Inflamación renal en el trasplante: ¿Existen biomarcadores?

Alberto Ortiz
Nefrología, IIS-FJD
Coordinador REDINREN

20 years ago…..

American Journal of Pathology, Vol. 148, No. 1, January 1996

Interferon-Inducible Protein-10 Is Highly Expressed in Rats with Experimental Nephrosis

Marta Gómez-Chiarri,* Alberto Ortiz,*
Silvia González-Cuadrado,* Daniel Serón,†
Steven N. Emancipator,‡
Thomas A. Hamilton,§ Antonio Barat,*
Juan José Plaza,* Eva González,* and
Jesús Egido*

We conclude that **IP-10** is highly inducible in vitro and in vivo in **resident glomerular and tubulointerstitial** cells.

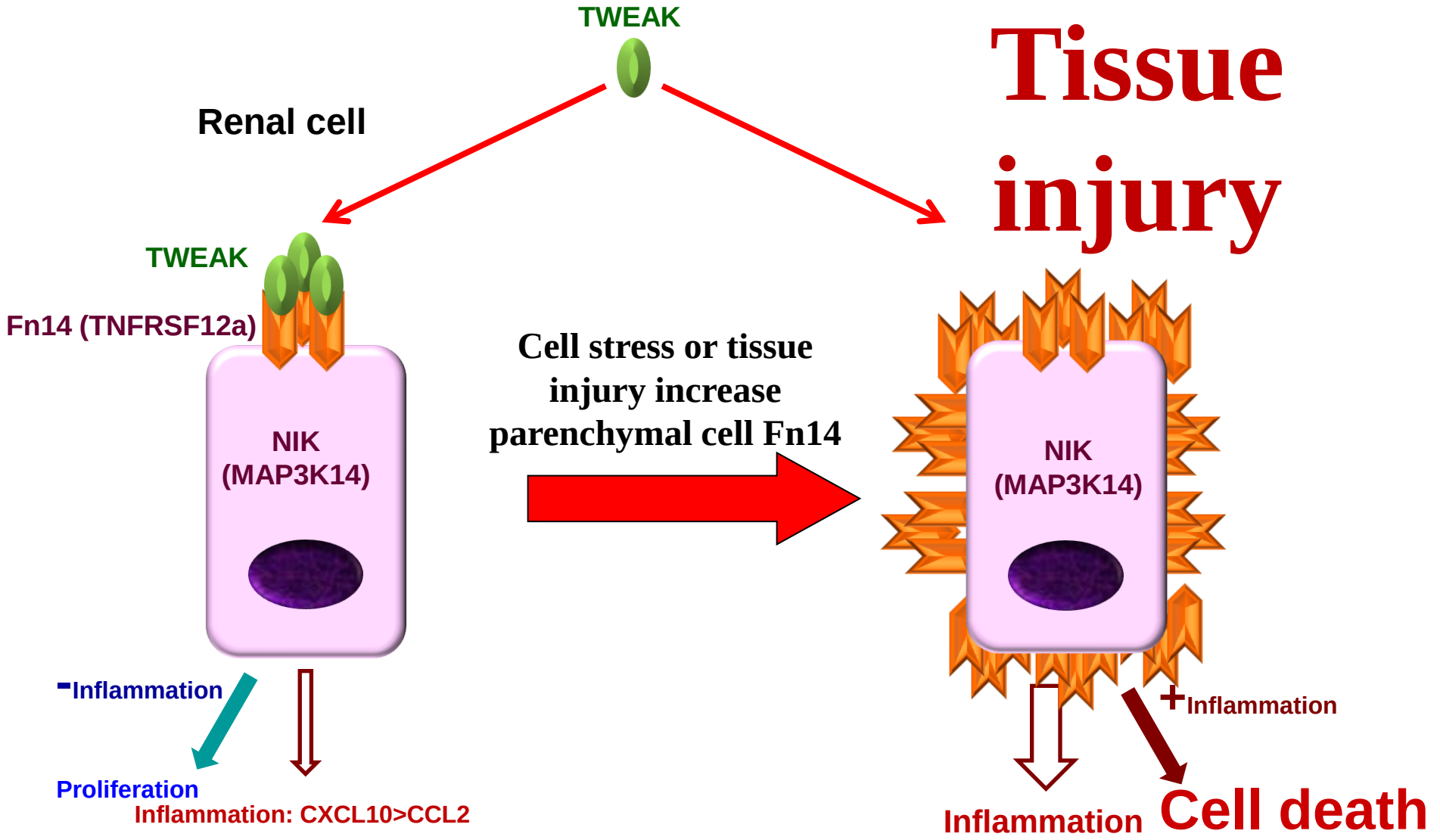
Back to the future…. October 21, 2015

- Murine tubular cells exposed to **TWEAK**
  - FC FDR
  - 2,8 0,02 **Cxcl10/IP10** (ranked 23 of 20,000)
  - 2,6 0,18 Ccl2/MCP1
  - No change in CXCL9

- Murine **nephrotoxic** AKI
  - 4,45 0,005 **Cxcl10** (Ranked 24 of 20,000)
  - 2,29 0,022 Ccl2 (Ranked 169 of 20,000)

What is TWEAK?

