Circulating non-coding RNAs in kidney disease

Zhao Q.

CHAPTER 3

Association of circulating miRNAs and vascular injury markers with cardiac function in older patients reaching end-stage renal disease

Qiao Zhao[1,2], Sabine J.L. Nooren[1,2], Laurien Zijlstra[3], Wouter Jukema[3], Noeleen C. Berkhout-Byrne[1], Ton J. Rabelink[1,2], Anton Jan van Zonneveld[1,2], Marjolijn van Buren[1,4*], Simon Mooijaart[5,6*], Roel Bijkerk[1,2*].

[1]Department of Internal Medicine (Nephrology), [2]Einthoven Laboratory for Vascular and Regenerative Medicine, [3]Department of Cardiology. [4]Department of Nephrology, HAGA Hospital, The Hague, The Netherlands. [5]Department of Internal Medicine (Gerontology and Geriatrics), [6]Institute of Evidence-Based Medicine in Old Age, Leiden, the Netherlands. *Authors contributed equally.

Noncoding RNA. 2022 Jan 10;8(1):2.

Abstract

The prevalence of end-stage renal disease (ESRD) is rapidly increasing and mostly occurring in patients aged 60 years or older. The main cause of death in these patients is cardiovascular disease (CVD). Therefore, novel markers of vascular integrity may be of clinical value for identifying patients at high risk for CVD. As such, here we associated the levels of circulating angiogenic miRNAs, angiopoietin-2 (Ang-2) and asymmetric dimethylarginine (ADMA) with cardiac function in older patients with ESRD. 67 patients were included from 'The Cognitive decline in Older Patients with End stage renal disease' (COPE) prospective, multicentered cohort study. The circulating levels of selected angiogenic miRNAs, as well as the concentrations of Ang-2 and ADMA were measured and associated with measures of cardiac function: pulse wave velocity (PWV), ejection fraction (EF) and cardiac index (CI). We found Ang-2 and miR-27a to be strongly correlated to the PWV, while Ang-2 also associated with ejection fraction. In addition, we determined the association between the vascular injury markers and specific heart conditions and observed that ESRD patients with coronary heart disease have significantly higher levels of circulating ADMA and miR-27a. Finally, circulating levels of miR-27a were significantly higher in patients with atrial fibrillation. Taken together, elderly with ESRD display altered levels of vascular injury markers in relation to an impaired cardiac function that may serve to identify individuals at risk of CVD, as well as give insight into the underlying (vascular) pathophysiology.

Introduction

Chronic kidney disease (CKD) has a worldwide prevalence of about 10% which is rapidly increasing.[1-3] Eventually, these patients may develop end stage renal disease (ESRD) and become dependent on renal replacement therapy (transplantation or dialysis). Currently, half of all new ESRD patients are aged 60 years or older.[4,5] These older patients with ESRD have an increased risk for adverse health events, such as cognitive impairment [6,7] and cardiovascular disease (CVD).[8-11] The 1-year probability for an elderly with chronic kidney disease (CKD) on developing CVD is 3.3% greater than that of the normal population,[9] with the risks for elderly with ESRD being even higher,[12] while premature CVD is the main cause of death in these patients.[8,11,13,14] The high incidence of CVD in ESRD relates to CKD-induced alterations to the (micro)vasculature,[15] especially in the heart,[13] affecting cardiovascular structure and function as defined by arterial stiffness (pulse wave velocity (PWV)), and by the systolic heart function (cardiac index (CI) and the ejection fraction (EF)).[16,17] However, the exact underlying

pathophysiological mechanisms behind the impaired cardiovascular structure and function and the reason why ESRD patients develop CVD at different rates or to a different extent remains unclear.

Vascular injury related factors such as Angiopoietin-2 (Ang-2) and asymmetric dimethylarginine (ADMA), together with (circulating) angiogenic miRNAs play a major role in mediating vascular injury or maintaining microvascular integrity.[18-22] As such, assessing their circulating levels in disease may provide insight into the underlying vascular pathophysiology, as well as provide potential markers for development of (micro)vascular injury and CVD.[22-26] E.g. it has been shown that circulating miRNAs can serve as biomarkers for myocardial infarction (miR-208)[27] and heart failure (miR-423).[28] In this study, we aimed to investigate whether circulating angiogenic miRNAs and the vascular injury markers Ang-2 and ADMA are also directly correlated with cardiovascular structure and function in older patients with ESRD (study overview in Figure 1).

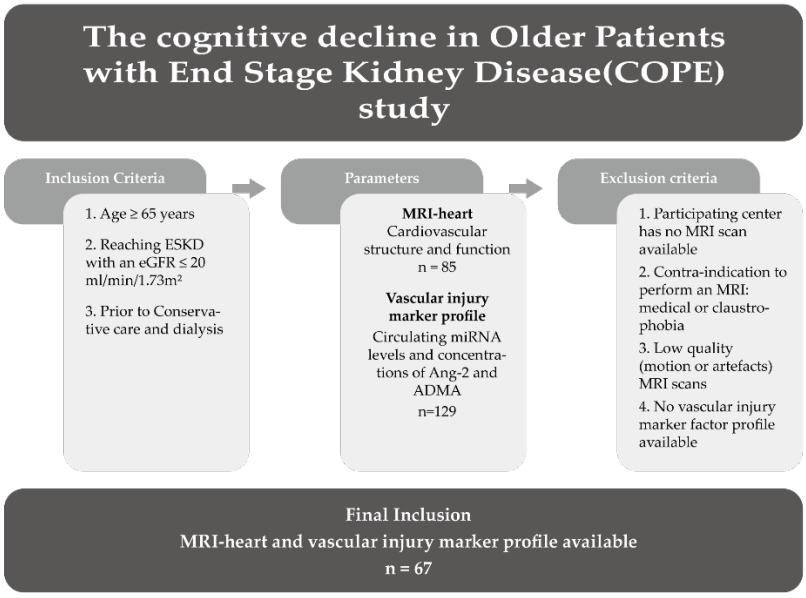


Figure 1. Flow Chart of study population selected from the COPE study. The COPE study contained 157 older patients with ESKD, from which multiple parameters were measured. This study only included the patients with available MRI scans of the heart and with a vascular injury marker profile. Other patients were excluded, which resulted in a study population of 67 patients.

