Circulating angiopoietin-2 and angiogenic microRNAs associate with cerebral small vessel disease and cognitive decline in older patients reaching end-stage renal disease

Bijkerk, R.; Kallenberg, M.H.; Zijlstra, L.E.; Berg, B.M. van den; Bresser, J. de; Hammer, S.; ... ; Mooijaart, S.

Nephrol Dial Transplant (2022) 37: 498–506
doi: 10.1093/ndt/gfaa370
Advance Access publication 23 December 2020

ORIGINAL ARTICLE

Circulating angiopoietin-2 and angiogenic microRNAs associate with cerebral small vessel disease and cognitive decline in older patients reaching end-stage renal disease

Roel Bijkerk [1,2], Marije H. Kallenberg[1,3], Laurien E. Zijlstra[4], Bernard M. van den Berg [1,2], Jeroen de Bresser[5], Sebastiaan Hammer[6], Esther E. Bron[7], Hakim Achterberg[7], Mark A. van Buchem[5], Noeleen C. Berkhout-Byrne[1], Willem Jan W. Bos[1,8], Diana van Heemst[3], Ton J. Rabelink[1,2], Anton Jan van Zonneveld[1,2], Marjolijn van Buren[1,9,*] and Simon Mooijaart[3,10,*]

[1]Department of Internal Medicine (Nephrology), Leiden University Medical Center, Leiden, The Netherlands, [2]Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands, [3]Department of Internal Medicine (Gerontology and Geriatrics), Leiden University Medical Center, Leiden, The Netherlands, [4]Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands, [5]Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands, [6]Department of Radiology, Haga Hospital, The Hague, The Netherlands, [7]Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands, [8]Department of Internal Medicine, St Antonius Hospital, Nieuwegein, The Netherlands, [9]Department of Nephrology, Haga Hospital, The Hague, The Netherlands and [10]Institute of Evidence-Based Medicine in Old Age, Leiden, The Netherlands

*These authors contributed equally to this work.
Correspondence to: Roel Bijkerk; E-mail: r.bijkerk@lumc.nl

ABSTRACT

Background. The prevalence of end-stage renal disease (ESRD) is increasing worldwide, with the majority of new ESRD cases diagnosed in patients >60 years of age. These older patients are at increased risk for impaired cognitive functioning, potentially through cerebral small vessel disease (SVD). Novel markers of vascular integrity may be of clinical value for identifying patients at high risk for cognitive impairment.

Methods. We aimed to associate the levels of angiopoietin-2 (Ang-2), asymmetric dimethylarginine and a selection of eight circulating angiogenic microRNAs (miRNAs) with SVD and cognitive impairment in older patients reaching ESRD that did not yet initiate renal replacement therapy ($n = 129$; mean age 75.3 years, mean eGFR 16.4 mL/min). We assessed brain magnetic resonance imaging changes of SVD (white matter hyperintensity volume, microbleeds and the presence of lacunes) and measures of cognition in domains of memory, psychomotor speed and executive function in a neuropsychological test battery.

Results. Older patients reaching ESRD showed an unfavourable angiogenic profile, as indicated by aberrant levels of Ang-2 and five angiogenic miRNAs (miR-27a, miR-126, miR-132, miR-223 and miR-326), compared with healthy persons and patients with diabetic nephropathy. Moreover, Ang-2 was associated with SVD and with the domains of psychomotor speed and executive function, while miR-223 and miR-29a were associated with memory function.

Conclusions. Taken together, these novel angiogenic markers might serve to identify older patients with ESRD at risk of cognitive decline, as well as provide insights into the underlying (vascular) pathophysiology.

Keywords: ageing, angiopoietin-2, cerebral small vessel disease, cognitive dysfunction, microRNAs

INTRODUCTION

The number of patients reaching end-stage renal disease (ESRD) is increasing worldwide, while half of the new ESRD cases are patients ≥60 years of age [1, 2]. These older patients are at increased risk for adverse health outcomes [3], including impaired cognitive functioning [4], which decreases quality of life and independently associates with increased mortality and other adverse health outcomes [5, 6]. However, the underlying pathophysiological mechanisms of cognitive impairment in this group of patients are not fully understood.

Diseases affecting small arteries and arterioles in the brain, also known as cerebral small vessel disease (SVD), play an important role in the development of cerebrovascular disease and cognitive impairment [7, 8]. A history of vascular disease is a determinant of cognitive impairment in patients reaching ESRD (before receiving renal replacement therapy) [9, 10] and SVD has been associated with impaired cognition [11]. Being exposed to high-volume blood flow, the brain and kidney share a high vulnerability for endothelial dysfunction and vascular

KEY LEARNING POINTS

What is already known about this subject?

- Older patients reaching end-stage renal disease (ESRD) are at increased risk for impaired cognitive functioning.
- The underlying pathophysiological mechanisms of cognitive impairment in this group of patients are not fully understood.
- Cerebral small vessel disease (SVD) plays an important role in the development of cerebrovascular disease and is associated with cognitive impairment.

What this study adds?

- Older patients reaching ESRD showed an unfavourable circulating angiogenic profile, as indicated by aberrant levels of angiopoietin-2 (Ang-2) and five angiogenic microRNAs (miRNAs).
- Ang-2 and miRNA levels associate with SVD and impaired cognition.

What impact this may have on practice or policy?

- These novel angiogenic markers might serve to identify older individuals with ESRD at risk of cognitive decline, as well as provide insights into the underlying (vascular) pathophysiology.
- Assessing and understanding these determinants of cognitive functioning in older patients reaching ESRD may prove beneficial in treatment decisions and may have implications for prevention of further cognitive decline.

damage [12]. Within the brain this can subsequently result in cognitive impairment [13]. Furthermore, accumulation of uraemic toxins [such as asymmetric dimethylarginine (ADMA)] may cause cerebral vascular injury and lead to neurodegenerative damage in the brain affecting the cognitive domains of attention and speed [14]. Microvascular destabilization factors such as angiopoietin-2 (Ang-2), together with (circulating) angiogenic microRNAs (miRNAs), play a major role in mediating vascular injury or maintaining microvascular integrity and have also been linked to endothelial dysfunction in cerebral SVD [15]. As such, they may provide insights into specific deregulated molecular mechanisms [16, 17], but also represent promising markers of microvascular disease [17–20]. Assessing and understanding these determinants of cognitive functioning in older patients reaching ESRD may prove beneficial in treatment decisions and may have implications for prevention of further cognitive decline.

