Clinical Kidney Journal, 2023, vol. 16, no. 8, 1278–1287

https://doi.org/10.1093/ckj/sfad051
Advance Access Publication Date: 20 March 2023
Original Article

ORIGINAL ARTICLE

Plasma glycocalyx pattern: a mirror of endothelial damage in chronic kidney disease

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ABSTRACT

Background. Endothelial damage and cardiovascular disease complicate chronic kidney disease (CKD). The increased atherogenicity observed in patients with CKD can be linked to microinflammation and endothelial damage. Circulating endothelial glycocalyx degradation products, such as perlecan and decorin, tend to be elevated in CKD. We aimed to explore the association between the plasma perlecan and decorin levels and this pro-inflammatory and atherogenic state by studying monocyte subpopulations and intracellular adhesion molecule (ICAM)-1 expression in patients with CKD.

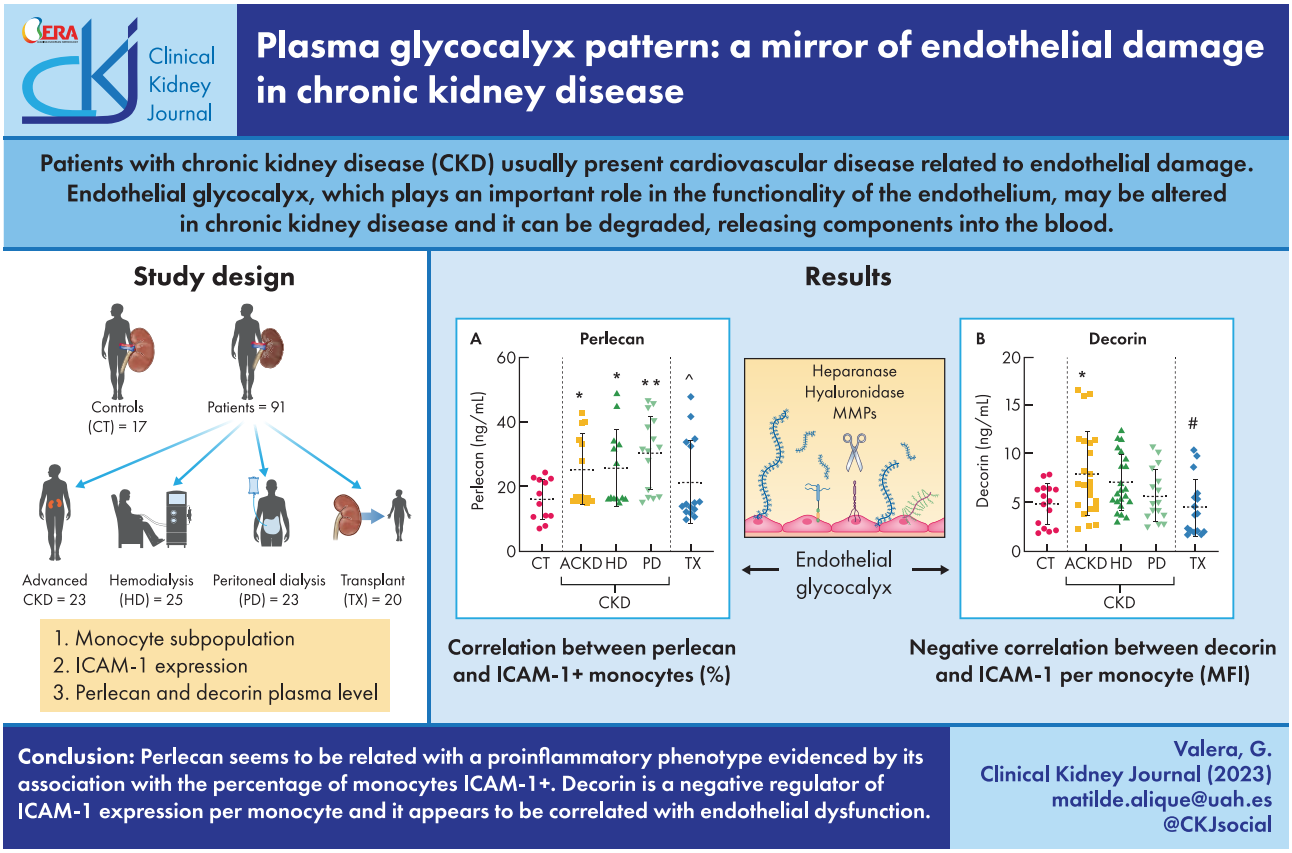
Methods. We studied 17 healthy controls, 23 patients with advanced CKD, 25 patients on haemodialysis, 23 patients on peritoneal dialysis and 20 patients who underwent kidney transplantation. Perlecan and decorin levels were evaluated using enzyme-linked immunosorbent assays, and the monocyte phenotype was analysed using direct immunofluorescence and flow cytometry.

Results. The plasma perlecan levels were higher in patients with CKD than in the healthy controls. These levels were associated with a higher prevalence of ICAM-1+ monocytes. Conversely, patients with advanced CKD (pre-dialysis) had higher plasma decorin levels, which were associated with a reduced ICAM-1 expression per monocyte.

Conclusions. Elevated perlecan levels in CKD may be associated with a higher prevalence of ICAM-1+ monocytes and a pro-inflammatory phenotype. Elevated decorin levels may act as a negative regulator of ICAM-1 expression in monocytes. Therefore, perlecan and decorin may be related to inflammation and monocyte activation in CKD and may act as potential markers of endothelial damage.

Received: 30.6.2022; Editorial decision: 3.3.2023

GRAPHICAL ABSTRACT



Keywords: chronic kidney disease, decorin, endothelial dysfunction, monocyte adhesion, perlecan

INTRODUCTION

Patients with chronic kidney disease (CKD) experience systemic inflammation; the origin of the inflammation is not fully understood, but the main cause may involve accumulation of uraemic toxins in the blood [1]. In patients with CKD, a modification changes the classical monocytes to intermediate and non-classical monocytes, which acquire a more senescent and pro-inflammatory phenotype. This conversion is predominant in patients undergoing haemodialysis (HD), and is accompanied by an increased expression of adhesion molecules. Among adhesion molecules, intercellular adhesion molecule (ICAM)-1 (also known as CD54) is relevant to extravasation and immune synapsis during antigen presentation as well as to a pro-inflammatory response [2, 3]. The abovementioned changes have been linked to atherosclerotic plaque development and a higher risk of cardiovascular disease (CVD) [4–9]. The prolonged persistence of inflammatory conditions favours CVD development, which is the leading cause of mortality and morbidity in patients with CKD [10]. The first step in CVD development is endothelial dysfunction [11, 12]. In CKD, endothelial dysfunction is caused by the accumulation of uraemic toxins that increase oxidative stress, thrombosis, inflammation, cell adhesion and endothelial permeabilization [13]. Endothelial dysfunction is associated with degradation of the endothelial glycocalyx (EG) [14, 15]. EG is located on the luminal face of endothelial cells and is fundamental for preserving the integrity of the vasculature, nitric oxide signalling, endothelial permeability, redox state regulation and cell adhesion [16]. EG mainly comprises glycoproteins and proteoglycans (such as perlecan, syndecans and glypicans) [14]. Perlecan is a heparan sulfate (HS) proteoglycan that is also a component of the extracellular matrix and basal membrane [17, 18]. Decorin, a small leucine-rich proteoglycan, is a component of the extracellular matrix and EG and is involved in regulating collagen fibril assembly, cell adhesion, angiogenesis, and modulation of growth factors and cytokine activity [19, 20].

