Supplementary Appendix

Supplementary Methods

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Figure S4. ROC curve analyses for each model for identifying Alb^{-}DKD with respect to subjects with Alb^{+}DKD

Supplementary Methods

At baseline, demography, smoking habits, age at diagnosis and DD, ongoing therapies, blood pressure (BP), BMI and waist circumference were recorded together with assessment of presence/severity of micro- and macro-vascular complications. Fasting blood samples were obtained to assess glucose, HbA1c, creatinine (modified Jaffe method, traceable to isotope dilution mass spectrometry), cystatin C (nephelometric assay), β2-microglobulin (β2m; chemiluminescence), total and HDL-cholesterol, triglycerides, uric acid, high-sensitive C-reactive protein (hsCRP) and fibrinogen. Hypertension was defined as systolic BP ≥140 and/or diastolic ≥90 mmHg and/or use of antihypertensive medications; dyslipidemia as LDL-cholesterol ≥100 mg/dL and/or HDL-cholesterol <40 in men and <50 mg/dL in women and/or triglycerides ≥150 mg/dL and/or use of lipid-lowering medications.

Ultrasound data acquisition

A single expert radiologist (V.N., from the Department of Radiology), blinded to the patient's clinical characteristics, performed all the ultrasound (US) examinations (2D and 3D) and evaluated the images for the 2D volumetric assessments. A second expert radiologist, also blinded to patient's characteristics and to 2D evaluations too, performed the 3D volumetric reconstructions. An ultrasound system equipped with a 5-1 MHz C5-1 array transducer with Pure-Wave crystal technology (iU22, Philips Ultrasound, Bothell, WA, USA) was used for both 2D US and color-doppler (CD) US (CDUS) examinations. All examinations started with preoperative arterial CDUS including aorta and renal arteries evaluation with visualization of the anatomy (B-mode imaging) and blood supply (CD imaging). The examination was performed with the patient in supine position, anterior approach and transverse scan on the epigastric region to visualize the renal artery at the ostium and in its proximal tract; then, the examination went on with the patient in lateral decubitus position, posterior-lateral translumbar approach and coronal scan to visualize, together with the kidney, the middle-distal tract of the renal artery and its branches. The presence of renal artery stenosis has been evaluated taking into account of the renal artery peak systolic velocity, the renal/aortic ratio and the occurrence of loss of the early systolic peak distally to the stenosis. The presence and the spectral flow curve of accessory arteries was also evaluated.

The intrarenal resistive index recording

To record the intrarenal resistive index (RI), the translumbar approach with the patient in lateral decubitus was preferred. First, the interlobar arteries were identified with CD and then, after the regulation of the angle of insonation <45°, the regulation of high color gain values and low Pulse Repetition Frequency values ≤800 Hz for the CD function and ≤3 KHz for the spectral trace selection, the spectral analysis for the velocimetric assessment of the renal flow was detected. The RI was automatically calculated and displayed on the screen. The procedures and recording of RI were repeated three times for each kidney in three

different positions (superior, mid, and inferior) for each of the two kidneys [1,2]. Applying spectral Doppler to renal artery and selected interlobular arteries, peak systolic velocities, resistive index and acceleration curves can be estimated. A peak systolic velocity of the renal artery >180 cm/s, associated with a renal-aortic ratio >3.5, was considered able to identify a >60% renal artery stenosis, whereas a >70% RI (calculated from peak systolic and end diastolic velocity) was taken as indicative of abnormal renovascular resistance [3].

The 2D US examination

A 2D US examination was performed after the vascular evaluation of each kidney. Three measurements of the cortical thickness, the maximum upper pole-to-lower pole renal diameter in longitudinal scan and midsagittal plane, and of antero-posterior and latero-lateral diameters orthogonal to this plane at the level of the renal hilum of each kidney were performed in a lateral decubitus position usually alongside the posterior axillary line. Three volume estimates of the whole kidney and of the renal sinus area were calculated applying the prolate ellipsoid formula of [volume = length x width x depth x π/6] and recorded in mL. The parenchymal volume was obtained subtracting sinus volume to total renal volume.