Results

Patient cohort description

67 patients of the COPE study had a cardiac MRI scan and microRNA profile available and were included in this study (Figure 1). Table 1 shows the baseline clinical characteristics of this study population. The mean (± SD) age of this population was 75.1 years (± 6.6 years) and 44 (65.7%) participants were male. At baseline, the mean (± SD) eGFR was 16.0 ml/min/1.73m2 (± 4.0 ml/min/1.73m2) and the primary kidney disease had a vascular cause, mainly diabetes or hypertension, in 41 (61.2%) participants. The median [IQR] of the cardiovascular function parameters was 9.8 m/s [8.0 – 13.7] for the pulse wave velocity (PWV), 61% [51 – 66] for the ejection fraction (EF) and 2.5 L/min/m2 [2.1 – 3.0] for the cardiac index (CI). Cardiac or vascular conditions, shown in the comorbidities section of Table 1, were seen in 59.7% of the study population (40 participants).

Associations between cardiac function and vascular injury markers

Table 2 shows the correlations and associations between the cardiac function parameters and the vascular injury markers, namely circulating angiogenic miRNAs, angiopoietin II (Ang-2) and asymmetric dimethylarginine (ADMA).

To assess associations, we first used cut-off values to divide the cardiac parameters into two dichotomized groups and tested whether the concentration or levels of the vascular injury markers were significantly different between the two groups. Second, we assessed the (continuous) correlation between injury marker and cardiac function parameters.

For the dichotomized groups, we observed that for PWV, the circulating levels of angiogenic miR-27a were significantly lower in the group with a PWV > 10 m/s (P = 0.012), while circulating levels of Ang-2 showed a trend towards upregulation in the high PWV group, but this was not statistically significant (P = 0.083). No statistically significant differences were found in the dichotomized CI groups and EF groups. However, we found a trend of higher circulating miR-326 levels in the group with EF<50% ((P = 0.061).

For the continuous correlation assessment, two statistically significant correlations between the pulse wave velocity and the vascular injury markers were discovered, namely with Ang-2 and miR-27a. A higher PWV, which indicates a higher aortic stiffness, was strongly correlated with higher serum Ang-2 concentrations (r = 0.45, P = 0.000) and with lower

circulating levels of miR-27a (r = -0.389, P = 0.001). Furthermore, the circulating levels of Ang-2 were found to be negatively correlated with ejection fraction (r = -0.250, P = 0.035). In addition, we found a trend towards an inverse correlation of miR-326 with EF ((P = 0.079).

Next, we aimed to assess whether these statistically significant correlations were particularly pronounced in the two separate groups (i.e. PWV either higher or lower than 10 m/s; EF either higher or lower than 50%). As such, when the miR-27a and Ang-2 correlations were tested in the group with a relatively good PWV (≤ 10 m/s) or bad PWV (> 10 m/s) separately, the discovered correlation between PWV and Ang-2 and correlation between PWV and miR-27a remained significant (r = 0.583, P = 0.001; r= - 0.379, P = 0.033 respectively) within the group with a PWV > 10 m/s (Figure 2A-B). Interestingly, when testing the discovered correlations between the two mentioned vascular injury markers and PWV within the group with a PWV ≤ 10 m/s, the correlation between PWV and Ang-2 or miR-27a was not statistically significant anymore (r = 0.016, P = 0.931; r = -0.232, P = 0.187, respectively). The correlation between Ang-2 and EF was no longer statistically significant when analyzed in the EF< or >50^% groups separately (Figure 2C).