However, so far no studies have been performed on circulating levels of ADMA, Ang-2 and angiogenic miRNAs in the older ESRD population or their relationship with SVD and cognitive impairment in this population. The aim of this study was to determine the levels of ADMA, Ang-2 and angiogenic miRNAs and their association with brain magnetic resonance imaging (MRI) markers of SVD and cognitive impairment in older patients reaching ESRD.

MATERIALS AND METHODS

Patient cohort

The full design, methods and baseline characteristics of the Cognitive decline in Older Patients with End-stage renal disease (COPE) study have been published previously [10, 21]. The patients selected for this study were those patients for which a cardiac MRI was available. In brief, the COPE study is a prospective, multicentre cohort study based in the Netherlands comprising patients ≥65 years of age reaching ESRD [estimated glomerular filtration rate (eGFR) ≤20 mL/min/1.73 m^2] before receiving renal replacement therapy. Patients who were illiterate were excluded. As part of the pre-dialysis nephrogeriatric workup, a physical examination, comprehensive geriatric assessment, brain MRI and neuropsychological testing were performed. Written informed consent was obtained from all patients. The study protocol was approved by the medical ethics committee (approval number NL46389.058.13) of all centres that participated [Leiden University Medical Centre, Haga Hospital (The Hague), Dialysis Centre Zoetermeer (Zoetermeer; part of the Haga Hospital) and Reinier de Graaf Groep (Delft)].

Renal care. eGFR was estimated using the Modification of Diet in Renal Disease or Chronic Kidney Disease Epidemiology Collaboration equation depending on the time of inclusion or protocol used in the different hospitals. Vascular or non-vascular cause of kidney disease was defined based on the European Renal Association–European Dialysis and Transplant Association primary renal diagnosis code, which was determined by the treating nephrologist. A history of vascular disease was defined as the presence of myocardial infarction and/or cerebral vascular incident (CVA) and/or peripheral vascular disease.

Neuropsychological testing. A standardized neuropsychological test battery was administered by trained geriatric or dialysis nurses. The tests were designed to assess different domains

of cognitive functioning, including global cognition, visuoconstruction, memory, psychomotor speed and executive function, which have been successfully used in several study cohorts [22–24]. Global cognition was tested by the Mini-Mental State Examination (MMSE), which ranges from 0 to 30 points, with higher scores representing better cognitive performance [25]. Clock drawing was used to determine visuoconstructive abilities and executive function, with scores ranging from 0 to 14 points, where higher scores indicate better performance [26]. Memory was tested using the 15-word verbal learning test (15WVLT), where both immediate (total score after five trials) and delayed recall were used, with higher scores representing better function [27]. To test for long-term memory reproduction, we used the Visual Attention Test, with better function indicated by higher scores [28]. To test for psychomotor speed, the Letter Digit Substitution Test (LDST) was used. The number of correct substitutions in 60 s was used, with higher scores representing better function [29]. Executive function was assessed with the Trail Making Test A and B (TMT-A and -B), with lower scores representing better function [30]. To distinguish between cognitive flexibility and processing speed as the explanation for the test result, the score on the TMT-B was corrected for the score on the TMT-A. In addition, the Stroop Colour Word Test (SCWT) was used to assess executive function, with lower scores representing better function [31]. To distinguish between cognitive inhibition and processing speed as an explanation of this test result, the SCWT III score (interference card) was corrected for the SCWT II score (colour naming card). The 15WVLT, TMT-B and LDST were used for the association analyses as representative tests for the different neuropsychological domains of memory, executive function and psychomotor speed, respectively.

MRI. In 70 patients, a brain MRI scan was performed. Patients were excluded if metal was present in their body, if they suffered from claustrophobia, if no consent was given for MRI or MRI was not available at the particular medical centre. As previously published, the MRI protocol consisted of three-dimensional (3D) fluid-attenuated inversion recovery, 3D T1-weighted, T2-weighted turbo spin echo images, diffusion-weighted imaging and susceptibility-weighted imaging sequences [21]. White matter hyperintensity (WMH) volume and intracranial volume (in mL) were determined by an automated method (Quantib Brain, Quantib, Rotterdam, The Netherlands) after manual segmentation of infarcts and other pathologies. WMH volume served as a marker of cerebral SVD. The presence of lacunes and microbleeds was scored by a radiologist according to the Standards for Reporting Vascular Changes on Neuroimaging criteria [32]. In addition, we applied the Scheltens scale to evaluate WMH [33]. Of note, this method was chosen over the Fazekas scale because the Scheltens scale provides a more detailed visual scale.

Reference cohorts. To compare our miRNA measurements with healthy controls and patients with diabetic nephropathy (DN) as a 'young' CKD group, we used archival data on circulating miRNAs from a patient study that was set up at the Leiden University Medical Centre, as previously described [20].

Laboratory assessments

For miRNA analysis, plasma was harvested by centrifugation of ethylenediaminetetraacetic acid–anticoagulated blood for 10 min at 3000 rpm and subsequently stored at −80°C. At the same time, blood was collected for analysis of serum Ang-2 and ADMA. Both Ang-2 and ADMA were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacturer.

RNA isolation and measurements

Total RNA was isolated from 200 µL of plasma followed by miRNA profiling for miR-27a, miR-29a, miR-126, miR-132, miR-137, miR-192, miR-223 and miR-326 using reverse transcription quantitative polymerase chain reaction (miRCURY LNA Universal RT microRNA polymerase chain reaction) performed by Exiqon (Vaedbek, Denmark). For the RNA extraction of plasma, two pre-mixed RNA spike-ins (UniSp2 and UniSp4), each at different concentrations in 100-fold increments, were added. This set of spike-ins was intended as an RNA isolation control and to ensure that the quality of the input RNA was sufficiently high for effective amplification. For the reverse transcription step, one spike-in (UniSp6) was added. This control was used to confirm that the reverse transcription and amplification occurs with equal efficiency in all samples, indicating similar and sufficient quality of the RNA. For normalization of the data we applied the median of the assays detected in all samples ($n = 129$ samples), as this was found to be the most stable normalizer. For comparison with a previous cohort [20] (Figure 1), data were normalized to miR-16. miR-16 was used since we observed a strong correlation between the median of all samples (the most preferable normalization method, but not applicable in our comparison to other cohorts) and miR-16 ($r = 0.92$, $P < 0.0001$), suggesting that miR-16 is suitable as a normalizer in this study. Moreover, in the original study that described the DN group and healthy controls (where we compared our results), we used miR-16 as a normalizer after careful evaluation of their levels in different samples and different groups and found it the most stable normalizer [20].

