

Future laboratory markers of diabetic kidney injury

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Outline: Future laboratory markers of diabetic kidney injury

1. Why do we need better biomarkers of kidney injury in diabetes?
2. Serum cystatin C as a marker of an early decline in GFR
3. Other novel serum markers of an early decline in GFR
4. Urinary tubular injury biomarkers of diabetic kidney injury
5. Proteomic identification of urinary biomarkers of diabetic kidney injury

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Why do we need accurate and simple makers of kidney injury in people with diabetes?

- Diabetes is the leading cause of end-stage kidney disease in the Western World
- The traditional method for screening people with diabetes to determine risk for a progressive decline in GFR (**measuring urinary albumin**) lacks specificity and sensitivity

Perspectives in Diabetes

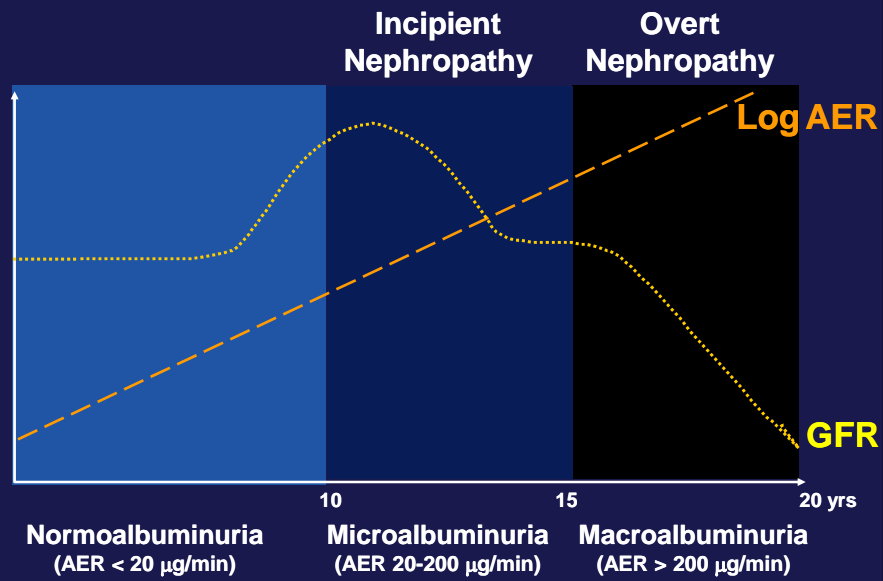
The Need for Early Predictors of Diabetic Nephropathy Risk

Is Albumin Excretion Rate Sufficient?

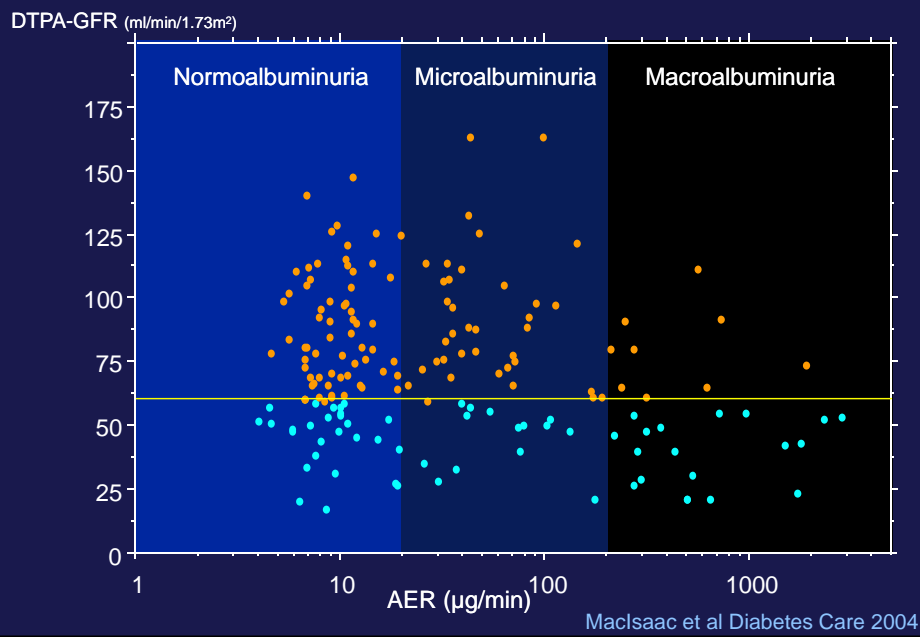
M. Luiza Caramori, Paola Fioretto, and Michael Mauceri