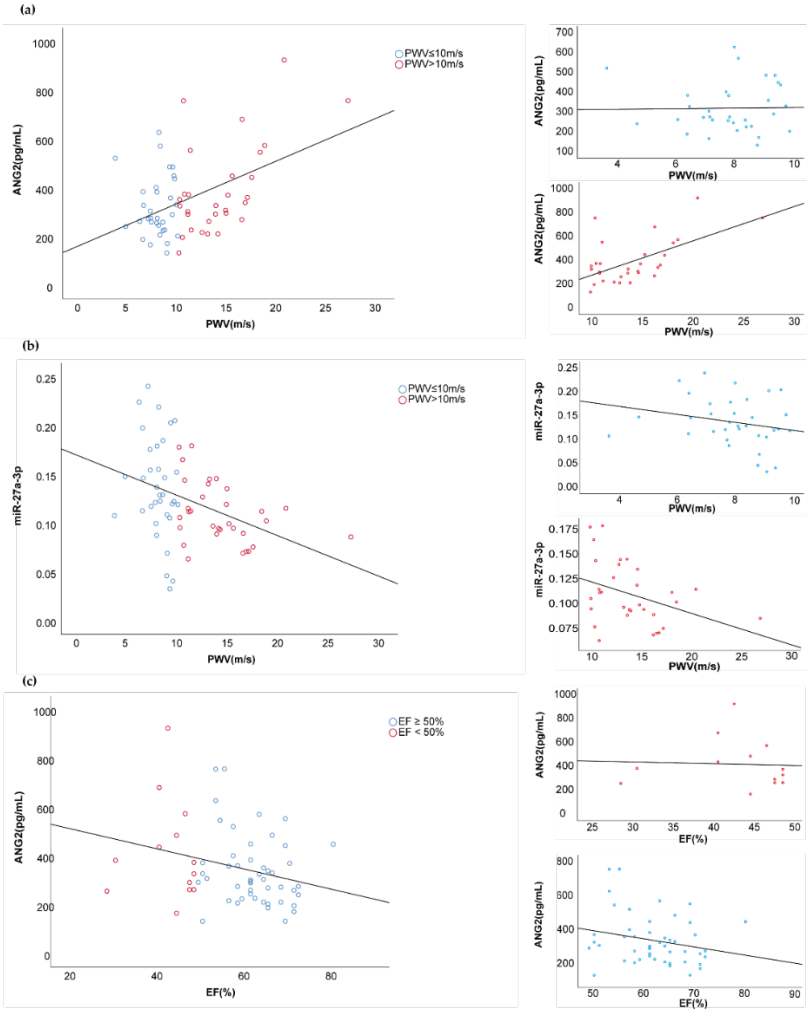


Figure 2. Correlations between PWV and the vascular injury markers Ang-2 and miR-27. Blue dots represent better cardiovascular condition patients whose PWV ≤ 10 m/s and red dots represent worse cardiac condition patients whose PWV > 10 m/s. (a) PWV was significantly correlated with serum Ang-2 concentrations (R = 0.45, P = 0.000) and the correlation between PWV and Ang-2 remained significant (R = 0.583, P = 0.001) when PWV > 10 m/s, while not existed (R = 0.016, P = 0.913) when PWV ≤ 10 m/s. (b) Higher PWV was significantly correlated with miR-27 (R = -0.389, P = 0.001) and correlation remained significant (R= - 0.379, P = 0.033 respectively) within the group with a PWV > 10 m/s. There is no significant correlation between PWV and miR-27 (R = -0.232, P = 0.102) when PWV < 10 m/s. (c) EF was significantly correlated with Ang-2 (R = -0.250, P = 0.035) but the correlation was not significant either in the group with EF ≥ 50% (R= - 0.229, P = 0.113 respectively) or in the group with EF < 50% (R = -0.046, P = 0.880).

These findings prompted us to test if the other markers correlated with specific parameters in specific dichotomized groups. Indeed, we observed miR-132 to be positively correlated with PWV within the group of PWV > 10 m/s (R = 0.371, P = 0.043), while miR-223 negatively correlated with CI within the group of CI ≤ 2.2 L/min/m2 (R= -0.440, P = 0.046) (Supplementary Figure 1). No other significant correlations or associations were observed between these vascular injury markers and the cardiac index (CI), nor with the ejection fraction (EF) (Supplementary table 1).

Associations between heart conditions and vascular injury markers

To investigate whether the vascular injury markers were also associated with common heart conditions, we compared the concentrations and levels of the vascular injury markers in elderly with ESRD with or without a specific heart/vascular condition: heart failure (HF), coronary heart disease (CHD), left ventricular hypertrophy (LVH) or atrial fibrillation (AF) (Figure 3). No significant differences were seen in the circulating miRNA levels of miRNA-126, -132, -192, -29a and -326 between elderly with ESRD and any of the conditions.

In contrast, the concentration of the vascular injury marker asymmetric dimethylarginine (ADMA) was significantly increased in elderly with ESRD and CHD (P = 0.027). Moreover, the circulating angiogenic miR-27a was significantly higher in elderly with ESRD and either CHD (P = 0.044) or AF (P = 0.016) compared to those patients with ESRD without this condition. In addition, we found trends for associations of Ang-2 with HF (P=0.081), miR-223 with CHD (P=0.058) and miR-326 with AF (P=0.089).

Discussion

This study revealed that, in older patients reaching end stage renal disease, several of the investigated vascular injury markers are associated with markers of cardiovascular structure and function. Specifically, the main findings were 1) a higher PWV is positively correlated with Ang-2 and negatively correlated with circulating miR-27a, 2) a lower EF is negatively correlated with Ang-2 levels, 3) elderly with ESRD and either AF or CHD had significantly higher levels of circulating angiogenic miR-27a and 4) significantly increased concentration of ADMA in elderly with ESRD and CHD.

A higher PWV, which is associated with an increased arterial stiffness, was significantly positively correlated with the vascular injury markers Ang-2 and negatively with miR-27a.

When measuring these correlations within the high (> 10 m/s) and low (≤ 10/s) groups separately, these correlations were even stronger (as illustrated by a higher r) within the high PWV group, suggesting the discovered correlations are mostly based on the group with a high PWV (> 10 m/s). This could possibly be explained by the Windkessel effect of the arteries that is compromised in patients with arterial stiffness. The Windkessel effect of normal elastic arteries, such as the aorta, decreases the pulsatility of the blood pump out of the heart, converts it into a more constant outflow and thereby prevents damage to the microvasculature.[17] As this effect is compromised in patients with a PWV > 10 m/s, their microvasculature has to cope with a higher pulsatility, which could initiate the local endothelial cells to start producing more Ang-2.[29,30] Indeed, Ang-2 was experimentally demonstrated to be involved in arterial stiffness[31]. Similarly, low miR-27a levels were demonstrated to be causally involved in vascular remodeling[32] and vascular calcification[33] via regulating vascular smooth muscle cell phenotype, potentially explaining its link we observed here with PWV. Interestingly, miR-27a has been shown to inhibit the production of angiotensin-converting enzyme (ACE),[34,35] which converts angiotensin-1 into angiotensin-2, while angiotensin-2 can stimulate the expression of angiopoietin-2(Ang-2),[36] suggesting a possible direct link between Ang-2 and circulating miR-27a levels. Taken together, a high concentration of Ang-2, or low miR-27a levels, could potentially serve as a biomarker for aortic stiffness and may reflect underlying pathophysiology.

