



Christian
Doppler
Laboratory

for
Cardiac and Thoracic
Diagnosis & Regeneration

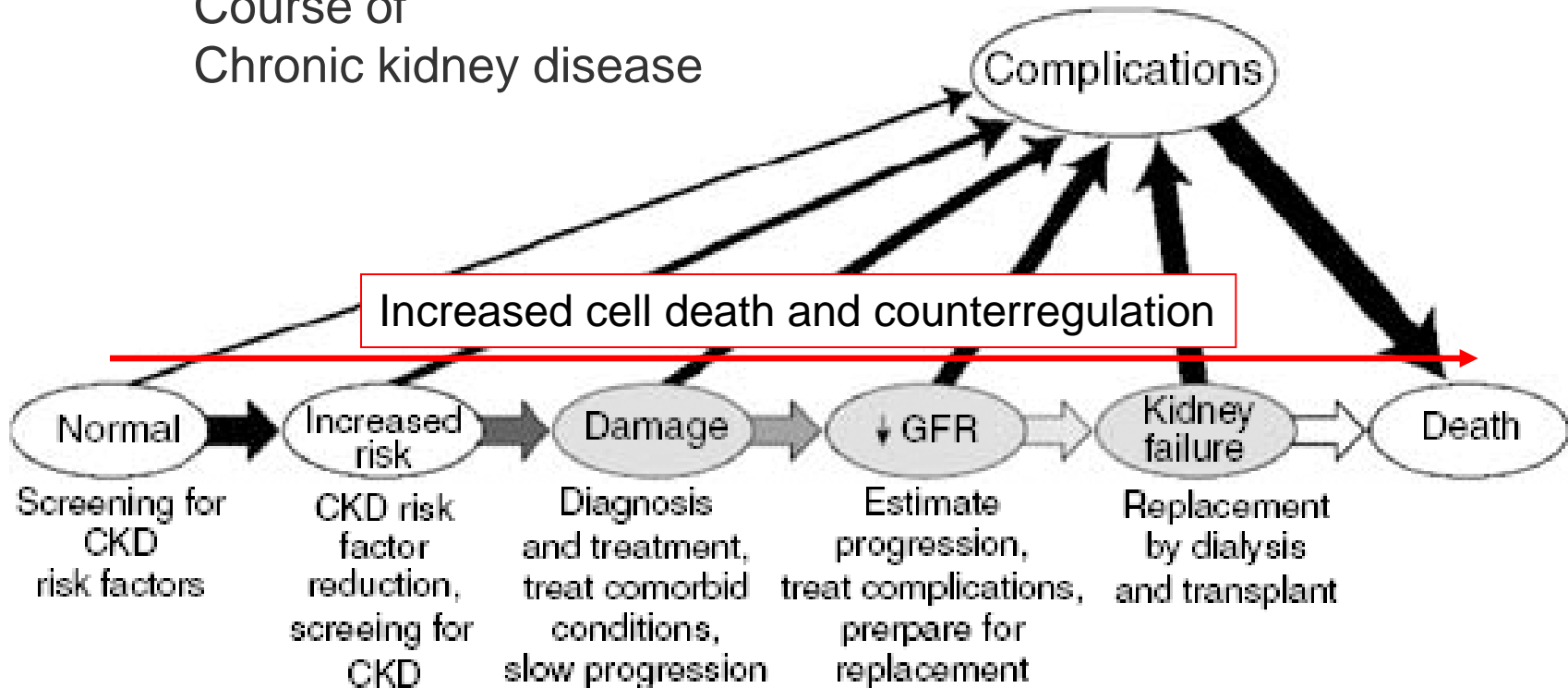


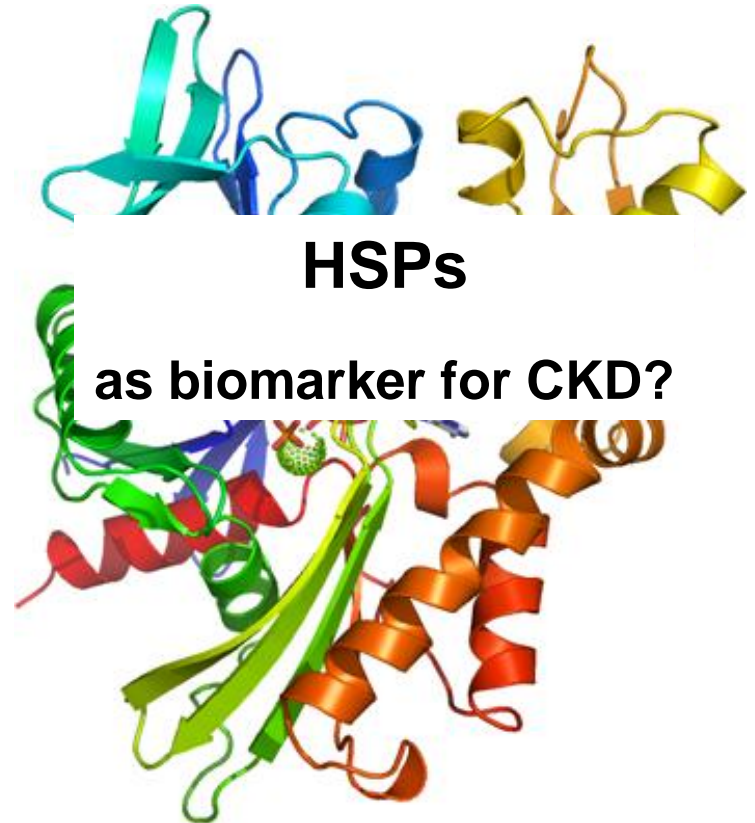
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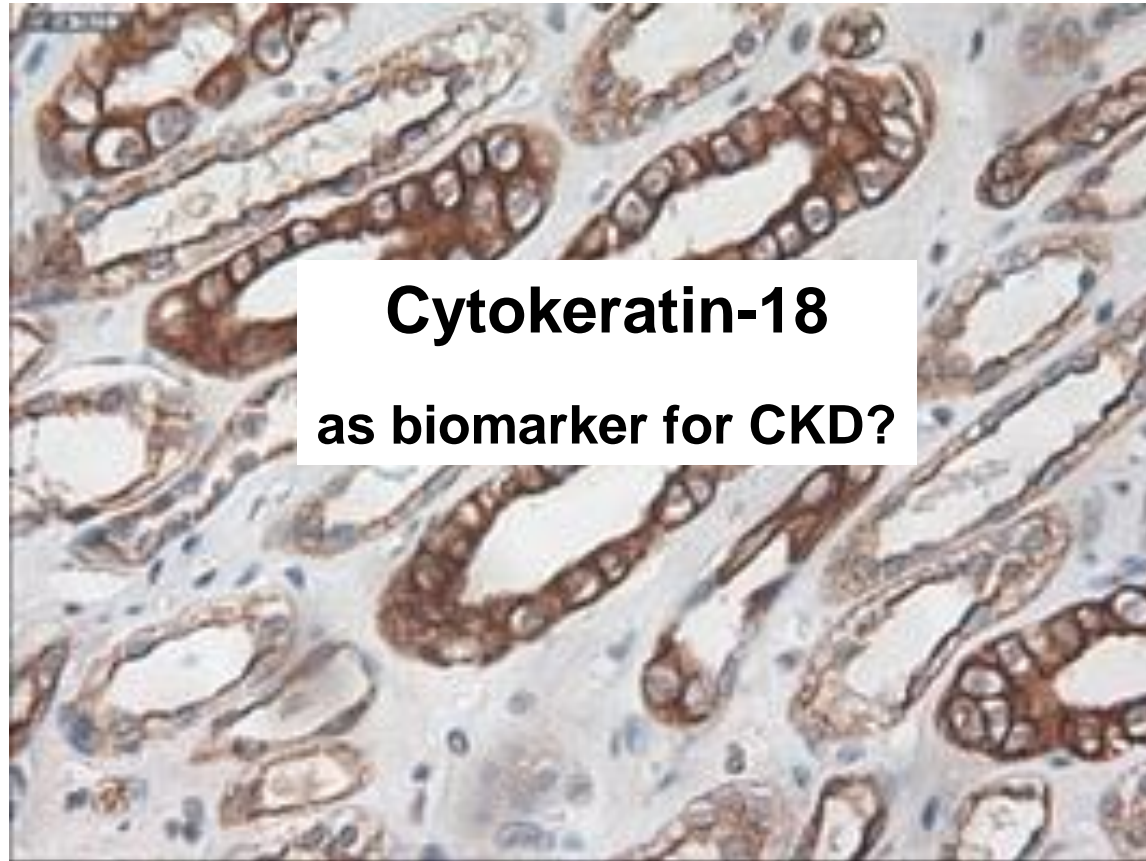
Potential biomarkers for chronic kidney disease