Diabetes 49:1399-1408, 2000

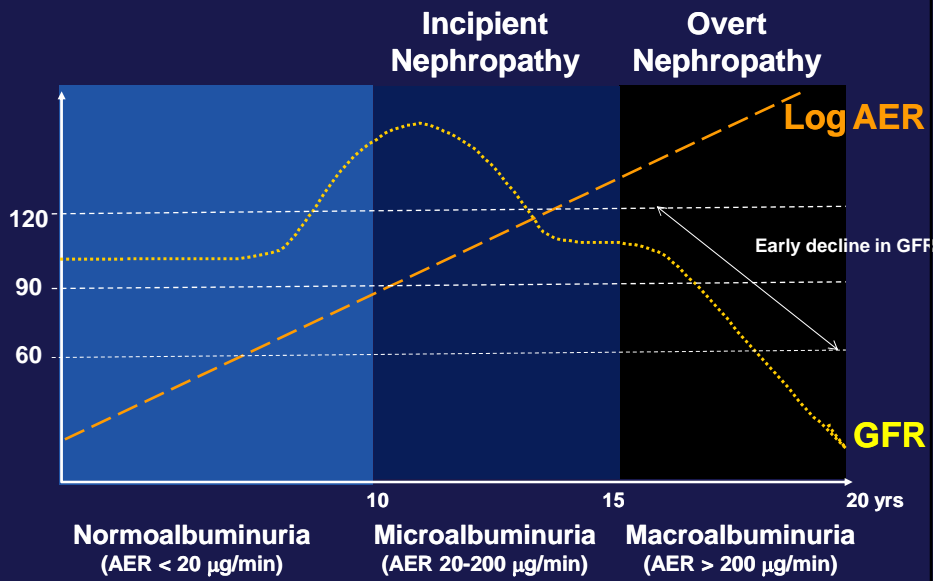
Evolution of Diabetic Nephropathy



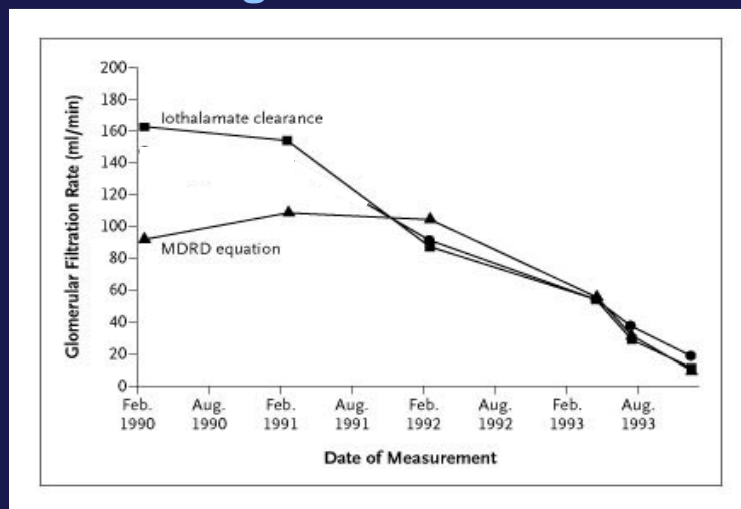
AER vs DTPA-GFR in Type 2 Diabetes



Evolution of Diabetic Nephropathy



eGFR-MDRD underestimates high normal GFR



Perkins et al NEJM 2005, 353, 842-844
Perkins, B. A. et al. J Am Soc Nephrol 2005;16:1404-1412