EG disruption plays a crucial role in several pathogenic conditions. For example, loss of hyaluronan due to glycocalyx degradation is associated with endothelial dysfunction in many diseases, such as diabetes, hypertension, atherosclerosis and dyslipidaemia [21]. In pathological conditions such as inflammatory and ischaemic injuries, the EG may be damaged and lose thickness due to protease activation. Proteases, such as metalloproteinases (MMPs) and hyaluronidases, are released as part of the senescent-associated secretory phenotype (SASP). This is common among patients with CKD and related to the observed premature ageing. The activity of these enzymes is associated with an increase in the reactive oxygen species (ROS) and pro-inflammatory cytokines [22–25]. Increased ROS levels and inflammatory markers have been observed in patients with CKD [11, 23–26]. The main components of the EG are

syndecan-1 and hyaluronan, which appear elevated in the plasma of patients with CKD (mainly those undergoing dialysis). This elevation is correlated with an increase in the endothelial dysfunction markers [27–29]. When the glycocalyx is released, the endothelial cell surface is exposed and may be easily damaged, which can lead to CVD development [27, 30]. Thus, we studied other glycocalyx components, namely decorin and perlecan, which have not been studied before. These molecules could be implicated in atherosclerotic plaque formation and vascular calcification, which may be related to CVD development [31–33].

Furthermore, information regarding the effect of CKD treatments on EG and the possible involvement of EG degradation in the physiopathology of CKD remains lacking. Therefore, we hypothesized that endothelial damage in patients with CKD, an antecedent of CVD, may be related to EG degradation. Our proposed mechanism is that inflammatory conditions, oxidative stress and uraemic toxins affect the endothelium leading to SASP acquisition and subsequent release proteases that degrade the EG; the resulting degradation products could increase in the plasma of patients with CKD. Furthermore, this damage might be related to inflammation and monocytes' adhesion to the endothelium because inflammation and the increased cellular senescence provoked by uraemic toxins are factors that activate the release of enzymes that degrade the EG and extracellular matrix.

This study aimed to assess the potential of perlecan and decorin as endothelial damage markers. The sub-objectives were as follows: (i) to compare the plasma perlecan and decorin levels among patients with CKD receiving different treatments (such differences may affect the parameters studied); (ii) to evaluate the relationship of plasma perlecan and decorin with immunological alterations by analysing the phenotypes of monocyte subpopulations; and (iii) to evaluate the relationship of plasma perlecan and decorin with ICAM-1 expression in different monocyte subpopulations to assess their adhesion to the endothelium.

MATERIALS AND METHODS

Study population and sample recollection

This cross-sectional study included 108 participants. These comprised 17 healthy controls (CT); 20 patients who underwent kidney transplantation (TX); 23 patients with stage 4 or 5 advanced CKD (ACKD); and 25 and 23 patients undergoing HD and peritoneal dialysis (PD), respectively, for at least 6 months. Those with neoplasms, active infections and autoimmune diseases were excluded. The Ethics Committee of the i+12 Health Research Institute approved the realization of this study, and all procedures were carried out according to the guidelines set by the World Medical Association. In addition, the study complied with the standards set by the Helsinki Declaration of 1975, as revised in 2013.

Patients were recruited from the Department of Nephrology Service of 'Hospital 12 de Octubre', Madrid. Studies involving human participants were reviewed and approved by the 'Comité de ética de la Investigation of Hospital Universitario 12 de Octubre'. All participants provided written informed consent to participate in this study. Peripheral blood samples were obtained in tubes with ethylenediaminetetraacetic acid and transported to the laboratory at 4°C to be processed within 24 h. Patients undergoing dialysis were sampled during their short weekday cycle before the dialysis session began.

Determination of monocyte subpopulations by flow cytometry

Quantification and characterization of monocyte subpopulations [classical monocytes (CD14++, CD16–), which are the most abundant in the peripheral blood of healthy individuals; intermediate monocytes (CD14++, CD16+); and non-classical monocytes (CD14+, CD16+)] were performed using direct immunofluorescence of peripheral whole blood. The results are expressed as percentages, absolute values and fluorescence intensities (MFI). Fluorochrome-conjugated monoclonal antibodies were used against CD14 (TuK4, TRI-COLOR®, Invitrogen), CD16 (3G8, FITC, Invitrogen) and ICAM-1/CD54 (MEM -111, PE, Invitrogen). Our previous paper has summarized this method [7]. The percentages, absolute values and MFI were measured.

Perlecan and decorin enzyme-linked immunosorbent assay

The plasma perlecan and decorin levels were analysed using a sandwich enzyme-linked immunosorbent assay (ELISA). Peripheral blood plasma was isolated by centrifugation and stored at –80°C. Plasma samples were centrifuged at 2000*g* for 10 min, and the supernatant was used to prepare the test dilutions. The Human HSPG2 SimpleStep ELISA® Kit (Abcam, cat #ab274393) was used for perlecan quantification (1:7 plasma dilution). The Decorin Human ELISA Kit (Abcam, cat #ab99998) was used for decorin quantification (1:120 plasma dilution). The experiments were performed in accordance with the manufacturer's instructions. Previous studies using these kits have been referenced in the Supplementary data.

Statistical analysis

We used SPSS 21.0 for statistical analysis and GraphPad Prism 8.0.2 for graphical representation. Data are expressed as mean ± standard deviation. Data normality was assessed using a one-sample Kolmogorov–Smirnov test. Parametric variables were evaluated using a one-way analysis of variance (ANOVA), followed by the Bonferroni or T3 Dunnet *post hoc* analyses depending on the homogeneity of the variances (analysed by the Levene's test). Non-parametric variables were analysed using the Kruskal–Wallis test. Finally, a correlation analysis was performed using Pearson's or Spearman's correlation tests. Results of the chi-square test, performed for qualitative data, are expressed as relative and absolute frequencies. $P < .05$ denoted statistical significance.

RESULTS

Demographic and clinical characteristics of the population studied

The baseline characteristics of CTs and patients with CKD are shown in Table 1. There were no statistically significant differences in the age, sex, smoking habits and chronic heart failure incidence among the study groups. Diabetes prevalence was higher in the TX group than in the CT and HD groups. The percentage of patients with cardiopathy was higher in the ACKD, HD and PD groups than in the CT group. Patients with TX had a the highest prevalence of acute cardiovascular accidents and vasculopathy.