It is of interest that we previously associated the cardiovascular structure and function, as well as vascular injury markers, as determined in this cohort, with measures of cognitive function.[7,26] There we observed strong correlations of Ang-2[26] and PWV[7] with cognitive function in the domains of executive function and psychomotor speed, as well as a correlation between miR-27a and executive function.[26] Given that we in the current study observed Ang-2 and miR-27a to associate with PWV, these separate findings suggest an interesting link for the presumed heart-kidney-brain axis. Similarly, we here found miR-223 to correlate to cardiac index (only in the low cardiac index group, Supplementary Figure 1), while we previously demonstrated that both miR-223 and cardiac index associated with memory function.[7,26] It may be interesting to perform additional studies to investigate causal roles for these connections.

We also observed that miR-27a levels were higher in older patients with ESRD and AF or CHD. This appears in line with previous findings that miR-27a could potentially be a biomarker for atherosclerosis as its levels correlated with the progression of atherosclerosis.[37,38] Moreover, several studies found that an increased arterial stiffness was

significantly correlated with the presence of AF.[39,40] However, we observed a negative correlation between miR-27a levels and arterial stiffness (PWV), thus contradicting a direct link in our studies between miR-27a, PWV and AF. Dedicated studies are therefore necessary to clarify the exact link between miR-27a and the development of different cardiovascular diseases. It should also be noted that our analysis of vascular injury markers in relation to these specific heart conditions should be carefully interpreted as group sizes for these conditions involve small groups (e.g. number of ESRD patients with CHD is only 14).

Another limitation of this study was that we could not determine the effect of the different medications on the vascular injury marker levels or concentration. Almost 93% of all participants used 5 or more medications, including anti-hypertensive drugs, anti-coagulants and cholesterol lowering drugs. Further research could investigate whether these drugs could influence the concentrations or levels of these vascular injury markers.

Taken together, this study shows the potential of circulating angiogenic miRNAs, Ang-2 and ADMA to serve as biomarkers for cardiovascular structure and function in older patients reaching end stage renal disease. Moreover, further research into the found correlations (in particular with Ang-2 and miR-27a) could give more insight in the role of the vascular injury markers in impaired cardiac function.

Materials and Methods

Patient Cohort

Patient data was gathered from 'The Cognitive decline in Older Patients with End stage renal disease' (COPE) prospective, multicentered cohort study. The design and rational behind the COPE study with all in- and exclusion criteria, has been published previously.[41] In brief, older patients with an age above 65, suffering from chronic kidney disease stage 4 or 5 (eGFR ≤ 20 ml/min/1.73m2) and prior to any conservative care or dialysis, were included. To study the association between circulating miRNAs and alterations in cardiovascular structure and function, blood samples and cardiac magnetic resonance imaging (MRI) scans were taken. Patients without a cardiac MRI, either due to the lack of an available MRI at the participating center, contra-indications to perform an MRI or an unusable MRI due to motion or artefacts, were excluded from this study (Figure 1). All included patients signed a written informed consent and the medical ethics committees (METC) approved the study

protocol of all the four participating centers (Leiden University Medical Center [Leiden, LUMC], HAGA hospital [the Hague], HAGA hospital [Zoetermeer, dialysis center] and Renier de Graaf Groups [Delft]).

Renal care

All participating centers used either the Chronic Kidney Disease Epidemiology Collaboration (CKD-epi)[42] or the Modification of Diet in Renal Disease (MDRD)[43] to estimate the patient's estimated glomerular filtration rate (eGFR). Based on the ERA-EDTA primary renal diagnosis code, patients were divided by the cause of their kidney disease, either non-vascular or vascular (mostly diabetes or hypertension).

Magnetic resonance imaging (MRI)

All cardiac MRI scans were made on a 3T Philips Achieva MRI scanner (Philips, Best, The Netherlands) with an 8-channel receive coil.

Cardiovascular structure and function

Phase contrast MRI scans, which visualize moving fluid, were used to determine the pulse wave velocity (PWV), which was required to measure the aortic stiffness of the patients.[41] Moreover, turbo field echo (TFE) multi-slice multi-phase cine-imaging of the left ventricle was made to measure the ejection fraction (EF) and cardiac index (CI), the two parameters of the cardiac systolic function. The EF is the percentage of blood leaving the left ventricle each time the heart contracts (stroke volume (SV) / end-diastolic volume (EDSV) * 100%). The CI is expressed in l/min/m2 and determined by correcting the cardiac output (CO, stroke volume (SV) * heart rate (HR)) for the body surface area (BSA) with the Du Bois formula.[44] For only one patient, the CI could not be determined, due to a low-quality MRI scan. PWV and EF were available for all 67 patients.

Angiopoietin-2 II (Ang-2) and asymmetric dimethylarginine (ADMA)

An ELISA (R&D Systems, Minneapolis, MN, USA) was performed to measure the serum concentrations of the vascular injury markers Ang-2 and ADMA.

Circulating angiogenic miRNAs

Patients EDTA-anti-coagulated blood was centrifugated for 10 minutes at 3000 rpm to harvest plasma from the 67 patients for the miRNA analysis. After sample collection the

plasma was stored at -80˚C. MicroRNA profiling was performed by Exigon (Vaedbek, Denmark) with the total amount of RNA isolated from 200 µL by using RT-qPCR (miRCURY LNA Universal RT microRNA PCR). Selection of the miRNAs to be tested (miR-27a, miR-29a, miR-126, miR-132, miR-192, miR-223 and miR-326) was based on a previous study,[26] which related these miRNAs to an impaired cognitive function and vascular injury. For normalization of the data, we have applied the median of the assays detected in all samples as this was found to be the most stable normalizer.[26]

Statistical analysis

All data analyses were performed with IBM SPSS Statistics version 25. The cut-off values to divide the cardiac parameters PWV, EF and CI into two dichotomized groups are based on current guidelines.[45,46] The presence of aortic stiffness was determined by a PWV > 10m/s and a bad EF and CI characterized by < 50% and ≤ 2.2 l/min/m2 respectively. Categorical data are given in numbers with percentages and all continuous data are presented as mean ± standard deviation (SD) or median ± interquartile range (IQR). Independent-sample T tests were used to assess the baseline differences between the cardiac parameters. Correlation models were used to assess the correlation between the circulating miRNA levels and alterations in cardiac structure and function. Through testing it was found that gender and age did not represent significant confounders, and as such no adjustments were performed for these parameters. Statistical significance was considered with p-values < 0.05.