Diana Leberherz-Eichinger
3rd of october

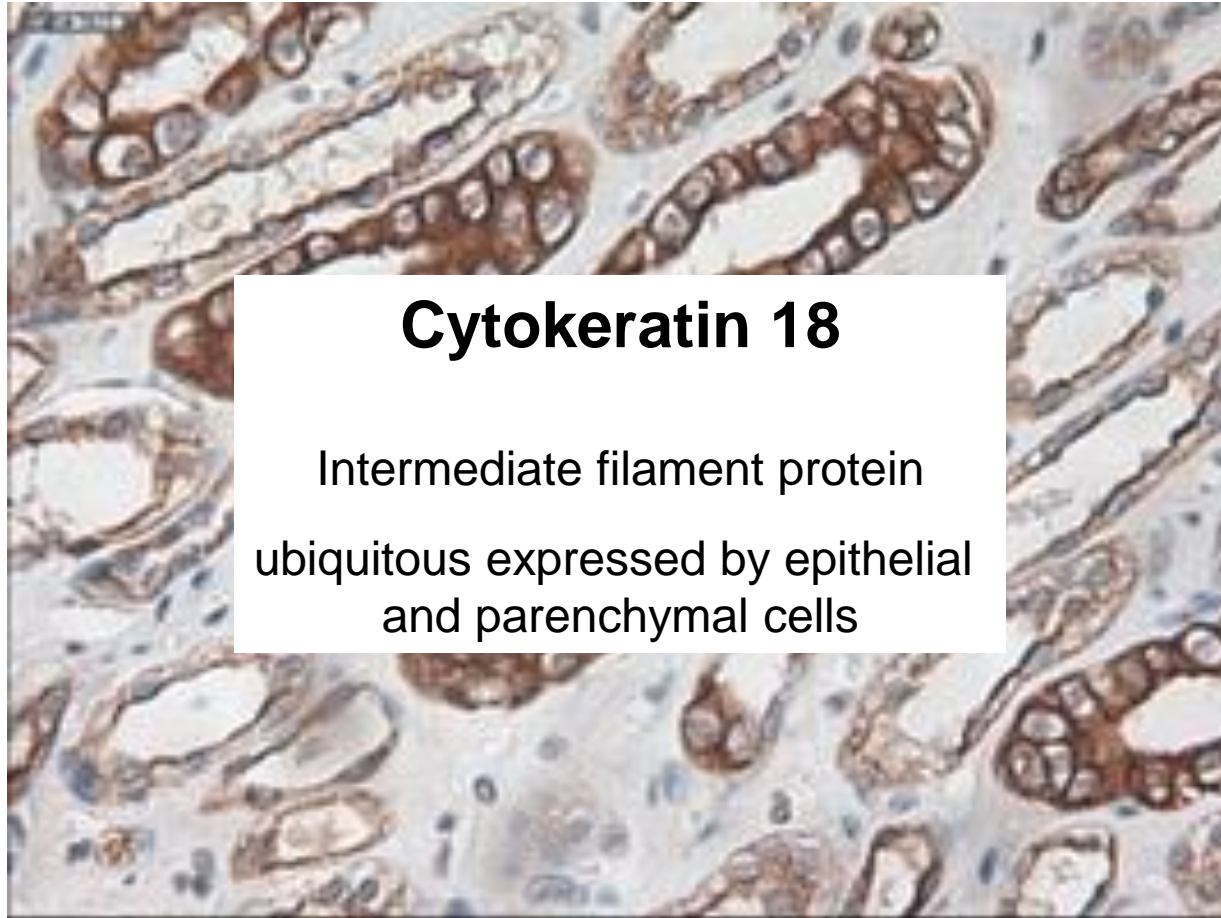
Course of Chronic kidney disease



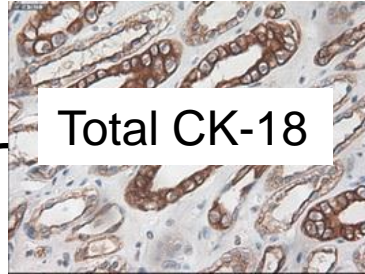




http://www.novusbio.com/Cytokeratin-18-Antibody-1E1_NBP1-47817/workingwith.html



Background – CK18



Necrosis

total CK-18 ↑



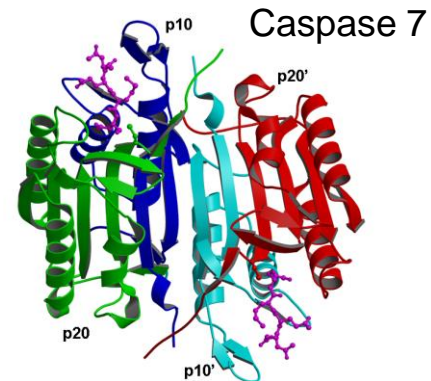
Serum levels





Apoptosis

total CK-18 cleaved by caspases 3, 7 and 9



<http://asterix.cs.gsu.edu/~weber/caspase.html>

Necrosis

total CK-18 ↑



<http://www.hg-kempen.de/bilder/Blutrohrchen.jpg>

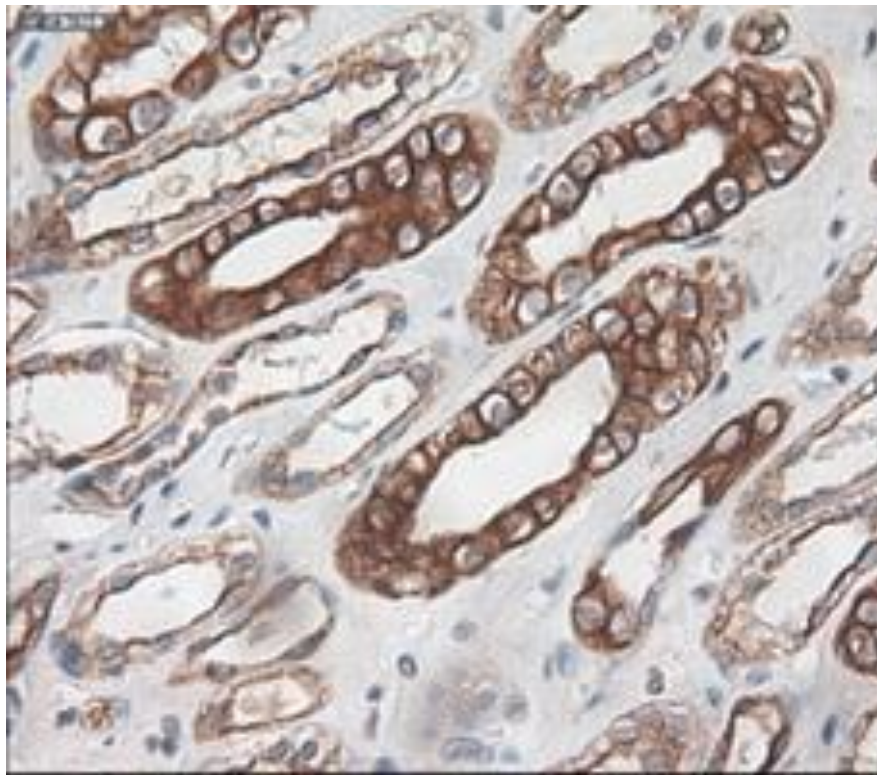
Caspase cleaved CK-18 ↑

CK -18 as biomarker for CKD?