TNF superfamily cytokine

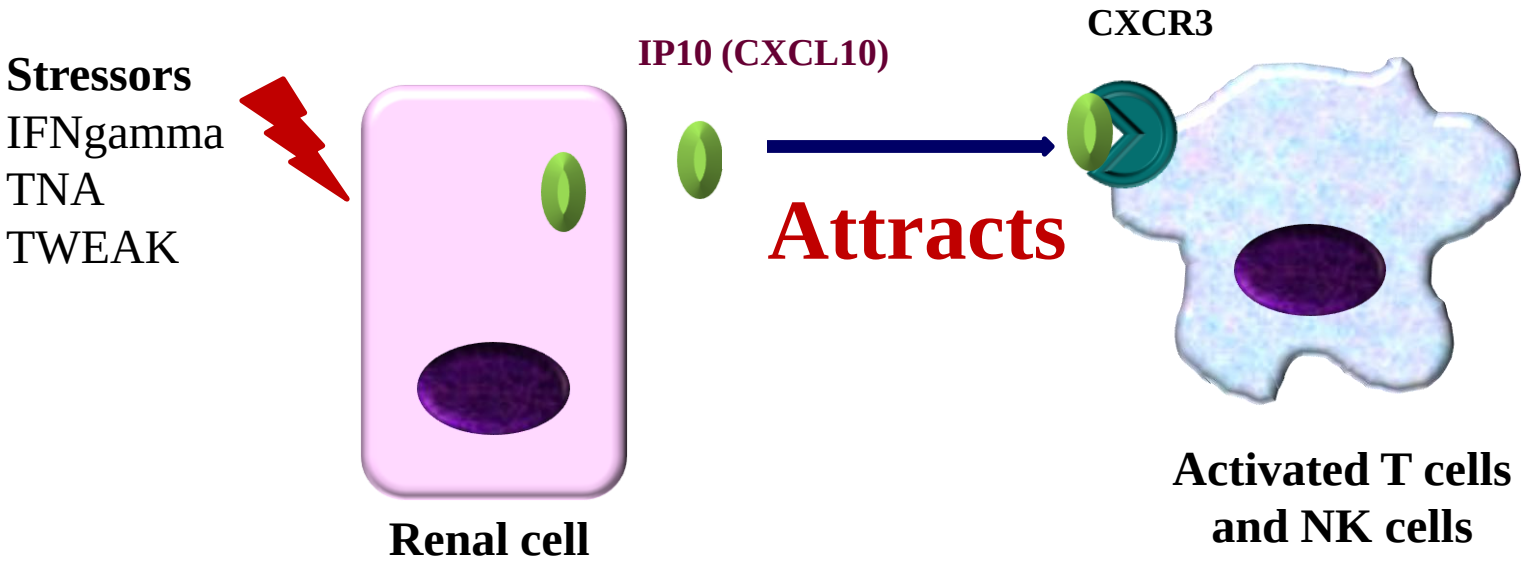


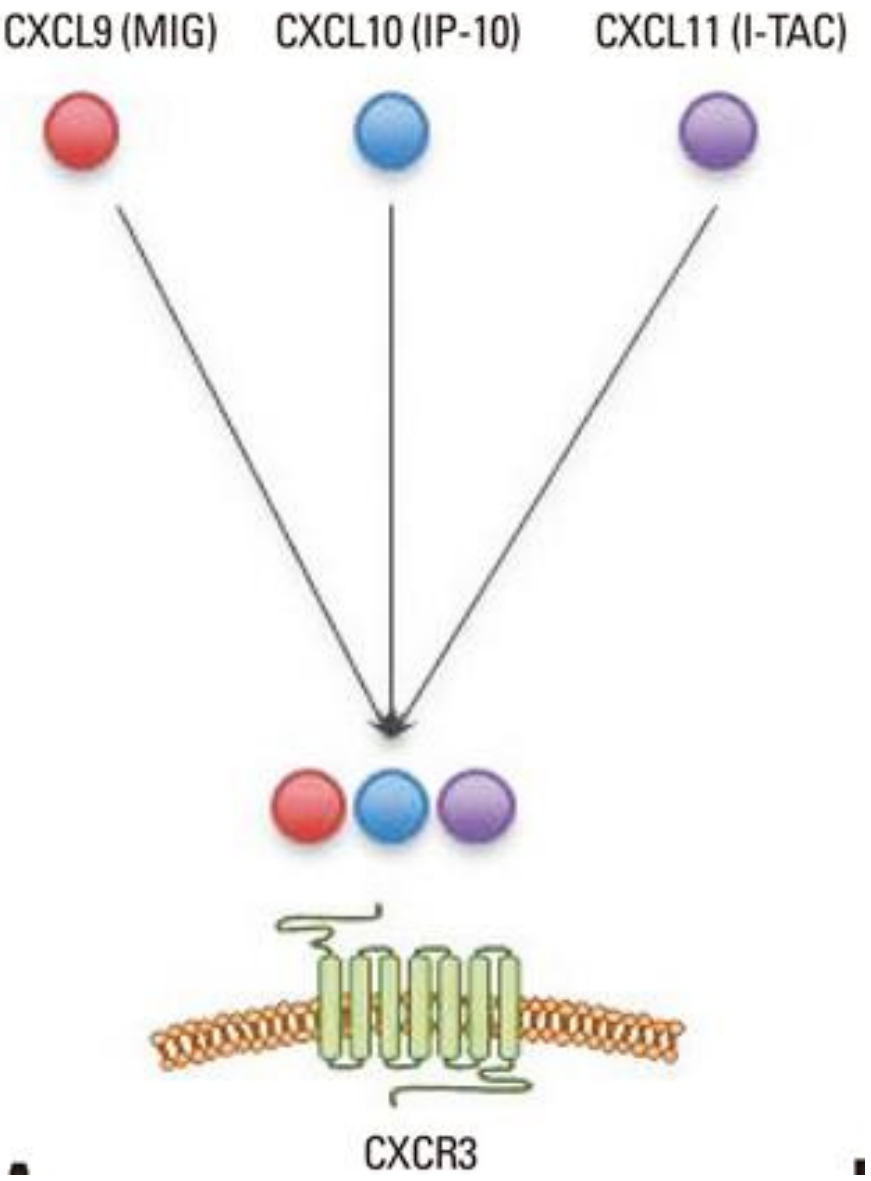
Sanz et al. NDT 2013

- **2015**: In search for a **molecular fingerprint** for **MAP3K14 activation** in the kidney

What is IP-10 (CXCL10)?

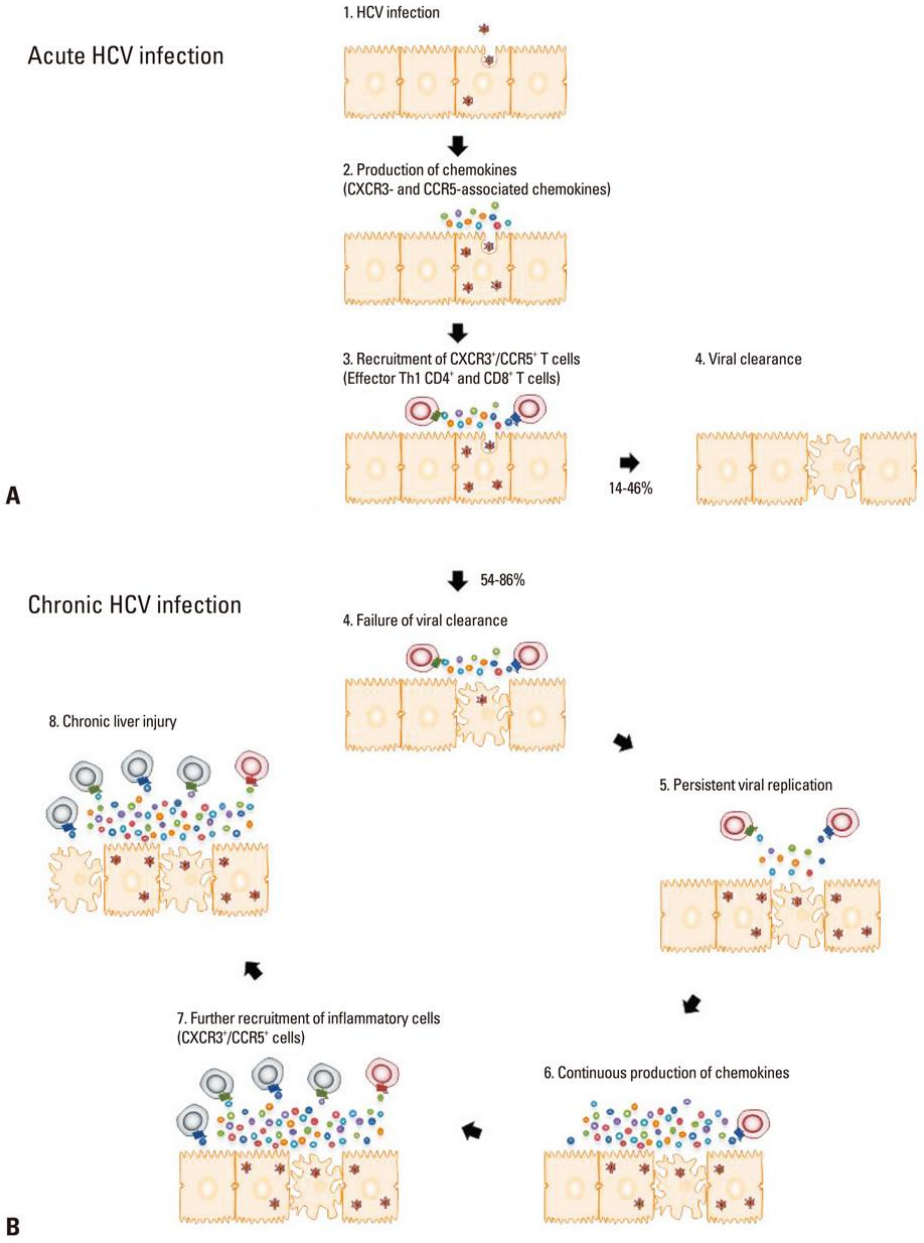
- **Chemokine:** Interferon gamma-induced protein 10 (IP-10), C-X-C motif chemokine 10 (CXCL10)
- An **amplifier** of the inflammatory response
- Secreted by **renal cells** and **monocytes** in response to diverse cytokines and other stressors
- Activates the **CXCR3** chemokine receptor