Possible approaches to improving estimates of GFR in diabetes before stage 3 CKD is reached

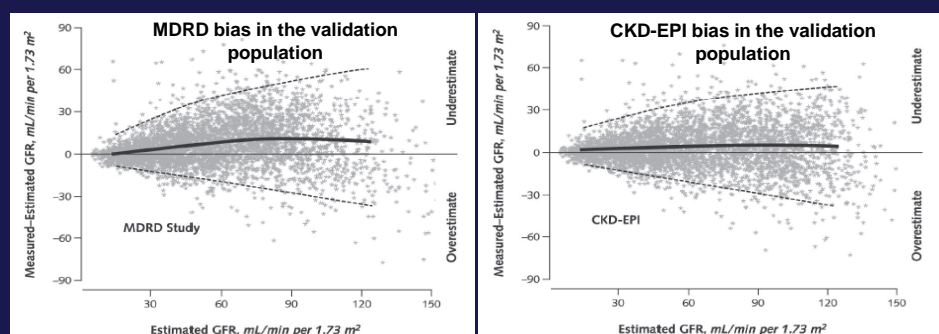
1. Improve the bias and accuracy of creatinine based methods but still need to include extra-renal factors such as age and gender
2. Use alternative markers that are not affected by extra-renal factors

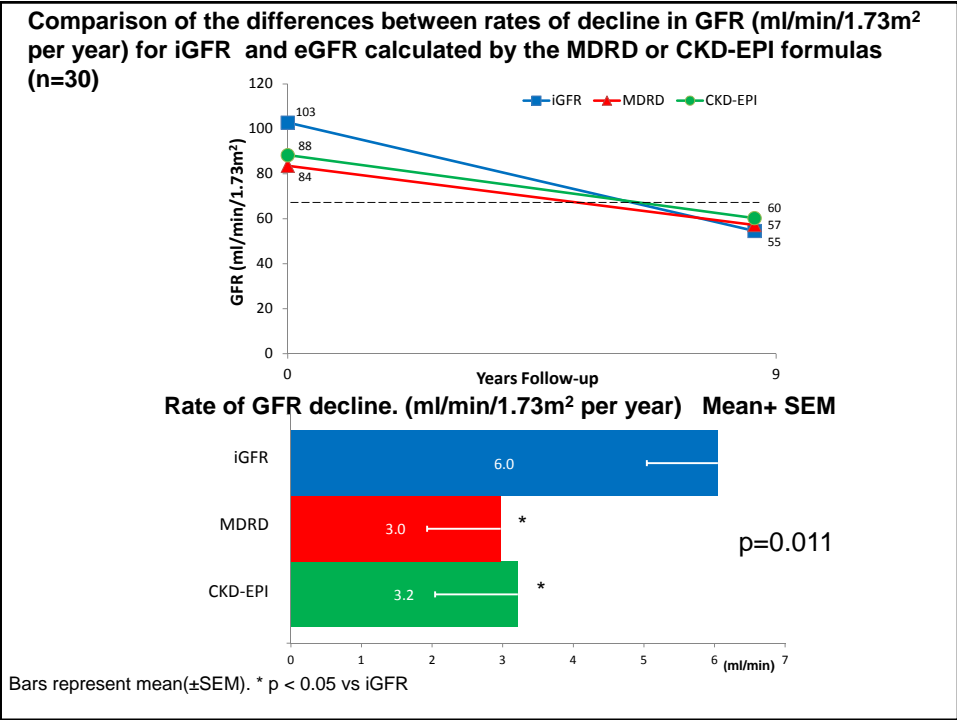
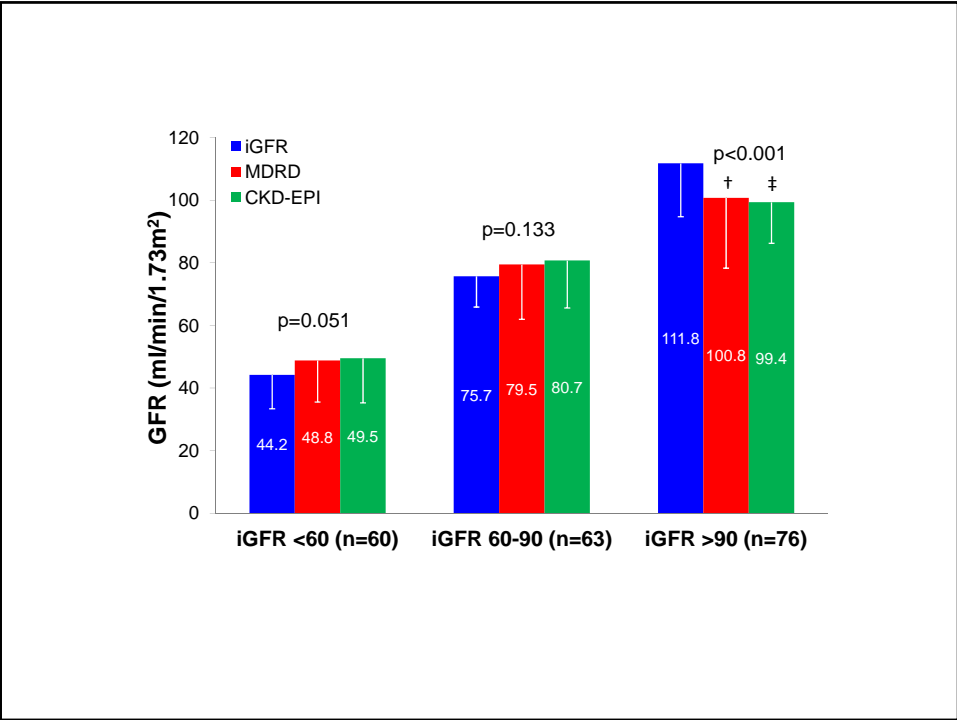
A New Equation to Estimate Glomerular Filtration Rate

Andrew S. Levey, MD; Lesley A. Stevens, MD, MS; Christopher H. Schmid, PhD; Yaping (Lucy) Zhang, MS; Alejandro F. Castro III, MPH; Harold I. Feldman, MD, MSCE; John W. Kusek, PhD; Paul Eggers, PhD; Frederick Van Lente, PhD; Tom