The 3D US examination

The same radiologist performed 3D-ultrasound examination with a Philips X6-1 MHz xMATRIX array transducer with Pure-Wave crystal technology immediately after 2D sonography and CDUS examination. The volumetric probe is an electronic volumetric data acquisition probe with piezoelectric crystals moving by selection of an angle of acquisition up to 90°. In the pre-scan acquisition phase, the better plane and transducer placement for the volume acquisition were established. The adjustment of the acquisition velocity with respect to the resolution of the image was set in favor of the latter. Three volume acquisitions for each kidney were recorded with a 1- to 2-second breath-hold to minimize motion artifacts. The acquired volume data were automatically stored on the hard disk of a separate workstation for further analysis with a 3D sonographic software (Q-Lab 10.5, GI 3DQ, Philips Electronics, Andover, MA, USA). With this software, using a segmentation technique, the second expert operator, blinded to the patient's clinical characteristics, visualized the kidneys in three orthogonal planes and calculated three renal and sinus volumes with the stacked ellipses method for each kidney. In the midsagittal acquisition plane, the upper and lower poles were well visualized on the monitor rotating the three planes, and 15 to 18 slices perpendicular to the long axis of the kidney were defined in order to semi-automatically trace the boundary of each slice in the axial reformation plane. The same procedure was repeated for the sinus bordering the hyperechoic calyxes. If cysts were present, their contour was traced in order to exclude them in the volume calculation. Each renal and sinus volume were then automatically calculated by the Q-Lab software and reported in mL. On average, 2 minutes were required to determine the total volume of a single kidney and

other 2 minutes for sinus volume with this method. In this case as well, the parenchymal volume was calculated as the difference between total renal volume and sinus volume.

References

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Supplementary Tables

Table S1 – Assessment of phenotypes by GFR estimated with the creatinine and cystatin C based CKD-EPI formula.

		GFR estimated with the creatinine-based CKD-EPI formula				
		No-DKD	**Alb$^+$**	**Alb$^-$DKD**	**Alb$^+$DKD**	Total
GFR estimated with the creatinine and cystatin C based CKD-EPI formula	**No-DKD**	64 (25.0%)	0	9 (3.5%)	0	73 (28.5%)
	Alb$^+$	0	54 (21.1%)	0	3 (1.2%)	57 (22.3%)
	Alb$^-$DKD	3 (1.2%)	0	53 (20.7%)	0	56 (21.9%)
	Alb$^+$DKD	0	9 (3.5%)	0	61 (23.8%)	70 (27.3%)
	Total	67 (26.2%)	63 (24.6%)	62 (24.2%)	64 (25.0%)	256

Table S2 – Assessment of phenotypes by GFR estimated with the β2-microglobulin based CKD-EPI formula.

		GFR estimated with the creatinine-based CKD-EPI formula				
		No-DKD	**Alb$^+$**	**Alb$^-$DKD**	**Alb$^+$DKD**	Total
GFR estimated with β2-microglobulin formula	**No-DKD**	58 (22.7%)	0	12 (4.7%)	0	70 (27.3%)
	Alb$^+$	0	41 (16.0%)	0	6 (2.3%)	47 (18.4%)
	Alb$^-$DKD	9 (3.5%)	0	50 (19.5%)	0	59 (23.0%)
	Alb$^+$DKD	0	22 (8.6%)	0	58 (22.7%)	80 (31.3%)
	Total	67 (26.2%)	63 (24.6%)	62 (24.2%)	64 (25.0%)	256

Table S3 - Absolute and adjusted kidney volumes related parameters in the whole study cohort.

	Median	**IQR**
Resistive index*		
Right kidney	0.76	0.71-0.82
Left kidney	0.76	0.71-0.83
mRI	0.77	0.71-0.82
mRI >0.70	n. 202	78.9%
Kidney lenghts		
Right kidney, mm	111.7	104.6-118.4
Left kidney, mm	115.4	108.4-124.3
Kidney cortical thicknesses		
Right kidney, mm	19.0	17.0-22.0
Left kidney, mm	18.9	16.4-21.6
aTRV2D, mL/m^2	171.8	144.1-203.0
aTRV3D, mL/m^2	171.9	152.4-198.7
aPRV2D, mL/m^2	140.1	115.9-167.9
aPRV3D, mL/m^2	142.4	124.5-161.4

*The difference in RI values of the two kidneys was 0.03, which corresponds to 3.9% of mRI 0.77.

Table S4 – Main kidney ultrasound parameters by DKD Phenotypes established by $eGFR_{creat_cysC}$.