comparison

CK - 18

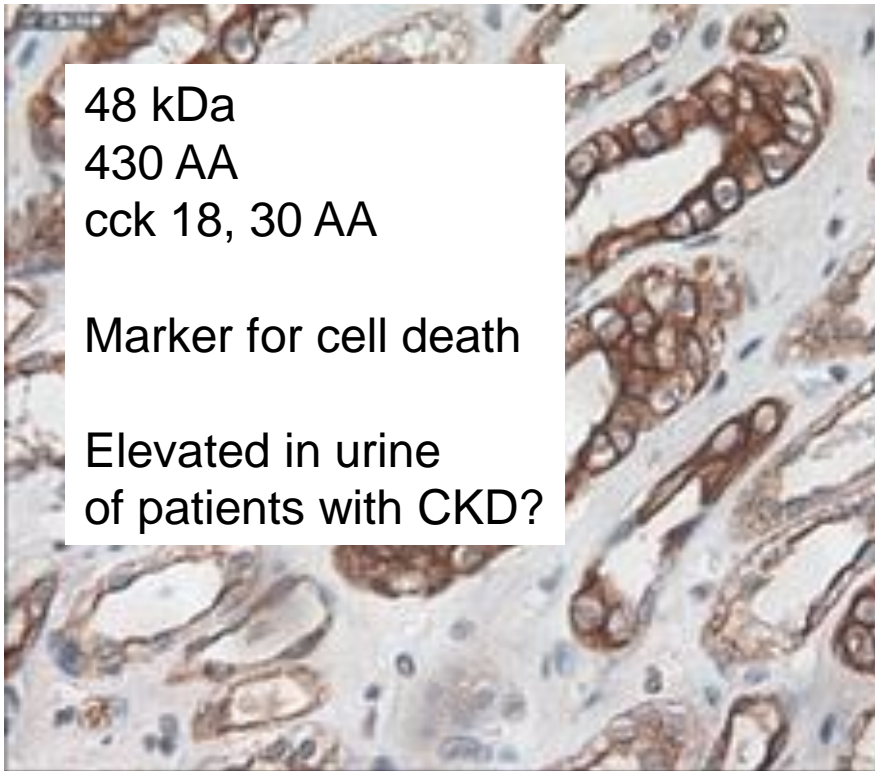
Albumin



CK -18 as biomarker for CKD? comparison

CK - 18

Albumin



48 kDa
430 AA
cck 18, 30 AA

Marker for cell death

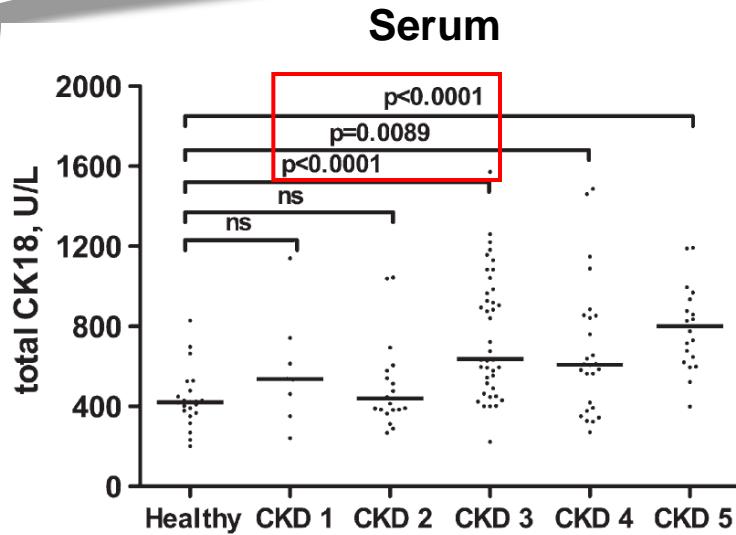
Elevated in urine
of patients with CKD?



66 kDa
585 AA
Mikroalbuminurie: 7 %

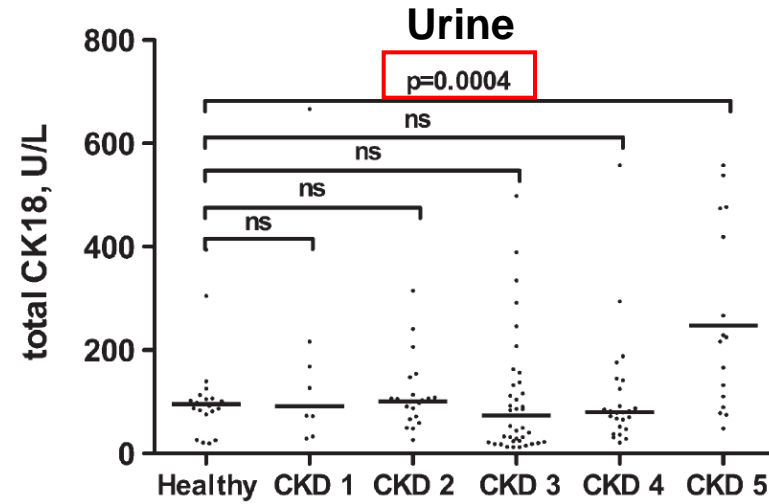
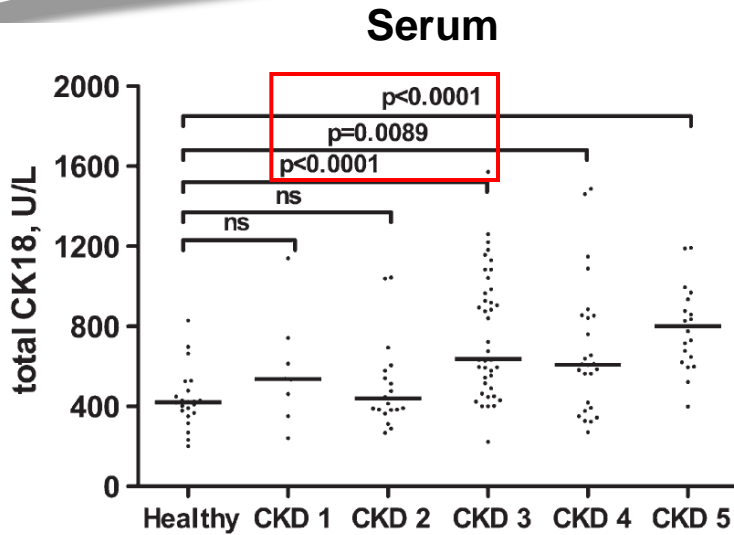
Results – CK18