Table 1: Baseline characteristics of the study population.

	CT	ACKD	HD	PD	TX
n	17	23	25	23	20
Age (years), mean ± SD	51.05 ± 16.01	60.68 ± 18.57	60.21 ± 15	53.02 ± 14.76	56.61 ± 13.87
Women, *n* (%)	8 (47.1)	7 (30.4)	6 (24)	13 (56.5)	6 (30)
Diabetes, *n* (%)	2 (11.8)	11 (47.8)	4 (16)	4 (17.4)	11 (55)$^{*,\$}$
Smoking, *n* (%)	4 (23.5)	7 (30.4)	8 (32)	1 (4.3)	5 (25)
Cardiopathy, *n* (%)	0	11 (47.8)**	9 (36)***	7 (30.4)**	8 (40)***
ACVA, *n* (%)	0	4 (17.4)	2 (8)	3 (13)	4 (20)$^{*,\$}$
Vasculopathy, *n* (%)	0	0	5 (20)	2 (8.7)	9 (47.4)$^{***,\#\#\#,\sim}$
CHF, *n* (%)	0	2 (8.7)	6 (24)	1 (4.3)	1 (11.1)
eGFR (mL/min)		14.04 ± 4.9	7.3 ± 4.1	7.1 ± 2.6	48.7 ± 19.7
Average time in the current phase (years)			3.46 ± 3.99	1.13 ± 0.84	5.95 ± 3.53

ACVA: acute cardiovascular accident; CHF: chronic heart failure; eGFR: estimated glomerular filtration rate; SD: standard deviation.
Chi-squared test; statistical significance was set as $^{*}P < .05$, $^{***}P < .001$ vs CT, $^{\#\#\#}P < .001$ vs ACKD, $^{\$}P < .05$ vs HD and $^{\sim}P < .01$ vs PD.

Table 2: Characterization of monocytes and extracellular vesicles in the patients.

	CT	ACKD	HD	PD	TX
Classical monocytes (%)	86.02 ± 7.63	90.24 ± 4.48	78.83 ± 10.15$^{*,\#\#\#}$	88.7 ± 4.87$^{\$\$}$	79.94 ± 15.93$^{\#}$
Intermediate monocytes (%)	7.01 ± 4.48	5.72 ± 2.98	13.12 ± 6.84$^{**,\#\#}$	6.25 ± 3.62$^{\$\$}$	13.45 ± 15
Non-classical monocytes (%)	6.97 ± 4.03	4.04 ± 1.97	8.05 ± 5.05	5.05 ± 2.9	6.61 ± 3.59
Classical monocytes ICAM-1+ (%)	84.08 ± 8.8	85.79 ± 11.71	83.46 ± 13.59	89.22 ± 8.6	89.27 ± 11.4
Intermediate monocytes ICAM-1+ (%)	91.64 ± 6.24	94.24 ± 8.66	94.99 ± 6.29	97.75 ± 3.22	94.86 ± 7.88
Non-classical monocytes ICAM-1+ (%)	81.45 ± 18.58	97.66 ± 10.57	88–04 ± 13.89	87.18 ± 15.75	87.11 ± 18.38
Expression of ICAM-1 in classical monocytes (MFI)	124.5 ± 28.91	131.89 ± 38.18	194.47 ± 63.78$^{**,\#\#}$	153.67 ± 36.88	168.48 ± 58.25
Expression of ICAM-1 in intermediate monocytes (MFI)	222.06 ± 68.76	222.15 ± 81.87	329.21 ± 102.6$^{**,\#\#}$	249.23 ± 78.44$^{\$}$	290.13 ± 99.67
Expression of ICAM-1 in non-classical monocytes (MFI)	143.82 ± 52.09	172.40 ± 65.35	214.11 ± 79.24*	195.13 ± 59.08	155.6 ± 59.45

Data are presented as mean ± standard deviation.
ANOVA (percentage of non-classical monocytes, percentage of classical monocytes ICAM-1+, amount of ICAM-1 in classical, intermediate and non-classical monocytes) or the Kolmogorov–Smirnov test were used depending on the distribution of the variable. Statistical significance was set at $^{*}P < .05$, $^{**}P < .01$ vs CT, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$ vs ACKD, $^{\$}P < .05$ and $^{\$\$}P < .01$ vs HD.

Pro-inflammatory phenotype in patients with CKD represented by changes in the monocyte subpopulations

The monocyte subpopulations and their ICAM-1 expression levels were analysed (Table 2). Briefly, the percentage of classical monocytes was lower in the HD group than in the ACKD and PD groups. Conversely, the percentage of intermediate monocytes was higher in the HD group than in the CT, ACKD and PD groups. Additionally, the expression of ICAM-1 per monocyte was the highest in the HD group.

Plasma perlecan and decorin levels were elevated in patients with CKD

The plasma perlecan levels were higher in ACKD, HD and PD groups than in the CT group (Fig. 1A). The plasma decorin levels were higher in the ACKD group than in the CT group. However, no statistically significant differences in the decorin levels were observed between the CT and the HD, PD or TX groups. The decorin levels were lower in the TX group than in the ACKD group (Fig. 1B).

Correlation between plasma perlecan levels and ICAM-1 expression in different monocyte subpopulations

Possible associations between EG degradation and the monocyte phenotype in patients with CKD (i.e. the ACKD, HD and PD groups) were analysed. No correlations were found between the plasma perlecan levels and the numbers or percentages of classical, intermediate and non-classical monocytes. Nevertheless, the plasma perlecan levels were positively correlated with the percentages of ICAM-1+ classical and non-classical monocytes, but not with the percentage of ICAM-1+ intermediate monocytes, in CKD (Fig. 2A and B). The positive correlation was mainly due to the inclusion of patients undergoing dialysis, in whom the same results were observed (Fig. 3). No correlation was found between plasma perlecan levels and ICAM-1 expression per monocyte in CKD (Supplementary data, Table S1).

Correlation between plasma decorin levels and ICAM-1 expression in different monocyte subpopulations

The plasma decorin levels were not correlated with the percentages of classical, intermediate and non-classical monocytes or ICAM-1+ monocytes. However, the plasma decorin levels were negatively correlated with the amount of expression (MFI) of ICAM-1 per classical (Fig. 4A), intermediate (Fig. 4B) and non-classical (Fig. 4C) monocyte in the ACKD, HD and PD groups. The same negative correlation was observed for all monocyte subpopulations in the ACKD group (Fig. 5A–C); however, it was only observed for classical (Fig. 5D) and intermediate monocytes (Fig. 5E) in the HD group.