References

1. Collaboration, G.B.D.C.K.D. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **395**, 709-733 (2020).
2. Geluk, C.A., Tio, R.A., Tijssen, J.G.*, et al.* Clinical characteristics, cardiac events and coronary angiographic findings in the prospective PREVEND cohort: an observational study. *Netherlands heart journal : monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation* **15**, 133-141 (2007).
3. Kalantar-Zadeh, K., Jafar, T.H., Nitsch, D., Neuen, B.L. & Perkovic, V. Chronic kidney disease. *Lancet* **398**, 786-802 (2021).
4. Kurella, M., Chertow, G.M., Luan, J. & Yaffe, K.J.J.o.t.A.G.S. Cognitive impairment in chronic kidney disease. **52**, 1863-1869 (2004).
5. Pippias, M., Jager, K.J., Kramer, A.*, et al.* The changing trends and outcomes in renal replacement therapy: data from the ERA-EDTA Registry. *Nephrol Dial Transplant* **31**, 831-841 (2016).
6. Drew, D.A., Weiner, D.E., Tighiouart, H.*, et al.* Cognitive decline and its risk factors in prevalent hemodialysis patients. **69**, 780-787 (2017).
7. Zijlstra, L.E., Trompet, S., Jukema, J.W.*, et al.* Association of cardiovascular structure and function with cerebrovascular changes and cognitive function in older patients with end-stage renal disease. **12**, 1496 (2020).
8. Levey, A.S. & Coresh, J. Chronic kidney disease. *Lancet* **379**, 165-180 (2012).
9. Grams, M.E., Yang, W., Rebholz, C.M.*, et al.* Risks of adverse events in advanced CKD: the chronic renal insufficiency cohort (CRIC) study. **70**, 337-346 (2017).
10. Ikram, M.A., Vernooij, M.W., Hofman, A.*, et al.* Kidney function is related to cerebral small vessel disease. **39**, 55-61 (2008).
11. Foley, R.N., Parfrey, P.S. & Sarnak, M.J.J.o.t.A.S.o.N.J. Epidemiology of cardiovascular disease in chronic renal disease. **9**, S16-23 (1998).
12. Lancet, C.K.D.P.C.J.T. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. **375**, 2073-2081 (2010).
13. Bozbas, H., Pirat, B., Demirtas, S.*, et al.* Evaluation of coronary microvascular function in patients with end-stage renal disease, and renal allograft recipients. **202**, 498-504 (2009).
14. Gradaus, F., Ivens, K., Peters, A.J.*, et al.* Angiographic progression of coronary artery disease in patients with end-stage renal disease. **16**, 1198-1202 (2001).
15. Ooi, Q.L., Tow, F.K.N.-F.H., Deva, R.*, et al.* The microvasculature in chronic kidney disease. **6**, 1872-1878 (2011).
16. de Roos, A., van der Grond, J., Mitchell, G. & Westenberg, J.J.C. Magnetic resonance imaging of cardiovascular function and the brain: is dementia a cardiovascular-driven disease? **135**, 2178-2195 (2017).
17. Mitchell, G.F., van Buchem, M.A., Sigurdsson, S.*, et al.* Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility–Reykjavik study. **134**, 3398-3407 (2011).
18. Bijkerk, R., van Solingen, C., de Boer, H.C.*, et al.* Hematopoietic microRNA-126 protects against renal ischemia/reperfusion injury by promoting vascular integrity. *J Am Soc Nephrol* **25**, 1710-1722 (2014).
19. Anand, S., Majeti, B.K., Acevedo, L.M.*, et al.* MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. *Nat Med* **16**, 909-914 (2010).
20. Creemers, E.E., Tijsen, A.J. & Pinto, Y.M. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Circ Res* **110**, 483-495 (2012).

