Clinical Kidney Journal, 2023, vol. 16, no. 2, 384–393

https:/doi.org/10.1093/ckj/sfac229
Advance Access Publication Date: 19 October 2022
Original Article

ORIGINAL ARTICLE

Microvascular perfusion, perfused boundary region and glycocalyx shedding in patients with autosomal dominant polycystic kidney disease: results from the GlycoScore III study

Alexander Fuchs [1], Jennifer Dederichs [1,2], Sita Arjune [2], Polina Todorova [2], Fabian Wöstmann [2], Philipp Antczak [2], Anja Illerhaus [3], Birgit Gathof [4], Franziska Grundmann [2], Roman-Ulrich Müller [2,5,*] and Thorsten Annecke [1,6,*]

[1]University of Cologne, Faculty of Medicine and University Hospital of Cologne, Department of Anaesthesiology and Intensive Care Medicine, Cologne, Germany, [2]University of Cologne, Faculty of Medicine and University Hospital of Cologne, Department II of Internal Medicine and Center for Molecular Medicine Cologne, Cologne, Germany, [3]University of Cologne, Faculty of Medicine and University Hospital of Cologne, Department of Dermatology, Cologne, Germany, [4]University of Cologne, Faculty of Medicine and University Hospital of Cologne, Institute of Transfusion Medicine, Cologne, Germany, [5]CECAD, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany and [6]University of Witten/Herdecke, Anaesthesiology II, Kliniken der Stadt Köln gGmbH, Department of Anaesthesiology and Intensive Care Medicine, Merheim Medical Center, Cologne, Germany

*These authors contributed equally.
Correspondence to: Roman-Ulrich Müller; E-mail: roman-ulrich.mueller@uk-koeln.de

ABSTRACT

Background. Vascular abnormalities and endothelial dysfunction are part of the spectrum of autosomal dominant polycystic kidney disease (ADPKD). The mechanisms behind these manifestations, including potential effects on the endothelial surface layer (ESL) and glycocalyx integrity, remain unknown.

Methods. Forty-five ambulatory adult patients with ADPKD were enrolled in this prospective, observational, cross-sectional, single-centre study. Fifty-one healthy volunteers served as a control group. All participants underwent real-time microvascular perfusion measurements of the sublingual microcirculation using sidestream dark field imaging. After image acquisition, the perfused boundary region (PBR), an inverse parameter for red blood cell (RBC) penetration into the ESL, was automatically calculated. Microvascular perfusion was assessed by RBC filling and capillary density. Concentrations of circulating glycocalyx components were determined by enzyme-linked immunosorbent assay.

Results. ADPKD patients showed a significantly larger PBR compared with healthy controls (2.09 ± 0.23 µm versus 1.79 ± 0.25 µm; $P < .001$). This was accompanied by significantly lower RBC filling (70.4 ± 5.0% versus 77.9 ± 5.4%;

Received: 30.5.2022; Editorial decision: 14.10.2022

$P < .001$) as well as a higher valid capillary density {318/mm^2 [interquartile range (IQR) 269–380] versus 273/mm^2 [230–327]; $P = .007$}. Significantly higher plasma concentrations of heparan sulphate (1625 ± 807 ng/ml versus 1329 ± 316 ng/ml; $P = .034$), hyaluronan (111 ng/ml [IQR 79–132] versus 92 ng/ml [82–98]; $P = .042$) and syndecan-1 were noted in ADPKD patients compared with healthy controls (35 ng/ml [IQR 27–57] versus 29 ng/ml [23–42]; $P = .035$).
Conclusions. Dimensions and integrity of the ESL are impaired in ADPKD patients. Increased capillary density may be a compensatory mechanism for vascular dysfunction to ensure sufficient tissue perfusion and oxygenation.

LAY SUMMARY

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic disorder leading to kidney failure. However, ADPKD not only affects the kidney, but also involves other organ systems, including the vasculature. The pathogenesis of vascular disease is not well understood. Here we examined changes in the inner-most layer of blood vessels in ADPKD patients and found significant alterations of the endothelial surface layer (ESL). Considering the importance of the ESL in the integrity of vascular function, this finding is of great interest for ADPKD research.

Keywords: ADPKD, endothelium, glycocalyx, intravital microscopy, microcirculation

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent genetic cause of kidney failure in adults [1]. Apart from its renal manifestation with a gradual loss of kidney function, it is considered a systemic disease. Cardiovascular complications are a major cause of morbidity and mortality in chronic kidney disease (CKD) in general and in ADPKD in particular [2]. In ADPKD, such complications are preceded by arterial hypertension and early vascular changes, even with normal blood pressure (BP), are occurring at a young age [3]. Endothelial dysfunction, as reflected by a decreased flow-mediated dilatation of the brachial artery, is already evident at an early stage in ADPKD patients [4] and has been linked to oxidative stress and vascular inflammation [5]. The vascular endothelium plays a pivotal role in regulating microvascular function and perfusion. On its luminal site it holds the glycocalyx, which is comprised of proteoglycans and glycoconjugates. Together with associated soluble plasma proteins it forms the dynamic and gel-like cover called the endothelial surface layer (ESL), which is involved in mechanotransduction, haemostasis, signalling and blood cell–vessel wall interactions [6]. Shedding of glycocalyx components has been described in a variety of medical conditions [7–9], including sepsis [10], diabetes [11] and CKD [12, 13].