DISCUSSION

The EG covers the surface of all healthy endothelial structures; it regulates microvascular tone and endothelial permeability, maintains the endothelial barrier, regulates leukocyte

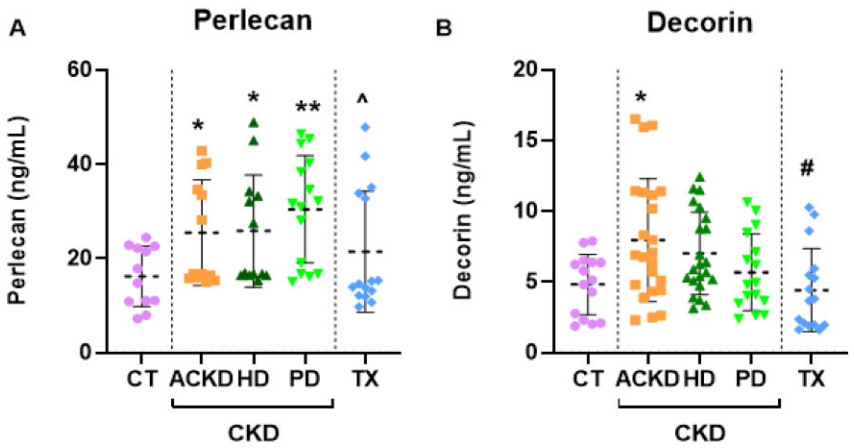


Figure 1: (**A**) Perlecan plasma levels (ng/mL) and (**B**) decorin plasma levels (ng/mL) in CT, patients with ACKD, patients undergoing HD, patients undergoing PD and patients who underwent TX. $^{*}P < .05$, $^{**}P < .01$ vs CT, $^{\#}P < .05$ vs ACKD, $\hat{}P < .05$ vs PD. Statistical analysis: Kruskal–Wallis test for perlecan (non-parametric) and a one-way ANOVA (T3 Dunnet *post hoc* test) for decorin (parametric).

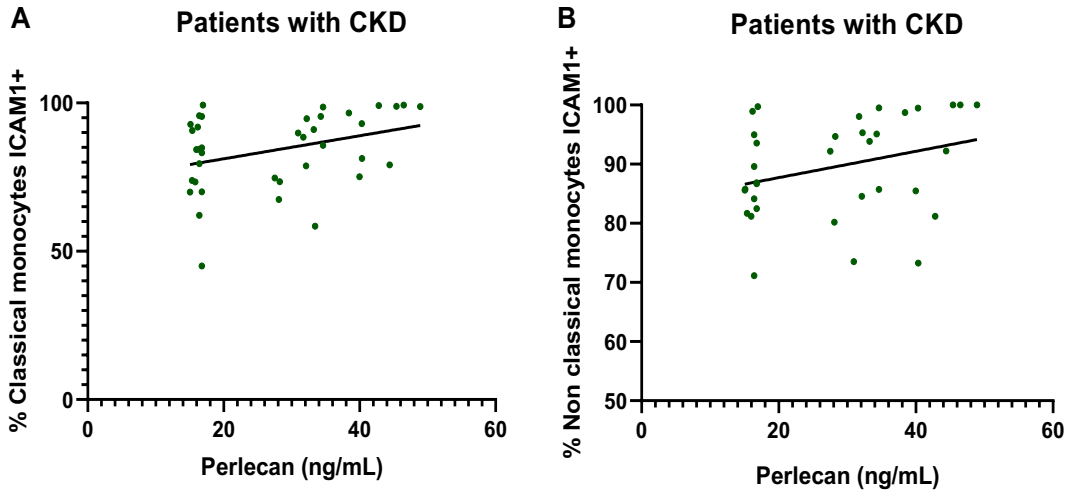


Figure 2: (**A**) Correlation between plasma perlecan levels and the percentage of classical monocytes expressing CD54 (CD14++, CD16–, ICAM-1+). (**B**) Percentage of non-classical monocytes expressing ICAM-1 (CD14+, CD16+, ICAM-1+) in patients with CKD (ACKD, HD and PD). A Spearman correlation test was performed.

adhesion/migration and inhibits intravascular thrombosis [34]. Several studies have suggested an association between EG degradation and acute kidney injury, CKD and CVD [35–37]. Alterations in EG structure cause endothelial dysfunction and exacerbate peripheral vascular disease [34]. In CVD, endothelial damage caused by inflammation and oxidative stress is reflected by changes in the expression of different molecules on the endothelial surface. The EG plays an essential role in mediating vascular signalling. Thus, when the EG is damaged, the endothelium tends to be more permeable; this facilitates the penetration and adhesion of cells with a pro-inflammatory profile, platelets and low-density lipoproteins (LDL), leading to atherosclerotic plaque development and thrombosis [38]. The plasma levels of some EG degradation products increase 7 days after an ischaemic stroke [39]. The plasma levels of EG components syndecan-1 and hyaluronan reportedly increase with the reduction of the estimated glomerular filtration rate in patients with CKD [40]. Syndecan-1 and hyaluronan increased in dialysis patients, followed by ACKD and TX. It is related to endothelial dysfunction markers and uraemic toxins levels [26, 39]. Patients with autosomal dominant polycystic kidney disease have a thinner glycocalyx than healthy people. They also have higher levels of plasma hyaluronan and syndecan-1 [41]. The lack of information on the role played by decorin and perlecan (EG molecules implicated in atherosclerotic plaque development [31–33]) in CKD motivated us to study these molecules as possible markers of endothelial damage.

In our study, the plasma perlecan levels were higher in the ACKD, HD and PD groups than in the CT group. This suggests that patients with CKD have EG damage, possibly due to accumulation of uraemic toxins in the blood; this explains why the plasma perlecan level returns to its optimal range once the patients undergo TX [40, 42]. This hypothesis is supported by the results of previous studies which demonstrated that increased release of heparinase in kidney diseases is involved in its development [17]. Heparinase degrades HS chains in low sulfidation sites, facilitating the release of perlecan into the bloodstream. Higher heparinase levels are found in the urine of patients with CKD and are related to severe proteinuria and a decreased renal function [43].

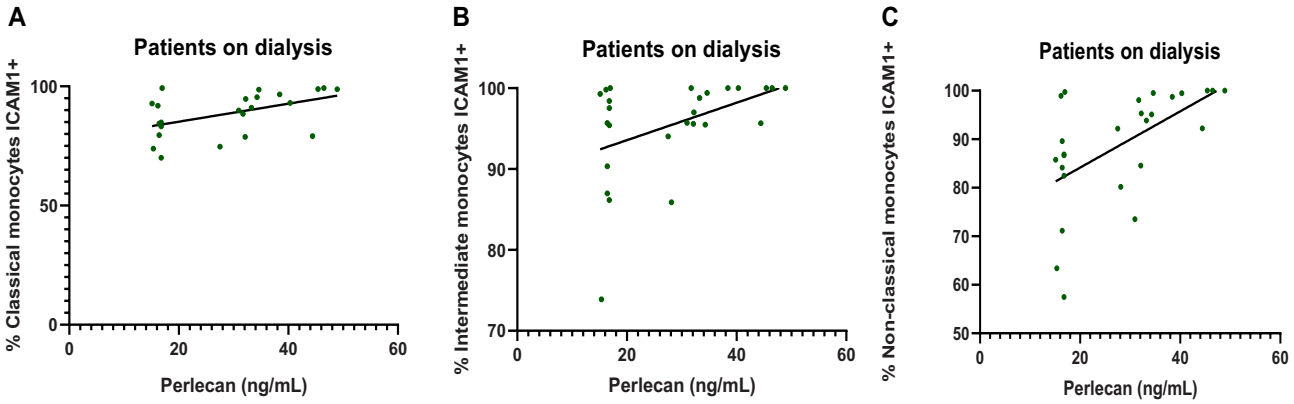


Figure 3: Correlation between plasma levels of perlecan and the percentage of (**A**) classical monocytes expressing CD54 (CD14++, CD16–, ICAM-1+), (**B**) intermediate monocytes expressing ICAM-1 (CD14++, CD16+, ICAM-1+) and (**C**) non-classical monocytes expressing CD54 (CD14+, CD16+, ICAM-1+) in patients undergoing dialysis (HD and PD). A Spearman correlation test was performed.