total CK -18 levels
in 120 patients + 20 controls



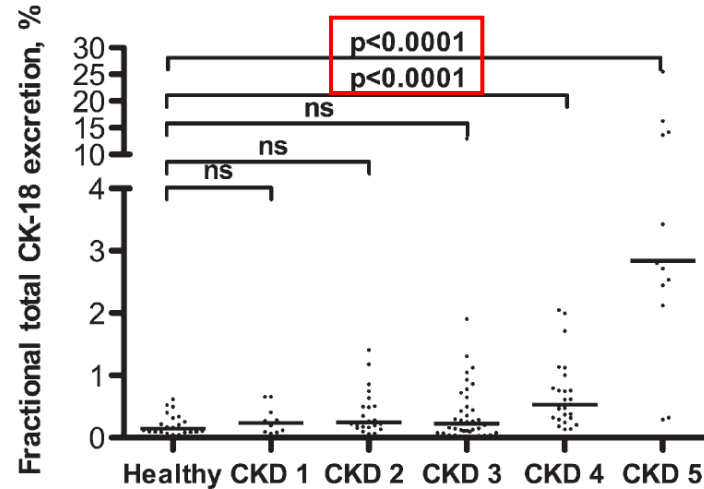
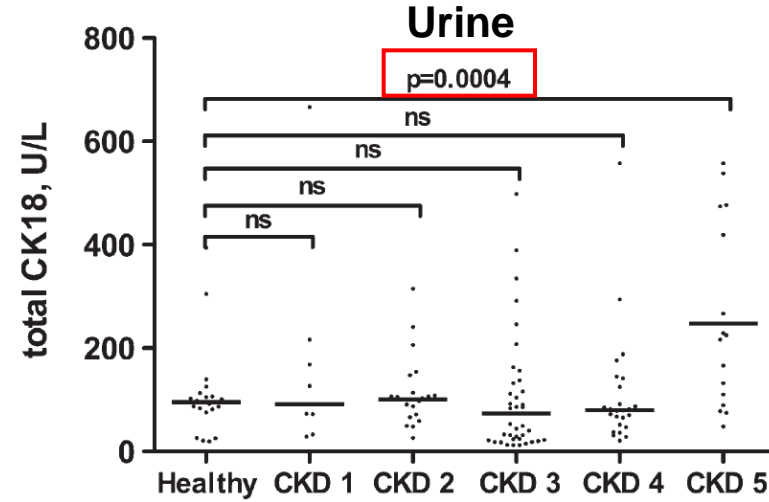
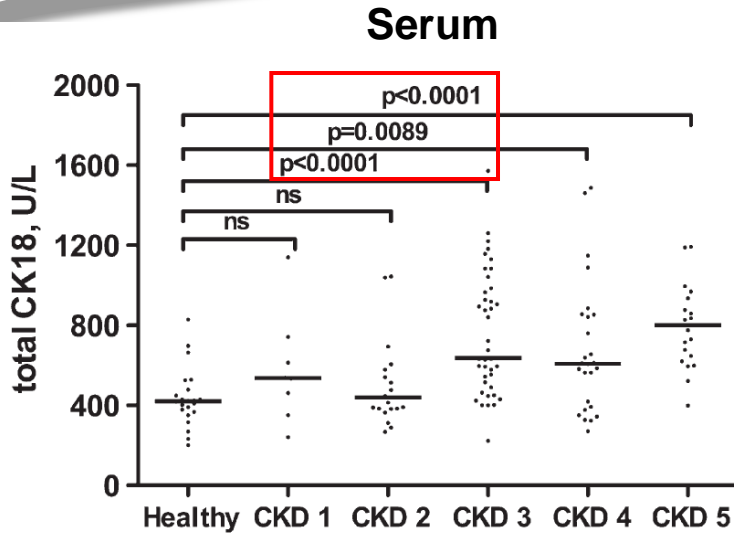
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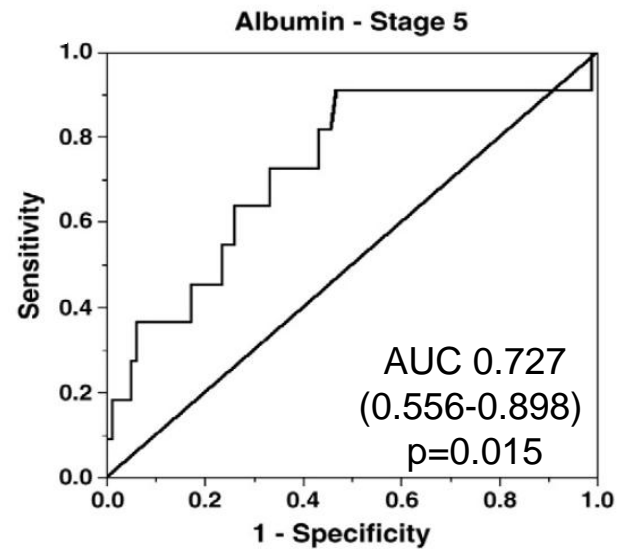
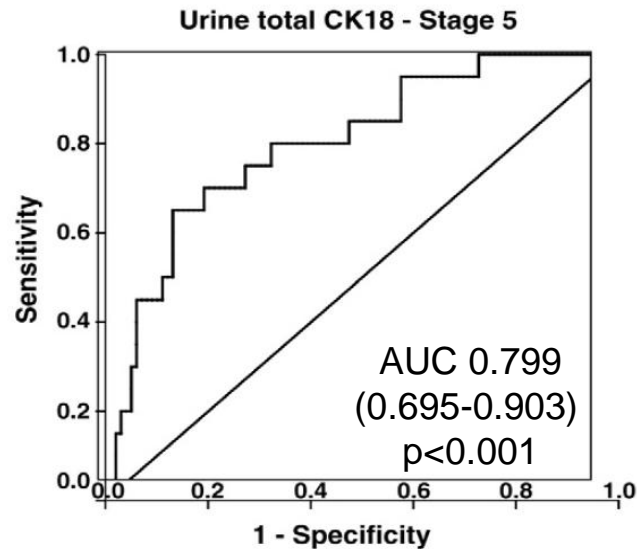
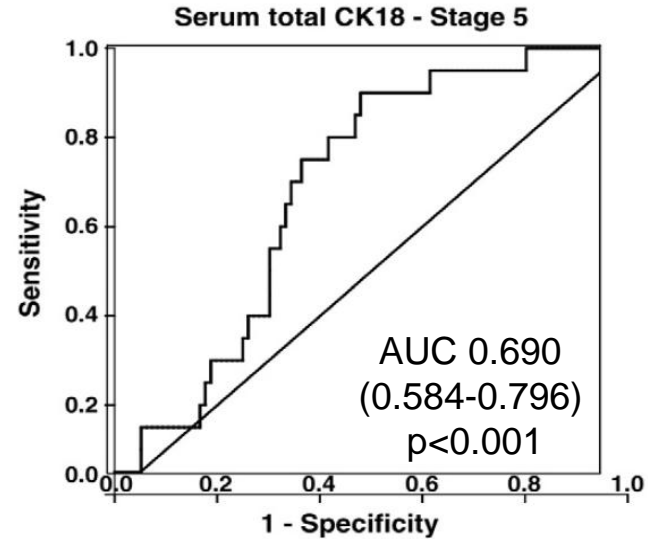
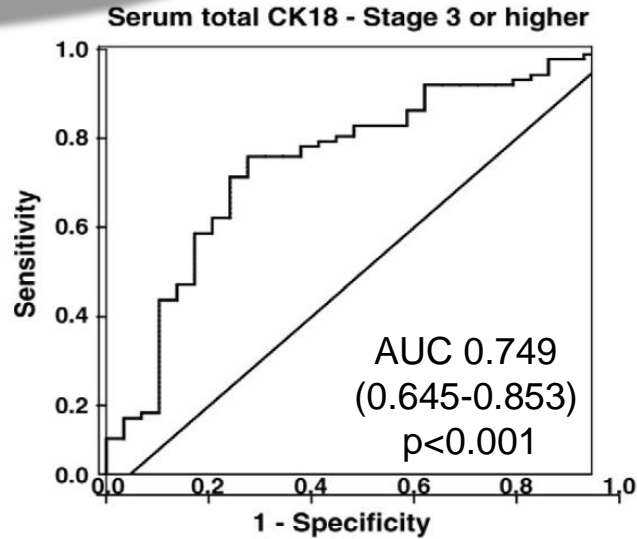
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Results – CK18

ROC curve analysis total CK-18 levels



Findings in patients with CKD - summary

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- caspase cleaved CK-18 **NOT elevated** in serum or urine

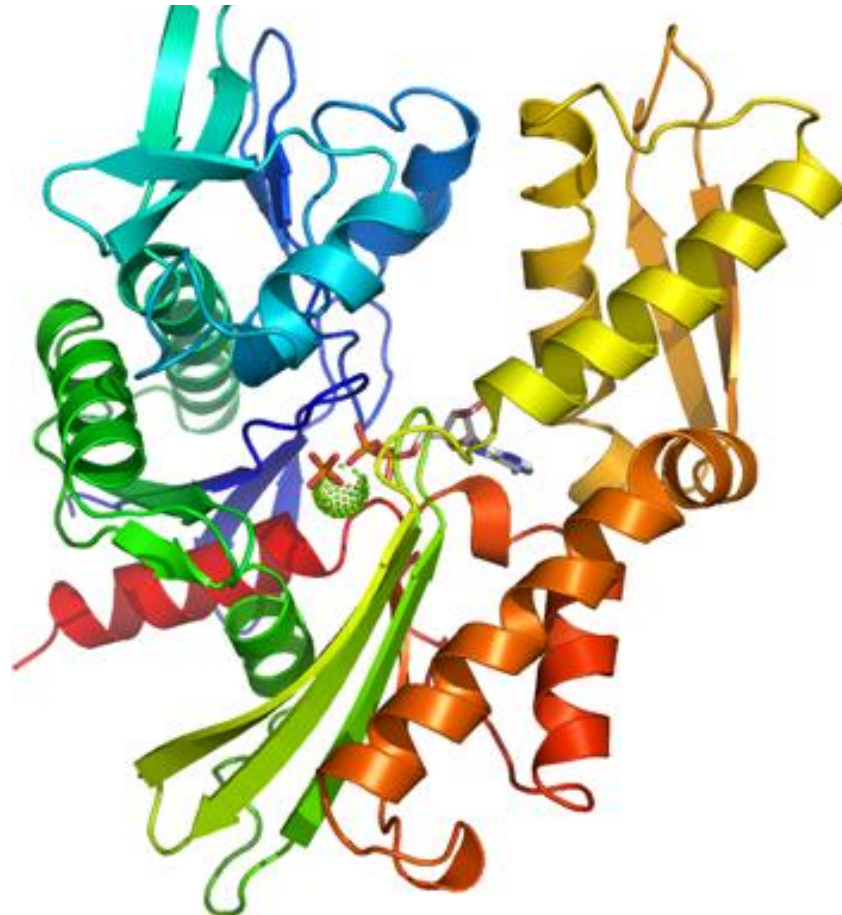
Findings in patients with CKD - summary

- caspase cleaved CK-18 **NOT elevated** in serum or urine
- total CK-18 significant **elevated** in serum and urine