Greene, PhD; and Josef Coresh, MD, PhD, MHS, for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration)*
Ann Intern Med. 2009;150:604-612.

	MDRD	CKD-EPI
Development population (n)	1628	5504
Age (y)	51±13	47±15
Measured GFR (ml/min/1.73m ²)	40±21	68±40
Diabetes (%)	6	29
Bias*: mGFR-eGFR (ml/min/1.73m ²)	5.5	2.5

*Validated in a population of 3896 subjects with a mGFR = 68 ± 36 ml/min/1.73m²

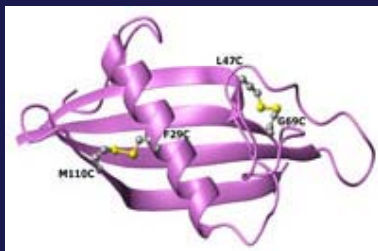




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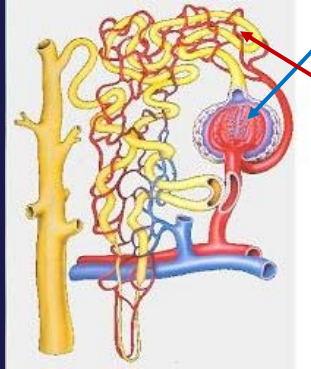
Cystatin C



- A nonglycosylated, low molecular mass (13.4 kDa) protein
- Contains 120 amino acids
- A proteinase inhibitor produced by all nucleated cells.