Selection of miRNAs

miRNAs to be assessed (miR-27a, miR-29a, miR-126, miR-132, miR-137, miR-192, miR-223 and miR-326) were selected based on their previously determined relation to vascular injury and cognitive dysfunction (Supplementary data, Table S1).

Statistical analyses

Patient characteristics are presented as mean with standard deviation (SD) when normally distributed. In the case of a skewed distribution, data are presented as median with interquartile range (IQR) or as number (n) with percentage (%). Function in the different cognitive domains (memory, executive function and psychomotor speed) is indicated as percentiles (mean with IQR). miRNA, ADMA and Ang-2 levels are stratified in tertiles and mean scores are presented as mean [standard error of the mean (SEM)]. Associations between miRNA, ADMA and Ang-2 levels as continuous variables with measures from the brain MRI measurements and different cognitive tests

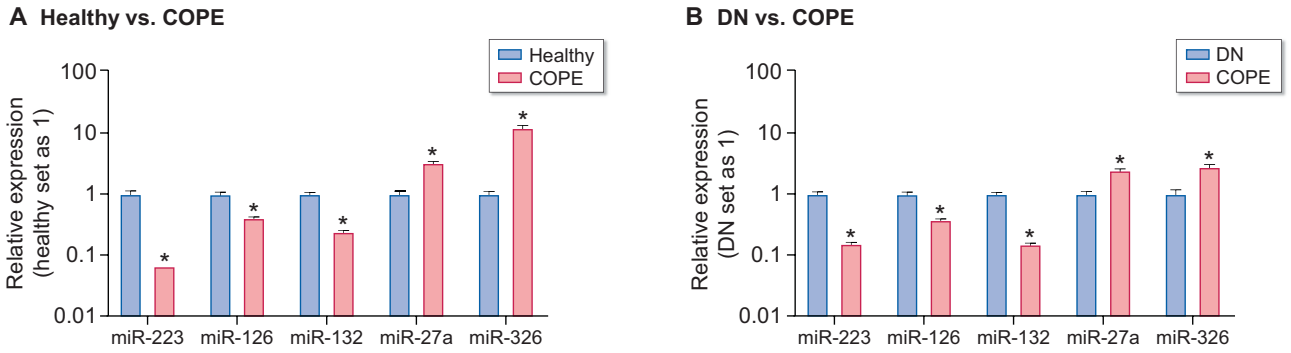

FIGURE 1: Comparison of miRNA levels in older patients with ESRD with (younger) healthy people and patients with DN. (**A**) Comparison with younger healthy people. (**B**) Comparison with younger patients with DN. Values were normalized to miR-16. miRNAs included in this analysis were those for which data were available from the patient cohort with healthy controls and patients with DN. Data are represented as mean ± SEM.

were assessed via linear regression analyses adjusted for age and gender. All analyses were carried out using SPSS version 23 (IBM, Armonk, NY, USA).

RESULTS

Patient cohort description

The clinical characteristics of the study population are presented in Table 1. The study population consisted of 129 participants with a median age of 75.3 years and 85 (65.9%) participants were male. At study enrolment, the mean eGFR was 16.4 mL/min (SD 4.3) and the mean decline in eGFR in the past 3 years was 9.2 mL/min (SD 7.3). In 80 (62.0%) patients the primary kidney disease had a vascular cause, mainly hypertension or diabetes mellitus. Fifty-nine participants (45.7%) had a history of vascular disease, defined by the presence of myocardial infarction and/or CVA and/or peripheral vascular disease.

In Table 1 we also report the performance on global cognitive function and different cognitive domains for this study population. The population had a median MMSE score of 28 out of 30 points (IQR 27–29). Nine patients had an MMSE score <25 (7.1%), a previously identified significant cut-off value to diagnose cognitive dysfunction [34]. The mean score on the immediate memory test (15WVLT) was 30.7 words remembered (SD 9.8). The mean score on the executive function test (TMT-B) was 178.1 s (SD 76.0). The mean score on psychomotor speed (LDST) was 21.8 correct substitutions (SD 6.8).

miRNA, Ang-2 and ADMA measurements in older patients reaching ESRD

To identify miRNAs that may associate with cognitive decline we selected miRNAs that were previously described to strongly associate with vascular injury and cognition (Supplementary data, Table S1; detailed description can be found in the Materials and methods section). As such, we selected miR-27a, miR-29a, miR-126, miR-132, miR-137, miR-192, miR-223 and miR-326 as well as vascular injury marker Ang-2 and ADMA. Table 2 summarizes these measurements in our cohort of 129 patients.

Comparison with younger healthy controls and CKD patients

The miRNA levels in our cohort were first compared with the miRNA levels measured in a healthy (young) control group ($n = 19$, mean age 44 years) as well as in a group of patients with DN ($n = 21$, mean age 44 years, mean eGFR 17 mL/min) from an earlier study [20] (patient characteristics are described in Table 3). For this comparison, data were available for miR-223, miR-126, miR-132, miR-27a and miR-326, as these were previously assessed (levels normalized to stable miR-16), and we found that the patients from this study have strongly altered miRNA levels as compared with both healthy young individuals and patients with DN (Figure 1; these graphs are presented with individual data points in Supplementary data, Figure S1). Specifically, we found lower levels of miR-223, miR-126 and miR-132, while miR-27a and miR-326 displayed higher levels in older patients, confirming previous reports that renal failure associated with vascular injury was linked to this change in circulating miRNA levels [20]. In addition, mean Ang-2 levels were 1901 and 5871 pg/mL in the healthy controls and patients with DN, respectively. Here, mean Ang-2 levels were 3608 pg/mL, indicating that Ang-2 levels in these older patients reaching ESRD are higher than normal, but not as high as in a group of (younger) DN patients. Next we compared the observed ADMA levels to reference values, indicating that 0.74 µmol/L is higher than observed in healthy individuals but comparable to other ESRD patient groups [35, 36].