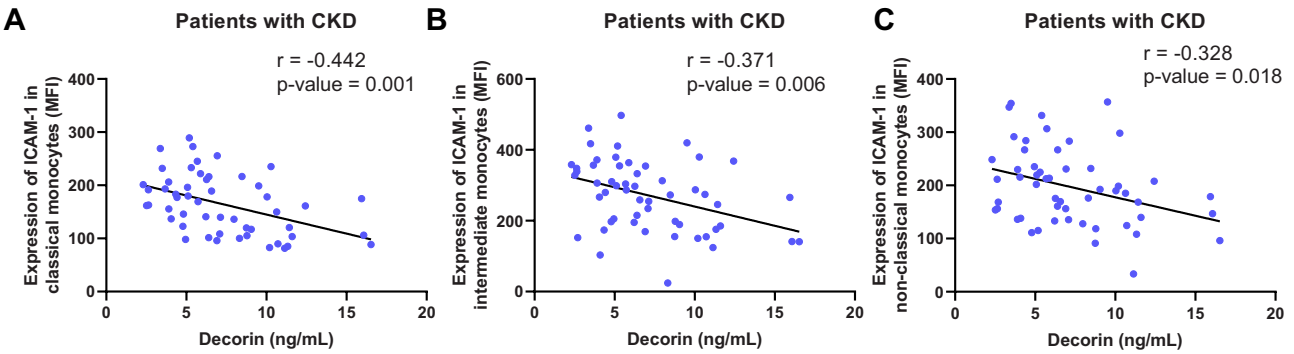


Figure 4: Correlation between plasma decorin levels and the amount of expression of ICAM-1 in (**A**) classical monocytes (CD14++, CD16–, ICAM-1+), (**B**) intermediate monocytes (CD14++, CD16+, ICAM-1+) and (**C**) non-classical monocytes (CD14+, CD16+, ICAM-1+) in patients with CKD (ACKD, HD and PD). Pearson's correlation test was performed.

Compared with the CT group, the plasma decorin levels were significantly higher in the ACKD group and slightly higher (not significant) in the HD group. Patients with ACKD have higher decorin levels because they accumulate higher amounts of uraemic toxins due to declining renal function without renal substitution therapy [28]. Furthermore, higher plasma decorin levels may be linked to atheromatic plaque formation and CVD development, owing to decorin's ability to interact with LDL and collagen [31].

Comparing our results with those in which syndecan-1 and hyaluronan were studied, we can hypothesize that depending on the treatment received (ACKD, HD, DP), different enzymes activate EG degradation, resulting in the distinct release of these molecules. However, in the end, there is a shedding of the EG in patients with CKD which may indicate endothelial damage. Nevertheless, this should be further studied.

Glycocalyx components, such as perlecan and decorin, are released into the blood owing to the activity of MMPs during inflammatory conditions and other pathological diseases [22, 39, 44]. These molecules can modify biochemical pathways, which may increase the risk of CKD. For example, free perlecan and decorin can be recognized by damage-associated molecule pattern receptors (such as toll-like receptors 2 and 4), which cause inflammation when activated [45]. The pro-inflammatory phenotype is also influenced by the SASP, common among patients with CKD. It is characterized by the release of pro-inflammatory cytokines and chemokines, growth factors and proteases (such as MMPs, which can also degrade the extracellular matrix and EG) [24, 25]. These pro-inflammatory conditions promote cellular senescence, endothelial dysfunction and CVD development in patients with CKD [11, 12].

Alterations in monocyte subpopulations indicate pro-inflammatory conditions [4, 5, 8, 9]. Herein, the percentage of classical monocytes was lower and that of intermediate monocytes was higher in the HD group than in the CT group. Intermediate and non-classical monocytes are considered senescent monocytes that are pro-inflammatory and proatherogenic (phenotypically similar to SASP); thus, they can lead to CVD development [7, 8]. In the HD group, a higher expression of ICAM-1 per monocyte was observed without any changes in the percentage of ICAM-1+ monocytes. ICAM-1+ monocytes are considered to have a pro-inflammatory phenotype that enables them to participate in extravasation, inflammation and immune synapsis during antigen presentation [2, 3].

Perlecan levels were positively correlated with the percentage of monocytes expressing ICAM-1. Contrastingly, decorin levels were negatively correlated with the amount (MFI) of ICAM-1 per monocyte. This finding corroborates other studies that showed that decorin is a negative regulator of ICAM-1 expression; however, the altered pathway remains undescribed [46].

