7-28-2014

Endothelial function in patients with familial Mediterranean fever-related amyloidosis and association with cardiovascular events

Mahmut I. Yilmaz

Erkan Demirkaya
Gulhane Military Medical Academy, erkan.demirkaya@lhsc.on.ca

Cengizhan Acikel
RandD Engineer

Mehmet Saldir
Gulhane Military Medical Academy

Servet Akar
Eylül University

See next page for additional authors

RHEUMATOLOGY

Rheumatology 2014;53:2002–2008
doi:10.1093/rheumatology/keu231
Advance Access publication 6 June 2014

Original article

Endothelial function in patients with familial Mediterranean fever-related amyloidosis and association with cardiovascular events

Mahmut I. Yilmaz[1], Erkan Demirkaya[2,3], Cengizhan Acikel[3], Mehmet Saldir[2], Servet Akar[4], Tuncer Cayci[5], Mutlu Saglam[6], Hilmi U. Unal[1], Mahmut Gok[1], Adem Polat[2,3], Hakkı Cetinkaya[1], Tayfun Eyileten[1], Sebahattin Sari[6], Ali O. Yildirim[1], Alper Sonmez[7], Yusuf Oguz[1], Abdulgaffar Vural[1], Seza Ozen[8] and Juan Jesús Carrero[9,10]

Abstract

Objectives. Secondary amyloidosis is the most important complication of FMF and endothelial function is more severely impaired. Elevated asymmetric dimethyl arginine (ADMA) may mediate the excess cardiovascular disease (CVD) risk of this group. We aimed to compare endothelial function characteristics, including ADMA, in patients with FMF-related amyloidosis and primary glomerulopathies and to define risk factors for a CVD event.

Methods. We undertook a cross-sectional study with prospective follow-up including consecutive patients with FMF-related amyloidosis ($n = 98$) or other non-diabetic glomerulopathies ($n = 102$). All patients had nephrotic-range proteinuria and normal glomerular filtration rate. Flow-mediated dilatation (FMD) was assessed and ADMA levels, CRP and pentraxin 3 (PTX3) were determined. Patients were followed for cardiovascular events.

Results. Amyloidosis patients secondary to FMF showed higher levels of ADMA, CRP and PTX3 and lower FMD as compared with patients with other glomerulopathies. Cardiovascular events ($n = 54$) were registered during 3 years of follow-up. Increased ADMA levels and lower FMD were observed in patients with cardiovascular risk in both groups, but especially in individuals with amyloidosis.

Conclusion. Patients with FMF-related amyloidosis have increased CVD event risk, probably related to the high ADMA levels, elevated inflammatory markers and decreased FMD measures observed in these patients.

Key words: familiar Mediterranean fever, amyloidosis, proteinuria, flow-mediated dilatation, vascular damage.

CLINICAL SCIENCE

Introduction

FMF is an autosomal recessive disorder common in eastern Mediterranean countries such as Turkey and is associated with the development of secondary amyloidosis [1]. Secondary amyloidosis in turn becomes a prevalent cause of proteinuria and chronic kidney disease (CKD) in this geographical region [2], with a prognosis worse than other CKD aetiologies [3–5].

We have previously showed that endothelial function is more severely impaired in patients with amyloidosis than in individuals with other glomerulopathies [3]. At the same time, asymmetric dimethyl arginine (ADMA), considered to

[1]Department of Nephrology, [2]Department of Pediatric Rheumatology, Gülhane School of Medicine, [3]FMF Arthritis Vasculitis and Orphan Disease Research Center, Institute of Health Sciences, R&D Center, Ankara, [4]Department of Rheumatology, Eylül University, Izmir, [5]Department of Biochemistry, [6]Department of Radiology, [7]Department of Endocrinology, Gülhane School of Medicine, [8]Department of Pediatric Rheumatology, Hacettepe University, Ankara, Turkey, [9]Center for Molecular Medicine and [10]Division of Renal Medicine, Karolinska Institutet, Stockholm, Sweden.

Submitted 24 January 2014; revised version accepted 2 April 2014.

Correspondence to: Erkan Demirkaya, Gülhane Military Medical Academy, School of Medicine, FMF Arthritis Vasculitis and Orphan Disease Research Center, Institute of Health Sciences, 06018 Etlik, Ankara, Turkey. E-mail: erkandemirkaya@yahoo.com

be a causative factor in endothelial dysfunction (ED) [4, 5], is also increased in amyloidosis patients [3, 6].

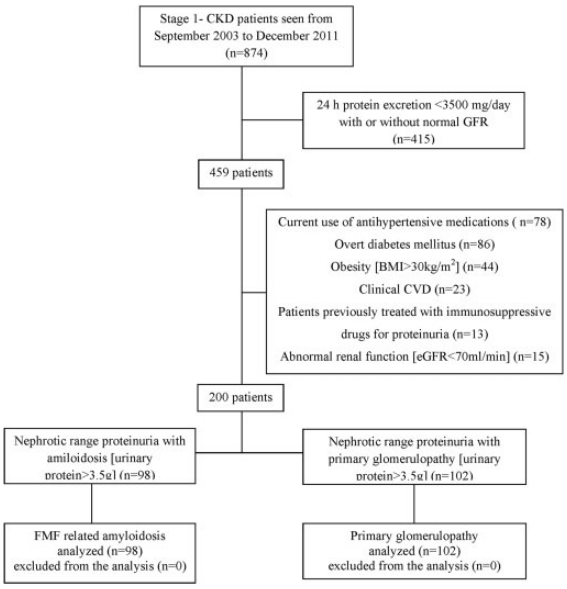
In this study we aimed (i) to discern the clinical, biochemical and endothelial function characteristics of nephrotic syndrome patients with FMF-related amyloidosis as their primary aetiology and (ii) to evaluate the possible association between ADMA levels and impaired FMD response with the cardiovascular disease (CVD) risk in these individuals. We studied these issues in, to the best of our knowledge, the largest collection of nephrotic syndrome patients with amyloidosis reported so far.

Patients and methods

Patients

In this case cohort study, patients were recruited from the renal outpatient clinic at Gülhane School of Medicine, Ankara, Turkey, between September 2003 and December 2011. Patients who had glomerulopathy were followed by a comprehensive patient-based registry that was established at Gülhane School of Medicine in 2003 and is described elsewhere in more detail [3]. Among a referred population of 874 patients without established renal disease, 459 had isolated proteinuria [24 h protein excretion >3500 mg/day with normal glomerular filtration rate (GFR)] (Fig. 1). Exclusion criteria were untreated hypertension [according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VII) criteria [7] and/or the current use of antihypertensive medication ($n = 78$)], overt diabetes mellitus ($n = 86$), obesity [BMI > 30 kg/m^2 ($n = 44$)], clinical history of CVD [defined as the presence or history of ischaemic heart disease, peripheral vascular disease and/or a cerebrovascular event ($n = 23$)] and treatment with immunosuppressive drugs for proteinuria ($n = 13$). Among patients with amyloidosis, we also excluded those with abnormal renal function [estimated GFR (eGFR) <70 ml/min ($n = 15$)]. In all, 200 patients were eligible for inclusion in the study [61.5% male, mean age 32 years (S.D. 5), all Caucasian] with an overall median follow-up period of 39 months (range 6–47). All patients had nephrotic-range proteinuria and normal GFR. A kidney biopsy was performed in 142 patients on admission, while the 58 remaining patients had their biopsy before referral. FMF-related amyloidosis was diagnosed by a positive staining pattern with Congo red dye. According to the renal biopsy results and standard histology criteria [8], all recruited patients were assigned to two possible groups: FMF-related amyloidosis ($n = 98$) and those with other primary glomerulopathies (PGs; $n = 102$), respectively. The ethics committee of the Gülhane School of Medicine approved the study and informed consent was obtained from each subject.

Fig. 1 Flowchart of the patients



Procedures

All enrolled subjects were evaluated by standard physical examination and routine biochemical laboratory tests, including liver and kidney function tests and 24-h urinary protein measurements. Venous blood samples were taken and flow-mediated dilatation (FMD) assessment was performed following a 2-week washout period, during which time no vasoactive drugs (including colchicine) were given.

Laboratory and imaging procedures

Arterial blood pressure (BP) was measured three times in the morning after a 15-min resting period and the mean values were calculated. Blood sampling was done after an overnight fast. Fasting plasma glucose (FPG), total protein, serum albumin, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined by enzymic colorimetry on an Olympus AU 600 autoanalyser using reagents from Olympus Diagnostics (Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald's formula [9]. Twenty-four-hour proteinuria was determined by a turbidimetric test using trichloroacetic acid. The serum basal insulin value was determined using a coated tube method (DPC, Los Angeles, CA, USA). Renal function was estimated by the modified modification of diet in renal disease formula (in ml/min) and expressed per 1.73 m^2 of body surface area [10]. Homeostasis model assessment (HOMA) was computed as HOMA insulin resistance (HOMA-IR) = FPG (mg/dl) × immunoreactive insulin (μIU/ml)/405 [11].

High-sensitivity CRP levels in serum (hsCRP) were determined by the turbidimetric fixed rate method by an automated analyser (AU-2700; Olympus, Mishima, Japan). The levels of pentraxin 3 (PTX3) were measured from frozen serum using a commercial ELISA kit (Quantakine DPTX 30; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Measurements of ADMA and SDMA

Measurements of serum ADMA and symmetric dimethyl arginine (SDMA) were done using HPLC, as described by Chen *et al.* [12]. In brief, to 1 ml of serum, 20 mg of 5-sulphosalicylic acid was added and the mixture was left in an ice bath for 10 min. The precipitated protein was removed by centrifugation at 2000 *g* for 10 min. Ten microlitres of the supernatant filtered through a 0.2 μm filter was mixed with 100 μl of derivatization reagent (prepared by dissolving 10 mg *o*-phthaldialdehyde in 0.5 ml of methanol, 2 ml of 0.4 M borate buffer (pH 10.0) and 30 μl of 2-mercaptoethanol) and then injected into the chromatographic system. Separation of ADMA was achieved with a 150 × 4 mm inside diameter (I.D.) Nova-pak C18 column with a particle size of 5 μm (Millipore, Milford, MA, USA) using 50 mM sodium acetate (pH 6.8), methanol and tetrahydrofurane as the mobile phase (A, 82:17:1; B, 22:77:1) at a flow rate of 1.0 ml/min. The areas of peaks detected by the fluorescent detector (Ex: 338 nm; Em: 425 nm) were used as quantification. The variability of the method was <7% and the detection limit of the assay was 0.01 μM.

Ultrasonic measurements

We determined ED according to Celermajer *et al.* [13]. Measurements were made by a single observer using an ATL 5000 US system (Advanced Technology Laboratories, Bothell, WA, USA) with a 12 MHz probe. The subjects remained at rest in the supine position for at least 15 min before the examination started. The subject's arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single two-dimensional (2D) frames. All US images were recorded on videotape for subsequent blinded analysis. A pneumatic tourniquet was then inflated to 200 mmHg with obliteration of the radial pulse. After 5 min the cuff was deflated. Flow measurements were made 60 sec after deflation. The maximum FMD (FMD_{max}) diameters were calculated as the average of three consecutive maximum diameter measurements. The percentage change in average FMD_{max} diameter compared with baseline resting diameter was defined as the flow-mediated diameter change. The intraobserver coefficient of variation for FMD was 5.1%.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 15.0 (SPSS, Chicago, IL, USA). Descriptive statistics were represented as frequency and percentage for categorical variables and as median (minimum–maximum) for continuous variables, as appropriate. Distributions of variables were evaluated by a one-sample Kolmogorov–Smirnov test. Independent samples *t* test or Mann–Whitney *U* test was used to compare continuous variables between groups. Categorical variables were compared by chi-square test.

Multivariate logistic regression analysis was performed to estimate the factors associated with FMF amyloidosis. Variables that had *P*-values ⩽0.20 in the univariate analysis were included in the multivariate analysis. The backward elimination method was used to select entry predictor variables. The success of the final model was evaluated by using −2 log likelihood ratio values.

Univariate survival analysis was performed to compare the CVD-free period by the Kaplan–Meier method and the differences between survival curves were evaluated using the log-rank test. The variables that had a statistically significant effect on the hazard ratio were included in the Cox proportional hazard method as a multivariate analysis. The model was then reduced by using the backward elimination method and the best-fitting models were reported.

Results

There were 102 patients [62 male (60.8%), median age 34 years (range 19–56)] with proteinuria due to PG and a further 98 patients [61 male (62.2%), median age 32 years (range 18–41)] with amyloidosis due to FMF. Reported symptoms of the patients with FMF-related amyloidosis who were being treated with colchicine were fever (36.7%), abdominal pain (33.7%), myalgia (30.6%), arthritis (28.6%), pleuritis (17.3%), erysipelas (14.3%) and peritonitis (10.2%). A family history of FMF or amyloidosis was present in 28.6% and 21.4% of the patients, respectively. As a complication of FMF, appendectomy was performed in nearly a quarter of patients (24.5%). Starting with the most frequently reported, the primary aetiology of proteinuria according to renal biopsy in the non-amyloidosis patients was minimal change in disease (23.5%), focal segmental glomerulosclerosis (19.6%), membranous nephropathy (15.7%), membranoproliferative glomerulonephritis (12.7%), IgA nephropathy (8.8%), secondary focal segmental glomerulosclerosis (7.8%), mesangial proliferation (5.9%) and LN (5.9%).

Clinical and laboratory characteristics

Comparison of clinical and laboratory features between the two study groups is presented in Table 1. There was a statistically significant difference in terms of age ($P = 0.005$): median age at diagnosis in patients with FMF-related amyloidosis was 16 years (range 6–25) and 71.4% of patients were ⩽18 years at the time of diagnosis of amyloidosis. BMI as well as systolic and diastolic BP were similar between the groups. The groups differed, however, in their serum albumin levels and most of the metabolic markers of blood lipids. In addition, patients with amyloidosis showed higher levels of insulin ($P < 0.01$), HOMA ($P < 0.01$) and FPG ($P = 0.02$).

Furthermore, whereas levels of ADMA and SDMA were significantly higher ($P < 0.001$), the FMD percentage ($P < 0.001$) was significantly lower in the FMF-related amyloidosis group (Table 1). Inflammatory markers such as hsCRP and PTX3 were also higher in this group. Multivariate factors associated with the presence of amyloidosis are shown in Table 2. After stepwise exclusion, triglyceridaemia and higher ADMA levels were found

Table 1 Clinical and biochemical characteristics of both study groups

	PGs		FMF-related amyloidosis		
	Median	Range	Median	Range	*P*-value[a]
Sex, female/male	40/62		37/61		0.83
Age, years	34.0	19.0–56.0	32.0	18.0–41.0	0.005
BMI, kg/m^2	27.0	21.2–30.0	26.0	19.0–32.0	0.33
Systolic BP, mmHg	132.0	111.0–141.0	132.0	112.0–138.0	0.69
Diastolic BP, mmHg	82.0	80.0–91.0	84.0	78.0–92.0	0.89
eGFR, ml/min/1.73 m^2	89.0	78.0–107.0	93.0	78.0–107.0	0.005
Follow-up time, months	39.0	8.0–44.0	40.0	6.0–47.0	0.82
Albumin, g/dl	3.7	2.0–4.8	3.2	1.8–4.6	<0.001
Triglyceride, mg/dl	183.0	78.0–256.0	215.0	21.0–365.0	<0.001
Total cholesterol, mg/dl	266.0	156.0–356.0	273.5	178.0–366.0	0.02
LDL cholesterol, mg/dl	153.5	69.0–221.0	158.0	100.0–242.0	0.012
HDL cholesterol, mg/dl	45.0	28.0–54.0	45.0	29.0–54.0	0.21
ADMA, μmol/l	2.5	1.1–5.0	3.8	2.3–6.4	<0.001
SDMA, μmol/l	3.0	1.4–5.0	3.9	1.7–6.3	<0.001
PTX-3, ng/ml	6.9	1.2–32.9	13.0	2.1–45.0	<0.001
CRP, mg/l	14.0	4.5–22.0	14.3	5.0–25.0	0.008
FMD, %	6.8	5.0–8.4	6.0	4.2–8.0	<0.001
Insulin, μIU/ml	11.0	6.2–34.0	13.0	4.7–42.0	0.03
Glucose, mg/dl	80.0	50.0–112.0	88.0	50.0–105.0	0.02
HOMA	2.2	1.0–6.1	2.7	1.0–10.4	0.003

[a]Mann–Whitney *U* test. ADMA: asymmetric dimethyl arginine; SDMA: symmetric dimethyl arginine; FMD: flow-mediated dilatation; eGFR: estimated glomerular filtration rat; PTX3: pentraxin 3; HOMA: homeostasis model assessment; PGs: primary glomerulopathies; BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 2 Multivariate analysis of risk factors associated with amyloidosis

	β	*P*-value	Odds ratio	95% CI
Triglycerides, mg/dl	0.01	0.02	1.01	1.002, 1.02
ADMA, μmol/l	1.98	<0.001	7.27	3.93, 13.45
Albumin, g/dl	−0.81	0.007	0.45	0.50, 0.81

$r^2 = 0.562$. Full model included ADMA, FMD, LDL, age, gender, SBP, SDMA, PTX3, CRP, HOMA and GFR in multivariate analysis. ADMA: asymmetric dimethyl arginine; SDMA: symmetric dimethyl arginine; FMD: flow-mediated dilatation; GFR: glomerular filtration rate; PTX3: pentraxin 3; HOMA: homeostasis model assessment; SBP: systemic blood pressure.

along with the presence of amyloidosis, while albuminaemia was a negative predictor.

Follow-up for CVD events

CVD-free survival time was calculated from the first admission to our centre, with no loss of follow-up. The median follow-up time for patients with FMF-related amyloidosis was 40 months (range 6–47), while follow-up for patients with other PGs was 39 months (range 8–44). For the purpose of this study, a combined endpoint of fatal and non-fatal cardiovascular events was created. In the FMF-related amyloidosis group, cardiovascular events were registered in 25 patients: 7 patients had one or more myocardial infarctions, 9 had angina pectoris, 2 had percutaneous transluminal coronary angiography and 4 had left ventricular dysfunction. Two patients suffered cerebrovascular disease and 1 had signs of peripheral atherothrombotic vascular disease. In addition, 11 deaths due to CVD were registered as a result of coronary heart disease ($n = 6$), sudden death ($n = 3$) and stroke ($n = 2$).

In patients with other PGs, cardiovascular events were registered in 13 individuals: 3 had one or more myocardial infarctions, 4 had angina pectoris, 1 had percutaneous transluminal coronary angiography and 2 had left ventricular dysfunction. Two patients suffered cerebrovascular disease and one had signs of peripheral atherothrombotic vascular disease. In addition, five deaths due to CVD were registered as a result of coronary heart disease ($n = 3$), sudden death ($n = 1$) and stroke ($n = 1$).

Kaplan–Meier curves

According to the Kaplan–Meier survival analyses, patients with FMF-related amyloidosis were more likely to suffer a cardiovascular event (combining fatal and non-fatal CV events) (Fig. 2). The 3-year survival probability was 79% for the PG group and 60% for the amyloidosis group.

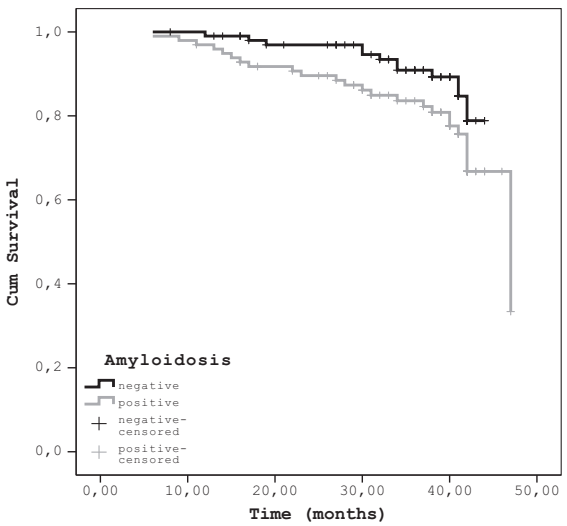
Given the relative rarity of amyloidosis patients, Table 3 compares the patient characteristics of these individuals depending on whether they suffer a cardiovascular event during follow-up. Both ADMA and SDMA were higher

Table 3 Risk factors associated with the development of a cardiovascular event during follow-up in nephrotic syndrome patients with FMF-related amyloidosis ($n = 98$)

	CVE negative		CVE positive		
	Median	Range	Median	Range	*P*-value[a]
Sex, female/male	26/47		11/14		0.455
Total cholesterol, mg/dl	278.0	187.0–366.0	267.0	178.0–366.0	0.083
Triglyceride, mg/dl	222.0	21.0–365.0	214.0	111.0–253.0	0.429
LDL cholesterol, mg/dl	158.0	100.0–198.0	163.0	122.0–242.0	0.133
HDL cholesterol, mg/dl	44.0	29.0–54.0	45.0	36.0–54.0	0.210
Age, years	32.0	18.0–41.0	33.0	19.0–36.0	0.987
BMI, kg/m^2	26.0	19.0–32.0	27.0	23.5–32.0	0.375
Systolic BP, mmHg	132.0	112.0–138.0	132.0	112.0–136.0	0.468
Diastolic BP, mmHg	84.0	78.0–92.0	82.0	78.0–92.0	0.770
ADMA, μmol/l	3.4	2.3–4.8	4.9	3.4–6.4	0.000
SDMA, μmol/l	3.7	1.7–5.5	4.9	3.5–6.3	0.000
PTX3, ng/ml	12.2	2.1–26.2	20.0	10.1–45.0	0.000
FMD, %	6.3	4.7–8.0	5.0	4.2–6.0	0.000
CRP, mg/l	14.0	5.0–21.0	18.0	9.0–25.0	0.000
Insulin, μIU/ml	12.0	4.7–38.8	16.0	6.2–42.0	0.076
HOMA	2.5	1.0–8.4	3.6	1.3–10.4	0.032
Albumin, g/dl	3.2	2.0–4.6	3.0	1.8–4.6	0.749
Glucose, mg/dl	88.0	50.0–105.0	88.0	67.0–105.0	0.419
eGFR, ml/min/1.73 m^2	90.0	78.0–107.0	98.0	82.0–106.0	0.011

[a]Mann–Whitney *U* test. CVE: cardiovascular event; CVD: cardiovascular disease; ADMA: asymmetric dimethyl arginine; SDMA: symmetric dimethyl arginine; PTX3: pentraxin 3; FMD: flow-mediated dilatation; eGFR: estimated glomerular filtration rate; HOMA: homeostasis model assessment; BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Fig. 2 Comparison of cardiovascular disease-free survival periods between patients with FMF-related amyloidosis or primary glomerulopathy



CVD: cardiovascular disease.

while the FMD percentage was lower in patients positive for CVD during follow-up. In addition, serum levels of hsCRP, PTX3 and HOMA values were significantly higher among those suffering a cardiovascular event.

Risk factors associated with the probability of suffering a cardiovascular event during follow-up were evaluated by Cox regression analysis and are presented in Table 4. When studying all patients together, age, inflammation, FMD and ADMA levels were factors independently contributing to the risk of cardiovascular events. When studying risk factors in each aetiology group separately, ADMA and FMD levels arose as the strongest predictors of cardiovascular risk.

Discussion

The results of the present study show that FMF patients who develop nephrotic syndrome due to amyloidosis carry a higher risk for cardiovascular morbidity when compared with other nephrotics. In our survey, patients in the group of FMF-related amyloidosis have a higher risk of CV events than those with other PGs. The same group also has increased ADMA synthesis and inflammatory markers, as well as worse endothelial function, all of which may mediate this excess cardiovascular risk. It is tempting to speculate that the increased inflammation is making the endothelium vulnerable to injury in these patients.

Systemic AA amyloidosis is a long-recognized, severe complication of several chronic inflammatory diseases. All the amyloidosis patients involved in this study had FMF, a recessive disorder relatively more common in Mediterranean countries such as ours. Renal involvement usually follows in FMF-related amyloidosis, which is thus a

Table 4 Multivariate analysis of factors associated with the risk of suffering a cardiovascular event

		Hazard ratio	95% CI		*P*-value
			Lower	Upper	
In all patients ($n = 200$)	ADMA, μmol/l	2.125	1.311	3.445	0.002
	FMD, %	0.341	0.195	0.595	<0.001
	Age, years	1.083	1.004	1.167	0.04
	PTX3, ng/ml	1.045	1.009	1.082	0.01
In patients with PG ($n = 102$)	ADMA, μmol/l	3.241	1.358	7.733	0.008
	FMD, %	0.288	0.102	0.811	0.018
In patients with FMF-related amyloidosis ($n = 98$)	ADMA, μmol/l	4.952	2.420	10.134	0.000
	FMD, %	0.243	0.107	0.554	0.001

ADMA: asymmetric dimethyl arginine; FMD: flow-mediated dilatation; PTX3: pentraxin 3; PG: primary glomerulopathy.

prevalent cause of micro and macroproteinuria and CKD in this geographic region [2]. In >90% of such patients, a clinical scenario of proteinuria, nephrotic syndrome and/or renal dysfunction is usually present [5]. Previous studies have described the increased cardiovascular morbidity risk associated with amyloidosis in individuals with end-stage renal disease [14–16]. Furthermore, Celik *et al*. [17] reported increased cardiovascular mortality in the course of renal transplantation in patients with amyloidosis when compared with patients having non-amyloid renal disease [5]. Two other case series have described the increased cardiovascular risk of dialysis patients with amyloidosis secondary to RA [18, 19]. Thus far, no prospective study had been performed to compare the cardiovascular mortality and morbidity of early CKD patients with FMF-related amyloidosis, perhaps given the difficulty of gathering a sufficient sample size in this relatively rare condition. Thus, and to the best of our knowledge, ours represents the largest study of FMF-related amyloidosis patients of this kind. We show that this aetiology associates with an increased cardiovascular risk as compared with other CKD aetiologies within the same proteinuric range.

Proteinuria is indeed a hallmark of renal disease, but also a significant cardiovascular risk factor [20]. Increased inflammatory activity, insulin resistance and dyslipidaemia are thought to be significant contributors to the cardiovascular damage associated with proteinuria [14–16]. However, ED is the main mechanism involved in the time course of vascular disease [4, 5]. Expanding previous reports [3, 6, 21], we show in our study that patients with nephrotic syndrome due to FMF-related amyloidosis suffer from ED and present with increased levels of ADMA and inflammatory biomarkers as compared with other aetiologies. In the general population, ADMA levels are independently associated with ED [22] and have been reported to be elevated in patients with CVD [23]. Because the kidneys provide a significant route for clearance of methylarginines, it is perhaps not surprising that, as in more severe CKD stages [24, 25], ADMA concentrations are increased in patients even at this early stage in our study. Inflammation, defined here as elevated serum CRP and PTX3, is also known to influence ADMA and has been proposed to be one factor linking ADMA to CVD [26]. Because ADMA in this study was independently associated with the presence of amyloidosis, and together with FMD derangements was a predictor of cardiovascular outcomes, we hypothesize that ED resulting from increased ADMA concentrations may be one factor contributing to the elevated risk of CVD in this patient group.

There are several limitations to this study. The observational design may preclude any conclusion about causality, and the strict inclusion criteria, albeit important for the research question addressed, produce a patient group exempted from co-morbidities and certain medications that may not be representative of all nephrotic syndrome patients. Moreover, we do not have genotype results for the entire group of patients in FMF-related amyloidosis. Also, although we measured circulating methylarginines, it is not known whether these are biologically active in themselves or just a reflection of high intracellular levels. Finally, we did not evaluate the environmental confounders that may also have influenced the clinical and laboratory findings.

To conclude, our data suggest that FMF-related amyloidosis as a cause of nephrotic syndrome heralds a poor prognosis and is accompanied by ED, higher ADMA levels and inflammation. However, it is hoped that effective control of inflammation and disease in these patients will save them from these devastating complications.

Rheumatology key messages

- Patients with FMF-related amyloidosis have increased cardiovascular disease event risk.
- Prognosis is poor in patients with nephrotic-range proteinuria due to FMF-related amyloidosis.
- High asymmetric dimethyl arginine levels may be related to poor prognosis in patients with amyloidosis.

Acknowledgements

J.J.C. acknowledges grant support from the Swedish Medical Research Council and the Centre for Gender Medicine at Karolinska Institutet, Stockholm, Sweden.

The authors would like to express their sincere appreciation to the FAVOR (FMF Arthritis Vasculitis and Orphan Diseases Research; www.favor.org.tr) web registries at the Gülhane School of Medicine, Institute of Health Sciences, for their epidemiological and statistical support and invaluable guidance in the preparation of this manuscript.

Disclosure statement: S.O. has acted as consultant for Novartis Turkey. All other authors have declared no conflicts of interest.

References

1 Kasifoglu T, Bilge SY, Sari I *et al*. Amyloidosis and its related factors in Turkish patients with familial Mediterranean fever: a multicentre study. Rheumatology 2014;53:741–5.

2 Tunca M, Akar S, Onen F *et al*. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. Medicine 2005;84:1–11.

3 Yilmaz MI, Sonmez A, Saglam M *et al*. ADMA levels correlate with proteinuria, secondary amyloidosis, and endothelial dysfunction. J Am Soc Nephrol 2008;19:388–95.

4 Cooke JP. Does ADMA cause endothelial dysfunction? Arterioscler Thromb Vasc Biol 2000;20:2032–7.

5 Ozen S. Renal amyloidosis in familial Mediterranean fever. Kidney Int 2004;65:1118–27.

6 Caglar K, Yilmaz MI, Sonmez A *et al*. ADMA, proteinuria, and insulin resistance in non-diabetic stage I chronic kidney disease. Kidney Int 2006;70:781–7.

7 Chobanian AV, Bakris GL, Black HR *et al*. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560–72.

8 Westermark P, Benson MD, Buxbaum JN *et al*. Amyloid: toward terminology clarification. Report from the Nomenclature Committee of the International Society of Amyloidosis. Amyloid 2005;12:1–4.

9 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.

10 Levey AS, Bosch JP, Lewis JB *et al*. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461–70.

11 Matthews DR, Hosker JP, Rudenski AS *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.

12 Chen BM, Xia LW, Zhao RQ. Determination of N(G),N(G)-dimethylarginine in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;692:467–71.

13 Celermajer DS, Sorensen KE, Gooch VM *et al*. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111–5.

14 Watts GF, Herrmann S, Dogra GK *et al*. Vascular function of the peripheral circulation in patients with nephrosis. Kidney Int 2001;60:182–9.

15 Festa A, D'Agostino R, Howard G *et al*. Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: the insulin resistance atherosclerosis study. Kidney Int 2000;58:1703–10.

16 Barzilay JI, Peterson D, Cushman M *et al*. The relationship of cardiovascular risk factors to microalbuminuria in older adults with or without diabetes mellitus or hypertension: the cardiovascular health study. Am J Kidney Dis 2004;44:25–34.

17 Celik A, Saglam F, Dolek D *et al*. Outcome of kidney transplantation for renal amyloidosis: a single-center experience. Transplant Proc 2006;38:435–9.

18 Hezemans RL, Krediet RT, Arisz L. Dialysis treatment in patients with rheumatoid arthritis. Neth J Med 1995;47:6–11.

19 Sanai T, Nanishi F, Nagata M *et al*. Role of amyloidosis in determining the prognosis of dialyzed patients with rheumatoid arthritis. Rheumatol Int 2007;27:363–7.

20 Haraldsson B, Nyström J, Deen WM. Properties of the glomerular barrier and mechanisms of proteinuria. Physiol Rev 2008;88:451–87.

21 Lachmann HJ, Goodman HJB, Gilbertson JA *et al*. Natural history and outcome in systemic AA amyloidosis. N Engl J Med 2007;356:2361–71.

22 Perticone F, Sciacqua A, Maio R *et al*. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. J Am Coll Cardiol 2005;46:518–23.

23 Fliser D. Asymmetric dimethylarginine (ADMA): the silent transition from an 'uraemic toxin' to a global cardiovascular risk molecule. Eur J Clin Invest 2005;35:71–9.

24 Kielstein JT, Boger RH, Bode-Boger SM *et al*. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. J Am Soc Nephrol 2002;13:170–6.

25 Aslam S, Santha T, Leone A, Wilcox C. Effects of amlodipine and valsartan on oxidative stress and plasma methylarginines in end-stage renal disease patients on hemodialysis. Kidney Int 2006;70:2109–15.

26 Zoccali C, Benedetto FA, Maas R *et al*. Asymmetric dimethylarginine, CRP, and carotid intima-media thickness in end-stage renal disease. J Am Soc Nephrol 2002;13:490–6.