Specific miRNAs and Ang-2 associate with cognitive function

Next we aimed to determine whether circulating miRNA, Ang-2 and ADMA levels associated with cognitive function in different cognitive domains. To that end we grouped the ESRD patients based on their ADMA, Ang-2 and miRNA levels in tertiles (Tertile 1, lowest levels; Tertile 3, highest levels) and plotted the cognitive test scores against the tertiles of these vascular markers (Figure 2; these graphs are presented with individual data points in Supplementary data, Figure S2). We found lower levels of miR-223 ($P = 0.006$) and higher levels of miR-29a

Table 1. Baseline characteristics of the included study population

Patient characteristics	Values
Patients, *N*	129
Age (years), median (IQR)	75.3 (70.7–81.0)
Gender (male), *n* (%)	85 (65.9)
Caucasian origin, *n* (%)	113 (87.6)
Married/living together, *n* (%)	83 (64.3)
Higher educational level, *n* (%)	35 (27.1)
Current smoking, *n* (%)	18 (14.0)
Alcohol consumption, *n* (%)	69 (53.5)
BMI, median (IQR)	27.7 (24.7–31.8)
Systolic blood pressure (mmHg), mean (SD)	148 (24)
Diastolic blood pressure (mmHg), mean (SD)	81 (13)
Heart rate (bpm), mean (SD)	74 (15)
Disease specific	
eGFR at study enrolment (mL/min/1.73 m^2), mean (SD)	16.4 (4.3)
Δ eGFR[a] (mL/min/1.73 m^2), mean (SD)	9.2 (7.3)
Primary kidney disease, *n* (%)	
Non-vascular cause	47 (36.4)
Vascular cause	80 (62.0)
Diabetes mellitus	56 (43.4)
History of malignancy	41 (31.8)
History of vascular disease	59 (45.7%)
Ankle–brachial index (right), mean (SD)	0.93 (0.22)
Biochemical	
Haemoglobin (mmol/L), mean (SD)	7.5 (0.9)
Cholesterol (mmol/L), mean (SD)	4.6 (1.2)
Triglycerides (mmol/L), mean (SD)	2.0 (1.2)
HDL (mmol/L), mean (SD)	1.2 (0.4)
LDL (mmol/L), mean (SD)	2.6 (0.9)
Urea (mmol/L), mean (SD)	20.7 (6.1)
Calcium (albumin corrected; mmol/L), mean (SD)	2.4 (0.1)
Phosphate (mmol/L), mean (SD)	1.3 (0.2)
PTH (pg/mL), mean (SD)	16.0 (10.4)
Glucose (mmol/L), mean (SD)	6.5 (2.1)
Medication use, *n* (%)	
Polypharmacy (the use of ≥5 medications)	118 (91.5)
Glucose-lowering medication	48 (37.2)
Antihypertensive medication	119 (92.2)
Diuretics	76 (58.9)
Cholesterol-lowering drugs	95 (73.6)
Vitamin D supplement	108 (83.7)
Performance on the different cognitive domains	Score
MMSE score (points), median (IQR)	28 (27–29)
Visuoconstruction	
Clock drawing, mean (IQR)	12 (11–13)
Memory	
15WVLT (words remembered)	
Immediate recall score, mean (SD)	30.7 (9.8)
Delayed recall score, mean (SD)	5.7 (3.0)
Visual Association Test (pictures remembered), median (IQR)	11 (11–12)
Executive function	
TMT-B (s), mean (SD)[b]	178.1 (76.0)
SCWT III, mean (SD)	172.9 (81.0)
SCWT III, corrected for SCWT II (s), mean (SD)	88.9 (72.8)
Psychomotor speed	
LDST (correct in 60 s), mean (SD)	21.8 (6.8)
TMT-A (s), mean (SD)	70.4 (39.6)
SCWT II (s), mean (SD)	84 (29.9)

[a]ΔeGFR, difference between eGFR 3 years before and at study enrolment. Primary kidney disease unknown = 2.
[b]16 patients did not complete the total test. They have been assigned the maximum number of 300 s.
BMI, body mass index.

Table 2. Levels of vascular markers in older patients with ESRD (*N* = 129)

Markers	Values
ADMA (μmol/L), mean (SD)	0.74 (0.21)
Ang-2, mean (SD), pg/mL	3608 (1501)
miRNAs (relative expression),[a] mean (SD)	
miR-126	0.933 (0.275)
miR-132	0.010 (0.006)
miR-137	ND
miR-192	0.009 (0.016)
miR-223	3.713 (1.325)
miR-27a	0.118 (0.046)
miR-29a	0.026 (0.026)
miR-326	0.006 (0.006)

[a]miRNA values are median-normalized and indicate a relative expression to the median, indicating that among miRNAs, miR-223 has the highest expression levels. ND, not determinable.

(P = 0.011) significantly associated with worse memory function (15WVLT), while higher Ang-2 (P = 0.004) associated with worse psychomotor speed (LDST). In addition, we found several close to statistically significant associations; lower miR-27a associated with worse memory (15WVLT; P = 0.067) and executive function (TMT-B; P = 0.067), while higher Ang-2 (P = 0.057) and lower miR-326 (P = 0.08) associated with worse executive function (TMT-B).

Ang-2 associates with imaging parameters of cerebral SVD

Given that it was previously demonstrated that imaging parameters of SVD are associated with measures of cognitive dysfunction, we assessed the relation of circulating vascular markers with MRI parameters of SVD. Figure 3A indicates both the qualitative and quantitative data from 93 and 70 patients, respectively, for which MRI data were available. Microbleeds were present in 56 patients (60.2%); the presence of lacunes of presumed vascular origin was observed in 44 participants (48%). The Scheltens score of the white matter hyperintensities (WMHs) was on average 15.8. The median of the quantitative measurement of the WMH was 3.16 mL. We observed no relation of ADMA, Ang-2 or miRNA levels with microbleeds or lacunes (data not shown). In contrast, Ang-2 (P = 0.08, $r = 0.21$) and miR-29 (P = 0.02, $r = 0.28$) were both (close to) significantly positively correlated with the Scheltens WMH score. Moreover, Figure 3B (individual data points in Supplementary data, Figure S3) illustrates that when we analysed quantitative measures of WMH, while no relationship was found for miRNAs, Ang-2 levels significantly correlated with quantitative WMH scores on MRI (P = 0.016).