- Physiological role is to regulate proteinases leaked from dying or diseased cells.

Cystatin C



- Cystatin C is freely filtered by the glomerulus.
- Almost completely reabsorbed and catabolized by the proximal tubular cells.
- No urinary excretion of Cystatin C in the healthy state.
- "Ideal marker of GFR?"

Cystatin C as a marker of GFR

- Cystatin C first proposed as an endogenous serum marker of glomerular filtration rate. Simonsen O, Grubb A, Thysell H. The blood serum concentration of cystatin C as a measure of the glomerular filtration rate. Scand J Clin Lab Invest, 45:97-101, 1985.
- Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis (46 studies/4500 subjects). Dharnidhaka VR, Kwon C, Stevens G. Am J Kidney Dis. 2002 Aug;40(2):221-6.
- Approved by the FDA as an alternative measure of kidney function in 2004

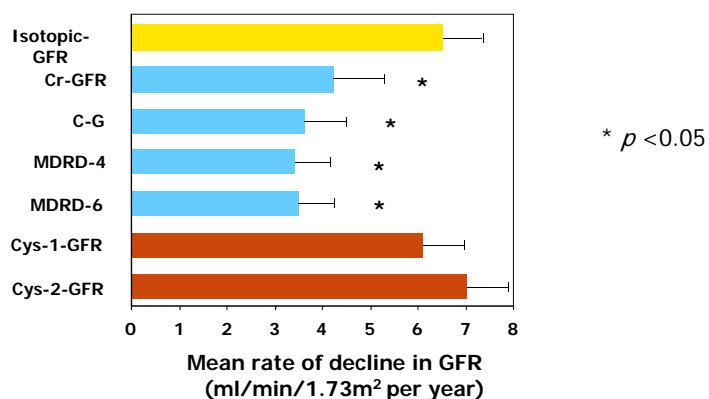
Methods for measuring cystatin C

- Particle-enhanced nephelometric immunoassay (PENIA)
 - Particle-enhanced turbidimetric immunoassay (PETIA)
 - ELISA
- Correlation between GFR and the reciprocal of cystatin C was significantly stronger by the PENIA method
 - PETIA produces reference values that are 20%–30% higher than those from the PENIA

Dharnidharka et al. AJKD 2002

Rates of change in GFR as measured by the indirect estimates and the reference method in the 19 subjects (type 1 diabetes) with declining GFR (rate of decline ≥ 3.3 ml/min/1.73m² per year)

initial iGFR 105 & final 52 iGFR ml/min/1.73m²



Cys-1-GFR = 86.7/Cystatin C-4.2
Cys-2-GFR = 100/Cystatin C

Premaratne, MacIsaac & Jerums, Diabetes Care 2008

Non-GFR influences on Cystatin C levels

- Inflammation
- Malignancy
- Corticosteroid use
- Thyroid function abnormalities
- Others?
 - Age?
 - Gender?
 - weight?
 - Smoking?
 - CRP?
 - Fenofibrate?

Systematic Shifts in Cystatin C Between 2006 and 2010 (Using the Dade-Behring assay)

David M. Maahs,^{**} Diana Jalal,[†] Kim McFann,^{*} Marian Rewers,^{**} and Janet K. Snell-Bergeon^{**}

Clinical Journal of the American Society of Nephrology 2011, 6, 1952

Table 1. Mean cystatin C by visit in all subjects with three completed values

Visit	T1D (n = 449)	non-DM (n = 535)	All (n = 984)
1	0.81 ± 0.25 [0.51 to 3.41]	0.78 ± 0.10 [0.42 to 1.14]	↓ 0.80 ± 0.18 [0.42 to 3.41] ↓ 0.75 ± 0.21 [0.39 to 3.77] ↓ 0.69 ± 0.22 [0.39 to 3.79]
2	0.78 ± 0.28 [0.46 to 3.77]	0.73 ± 0.10 [0.39 to 1.38]	
3	0.72 ± 0.30 [0.39 to 3.79]	0.66 ± 0.11 [0.41 to 1.41]	

Mean ± SD [min to max]. T1D, type 1 diabetes; non-DM, nondiabetics.