	No-DKD (n=73)	**Alb$^+$ (n=57)**	**Alb$^-$DKD (n=56)**	**Alb$^+$DKD (n=70)**	***P***
RI right	0.72 (0.68-0.76)	0.78 (0.71-0.84)***	0.78 (0.72-0.86)***	0.79 (0.76-0.85)***	<0.0001
RI left	0.73 (0.69-0.77)	0.76 (0.71-0.84)**	0.78 (0.73-0.85)***	0.79 (0.72-0.84)***	<0.0001
***m*RI**	0.73 (0.68-0.77)	0.77 (0.71-0.84)***	0.78 (0.73-0.85)***	0.80 (0.72-0.84)***	<0.0001
mRI >0.70, n (%)	50 (68.5)	47 (82.5)	48 (85.7)	57 (81.4)	0.071
Left kidney length, mm	117.50 (109.45-125.47)	120.21 (114.11-127.37)	108.86** ††† (103.75-118.21)	114.11†† (106.86-122.00)	<0.0001
Left kidney cortical thickness, mm	19.93 (17.83-22.84)	21.80 (19.08-23.09)	16.86*** ††† (14.68-19.61)	17.41*** ††† (15.83-19.49)	<0.0001
Right kidney length, mm	113.40 (107.36-120.36)	116.24* (109.78-125.92)	107.97** ††† (100.92-113.40)	110.08†† ‡ (103.86-117.31)	<0.0001
Right kidney cortical thickness, mm	20.24 (17.30-22.54)	21.23* (19.13-23.56)	17.26*** ††† (15.01-19.36)	18.38* ††† (16.51-20.65)	<0.0001
***a*TRV2D, mL/m^2**	182.85 (158.32-213.73)	194.41 (175.32-235.15)	148.66*** ††† (124.75-169.84)	156.15*** ††† (132.86-184.27)	<0.0001
***a*PRV2D, mL/m^2**	151.17 (125.77-173.70)	170.50* (143.80-194.39)	115.62*** ††† (101.58-134.64)	126.00*** ††† ‡ (105.18-148.95)	<0.0001
***a*TRV3D, mL/m^2**	177.33 (154.18-202.45)	198.38** (180.70-224.69)	156.36*** ††† (137.16-169.95)	162.25* ††† ‡ (145.08-187.14)	<0.0001
***a*PRV3D, mL/m^2**	149.50 (126.08-159.26)	163.71** (147.54-184.53)	127.66*** ††† (108.65-141.71)	137.34* ††† ‡ (121.26-150.26)	<0.0001

* P < 0.05; ** P < 0.01; *** P < 0.001 vs No-DKD.

† P < 0.05; †† P < 0.01; ††† P < 0.001 vs Alb$^+$.

‡ P < 0.05; ‡‡ P < 0.01; ‡‡‡ P < 0.001 vs Alb$^-$DKD.

Table S5 – Main kidney ultrasound parameters by DKD Phenotypes established by $eGFR_{\beta 2m}$.

	No-DKD (n=73)	Alb^{+} (n=57)	Alb^{-}DKD (n=56)	Alb^{+}DKD (n=70)	*P*
RI right	0.72 (0.67-0.76)	0.77 (0.70-0.82)**	0.79 (0.72-0.85)***	0.80 (0.76-0.85)***	<0.0001
RI left	0.73 (0.68-0.76)	0.76 (0.71-0.84)**	0.78 (0.73-0.84)***	0.79 (0.72-0.84)***	<0.0001
***m*RI**	0.73 (0.68-0.77)	0.77 (0.71-0.83)**	0.78 (0.74-0.83)***	0.80 (0.73-0.84)***	<0.0001
mRI >0.70, n (%)	45 (64.3)	37 (78.7)	53 (89.8)***	67 (83.8)**	0.002
Left kidney length, mm	117.21 (109.03-125.55)	118.77 (110.79-127.34)	109.78*** ††† (103.70-118.83)	116.49† ‡ (108.60-123.08)	0.001
Left kidney cortical thickness, mm	19.64 (17.84-22.80)	21.51 (18.50-22.55)	16.86*** ††† (15.27-19.78)	17.77*** ††† (16.10-20.60)	<0.0001
Right kidney length, mm	113.21 (105.11-120.33)	114.35 (109.57-124.03)	108.30** ††† (101.60-113.58)	112.75‡ (104.32-119.47)	<0.0001
Right kidney cortical thickness, mm	20.23 (17.56-22.58)	20.69 (18.29-22.74)	17.26*** ††† (15.48-19.80)	18.89† ‡‡ (16.97-21.60)	<0.0001
aTRV2D, mL/m²	181.80 (156.65-213.32)	192.64 (170.93-235.39)	148.95*** ††† (124.61-174.96)	166.39* ††† ‡ (138.07-194.79)	<0.0001
aPRV2D, mL/m²	150.20 (124.01-173.08)	166.93* (139.96-194.98)	118.08*** ††† (102.12-137.44)	130.21** ††† ‡ (109.59-162.65)	<0.0001
aTRV3D, mL/m²	177.25 (154.59-203.84)	197.52** (178.90-215.84)	157.15*** ††† (138.93-171.36)	169.56††† ‡‡ (150.45-195.00)	<0.0001
aPRV3D, mL/m²	149.51 (126.40-160.01)	163.35** (147.35-183.13)	127.45*** ††† (108.66-141.48)	138.60††† ‡‡ (123.27-155.98)	<0.0001

* P < 0.05; ** P < 0.01; *** P < 0.001 vs No-DKD.