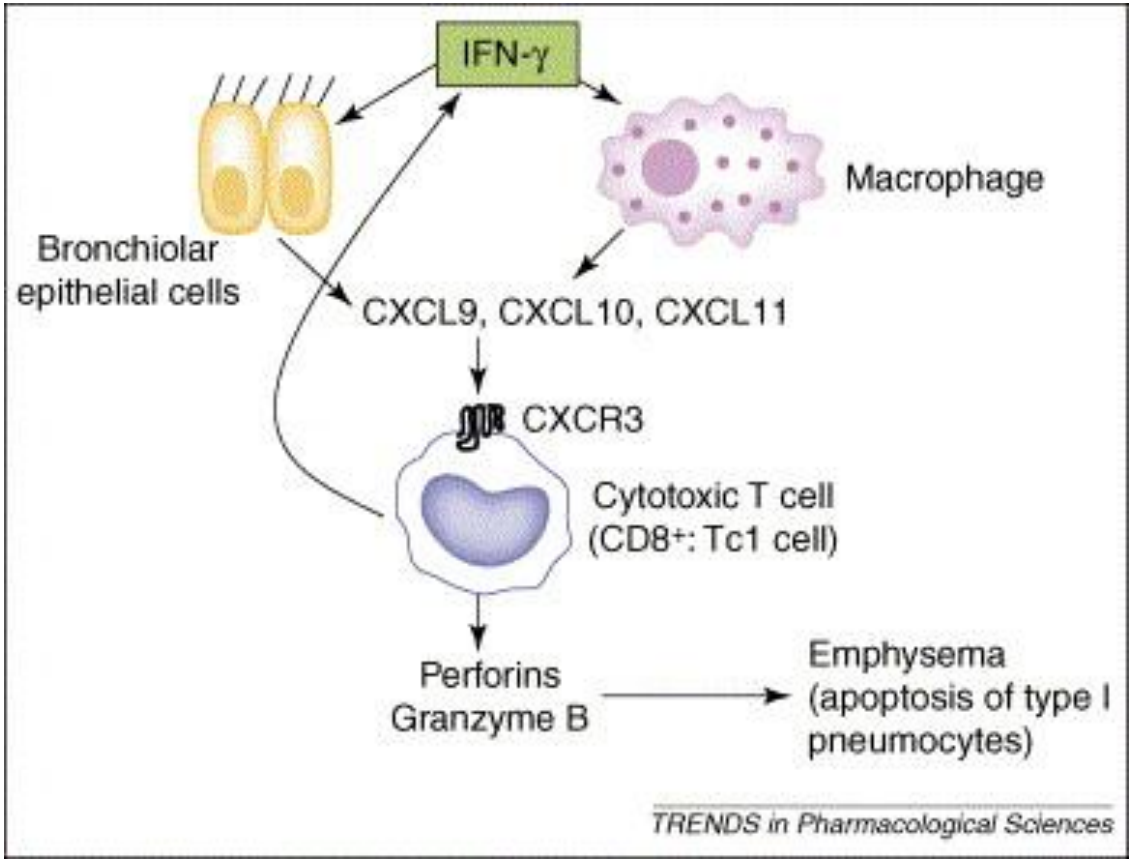
http://www.eymj.org/ViewImage.php?Type=F&aid=342487&id=F2&afn=69_YMJ_52_6_871&fn=ymj-52-871-g002_0069YMJ

HVC infection



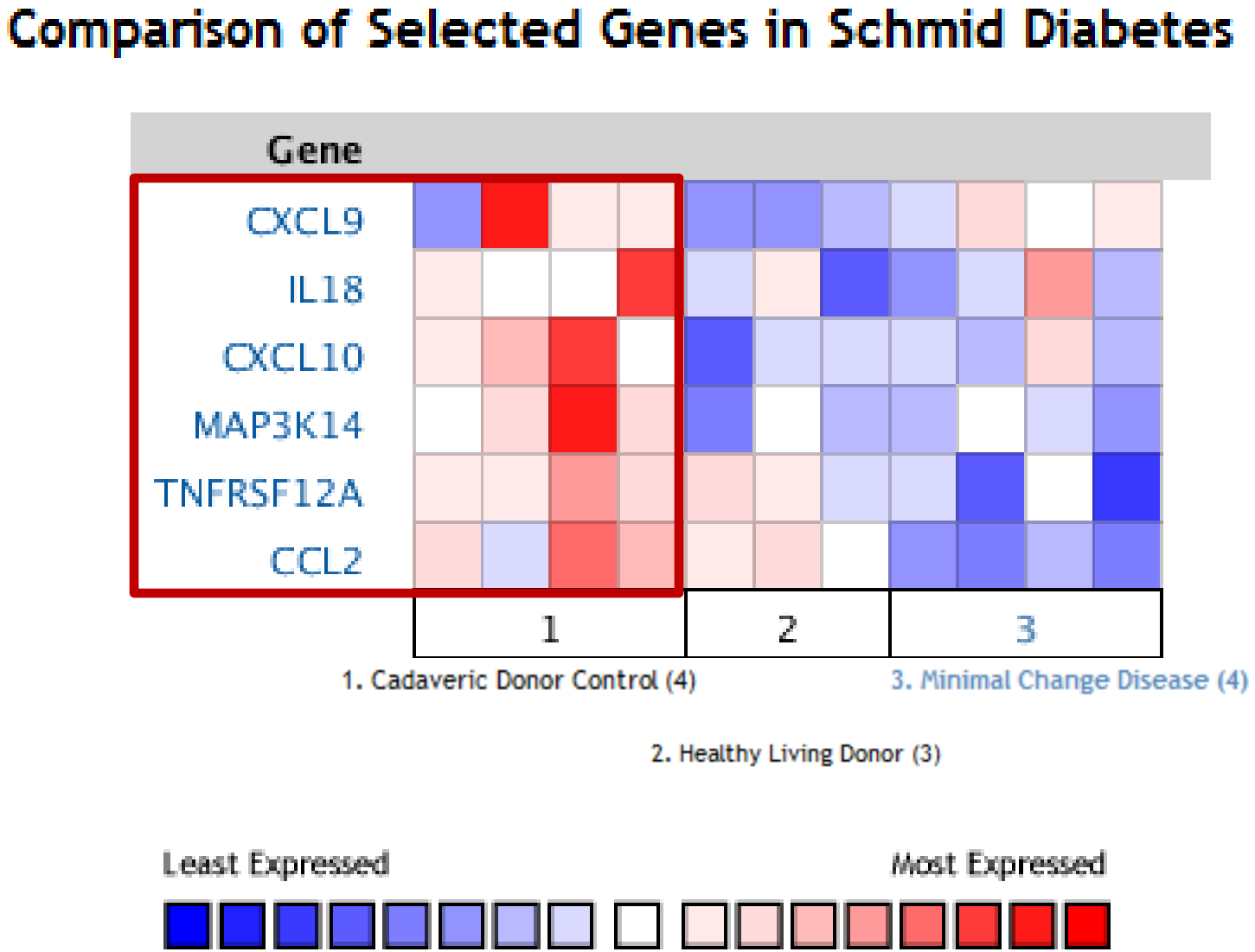
http://www.eymj.org/ViewImage.php?Type=F&aid=342487&id=F2&afn=69_YMJ_52_6_871&fn=ymj-52-871-g002_0069YMJ