Table 2. Mean cystatin C re-runs by visit

Visit	Original	Re-Run	Difference	P
1 (n = 189)	0.82 ± 0.29 [0.51 to 3.41]	0.70 ± 0.27 [0.42 to 3.15]	0.13 ± 0.04 [0.06 to 0.30]	<0.01
2 (n = 195)	0.79 ± 0.35 [0.51 to 4.17]	0.71 ± 0.34 [0.43 to 3.89]	0.08 ± 0.04 [-0.04 to 0.28]	<0.01

Mean ± SD [min to max] or for difference mean ± SD [min to max].

The Impact of Interlaboratory Differences in Cystatin C Assay Measurement on Glomerular Filtration Rate Estimation

Clinical Journal of the American Society of Nephrology 2011, 6, 2150

Christine A. White,^a Andrew D. Rule,^a Christine P. Collier,^a Ayub Akbar,^a John C. Lieske,^{all} Nathalie Lepage,^a Steve Doucette,^{**} and Greg A. Knoll^{**}

Table 1. Interlaboratory CysC Differences^a

	Mean Mayo Clinic CysC ± SD (range)	Mean CHEO CysC ± SD (range)	Mean Absolute Difference (95% CI) ^b	Mean % Difference (95% CI) ^c
Whole cohort (n = 94)	1.58 ± 0.61 (0.65 to 3.82)	1.41 ± 0.53 (0.63–3.15)	-0.17 (-0.21, -0.13) ^d	-9.9 (7.6, 12.2) ^d
CysC <1.41 mg/L (n = 47)	1.14 ± 0.24	1.01 ± 0.19	-0.12 (-0.17, -0.07) ^d	-9.7 (-6.2, -13.1) ^d
CysC ≥1.41 mg/L (n = 47)	2.03 ± 0.53	1.81 ± 0.44	-0.22 (-0.29, -0.16) ^d	-10.1 (-7.0, -13.2) ^d

^a Expressed as mg/L.
^b Absolute difference = CHEO CysC × Mayo Clinic CysC Mean.
^c % difference = (CHEO CysC - Mayo Clinic CysC)/Mayo Clinic CysC × 100.
^d P < 0.0001.

Table 2. GFR Estimates and Difference in Estimates^a

	Mean eGFR (Mayo Clinic CysC) ± SD	Mean eGFR (CHEO CysC) ± SD	Mean Absolute Difference (95% CI) ^b	Mean % Difference (95% CI) ^c
Whole cohort (n = 94)	52.9 ± 21.9	60.1 ± 24.7	7.2 (5.2, 9.3) ^d	15.3 (11.3, 19.4) ^d
CysC <1.41 mg/L (n = 47)	69.8 ± 17.6	79.2 ± 19.7	9.5 (5.8, 13.1) ^d	15.7 (8.9, 22.4) ^d
CysC ≥1.41 mg/L (n = 47)	36.0 ± 8.9	41.0 ± 10.1	5.0 (3.5, 6.5) ^d	15.0 (10.8, 19.2) ^d

CysC, cystatin C; CHEO, Children's Hospital of Eastern Ontario; CI, confidence interval.
^a Expressed as ml/min per 1.73 m².
^b Absolute difference = CHEO eGFR - Mayo Clinic eGFR.
^c % difference = (CHEO eGFR - Mayo Clinic eGFR)/Mayo Clinic eGFR × 100.
^d P < 0.0001.

