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NEPHROLOGY - ORIGINAL PAPER

Pentosidine in chronic hemodialysis patients: relation with arteriovenous fistula morphology and function

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Abstract

Purpose It has been suggested that advanced glycation end products (AGEs) are involved in atherogenesis, vascular calcification and remodeling, including neointimal hyperplasia, in renal and non-renal patients. Their relevance for arteriovenous fistula (AVF) function has been poorly studied to date, with only one clinical study addressing the issue of thrombosis of vascular access in relation to AGEs in dialysis patients. We aimed to evaluate the relationship between serum pentosidine and AVF morphology and function.

Methods Eighty-eighth hemodialysis patients with patent native AVF were included. Ultrasound examination of AVF evaluated blood flow in the brachial artery, resistivity index (RI), the diameter of the vessels and the presence of stenosis. AVF and cardiovascular history were recorded, routine clinical and laboratory evaluation was performed and serum pentosidine was assessed.

Results Forty-eight patients (54.54%) had AVF stenosis. Pentosidine correlated in univariate analysis with cholesterol ($r=0.270$, $p=0.01$), triglycerides ($r=0.309$, $p=0.003$), calcium ($r=0.040$, $p<0.001$) and inversely to dialysis vintage ($r=-0.453$, $p<0.001$), access vintage ($r=-0.432$, $p=0.001$), phosphate ($r=-0.211$, $p=0.04$), parathyroid hormone ($r=-0.211$, $p=0.04$), urea ($r=-0.230$, $p=0.03$), residual diameter of AVF ($r=-0.023$, $p=0.03$). In multivariate regression calcium ($p=0.006$), access vintage ($p=0.03$), and residual diameter of AVF vein ($p=0.02$) remain significantly linked to pentosidine. Patients with pentosidine above median had higher cholesterol (179.91 vs. 160.97, $p=0.04$), triglycerides (187.18 vs. 129.31, $p=0.002$) and higher prevalence of hypertension (93.70% vs. 84.10%, $p=0.02$).

Conclusions Our study suggests that pentosidine could be associated to vascular access morphology and function in dialysis patients.

Keywords Arteriovenous fistula · Hemodialysis · Advanced glycation end product · Stenosis · Pentosidine

Introduction

Advanced glycation end products (AGEs) are proteins generated by a series of chemical processes termed the Maillard reaction [1, 2]. Carbohydrates are linked non-enzymatically with amino groups of proteins and further undergo reactions that lead to the formation of reversible Schiff base adducts and finally the generation of more stable products (Amadori products) [3]. Subsequently, complex rearrangements occur, including intermolecular cross-link formation, oxidation mediated cleavage with the generation of AGEs. Importantly, the formation of glycoxidation products such as *N*-carboxymethyllysine (CML) and pentosidine is considered to be the result of a chemical reaction dependent on the

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concentration of both carbohydrate precursors and reactive oxygen species (oxidative stress) [4].

Increased levels of AGEs are, therefore, found in diabetic patients, in whom the accumulation of AGEs may be attributable to both glycative and oxidative stress. However, in renal patients, increased levels of AGEs are, also, repeatedly described [5], and are inversely related to the decline of renal function, with the highest levels reported at the onset of dialysis treatment [6]. This is nevertheless much more than a passive accumulation process as increased AGEs in end-stage renal disease (ESRD) are linked to a multitude of pathological processes, including reduced metabolic clearance, increased oxidative stress in addition to a higher rate of reactive carbonyl compound formation [7].

Accumulation of AGEs is reflected by serum levels of various molecules with pentosidine being a relevant marker of the overall level of AGEs [8]. Tissue deposition can also be evaluated; tissue bound AGEs are important as they are less readily cleared and can exert specific effects. Tissue levels of pentosidine are generally well correlated to serum values [9].

AGEs are involved in atherogenesis, vascular calcification and remodeling, including neointimal hyperplasia (NIH) in renal and non-renal patients [10, 11]. Their relevance for arteriovenous fistula (AVF) function and patency has been poorly investigated to date, with only one clinical study addressing the issue of thrombosis of vascular access in relation to AGEs in dialysis patients [12].

As vascular access patency is very important for the prognosis of dialysis patients and mechanisms leading to AVF dysfunction and loss are not completely understood, we aimed to explore the relationship between AGE compounds (evaluated by serum pentosidine levels) and vascular access function and morphology (assessed by ultrasound examination) in a cohort of prevalent hemodialysis patients.

Materials and methods

We conducted an observational cross-sectional clinical study in a dialysis centre of Cluj-Napoca, Romania, between August 2016 and April 2017. All the patients signed an informed consent and the study was approved by the local Ethics Committee. Eighty-eight ($n = 88$) patients were included in the current study.

Inclusion criteria were prevalent patients treated with chronic hemodialysis or hemodiafiltration three times a week; the presence of a functional AVF.