† P < 0.05; †† P < 0.01; ††† P < 0.001 vs Alb^{+}.

‡ P < 0.05; ‡‡ P < 0.01; ‡‡‡ P < 0.001 vs Alb^{-}DKD.

Table S6 – Independent correlates of Alb^{+}, $Alb^{-}DKD$ and $Alb^{+}DKD$ vs. No-DKD.

	Alb^{+}			$Alb^{-}DKD$			$Alb^{+}DKD$		
	OR	**95%CI**	**p**	**OR**	**95%CI**	**p**	***OR***	**95%CI**	**p**
Model 1 (inclusion of mRI)									
Age, 1 year	--	--	--	1.17	1.08-1.29	*<0.0001*	--	--	--
Smoking habits			*0.021*	--	--	--	--	--	--
No smokers	1								
Ex-smokers	0.43	0.13-1.43	*0.169*						
Active smokers	3.65	0.89-15.01	*0.073*						
SBP, 1 mmHg	--	--	--	--	--	--	1.04	1.01-1.08	0.022
HbA1c, 1%	--	--	--	--	--	--	1.95	0.98-3.89	*0.057*
HDL cholesterol, 1 mg/dL	0.94	0.90-0.98	*0.005*	0.96	0.93-1.00	*0.050*	--	--	--
Triglycerides, 10 mg/dL	--	--	--	--	--	--	1.12	1.04-1.21	*0.004*
Uric acid, 1 mg/dL	1.69	1.10-2.58	*0.016*	1.65	1.16-2.35	*0.005*	4.25	2.18-8.27	*<0.0001*
hsCRP, 1 mg/dL	0.71	0.56-0.90	*0.004*	--	--	--	--	--	--
Fibrinogen, 1 mg/dL	1.02	1.01-1.03	*<0.0001*	--	--	--	1.01	1.01-1.02	*<0.0001*
RAAS blockers	7.23	2.24-23.28	*0.001*	3.25	1.21-8.72	*0.019*	--	--	--
Retinopathy (no advanced or advanced)	4.28	1.30-14.03	*0.017*	--	--	--	4.43	1.06-18.59	*0.042*
mRI, 0.1	2.92	1.13-7.55	*0.027*	1.91	1.00-3.66	0.050	2.83	1.21-6.61	*0.016*
Other variables included in the model, but not in equation	Age, diabetes duration, SBP, DBP, HbA1c, triglycerides			Diabetes duration, smoking habits, SBP, DBP, HbA1c, triglycerides, hsCRP, fibrinogen, retinopathy			Age, diabetes duration, smoking habits, DBP, HDL cholesterol, hsCRP, RAAS blockers		
Model 2 (inclusion of mRI and aPRV3D)									
Age, 1 year	--	--	--	1.21	1.10-1.34	*<0.0001*	--	--	--
Smoking habits			*0.021*	--	--	--	--	--	--
No smokers	1								
Ex-smokers	0.43	0.13-1.43	*0.169*						
Active smokers	3.65	0.89-15.01	*0.073*						
SBP, 1 mmHg	--	--	--	--	--	--	1.04	1.01-1.08	0.030
HbA1c, 1%	--	--	--	--	--	--	2.58	1.17-5.67	0.018
HDL cholesterol, 1 mg/dL	0.94	0.90-0.98	*0.005*	0.96	0.92-0.99	*0.025*	--	--	--
Triglycerides, 10 mg/dL	--	--	--	--	--	--	1.16	1.05-1.28	*0.003*
Uric acid, 1 mg/dL	1.69	1.10-2.58	*0.016*	1.46	1.02-2.10	*0.041*	4.53	2.23-9.18	*<0.0001*
hsCRP, 1 mg/dL	0.71	0.56-0.90	*0.004*	--	--	--	--	--	--
Fibrinogen, 1 mg/dL	1.02	1.01-1.03	*<0.0001*	--	--	--	1.02	1.01-1.02	*<0.0001*