Findings in patients with CKD - summary

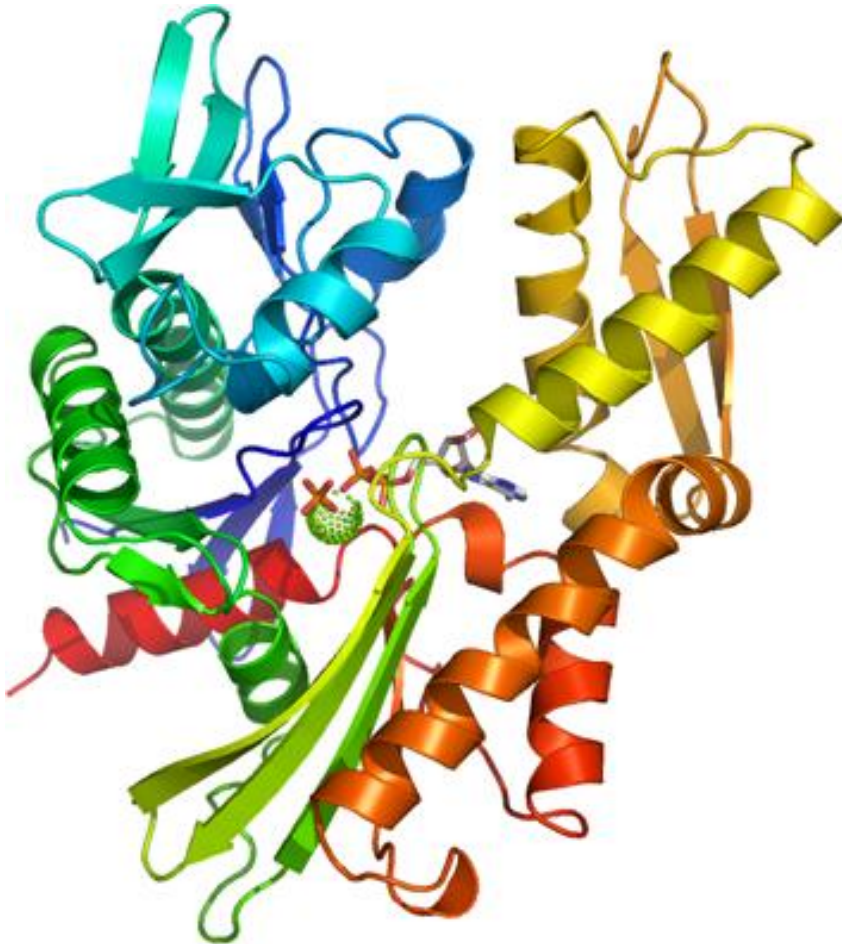
- caspase cleaved CK-18 **NOT elevated** in serum or urine
 - total CK-18 significant **elevated** in serum and urine
- indication for increased necrotic cell death

Heat shock proteins as biomarkers for CKD?