Exclusion criteria were evidence of significant inflammatory syndrome (acute infection); patients with central venous catheters (temporary or permanent).

Type and history of vascular access, the presence of atherosclerosis-related cardiovascular disease (defined as ischemic heart disease, history of stroke, peripheral arterial disease identified in patient's records), the presence of diabetes mellitus, hypertension, certain specific treatment regimens (phosphate-binders, hypolipemiant medication) were recorded. Body mass index was calculated by dividing weight (in kilograms) by height squared (in meters). General and specific laboratory data were measured before midweek dialysis session, with patient at rest. Blood was collected and shipped to an authorized laboratory immediately were the following biochemical parameter were determined: creatinine, urea, phosphorus, total calcium, total cholesterol, triglycerides (spectrophotometry), parathyroid hormone (PTH) using electrochemiluminescence immunoassay and parameters of dialysis efficiency (K_t/V) using Adimea method based on the principles of spectroscopy for determining the reduction in the molar concentration of urinary excreted substances in the dialysate drain. A light source transmits ultraviolet light through the dialysate. The particles contained in the dialysate, which were removed from the plasma during dialysis, absorb the light. This absorption is measured by a sensor [13]. At the same time blood was drawn for pentosidine measurement as follows: 5 ml venous blood was collected in EDTA-containing vacutainer tubes. The samples were protected with cold chain, centrifuged at 3000 rpm for 15 min, and kept at − 80 °C until the analysis. Plasma levels of Pentosidine were analyzed in the serum with an enzyme-linked immunosorbent assay (Human Pentosidine Elisa kit, My BioSource.com, San Diego, USA). This assay has high sensitivity (lower limit of detection 0.1 ng/mL) and exhibits no detectable cross-reactivity with other relevant proteins. Calibration and standardization of the assays were performed according to the manufacturer's protocol.

Clinical and ultrasound evaluation of patients was performed in one of the dialysis days, prior to start of the dialysis session.

Color Doppler ultrasonography studies were performed by a single operator using General Electric Logiq e BT12 machines (linear transducer 10 MHz frequency). The diameters of the anastomosis and residual diameter of the venous outflow were measured. The estimation of the vessel caliber was performed in transversal section. Presence of stenosis, calcification and aneurisms in the puncture areas (defined as increase in vessel section > 10 mm), brachial resistivity index (RI) and blood flow were noted. After the measurement of the brachial artery diameter we performed a longitudinal scan of the artery recording the Doppler velocity spectrum, in the lower third of the brachial artery at the elbow, in an area where superposition from other vessels (vein) was minimal with an insonation angle ≤ 60° and sample volume between 50 and 70% of the lumen, using a minimum of five cardiac circles, but using all the cycles available on the screen, depending on heart-rate. Blood flow (Q) was measured automatically (software of General Electric Logiq

BT12 machines) according to the following principle: $Q = r^2 \times 3.14 \times V_m \times 60$, where r is the radius of the vessel, Vm is the mean velocity. RI was measured in the brachial artery according to the following formula: $RI = (V_{max} - V_{min})/V_{max}$, where V_{max} represents the peak systolic velocity and V_{min} represents the end diastolic velocity [14]. The stenosis was defined as diameter narrowing more than 50% and an increase in peak systolic velocities (PSV) ratio (PSV in the stenotic area/PSV upstream the stenotic area) > 3:1 in the anastomotic area or > 2:1 in the draining vein [15–17]. The diagnostic criteria vary in accordance with the location of the stenosis. The site of stenotic lesion was classified in 4 categories based upon location: juxta-anastomotic vein (the first 2 cm downstream from the arterial anastomosis), venous outflow > 2 cm from anastomosis (cannulation zone), distal outflow (defined as above elbow joint for radial-cephalic fistulae and above mid-humerus for brachial fistulae) and cephalic arch stenosis [14, 18].

Intima media thickness was measured near and far walls on the left and right common carotid arteries, 2–3 cm proximal to bifurcation, in a longitudinal section and was expressed as a mean of three consecutive measures.

Statistical analysis was performed using SPSS 19.0, and Microsoft EXCEL software. Kolmogorov–Smirnov test or Shapiro–Wilk test were used to assess the normal distribution of data. Continuous variables were defined as mean ± standard deviation or median (inter-quartile range); categorical variables were given as percentages. Student´s *t* test or Mann–Whitney *U* test was used for the comparison of two means of independent samples. For comparison of categorical variables, Chi-square test or Fisher exact test was employed. Pearson's or Spearman correlation coefficient (*r*) and linear univariate regression were used for identifying correlation between two continuous variables. Multivariate linear regression using the enter method was performed to. The differences were considered statistically significant at $p < 0.05$.