RAAS blockers	7.23	2.24-23.28	*0.001*	4.06	1.39-11.86	*0.011*	--	--	--
Retinopathy (no advanced or advanced)	4.28	1.30-14.03	*0.017*	--	--	--	3.70	0.80-17.17	*0.095*
mRI, 0.1	2.92	1.13-7.55	*0.027*	--	--	--	2.33	0.98-5.52	*0.055*
aPRV3D, mL/m²	--	--	--	0.96	0.94-0.98	*<0.0001*	0.96	0.93-0.99	*0.006*
Other variables included in the model, but not in equation	Age, diabetes duration, SBP, DBP, HbA1c, triglycerides, aPRV3D			Diabetes duration, smoking habits, HbA1c, SBP, DBP, triglycerides, hsCRP, fibrinogen, retinopathy, mRI			Age, diabetes duration, smoking habits, DBP, HDL cholesterol, hsCRP, RAAS blockers, retinopathy		

Table S7 – Independent correlates of $Alb^{+}DKD$ vs. $Alb^{-}DKD$.

	$Alb^{+}DKD$ vs. $Alb^{-}DKD$		
	OR	**95%CI**	**p**
Model 1 (inclusion of mRI)			
Age, 1 year	0.93	0.86-1.00	*0.042*
Diabetes duration, 1 year	1.05	1.00-1.10	*0.064*
SBP, 1 mmHg	1.03	1.01-1.06	*0.007*
Triglycerides, 10 mg/dL	1.07	1.01-1.13	*0.018*
Fibrinogen, 1 mg/dl	1.01	1.01-1.02	*<0.0001*
Retinopathy (no advanced or advanced)	--	--	--
mRI, x 0.1	--	--	--
Other variables included in the model, but not in equation	Smoking habits, HbA1c, DBP, uric acid, HDL cholesterol, hsCRP, RAAS blockers, retinopathy, mRI		
Model 2 (inclusion of mIR and aPRV3D)			
Age, 1 year	--	--	--
SBP, 1 mmHg	1.03	1.01-1.06	*0.011*
Triglycerides, 10 mg/dL	1.07	1.01-1.12	*0.022*
Fibrinogen, 1 mg/dL	1.01	1.01-1.02	*<0.0001*
Retinopathy (no advanced or advanced)	2.14	0.88-5.20	*0.094*
mRI, x 0.1	--	--	--
aPRV3D, mL/m^2	1.03	1.01-1.05	0.014
Other variables included in the model, but not in equation	Age, diabetes duration, smoking habits, HbA1c, DBP, uric acid, HDL cholesterol, hsCRP, RAAS blockers, mRI		

Table S8 – ROC curve analyses for each selected US parameter

	Alb$^+$ vs No-DKD					
	AUC	**95%CI**	**p**	**Cutoff value**	**Sensitivity (%)**	**Specificity (%)**
mRI	0.656	0.568-0.737	*0.0012*	>0.81	33.3	95.5
aTRV3D	0.619	0.530-0.703	*0.016*	>187.9	65.1	59.7
aPRV3D	0.630	0.541-0.713	*0.008*	>161.8	54.0	77.6

No significant differences among AUCs

	Alb$^-$DKD vs. No-DKD					
mRI	0.712	0.626-0.788	*<0.0001*	>0.76	62.9	71.6
aTRV3D	0.789	0.709-0.856	*<0.0001*	<172.3	85.5	67.2
aPRV3D	0.775	0.693-0.844	*<0.0001*	<146.9	91.9	61.2

No significant differences among AUCs

	Alb$^+$DKD vs. No-DKD					
mRI	0.770*	0.688-0.839	*<0.0001*	>0.78	65.6	86.6
aTRV3D	0.655	0.567-0.735	*0.0013*	<170.4	59.4	70.2
aPRV3D	0.643	0.555-0.725	*0.0032*	<149.3	71.9	56.7

*Marginally higher than $a\text{TRV}_{3D}$ (p=0.079) and $a\text{PRV}_{3D}$ (p=0.051)

	Alb$^-$DKD vs. Alb$^+$DKD					
mRI	0.573	0.481-0.660	*0.163*			
aTRV3D	0.629	0.538-0.713	*0.010*	<173.2	87.1	40.6
aPRV3D	0.645	0.555-0.729	*0.003*	<146.9	91.9	34.4

No significant differences among AUCs

Comparing Alb$^+$ with No-DKD, mRI showed the highest ability to identify subjects with Alb$^+$ (AUC = 0.656); however, we did not find any difference among AUCs of the three US parameters. Similarly, no differences have been demonstrated among AUCs for Alb$^-$DKD with the best performance for aTRV3D (AUC = 0.789). For Alb$^+$DKD, mIR had the highest AUC (0.770), marginally significantly higher than AUCs of both aTRV3D and aPRV3D.