CXCL10 in non-infectious tissue injury: emphysema

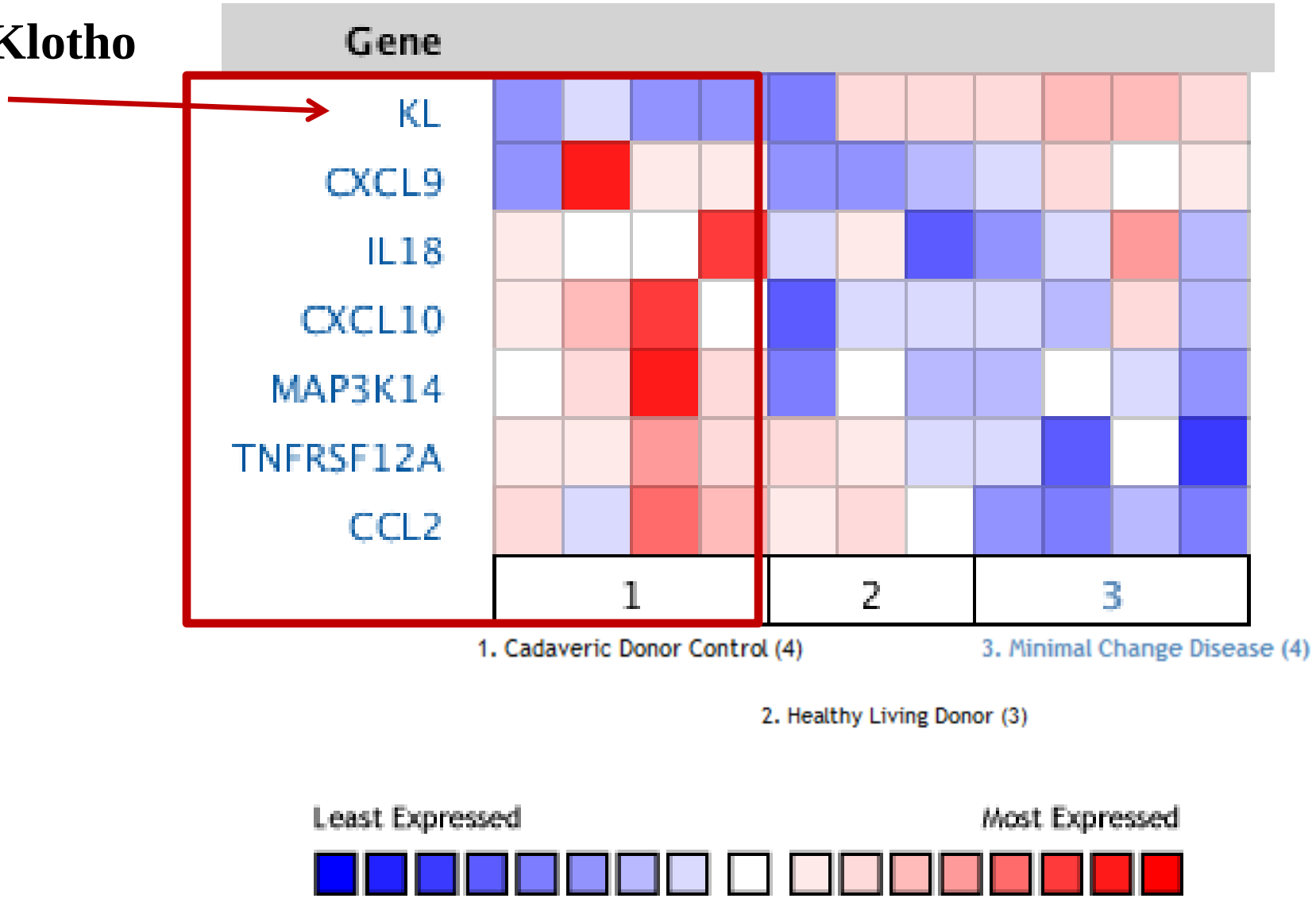


Suthanthiran M, et al. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. N Engl J Med 2013;369:20-31

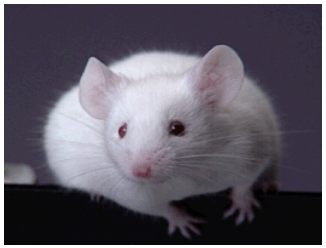
Inflammation in the transplanted kidney starts before implantation

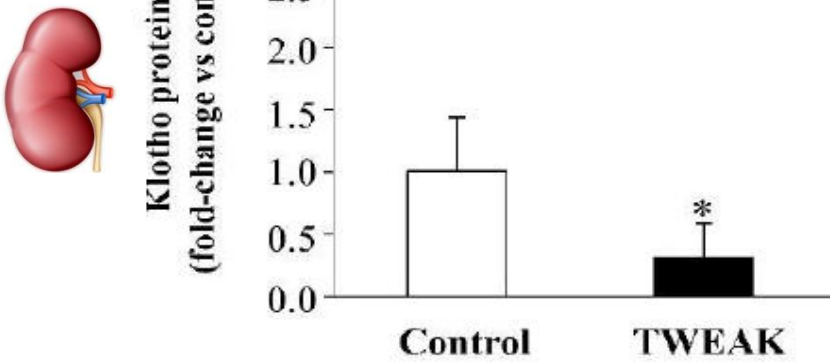


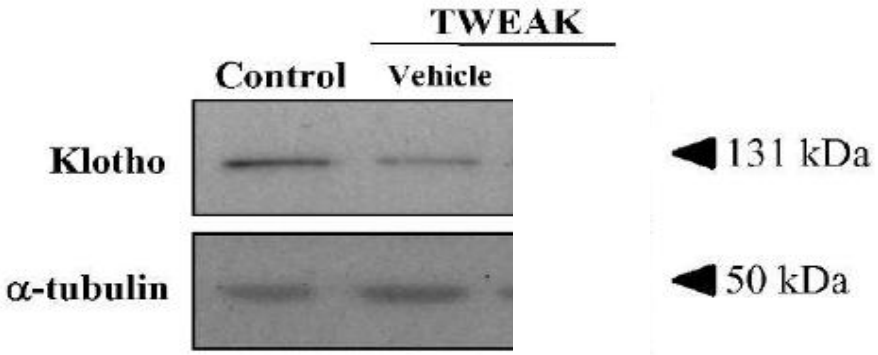
Klotho


Gene
KL
CXCL9
IL18
CXCL10
MAP3K14
TNFRSF12A
CCL2
1
2
3
1. Cadaveric Donor Control (4)
2. Healthy Living Donor (3)
3. Minimal Change Disease (4)
Least Expressed
Most Expressed

Inflammation (TWEAK) downregulates kidney Klotho in healthy mice







Moreno et al. JASN 2011

Klotho: a kidney factor with anti-aging and nephroprotective functions

1. **Protects from excess phosphate**
2. **Multiple (Glycosidase activity)**
    1. Anti-oxidant
    2. Anti-inflammatory
    3. Anti-fibrosis
    4. Interferes with insulin/IGF-1 signaling...

EDITORIAL www.jasn.org

Klotho to Treat Kidney Fibrosis

Maria D. Sanchez-Niño,[*†] Ana B. Sanz,[†‡] and Alberto Ortiz[†‡§‖]

J Am Soc Nephrol 2013

Inflammation biomarkers and kidney Tx

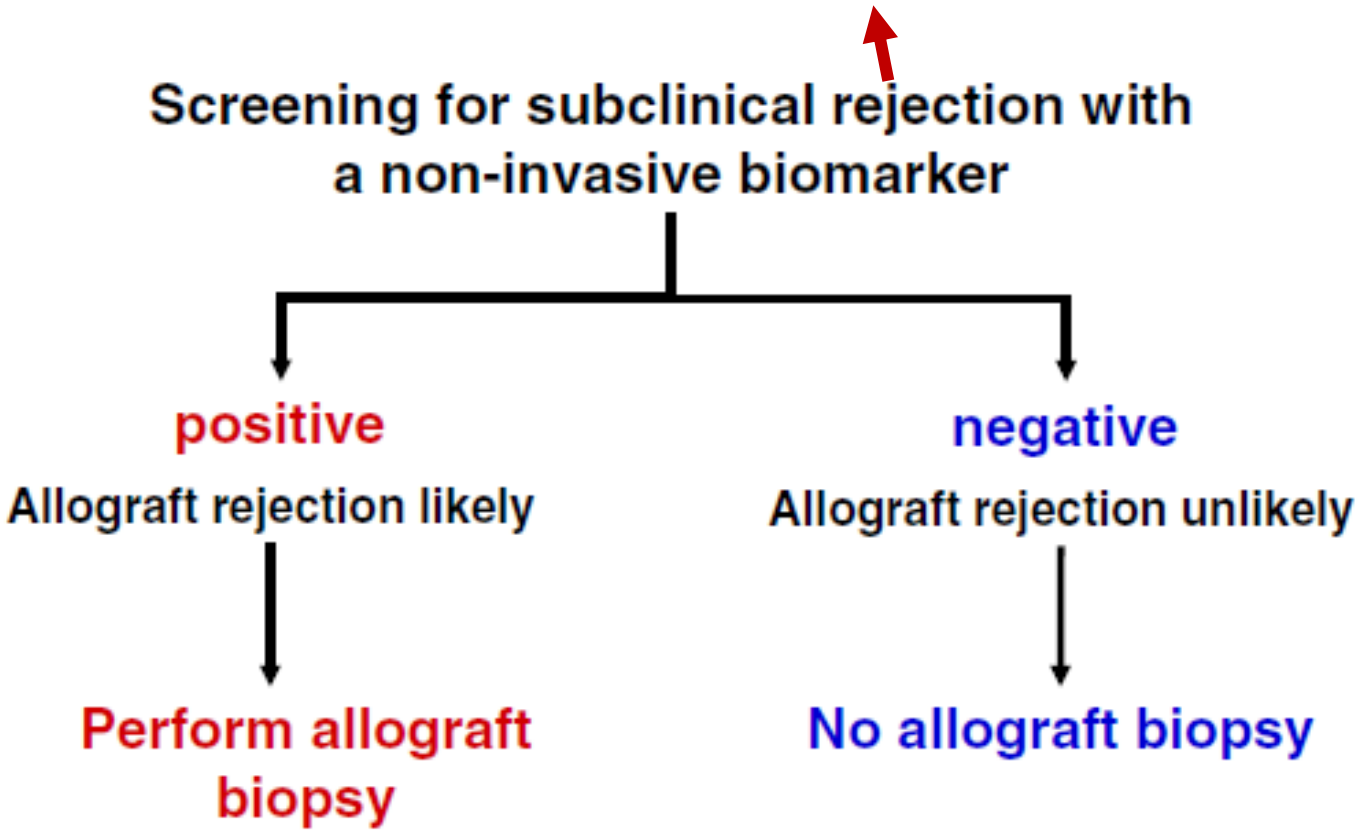
What we have

Observational associations between biomarkers and outcomes

What we need

Clinical trials that explore outcomes of biomarker-guided or biomarker-targeted therapy/intervention