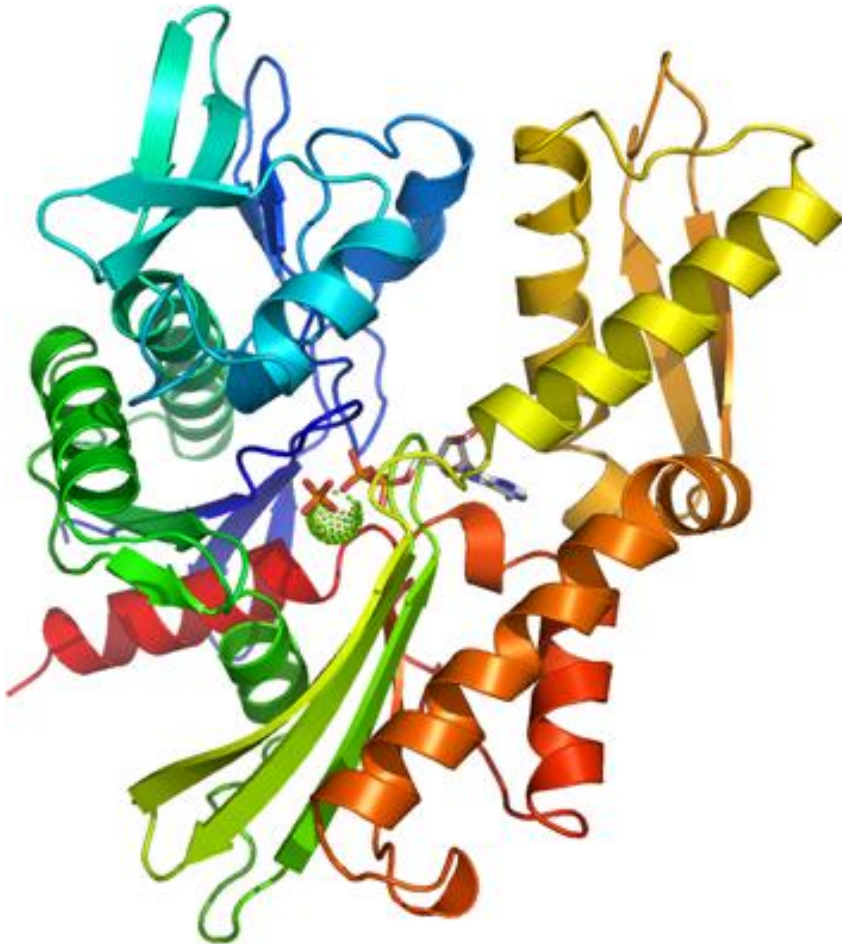


Heat shock proteins

- Cellular defense mechanisms
- Inhibit apoptotic pathways



Heat shock proteins

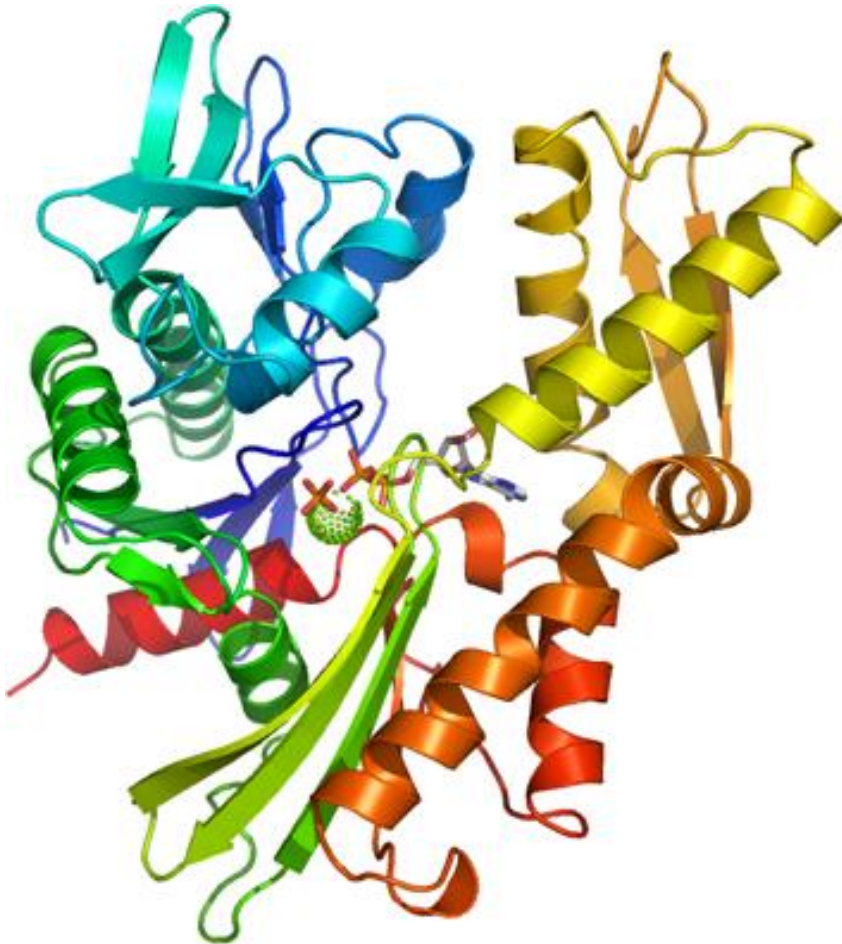


- Cellular defense mechanisms
- Inhibit apoptotic pathways

HSP27: 27kDa

- Inhibits caspase activation
- Anti-oxidant qualities

Heat shock proteins



- Cellular defense mechanisms
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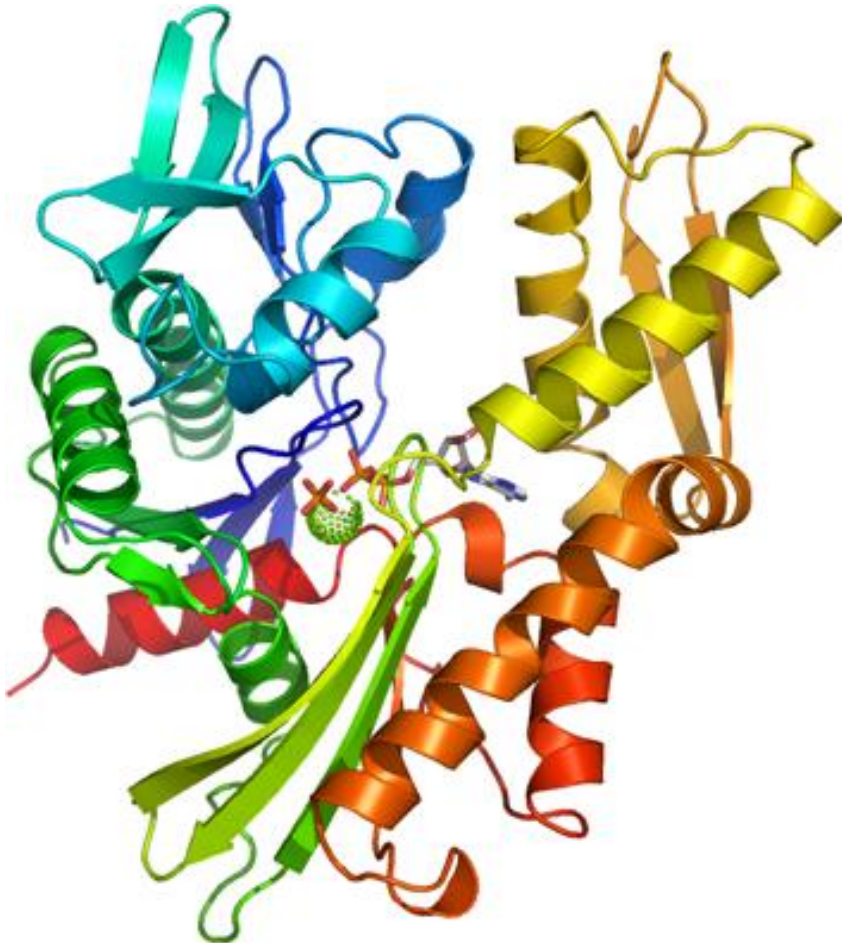
HSP27: 27kDa

- Inhibits caspase activation
- Anti-oxidant qualities

HSP70: 70kDa

- Inhibits intrinsic and extrinsic pathways
- Inhibits cell damage by urea *in vitro*

Heat shock proteins



- Cellular defense mechanisms
- Inhibit apoptotic pathways

HSP27: 27kDa

- Inhibits caspase activation
- Anti-oxidant qualities

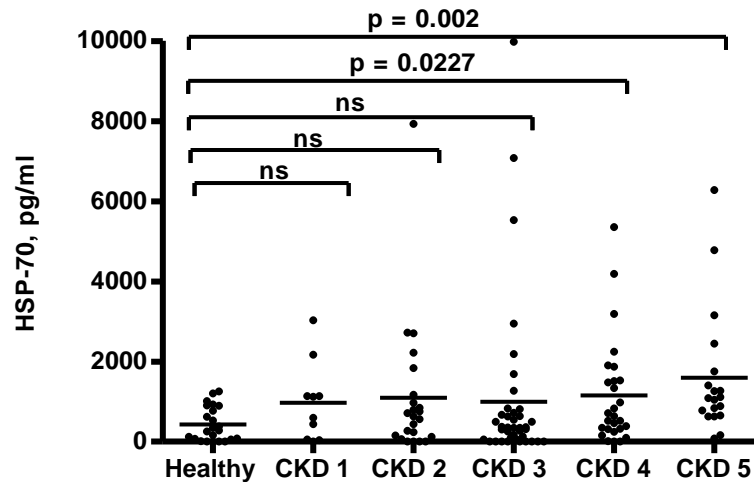
HSP70: 70kDa

- Inhibits intrinsic and extrinsic pathways
- Inhibits cell damage by urea *in vitro*
- Unchanged serum levels in children with CKD (Musial et al. 2010)
- Decreased expression in blood monocytes of adults with CKD (Marzec et al. 2009)

Results – HSP

HSP70 levels

Urine (119 patients, 23 controls)

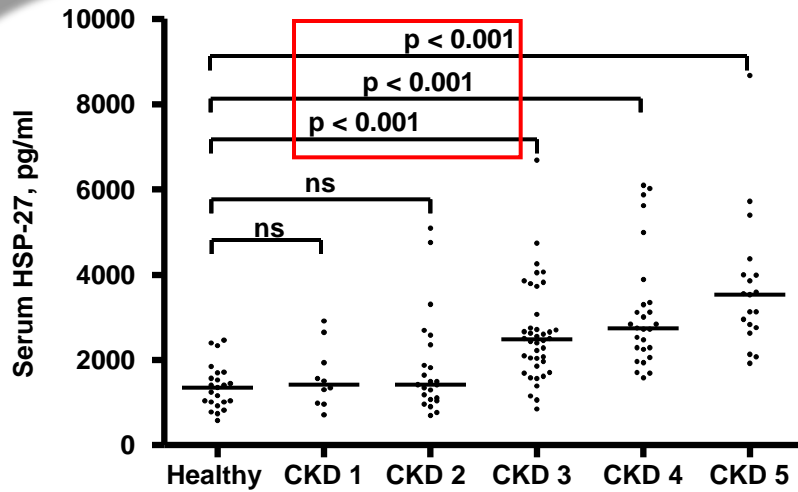


Serum levels: technical limitations,
data for remaining 42 patients not significant

Fractional excretion: significant elevated in stage 5 (p = 0.0027)

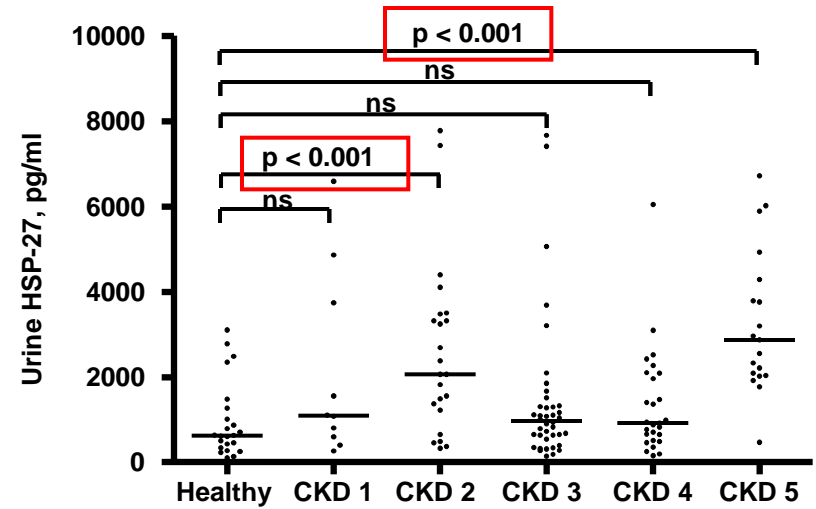
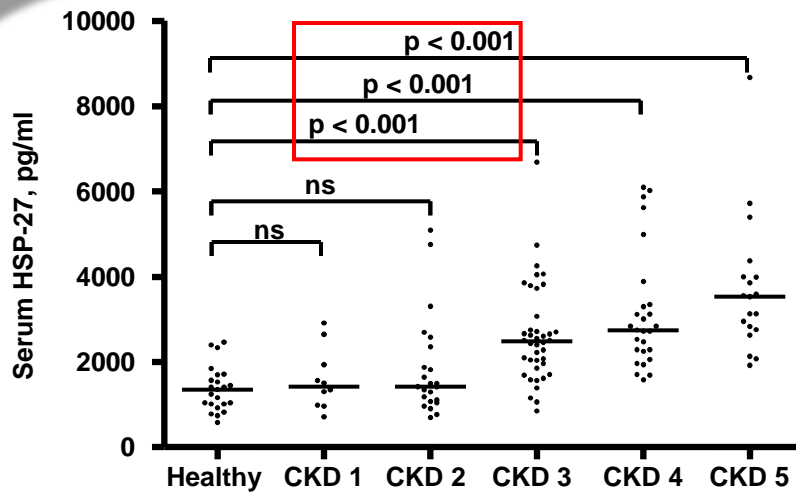
Results – HSP