Finally, in comparing Alb$^-$DKD with Alb$^+$DKD, the highest AUC was observed for aPRV3D (0.645), with no differences among AUCs of the three parameters.

Supplementary Figures

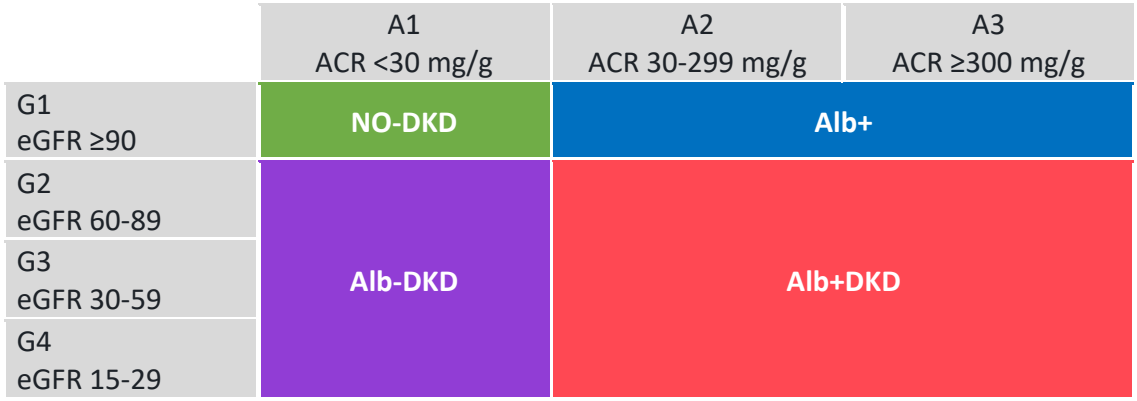


Figure S1 – Graphical representation of stratification of subjects according to ACR and eGFR categories (KDIGO) in DKD phenotypes