Inflammation as a surrogate marker



Hirt-Minkowski *et al. Canadian Journal of Kidney Health and Disease* (2015) 2:28

The NEW ENGLAND JOURNAL of MEDICINE 2013

ORIGINAL ARTICLE

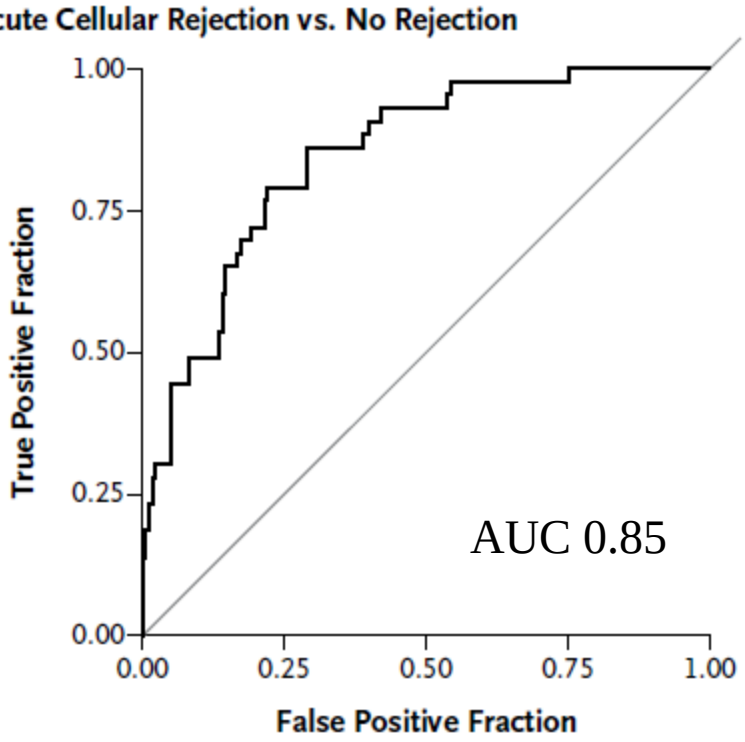
Urinary-Cell mRNA Profile and Acute Cellular Rejection in Kidney Allografts

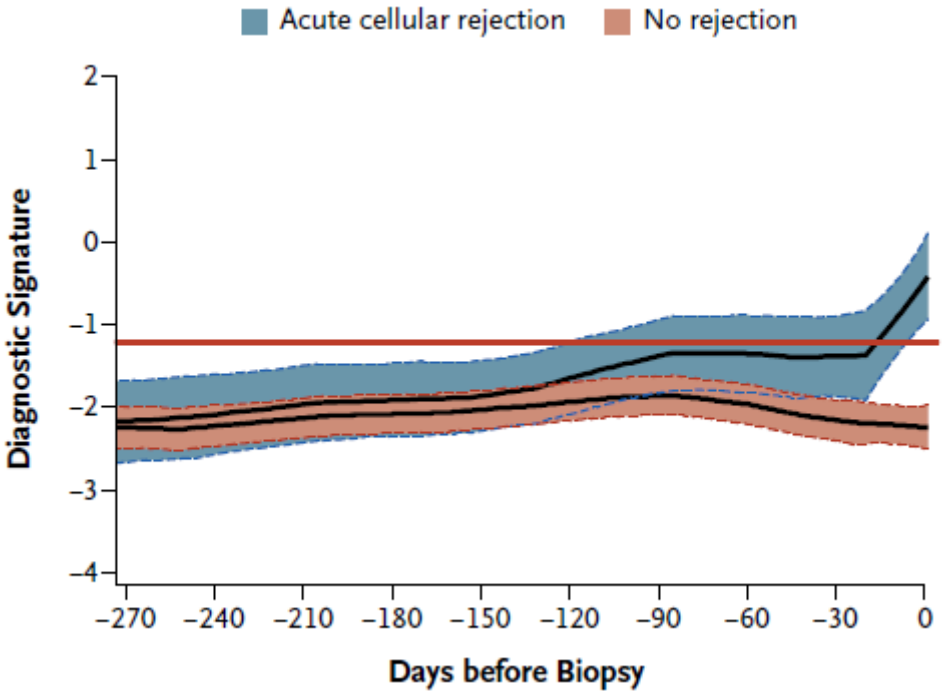
Manikkam Suthanthiran, M.D., Joseph E. Schwartz, Ph.D., Ruchuang Ding, M.D.,

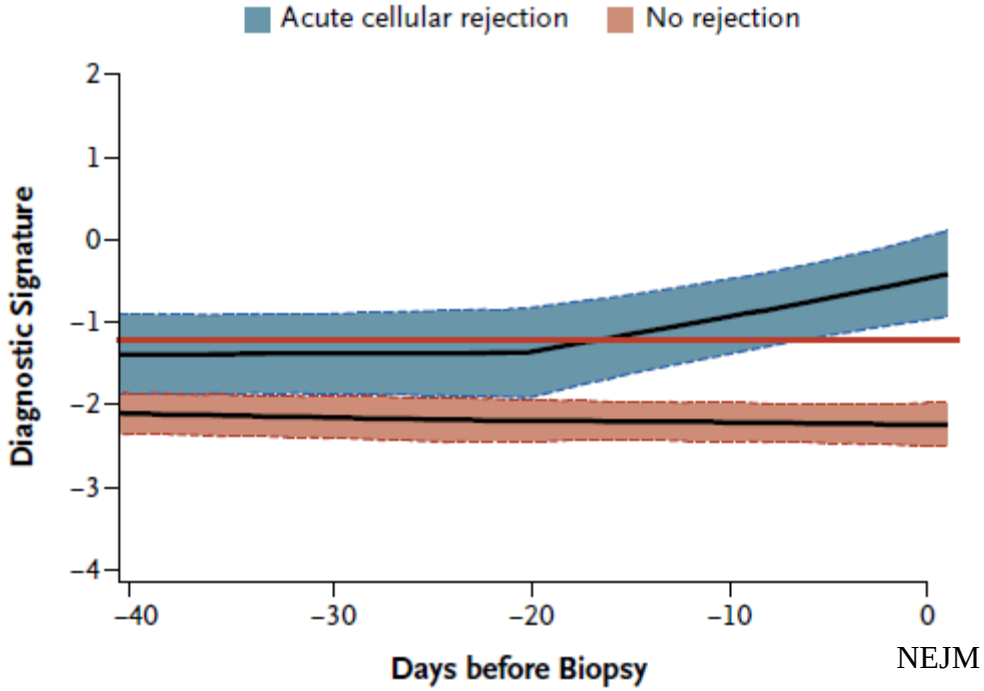
Urinary cell **mRNA** diagnostic signature:

$$-6.1487 + 0.8534 \log_{10}(\text{CD3}\varepsilon/18S) + 0.6376 \log_{10}(\text{IP-10}/18S) + 1.6464 \log_{10}(18S)$$