ESL dimensions can be estimated by calculating the perfused boundary region (PBR). The PBR is the depth of lateral erythrocyte penetration into the ESL and has emerged from intravital microscopy recordings, a non-invasive technique to study the microcirculation in regions that are easily accessible, e.g. the sublingual microvasculature. It has been recently employed in different clinical situations and conditions to assess the ESL [14–18]. The calculation of the PBR has previously been described in detail [19, 20].

The concentration of circulating glycocalyx components increases with progression of CKD and correlates with endothelial dysfunction [12], and patients with kidney failure show an increased PBR and circulating syndecan-1 levels [16]. However, ESL dimensions and its circulating components have not been investigated in ADPKD patients. We hypothesised that ADPKD patients—due to endothelial involvement in this disease—show an increased PBR (reduced ESL) accompanied by increased shedding of glycocalyx components, leading to higher plasma concentrations.

MATERIALS AND METHODS

Study design and recruitment

Real-time PBR measurements of the sublingual microcirculation and determination of soluble glycocalyx components were performed in GlycoScore III, a prospective, observational, cross-sectional, single-centre study in ADPKD patients. Ambulatory adult patients with ADPKD were recruited consecutively from the AD(H)PKD registry at the University Hospital of Cologne. Fifty-one healthy blood donors from a previously conducted study served as a control group [21]. The pre-donation data from the control group were included in the analysis. The protocol was approved by the local ethics committee of the University of Cologne (AZ 19-1646). The study was performed in accordance with the Helsinki Declaration and the Good Clinical Practice guidelines of the International Conference on Harmonization. All subjects gave written informed consent prior to any study-related procedures and blood collection. GlycoScore III is registered in the German Clinical Trials Register (www.drks.de; DRKS00022460).

Study endpoints

The primary endpoint was the dimension of the PBR of vessels in the sublingual microvasculature with a diameter of 5–25 µm. Secondary endpoints were the red blood cell (RBC) filling percentage and capillary density as parameters for quantification of microvascular perfusion. Laboratory secondary endpoints were plasma concentrations of heparan sulphate, hyaluronan and syndecan-1 as markers of glycocalyx degradation.

Study-related measurements: microcirculation

Participants underwent non-invasive imaging of the sublingual microvasculature via intravital microscopy using a handheld sidestream dark field videomicroscope (Capiscope HVCS Handheld Video Capillaroscopy System, KK Research

Technology, Devon, UK). After image acquisition, an analysis software automatically calculated the PBR in vessels with a diameter of 5–25 µm. Microvascular perfusion was measured by RBC filling percentage and capillary density. Each participant underwent two measurements from which the average PBR was calculated to provide a single PBR value per subject and measurement. The process of image acquisition and reproducibility of imaging and analysis via intravital microscopy and the above mentioned software have been described elsewhere [16–18]. The PBR is an inverse parameter for RBC penetration into ESL, and hence its thickness. Regarding this inverse relationship between the ESL and PBR, a low PBR reflects a thick ('healthy') ESL, while a high PBR reflects a thin ESL. The RBC filling percentage is the percentage of time in which valid vessel segments have RBCs present. The valid capillary density reflects the vessels <30 µm in the recordings that show a contrast above a defined threshold indicating they are functional, i.e. perfused [22]. It is reported as the number of perfused valid vessels per mm^2. RBC filling percentage and valid capillary density are estimates for microvascular perfusion [19, 23].

ESL properties and microvascular perfusion were analysed using GlycoCheck software (Microvascular Health Solutions, Orem, UT, USA). During acquisition, the software provides feedback regarding stability and focus and adjusts light intensity. This ensures that only adequate images are being recorded for automatic analysis, thus limiting interobserver variability.

Study-related measurements: blood analyses

Peripheral venous blood samples were collected using ethylenediaminetetraacetic acid as an anticoagulant. Samples for enzyme-linked immunosorbent assay (ELISA) were centrifuged (882 *g*, 7 min, room temperature), the supernatant plasma was then aliquoted and stored at −80°C until further analysis. ELISAs in duplicate were performed in accordance with the manufacturer's instructions to determine plasma concentrations of heparan sulphate (Cusabio Technology, Houston, TX, USA), hyaluronan (Echelon Biosciences, Salt Lake City, UT, USA) and syndecan-1 (Diaclone SAS, Besançon, France).