21. Kato, M., Castro, N.E. & Natarajan, R. MicroRNAs: potential mediators and biomarkers of diabetic complications. *Free Radic Biol Med* **64**, 85-94 (2013).
22. Khairoun, M., de Koning, E.J., van den Berg, B.M.*, et al.* Microvascular damage in type 1 diabetic patients is reversed in the first year after simultaneous pancreas-kidney transplantation. *Am J Transplant* **13**, 1272-1281 (2013).
23. Guay, C. & Regazzi, R. Circulating microRNAs as novel biomarkers for diabetes mellitus. *Nat Rev Endocrinol* **9**, 513-521 (2013).
24. Bijkerk, R., Florijn, B.W., Khairoun, M.*, et al.* Acute Rejection After Kidney Transplantation Associates With Circulating MicroRNAs and Vascular Injury. *Transplant Direct* **3**, e174 (2017).
25. Bijkerk, R., Duijs, J.M., Khairoun, M.*, et al.* Circulating microRNAs associate with diabetic nephropathy and systemic microvascular damage and normalize after simultaneous pancreas-kidney transplantation. *Am J Transplant* **15**, 1081-1090 (2015).
26. Bijkerk, R., Kallenberg, M.H., Zijlstra, L.E.*, et al.* Circulating angiopoietin-2 and angiogenic microRNAs associate with cerebral small vessel disease and cognitive decline in older patients reaching end stage renal disease. *Nephrol Dial Transplant* (2020).
27. Wang, G.-K., Zhu, J.-Q., Zhang, J.-T.*, et al.* Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. **31**, 659-666 (2010).
28. Tijsen, A.J., Creemers, E.E., Moerland, P.D.*, et al.* MiR423-5p as a circulating biomarker for heart failure. **106**, 1035-1039 (2010).
29. Grey, E., Bratteli, C., Glasser, S.P.*, et al.* Reduced small artery but not large artery elasticity is an independent risk marker for cardiovascular events. *Am J Hypertens* **16**, 265-269 (2003).
30. Nguyen, K.T., Donoghue, L., Giridharan, G.A.*, et al.* Acute Response of Human Aortic Endothelial Cells to Loss of Pulsatility as Seen during Cardiopulmonary Bypass. *Cells Tissues Organs*, 1-11 (2021).
31. Chang, F.C., Chiang, W.C., Tsai, M.H.*, et al.* Angiopoietin-2-induced arterial stiffness in CKD. *J Am Soc Nephrol* **25**, 1198-1209 (2014).
32. Sun, Y., Xiao, Y., Sun, H.*, et al.* miR-27a regulates vascular remodeling by targeting endothelial cells' apoptosis and interaction with vascular smooth muscle cells in aortic dissection. *Theranostics* **9**, 7961-7975 (2019).
33. Choe, N., Kwon, D.H., Ryu, J.*, et al.* miR-27a-3p Targets ATF3 to Reduce Calcium Deposition in Vascular Smooth Muscle Cells. *Mol Ther Nucleic Acids* **22**, 627-639 (2020).
34. Pacurari, M. & Tchounwou, P.B.J.I.j.o.i. Role of microRNAs in renin-angiotensin-aldosterone system-mediated cardiovascular inflammation and remodeling. **2015**(2015).
35. Chen, L.-J., Xu, R., Yu, H.-M., Chang, Q. & Zhong, J.-C.J.I.j.o.h. The ACE2/apelin signaling, microRNAs, and hypertension. **2015**(2015).
36. Otani, A., Takagi, H., Oh, H., Koyama, S. & Honda, Y.J.D. Angiotensin II induces expression of the Tie2 receptor ligand, angiopoietin-2, in bovine retinal endothelial cells. **50**, 867-875 (2001).
37. Chen, W.-J., Yin, K., Zhao, G.-J., Fu, Y.-C. & Tang, C.-K.J.A. The magic and mystery of microRNA-27 in atherosclerosis. **222**, 314-323 (2012).
38. Li, T., Cao, H., Zhuang, J.*, et al.* Identification of miR-130a, miR-27b and miR-210 as serum biomarkers for atherosclerosis obliterans. **412**, 66-70 (2011).
39. Lee, S.-H., Choi, S., Jung, J.-H. & Lee, N.J.A. Effects of atrial fibrillation on arterial stiffness in patients with hypertension. **59**, 459-463 (2008).
40. Chu, C.-Y., Lin, T.-H., Hsu, P.-C.*, et al.* Heart rate significantly influences the relationship between atrial fibrillation and arterial stiffness. **10**, 1295 (2013).

41. Berkhout-Byrne, N., Kallenberg, M.H., Gaasbeek, A.*, et al.* The Cognitive decline in Older Patients with End stage renal disease (COPE) study–rationale and design. **33**, 2057-2064 (2017).
42. van den Brand, J.A., van Boekel, G.A., Willems, H.L.*, et al.* Introduction of the CKD-EPI equation to estimate glomerular filtration rate in a Caucasian population. **26**, 3176-3181 (2011).
43. Levey, A.S., Bosch, J.P., Lewis, J.B.*, et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. **130**, 461-470 (1999).
44. Du Bois, D.J.N. A formula to estimate the approximate surface area if height and weight be known. **5**, 303-313 (1989).
45. Williams, B., Mancia, G., Spiering, W.*, et al.* 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). **39**, 3021-3104 (2018).
46. Ponikowski, P., Voors, A.A., Anker, S.D.*, et al.* 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. **74**, 1037-1147 (2016).

Table 1. Baseline characteristics of the study population (n=67)

Male gender, n (%)	44 (65.7)
Age, years; mean ± SD	75.1 ± 6.6
Body Mass Index (BMI); mean ± SD	27.9 ± 3.83
Race, Caucasian, n (%)	58 (86.6)
Higher educational level[1], n (%)	25 (37.4)
Primary kidney disease, n (%)	
Non-vascular cause	25 (37.3)
Vascular cause	41 (61.2)
Comorbidity, n (%)	
Diabetes mellitus	28 (41.8)
Peripheral vascular disease	10 (14.9)
Cerebral vascular accident	17 (25.4)
Heart failure	4 (6.0)
Coronary heart disease	15 (22.4)
Left ventricle hypertrophy	8 (11.9)
Atrial fibrillation	14 (20.9
Alcohol consumption	39 (58.2)
History of smoking	39 (72.2)
Medication use, n (%)	
Polypharmacy (the use of ≥ 5 medications)	62 (92.5)
Antihypertensive medication	62 (92.5)
Diuretics	38 (56.7)
Cholesterol-lowering	49 (73.1)
Anti-coagulants	50 (74.6)
Objective measures, mean ± SD	
Blood pressure (mmHg)	
Systolic	155.7 ± 21.7
Diastolic	81.6 ± 11.3
eGFR (mL/min/1.73 m2)	16.0 ± 4.0
Urea (mg/dL)	21.3 ± 6.6
Albumin (mg/24 hours)[2]	803 ± 956
Troponin-T (µg/L)	0.071 ± 0.117
NT-proBNP (ng/L)	792 ± 1155
Cholesterol	4.5 ± 1.1
HDL (mmol/L)	1.22 ± 0.39
LDL-cholesterol (mmol/L)	2.49 ± 0.80
Cardiovascular function, measured by MRI, median [IQR]	
Pulse wave velocity (m/s)	9.8 [8.0 – 13.7]
Ejection fraction (%)	61 [51 – 66]
Cardiac index (L/min/m2)	2.5 [2.1 – 3.0]

[1]Higher education level includes HAVO/WVO/HBO and university. [2] Missing values of 29 patients. Abbreviations: estimated glomerular filtration rate (eGFR); high-density-lipoprotein (HDL); interquartile range (IQR); low-density-lipoprotein (LDL); magnetic resonance imaging (MRI); N-terminal pro b-type natriuretic peptide (NT-proBNP); standard deviation (SD).

