# The urine biomarker panel [IGFBP7]x[TIMP-2] (NephroCheck® parameter) does not correlate with IGFBP7 and TIMP-2 gene expression in urinary sediment

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**Background:** Acute kidney injury (AKI) is frequently observed in serious infections, following nephrotoxic medication, surgery and trauma. Here we tested whether the detection of two recently identified biomarkers for AKI, Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) and Insulin-Like Growth Factor Binding Protein 7 (IGFBP7), depends on the expression of these proteins in cells of the urinary sediment.

**Method:** We collected urine samples of 24 kidney transplant recipients and 14 non-transplanted patients who all had AKI (stages 1-3 according to KDIGO), and measured [IGFBP7]x[TIMP-2] using the NephroCheck® Astute140 ™ meter. Concomitantly, we analyzed IGFBP7 and TIMP-2 mRNA expression by quantitative polymerase chain reaction (qPCR) from urinary sediment of the same patients, and correlated the results with [IGFBP7]x[TIMP-2] (protein), by linear regression analysis. We also determined the association between [IGFBP7]x[TIMP-2] and estimated glomerular filtration rate (eGFR), and between IGFBP7 and TIMP-2 mRNA expression and markers of inflammation. Light microscopy and confocal immunofluorescence served to illustrate changes in the urinary sediment over the time course of renal function improvement.

**Demographics and laboratory values**

| | All | Normal Range |
|---|---|---|
| Number of patients (%) | 38 (100) | |
| Transplant recipients (%) | 24 (63.16) | |
| Female (%) | 13 (34.21) | |
| Age [years] | 59±16 | |
| AKI stage I (%) | 12 (31.58) | |
| Transplant recipients | 10 | |
| AKI stage II (%) | 15 (39.47) | |
| Transplant recipients | 10 | |
| AKI stage III (%) | 11 (28.95) | |
| Transplant recipients | 4 | |
| Patients requiring haemodialysis (%) | 3 (7.89) | |
| Transplant recipients | 2 | |
| sCr [mg/dl] | 4.2±2.6 | 0.5-0.9 |
| eGFR [ml/min/1.73m$^2$] | 23.78±17.2 | >90 |
| NephroCheck® score [(ng/ml)$^2$/1000] | 1.2±2.68 | |
| CRP [mg/dl] | 5.9±7.9 | <0.5 |

Categorical variables are reported as counts and frequencies. Continues variables are reported as medians and ranges, or means ± standard deviations.

**Table 1.** Patients´ demographics and laboratory values.

| renal transplant | N |
|---|---|
| cause of AKI | |
| pyelonephritis | 9 |
| delayed graft function | 10 |
| rejection episode | 2 |
| wound infection | 1 |
| hypovolemic shock | 1 |
| kidney transplantation | 1 |
| | |
| non transplanted | N |
| cause of AKI | |
| chemotherapy induced kidney injury | 3 |
| sepsis | 3 |
| pyelonephritis | 1 |
| dehydration | 1 |
| hemolytic uremic syndrome | 1 |
| cardiac failure | 2 |

**Table 2.** Underlying diseases most likely causing AKI in renal transplant patients

**Conclusion:** The gene expression pattern of IGFBP7 and TIMP-2 from urinary sediment, which contains desquamated renal tubular epithelial cells, did not correlate with [IGFBP7]x[TIMP-2] protein, indicating that IGFBP7 and TIMP-2 measured in the NephroCheck® test originated predominantly from intact but stressed cells of the kidney itself.

**Figure 1 A.** Pearson square pairwise correlation of NephroCheck (NC)-score with relative change of TIMP-2 mRNA expression in urinary sediment cells taking three normal kidney tissues as reference level. **1 B.** Pearson square pairwise correlation of NC-score with relative IGFBP7 in urinary sediment cells taking three normal kidney tissues as reference level. **1 C.** Pearson square pairwise correlation of NC-score with serum creatinine levels (mg/dl). **1 D.** Pearson square pairwise correlation of NC-score with CRP (mg/dl).