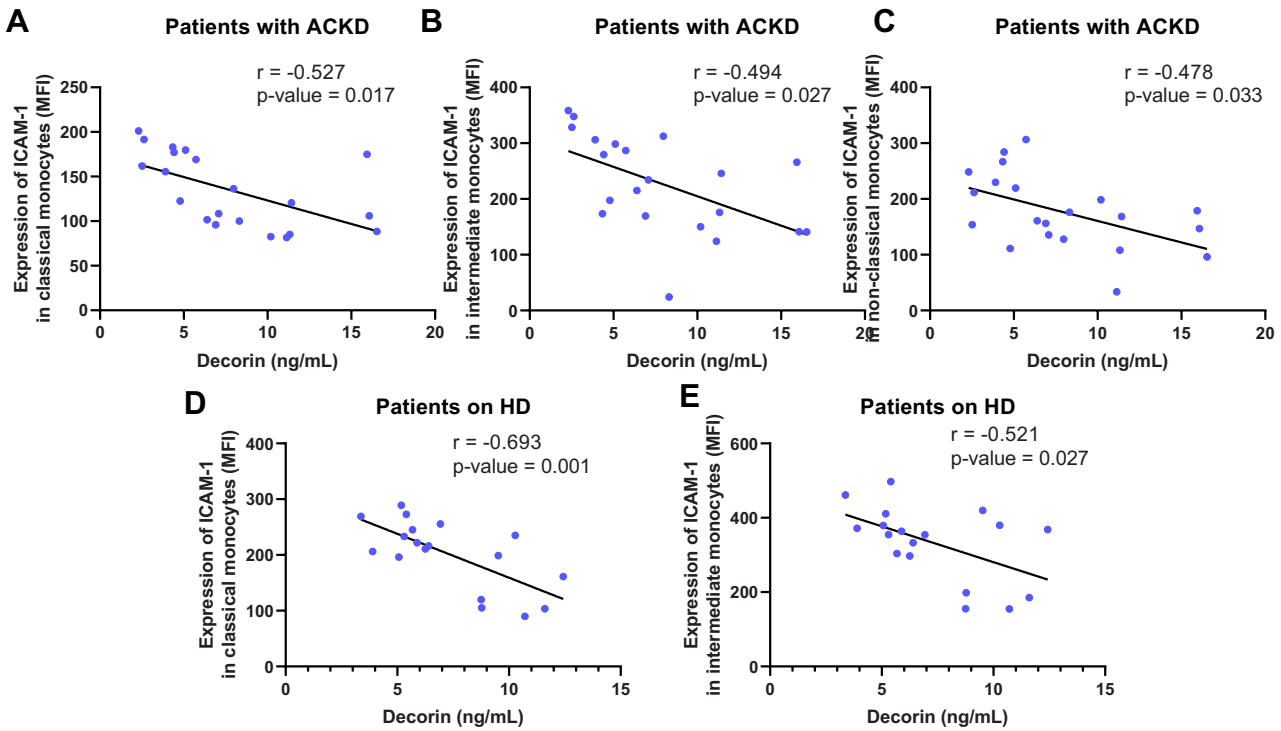


Figure 5: Correlation between plasma decorin levels and the amount of expression of ICAM-1 in (**A**) classical monocytes (CD14++, CD16–, ICAM-1+), (**B**) intermediate monocytes (CD14++, CD16+, ICAM-1+) and (**C**) non-classical monocytes (CD14+, CD16+, ICAM-1+) in patients with ACKD. HD was correlated with the plasma decorin levels and the amount of ICAM-1 in (**D**) classical monocytes and (**E**) intermediate monocytes. A Pearson's correlation test was performed.

Glycocalyx has an anti-adherent function due to its capacity to limit the interaction of leukocytes with the integrins and selectins present on the surface of endothelial cells. Studies have shown that the heparinase-mediated HS degradation and release of EG components imply the exposition of selectins and integrins, such as ICAM-1, facilitating their interaction with leukocytes [47, 48]. Therefore, we hypothesize that the reduction in ICAM-1 per monocyte could be caused by the reduced need for high ICAM-1 expression to achieve endothelial adhesion, owing to the exposure of their ligands. However, systemic inflammation (precisely, the increased release of pro-inflammatory factors such as cytokines) and the increase in the plasma perlecan levels activate immune cells (e.g. monocytes); this leads to an increase in the expression of endothelial adhesion molecules [49.

Water and salt retention have been linked to micro-inflammation and endothelial activation in patients with CKD [50. Salt retention is correlated with higher ICAM levels in the serum. Furthermore, salt and water retention have been proven to cause a collapse and disruption of the EG by reducing negatively charged HS residues [21]. Therefore, these factors could also cause the observed increase in the plasma perlecan and decorin levels; however, unlike uraemic toxins, water and salt retention are easier to manage using current treatments.

Based on a comparison of our findings with those of studies on syndecan-1 and hyaluronan, we can hypothesize that depending on the treatment received (ACKD, HD or PD), different enzymes activate EG degradation, resulting in the distinct release of these molecules. Furthermore, dialysis could lead to chronic inflammation caused by incompatibility with the hemofiltration membrane in HD or peritoneal irritation in PD [51, 52]. Consequently, the treatment could affect the monocyte subpopulations and the perlecan and decorin levels. Nevertheless, the outcome is shedding of the EG in patients with CKD, which may indicate endothelial damage.

Analysis of the plasma perlecan and decorin levels and their relationship with the expression of endothelial adhesion molecules (ICAM-1) can provide an overview of the endothelial status. The search for less invasive techniques to measure early prognostic and diagnostic markers of CVD in patients with CKD is necessary to improve their life expectancy and quality of life.

Our study has some limitations. Under normal conditions, perlecan and decorin should not appear in the urine and should not be filtered during dialysis. However, fragments of these molecules have been found in urine samples in cases of progressive proteinuria [53, 54]; this may mask the increased expression of these molecules in the plasma. Additionally, even though we assumed that these molecules occurred in the plasma due to glycocalyx shedding, they may also originate from the extracellular matrix or basement membrane (including the glomerular basement membrane). Nevertheless, the endothelium is directly in contact with the blood, so alterations in these parameters are probably highly related to the endothelial condition. If these molecules arose from other structures, an increase in their levels could point to a loss of the endothelial barrier function. After this proof of concept, we propose to investigate this issue further and provide additional evidence of an increase in these molecules as a consequence of endothelial damage and also elucidate the mechanism and the inflammatory pathways disrupted in these patients. To evaluate the effect of uraemic toxins in the endothelium, further *in vitro* studies are warranted. Furthermore, more patients should be analysed to validate our findings. Finally, as EG affects the endothelial function, we propose to study drugs that reduce EG degradation as possible therapies for patients with CKD.

In conclusion, plasma perlecan and decorin levels are higher in patients with CKD, possibly due to endothelial damage. The presence of these molecules in the plasma is seemingly associated with the monocyte adhesion capacity. The plasma perlecan levels correlated positively with the percentage of ICAM-1+ monocytes, while the plasma decorin levels correlated negatively with the expression of ICAM-1 per monocyte. These findings and the roles played by decorin and perlecan in atherosclerotic plaque formation make them interesting parameters to measure in patients with CKD. Nevertheless, their role as potential markers of endothelial damage has to be further investigated. The search for such endothelial damage markers could lead to affordable and minimally invasive prognostic and diagnostic techniques for CVD derived from CKD. Finally, we should consider their possible role as therapeutic targets.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

ACKNOWLEDGEMENTS

We thank the patients who participated in this study. We also thank the UCM cytometry service for its assistance.

FUNDING

Instituto de Salud Carlos III funded this study through the projects 'PI17/01029', 'PI19/00240' and 'PI20/01321' (co-funded by the European Regional Development Fund 'A way to make Europe'). Instituto de Salud Carlos III (ISCIII) FEDER funds the RICORS program to RICORS2040 (RD21/0005/0002), Ayuda Adicional-Excelencia Profesorado Programa Propio UAH, Comunidad de Madrid (CAM) CIFRA_COR-CM (P2022/BMD-7223), and Sociedad Española de Nefrología. A.F. was a fellow of the program 'Contratos Predoctorales de Investigación en Salud, Instituto de Salud Carlos III' (FI20/00 018). G.V. was issued a grant (number: PI20/01 321).