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High-Normal Serum Uric Acid Increases Risk of Early Progressive Renal Function Loss in Type 1 Diabetes

Results of a 6-year follow-up *Diabetes Care* 2011;33, 1337

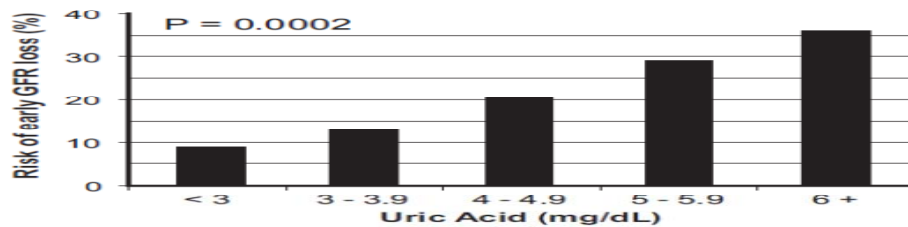


Table 2—Odds ratios for a 1.0 mg/dl increase in baseline serum uric acid concentration for the odds of developing selected renal outcomes during 4–6 years of follow-up

Renal outcome	Unadjusted measure of association (95% CI)	Adjusted measure of association (95% CI)*	Adjusted measure of association including adjustment for baseline level of outcome† (95% CI)
Early GFR loss	1.5 (1.3–1.9)	1.5 (1.2–1.9)	1.4 (1.1–1.8)
Progression of ACR	1.1 (0.9–1.4)	1.0 (0.8–1.3)	1.0 (0.8–1.3)
Regression of ACR	1.0 (0.8–1.1)	1.1 (1.0–1.4)	1.1 (1.0–1.4)

*Adjusted for ACR, sex, and A1C in models with outcome early GFR loss; adjusted for baseline GFR, cystatin, sex, and A1C in models with outcomes progression or regression of ACR. †Adjusted for baseline GFR, cystatin, ACR, sex, and A1C in models with outcome early GFR loss; adjusted for baseline ACR, GFR, cystatin, sex, and A1C in models with outcomes progression or regression of ACR.

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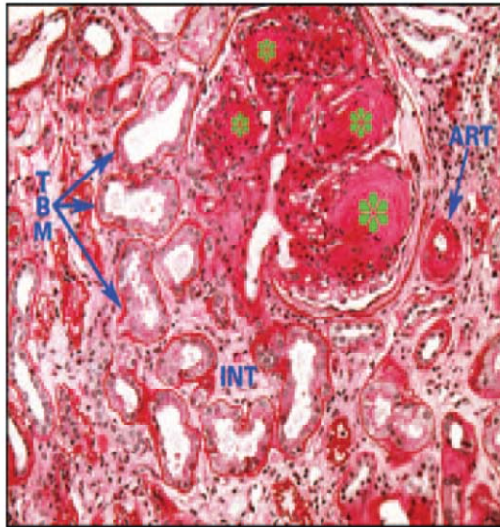


Figure 1 | Photomicrograph depicting concomitant involvement of glomerular and tubulointerstitial compartments in diabetic nephropathy. Asterisks represent the Kimmelstiel-Wilson nodular lesions in the affected glomerulus. The arteriole (ART) has undergone hyalinosis. The interstitium (INT) is expanded, and tubular basement membranes (TBM) are thickened.