Results

General characteristics, clinical and laboratory parameters of patients are presented in Table 1. The median age was 65 (51.25–70) years and 64.77% of the patients were male. Renal failure had various causes, but the major cause was diabetic nephropathy. Ultrasound evaluation of AVF revealed a high prevalence of AVF stenosis (54.54%). All patients had one stenotic lesion and the site of stenotic lesion was: 15.92% juxta-anastomotic vein, 13.64% venous outflow, 21.58% distal outflow and 3.40% cephalic arch. Pentosidine levels correlated significantly in univariate analysis with cholesterol, triglycerides and calcium. Pentosidine also correlated inversely with dialysis vintage, access vintage, serum phosphate, PTH, urea, residual diameter of AVF. Results are presented in Table 2.

Table 1 General characteristics of the patients

Parameter	All patients ($n = 88$)
Age (years)	65 (51.25–70)
Male, *n* (%)	57 (64.77)
Body mass index (kg/m^2)	27.38 ± 4.78
Hypertension, *n* (%)	80 (90.9)
Diabetes mellitus, *n* (%)	27 (30.68)
Radiocephalic AVF, *n* (%)	47 (53.40)
Brachiocephalic AVF, *n* (%)	31 (35.22)
Brachiobasilic AVF, *n* (%)	10 (11.36)
Brachial blood flow (ml/min)	923.50 (750–1148)
Brachial resistivity index	0.48 ± 0.12
Anastomosis diameter (mm)	5.75 (5.0–7.5)
Residual diameter (mm)	4.4 (3.1–6.5)
Aneurisms > 10, *n* (%)	54 (61.39)
Stenosis, *n* (%)	48 (54.54)
Intima-media thickness (mm)	1.09 ± 0.22
Duration of regular dialysis therapy (years)	5.00 (3–7.75)
Access vintage (years)	4.68 (2.00–6.00)
Phosphorus (mg/dl)	4.85 (4.12–5.77)
Calcium (mg/dl)	8.85 (7.80–9.20)
PTH (pg/ml)	278.5 (144.87–546.75)
Pentosidine (ng/ml)	43.95 (6.91–143.18)
Total cholesterol (mg/dl)	166.8 (144.5–192.30)
Triglycerides (mg/dl)	128.05 (97.35–189.40)
Urea (mg/dl)	127.6 ± 30.86
Creatinine (mg/dl)	8.53 ± 1.92
K_t/V	1.33 (1.26–1.52)
Calcification AVF, *n* (%)	66 (75)
Ischemic heart disease, *n* (%)	34 (38.63)
Peripheral vascular disease, *n* (%)	22 (25)
Cerebrovascular disease, *n* (%)	15 (17.04)
Antiplatelet therapy, *n* (%)	43 (48.86)
Anticoagulant therapy, *n* (%)	10 (11.36)
Phosphate binders, *n* (%)	71 (80.68)
Statins, *n* (%)	31 (35.22)

Values are expressed as mean ± SD for normally distributed variables and median (25th–75th percentiles) for the abnormally distributes ones

AVF arteriovenous fistula, *PTH* parathyroid hormone

In multivariate regression—enter method, with pentosidine as a dependent variable and using as independent variables those parameters which significantly correlated with pentosidine in univariate, calcium ($p = 0.006$), access vintage ($p = 0.03$), and residual diameter of AVF ($p = 0.02$) remained significant predictors of pentosidine (Table 3).

The patients were divided into two groups according to median value of pentosidine: patients with

Table 2 Correlations between the investigated parameters with pentosidine concentration

Parameter	*r*	*p*
Brachial blood flow (ml/min)	− 0.041	0.70
Brachial resistivity index	0.027	0.80
Anastomosis diameter (mm)	− 0.038	0.72
Residual diameter (mm)	− 0.223	0.03*
Duration of regular therapy with dialysis (years)	− 0.453	< 0.001*
Access vintage (years)	− 0.432	0.001*
PTH (pg/ml)	− 0.211	0.04*
Calcium (mg/dl)	0.401	< 0.001*
Phosphorus (mg/dl)	− 0.211	0.04*
Total cholesterol (mg/dl)	0.270	0.01*
Triglycerides (mg/dl)	0.309	0.003*
Urea (mg/dl)	− 0.230	0.03*
Creatinine (mg/dl)	− 0.185	0.08
K_t/V	0.069	0.52

AVF arteriovenous fistula, *PTH* parathyroid hormone

*Significance value

pentosidine < 43.92 ng/ml (Group 1; $n = 44$) and patients with pentosidine > 43.92 ng/ml (Group 2; $n = 44$). There was no difference in age, gender, diabetes, type of FAV, and body mass index between Group 1 and Group 2 (Table 4). The access vintage was significantly shorter in patients with pentosidine levels above median. The duration of regular dialysis therapy was also significantly smaller in group with pentosidine levels above median. There was no difference in medication use and incidence of cardiovascular diseases between the two groups. Biochemical tests showed that patients with pentosidine levels above median had higher level of cholesterol and higher level of triglycerides compared with patients with pentosidine level below median. Patients with pentosidine level above median had higher prevalence of hypertension. Patients with high pentosidine levels had higher prevalence of stenosis. Comparison of patients with and without stenosis is provided in Table 5.