DISCUSSION

Our study in older patients with ESRD shows that five selected circulating angiogenic miRNAs (miR-223, miR-126, miR-132, miR-27a and miR-326) and Ang-2 display altered (unfavourable) levels as compared with younger CKD patients and healthy controls. In addition, higher levels of Ang-2 and miR-29a and lower levels of miR-223, miR-27a and miR-326 are associated

Table 3. Characteristics of healthy controls and patients with DN

Patient characteristics	Controls ($n = 19$)	DN ($n = 21$)
Sex (male), *n* (%)	9 (47)	16 (76)
Age (years), mean ± SD	44 ± 11	44 ± 5
BMI (kg/m^2), mean ± SD	25.2 ± 3.8	25.4 ± 3.2
HbA1c (%), mean ± SD	–	8.9 ± 2.3
Glucose (mmol/L), mean ± SD	5.3 ± 1.0	13.8 ± 6.4
eGFR (mL/min/1.73 m^2), mean ± SD	93 ± 17	18 ± 7
Proteinuria (g/24 h), median (IQR)	–	0.72 (0.35–1.5)
Dialysis, *n* (%)	–	3 (14)
History of vascular disease, *n* (%)	0 (0)	4 (19)
Smoking, *n* (%)	0 (0)	0 (0)
Acetylsalicylic acid, *n* (%)	0 (0)	2 (10)
Antihypertensive drugs, *n* (%)		
ACE inhibitor	–	14 (67)
Angiotensin-II antagonist	––	13 (62)
β-blocker	–	9 (43)
Calcium antagonist	–	11 (52)
Diuretics	––	13 (62)

ACE, angiotensin-converting enzyme; hbA1c, haemoglobin A1c.

with worse cognitive function (either psychomotor speed, executive function or memory). Furthermore, while no relation was found with microbleeds or the presence of lacunes, Ang-2 and miR-29a positively correlated with the Scheltens WMH score, while Ang-2 levels also related to quantitative WMH volume on MRI as a marker of cerebral SVD.

We observed that higher Ang-2 levels associated with worse cognitive function in the domain of psychomotor speed, but we also found trends towards an association with worse memory and executive function scores. Interestingly, Ang-2 was also shown previously to associate with cognition in maintenance haemodialysis patients [37]. Ang-2 is known to destabilize vessels via negative interference with Ang-1-mediated Tie-2 signalling, which disrupts pericyte–endothelial cell interaction [38], resulting in abnormal microvascular remodelling and blood flow [39]. Moreover, circulating Ang-2 levels and a disturbed Ang-2/Ang-1 balance have been previously demonstrated to strongly associate with vascular injury in the settings of CKD, diabetes mellitus, acute coronary syndrome and other diseases characterized by endothelial dysfunction and microvascular inflammation [38, 40, 41]. Given the observed associations of Ang-2 with WMH (as an imaging parameter of SVD) and cognitive function in our study, this suggests that vascular injury may indeed be an important determinant of poor cognitive function, while Ang-2 may also serve as a biomarker for cognitive impairment in this population. This is also emphasized by our finding that Ang-2 associated with quantitative measures of WMH of presumed vascular origin, which are a manifestation of SVD on MRI [32]. Indeed, the association between WMH and cognitive dysfunction (among others with executive function) has been well-established [11, 42, 43]. However, we also find that Ang-2 levels are higher in (younger) DN patients, indicating that the suitability of Ang-2 as biomarker depends on patient characteristics and needs to be carefully evaluated.

Regarding the miRNAs, it is increasingly recognized that circulating miRNAs are involved in cell–cell communication and can be transferred to (vascular) target cells, thereby mediating vascular (dys)function [16]. Circulating miRNAs are carried in exosomes, lipoprotein complexes [mainly high-density lipoprotein (HDL)] or associated to RNA-binding proteins (Argonaute2). These carrier–miRNA complexes have been described as being transferred to and functional in target cells in various tissues [44, 45]. Furthermore, 'brain miRNAs' may be detected in the circulation, as the blood–brain barrier does not block the passage of miRNAs between cerebrospinal fluid and blood (although in lower concentrations) [46], while in pathological states, miRNAs can pass from the brain tissue into the blood through the injured blood–brain barrier [47]. As such, our hypothesis is that the circulating miRNAs we identified may play a role in the pathophysiology of cognitive decline and associated vascular injury in (older) ESRD patients. One such potentially relevant miRNA that we identified is miR-223, where we observed that lower levels of miR-223 were accompanied by impaired memory function. Interestingly, miR-223 has been described to be neuroprotective by targeting glutamate receptors while the absence of miR-223 leads to contextual memory deficits [48]. Other studies have implicated miR-223 in inflammation and vascular injury, suggesting that miR-223 may play a role in the link between vascular injury and inflammation in cognitive deficits [49, 50]. For example, the fact that miR-223 promotes microglia repair [51] could link its lower levels to ongoing injury (or lack of a reparative response), as microglia are intimately linked to memory [52]. We also observed an association of higher miR-29a with poorer memory function and higher WMH scores (Scheltens scale). This appears to be in contrast with previous studies that described lower miR-29a levels in Alzheimer's disease [53] and loss of miR-29a causing neuronal dysfunction [54]. Further studies are necessary to clarify the relations between differential miRNA levels and cognitive function.

We also found several non–statistically significant trends for an association of Ang-2 or miRNAs with cognitive function in the different domains of memory, executive function and psychomotor speed. These might reflect real associations, but the lack of statistical significance might be due to the relatively small group size or more likely due to the fact that our cohort comprises a rather homogeneous group of patients who already have severe kidney failure and comparable cognitive (dys)function. Differences in levels of Ang-2 and miRNAs might be greater if we were to compare these measurements to similarly aged healthy people or patients spanning a larger range of cognitive function. In fact, our comparison of the levels of five angiogenic miRNAs (miR-27a, miR-126, miR-132, miR-223 and miR-326) and Ang-2 in our population of older patients with ESRD with those in young healthy people, as well as with those in patients with DN, already indicate quite large differences in the levels of circulating miRNAs and Ang-2, although the group sizes of healthy controls and DN patients are limited. We previously also showed that cognitive function in the COPE cohort is impaired compared with people without kidney failure of the same age [10], suggesting the combination of old age and kidney failure contribute to changes in the levels of Ang-2 and circulating miRNAs. Furthermore, in that comparison, altered miR-223, miR-126, miR-326 and miR-27a