Statistical analysis

We planned this observational study to evaluate the difference in PBR in ADPKD patients compared with healthy controls. Based on data from our study group, the mean ± standard deviation (SD) PBR of the control group was 1.79 ± 0.25 µm. According to previously published data regarding the PBR in end-stage renal disease patients [16], a relative difference in PBR of 12.6% (or 0.23 µm in absolute terms) compared with controls could be detected. Since we did not expect such a compromised renal function in the ADPKD patients, but an increased PBR compared with controls, we estimated that the PBR difference would be about 30% smaller. Hence we aimed to detect a mean PBR difference of 0.16 µm (equivalent to a 9% relative difference in PBR) assuming an SD of 0.25 µm applying a two-sided Student's test with type I error protection of <0.05 and a power >0.80, which gave an estimated sample size of 40. In the sample size calculation, we set the control:experimental subjects ratio at 1.0. Continuous variables were assessed for normal distribution using histograms, quantile–quantile plots and the Shapiro–Wilk test. Analysis between the two groups was carried out using Student's unpaired *t*-test or the non-parametric Mann–Whitney U test, if normal distribution was not confirmed. Normally distributed variables are presented as mean ± SD; for non-normally distributed data the median and interquartile range (IQR) are given. We performed an age-matched subgroup analysis for the primary endpoint followed by splitting the data by gender, and a multivariate analysis of PBR against age, gender, group, smoking status and estimated glomerular filtration rate (eGFR). Spearman's correlation was used to test for independence between variables. The significance threshold was set at .05 (two-tailed). The calculations were performed with SPSS Statistics version 28 (IBM, Armonk, NY, USA) and visualisation with GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, CA, USA).

RESULTS

Study population

We recruited 45 patients with ADPKD from August to December 2020 from a single centre. A total of 51 healthy participants from a previously conducted study served as a control group [21]. Participants' demographic and clinical characteristics are presented in Table 1. ADPKD patients were significantly older, had a higher BMI and systolic and diastolic BP and a lower heart rate than healthy controls. The median time since diagnosis of ADPKD was 13 years. The eGFR, determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 equation was significantly lower in ADPKD patients. The majority of ADPKD patients were in CKD stage 2 or 3 (stage 1, $n = 5$; stage 2, $n = 13$; stage 3, $n = 23$; stage 4, $n = 4$). As expected, ADPKD patients were on prescribed medication much more frequently, with a specific focus on antihypertensives. A total of 26.7% were on targeted ADPKD therapy with tolvaptan. The majority of ADPKD patients were distributed across Mayo classes 1B, C and D, an important kidney volume–based indicator of disease severity [24]. No patient required kidney replacement therapy at the time of inclusion.

PBR dimension and perfusion measurements

There was a significantly larger PBR in ADPKD patients compared with healthy controls (2.09 ± 0.23 µm versus 1.79 ± 0.25 µm; $P < .001$). There was no significant correlation between PBR and eGFR in ADPKD patients (see Fig. 1). ADPKD patients showed a significantly lower RBC filling (70.4 ± 5.0% versus 77.9 ± 5.4%; $P < .001$) as well as a higher valid capillary density (318/mm^2 [IQR 269–380] versus 273/mm^2 [230–327]; $P = .007$) (Fig. 2). To detect potential age-related changes in PBR we performed a subgroup analysis of the healthy controls, comparing the PBR of the 16 oldest control subjects (mean age 48 years [IQR 41–54]) to the 35 younger subjects (mean age 28 years [IQR 24–30]); dimensions did not differ significantly (1.79 ± 0.19 µm versus 1.79 ± 0.27 µm; $P = .996$). In addition, we performed a subgroup analysis on 67 of 96 participants ($n = 34$ for ADKPD and $n = 33$ for healthy controls) that were matched by age (range ≥28–≤57 years) and found the same significant differences for PBR between ADPKD and healthy controls. Further splitting the data by gender showed that group remained the only significant factor for differences in PBR. We also performed a multivariate analysis of PBR against age, gender, group, smoking status and eGFR and found that group was the only significant coefficient within this analysis, which strengthens our findings (see Table 2).

Table 1: Participant characteristics.

Characteristics	ADPKD ($n = 45$)	Control (n = 51)	*P*-value
Age (years), median (IQR)	49 (37–56)	30 (25–39)	<.001
Female, *n* (%)	23 (51.1)	23 (45.1)	.556
BMI (kg/m^2), median (IQR)	26.0 (23.1–29.0)	24.4 (21.8–26.0)	.012
Years since ADPKD diagnosis, median (IQR)	13 (4–24)	–	
Heart rate (beats/min), median (IQR)	63 (59–73)	78 (70–90)	<.001
SpO_2 (%), median (IQR)	98 (97–99)	99 (98–99)	.285
Systolic BP (mmHg), median (IQR)	140 (131–153)	130 (120–140)	.001
Diastolic BP (mmHg), median (IQR)	89 (84–99)	80 (70–90)	<.001
Smoking status, *n* (%)			
Non-smoker	17 (37.8)	40 (78.4)	.245
Ex-smoker	20 (44.4)	2 (3.9)	<.001
Smoker	8 (17.8)	9 (17.6)	.245
Pack years, median (IQR)	1 (0–13)	0 (0–0)	<.001
Arterial hypertension, *n* (%)	42 (93.3)	0	<.001
Age of hypertension onset, *n* (%)			
Diagnosed at ≥35 years	20 (44.4)	–	
Diagnosed at <35 years	22 (48.9)	–	
Dyslipidaemia, *n* (%)	10 (22.2)	0	<.001
Diabetes mellitus, *n* (%)	0	0	
eGFR (ml/min/1.73 m^2; CKD-EPI 2009), median (IQR)	53 (39–76)	106 (93–119)	<.001
htTKV (ml/m), median (IQR)	787 (514–1185)	–	
MAYO class, *n* (%)			
1A	1 (2.3)	–	
1B	16 (36.4)	–	
1C	11 (25.0)	–	
1D	12 (27.3)	–	
1E	4 (9.1)	–	
Medical condition, *n* (%)			
Valvular heart disease	6 (13.3)	–	
Coronary artery disease	0	–	
Cardiomyopathy	1 (2.2)	–	
Atrial fibrillation	2 (4.4)	–	
Peripheral artery disease	1 (2.2)	–	
Intracerebral aneurysm	1 (2.2)	–	
Stroke	1 (2.2)	–	
Status post hypothyreosis	6 (13.3)	3 (5.9)	.508
Pollen allergy	0	3 (5.9)	.245
Medication, *n* (%)			
Tolvaptan	12 (26.7)	0	<.001
ACE-I	18 (40.0)	0	<.001
ARB	18 (40.0)	0	<.001
Calcium channel blocker	15 (33.3)	0	<.001
Diuretic	9 (20.0)	0	<.001
Beta-blocker	10 (22.2)	0	<.001
Statin	10 (22.2)	0	<.001
Acetylsalicylic acid	2 (4.4)	1 (2.0)	.598
Clopidogrel	1 (2.2)	0	.469
DOAC	2 (4.4)	0	.217
Allopurinol	5 (11.1)	0	.014
Vitamin D	11 (24.4)	0	<.001
L-thyroxine	6 (13.3)	3 (5.9)	.211
Contraceptive	2 (4.4)	5 (9.8)	.314
Proton pump inhibitor	3 (6.7)	0	.099