Only **83**% of samples **valid**

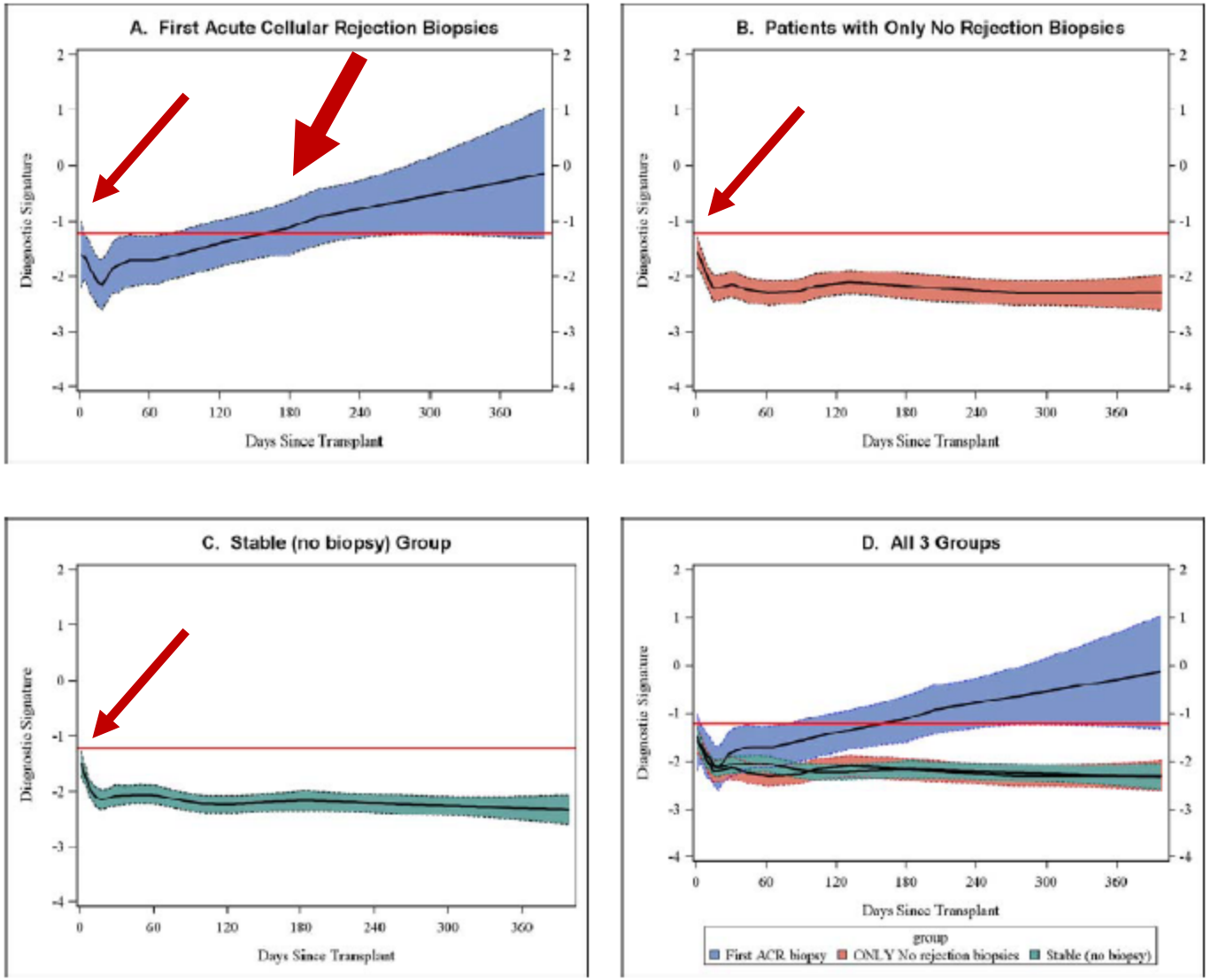



Acute cellular rejection
No rejection
Diagnostic Signature
2
1
0
−1
−2
−3
−4
−270
−240
−210
−180
−150
−120
−90
−60
−30
0
Days before Biopsy


Acute cellular rejection
No rejection
Diagnostic Signature
2
1
0
−1
−2
−3
−4
−40
−30
−20
−10
0
Days before Biopsy

NEJM 2013

Figure S2. Prospective Longitudinal Trajectory of Diagnostic Signature as a Function of Time since Kidney Transplantation*.



NEJM 2013

- This profile also elevated in **polyomavirus** BK infection

- AUC in external-validation data set decreased to 0.74, and the lower 95% CI limit was 0.61

NEJM 2013

2. Protein

American Journal of Transplantation 2013; 13: 2634–2644
Wiley Periodicals Inc.

doi: 10.1111/ajt.12426

Multicenter Validation of Urinary CXCL9 as a Risk-Stratifying Biomarker for Kidney Transplant Injury

D. E. Hricik[1], P. Nickerson[2], R. N. Formica[3],

Clinically indicated bx

Table 4: Logistic regression and bootstrap validation of urinary markers for diagnosing Banff ≥1A acute rejection[1]

Parameter estimates and tests			ROC-based discrimination measures			Positive/negative predictive value	
Univariate models							
CXCL9 mRNA	2.77 (1.59, 4.80)	0.0003	0.788	66.7	79.6	61.5	83.0
CXCL9 protein	3.40 (2.12, 5.47)	<0.0001	0.856	85.2	80.7	67.6	92.0
CXCL10 protein	3.25 (1.89, 5.57)	<0.0001	0.768	74.1	86.0	71.4	87.5

American Journal of Transplantation 2013; 13: 2634–2644
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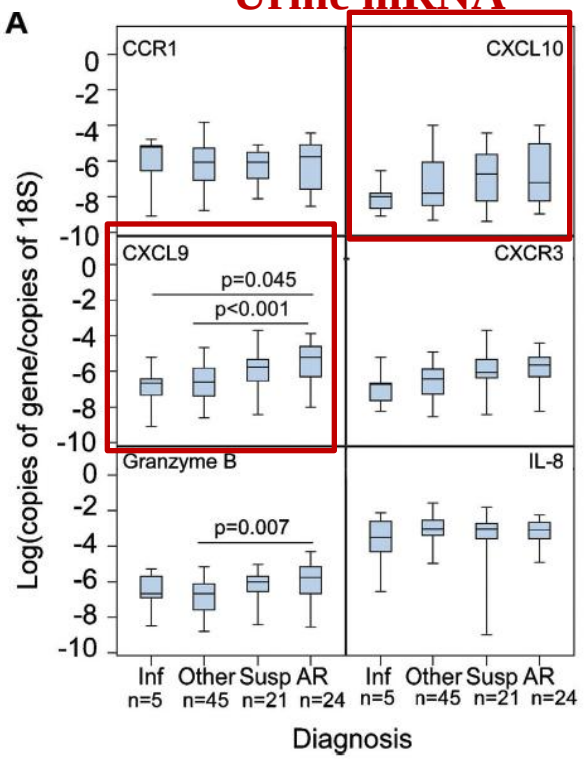
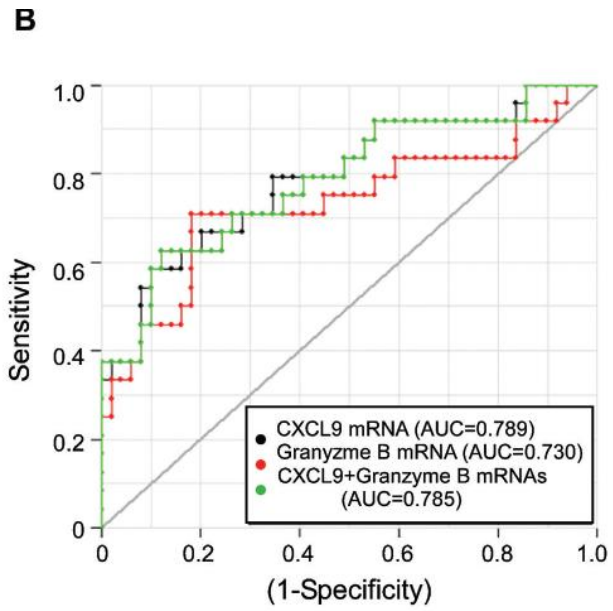
Multicenter Validation of Urinary CXCL9 as a Risk-Stratifying Biomarker for Kidney Transplant Injury

D. E. Hricik[1], P. Nickerson[2], R. N. Formica[3],

biopsy-proven acute rejection (AR) within the first 6 months posttransplant.