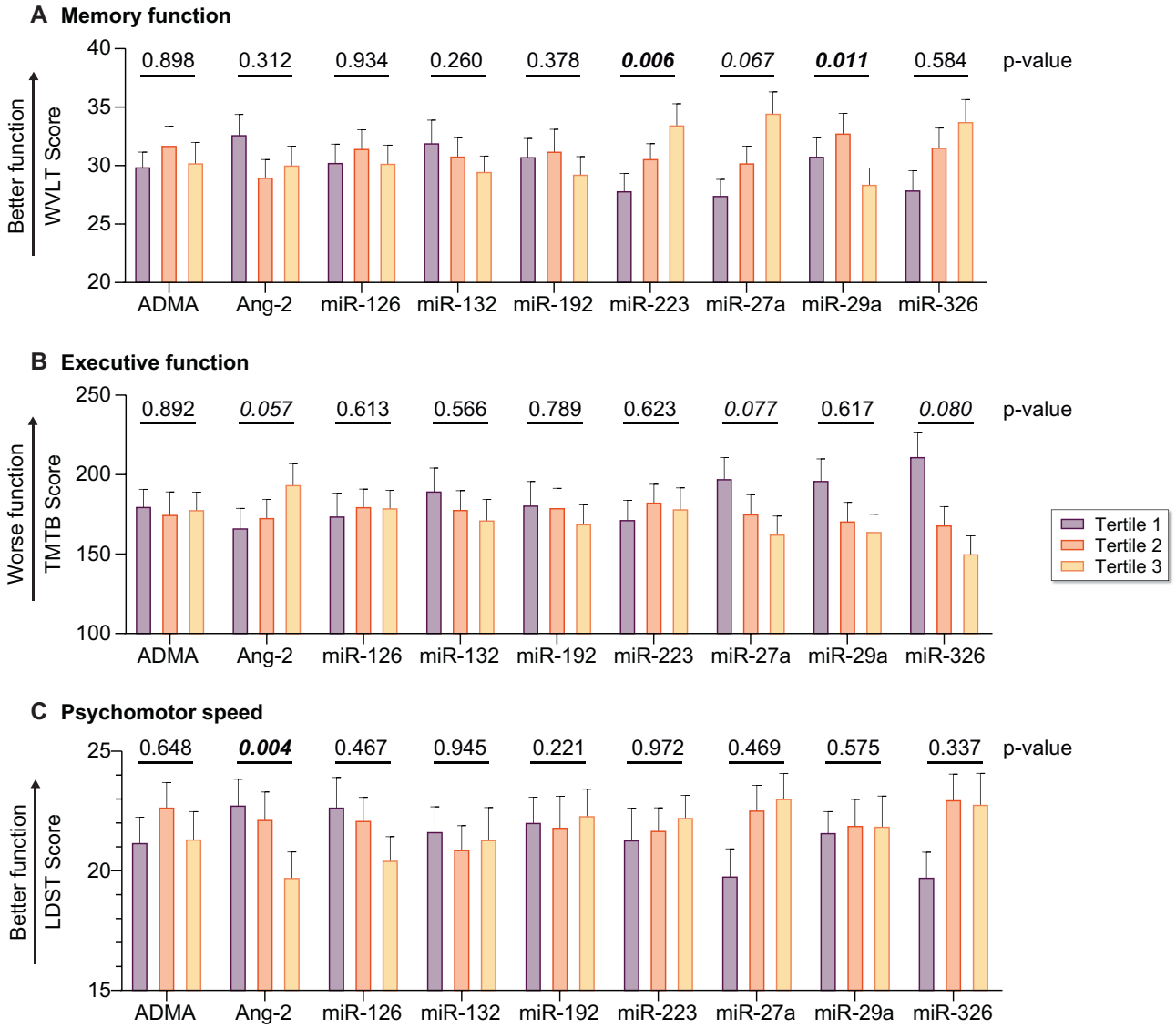

FIGURE 2: Association of miRNAs, Ang-2 and ADMA with cognitive function. ADMA, Ang-2 and miRNA values were divided into tertiles (Tertile 1, lowest levels; Tertile 3, highest levels) and plotted versus (A) 15WVLT score, (B) TMT-B score and (C) LDST score. P-values represent continuous correlation adjusted for sex and age. Data are represented as mean ± SEM.

levels correspond to their previously demonstrated relation with vascular injury [20]. Interestingly, circulating miR-126 levels have already been shown to decrease in older patients with advanced CKD stage [55], while plasma miR-126 levels have also been shown to increase with age [56], suggesting a CKD-dependent decrease. Moreover, miR-126 has been demonstrated to be deregulated in small brain vessels during CKD [57]. miR-132 levels, however, were lower, while miR-132 was expected to be higher when more vascular injury is present. On the other hand, lower miR-132 levels have been extensively associated with decreased cognition [58].

Taken together, although further studies are warranted to understand the exact relation between circulating Ang-2 and angiogenic miRNA levels, our data suggest that Ang-2 and miRNAs might serve as biomarkers of cognitive function in older patients reaching ESRD and may provide insights into the underlying (vascular) pathophysiology.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

FUNDING

R.B. is supported by a grant from the Dutch Kidney Foundation (16OKG16) and R.B. and A.J.V.Z. are supported by a grant from the European Foundation for the Study of Diabetes. Additional support was provided to A.J.V.Z by the Dutch Heart Foundation in the context of the CVON consortium RECONNECT and to M.J.P.V.O., M.A.V.B., E.E.B. and H.C.A. in the context of the CVON consortium Heart Brain Connection (CVON 2018-28 and 2012-06).

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and its online supplementary material.

A Patient characteristics cerebrovascular MRI

Qualitative MRI features, n	93
Presence of microbleeds, n (%)	56 (60.2%)
Presence of lacunar infarction, n (%)	44 (47.3%)
Total WMH (Scheltens score), mean (SD)	15.8 (7.6)

Quantitative MRI feature, n	70
White matter hyperintensities (mL), median (IQR)	3.16 (1.49–10.84)

B White matter hyperintensities

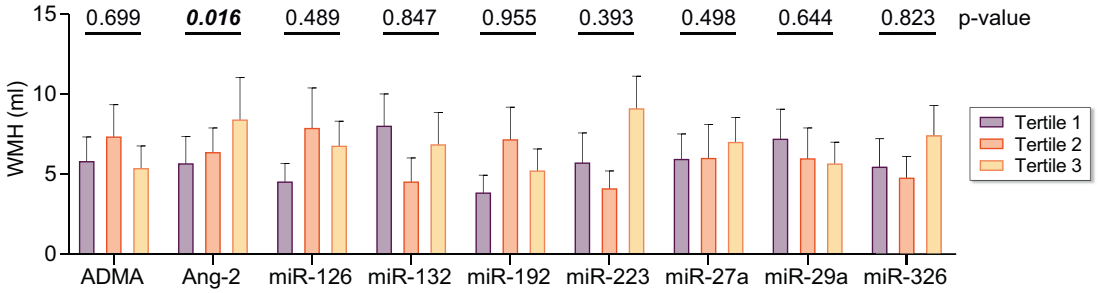

FIGURE 3: Association of miRNAs, Ang-2 and ADMA with MRI of cerebral SVD. (**A**) Data on qualitative ($n = 93$) and quantitative ($n = 70$) features of cerebrovascular MRI. (**B**) ADMA, Ang-2 and miRNA values were divided into tertiles (Tertile 1, lowest levels; Tertile 3, highest levels) and plotted versus WMH volume (quantitative data) as a measure of SVD. Numbers above the bars indicate P-values that represent continuous correlation adjusted for sex, age and intracranial volume. Data are represented as mean ± SEM.