Discussion

We chose to study pentosidine as marker of advanced glycosylation in our patients as it is considered, along CML, a good surrogate of carbohydrate-derived AGEs and assays are well standardized, with reproducible results [19]. Pentosidine, as other AGEs, accumulates in renal insufficiency, the highest levels being detected in dialysis patients [20]. Although we did not have a control group to compare with, levels of pentosidine in our hemodialysis patients are in line with those reported by other authors [21]. Pentosidine is removed by dialysis [22, 23] and several authors have described an inverse correlation of pentosidine with dialysis vintage. We confirm such findings with an inverse correlation of pentosidine in both univariate and multivariate analysis with time on dialysis.

The main goal of our study was to detect a possible link of pentosidine levels to vascular access morphology and function. Atherosclerosis, NIH and vascular calcification of arteriovenous fistula can pathogenetically be influenced by AGEs.

AGEs have been studied extensively with regard to their influence on atherosclerosis and its progression, in renal and non-renal patients: higher values are independent predictors for increase in intima media thickness, the occurrence of coronary events, cardiovascular mortality [24, 25]. There are several possible levels at which AGEs can promote the atherosclerotic process: stimulation of oxidative stress and promotion of leukocyte adhesion [26]. One important mechanism is mediated by cholesterol levels. Hypercholesterolemia might increase AGEs levels through a not yet defined mechanism. Also, hypercholesterolemia decreases

Table 3 Multivariate regression analysis with pentosidine as dependent variable

	Unstandardized coefficient	Standardized coefficient		*p*	95% confidence interval for *B*	
	B	SE	Beta		Lower bound	Upper bound
Calcium	104562.90	37326.78	0.30	0.006*	30265.82	178860
Access vintage	− 34968.29	16218.47	− 0.21	0.034*	− 67250.34	− 2686.23
Residual diameter	− 68533.52	30943.62	− 0.21	0.029*	− 130125.30	− 6941.78
Phosphorus	− 54474.58	54696.18	− 0.10	0.322	− 163344.60	54395.44
PTH	− 135.55	160.99	− 0.08	0.402	− 455.99	184.89
Urea	− 1297.44	2426.87	− 0.05	0.594	− 6128.01	3533.12
Cholesterol	2825.02	1571.23	0.17	0.076	− 302.44	5952.50

B unstandardized coefficient B, *SE* standard error, *CI* confidence interval, *AVF* arteriovenous fistula, *PTH* parathyroid hormone

*Significance value

Table 4 Comparison of patients according to pentosidine median

Parameter	Pentosidine < 43.95 ng/ml ($n = 44$)	Pentosidine > 43.95 ng/ml ($n = 44$)	p
Age (years)	63.00 (49.50–70.00)	65.50 (56.00–70.00)	0.39
Male, *n* (%)	29 (65.90)	28 (63.60)	0.82
Hypertension, *n* (%)	37 (84.10)	43 (97.7)	0.02*
Diabetes mellitus, *n* (%)	11 (25)	16 (36.40)	0.24
Ischemic heart disease, *n* (%)	16 (36.4)	18 (40.90)	0.66
Cerebrovascular disease, *n* (%)	7 (15.90)	8 (18.20)	0.77
Peripheral vascular disease, *n* (%)	13 (29.50)	9 (20.50)	0.32
Intima-media thickness (mm)	1.10 (0.97–1.29)	1.05 (0.96–1.10)	0.05
Brachial blood flow (ml/min)	950.50 (754.00–1128.00)	917.00 (698.00–1327.00)	0.95
Brachial resistivity index	0.48 ± 0.13	0.47 ± 0.12	0.75
Anastomosis diameter (mm)	5.75 (5.10–7.45)	5.85 (5.00–7.60)	0.50
Residual diameter (mm)	4.40 (3.30–6.55)	4.10 (2.60–6.20)	0.42
Duration of dialysis therapy (years)	8.30 ± 5.39	4.11 ± 2.78	< 0.001*
Access vintage (years)	5.00 (2.00–7.00)	2.00 (2.00–4.00)	0.001*
Brachiobasilic AVF, *n* (%)	2 (4.50)	8 (18.20)	0.09
Radiocephalic AVF, *n* (%)	24 (54.50)	23 (52.30)	0.10
Brachiocephalic AVF, *n* (%)	18 (40.90)	13 (29.50)	0.28
Stenosis, *n* (%)	23 (52.30)	25 (56.80)	0.41
PTH (pg/ml)	370.00 (144.45–600.50)	233.50 (144.50–404.00)	0.12
Calcium (mg/dl)	8.30 (6.42–9.20)	9.00 (8.70–9.20)	< 0.001*
Phosphorus (mg/dl)	4.90 (4.30–6.00)	4.80 (4.10–5.80)	0.14
Total cholesterol (mg/dl)	159.80 (141.25–180.85)	171.50 (147.00–210.30)	0.04*
Triglycerides (mg/dl)	116.50 (189.00–168.20)	168.35 (115.2–221.10)	0.002*
Calcification AVF, *n* (%)	35 (79.50)	31 (70.50)	0.32
Urea (mg/dl)	133.79 ± 34.77	121.46 ± 25.36	0.06
Creatinine (mg/dl)	8.85 ± 2.17	8.21 ± 1.59	0.12
Statin therapy, *n* (%)	15 (34.10)	16 (36.40)	0.81
Phosphate-binders therapy, *n* (%)	37 (84.10)	34 (77.30)	0.41
Antiplatelet therapy, *n* (%)	25 (56.80)	18 (40.90	0.13
Anticoagulant therapy, *n* (%)	3 (6.80)	7 (15.90)	0.10