Name	Abbreviation	Comment
Neutrophil gelatinase-associated lipocalin	NGAL	Produced by epithelial cells and neutrophils Produced in renal tubules in response to structural kidney injury A marker of functioning tubular mass.
Kidney injury molecule-1	KIM-1	A transmembrane glycoprotein expressed in proximal tubular cells. A marker of tubular damage in various chronic kidney diseases

Tubular markers do not predict the decline in glomerular filtration rate in type 1 diabetic patients with overt nephropathy

Stine E. Nielsen¹, Steen Andersen¹, Dietmar Zdunek², Georg Hess³, Hans-Henrik Parving^{4,5} and Peter Rossing¹

Kidney International, 79, 1113

Table 1 | Yearly decline in GFR according to baseline levels of markers of tubular damage

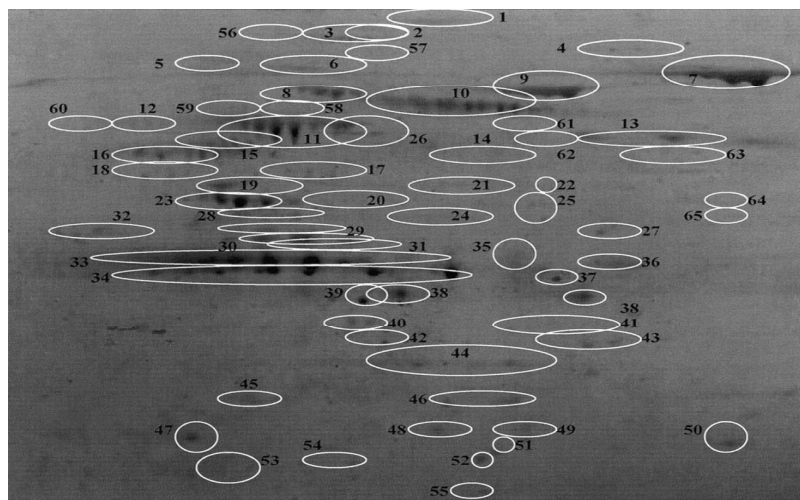
	GFR decline (mean, s.d.)/year ml/min per 1.73 m ²				P-value ANOVA/(lower vs upper quartile)
	First quartile	Second quartile	Third quartile	Fourth quartile	
u-NGAL*	2.6 (1.9)	4.0 (3.7)	3.9 (3.3)	6.8 (4.4)	0.037/0.01
u-KIM1*	2.6 (2.8)	4.1 (2.9)	5.2 (4.7)	5.5 (3.7)	0.20/0.04

No significant association between tubular markers and decline in GFR after adjustment for known progression promoters

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Urinary proteomic profile of type 2 diabetic patients with macroalbuminuria as determined by two-dimensional differential in-gel electrophoresis (DIGE)-after depletion of albumin, IgG, IgA, anti-trypsin, transferrin & haptoglobin



Proteomic Identification of Urinary Biomarkers of Diabetic Nephropathy
Rao P V et al. Diabetes Care 2007;30:629-637

Urine proteins differentially expressed in type 2 diabetic patients with macroalbuminuria compared to those without albuminuria detected by 2-D DIGE . Protein identification was performed by liquid chromatography-tandem mass spectrometry

Swiss-Prot accession no.	Protein name	-Fold change
P04217	α_{1B} -Glycoprotein	6.95
P25311	α_2 -Glycoprotein, zinc	5.86
P02774	VDBP	4.84
P02765	α_2 -HS-glycoprotein	4.71
P06702	Calgranulin B	3.87
P01009	α_1 -Antitrypsin	2.89
P02790	Hemopexin	2.39
P02753	Plasma RBP	-1.52
P02760	AMBP protein	-1.61
P02647	ApoA-1	-3.20
P02766	Transthyretin	-4.32

Proteomic Identification of Urinary Biomarkers of Diabetic Nephropathy
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Conclusions

Cystatin C is a promising marker of GFR but assay standardisation and measurement reliability must be addressed

Elevated serum uric acid levels are a marker of risk for a progressive decline in GFR (do uric acid levels rise before a decline in GFR?)

NGAL & KIM-1 are promising tubular markers of an increased risk for a progressive decline in GFR, but do they provide additional prognostic information over and above that of known progression promoters?

Urinary proteomics may uncover novel markers of risk for a progressive decline in renal function

The End