HSP27 levels
in 119 patients + 23 controls



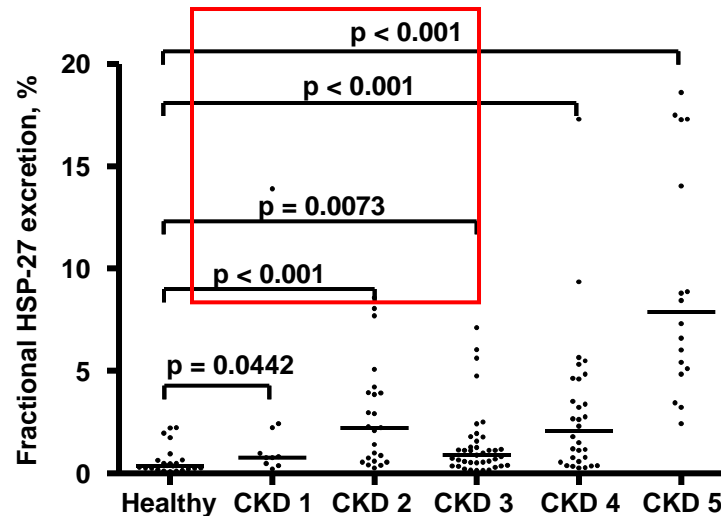
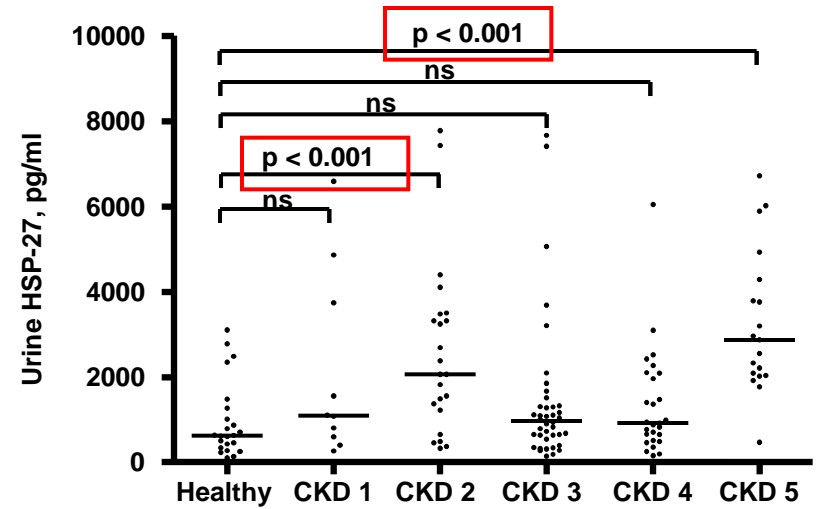
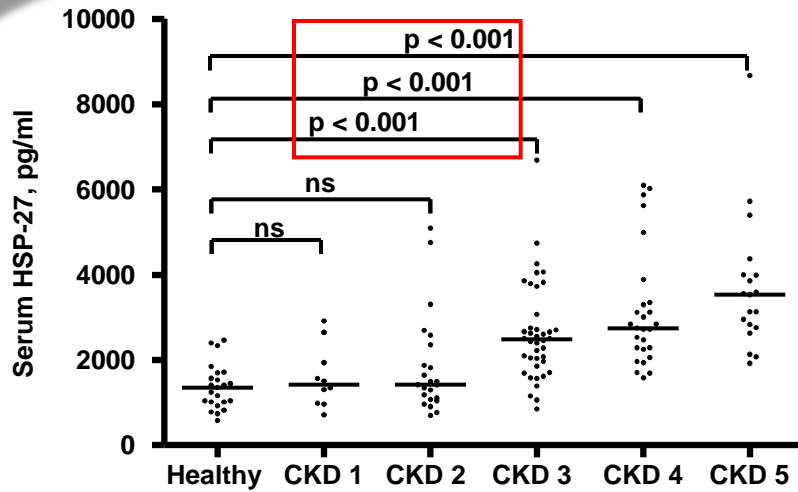
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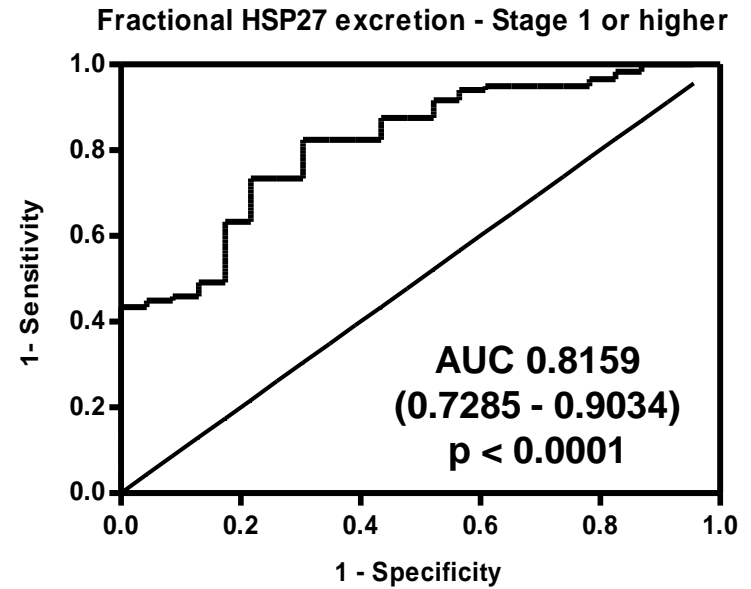
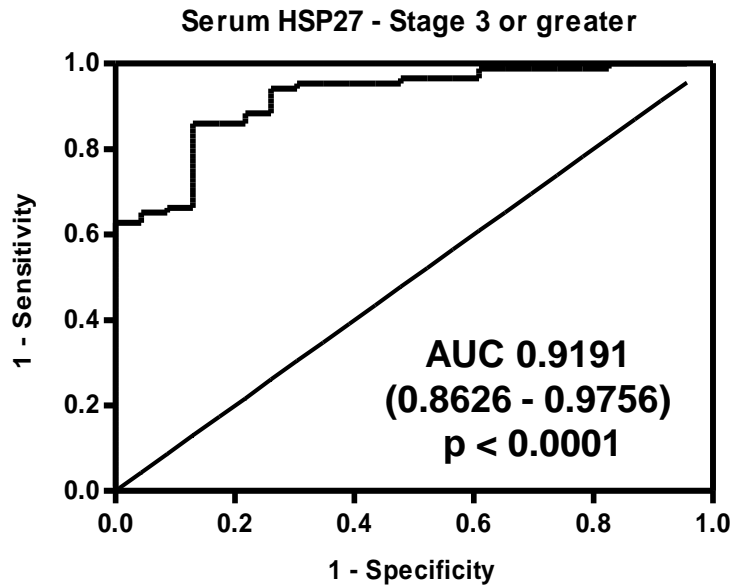
Results – HSP

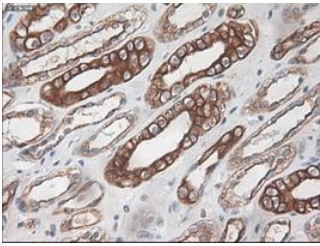
HSP27 levels in 119 patients + 23 controls