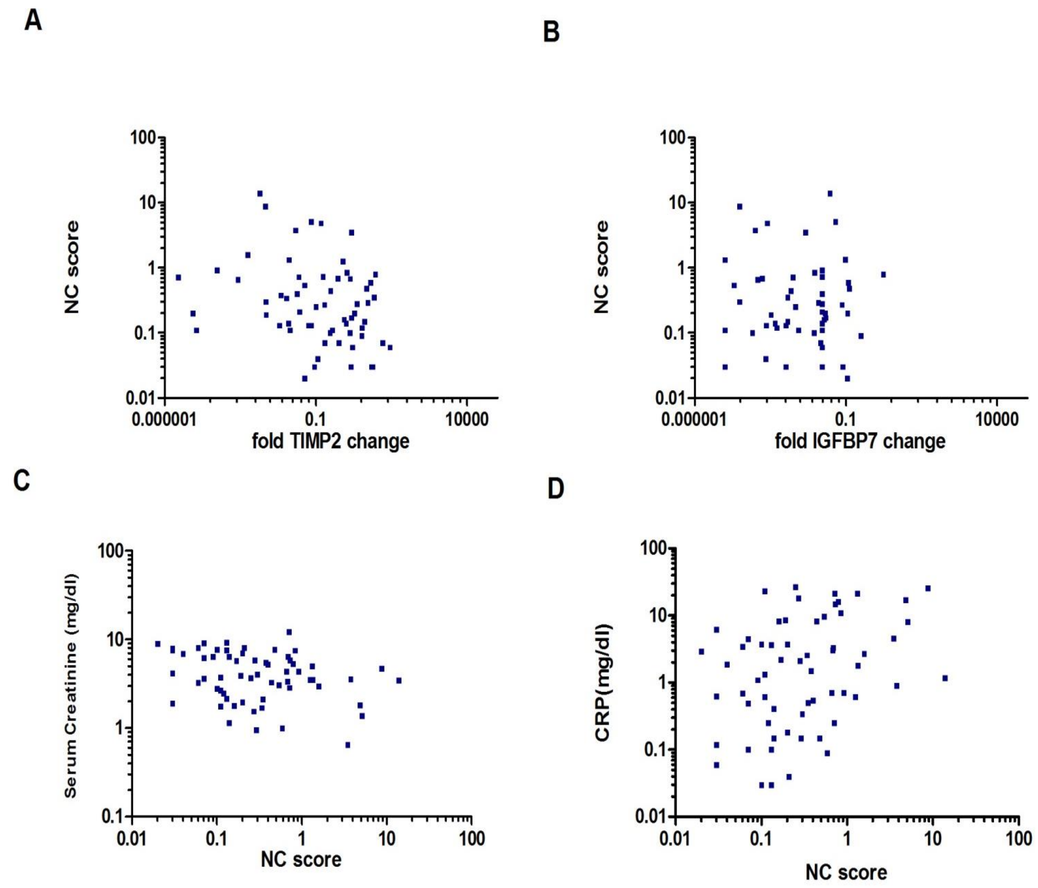


**Figure 2a)** A representative patient after kidney transplantation, but requiring hemodialysis (HD) throughout the first week post-transplant (delayed graft function). Improvement of renal function was accompanied by an increase of urinary sediment TIMP-2 expression, and decrease in NC score. 100 days after kidney transplantation the patient had a serum creatinine of 2.1 mg/dL.