ACE-I: angiotensin-converting enzyme inhibitor, ADPKD: autosomal dominant polycystic kidney disease, ARB: angiotensin receptor blocker, DOAC: directly acting oral anticoagulant, BMI: body mass index, BP: blood pressure, htTKV: height-adjusted total kidney volume, IQR: interquartile range, SpO_2: peripheral oxygen saturation.

Circulating glycocalyx components

Blood samples from 40 participants of each group were analysed. In the control group, five unusually high data points of syndecan-1 plasma concentration measurements were identified as obvious outliers applying the IQR rule. Those values exceeded the third quartile >3 IQRs and were excluded from analysis (see Fig. S1 in the supplementary material). No outliers were identified in the ADPKD group. Significantly higher plasma concentrations of heparan sulphate (1625 ± 807 ng/ml versus 1329 ± 316 ng/ml; $P = .034$), hyaluronan (111 ng/ml [IQR 79–132]

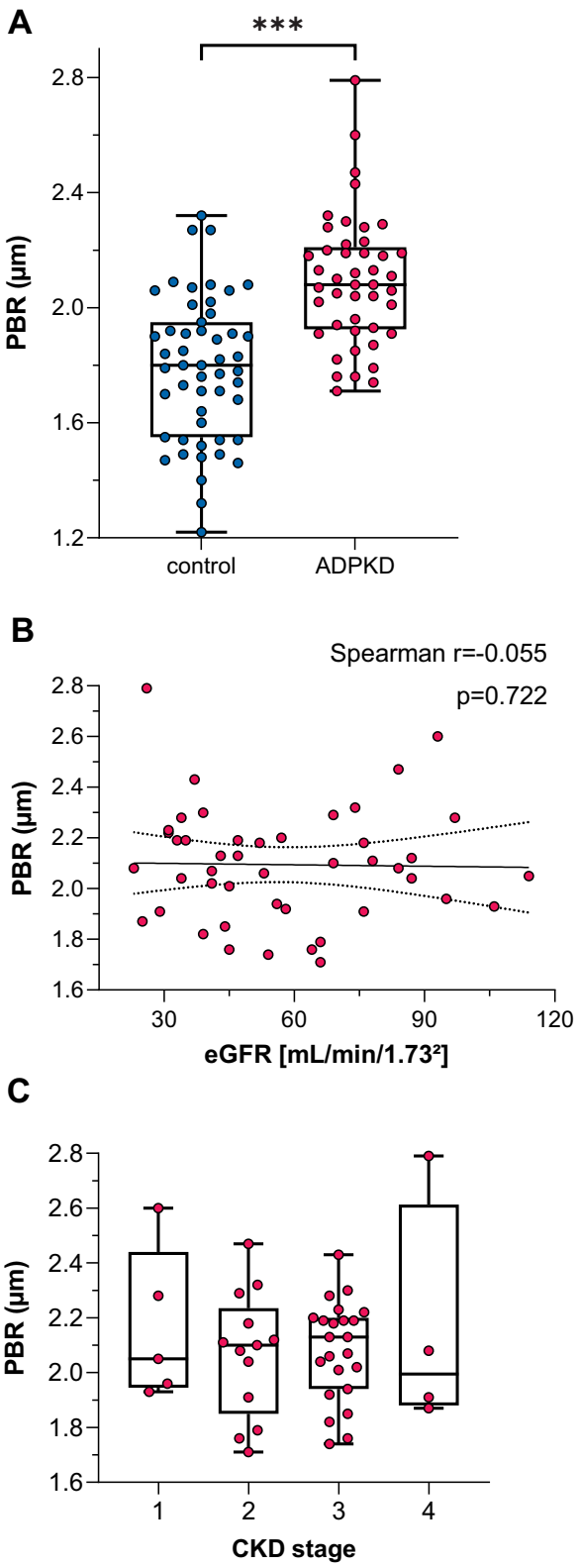

Figure 1: **(A)** PBR (µm) in vessels with a diameter of 5–25 µm as an inverse parameter for RBC penetration into the glycocalyx in ADPKD patients and healthy controls. **(B)** PBR (µm) plotted against eGFR (CKD-EPI 2009) in the ADPKD group. The 95% confidence bands of the best-fit line are shown. **(C)** PBR (µm) in ADPKD patients sorted by CKD stage. The whiskers extend from quartile 1 and quartile 3 to the minimum and maximum of the data sets. ***$P < .001$.

versus 92 ng/ml [82–98]; $P = .042$) and syndecan-1 were noted in ADPKD patients compared with healthy controls (35 ng/ml [IQR 27–57] versus 29 ng/ml [23–42]; $P = .035$) (see Fig. 3). In the control group there were no significant correlations between heparan sulphate, hyaluronan or syndecan-1 and eGFR. In the ADPKD group we found a strong inverse relationship between heparan sulphate and eGFR (Spearman's $r = -0.745$, $P < .001$), whereas such a relationship with eGFR was not observed for hyaluronan (Spearman's $r = -0.249$, $P = .122$) or syndecan-1 (Spearman's $r = 0.034$, $P = .833$) (see Fig. 4).

Relationship between endpoints and subgroup analyses