AUTHORS' CONTRIBUTIONS

M.A., J.C., C.Y., R.R., G.B. and E.M. conceived and designed the study. J.C. and E.M. selected the patients and collected clinical data. A.F. and G.V. analysed perlecan and decorin data and carried out the graphical design, statistical analysis and data interpretation. N.C., G.V., A.F., M.A., R.R. and J.C. performed monocyte experiments and analysis. G.V. and A.F. drafted the manuscript. N.C., M.A., J.C., R.R. and G.B. edited and revised the manuscript. All authors contributed to the article and approved the submitted version. The results presented in this paper have not been published previously in whole or in part, except in an abstract form.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

REFERENCES

1. Claro LM, Moreno-Amaral AN, Gadotti AC *et al*. The impact of uremic toxicity induced inflammatory response on the cardiovascular burden in chronic kidney disease. *Toxins* 2018;**10**:384. https://doi.org/10.3390/toxins10100384
2. Hubbard AK, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades. *Free Radical Biol Med* 2000;**28**:1379–86. https://doi.org/10.1016/S0891-5849(00)00223-9
3. Thornton J, McDaniel LS. THP-1 monocytes up-regulate intercellular adhesion molecule 1 in response to pneumolysin from *Streptococcus pneumoniae*. *Infect Immun* 2005;**73**:6493–8. https://doi.org/10.1128/IAI.73.10.6493-6498.2005
4. Ramirez R, Carracedo J, Soriano S *et al*. Stress-induced premature senescence in mononuclear cells from patients on long-term hemodialysis. *Am J Kidney Dis* 2005;**45**:353–9. https://doi.org/10.1053/j.ajkd.2004.10.022
5. Moens SJB, Verweij SL, van der Valk FM *et al*. Arterial and cellular inflammation in patients with CKD. *J Am Soc Nephrol* 2017;**28**:1278–85. https://doi.org/10.1681/ASN.2016030317
6. Ramírez R, Carracedo J, Merino A *et al*. CD14+CD16+ monocytes from chronic kidney disease patients exhibit increased adhesion ability to endothelial cells. *Contrib Nephrol* 2011;**171**:57–61. https://doi.org/10.1159/000327134
7. Ceprian N, Valera G, Caro J *et al*. Effect of kidney transplantation on accelerated immunosenescence and vascular changes induced by chronic kidney disease. *Front Med* 2021;**8**:705159. https://doi.org/10.3389/fmed.2021.705159
8. Carracedo J, Alique M, Vida C *et al*. Mechanisms of cardiovascular disorders in patients with chronic kidney disease: a process related to accelerated senescence. *Front Cell Dev Biol* 2020;**8**:705159. https://doi.org/10.3389/fcell.2020.00185
9. Merino A, Buendia P, Martin-Malo A *et al*. Senescent CD14(+)CD16(+) monocytes exhibit proinflammatory and proatherosclerotic activity. *J Immunol* 2011;**186**:1809–15. https://doi.org/10.4049/jimmunol.1001866
10. Ungvari Z, Tarantini S, Donato AJ *et al*. Mechanisms of vascular aging. *Circ Res* 2018;**123**:849–67. https://doi.org/10.1161/CIRCRESAHA.118.311378
11. Figuer A, Bodega G, Tato P *et al*. Premature aging in chronic kidney disease: the outcome of persistent inflammation beyond the bounds. *Int J Environ Res Public Health* 2021;**18**:8044. https://doi.org/10.3390/ijerph18158044
12. Daiber A, Steven S, Weber A *et al*. Targeting vascular (endothelial) dysfunction. *Br J Pharmacol* 2017;**174**:1591–619. https://doi.org/10.1111/bph.13517
13. da Cunha RS, Santos AF, Barreto FC *et al*. How do uremic toxins affect the endothelium? *Toxins* 2020;**12**:412. https://doi.org/10.3390/toxins12060412
14. Reitsma S, Slaaf DW, Vink H *et al*. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch* 2007;**454**:345–59. https://doi.org/10.1007/s00424-007-0212-8
15. Jedlicka J, Becker BF, Chappell D. Endothelial glycocalyx. *Crit Care Clin* 2020;**36**:217–32. https://doi.org/10.1016/j.ccc.2019.12.007
16. Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng* 2007;**9**:121–67. https://doi.org/10.1146/annurev.bioeng.9.060906.151959
17. Ferreras L, Moles A, Situmorang GR *et al*. Heparan sulfate in chronic kidney diseases: exploring the role of 3-O-sulfation. *Biochim Biophys Acta Gen Subj* 2019;**1863**:839–48. https://doi.org/10.1016/j.bbagen.2019.02.009

