predialysis patients whereas volume overload and anaemia are the most important potential determinants of impaired diastolic function in dialysis patients.

Whereas aortic stiffness attenuation with antihypertensives in ESRD can improve survival, (Guerin AP et al, 2001), effective treatment of HFpEF remains most challenging at present

(Redfield MM, 2016; Pfeffer MA et al, 2019). In view of these circumstances and our current results, future studies that determine whether impaired arterial function and glucose control in predialysis patients and adequate management of volume overload and anaemia (Wyatt CM et al 2016) among those on dialysis can prevent the development of diastolic dysfunction and HFpEF, are indicated.

In the **third study** of this thesis, we assessed the potential role of post transplantation anaemia and persistent secondary hyperparathyroidism in diastolic function among stable kidney transplant recipients. The reasons for this undertaking are given in the introduction of this thesis.

Post transplantation anaemia and persistent secondary hyperparathyroidism were present in 27.9% and 30.8% of kidney transplant recipients. Overweight or obesity was identified in 67.5% of them. Haemoglobin concentrations were inversely associated with E/e' whereas intact parathyroid hormone levels were directly related to E/e'. Adiposity measures including waist-height ratio, waist circumference and body mass index were each associated with both e' and E/A. The haemoglobin-E/e', intact parathyroid hormone-E/e', anthropometric measure-e' and anthropometric measure-E/A relationships were each independent of left ventricular mass index and cardiac preload and afterload measures. Estimated glomerular filtration rate was associated with E/e' but this relationship was no longer significant after adjustment for established confounders as well as haemoglobin and intact parathyroid hormone levels. In interaction analysis, transplant duration did not influence the haemoglobin-E/e' and intact parathyroid hormone-E/e'. Consequently, in stratified analysis the respective relationships were similar in those with a transplantation duration of <13.3 (median value in the whole group) and $\geq$13.3 years. However, transplant duration did impact the waist-height-e' (interaction p=0.03) relationship. Consequently, in stratified analysis, anthropometric measures were associated with e' and E/A in those with a transplant duration of $\geq$13.3 years but not shorter transplant duration.

What are the main implications of these findings? Firstly, a striking finding was that the associations of post transplantation anaemia, persistent secondary hyperparathyroidism and excess adiposity with diastolic function measures were consistently independent of cardiac preload and afterload parameters as well as left ventricular mass. This points towards potential direct effects of the respective risk factors on the myocardium in kidney transplant recipients as has been suggested in non-CKD persons and experimental models (Mistry N et al, 2018; Caramelo C et al, 2007; D'Amario D et al, 2019; Fujii H et al, 2018; Russo C et al, 2011; Libhaber CD et al, 2009).

Secondly, largely in keeping with previous reports (Gafter-Gvili A et al, 2019; Vanrentherghem Y et al, 2003), post transplantation anaemia was markedly prevalent and none of the affected patients were receiving iron or erythropoietin stimulating agent therapy. Haemoglobin levels were independently associated with E/e'. Persistent secondary hyperparathyroidism was equally prevalent and also independently associated with E/e'. Effective management strategies for this comorbidity have been reported (Santos RD et al, 2019). Treatment with the calcimimetic agent cinacalcet was shown to reduce not only left ventricular hypertrophy but also E/e' in dialysis patients (Choi SR et al, 2012). Finally, excess weight was frequently observed in our patients and, as applies to non-CKD persons (Nagueh S et al, 2016; Russo C et al, 2011; Libhaber CD et al, 2009), adiposity measures were associated with e' and E/A, particularly in those with long transplant duration. Excess adiposity is a documented risk factor for heart failure in kidney transplant recipients (Devine PA et al, 2019). Taken together, post transplantation anaemia, persistent secondary hyperparathyroidism and excess adiposity comprise frequent comorbidities that may be in need of more systematic management among kidney transplant recipients. Whether such interventions can prevent the development of HFpEF merits further study.

In the **fourth study** of this thesis, we hypothesized that the optimal haemoglobin target depends on its opposing effects on arterial stiffness and pressure pulsatility in CKD patients. We

simultaneously assessed the potential impact of haemoglobin concentrations on pulse wave velocity, wave reflection and pressure pulsatility as arterial function markers, as well as other hemodynamic characteristics in dialysis patients. The most striking and novel finding in this study was that haemoglobin concentrations were associated not only with arterial stiffness but also simultaneously with low pressure pulsatility measures including central systolic blood pressure, central and peripheral pulse pressure as well as forward wave pressure. In ROC curve analysis, the optimal haemoglobin concentration cut-off values in predicting arterial stiffness and increased central pulse pressure were remarkably similar at 10.95 g/dl and 10.85 g/dl respectively, and with clinically useful sensitivities, specificities and positive and negative predictive values. A haemoglobin value of >10.9 mg/dl was independently and strongly associated with both arterial stiffness (OR (95% CI) = 10.48 (1.57 – 70.08), p=0.02) and normal central pulse pressure (OR (95% CI) = 7.55 (1.58 – 36.03), p=0.01).