We could not detect a close correlation between PBR dimensions and the concentrations of glycocalyx shedding parameters (see Table S1 in the supplementary material). Regarding a correlation between age and PBR as well as the shedding parameters, only heparan sulphate and age showed a weak statistically significant relationship in the ADPKD group (see Fig. S2 in the supplementary material). PBR and the plasma concentrations of glycocalyx components showed no gender-related differences within the groups (see Fig. S3 in the supplementary material). There was no statistically significant difference regarding all primary and laboratory secondary endpoints in ADPKD patients with later onset of arterial hypertension (≥35 years) compared with early onset (<35 years) (see Fig. S4 in the supplementary material). The endpoints did not differ between the lower Mayo class patients (1A and B) and the higher Mayo class patients (1C, D and E) (see Fig. S5 in the supplementary material) and appeared not to be influenced by tolvaptan treatment (see Fig. S6 in the supplementary material).

DISCUSSION

In accordance with our primary hypothesis, we detected larger PBR dimensions in ADPKD patients compared with healthy controls, indicating deeper penetration of RBCs into the thinner ESL. This was accompanied by lower RBC filling and higher capillary density. Impairment of the ESL as measured by a larger PBR has been described in patients with kidney failure with or without the need for dialysis [16, 25]. However, other studies could not confirm a difference in PBR between stage 5 CKD patients before kidney transplantation and controls [13] or comparing groups of dialysis patients, CKD patients and kidney transplant recipients with controls [26]. To the best of our knowledge, ESL dimensions in ADPKD patients have not been investigated before.

We also observed higher concentrations of soluble glycocalyx constituents in ADPKD patients, which may indicate shedding of the endothelial glycocalyx and contribute to the impaired ESL dimensions.

Previous studies have shown that plasma concentrations of hyaluronan and syndecan-1 increased throughout CKD stages 3–5 and were elevated compared with apparently healthy controls [12] and were highest in the dialysis group, followed by the CKD and transplant groups compared with controls [26]. In our study, concentrations of heparan sulphate, hyaluronan and syndecan-1 were elevated in ADPKD patients. This may indicate that degradation of the glycocalyx in ADPKD does affect its major components (both glycosaminoglycans and proteoglycans) to the same extent and points towards an activation of different 'sheddases'. ESL integrity appears to be a crucial factor for vas-