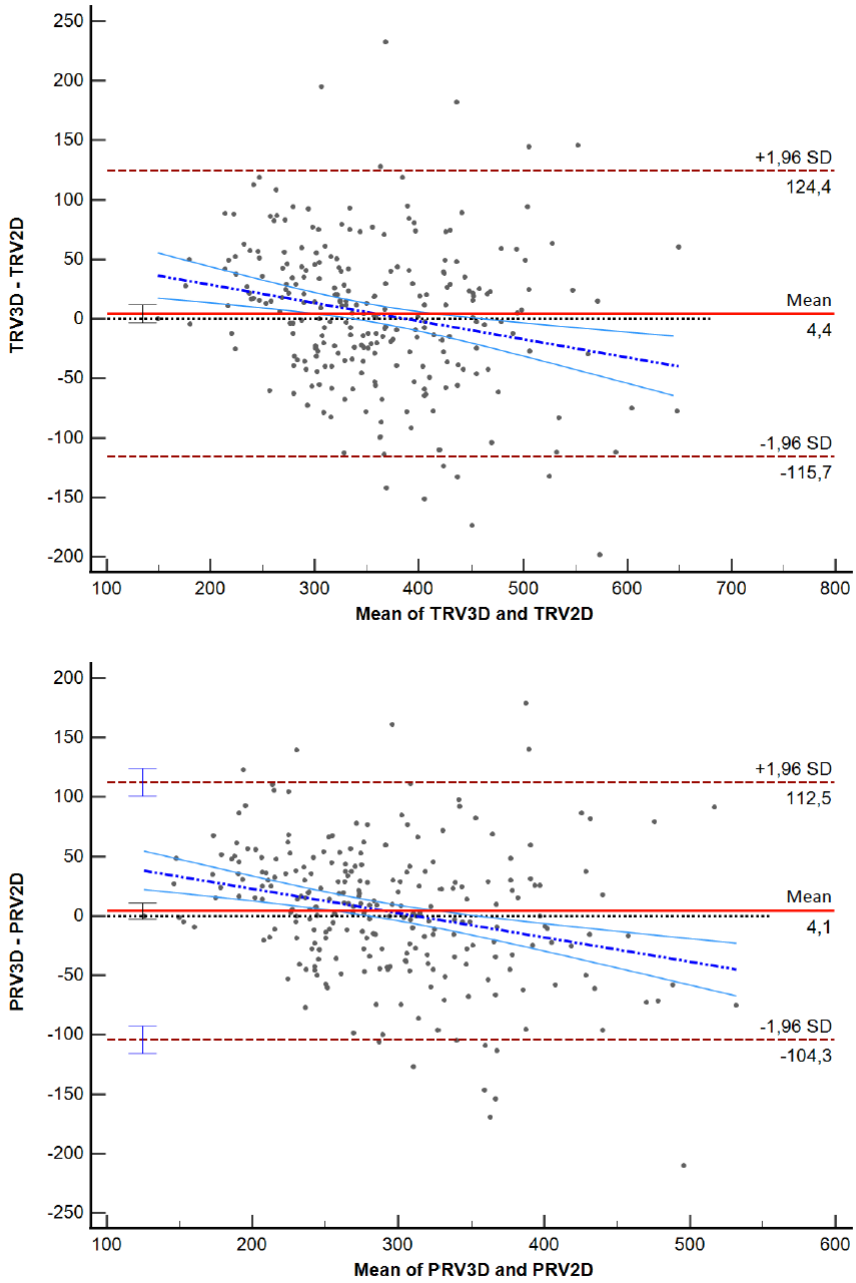
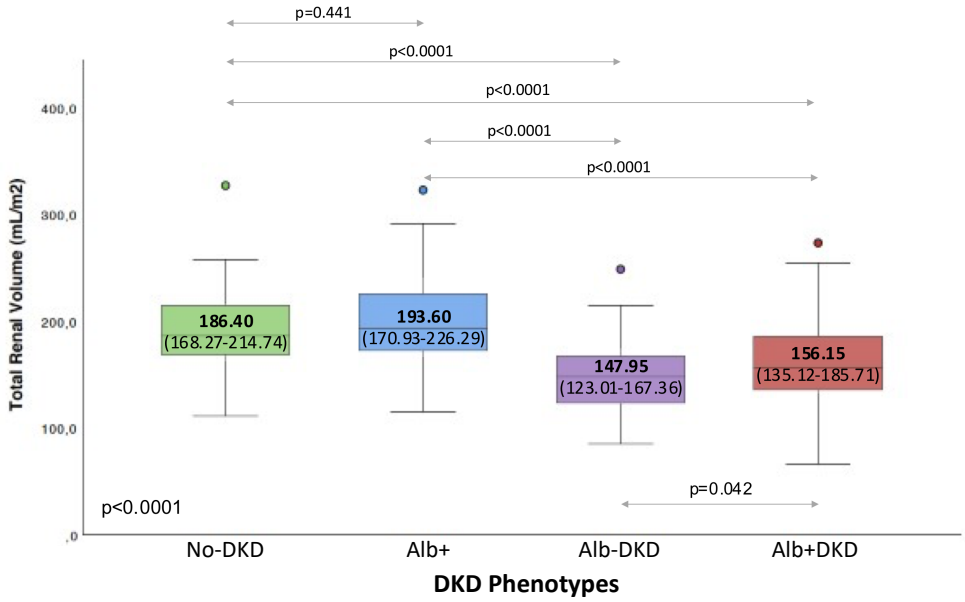


Figure S2 - Inter-method agreement of individual renal volume measurements. Bland-Altman plot for (A) total renal volume and (B) parenchymal renal volume. Solid lines: mean differences (bias) and 95% CI; dashed lines: lower and upper limits of agreement and 95% CI.