These findings have at least two potential implications. Firstly, they provide a potential novel mechanism that could explain why the optimal haemoglobin level is lower in at least dialysis patients as compared to in the non-CKD population. Secondly, due to previous trial designs, it remains uncertain as to whether targeting a haemoglobin level of 11.5 to 13 g/dl may still be justified in certain dialysis patients (Wyatt CM et al, 2016; Kidney Disease: Improving Global Outcomes (KDIGO) Anaemia Work Group, 2012). Our results obtained in ROC curve analysis argue against targeting a haemoglobin level that is larger than ~11 g/dl in dialysis patients.

Before getting to the conclusion of the discussion section of this thesis, some **interesting and important issues have arisen from the comments given by the reviewers on the current work**. In this regard, although patients with CKD are often reported to die from CVD before they develop ESRD, this was not the case in the recent Chronic Renal Insufficiency Cohort (Ku E et al, 2020). This study included 1650 black and 1638 white participants with a median follow-up of 7.1 years. The frequency of death and ESRD were 13% and 15%, respectively, in white patients, and 16% and 30%, respectively, in black participants.

The poorer performance of the Framingham score in identifying atherosclerotic CVD among our black CKD patients was also recognized in previous studies (Drawz PE, 2012; Weiner DE, 2007).

Our patients were all investigated after one day of no dialysis, i.e. not on a Monday as two days of no dialysis (after a weekend) could have impacted volume overload estimation.

NT-proBNP is probably cleared from the plasma by renal excretion and thereby results in higher circulating concentrations in relation to decreasing kidney function (Hall C, 2005).

We have used the term ‘pulsatility’ in our manuscripts. This represents the rhythmic increases and decreases in arterial pressure during repeated cardiac cycles.

With regard to paper 4, it is particularly cell-free haemoglobin and red blood cell microparticles rather than intracellular haemoglobin that acts as a nitric oxygen scavenger (Donadee et al, 2011). Hence, hemolysis is potentially most important in the present context. These issues should be considered in future studies on the effects of haemoglobin on vascular and cardiac function among CKD patients.

An initial power analysis had revealed that we needed to investigate 150 CKD patients to adequately identify changes in cardiac and vascular structure and function. Nevertheless, future studies will be needed to confirm our findings that were obtained in subgroup incuding sensitivity analyses.

All enrolled patients had been managed previously in our clinic and hence, none of them comprised new referrals. The first presenters on each day were consecutively enrolled. Only one of all invited patients refused participation.

All procedures in each of the studies were performed on a dialysis-free day. To determine that our findings on the potential effects of haemoglobin on aortic function are truly CKD specific as given in study 4, the respective relationships require future evaluation in non-CKD persons as we did not include a control group. In the respective study 4, we proposed that our findings were explainable on the basis of the reported nitric oxide scavenging effect of haemoglobin. However, haemoglobin also has antioxidant effects and both anaemia and high haemoglobin can have numerous other negative effects on haemodynamics. These issues should also be addressed in future studies.

In study 1, we found that in black CKD patients, the Framingham score was not related to carotid artery plaque presence. However, this finding requires cautious interpretation. Firstly, both employed measures have their potential shortcomings in predicting cardiovascular events in general and likely even more so in CKD patients. Moreover, black patients were younger and experienced higher blood pressure compared to their non-black counterparts, which may have impacted the variability of the Framingham score in the former group.

With regard to paper 3, anti-rejection drug use may have impacted vascular and/or cardiac function but our study design did not allow for addressing this issue.

In **conclusion**, black compared to other African predialysis patients experience an increased traditional and non-traditional cardiovascular risk factor burden and more impaired arterial and diastolic function. Once on dialysis, black African patients sustain less frequent cardiovascular event rates, which may represent a survival bias. The atherosclerotic burden is smaller in black compared to other African CKD patients but the Framingham score is unreliable in identifying those with very high risk atherosclerosis, which is nevertheless present in ~30% of them. Cardiovascular risk factors that are independently associated with markers of diastolic function include impaired arterial function and diabetes in predialyis patients, NT-pro-BNP levels as a

marker of volume overload, and low haemoglobin concentrations in dialysis patients and post transplantation anaemia and persistent secondary hyperparathyroidism in kidney disease transplant recipients. The optimal haemoglobin target in dialysis patients may be determined by its contrasting effects on aortic stiffness and pressure pulsatility. These findings have implications in our understanding of CVD risk and its management in CKD patients.

References

Caramelo C, Justo S, Gil P. Anemia in heart failure: pathophysiology, pathogenesis, treatment, and incognitae. Rev Esp Cardiol 2007;60:848-860.

Carnethon MR, Pu J, Howard G et al. Cardiovascular health in African Americans: A scientific statement from the American Heart Association. Circulation 2017:136:e393-e423.

Chirinos JA, Segers P, Hughes T, Townsend R. Large-artery stiffness in health and disease. J Am Coll Cardiol 2019;9:1237-63.

Choi AI, Rodriguez RA, Bacchetti P et al. White/black racial differences in risk of end-stage renal disease and death. Am J Med 2009;122:672-8.