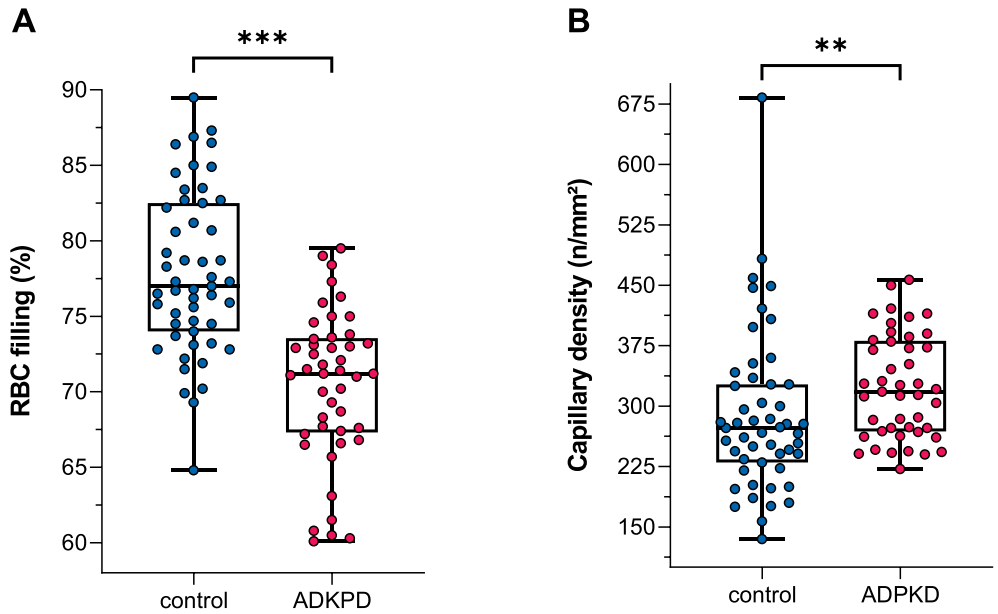

Figure 2: (A) RBC filling (%) and **(B)** capillary density (/mm²) in ADPKD patients and healthy controls. The whiskers extend from quartile 1 and quartile 3 to the minimum and maximum of the data sets. ***$P < .001$, **$P < .01$.

Table 2: Multivariate analysis of PBR.

Variables	Coefficient (β)	Standard error	*t*-value	*P*-value
(Intercept)	2.378	0.216	11.016	<.001
Age	−0.003	0.003	−1.090	.279
Gender, female	0.034	0.053	0.638	.525
Group, control	−0.214	0.081	−2.643	<.01
Smoker	−0.020	0.087	−0.228	.820
Non-smoker	−0.080	0.071	−1.126	.263
eGFR	−0.002	0.002	−1.308	.194

cular health, as glycocalyx degradation has been described as an early step in and driver for endothelial dysfunction and vascular complications in systemic inflammation, atherosclerosis and renal disease [7, 27, 28]. Since the ESL is dynamic and constantly adapting and our measurements describe one point in time, further investigation to confirm our results longitudinally is needed. Besides ESL dimensions, it is intriguing to speculate that the microvasculature of ADPKD patients shows a higher capillary density to compensate for lower RBC filling and ensure sufficient perfusion and oxygen delivery to tissues. However, investigation of the underlying mechanisms was beyond the scope of this investigation.

ADPKD is primarily caused by mutations in the *PKD-1* and *PKD-2* genes that encode the proteins polycystin-1 and polycystin-2, respectively. Polycystin-1 and -2 are expressed on the surface of renal tubular cells, but also in vascular smooth muscle and vascular endothelial cells [29–31]. Aside from promoting cyst formation, defects in these proteins are thought to be involved in oxidative stress, hypoxia and endothelial dysfunction [5].

In the control group, the third quartile for systolic BP was 140 mmHg and a higher heart rate as compared with the ADPKD patients was observed. We can confirm that no blood donor reported arterial hypertension or was on antihypertensive medication. The ADPKD patients as well as the healthy volunteers were recruited on site, at an ambulatory visit or before a blood donation. While we have extensive data regarding clinical characteristics for ADPKD patients, vital signs and biosamples of healthy volunteers were obtained at a single visit at the blood donation centre. Hence we cannot exclude a certain amount of excitement or even a 'white coat effect' in this situation that may have influenced the obtained vital signs. Since we did not repeat BP measurements at a follow-up in the control group, we were unable to meet the valid clinical standards for diagnosing arterial hypertension.

Obviously age is a possible confounder for our results, as there was a significant age difference between both groups because we did not control for age. Nevertheless, comparing the 16 oldest subjects of the control group with the remaining 35 younger subjects resulted in a median age difference of 20 years and showed no difference in PBR between the groups. To further examine the effect of age on PBR, an age-matched subgroup analysis was performed and showed the same significant differences for PBR between ADPKD and controls. These group differences persisted when the data set was split by gender. The data regarding the influence of age on the ESL in the literature are conflicting. There was no difference in PBR comparing healthy young men (mean age 24 years) with healthy older men (mean age 70 years) [32]. But in the same study, PBR was significantly larger when an age-matched group of older type 2 diabetes patients was compared with controls. In contrast, another study suggested that age does affect glycocalyx thickness and PBR in humans and male mice, with a PBR significantly larger in older (mean age 60 years) compared with younger participants (mean age 29 years) [33]. Interestingly, the PBR of CKD patients (median age 71 years) did not differ from that of younger controls (median age 36 years) [26] and a large multi-ethnic study did not show an association of age and PBR [34]. Taking all the evidence together, age per se seems not to be a relevant factor for PBR. Instead, older age inherently implicates a higher risk for the presence of damage to the ESL and provides a longer time interval of possible damaging factors, including arterial hypertension, diabetes and