Values are expressed as mean ± SD for normally distributed variables and median (25th–75th percentiles) for the abnormally distributes ones

AVF arteriovenous fistula, *PTH* parathyroid hormone

*Significance value

shedding of receptor for advanced glycation end products (RAGE) from the cell surface and thus decreases soluble receptors for advanced glycation end products (sRAGE). The latter acts as a decoy for AGEs and reduce their action. AGEs, on the other hand, promote glycation of low-density lipoprotein (LDL) which can no longer be recognized by its receptor [27]. In accordance with previous reports [27], we also found a statistically significant link of pentosidine to serum cholesterol and triglycerides in univariate regression, with cholesterol having a trend to be significant in multivariate regression also. When comparing patients according to pentosidine levels, those with pentosidine above median, have significantly higher cholesterol and triglycerides than those with pentosidine below median. Statin treatment might confound interaction of AGEs and cholesterol as it promotes sRAGE shedding [28]. In our patients, pentosidine levels were similar in patients with or without on statin treatment. Several clinical studies have reported that antiplatelet and anticoagulant therapy can improve AVF patency [29] but in our study there were no differences in the proportion of patients with or without such medication according to presence or absence of stenosis or pentosidine level. On the other hand, no significant differences were detected in the presence of atherosclerosis-related cardiovascular disease between the patients with high and low levels of pentosidine. However, is not unexpected considering the retrospective data collection. Furthermore, in our study we did not find a significant relation between plasma pentosidine levels and intima media thickness, probably due to a small number of patients.

Table 5 Comparison between patients with and without stenosis

Parameter	Stenosis ($n=48$)	Without stenosis ($n=40$)	p
Age (years)	60.50 (50.50–67.00)	66.00 (57.00–71.00)	0.05
Male, *n* (%)	28 (58.33)	29 (72.50)	0.17
Hypertension, *n* (%)	45 (93.75)	35 (87.50)	0.46
Diabetes mellitus, *n* (%)	15 (31.25)	12 (30.00)	0.90
Ischemic heart disease, *n* (%)	17 (35.42)	17 (42.50)	0.50
Cerebrovascular disease, *n* (%)	9 (18.75)	6 (15.00)	0.64
Peripheral vascular disease, *n* (%)	11(22.92)	11(27.50)	0.62
Intima-media thickness (mm)	1.12 (0.95–1.29)	1.00 (0.90–1.20)	0.07
Brachial blood flow (ml/min)	950.50 (715.00–1140.00)	869.00 (750.00–1189.00)	0.81
Brachial resistivity index	0.51 ± 0.13	0.43 ± 0.10	0.002*
Anastomosis diameter (mm)	6.00 (5.00–8.00)	5.65 (5.00–6.65)	0.39
Residual diameter (mm)	3.30 (2.60–4.10)	6.50 (5.40–7.60)	0.31
Duration of dialysis therapy (years)	5.00 (3.00–9.50)	4.50 (2.00–7.00)	0.37
Access vintage (years)	3.00 (2.00–6.00)	4.00 (2.00–6.50)	0.46
Brachiobasilic AVF, *n* (%)	6 (12.50)	4 (10.00)	0.29
Brachiocephalic AVF, *n* (%)	20 (41.67)	11 (27.50)	0.19
Radiocephalic AVF, *n* (%)	22 (45.83)	25 (62.50)	0.28
PTH (pg/ml)	267.00 (136.45–556.90)	314.05 (148.00–543.50)	0.78
Calcium (mg/dl)	8.80 (6.46–9.20)	8.95 (8.30–9.35)	0.60
Phosphorus (mg/dl)	4.85 (4.45–5.75)	4.85 (3.90–5.75)	0.41
Total cholesterol (mg/dl)	159.75 (124.65–185.35)	175.65 (153.10–196.25)	0.33
Triglycerides (mg/dl)	137.10 (93.50–190.20)	126.10 (103.10–188.25)	0.77
Calcification AVF, *n* (%)	40 (83.3)	26 (65.0)	0.04*
Urea (mg/dl)	132.93 ± 29.23	121.25 ± 31.92	0.08
Creatinine (mg/dl)	8.85 ± 1.75	8.15 ± 2.06	0.09
Pentosidine (ng/ml)	73.68 (9.47–143.78)	31.03 (5.18–143.46)	0.01*
K_t/V	1.39 (1.27–1.55)	1.31 (1.26–1.41)	0.04*
Statin therapy, *n* (%)	15 (31.25)	16 (40.00)	0.39
Phosphate-binders therapy, *n* (%)	38 (79.17)	33 (82.50)	0.69
Antiplatelet therapy, *n* (%)	25 (52.08)	18 (45.00)	0.50
Anticoagulant therapy, *n* (%)	6 (12.50)	4 (10.00)	0.75