18. Kinsella MG, Wight TN. Perlecan: an extracellular matrix heparan sulfate proteoglycan that regulates key events in vascular development and disease. In: Garg HG, Linhardt RJ, Hales CA (eds). *Chemistry and Biology of Heparin and Heparan Sulfate*. Amsterdam: Elsevier Science, 2005, chapt 22, 607–35.
19. Jarvelainen H, Sainio A, Wight TN. Pivotal role for decorin in angiogenesis. *Matrix Biol* 2015;**43**:15–26. https://doi.org/10.1016/j.matbio.2015.01.023
20. Fu L, Kim HN, Sterling JD *et al*. The role of the cell surface glycocalyx in drug delivery to and through the endothelium. *Adv Drug Deliv Rev* 2022;**184**:114195. https://doi.org/10.1016/j.addr.2022.114195
21. Yilmaz O, Afsar B, Ortiz A *et al*. The role of endothelial glycocalyx in health and disease. *Clin Kidney J* 2019;**12**:611–9. https://doi.org/10.1093/ckj/sfz042
22. Dogne S, Flamion B. Endothelial glycocalyx impairment in disease focus on hyaluronan shedding. *Am J Pathol* 2020;**190**:768–80. https://doi.org/10.1016/j.ajpath.2019.11.016
23. Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. *Crit Care* 2019;**23**:16. https://doi.org/10.1186/s13054-018-2292-6
24. Vida C, Oliva C, Yuste C *et al*. Oxidative stress in patients with advanced CKD and renal replacement therapy: the key role of peripheral blood leukocytes. *Antioxidants* 2021;**10**:1155. https://doi.org/10.3390/antiox10071155
25. Padberg J-S, Wiesinger A, di Marco GS *et al*. Damage of the endothelial glycocalyx in chronic kidney disease. *Atherosclerosis* 2014;**234**:335–43. https://doi.org/10.1016/j.atherosclerosis.2014.03.016
26. Vlahu CA, Lemkes BA, Struijk DG *et al*. Damage of the endothelial glycocalyx in dialysis patients. *J Am Soc Nephrol* 2012;**23**:1900–8. https://doi.org/10.1681/ASN.2011121181
27. Koch J, Hijmans RS, Builes MO *et al*. Direct evidence of endothelial dysfunction and glycocalyx loss in dermal biopsies of patients with chronic kidney disease and their association with markers of volume overload. *Front Cell Dev Biol* 2021;**9**:733015. https://doi.org/10.3389/fcell.2021.733015
28. Sampei S, Okada H, Tomita H *et al*. Endothelial glycocalyx disorders may be associated with extended inflammation during endotoxemia in a diabetic mouse model. *Front Cell Dev Biol* 2021;**9**:623582. https://doi.org/10.3389/fcell.2021.623582
29. Neufeld EB, Zadrozny LM, Phillips D *et al*. decorin and biglycan retain LDL in disease-prone valvular and aortic subendothelial intimal matrix. *Atherosclerosis* 2014;**233**:113–21. https://doi.org/10.1016/j.atherosclerosis.2013.12.038
30. Ng CY, Whitelock JM, Williams H *et al*. Macrophages bind LDL using heparan sulfate and the perlecan protein core. *J Biol Chem* 2021;**296**:100520. https://doi.org/10.1016/j.jbc.2021.100520
31. Fischer JW, Steitz SA, Johnson PY *et al*. Decorin promotes aortic smooth muscle cell calcification and colocalizes to calcified regions in human atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2004;**24**:2391–6. https://doi.org/10.1161/01.ATV.0000147029.63303.28
32. Tomita H, Suzuki K, Komatsu M *et al*. Editorial: vascular endothelial glycocalyx in cardiovascular disease. *Front Cardiovasc Med* 2022;**9**:952022. https://doi.org/10.3389/fcvm.2022.952022
33. Jourde-Chiche N, Fakhouri F, Dou L *et al*. Endothelium structure and function in kidney health and disease. *Nat Rev Nephrol* 2019;**15**:87–108. https://doi.org/10.1038/s41581-018-0098-z
34. Goligorsky MS, Sun D. Glycocalyx in endotoxemia and sepsis. *Am J Pathol* 2020;**190**:791–8. https://doi.org/10.1016/j.ajpath.2019.06.017
35. Tarbell JM, Cancel LM. The glycocalyx and its significance in human medicine. *J Intern Med* 2016;**280**:97–113. doi: https://doi.org/10.1111/joim.12465
36. Banerjee S, Mwangi JG, Stanley TK *et al*. Regeneration and assessment of the endothelial glycocalyx to address cardiovascular disease. *Ind Eng Chem Res* 2021;**60**:17328–47. https://doi.org/10.1021/acs.iecr.1c03074
37. DellaValle B, Hasseldam H, Johansen FF *et al*. Multiple soluble components of the glycocalyx are increased in patient plasma after ischemic stroke. *Stroke* 2019;**50**:2948–51. https://doi.org/10.1161/STROKEAHA.119.025953
38. Liew H, Roberts MA, Pope A *et al*. Endothelial glycocalyx damage in kidney disease correlates with uraemic toxins and endothelial dysfunction. *BMC Nephrol* 2021;**22**:21. https://doi.org/10.1186/s12882-020-02219-4
39. Liew H, Roberts MA, Mcmahon LP. Markers of the endothelial glycocalyx are improved following kidney transplantation. *Kidney Blood Press Res* 2021;**46**:581–7. https://doi.org/10.1159/000517317
40. Shafat I, Agbaria A, Boaz M *et al*. Elevated urine heparanase levels are associated with proteinuria and decreased renal allograft function. *PLoS One* 2012;**7**:e44076. https://doi.org/10.1371/journal.pone.0044076
41. Fuchs A, Dederichs J, Arjune S *et al*. Microvascular perfusion, perfused boundary region and glycocalyx shedding in patients with autosomal dominant polycystic kidney disease: results from the GlycoScore III study. *Clin Kidney J* 2023;**16**:384–93. https://doi.org/10.1093/ckj/sfac229
42. Huang X, Hu HR, Sun T *et al*. Plasma endothelial glycocalyx components as a potential biomarker for predicting the development of disseminated intravascular coagulation in patients with sepsis. *J Intensive Care Med* 2021;**36**:1286–95. https://doi.org/10.1177/0885066620949131
43. Goode KJ, Poon IKH, Phipps S *et al*. Soluble heparan sulfate fragments generated by heparanase trigger the release of pro-inflammatory cytokines through TLR-4. *Plos One* 2014;**9**:e109596.
44. Docherty MH, O'Sullivan ED, Bonventre JV *et al*. Cellular senescence in the kidney. *J Am Soc Nephrol* 2019;**30**:726–36. https://doi.org/10.1681/ASN.2018121251
45. Tan H, Xu J, Liu Y. Ageing, cellular senescence and chronic kidney disease: experimental evidence. *Curr Opin Nephrol Hypertens* 2022;**31**:235–43. https://doi.org/10.1097/MNH.0000000000000782.
46. Mao L, Yang J, Yue J *et al*. Decorin deficiency promotes epithelial-mesenchymal transition and colon cancer metastasis. *Matrix Biol* 2021;**95**:1–14. https://doi.org/10.1016/j.matbio.2020.10.001
47. Collins LE, Troeberg L. Heparan sulfate as a regulator of inflammation and immunity. *J Leukoc Biol* 2018;**105**:81–92. https://doi.org/10.1002/JLB.3RU0618-246R
48. Celie J, Beelen RHJ, van den Born J. Heparan sulfate proteoglycans in extravasation: assisting leukocyte guidance. *Front Biosci* 2009;**14**:4932–49. https://doi.org/10.2741/3578
49. Lino DOC, Freitas IA, Meneses GC *et al*. Interleukin-6 and adhesion molecules VCAM-1 and ICAM-1 as biomarkers of post-acute myocardial infarction heart failure. *Braz J Med Biol Res* 2019;**52**:e8658. https://doi.org/10.1590/1414-431x20198658

50. Mitsides N, Alsehli FMS, Mc Hough D *et al*. Salt and water retention is associated with microinflammation and endothelial injury in chronic kidney disease. *Nephron* 2019;**143**:234–42. https://doi.org/10.1159/000502011.
51. Memoli B, Minutolo R, Bisesti V *et al*. Changes of serum albumin and C-reactive protein are related to changes of interleukin-6 release by peripheral blood mononuclear cells in hemodialysis patients treated with different membranes. *Am J Kidney Dis* 2002;**39**:266–73. https://doi.org/10.1053/ajkd.2002.30545
52. Parikova A, Hruba P, Krediet RT *et al*. Long-term peritoneal dialysis treatment provokes activation of genes related to adaptive immunity. *Physiol Res* 2019;**68**:775–83. https://doi.org/10.33549/physiolres.934158
53. Oda O, Shinzato T, Ohbayashi K *et al*. Purification and characterization of perlecan fragment in urine of end-stage renal failure patients. *Clin Chim Acta* 1996;**255**:119–32. https://doi.org/10.1016/0009-8981(96)06395-4
54. Schaefer L, Raslik I, Grone HJ *et al*. Small proteoglycans in human diabetic nephropathy: discrepancy between glomerular expression and protein accumulation of decorin, biglycan, lumican, and fibromodulin. *FASEB J* 2001;**15**:559–61. https://doi.org/10.1096/fj.00-0493fje