AUTHORS' CONTRIBUTIONS

R.B. conducted experiments, acquired and analysed data and wrote the manuscript. M.H.K., L.E.Z, B.M.V.B., J.D.B., S.H., E.E.B., N.C.B.B. and H.A. acquired and analysed data. R.B., M.A.V.B., W.J.W.B., D.V.H., T.J.R., M.V.B. and S.M. conceptualized the study. R.B., A.J.V.Z., M.V.B. and S.M. contributed to the discussion and reviewed and edited the manuscript. R.B., M.V.B. and S.M. are guarantors of this work and, as such, had full access to all the data in the study and responsibility for the integrity of the data and the accuracy of the data analyses.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Pippias M, Jager KJ, Kramer A *et al.* The changing trends and outcomes in renal replacement therapy: data from the ERA-EDTA Registry. *Nephrol Dial Transplant* 2016; 31: 831–841
2. Gillespie BW, Morgenstern H, Hedgeman E *et al.* Nephrology care prior to end-stage renal disease and outcomes among new ESRD patients in the USA. *Clin Kidney J* 2015; 8: 772–780
3. Grams ME, Yang W, Rebholz CM *et al.* Risks of adverse events in advanced CKD: the Chronic Renal Insufficiency Cohort (CRIC) study. *Am J Kidney Dis* 2017; 70: 337–346
4. Drew DA, Weiner DE, Tighiouart H *et al.* Cognitive decline and its risk factors in prevalent hemodialysis patients. *Am J Kidney Dis* 2017; 69: 780–787
5. Drew DA, Weiner DE, Tighiouart H *et al.* Cognitive function and all-cause mortality in maintenance hemodialysis patients. *Am J Kidney Dis* 2015; 65: 303–311
6. Kallenberg MH, Kleinveld HA, Dekker FW *et al.* Functional and cognitive impairment, frailty, and adverse health outcomes in older patients reaching ESRD–a systematic review. *Clin J Am Soc Nephrol* 2016; 11: 1624–1639
7. Bugnicourt JM, Godefroy O, Chillon JM *et al.* Cognitive disorders and dementia in CKD: the neglected kidney-brain axis. *J Am Soc Nephrol* 2013; 24: 353–363
8. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010; 9: 689–701
9. Foster R, Walker S, Brar R *et al.* Cognitive impairment in advanced chronic kidney disease: the Canadian Frailty Observation and Interventions Trial. *Am J Nephrol* 2016; 44: 473–480
10. van Deudekom FJ, Kallenberg MH, Berkhout-Byrne NC *et al.* Patterns and characteristics of cognitive functioning in older patients approaching end stage kidney disease, the COPE-study. *BMC Nephrol* 2020; 21: 126
11. Prins ND, Scheltens P. White matter hyperintensities, cognitive impairment and dementia: an update. *Nat Rev Neurol* 2015; 11: 157–165
12. Mogi M, Horiuchi M. Clinical interaction between brain and kidney in small vessel disease. *Cardiol Res Pract* 2011; 2011: 306189
13. Bucur B, Madden DJ. Effects of adult age and blood pressure on executive function and speed of processing. *Exp Aging Res* 2010; 36: 153–168
14. Umans JG, Pliskin NH. Attention and mental processing speed in hemodialysis patients. *Am J Kidney Dis* 1998; 32: 749–751
15. van Kralingen JC, McFall A, Ord ENJ *et al.* Altered extracellular vesicle microRNA expression in ischemic stroke and small vessel disease. *Transl Stroke Res* 2019; 10: 495–508
16. Creemers EE, Tijsen AJ, Pinto YM. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Circ Res* 2012; 110: 483–495
17. Khairoun M, de Koning EJ, van den Berg BM *et al.* Microvascular damage in type 1 diabetic patients is reversed in the first year after simultaneous pancreas-kidney transplantation. *Am J Transplant* 2013; 13: 1272–1281
18. Guay C, Regazzi R. Circulating microRNAs as novel biomarkers for diabetes mellitus. *Nat Rev Endocrinol* 2013; 9: 513–521
19. Bijkerk R, Florijn BW, Khairoun M *et al.* Acute rejection after kidney transplantation associates with circulating microRNAs and vascular injury. *Transplant Direct* 2017; 3: e174
20. Bijkerk R, Duijs JM, Khairoun M *et al.* Circulating microRNAs associate with diabetic nephropathy and systemic microvascular damage and normalize after simultaneous pancreas-kidney transplantation. *Am J Transplant* 2015; 15: 1081–1090