Values are expressed as mean ± SD for normally distributed variables and median (25th–75th percentiles) for the abnormally distributes ones

AVF arteriovenous fistula, *PTH* parathyroid hormone

*Significance value

Another important pathologic vascular process, named neointimal hyperplasia (NIH) might be directly linked to AGEs and pentosidine. In animal models vascular injury is associated to neointimal proliferation and in the area of NIH one can observe increased expression of RAGE. This process can be reduced by addition of blocking antibodies, sRAGE or genetic manipulation (RAGE knock-out) [30]. The major cause of AVF stenosis is the development of NIH [31] and AGEs might play a role in this process: in samples from native AVF recovered from hemodialysis patients at the time of surgical revision or resection, CML was detectable in the area of NIH [32]. The mechanism through which AGEs influences NIH is not understood enough but non-enzimatic cross linking of collagen may play a role. A positive relationship of AGEs and matrix metalloproteinase 2 (MMP2) has been described [33] and it is known that MMP2 favors NIH [34]. In our study we found a statistically significant negative correlation between pentosidine concentration and residual diameter of AFV. NIH reduces the residual diameter of AVF and may impair the flow in the brachial artery [35]. Patients with high pentosidine levels had higher prevalence of stenosis. Comparison of patients with and without stenosis is provided in Table 5. Separate analysis of the relationship between pentosidine and residual diameter, brachial artery flow in stenosis patients was not relevant, probably due to the limited number of studied subjects. This would be, however, of interest given the high risk for graft loss in stenosis patients. Schwing et al. [12]

studied the predictive value of pentosidine for thrombosis of vascular access in hemodialysis patients and showed that an increased level of pentosidine is a significant predictor for vascular access thrombosis.

The third mechanism trough which AGEs can interfere with vascular access in dialysis patients is the promotion of vascular calcification. In our study we found a significant positive correlation of pentosidine to serum calcium levels in both univariate in multivariate analysis. In fact, in experimental settings, AGEs have been shown to activate osteoclast function [36] and to inhibit osteoblasts [37]. Previous studies have also validated a positive correlation of AGEs to serum calcium and pentosidine has been linked to vascular calcification in multiple settings (aortic, coronary), including in dialysis patients [38]. The positive link of pentosidine to serum calcium can explain the negative correlation that we found with serum PTH in our patients in univariate analysis, by means of calcium mediated PTH suppression. There is, however, an alternative explanation, as in cell culture direct inhibitory effects of AGEs have been observed in parathyroid cells [39]. There is only one another study addressing the link of pentosidine to PTH in ESRD. Similar to our findings, Zoccali et al. [40] highlighted the inverse correlation of serum pentosidine to PTH in hemodialysis patients.

Thus, our study suggests that increased AGEs could be associated to vascular access dysfunction in dialysis patients. This observational study does not allow for pathogenetic insights, although premises for AGEs to be involved in atherogenesis, NIH and vascular calcification exist. Our study did not evaluate sRAGE at this point, and thus we cannot appreciate a possible influence of the latter in the above-mentioned processes. Also, another shortcoming of our study is the relatively limited number of patients: larger studies including more homogenous population are needed to confirm our results.