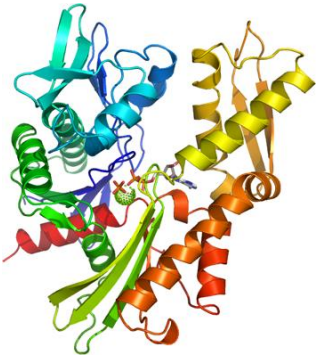
ROC curve analysis

HSP27





- total CK-18 significant **elevated** in serum and urine
→ indication for increased necrotic cell death



- Heat shock proteins significant **elevated** in serum and urine
→ counterregulation to increased systemic stress levels



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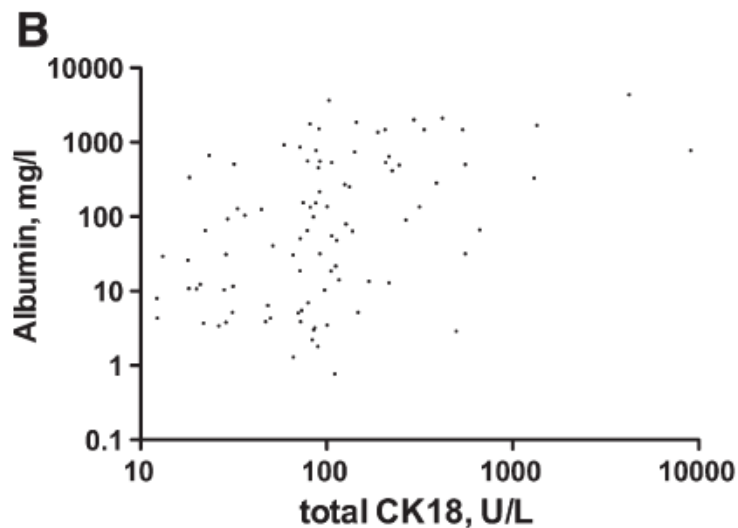
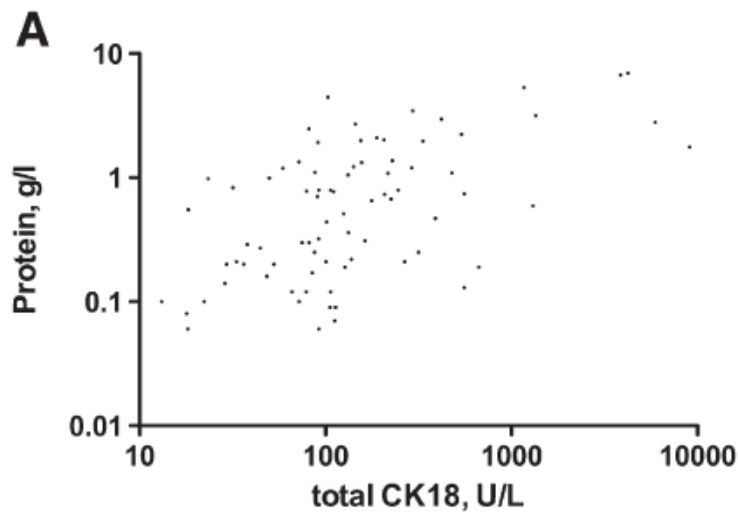
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Thank you for your attention

Potential biomarkers
for chronic kidney disease

	All patients	CKD 1	CKD 2	CKD 3	CKD 4	CKD 5	Controls
	120	10 (8.3%)	23 (19.2%)	41 (34.2%)	27 (22.5%)	19 (15.8%)	23
Age (years)	59 (19–88)	36 (19–61)	50 (19–80)	63 (23–78)	61 (29–88)	65 (20–81)	30 (21–67)
Gender (male/female)	67/53	7/3	9/14	27/14	15/12	9/10	15/8
Kidney disease							
Glomerulonephritis	30	4	7	13	7	3	
Polycystic kidney disease	16	2	3	8	2	1	
Vascular nephropathy	26	2	5	7	8	4	
Interstitial nephropathy	5		1	1	1	2	
Bilateral nephrectomy	2		1			1	
Urine stasis	5		1	1	1	2	
Unknown	36	2	5	11	8	6	
Serum creatinine (mg dL ⁻¹)	1.84 (0.72–6.88)	0.90 (0.72–1.03)	0.99 (0.77–1.52)	1.62 (1.02–2.34)	2.73 (2.04–3.89)	5.00 (3.47–6.88)	0.99 (0.77–1.20)
Blood urea nitrogen (mg dL ⁻¹)	31.9 (7.1–91.2)	12.6 (7.5–17.6)	13.9 (7.1–33.4)	30.5 (11.6–64.1)	51.2 (23.8–91.2)	63.3 (31.9–87.3)	13.1 (8.2–20)
Urine creatinine (mg dL ⁻¹)	69.2 (12.7–294.5)	69.1 (22.7–252.9)	77.3 (14.8–243.5)	78 (12.7–294.5)	60.9 (29.3–172.1)	41.65 (17.7–108.2)	
Urine urea (mg dL ⁻¹)	844 (247–2557)	948 (336–1814)	976 (247–2557)	923 (291–2464)	854 (267–1370)	647 (273–1381)	
Urine protein (g L ⁻¹)	0.2 (<0.05–6.94)	0.06 (<0.05–0.21)	0.1 (<0.05–4.45)	0.1 (<0.05–2.79)	0.29 (<0.05–3.46)	1.07 (0.05–6.94)	
GOT (U L ⁻¹)	22 (9–88)	24.5 (15–88)	22 (12–62)	26 (14–57)	22 (9–60)	18 (10–69)	
GPT (U L ⁻¹)	21 (<3–98)	29.5 (11–75)	20 (11–72)	25 (9–63)	17 (6–51)	15 (<3–98)	
Gamma-GT (U L ⁻¹)	28.5 (9–561)	38.5 (13–561)	25 (10–134)	36 (9–472)	27 (10–303)	24 (13–346)	
LDH (U L ⁻¹)	183.5 (81–333)	157.5 (147–289)	172 (129–297)	195 (92–333)	197 (81–285)	185 (109–259)	

Date are given as median with range; CKD, chronic kidney disease.



Correlation CK-18

	Total CK-18 serum	Total CK-18 urine
Age (120 pairs)	$r = 0.177, p = 0.06$	$r = 0.063, p = 0.495$
Estimated glomerular filtration rate (120 pairs)	$r = -0.2242, p = 0.0138$	$r = -0.228, p = 0.0125$
Amount of excreted urine, ml (33 pairs)	$r = 0.190, p = 0.2808$	$r = 0.121, p = 0.495$

Fig. 2. Panel A: Total urine protein and total urine CK-18 correlated significantly using Spearman's rank correlation coefficient (Spearman's $r = 0.5178, p < 0.0001, 119$ pairs). The X as well as the Y-axis is given as log scale. Panel B: Urine albumin and total urine CK-18 correlated significantly using Spearman's rank correlation coefficient (Spearman's $r = 0.4377, p < 0.0001, 119$ pairs). The X as well as the Y-axis is given as log scale.

Correlation of HSP serum and urine levels with clinical and kidney function parameters.

	HSP27 serum	HSP27 urine	HSP70 urine
Age (119 pairs)	$r = 0.3625, p < 0.0001$	$r = -0.1249, p = 0.1760$	$r = -0.04412, p = 0.6338$
Estimated glomerular filtration rate (119 pairs)	$r = -0.5414, p < 0.0001$	$r = -0.01213, p = 0.1887$	$r = -0.1770, p = 0.0541$
Total urine protein (118 pairs)	$r = 0.3599, p < 0.0001$	$r = 0.2364, p = 0.0100$	$r = 0.2187, p = 0.0174$
Urine albumin (92 pairs)	$r = 0.3244, p = 0.0016$	$r = 0.1935, p = 0.0645$	$r = 0.1055, p = 0.3170$
Creatinine clearance (33 pairs)	$r = -0.595, p = 0.0003$	$r = -0.2697, p = 0.129$	$r = -0.2635, p = 0.1385$
CRP (118 pairs)	$r = 0.2804, p = 0.0021$	$r = 0.04044, p = 0.6637$	$r = 0.01164, p = 0.9005$