Urine mRNA

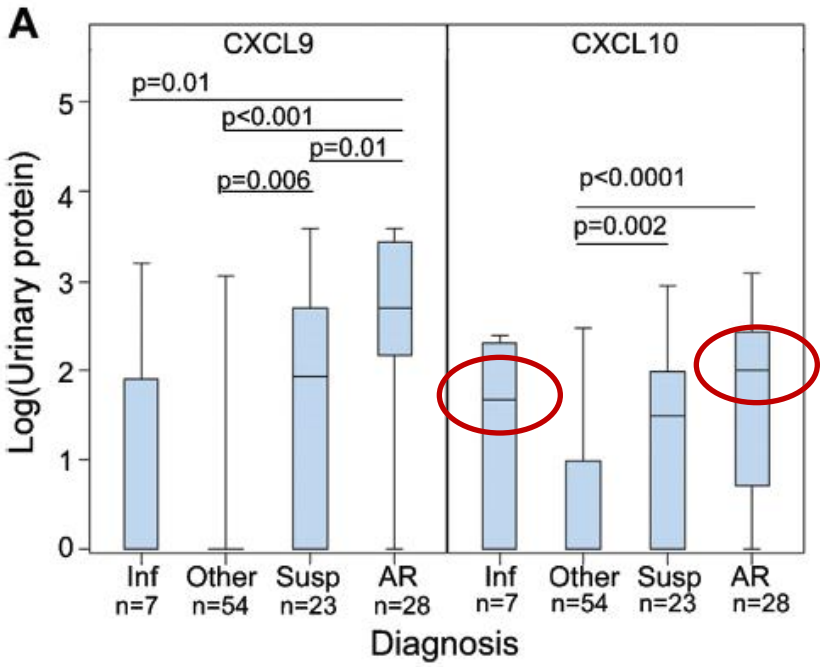


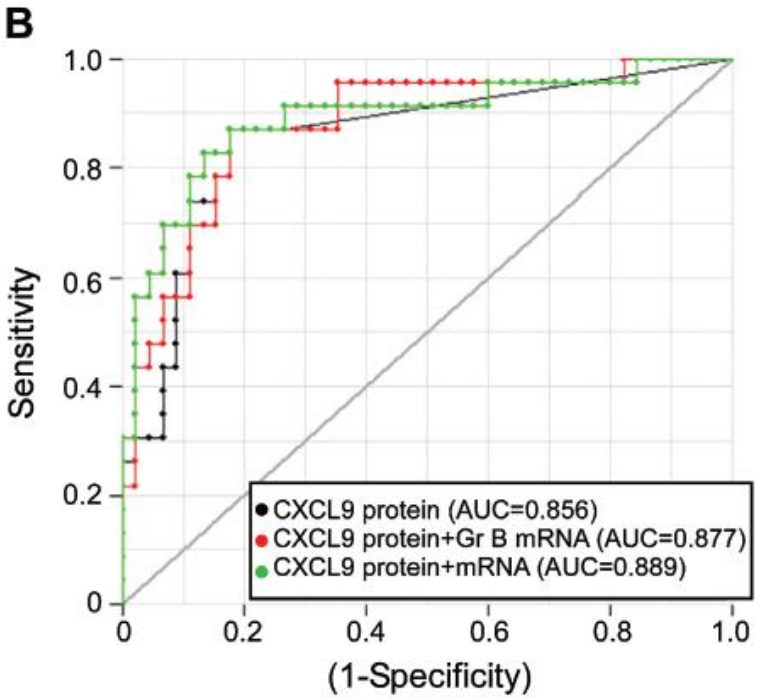


Infection (Inf), Banff grade suspicious rejection (Susp) Banff grade 1A AR or other/no rejection/infection (Other).

Urinary protein

biopsy-proven acute rejection (AR) within the first 6 months posttransplant.





Infection (Inf),
other/no rejection/infection (Other).
Banff grade suspicious rejection (Susp)
Banff grade 1A AR or

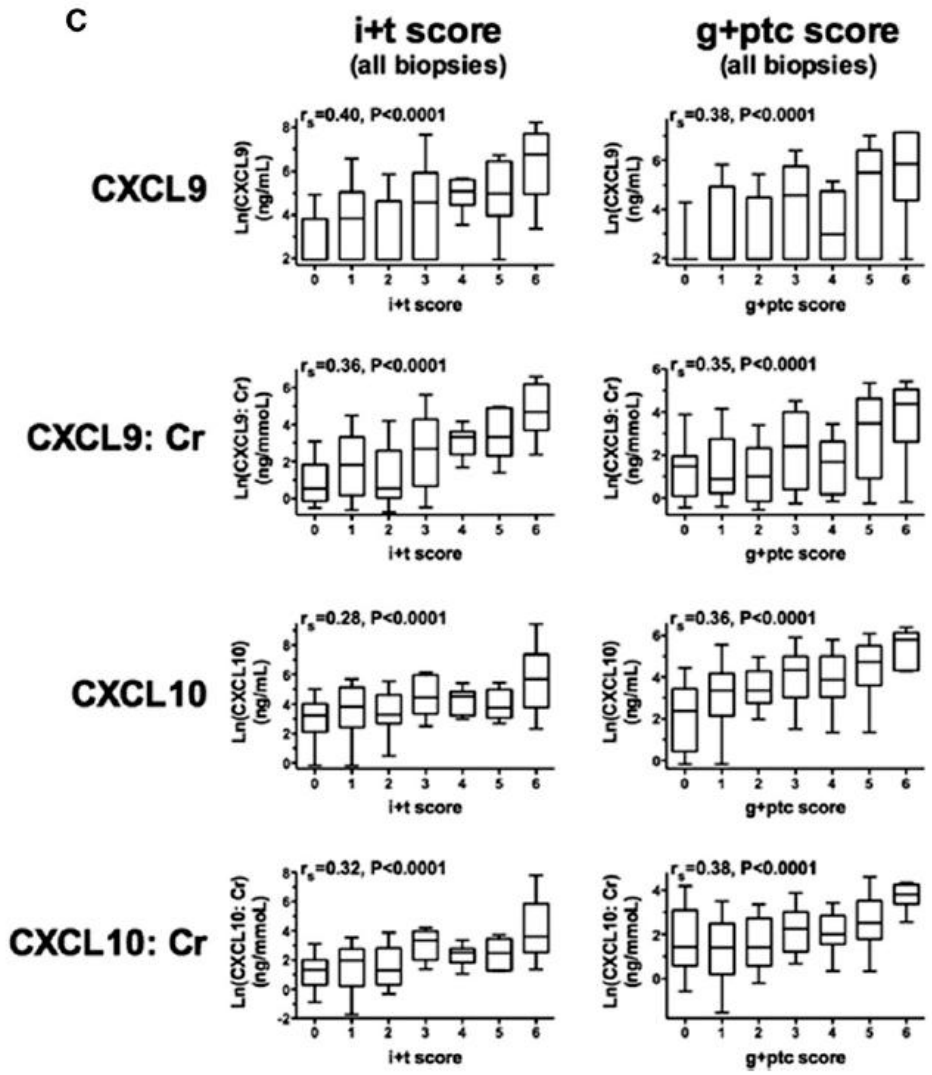
Hricik. AJT 2013

CLINICAL RESEARCH www.jasn.org

Urinary C-X-C Motif Chemokine 10 Independently Improves the Noninvasive Diagnosis of Antibody–Mediated Kidney Allograft Rejection

Marion Rabant,*†‡ Lucile Amrouche,* Xavier Lebreton,§‖ Florence Aulagnon,§ **2015**