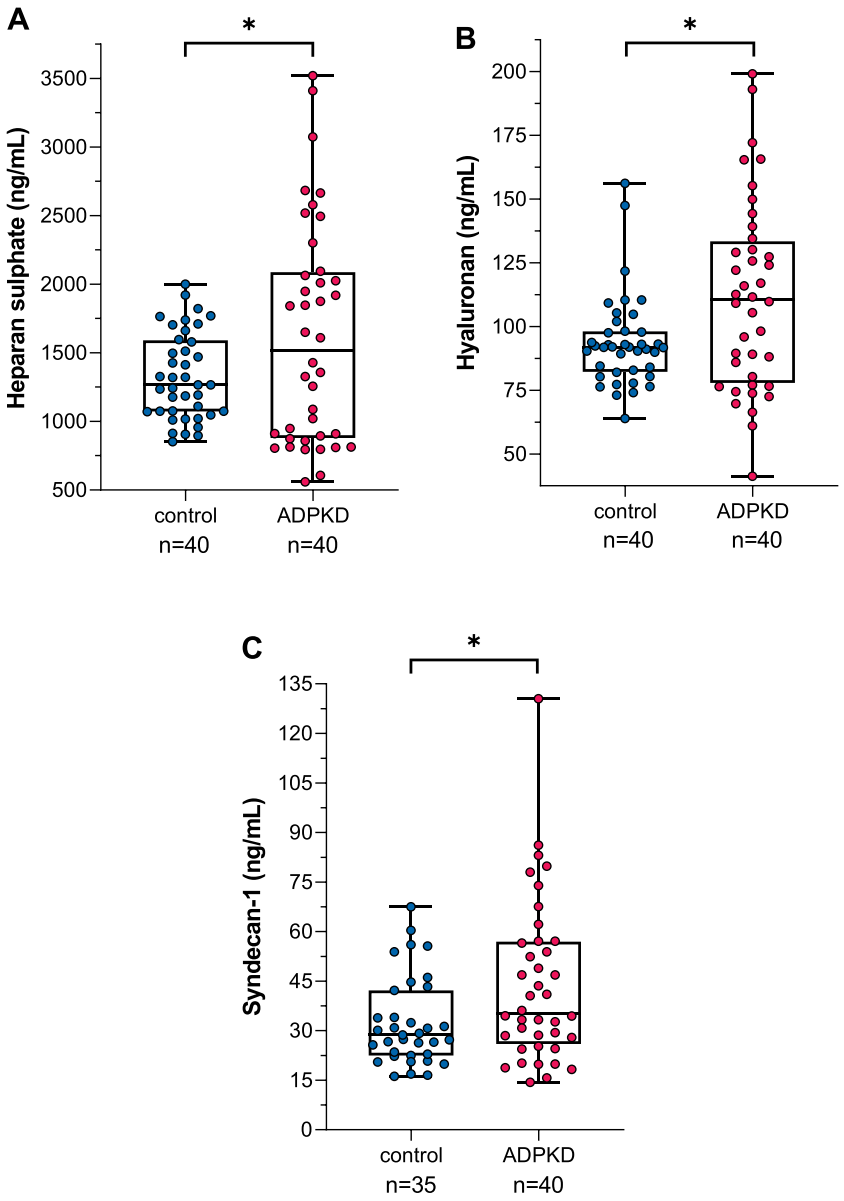

Figure 3: Plasma concentrations of **(A)** heparan sulphate, **(B)** hyaluronan and **(C)** syndecan-1 (each in ng/ml) in ADPKD patients and healthy controls. The whiskers extend from quartile 1 and quartile 3 to the minimum and maximum of the data sets. *$P < .05$.

smoking, to impact the composition, and hence the dimensions of the ESL and status of the microvasculature overall.

There is evidence that in addition to restoring GFR, kidney transplantation also has a positive effect on the ESL. In a cross-sectional study, the PBR in stable transplanted participants was similar to that of healthy controls [16]. This was later confirmed in a longitudinal study, in which PBR was restored 3 months after renal transplantation [13]. A previous study described an inverse correlation between PBR and eGFR, including measurements obtained in healthy controls, patients with kidney failure and patients with both normal and impaired kidney function after living donor transplantation [16]. We did not observe such a correlation between the eGFR and PBR in the ADPKD group. Since most CKD cohorts come with more confounders (e.g. comorbidities) than ADPKD as a genetic disorder, this may point towards the fact that eGFR itself is not one of the key drivers of changes in PBR. As a general limitation of our study, we must consider that PBR and perfusion measurements were performed only in sublingual microvessels and results in other parts of the vasculature may vary. However, our technique can provide real-time *in vivo* PBR measurements reflecting the dimensions of ESL in humans, not affected by staining techniques or limited by experimental conditions in laboratory animals.

CONCLUSION

To the best of our knowledge, this is the first study to assess ESL dimensions in ADPKD patients measured via intravital microscopy. Considering the importance of changes in the