a



b

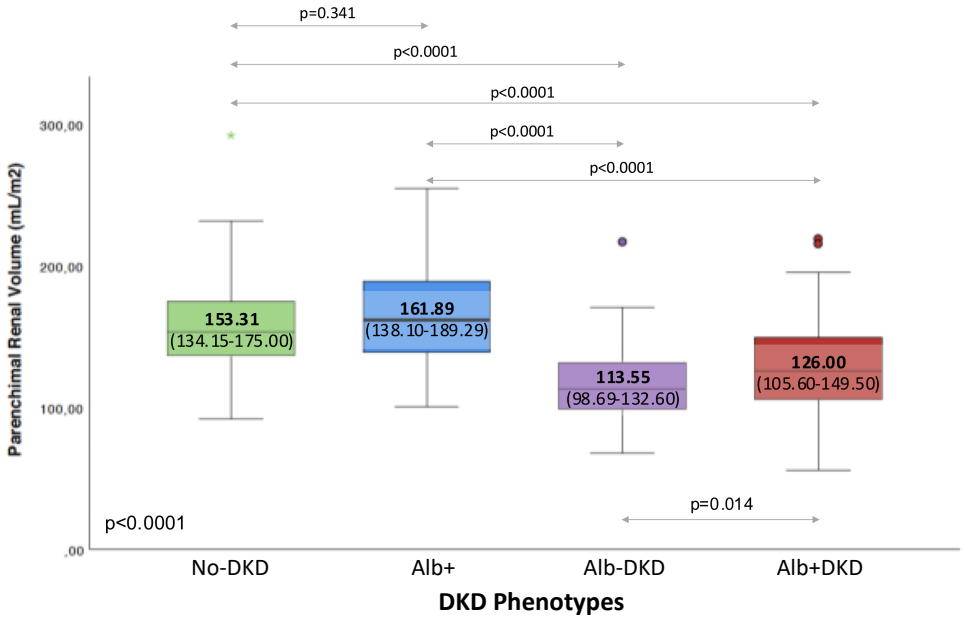


Figure S3 - BSA adjusted total and parenchymal renal volumes assessed by the 2D ultrasound technique in different DKD phenotypes (No-DKD, n = 67; Alb^{+}, n = 63; $Alb^{-}DKD$, n = 62; $Alb^{+}DKD$, n = 64): (**a**) aTRV2D; (**b**), aPRV2D. p values were calculated by Kruskal-Wallis one-way ANOVA tests. The horizontal line within each box represents the median, and the top and the bottom of the box represent the 75^{th} and 25^{th} percentiles, respectively. The whiskers indicate the maximum and minimum values, excluding outliers. Circles indicate outliers.

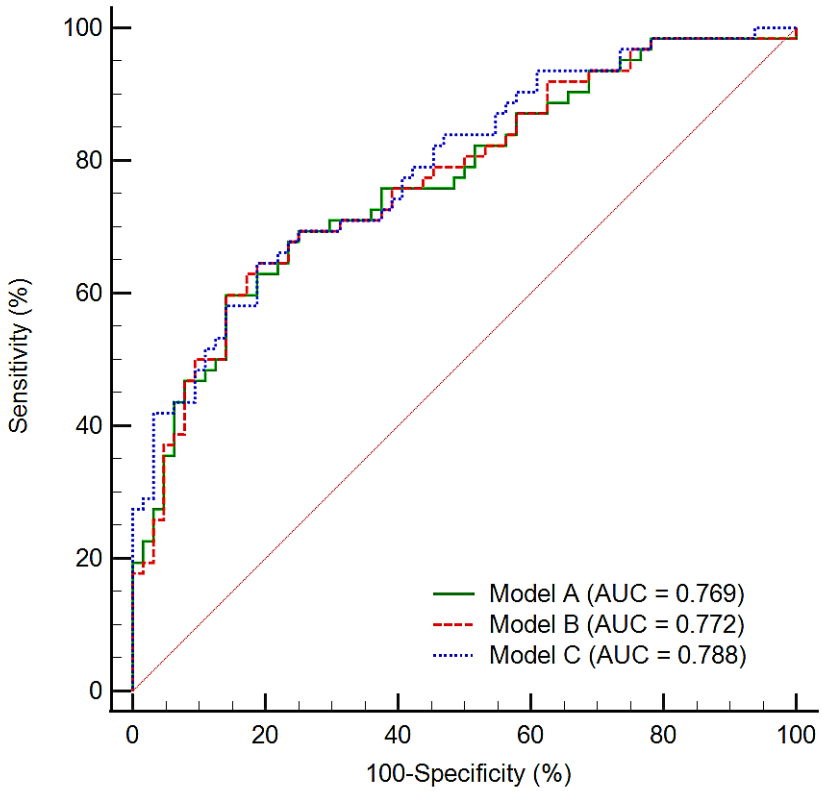


Figure S4 - ROC curve analyses for each model for identifying $Alb^{-}DKD$ with respect to subjects with $Alb^{+}DKD$. Model A: conventional risk factors for DKD (age, diabetes duration, smoking habits, BMI, SBP, HbA1c, total-cholesterol, HDL-cholesterol, triglycerides, and retinopathy); Model B: as Model A plus mRI; Model C: as Model A plus mRI and aPRV3D.

ΔAUC Model A vs Model B = 0.0023, p = 0.500; ΔAUC Model A vs Model C = 0.0184, p = 0.264; ΔAUC Model B vs Model C = 0.0161, p = 0.333.