Compliance with ethical standards

Conflict of interest Maria Ticala declares that she has no conflict of interest. Dacian Tirinescu declares that he has no conflict of interest. Crina Claudia Rusu declares that she has no conflict of interest. Diana Moldovan declares that she has no conflict of interest. Alina Ramona Potra declares that she has no conflict of interest. Anca Laura Coman declares that she has no conflict of interest. Cosmina Ioana Bondor declares that she has no conflict of interest. Livia Budisan declares that she has no conflict of interest. Ina Maria Kacso declares that she has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Qibin Z, Jennifer MA, Richard DS, John WB, Thomas OM (2009) A perspective on the maillard reaction and the analysis of protein glycation by mass spectrometry: probing the pathogenesis of chronic disease. J Proteome Res 8:754–769
2. Cho SJ, Roman G, Yeboah F, Konishi Y (2007) The road to advanced glycation end products: a mechanistic perspective. Curr Med Chem 14:1653–1671
3. Vistoli G, De Maddis D, Cipak A, Zarkovic N, Carini M, Aldini G (2013) Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radic Res 47:3–27
4. Kooman JP, Kotanko P, Schols AM, Shiels PG, Stenvinkel P (2014) Chronic kidney disease and premature ageing. Nat Rev Nephrol 10:732–742
5. Semba RD, Ferrucci L, Fink JC, Sun K, Beck J, Dalal M, Guralnik JM, Fried LP (2009) Advanced glycation end products and their circulating receptors and level of kidney function in older community-dwelling women. Am J Kidney Dis 53:51–58
6. Busch M, Franke S, Muller A, Wolf M, Gerth J, Ott U, Niwa T, Stein G (2004) Potential cardiovascular risk factors in chronic kidney disease: aGEs, total homocysteine and metabolites, and the C-reactive protein. Kidney Int 66:338–647
7. Vaziri ND (2004) Oxidative stress in uremia: nature, mechanisms, and potential consequences. Semin Nephrol 24:469–473
8. Miyata T, Ypersele Van, de Strihou C, Kurokawa K, Baynes JW (1999) Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. Kidney Int 55:389–399
9. Meerwaldt R, Graaff R, Oomen PHN, Links TP, Jager JJ, Alderson NL, Thorpe SR, Baynes JW, Gans ROB, Smit AJ (2004) Simple non-invasive assessment of advanced glycation end product accumulation. Diabetologia 47:1324–1330
10. Busch M, Franke S, Ruster C, Gunter W (2010) Advanced glycation end-products and the kidney. Eur J Clin Invest 40:742–755
11. Stinghen AE, Massy ZA, Vlassara H, Striker GE, Boullier A (2016) Uremic toxicity of advanced glycation end products in CKD. J Am Soc Nephrol 27:354–370
12. Schwing WD, Erhard P, Holloman CK, Weigel KA, Blankschaen SC, Anderson JM, Siegel CT, Seaman DS, Valente JF, DeOreo PB (2004) Thrombotic events and markers of oxidation and inflammation in hemodialysis. Hemodial Int 8:338–343
13. Uhlin F, Fridolin I, Magnusson M, Linberg L-G (2006) Dialysis dose (K_t/V) and clearance variation sensitivity using measurement of ultraviolet absorbance (on-line), blood urea, dialysate urea and ionic dialysance. Nephrol Dial Transpl 21:2225–2231
14. Napoli M, Russo F, Pati C (2011) AVF Monitoring. In: Napoli M (ed) echo color doppler & vascular accesses for hemodialysis, 1st edn. Wichtig, Milano, pp 46–62
15. National Kidney Foundation (2006) KDOQI Clinical practice guidelines for vascular access. Am J Kidney Dis. https://doi.org/10.1053/J.ajkd.2006.04.029
16. Tordoir J, Cannaud B, Haage P et al (2007) EBPG on vascular access. Nephrol Dial Transpl 22:88–117
17. Schmidli J, Widmer M, Basile C et al (2018) Vascular access: clinical practice guidelines of the European Society for vascular surgery. Eur J Vasc Endovasc Surg 55:757–818
18. Clark TW, Hirsch DA, Jindal KJ, Veugelers PJ, Leblanc J (2002) Outcome and prognostic factors of restenosis after percutaneos treatment of native hemodialysis fistulas. J Vasc Interv Radiol 13:51–59
19. Miyata T, Ueda Y, Yasuda Y, Izuhara Y, Wada T, Jadoul M, Saito A, Kurokawa K, Ypersele Van, de Strihou C (1998) Accumulation of carbonyls accelerates the formation of pentosidine, an advanced