21. Berkhout-Byrne N, Kallenberg MH, Gaasbeek A *et al.* The Cognitive decline in Older Patients with End stage renal disease (COPE) study – rationale and design. *Curr Med Res Opin* 2017; 33: 2057–2064
22. Houx PJ, Shepherd J, Blauw GJ *et al.* Testing cognitive function in elderly populations: the PROSPER study. PROspective Study of Pravastatin in the Elderly at Risk. *J Neurol Neurosurg Psychiatry* 2002; 73: 385–389
23. van Exel E, Gussekloo J, Houx P *et al.* Atherosclerosis and cognitive impairment are linked in the elderly. The Leiden 85-plus Study. *Atherosclerosis* 2002; 165: 353–359
24. Moonen JE, Foster-Dingley JC, de Ruijter W *et al.* Effect of discontinuation of antihypertensive treatment in elderly people on cognitive functioning–the DANTE study Leiden: a randomized clinical trial. *JAMA Intern Med* 2015; 175: 1622–1630
25. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189–198
26. Adunsky A, Fleissig Y, Levenkrohn S *et al.* A comparative study of Mini-Mental Test, Clock Drawing task and Cognitive-FIM in evaluating functional outcome of elderly hip fracture patients. *Clin Rehabil* 2002; 16: 414–419
27. Brand N, Jolles J. Learning and retrieval rate of words presented auditorily and visually. *J Gen Psychol* 1985; 112: 201–210
28. Lindeboom J, Schmand B, Tulner L *et al.* Visual association test to detect early dementia of the Alzheimer type. *J Neurol Neurosurg Psychiatry* 2002; 73: 126–133
29. van Hoof JJ, Jogems-Kosterman BJ, Sabbe BG *et al.* Differentiation of cognitive and motor slowing in the Digit Symbol Test (DST): differences between depression and schizophrenia. *J Psychiatr Res* 1998; 32: 99–103
30. Reitan RM. The relation of the trail making test to organic brain damage. *J Consult Psychol* 1955; 19: 393–394
31. Van der Elst W, Van Boxtel MP, Van Breukelen GJ *et al.* The Stroop color-word test: influence of age, sex, and education; and normative data for a large sample across the adult age range. *Assessment* 2006; 13: 62–79
32. Wardlaw JM, Smith EE, Biessels GJ *et al.* Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013; 12: 822–838
33. Scheltens P, Barkhof F, Leys D *et al.* A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. *J Neurol Sci* 1993; 114: 7–12
34. Creavin ST, Wisniewski S, Noel-Storr AH *et al.* Mini-Mental State Examination (MMSE) for the detection of dementia in clinically unevaluated people aged 65 and over in community and primary care populations. *Cochrane Database Syst Rev* 2016; 1: CD011145
35. Choi HR, Lee SW, Jeon DH *et al.* Association between estimated glomerular filtration rate (eGFR) and asymmetric dimethylarginine (ADMA) concentrations among the elderly in a rural community: a cross-sectional study. *BMC Geriatr* 2019; 19: 370
36. MacAllister RJ, Rambausek MH, Vallance P *et al.* Concentration of dimethyl-L-arginine in the plasma of patients with end-stage renal failure. *Nephrol Dial Transplant* 1996; 11: 2449–2452
37. Freire de Medeiros CMM, Diogenes da Silva BR, Costa BG *et al.* Cognitive impairment, endothelial biomarkers and mortality in maintenance haemodialysis patients: a prospective cohort study. *Nephrol Dial Transplant* 2020; 35: 1779–1785
38. Cai J, Kehoe O, Smith GM *et al.* The angiopoietin/Tie-2 system regulates pericyte survival and recruitment in diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2008; 49: 2163–2171
39. Fagiani E, Christofori G. Angiopoietins in angiogenesis. *Cancer Lett* 2013; 328: 18–26
40. David S, Kumpers P, Lukasz A *et al.* Circulating angiopoietin-2 levels increase with progress of chronic kidney disease. *Nephrol Dial Transplant* 2010; 25: 2571–2576
41. David S, Kumpers P, Hellpap J *et al.* Angiopoietin 2 and cardiovascular disease in dialysis and kidney transplantation. *Am J Kidney Dis* 2009; 53: 770–778
42. Kloppenborg RP, Nederkoorn PJ, Geerlings MI *et al.* Presence and progression of white matter hyperintensities and cognition: a meta-analysis. *Neurology* 2014; 82: 2127–2138
43. Lampe L, Kharabian-Masouleh S, Kynast J *et al.* Lesion location matters: the relationships between white matter hyperintensities on cognition in the healthy elderly. *J Cereb Blood Flow Metab* 2019; 39: 36–43
44. Thomou T, Mori MA, Dreyfuss JM *et al.* Adipose-derived circulating miRNAs regulate gene expression in other tissues. *Nature* 2017; 542: 450–455
45. Karpman D, Stahl AL, Arvidsson I. Extracellular vesicles in renal disease. *Nat Rev Nephrol* 2017; 13: 545–562
46. Stoicea N, Du A, Lakis DC *et al.* The miRNA journey from theory to practice as a CNS biomarker. *Front Genet* 2016; 7: 11
47. Toyama K, Spin JM, Tsao PS. Role of microRNAs on blood brain barrier dysfunction in vascular cognitive impairment. *Curr Drug Deliv* 2017; 14: 744–757
48. Harraz MM, Eacker SM, Wang X *et al.* MicroRNA-223 is neuroprotective by targeting glutamate receptors. *Proc Natl Acad Sci USA* 2012; 109: 18962–18967
49. Shi L, Fisslthaler B, Zippel N *et al.* MicroRNA-223 antagonizes angiogenesis by targeting beta1 integrin and preventing growth factor signaling in endothelial cells. *Circ Res* 2013; 113: 1320–1330
50. Selles MC, Oliveira MM, Ferreira ST. Brain inflammation connects cognitive and non-cognitive symptoms in Alzheimer's disease. *J Alzheimers Dis* 2018; 64: S313–S327
51. Galloway DA, Blandford SN, Berry T *et al.* miR-223 promotes regenerative myeloid cell phenotype and function in the demyelinated central nervous system. *Glia* 2019; 67: 857–869
52. Williamson LL, Sholar PW, Mistry RS *et al.* Microglia and memory: modulation by early-life infection. *J Neurosci* 2011; 31: 15511–15521
53. Shioya M, Obayashi S, Tabunoki H *et al.* Aberrant microRNA expression in the brains of neurodegenerative diseases: miR-29a decreased in Alzheimer disease brains targets neurone navigator 3. *Neuropathol Appl Neurobiol* 2010; 36: 320–330
54. Roshan R, Shridhar S, Sarangdhar MA *et al.* Brain-specific knockdown of miR-29 results in neuronal cell death and ataxia in mice. *RNA* 2014; 20: 1287–1297
55. Fourdinier O, Schepers E, Metzinger-Le Meuth V *et al.* Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. *Sci Rep* 2019; 9: 4477
56. Olivieri F, Bonafe M, Spazzafumo L *et al.* Age- and glycemia-related miR-126-3p levels in plasma and endothelial cells. *Aging (Albany NY)* 2014; 6: 771–787
57. Metzinger-Le Meuth V, Andrianome S, Chillon JM *et al.* microRNAs are dysregulated in the cerebral microvasculature of CKD mice. *Front Biosci* 2014; E6: 80–88
58. Hansen KF, Karelina K, Sakamoto K *et al.* miRNA-132: a dynamic regulator of cognitive capacity. *Brain Struct Funct* 2013; 218: 817–831

Received: 13.10.2020; Editorial decision: 8.12.2020