Choi SR, Lim JH, Kim MY et al. Cinacalcet improves endothelial dysfunction and cardiac hypertrophy in patients on hemodialysis with secondary hyperparathyroidism. Nephron Clin Pract 2012;122(1-2):1-8.

D'Amario D, Migliaro S, Borovac JA et al. Microvascular dysfunction in heart failure with preserved ejection fraction. Front Physiol 2019;10:1347. doi: 10.3389/fphys.2019.01347. eCollection 2019.

Devine PA, Courtney AE, Maxwell AP. Cardiovascular risk in renal transplant recipients. J Nephrol 2019;32(3):389-399.

Donadee C, Raat NJH, Kanias T et al. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 2011;124:465-476.

Drawz PE, Baraniuk S, Davis BR et al. Cardiovascular risk assessment: addition of CKD and race to the Framingham equation. Am Heart J 2012;164:925-931.

Drueke TB, Massy ZA. Atherosclerosis in CKD: differences from the general population. Nat Rev Nephrol 2010;6:723-35.

Gafter-Gvili A, Gafter U. Posttransplantation anemia in kidney transplant recipients. Acta Haematol 2019;142(1):37-43.

Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. Circulation 2001;103:987-92.

Hall C. NT-ProBNP: the mechanism behind the marker. J Card Fail 2005;11 (5 Suppl):S81-S83.

Hounkpatin HO, Fraser SDS, Honney R, Dreyer G, Brettle A, Roderick PJ. Ethnic minority disparities in progression and mortality of pre-dialysis chronic kidney disease: a systematic scoping review. BMC Nephrol (2020) 21:217.

Kidney Disease: Improving. Global Outcomes (KDIGO) Anemia Work Group. KDIGO clinical practice guideline for anemia in chronic kidney disease. Kidney Int (Suppl) 2012;2:279-335.

Ku E, Yang W, McCulloch CE et al. Race and mortality in CKD and dialysis: findings from the Chronic Renal Insufficiency Cohort (CRIC) Study. Am J kidney Dis 2020;75:394-403.

Libhaber CD, Norton GR, Majane OHI et al. Contribution of central and general adiposity to abnormal left ventricular diastolic function in a community sample with high prevalence of obesity. Am J Cardiol 2009;104(11):1527-1233.

Mistry N, Mazer CD, Sled JG, Lazarus AH, Cahill LS, et al. Red blood cell antibody-induced anemia causes differential degrees of tissue hypoxia in kidney and brain. Am J Physiol Reg Integr Comp Physiol 2018;314:R611-R622.

Nagueh SF, Smiseth OA, Appleton CP et al. Recommendations for the evaluation of ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016;29:277-314.

Peterson VR, Woodiwiss AJ, Libhaber CD, Raymond A, Sareli P, Norton GR. Cardiac diastolic dysfunction is associated with aortic wave reflection, but not stiffness in a predominantly young-to-middle-aged community sample. Am J Hypertens 2016;29:1148-57.

Pillay-van Wyk V, Msemburi W, Laubscher R et al. Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. Lancet 2016;4:e642-e653.

Redfield MM. Heart failure with preserved ejection fraction. N Engl J Med 2016;375:1868-77.

Pfeffer MA, Shah AM, Bolaug BA. Heart failure with preserved ejection fraction. In perspective. Circ Res 2019;124:1598-617.

Russo C, Jin Z, Homma S et al. Effect of obesity and overweight on left ventricular diastolic function: a community-based study in an elderly cohort. J Am Coll Cardiol 2011;57(12):1368-1374.

Santos RD, Rossi A, Coyne D, Maw TT. Management of post-transplant hyperparathyroidism and bone disease. Drugs 2019;79(5):501-513.

Solomon A, Stanwix AE, Castañeda S. Points to consider in cardiovascular disease risk management among patients with rheumatoid arthritis living in South Africa, an unequal middle income country. BMC Rheumatol. 2020 Jun 16;4:42. doi: 10.1186/s41927-020-00139-2. eCollection 2020.PMID: 32550295.

Vanrenterghem Y, Ponticelli C, Morales JM et al. Prevalence and management of anemia in renal transplant recipients: a European survey. Am J Transplant 2003;3(7):835-845.

Wang X, Shapiro JI. Evolving concepts in the pathogenesis of uraemic cardiomyopathy. Nat Rev 2019;15:159-74.

Wiener DE, Tighiouart H, Griffith JL et al. Kidney disease, Framingham risk score, and cardiac and mortality outcomes. Am J Med 2007;120:552.e1-8.

Zanoli L, Lentini P, Briet M et al. Arterial stiffness in the heart disease of CKD. J Am Soc Nephrol 2019;30:918-28.

R14/49 Dr Hon-Chun Hsu

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150843

NAME: (Principal Investigator)	Dr Hon-Chun Hsu
DEPARTMENT:	School of Physiology Milpark Hospital
PROJECT TITLE:	Cardiovascular Disease and its Risk Factors in South African Chronic Kidney Disease Patients from Developing and Developed Population
DATE CONSIDERED:	28/08/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Patrick Dessein and Prof Gavin Norton

APPROVED BY: Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 16/09/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature Date 18/9/2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES