CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

DOCTORAL SCHOOL

FIELD OF MEDICINE

Impact of Oxidative Stress on the Maturation Process of Native Arteriovenous Fistulas in Chronic Kidney Disease Patients

DOCTORAL THESIS SUMMARY

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2024

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List of published scientific papers

1. Vinereanu V, Peride I, David C, Radulescu D, Turcu FL, Jinga M, Ferechide D, Niculae A. The Effect of Altered Redox Homeostasis on Vascular Wall Elasticity in Patients with Chronic Kidney Disease. Rev. Chim.[internet]. 2017 Jun;68(6):1216-122. https://doi.org/10.37358/RC.17.6.5644

2. Vinereanu I-V, Peride I, Niculae A, Tiron AT, Caragheorgheopol A, Manda D, Checherita IA. The Relationship between Advanced Oxidation Protein Products, Vascular Calcifications and Arterial Stiffness in Predialysis Chronic Kidney Disease Patients. Medicina. 2021;57(5):452. https://doi.org/10.3390/medicina57050452

Introduction

Chronic kidney disease is a pathology with a significant impact on the cardiovascular system, as evidenced by high morbidity and mortality rates, especially in patients in advanced stages of renal function decline and particularly in those requiring renal replacement therapies such as hemodialysis or peritoneal dialysis.

This predisposition to developing cardiovascular complications is not fully explained by traditional risk factors. Therefore, to fully understand and treat chronic kidney disease, it is necessary to identify specific agents that contribute to the accelerated deterioration of the vascular bed, such as secondary hyperparathyroidism, chronic anemia, or oxidative stress. The field of research into oxidative stress in the context of chronic kidney disease has the potential to offer explanations that enhance our understanding of pathogenesis, but currently does not identify viable therapeutic options to improve medium- and long-term survival.

The native arteriovenous fistula represents the best option for creating vascular access. Studies conducted over time consistently highlight the significant benefits of this type of vascular approach compared to other access methods (temporary/permanent central venous catheters, arteriovenous grafts).

Despite the obvious advantages, native arteriovenous fistulas are characterized by mediocre patency rates, so few patients truly benefit from them. The maturation process is extremely complex and dependent on many factors. Unlike the failure of creation, where the surgeon's experience plays an important role, the success of the maturation process significantly depends on vascular quality.

Globally, the main causes of chronic kidney disease and progression to renal failure remain hypertension and diabetes mellitus, conditions known for their substantial negative impact on vascular wall quality by promoting the progression of systemic atherosclerosis. Secondary hyperparathyroidism, which becomes apparent in the advanced stages of chronic kidney disease, contributes to decreased vascular compliance through medial calcification. Thus, the adaptive changes in the venous wall necessary for the development of vascular access cannot occur under optimal conditions.

The success of the maturation process also depends on how the vasculature reacts to the changes in flow and pressure induced after the creation of the anastomosis. The arterialization process of the anastomosed vein results from complex interactions between the cells that make up the vascular wall structure, interactions that sometimes go awry under the influence of the uremic environment and its associated oxidative stress.

The studies conducted as part of this doctoral thesis are among the first to clinically evaluate the effects of oxidative stress on the cardiovascular system and the maturation process of native arteriovenous fistulas, including vascular stiffness indicators.

In the general part of the doctoral thesis, a synthetic presentation of the currently available data on the imbalance of redox homeostasis was carried out, highlighting the relevant aspects concerning the role of uremic toxins in inducing and maintaining oxidative stress and their influence on the quality and compliance of vascular structures. Additionally, an overview of the available data on native arteriovenous fistulas was presented, with emphasis on the cellular biology mechanisms involved in the maturation process and the influence of oxidative stress, concluding with a brief description of the complications that may arise from creating this type of vascular access.

The first study aimed to evaluate the correlations between the level of oxidative stress, assessed by determining advanced oxidation protein products (AOPP), vascular stiffness indicators, and the success of the maturation process of arteriovenous fistulas, integrating these data with clinico-biological parameters commonly used to evaluate patients with advanced stages of chronic kidney disease.

The second doctoral research aimed to assess the level of oxidative stress in pre-dialysis stage chronic kidney disease patients and its correlation with the presence of vascular and valvular calcifications as well as vascular stiffness indicators.

The methodology of the first study involved the prospective inclusion of patients scheduled for surgical intervention to create the arteriovenous fistula and their monitoring over a period of two months or until the start of hemodialysis sessions, to establish the success of the surgical intervention and vascular access patency based on functional criteria. In the second study, the

evaluation was cross-sectional, including known chronic kidney disease patients in pre-dialysis stages, aiming to identify vascular calcifications using simple radiographs and valvular calcifications using Doppler echocardiography. Descriptive and inferential statistical methods were applied to achieve the proposed objectives.

The results obtained contribute to the current understanding of the role of oxidative stress in the pathogenesis of cardiovascular complications associated with chronic kidney disease, especially in patients in advanced stages of renal function decline.

The limitations of this doctoral research are the consequences of several factors, primarily the small number of patients included in each study. Additionally, using more oxidative stress indicators could have provided further insights into the pathogenic process generating reactive oxygen species. Moreover, in the first study, the use of Doppler ultrasound for periodic non-invasive monitoring of the maturation process would have been advantageous compared to the functional criteria used. Among the disadvantages of the research methodology applied in the second study is the selection bias, with patients coming from a single nephrology clinic of an emergency hospital.

I. General Part

1. Oxidative Stress: Definition, Endogenous and Exogenous Sources of Free Radicals, Antioxidant Mechanisms, Endogenous and Exogenous Antioxidants

1.1. Definition

Oxidative stress occurs when the balance between pro-oxidant free radicals and antioxidant substances is disturbed in favor of reactive oxygen species (ROS), either through their accumulation or due to insufficient degradation at the cellular metabolism level (Daenen et al., 2019).

1.2. Endogenous Sources of Reactive Oxygen Species

Reactive oxygen species result from the normal activity of cells and are involved in essential processes such as cellular signaling, the immune system's response against pathogens, or

as a result of mitochondrial activity. Sources include the mitochondrial electron transport chain, peroxisomes, the endoplasmic reticulum, and NADPH oxidase.

1.3. Exogenous Sources of ROS

Oxidative stress can be promoted by external factors either directly through the intake of molecules with oxidant potential or indirectly through agents that trigger free radical-generating processes within the body. Examples include exposure to metals such as lead, mercury, cadmium, arsenic; smoke from tobacco burning; ultraviolet radiation, ionizing radiation such as X-rays or gamma rays; ethyl alcohol; diets high in saturated fatty acids and rich in sugars (Prasad & Dhar, 2014); and infections that cause oxidative stress by activating the immune system.

1.4. Endogenous and Exogenous Antioxidants

1.4.1. Major Non-Enzymatic Endogenous Antioxidants

Major non-enzymatic endogenous antioxidants include ferritin, transferrin, ceruloplasmin, lactoferrin, haptoglobin, hemopexin, albumin, glutathione, and ubiquinol (Mirończuk-Chodakowska et al., 2018).

1.4.2. Main Endogenous Enzymatic Antioxidants

- **Superoxide Dismutase (SOD)** catalyzes the conversion of superoxide anion into molecular oxygen and hydrogen peroxide. Subsequently, the resulting peroxide is neutralized by other antioxidant enzymes such as catalase or glutathione peroxidase (Younus, 2018). Depending on the metallic cofactor involved in redox reactions, superoxide dismutases have three isoforms: Cu,Zn-SOD, Mn-SOD, and extracellular SOD (Miller, 2001).
- **Catalase (CAT)** is a tetrameric antioxidant enzyme of 240 kDa that works in synergy with superoxide dismutase to neutralize hydrogen peroxide, thus generating water and molecular oxygen (Matés et al., 1999).

- **Glutathione Peroxidase (GPx)** consists of eight isoenzymes with antioxidant effects by neutralizing hydrogen peroxide and organic hydroperoxides (Zhao et al., 2019), most of which achieve this in the presence of glutathione (Pei et al., 2023).

1.4.3. Exogenous Antioxidants

Exogenous antioxidants include vitamins C and E, beta-carotene, flavonoids, selenium, and polyphenols.

1.5. The Role of Uremic Toxins in the Onset of Oxidative Stress and Their Effect on Vascular Elasticity

Uremic toxins are substances that accumulate in the body as kidney function deteriorates. They are classified by the European Uremic Toxin Work Group into three categories (Rosner et al., 2021): small (<500 Da) water-soluble molecules that are dialyzable, medium molecules (>500 Da) dialyzable using special membranes with sufficiently large pores, and protein-bound molecules that have a low molecular mass but are difficult to remove through dialysis. Urea is the most well-known uremic toxin. High serum urea levels in chronic kidney disease patients alter the structure and function of proteins through carbamylation (Jaisson et al., 2011). Doue et al. demonstrated in murine models that carbamylation of elastin fibers in the vascular walls led to their stiffening (Doué et al., 2021). Other uremic toxins relevant for their role in vascular complications include indoxyl sulfate, p-cresyl sulfate, advanced glycation end products (AGE), asymmetric dimethylarginine (ADMA), and FGF-23.

2. Native Arteriovenous Fistula

2.1. Definition

The native arteriovenous fistula (AVF) is the preferred type of vascular access for patients needing chronic hemodialysis. It is created by anastomosing an artery to a vein of the superficial venous system, usually in the upper limb.

2.2. Types of Native Arteriovenous Fistulas

- Radiocephalic Cimino-Brescia fistula
- Brachiocephalic Kaufmann fistula
- Brachiobasilic fistula

2.3. Definition of a Mature Fistula

Currently, there is no clear definition of a mature fistula, leading to highly heterogeneous data regarding maturation and failure rates. The most known definition is the "rule of sixes" proposed in the 2006 NKF-KDOQI guidelines.

2.4. The Maturation Process of the Native Arteriovenous Fistula

The maturation process of the native arteriovenous fistula is extremely complex and involves all structures of the vascular wall. Particularly relevant are the stepwise reorganization processes of the extracellular matrix to facilitate the dilation of the venous segment and the phenotypic transitions undergone by vascular smooth muscle cells under the influence of paracrine signaling pathways initially triggered by endothelial cells.

2.5. Neointimal Hyperplasia

Neointimal hyperplasia is a vascular endothelial response pattern to injuries caused by various etiological agents. It involves the localization and proliferation of smooth muscle cells or contractile property cells at the intima level, resulting in a progressive reduction of vessel permeability (Bonatti et al., 2005). The prognosis of AVF is partially dictated by both hyperplastic lesions resulting from the intervention and pre-existing lesions.

2.6. Influence of Reactive Oxygen Species and Oxidative Stress on the Maturation Process of the Native Arteriovenous Fistula

Reactive oxygen species affect the maturation process by depleting local antioxidants, uncoupling nitric oxide synthase, and generating new ROS (ONOO- and lipid peroxides). They induce the synthesis of mitogenic factors such as PDGF, ET-1, TGF-β, promoting lumen narrowing through neointimal hyperplastic lesions, and cause activation of a synthetic and osteoblast-like phenotype in VSMCs (Weiss et al., 2001; Hu et al., 2022b).

2.7. Complications of Native Arteriovenous Fistulas: infection, steal syndrome, thrombosis, aneurysmal development, venous hypertension, neurological complications

II. Personal Contributions

II.3. Hypothesis and General Objectives

II.3.1. Hypothesis

Chronic kidney disease (CKD) has a significant impact on cardiovascular health, often leading to increased morbidity and mortality among affected individuals. It is associated with dyslipidemia, inflammation, and oxidative stress, all of which exacerbate vascular bed deterioration and contribute to the failure of native AVF maturation. The pro-oxidant environment affects the vascular remodeling mechanisms necessary for the arterialization process of the vein by depleting local vasodilators and inducing phenotypic changes that contribute, among other things, to the development of neointima.

II.3.2. General Objectives

The first study aimed to evaluate the relationship between oxidative stress and the success of the maturation process of the arteriovenous fistula, integrating parameters characterizing vascular stiffness and commonly used clinico-biological data for monitoring patients with advanced stages of chronic kidney disease. The second study started from the premise that vascular calcifications appear even in the pre-dialysis stages of chronic kidney disease and explored their relationship with the level of oxidative stress. Similar to the first study, it aimed to correlate these data with vascular stiffness indicators and usual clinico-biological parameters.

Methodology of the Research

The first study was conducted using a prospective observational model and included 37 adult patients diagnosed with stage G5 and G5D chronic kidney disease. The second study, a cross-sectional type, evaluated a cohort of 46 patients diagnosed with stage G3-G5 chronic kidney disease. Patient enrollment was carried out after signing the informed consent form, and all study procedures were conducted in accordance with the principles of the Helsinki Declaration. The

protocol was approved by the Ethics Committee of the "Carol Davila" University of Medicine and Pharmacy, Bucharest (No. 124/14.06.2017).

Data collected at the time of inclusion in the study were obtained from patient medical history documents (previous discharge tickets, medical tests, specialist outpatient consultations, etc.), observation sheets during hospitalization, and dialysis session protocols (where applicable). Vascular stiffness parameters were evaluated at the time of enrollment, determined using a standardized protocol specific to each evaluation method (sphygmomanometric or applanation tonometry).

In the first study, the definition of a mature fistula was based on functional criteria from the HFM study: a fistula that can be punctured with two needles in 75% of dialysis sessions over a four-week interval, with an average flow of 300 ml/min in four consecutive sessions or Kt/V>1.4 or URR>70%, and the first of these four sessions must occur within the first nine months of creating the vascular access or within the first four weeks of starting hemodialysis (Robbin et al., 2018).

In the second study, vascular calcifications were detected using simple radiographs, and valvular calcifications were diagnosed through Doppler echocardiography. Plasma levels of advanced oxidation protein products were determined using a reagent kit produced and commercialized by Immundiagnostik, Germany.

The information thus obtained was centralized into a database using Microsoft Excel 2016/2019, and statistical analysis was performed using IBM SPSS 20. Discrete variables were presented as frequencies. The normal distribution of continuous variables was evaluated using the Shapiro-Wilk test. For continuous variables following a normal distribution, dispersion was described using the mean (M) and standard deviation (SD), while others were characterized by the median (Md) and interquartile range (IQR). The following tests were used to assess statistical significance: $\chi 2$ test, T-Student test, or Mann-Whitney-U test. Correlations between continuous variables were evaluated using Pearson (r) or Spearman (rho) coefficients. Predictors were identified using multivariate linear or logistic regression models, and their discriminatory capacity

was subsequently evaluated using ROC curves. The threshold for statistical significance was set at $p<0.05$.

Study 1: Impact of Oxidative Stress on the Maturation Process of Native Arteriovenous Fistulas

Introduction

The primary objectives of this study were:

- To evaluate the relevance of advanced oxidation protein products (AOPP) as a predictive factor for the failure of native arteriovenous fistula (AVF) maturation.
- To assess the relevance of vascular stiffness parameters (PWV, pAug, AIX) as predictive factors for the failure of native AVF maturation and identify correlations with AOPP.
- To identify other clinico-biological parameters associated with native AVF failure.

Material and Method

This is a prospective observational cohort study that included 37 adult patients (minimum age 18 years) diagnosed with KDIGO stage G5 or G5D chronic kidney disease, who were hemodynamically and respiratorily stable at the time of inclusion. Data collected included age, gender, anthropometric data (weight, height), history of chronic kidney disease, history of associated pathologies, administered medication, usual biological tests (complete blood count, blood glucose, urea, serum creatinine, uric acid, total cholesterol, total serum calcium, serum phosphorus, PTH, serum bicarbonate, serum albumin, total proteins, ESR, fibrinogen, C-reactive protein), and a blood sample for determining AOPP. Vascular stiffness was assessed using the Sphygmocor CP device (Atcor Medical).

Results

Characteristics of the Studied Group

A total of 37 patients were enrolled: 19 men (51.4%) and 18 women (48.6%), with average ages of 56.1 years (±15.49) for men and 63.5 years (±12.9) for women. Twenty-five patients (67%) developed mature fistulas that could be used for hemodialysis sessions. The most common type of fistula was the brachiocephalic (24, 64.9%), especially among male patients (p=0.046). There were no statistically significant differences in failure rates between genders (p=0.556). At the time of enrollment, the majority of patients (22, 59.5%) were not undergoing hemodialysis.

Advanced Oxidation Protein Products and Vascular Stiffness

The mean value of advanced oxidation protein products was 32.22 µmol/L, not influenced by gender (p=0.563) or hemodialysis. AOPP negatively correlated with eGFR (p=0.001) and positively with serum creatinine (p=0.003). AOPP varied linearly and positively with PWV (p=0.02). In gender-specific analysis, AOPP significantly and positively correlated with age (p=0.026) and PWV (p=0.036) among female patients. PWV (p=0.433) and AIX were higher in male patients, but without statistical significance (p=0.685). PWV (p=0.001), pAug (p=0.02), and AIX (p=0.001) varied linearly and positively with the age of the patients.

Among female patients, PWV varied linearly and positively with age (p=0.003) and negatively with serum albumin levels (p=0.001) and total proteins (p=0.026). Multiple linear regression analysis highlighted serum albumin levels as a negative predictor for pulse wave velocity ($p<0.01$), while age was a significant positive predictor for pulse wave velocity in male patients (p=0.002). pAug varied positively with C-reactive protein (p=0.039), and AIX negatively correlated with serum protein levels (p=0.11). Among men, pAug varied linearly with age (p=0.028) and uric acid levels (p=0.051). AIX significantly correlated with age (p=0.01) and uric acid levels (p=0.03). Across the entire cohort, multivariate linear regression models identified age and serum uric acid levels as significant predictors for pAug (p=0.007), and age as a significant predictor for AIX (p=0.01).

Failure of the Maturation Process

AOPP levels were not significantly higher in patients with failed AVFs (p=0.46). However, PWV (p=0.05) and pAug (p=0.04) were significantly higher among patients whose arteriovenous fistulas did not mature, though they were not significant predictors in the multivariate logistic

regression model (p=0.634). pAug was significantly higher in female patients with failed AVFs (p=0.01). In male patients with undeveloped AVFs, BMI (p=0.045) and uric acid levels (p=0.007) were significantly higher, with uric acid being a significant predictor in the multivariate logistic regression model (p=0.050). Across the entire cohort, uric acid was significantly higher in patients whose AVFs did not mature (p=0.001), serving as a significant predictor for maturation failure (p=0.012). Hemodialysis patients whose fistulas did not develop had higher average ages (p=0.028) as well as higher PWV (p=0.050) and uric acid levels (p=0.002).

The ROC curve for uric acid levels in the entire cohort identified a value of 7.83 mg/dl as having good discriminatory capacity (Sn=75%, Sp=76%) for identifying patients with failed AVFs, while a value of 8.36 mg/dl had Sn=50% and Sp=84% (p=0.003). Among the stiffness indicators, only pAug showed moderate discriminatory capacity at the threshold value of 10.5 mmHg (Sn=75%, Sp=66%, p=0.044). For females, the threshold of 7.33 mg/dl for uric acid presented a high discriminatory capacity (Sn=80%, Sp=76.9%), and for males, a value of 8.9 mg/dl showed Sn=71% and Sp=83% (p=0.043). Regarding pAug, a value of 11.5 mmHg had Sn=100% and Sp=76.9% for identifying female patients at high risk of AVF development failure (p=0.014). AOPP did not show utility for identifying at-risk patients of either gender (p=0.805). In hemodialysis patients, a cut-off value of 7.7 mg/dl for uric acid (p=0.014, Sn=80%, Sp=80%) and 9.2 m/s for PWV (p=0.037, Sn=80%, Sp=70%) were effective indicators for predicting fistula failure.

Discussion

Statistical analysis revealed significant correlations of AOPP with serum creatinine levels and eGFR, in line with current literature (Witko-Sarsat et al., 2003a). In the analyzed cohort, AOPP is associated with vascular stiffness, a relationship currently underexplored. Other studies show high AOPP values in patients with carotid atheromatosis, abdominal aortic aneurysm, or aorto-iliac occlusive disease. The highest correlation coefficient between AOPP and PWV was found among female patients. In the current cohort, serum albumin and total protein levels seem to vary linearly with vascular stiffness parameters: in female patients, albumin levels negatively correlate and are a significant predictor for PWV; the correlation between augmentation index (AIX) and serum protein levels supports the argument that nutritional status influences vascular stiffness

parameters and, consequently, can contribute to the failure of native AVF maturation (Gu et al., 2008; Mahmoud et al., 2021; Tang et al., 2009). AOPP is not associated with maturation failure.

Mean values for pulse wave velocity and augmentation pressure were significantly higher in the group with failed fistulas, although they were not significant predictors. The literature presents contradictory data. Masengu et al. did not identify a relationship between PWV, augmentation index, and the success of the maturation process, while McGrogan suggests that those with primary maturation failure have higher vascular stiffness (Masengu et al., 2016; McGrogan et al., 2018). In this study cohort, we also identified cut-off values for pAug and PWV that might be useful for identifying high-risk patients among those undergoing hemodialysis as well as female patients. The lack of congruence likely results from different methodologies and the absence of a universally accepted definition for a mature fistula.

Uric acid was significantly higher in the group of patients whose fistulas did not develop, including hemodialysis patients, and was a predictive value for maturation failure in men. In the context of chronic kidney disease, uric acid contributes, among other things, to amplifying oxidative stress and endothelial dysfunction by inhibiting eNOS (Kanbay et al., 2013; LI et al., 2016; Wu et al., 2016).

Study 2: The Relevance of Oxidative Stress in the Pathogenesis of Vascular Calcifications in Pre-Dialysis Chronic Kidney Disease Patients

Introduction

The primary objectives of this study were to investigate the relationship between AOPP, vascular and valvular calcifications, the relationship with parameters used for arterial stiffness evaluation, and potential correlations with routine clinico-biological indicators used for evaluating chronic kidney disease patients.

Material and Method

This is a cross-sectional clinical study conducted on a cohort of adult patients with chronic kidney disease stages G3-G5. Collected data included age, gender, history of chronic kidney

disease, medical history related to diabetes mellitus, hypertension, peripheral artery disease, weight, height, blood pressure, vascular stiffness parameters (PWV, pAug, AIX) using the sphygmomanometric method, and laboratory data (complete blood count, glycated hemoglobin in known diabetes patients, serum creatinine, serum urea, uric acid, complete lipid profile, inflammation parameters, phosphate-calcium metabolism parameters, protein levels, albumin levels, proteinuria, and albuminuria). Advanced oxidation protein products were determined spectrophotometrically using a reagent kit from Immundiagnostik, Bensheim (Germany). Vascular calcifications were quantified on hand and pelvis radiographs using the protocol proposed by Adragao et al. (Adragao et al., 2004). Valvular calcifications were identified at the mitral and aortic levels through Doppler echocardiography.

Results

The study included 46 patients diagnosed with CKD G3-G5, of whom 22 were women and 24 were men, with an average age of 65.07 ± 13.89 years and a median filtration rate of 10 ml/min/1.73 m² (IQR=9.86). Hypertensive patients represented 78.3% of the cohort, and those with diabetes mellitus 43.5%. Among those included, 7 patients did not consent to radiographs and 4 refused echocardiography. AOPP values ranged from 9.9 to 45.78 µmol/L, being slightly higher among female patients. Radiologically visible vascular calcifications were present in 29 patients (63%), 24 (52.1%) had valvular calcifications, 16 had both types of calcifications, and 2 had no calcifications. There were no statistically significant differences between genders regarding the presence of vascular (p=0.9) or valvular (p=0.791) calcifications. The average age did not differ significantly between patients with and without vascular (p=0.3) or valvular (p=0.07) calcifications. The presence of valvular (p=0.89) or vascular (p=0.31) calcifications did not correlate with AOPP levels.

In patients with type 2 diabetes who had valvular calcifications on echocardiography, PWV values frequently exceeded the cut-off value of 10 m/s proposed in the European Society of Cardiology guidelines (N=19, χ^2=4.232, p=0.04) (Williams et al., 2018). Additionally, the average age was higher among patients with valvular calcifications (p=0.017), as well as serum uric acid levels (p=0.046).

Among male patients, systolic blood pressure (p=0.004) and mean arterial pressure (p=0.004) were significantly higher compared to female patients. PWV did not show statistically significant differences between genders (p=0.303).

The relationship between AOPP and PWV was characterized by a weak correlation coefficient in the entire cohort (p=0.02), but this was amplified when controlled for serum creatinine (p=0.01) and eGFR (p=0.02). Pulse pressure did not correlate with AOPP in the entire cohort (p=0.19).

In women, AOPP varied positively linearly with PWV (p=0.002), AIX (p=0.037), and PP (p=0.019). In men, the linear relationship between AOPP and PWV did not hold (p=0.798).

To identify the strongest predictors of PWV in the entire cohort, three hierarchical logistic regression models were tested with AOPP and eGFR as common independent variables, successively adding additional variables (systolic pressure, pulse pressure, and augmentation index) to avoid collinearity. The model including pulse pressure explained 34% of PWV variability, with pulse pressure (β=0.46) and AOPP (β=0.24) having the highest weights (Table 1). Notably, in patients with type 2 diabetes, AOPP significantly correlated with pulse wave velocity (r=0.486, p=0.030) and age (r=0.479, p=0.033).

					Caracteristicile modelului		
		Coeficient standardizat	**β-**	**Valoare T Valoare p**	**Coeficient R**	**Valoarea F**	**Valoarea p**
MODELUL 1	AOPP	0.316	2.306	0.02	0.446	4.858	0.03
	RFGe	0.336	2.457	0.018			
MODELUL 2	AOPP	0.245	2.003	0.05	0.62	8.87	< 0.01
	RFGe	0.20	1.579	NS			
	PP	0.466	3.605	0.01			
MODELUL 3	AOPP	0.318	2.4	0.01	0.537	5.671	0.002
	RFGe	0.283	2.1	0.03			
	$PA_{sistolică}$	0.304	2.3	0.02			
MODELUL 4	AOPP	0.27	2.05	0.04	0.539	5.720	0.02
	RFGe	0.35	2.684	0.06			

	PAug	0.306	2.327	0.02

Table 1 Hierarchical Linear Regression Models. AOPP – Advanced Oxidation Protein Products; eGFR – Estimated Glomerular Filtration Rate; BP – Blood Pressure; PP – Pulse Pressure;

These findings underline the complex interplay between oxidative stress, arterial stiffness, and vascular calcifications, particularly in chronic kidney disease patients, and suggest that oxidative stress markers like AOPP, along with traditional cardiovascular risk factors, could be useful in predicting vascular complications.

Multivariate linear regression analysis identifies age ($p<0.01$) and uric acid level ($p=0.04$) as the most important predictors of pulse wave velocity (PWV) in male patients. For female patients, regression analysis highlights AOPP ($p=0.008$) and age ($p=0.003$) as significant predictors. Age is a significant predictor of PWV in both genders.

In the entire cohort, pAug significantly positively correlates with uric acid level ($p=0.015$) and negatively with HDL cholesterol level ($p=0.049$). There is also a weak positive correlation with proteinuria, nearing statistical significance ($r=-0.285$, $p=0.055$). Multivariate linear regression analysis identifies uric acid level and proteinuria as statistically significant predictors of pAug.

In gender-specific analysis, augmentation pressure varies linearly and significantly with albumin level ($r=-0.576$, $p=0.006$), total protein level ($r=-0.512$, $p=0.015$), and uric acid level ($r=0.714$, $p=0.0001$) in female patients. In male patients, 24-hour proteinuria correlates with pAug ($r=0.568$, $p=0.007$) (Figures 5 and 6).

In multivariate linear regression analysis including albumin level, protein level, serum uric acid level, and 24-hour proteinuria, the following predictors of augmentation pressure were identified: in female patients, uric acid level ($p=0.002$) was a significant predictor in all three models; in male patients, only 24-hour proteinuria ($p=0.012$) was a significant predictor of pAug. Uric acid is a significant predictor of augmentation pressure in all tested regression models for the entire cohort.

Patients with isolated systolic hypertension had higher proteinuria ($t(37)=2.67$, $p=0.01$) and PWV ($t(44)=2.16$, $p=0.036$). AOPP does not vary linearly with creatinine, urea, uric acid, or eGFR but positively correlates with glycated hemoglobin in the entire cohort. Regarding lipid profile, AOPP negatively correlates with HDL cholesterol ($p=0.0042$) and positively with total cholesterol/HDL cholesterol ($p=0.05$) and LDL cholesterol/HDL cholesterol ($p=0.038$). Among patients with hypertension, AOPP significantly correlates with LDL cholesterol ($p=0.008$) and the LDL/HDL ratio ($p=0.010$). Phospho-calcium metabolism parameters do not correlate with AOPP in the analyzed cohort.

Regarding systemic inflammation indicators, AOPP positively correlates with C-reactive protein and the derived NLR (neutrophil-to-lymphocyte ratio). NLR positively correlates with PWV, age, C-reactive protein, and the platelet-to-lymphocyte ratio. Average values of these indicators were similar regardless of the presence of vascular or valvular calcifications.

Discussion

The aim of this cross-sectional study was to examine the relationship between advanced oxidation protein products, vascular or valvular calcifications, and vascular stiffness indicators, as well as potential correlations with routine bioumoral parameters in a cohort of known chronic kidney disease patients, most of whom were in relatively advanced stages of renal function decline (median eGFR 10 ml/min/1.73 m^2), but before starting renal replacement therapies.

The prevalence of radiologically detectable vascular calcifications was 74.3%. Comparatively, Toussaint et al., in their cohort of 48 pre-dialysis patients, report a prevalence of 90% for abdominal aorta calcifications and 60% for superficial femoral artery calcifications (Toussaint et al., 2007). Data on the prevalence of valvular calcifications vary substantially depending on the study methodology, ranging from 27-100%, with identification typically done through computed tomography (Hutcheson & Goettsch, 2023). In the current cohort, the prevalence was 57.1%, with calcifications identified using echocardiography (Vinereanu et al., 2021).

AOPP does not correlate with the presence of valvular or vascular calcifications, suggesting that the factors contributing to their development do not depend on the mechanisms

generating advanced oxidation protein products. Comparatively, Lin L et al. identified significantly higher values in a cohort of uremic patients with aortic or coronary calcifications detected by tomography (Lin et al., 2017); Gryszczyńska B et al. found much higher AOPP levels in patients with abdominal aortic aneurysm and aorto-iliac disease than in chronic kidney disease patients (Gryszczyńska et al., 2017). The discrepancy likely results from different methodologies, with the cited studies using CT scans to identify vascular calcifications (Gaibazzi et al., 2014; Vinereanu et al., 2021).

The relationship between AOPP and vascular stiffness in chronic kidney disease patients remains underexplored. In the current study, AOPP correlates with PWV but not with pulse pressure, considered a surrogate stiffness indicator (Kass, 2002). The linear regression model highlighted that pulse pressure, augmentation pressure, systolic pressure, AOPP, and eGFR are significant predictors of PWV, with pulse pressure, advanced oxidation protein products, and estimated filtration rate together having the greatest weight in PWV variability (Vinereanu et al., 2021).

No significant association was identified between AOPP and eGFR or serum creatinine, which is somewhat surprising given that the accumulation of uremic toxins in the context of declining renal function contributes to generating a pro-oxidant internal environment. Although Witko-Sarsat et al. argue that AOPP can be considered genuine uremic toxins with pro-inflammatory effects (Witko-Sarsat et al., 2003a), clinical study results are divergent, with some authors reporting significant correlations between serum creatinine and/or eGFR and AOPP, while others refute this aspect (Colombo et al., 2019; Furuya et al., 2009; Huang et al., 2013; Liang et al., 2012; Witko-Sarsat et al., 2003b; Vinereanu et al., 2021).

Elevated AOPP levels are associated with an atherogenic lipid profile (Vinereanu et al., 2021). In the studied cohort, advanced oxidation protein products correlated with C-reactive protein but not with other systemic inflammation parameters, ESR, and fibrinogen. Additionally, the neutrophil-to-lymphocyte ratio significantly and linearly correlated with pulse wave velocity, a finding also identified in other studies (Afari & Bhat, 2016). These data suggest that chronic inflammatory status contributes to increased vascular stiffness (Vinereanu et al., 2021).

Conclusions and Personal Contributions

The study of oxidative stress could provide essential insights into the significant cardiovascular risk that chronic kidney disease (CKD) patients face and help identify new therapeutic means to improve the unassisted maturation rates of native arteriovenous fistulas (AVFs). Currently, the available clinical indicators for assessing oxidative stress are limited, and heterogeneous research methodologies have produced discrepant and difficult-to-replicate results over time.

First Study Contributions: In the first study, we explored the hypothesis regarding the adverse impact of oxidative stress on the maturation process of AVFs, considering vascular stiffness indicators obtained through applanation tonometry. While numerous in vitro studies in the current literature demonstrate the importance of redox homeostasis imbalance for the failure of AVF maturation, this work is the first to explore the relationship between advanced oxidation protein products (AOPP), vascular stiffness, and AVF maturation failure in a clinical context. The results of this doctoral research contribute to the current understanding of the relationship between oxidative stress and the maturation process of native AVFs in several ways:

- **AOPP and Vascular Stiffness:** Although AOPP is not a useful parameter for predicting AVF maturation failure, it is associated with increased vascular stiffness in CKD patients.
- **Pulse Wave Velocity and Augmentation Pressure:** Pulse wave velocity (PWV) and augmentation pressure (pAug) are associated with AVF maturation failure, suggesting their utility in prediction algorithms for AVF success.
- **Uric Acid:** The study highlights the role of uric acid as a predictor of AVF failure, even among hemodialysis patients, a topic insufficiently addressed in the literature.
- **Nutritional Status and Cardiovascular Compliance:** The results corroborate the available data on the implications of poor nutritional status on cardiovascular compliance by identifying serum albumin as a predictor of high PWV, especially in female CKD patients.

Second Study Contributions: In the second study, we clinically explored the hypothesis that a pro-oxidant environment, as encountered in CKD, contributes to vascular stiffening through

media calcification even in the pre-dialysis stage, based on literature data mainly derived from in vitro studies. The results showed that:

- **AOPP and Vascular/Valvular Calcifications:** AOPP levels do not specifically associate with the presence of radiologically and echographically detectable vascular or valvular calcifications. However, they underscore the link between this indicator and vascular stiffness parameters, as well as its predictive capacity for PWV value before the initiation of renal replacement therapies.
- **AOPP and Arterial Stiffness:** The relationship between AOPP levels and arterial stiffness is currently insufficiently explored, and the study results suggest the utility of including an oxidative stress indicator in cardiovascular risk prediction models for CKD patients.

Limitations

The limitations of this doctoral research are the result of several factors, primarily:

- **Sample Size:** The small number of patients included in each study.
- **Oxidative Stress Indicators:** Using more oxidative stress indicators could have provided additional insights into the pathogenic process generating reactive oxygen species.
- **Monitoring Techniques:** In the first study, the use of Doppler ultrasound for periodic non-invasive monitoring of the maturation process would have been advantageous compared to the functional criteria used.
- **Selection Bias:** In the second study, the selection bias stems from including patients from a single nephrology clinic of an emergency hospital.

Despite these limitations, the findings contribute significantly to the current knowledge and suggest further research avenues to improve the clinical outcomes for CKD patients, particularly regarding AVF maturation and cardiovascular risk assessment.

Selective bilbiography

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