glycation end product: carbonyl stress in uremia. J Am Soc Nephrol 9:2349–2356
20. Raj DS, Choudhury D, Welbourne TC, Levi M (2000) Advanced glycation end products: a Nephrologist's perspective. Am J Kidney Dis 35:365–380
21. Suliman ME, Heimburger O, Barany P, Anderstam B, Pecoits-Filho R, Ayala RE, Qureshi AR, Ekholm FI, Lindholm B, Stenvinkel P (2003) Plasma pentosidine is associated with inflammation and malnutrition in end-stage renal disease patients starting on dialysis therapy. J Am Soc Nephrol 14:1614–1622
22. Stein G, Franke S, Mahiout A, Schneider S, Sperschneider H, Borst S, Vienken J (2001) Influence of dialysis modalities on serum AGE levels in end-stage renal disease patients. Nephrol Dial Transplant 16:999–1008
23. Jadoul M, Ueda Y, Yasuda Y, Saito A, Robert A, Ishida N, Kurokawa K, de Strihou CV, Miyata T (1999) Influence of hemodialysis membrane type on pentosidine plasma level, a marker of "carbonyl stress". Kidney Int 55:2487–2492
24. Nishizawa Y, Koyama H, Inaba M (2012) AGEs and cardiovascular diseases in patients with end-stage renal diseases. J Ren Nutr 22:128–133
25. Ueno H, Koyama H, Fukumoto S, Tanaka S, Shoji T, Shoji T, Emoto M, Tahara H, Inaba M, Kakiya R, Tabata T, Miyata T, Nishizawa Y (2011) Advanced glycation end products, carotid atherosclerosis, and circulating endothelial progenitor cells in patients with end-stage renal disease. Metabolism 60:453–459
26. Suliman ME, Stenvinkel P, Jogestrand T, Maruyama Y, Qureshi AR, Bárány P, Heimbürger O, Lindholm B (2006) Plasma pentosidine and total homocysteine levels in relation to change in common carotid intima-media area in the first year of dialysis therapy. Clin Nephrol 66:418–425
27. McNair E, Qureshi M, Prasad K, Pearce C (2016) Atherosclerosis and the Hypercholesterolemic AGE–RAGE Axis. Int J Angiol 25:110–116
28. Machowska A, Carrero JJ, Lindholm B, Stenvinkel P (2016) Therapeutics targeting persistent inflammation in chronic kidney disease. Transl Res 167:204–213
29. Yung-Ho H, Yu-Chun Y, Yi-Chun L, Li-Chin S (2018) Antiplatelet agents maintain arteriovenous fistula and graft function in patients receiving hemodialysis: a nationwide case–control study. PLOS One. https://doi.org/10.1371/journal.pone.0206011
30. Sakaguchi T, Yan SF, Yan SD, Belov D, Rong LL, Sousa M, Andrassy M, Marso SP, Duda S, Arnold B, Liliensiek B, Nawroth PP, Stern DM, Schmidt AM, Naka Y (2003) Central role of RAGE-dependent neointimal expansion in arterial restenosis. J Clin Invest 111:959–972
31. Roy-Chaudhury P, Sukhatme VP, Cheung AK (2006) Hemodialysis vascular access dysfunction: a cellular and molecular viewpoint. J Am Soc Nephrol 17:1112–1127
32. Weiss MF, Scivittaro V, Anderson JM (2001) Oxidative stress and increased expression of growth factors in the lesions of failed hemodialysis access. Am J Kidney Dis 37:970–980
33. Du H, Li P, Wang J, Qing X, Li W (2012) The interaction of amyloid β and the receptor for advanced glycation end products induces matrixmetalloproteinase-2 expression in brain endothelial cells. Cell Mol Neurobiol 32:141–147
34. Tirinescu DC, Tomuleasa C, Pop L, Bondor CI, Vlăduţiu DŞ, Paţiu IM, Rusu CC, Moldovan DT, Potra A, Kacsó IM (2017) Matrix-metalloproteinase-2 predicts arteriovenous fistula failure in hemodialysis patients. Ther Apher Dial 21:586–591
35. Smith GE, Gohil R, Chetter IC (2012) Factors affecting the patency of arteriovenous fistulas for dialysis access. J Vasc Surg 55:849–855
36. Zhou Z, Immel D, Xia Xi C, Bierhaus A, Lin Mei XF, Nawroth P, Stern DM, Xiong WC (2006) Regulation of osteoclast function and bone mass by RAGE. J Exp Med 203:1067–1080
37. Sanguineti R, Storace D, Monacelli F, Federici A, Odett P (2008) Pentosidine effects on human osteoblasts in vitro ann. N Y Acad Sci 1126:166–172
38. Kakuta T, Tanaka R, Hyodo T, Suzuki H, Kanai G, Nagaoka M, Takahashi H, Hirawa M, Oogushi Y, Miyata T, Kobayashi H, Fukagawa M, Saito A (2011) Effect of sevelamer and calcium-based phosphate binders on coronary artery calcification and accumulation of circulating advanced glycation end products in hemodialysis patients. Am J Kidney Dis 57:422–431
39. Yamamoto T, Ozono K, Miyauchi A, Kasayama S, Kojima Y, Shima M, Okada S (2001) Role of advanced glycation end products in adynamic bone disease in patients with diabetic nephropathy. Am J Kidney Dis 38:161–164
40. Panuccio V, Mallamaci F, Tripepi G, Parlongo S, Cutrupi P, Asahi K, Miyata T, Zoccali C (2002) Low parathyroid hormone and pentosidine in hemodialysis patients. Am J Kidney Dis 40:810–815