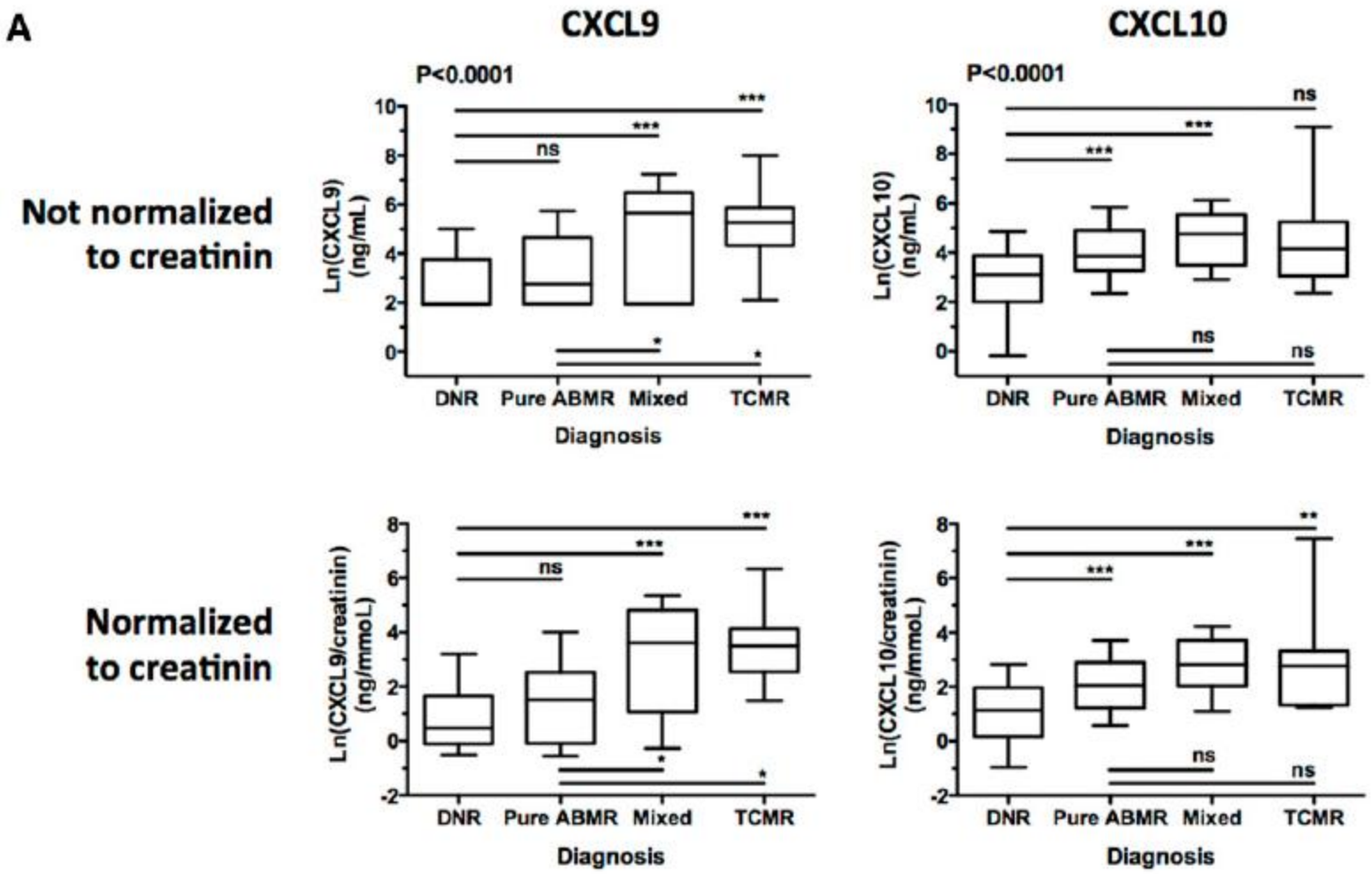
Clinically indicated bx
BK infection **excluded**



Urinary CXCL9:Cr and CXCL10:Cr **correlated** with the extent of **tubulointerstitial and microvascular inflammation.**

CXCL10:Cr diagnosed T cell mediated **rejection** (AUC=0.80; 95% CI 0.68 to 0.92) and ABMR (AUC=0.76; 95% CI, 0.69 to 0.82; P,0.001)

Rabant JASN 2015



[3.6%]). The other biopsies, subsequently classified as dysfunction with no rejection (DNR), revealed acute tubular necrosis/minimal lesions (n=43 [15%]), isolated interstitial fibrosis/tubular atrophy (n=140 [50%]), borderline lesions (n=17 [6%]), or a primary diagnosis of recurrent disease (n=3 [1%]) (Table 2).

Rabant JASN 2015

Urinary CXCL9/CXCL10 summary

Table 1 Diagnostic performance of urinary CXCR3 chemokine proteins

Biomarker				Acute cellular rejection [g]				Subclinical rejection			
CXCL9	Population	Study Design	n [a, b]	AUC	Sensitivity	Specificity	Ref	AUC	Sensitivity	Specificity	Ref
	Multi-center [c]	Prospective, observational cohort	280	0.86	0.87	0.82	26	*Subclinical group too small for AUC*			
	Single center	Prospective, observational cohort	69	NR	0.93	0.89	44	*Subclinical group too small for AUC* [e]			
	Single center	Case control	125	0.87	0.86	0.80	45	*Subclinical group too small for AUC*			
	Two centers	Case control	88					0.78	0.86	0.64	27
	Single center	Case control	113	0.92	0.85	0.90	42				
	Single center	Case control	99	NR	0.80	0.94	43				
	Single center	Case control	201	0.90	0.84	0.83	46				
	Single center [f]	Case control	213	0.91	0.90	0.84	47				
CXCL10	Population	Study Design	n [a, b]	AUC	Sensitivity	Specificity	Ref	AUC	Sensitivity	Specificity	Ref
	Multi-center [c]	Prospective, observational cohort	280	0.77	0.74	0.86	26	*Subclinical group too small for AUC*			
	Single center	Prospective, observational cohort	213	0.74	0.63	0.80	25	0.69	0.61	0.72	25
	Two centers	Case control	88					0.79	0.68	0.90	27
	Single center	Case control	91	0.84	0.78	0.59	28	0.85	0.73	0.73	28
	Single center [d]	Case control	51	0.88	0.77	0.60	52	0.81	0.59	0.67	52
	Single center	Case control	54					0.77 [e]	0.62	0.95	48
	Single center [c]	Case control	125	0.83	0.80	0.76	45	*Subclinical group too small for AUC*			
	Single center	Case control	113	0.93	0.89	0.81	42				
	Single center	Case control	99	NR	0.86	0.91	43				
	Single center	Case control	201	0.81	0.65	0.97	46				
	Single center [f]	Case control	213	0.80	0.70	0.76	47				

[a] Numbers of patients (not urine numbers), some studies are confounded with repeated measures; [b] Numbers in the AUC analysis, not the overall study population; [c] Pediatric population included; [d] Pediatric population only; [e] Subclinical refers to the AUC 4-5 days prior to clinical rejection for these studies; [f] Rabant et al. performed a prospective observational study of patients with indication biopsies – the data reported here are for the TCMR sub-group analysis; [g] Rejection is scored by the Banff criteria, but the definitions used vary by study. *NR* not reported

CXCL9

Population	Study Design	n [a, b]	Acute cellular rejection [g] AUC	Sensitivity	Specificity	Ref	Subclinical rejection AUC	Sensitivity	Specificity	Ref
Multi-center [c]	Prospective, observational cohort	280	0.86	0.87	0.82	26	*Subclinical group too small for AUC*			
Single center	Prospective, observational cohort	69	NR	0.93	0.89	44	*Subclinical group too small for AUC* [e]			

High at 6 m predicts 24 mo renal function

CXCL10

Population	Study Design	n [a, b]	AUC	Sensitivity	Specificity	Ref	AUC	Sensitivity	Specificity	Ref
Multi-center [c]	Prospective, observational cohort	280	0.77	0.74	0.86	26	*Subclinical group too small for AUC*			
Single center	Prospective, observational cohort	213	0.74	0.63	0.80	25	0.69	0.61	0.72	25

Persistently high associated with early development of IF/TA

Summary CXCL9/CXCL10

- urinary CXCR3 chemokines (i.e. CXCL9 and CXCL10) are promising for detecting subclinical inflammation:
  - increase up to 30 days prior to biopsy-proven acute rejection
  - decrease in response to anti-rejection therapy
  - Predict subsequent allograft dysfunction.

- Simple to measure by ELISA
- May increase with infection

Hirt-Minkowski *et al. Canadian Journal of Kidney Health and Disease* (2015) 2:28

Take home message

- Inflammation is present in most grafts before grafting
- Biomarker studies to date have been mostly **hypothesis-driven**
  - Non-biased assessment may identify better biomarkers
- Both **urinary cell mRNA** analysis and assessment of **urinary cytokine** hold promise for the early identification of subclinical inflammation/rejection (**infection**!!)
  - mRNA studies technically more difficult
  - A single molecule unlikely to provide precise information, but their combination does not add information
- We have observational studies of associations. We need **interventional** studies in which decision-making is based on biomarker results (e.g. to biopsy or not to biopsy; intervention for subclinical inflammation?) before their widespread implementation