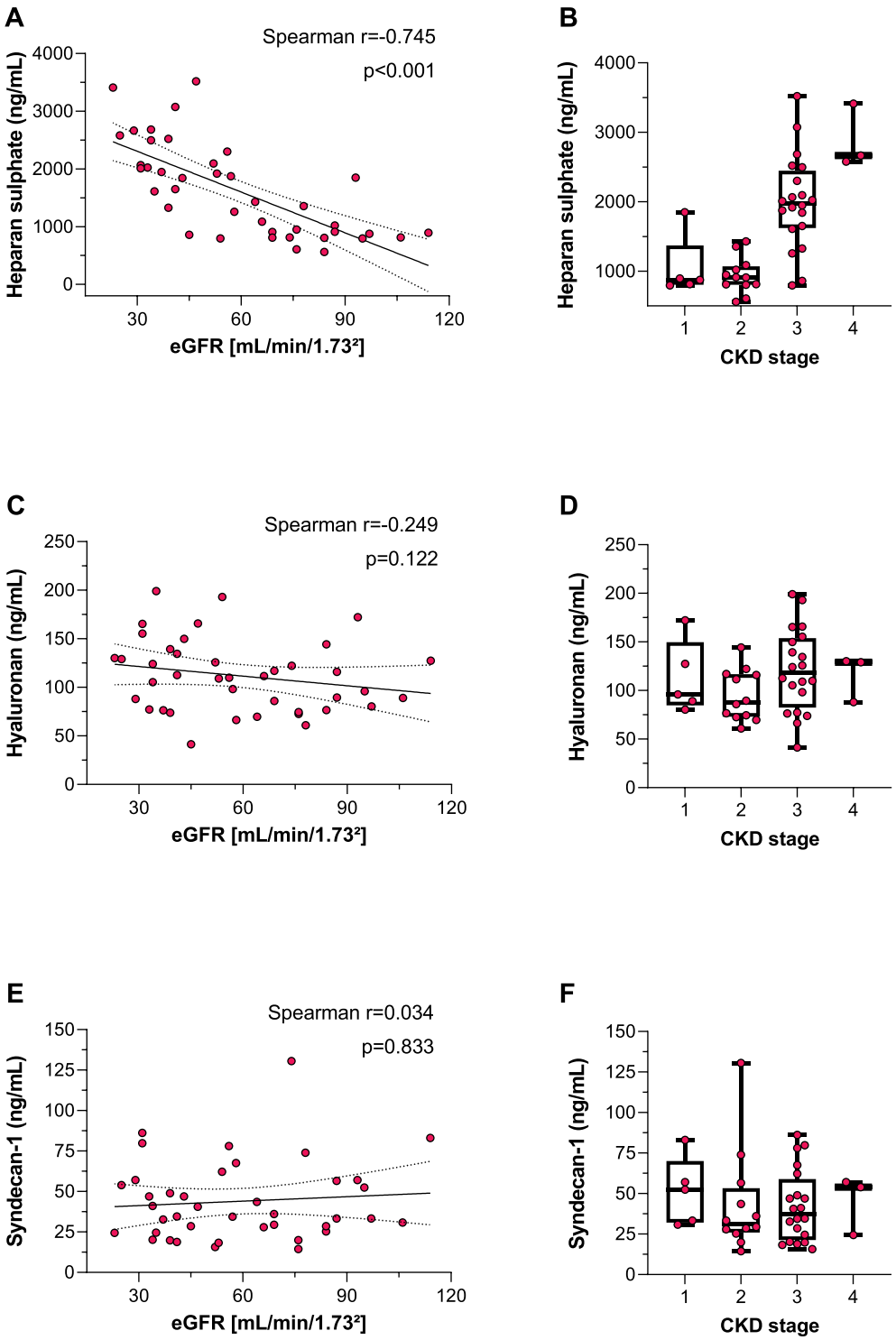

Figure 4: Plasma concentrations of **(A)** heparan sulphate, **(C)** hyaluronan and **(E)** syndecan-1 (each in ng/ml) plotted against eGFR (CKD-EPI 2009) and sorted by CKD stage **(B, D,** and **F**, respectively) in ADPKD patients. The 95% confidence bands of the best-fit line are shown. The whiskers extend from quartile 1 and quartile 3 to the minimum and maximum of the data sets.

microvasculature for cardiovascular as well as renal outcome, it is intriguing to speculate that the changes observed may be mechanistically involved in disease progression. However, the endothelium and endothelial surface layers in the body vary in composition between vessel type and tissues. We must be cautious in transferring our results to every tissue and organ. Hence further investigation of the microvasculature in ADPKD patients as well as mechanistic studies in animal models are needed to obtain a full understanding of the link between ESL defects and outcome.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

ACKNOWLEDGEMENTS

This study was approved by the Ethics Committee of the University of Cologne. We thank Israa Karimov for processing the blood samples and performing ELISA measurements and the participants for supporting this study. We would also like to thank Cornelia Böhme for excellent support regarding recruitment from the AD(H)PKD Registry and Silke Leja, Sabine Groß and Gerda Klasen for excellent support during the blood bank study.

FUNDING

This work was supported by research grants from the Anästhesiologie–Forschung und Fortbildung e.V. (to T.A.) and partially by research grants from the Koeln Fortune Program, Faculty of Medicine, University of Cologne, Germany (216/2016) and from the German Research Foundation (DFG; RA 2838/2-1; to A.I.). R.-U.M. was supported by the Ministery of Science North Rhine-Westphalia Nachwuchsgruppen.NRW 2015-2021) and received further funding from the German Research Foundation (KFO329, MU3629/2-1, MU 3629/6-1). We acknowledge the support for the article processing charge from the German Research Foundation (DFG; 491454339).

AUTHORS' CONTRIBUTIONS

All authors take responsibility for all aspects of the reliability and freedom from bias of the presented data and their discussed interpretation and were involved in the drafting and critical revision of the manuscript. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The results presented in this article have not been published previously in whole or part. The Department II of Internal Medicine, University Hospital Cologne received research funding from Otsuka Pharmaceuticals. The authors have no conflicts of interest to declare.

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