Table 2. Associations between cardiovascular function parameters and vascular injury markers.

	Better cardiovascular function	**Worse cardiovascular function**			
			Correlation		**t-test**
	Pulse wave velocity ≤ 10 m/s (n = 35)	**Pulse wave velocity > 10 m/s (n = 32)**	**P value**	**R**	**P value**
ADMA	0.70 ± 0.19	0.73 ± 0.20	0.787	0.034	0.547
Ang-2	319.6 ± 123.7	390.8 ± 189.4	0.000*	0.451	0.083#
miR-126	0.947 ± 0.324	0.963 ± 0.237	0.849	-0.013	0.818
miR-132	0.011 ± 0.008	0.011 ± 0.006	0.169	0.190	0.970
miR-192	0.009 ± 0.014	0.007 ± 0.006	0.999	0.000	0.561
miR-223	3.850 ± 1.442	3.784 ± 1.388	0.217	-0.149	0.849
miR-27	0.137 ± 0.051	0.110 ± 0.031	0.001*	-0.389	0.012*
miR-29	0.027 ± 0.032	0.027 ± 0.033	0.645	0.112	0.991
miR-326	0.008 ± 0.008	0.006 ± 0.004	0.798	0.006	0.241
	Ejection fraction ≥ 50 % (n = 53)	**Ejection fraction < 50 % (n = 14)**			
ADMA	0.72 ± 0.20	0.70 ± 0.16	0.530	0.076	0.721
Ang-2	337.3 ± 144.9	417.1 ± 208.1	0.035*	-0.250	0.114
miR-126	0.944 ± 0.294	0.996 ± 0.245	0.463	-0.098	0.555
miR-132	0.011 ± 0.007	0.011 ± 0.008	0.857	-0.027	0.813
miR-192	0.008 ± 0.012	0.008 ± 0.005	0.941	-0.047	0.946
miR-223	3.766 ± 1.406	4.036 ± 1.442	0.431	-0.152	0.539
miR-27	0.125 ± 0.043	0.120 ± 0.054	0.779	0.000	0.754
miR-29	0.029 ± 0.035	0.024± 0.014	0.243	0.110	0.665
miR-326	0.006 ± 0.006	0.010 ± 0.009	0.079#	-0.216	0.061#
	Cardiac index > 2.2 L/min/m^2 (n = 45)	**Cardiac index ≤ 2.2 L/min/m^2 (n = 21)**			
ADMA	0.74 ± 0.22	0.68 ± 0.14	0.269	0.143	0.257
Ang-2	339.2 ± 166.7	379.8 ± 153.9	0.817	-0.046	0.364
miR-126	0.930 ± 0.264	1.018 ± 0.322	0.479	-0.091	0.246
miR-132	0.011 ± 0.007	0.011 ± 0.007	0.548	-0.073	0.802
miR-192	0.009 ± 0.012	0.007 ± 0.007	0.987	0.010	0.555
miR-223	3.750 ± 1.312	4.018 ± 1.621	0.960	0.024	0.476
miR-27	0.125 ± 0.043	0.121 ± 0.049	0.972	0.019	0.751
miR-29	0.026 ± 0.030	0.030 ± 0.039	0.940	0.027	0.658
miR-326	0.007 ± 0.008	0.006 ± 0.004	0.931	-0.009	0.301

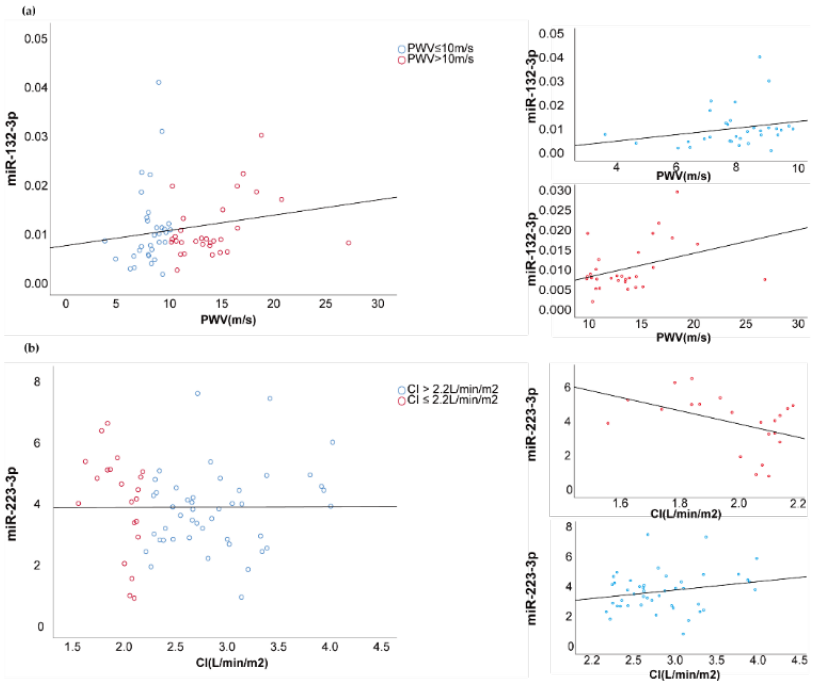
Bivariate correlation models were used for the correlation p-values. To compare the vascular injury marker concentrations / circulating levels between the low versus high PWV, EF and CI groups, independent-samples T tests were used for the p-values. Unit of ADMA is umol/L and unit of Ang-2 is pg/mL. *Indicates statistically significant values (P<0.05), #indicates trends (P<0.10).

Table 3. Associations between cardiac conditions and the vascular injury markers.