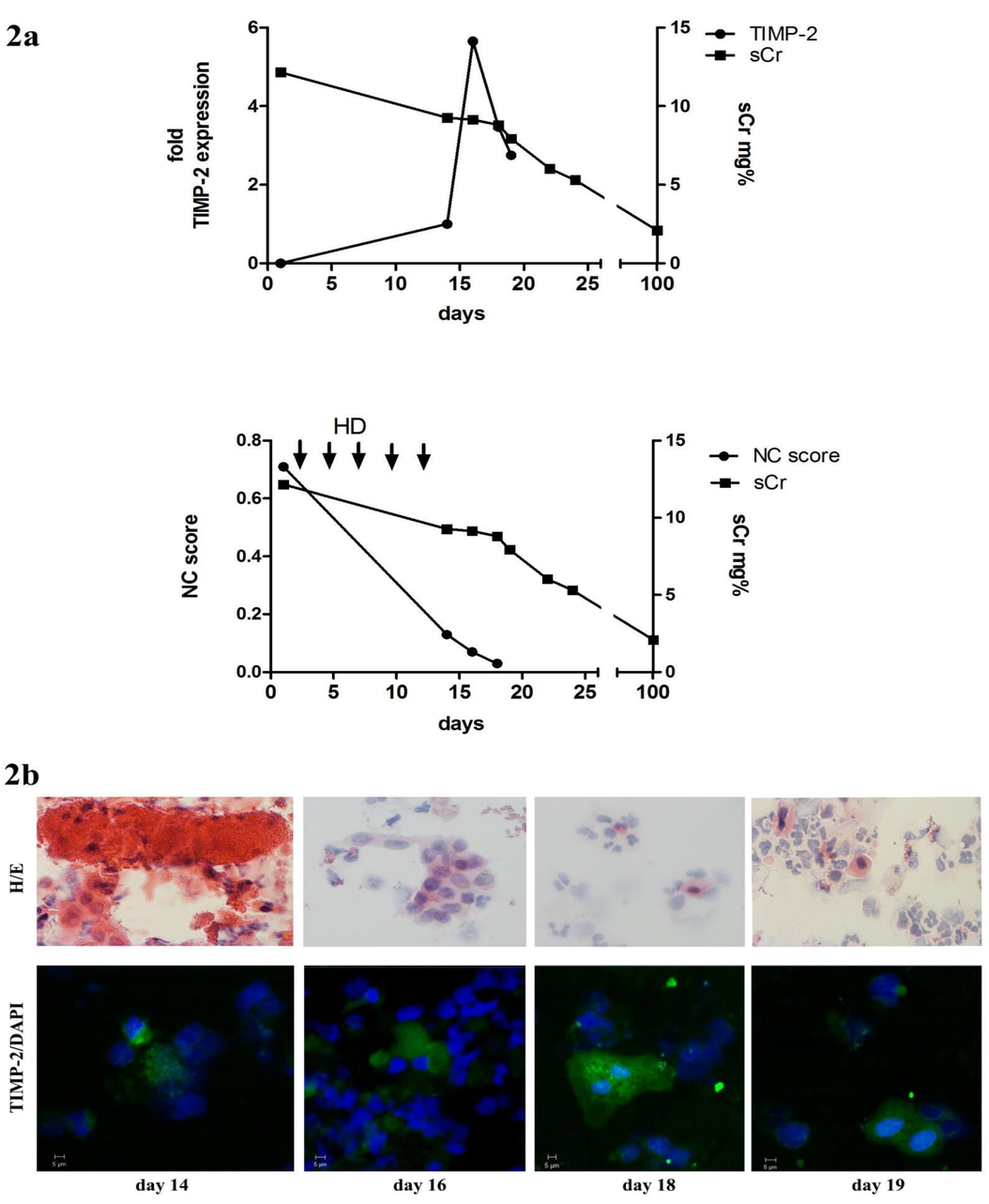


**Figure 2b)** HE (upper panel) and confocal immunofluorescence staining (lower panel) of urine sediment from the representative patient with delayed graft function. Many morphologically disintegrated cells including casts appeared in urine (day 14). This changed rapidly the following days, cell number in urine increased from day 14 to day 17. Furthermore, the percentage of TIMP-2 positive staining cells increased with presence of a mitosis (day 18) while the number of disintegrated cells decreased, accompanied by rapid fall in NC-score and decrease in sCr.


DOI: 10.3252/pso.eu.54ERA.2017