	ESRD without HF (n=62)		ESRD with HF (n=4)		p value
	mean	SD	mean	SD	
ADMA	0.712	0.195	0.824	0.198	0.273
ANG2	344.666	157.669	490.254	179.785	0.081#
miR-126	0.952	0.286	0.995	0.292	0.772
miR-132	0.011	0.007	0.009	0.005	0.682
miR-192	0.008	0.011	0.009	0.006	0.901
miR-223	3.790	1.365	4.278	2.163	0.505
miR-27	0.122	0.043	0.156	0.056	0.133
miR-29	0.028	0.033	0.018	0.015	0.576
miR-326	0.006	0.006	0.012	0.015	0.532
	ESRD without CHD (n=52)		ESRD with CHD (n=14)		p value
	mean	SD	mean	SD	
ADMA	0.691	0.174	0.816	0.237	0.027*
ANG2	350.111	161.698	367.596	166.662	0.725
miR-126	0.947	0.302	0.980	0.218	0.692
miR-132	0.012	0.008	0.008	0.004	0.086
miR-192	0.009	0.011	0.007	0.010	0.684
miR-223	3.644	1.412	4.424	1.246	0.058#
miR-27	0.118	0.043	0.145	0.045	0.044*
miR-29	0.030	0.035	0.017	0.014	0.178
miR-326	0.007	0.006	0.007	0.008	0.958
	ESRD without LVH (n=58)		ESRD with LVH (n=8)		p value
	mean	SD	mean	SD	
ADMA	0.728	0.200	0.651	0.155	0.299
ANG2	359.281	168.427	305.318	58.771	0.442
miR-126	0.935	0.273	1.098	0.340	0.128
miR-132	0.011	0.008	0.011	0.005	0.961
miR-192	0.007	0.006	0.016	0.025	0.331
miR-223	3.858	1.425	3.525	1.303	0.534
miR-27	0.126	0.046	0.107	0.023	0.25
miR-29	0.028	0.034	0.022	0.019	0.618
miR-326	0.007	0.007	0.004	0.002	0.27
	ESRD without AF (n=52)		ESRD with AF (n=14)		p value
	mean	SD	mean	SD	
ADMA	0.704	0.190	0.775	0.212	0.233
ANG2	329.794	125.840	437.253	235.965	0.122
miR-126	0.959	0.293	0.937	0.254	0.797
miR-132	0.011	0.008	0.010	0.005	0.624
miR-192	0.009	0.012	0.007	0.006	0.594
miR-223	3.681	1.265	4.339	1.807	0.121
miR-27	0.117	0.040	0.149	0.053	0.016*
miR-29	0.025	0.028	0.035	0.046	0.321
miR-326	0.006	0.006	0.010	0.009	0.089#

Independent-samples T tests were used for calculating p-values. Unit of ADMA is umol/L and unit of Ang-2 is pg/mL. *Indicates statistically significant values (P<0.05), #indicates trends (P<0.10).

Supplementary figure 1



Supplementary figure 1. Blue dots represent better cardiovascular condition patients whose PWV ≤ 10 m/s or CI > 2.2 L/min/m^2, and red dots represent worse cardiac condition patients whose PWV > 10 m/s or CI ≤2.2 L/min/m^2. (a) PWV was significantly correlated with miR-132 (R = 0.371, P = 0.043) when PWV > 10 m/s, while not existed (R = 0.230, P = 0.205) when PWV ≤ 10 m/s. (b) CI was negatively correlated with miR-223 (R= -0.440, P = 0.046) within the group with a CI ≤2.2 L/min/m^2. There is no significant correlation between CI and miR-223 (R = 0.220, P = 0.147) when CI > 2.2 L/min/m^2.

Supplementary table 1. Correlations between vascular injury markers and cardiovascular function parameters within dichotomized groups.

	Pulse wave velocity ≤ 10 m/s n = 35		**Pulse wave velocity > 10 m/s n = 32**	
	R	P value	R	P value
ADMA	0.062	0.725	-0.075	0.681
ANG2	0.016	0.931	0.583	0.001**
miR-126	-0.023	0.893	0.019	0.916
miR-132	0.230	0.205	0.371	0.043*
miR-192	0.016	0.931	0.224	0.233
miR-223	-0.233	0.178	-0.225	0.215
miR-27	-0.232	0.187	-0.379	0.033*
miR-29	0.085	0.626	0.102	0.578
miR-326	0.163	0.366	0.164	0.425
	Ejection fraction ≥ 50 % n = 54		**Ejection fraction < 50 % n = 13**	
	R	P value	R	P value
ADMA	0.045	0.744	0.199	0.515
ANG2	-0.229	0.113	-0.046	0.880
miR-126	-0.104	0.453	0.211	0.488
miR-132	0.004	0.978	-0.022	0.945
miR-192	-0.027	0.856	0.209	0.494
miR-223	-0.119	0.390	0.192	0.529
miR-27	-0.006	0.967	0.063	0.846
miR-29	0.176	0.203	0.026	0.932
miR-326	-0.110	0.452	0.022	0.953
	Cardiac index > 2.2 L/min/m² n = 45		**Cardiac index ≤ 2.2 L/min/m² n = 21**	
	R	P value	R	P value
ADMA	0.064	0.678	-0.019	0.934
ANG2	0.064	0.690	0.215	0.362
miR-126	0.069	0.654	-0.218	0.342
miR-132	-0.154	0.337	0.405	0.076
miR-192	-0.093	0.561	0.094	0.702
miR-223	0.220	0.147	-0.440	0.046*
miR-27	0.001	0.993	-0.244	0.301
miR-29	0.032	0.834	0.290	0.202
miR-326	-0.140	0.396	0.078	0.752

Bivariate correlation models were used for the correlation p-values. Numbers of patients in each group is showed by n. * $p < 0.05$, ** $p < 0.01$