# RNA profiling of urinary extracellular vesicles (EV) identifies T cell-mediated rejection (TCMR) in kidney transplantation

**Sergio Dellepiane[1], Vincenzo Cantaluppi[1], Masato Mitsuhashi[2], Taku Murakami[2], Alfredo Benso[3], Luigi Biancone[1], Giovanni Camussi[1]**

[1]Nephrology, Dialysis and "A. Vercellone" Kidney Transplantation Unit, University of Torino, "Città della Salute e della Scienza-Molinette" Hospital, Torino, Italy;
[2]Hitachi Chemical Research Center, Inc., Irvine, CA (USA);
[3] Department of Computer and Control Engineering, Polytechnic of Torino, Italy

## BACKGROUND AND AIM

T cell-mediated rejection (TCMR) is a leading cause of loss of kidney transplant (KT) function. Several urine and plasma biomarkers, including Neutrophil Gelatinase Associated Lipocalin (NGAL), have been proposed for non invasive diagnosis of TCMR[1]. However, graft biopsy is still necessary in the clinical practice. Urinary extracellular vesicles (EV) may have a role as early and accurate biomarkers of TCMR. EV are nanoparticles, involved in cell-to-cell communication, able to shuttle specific proteins, lipids and nucleic acids including mRNA and microRNA (miRNA). In this study, we evaluated the size and the concentration of urinary EV from KT patients. Moreover, we analyzed EV mRNA/microRNA profiling as potential noninvasive markers of TCMR.

**Fig 1**

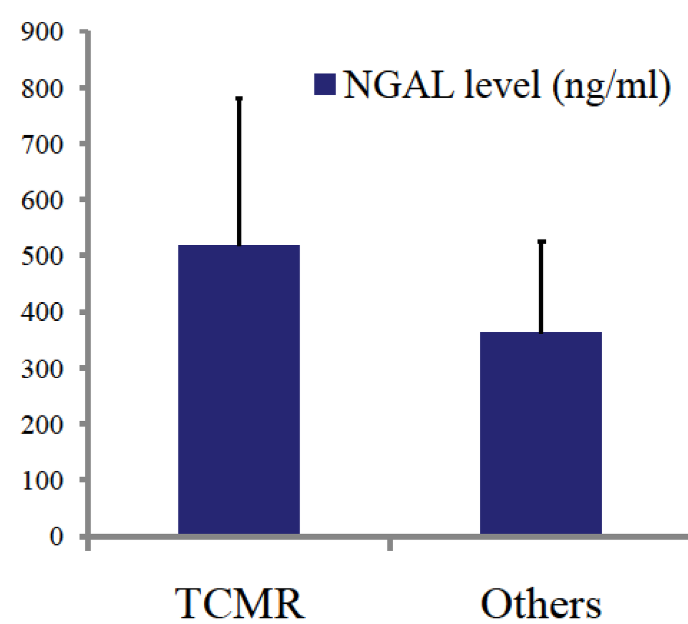


**Fig 2**

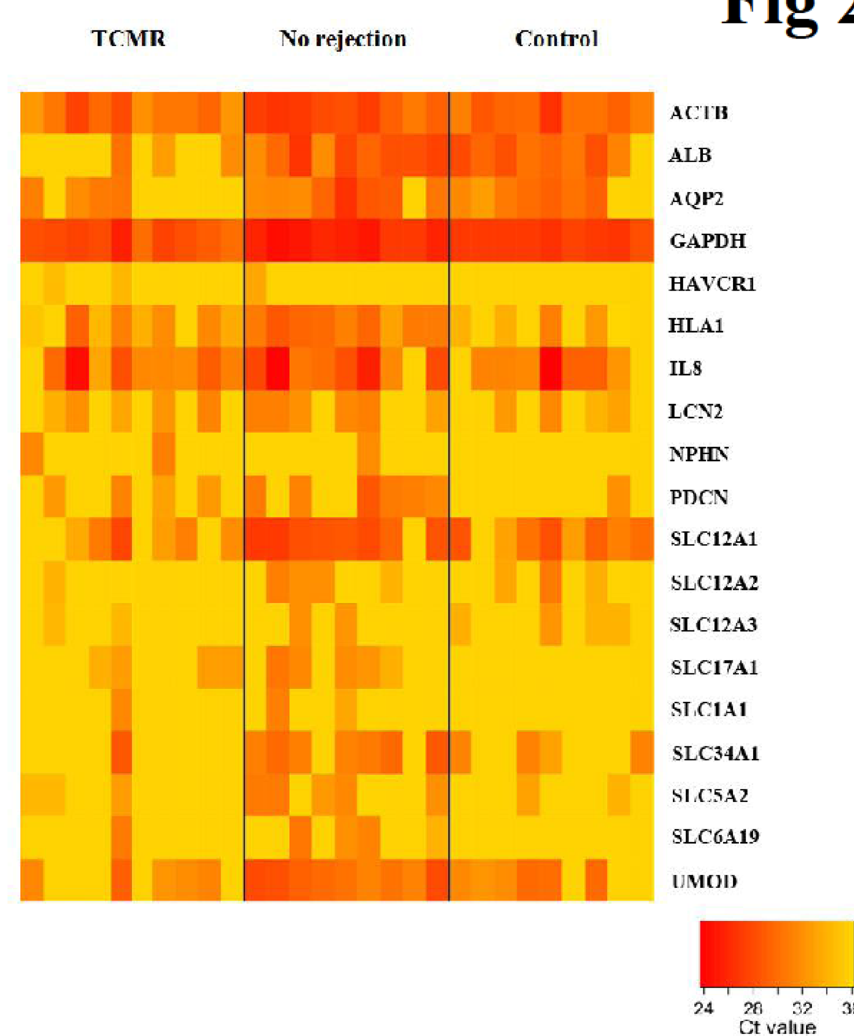


**Fig 3**

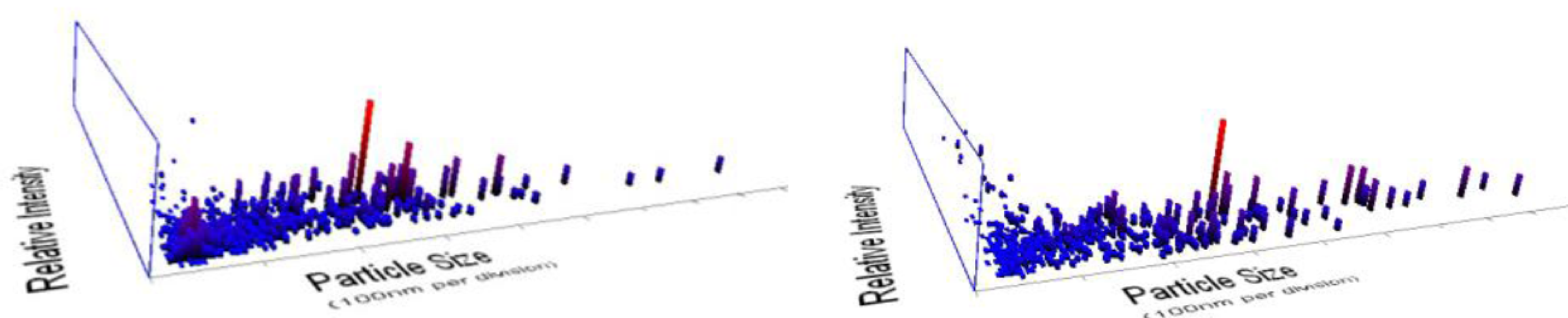


**Fig 4**

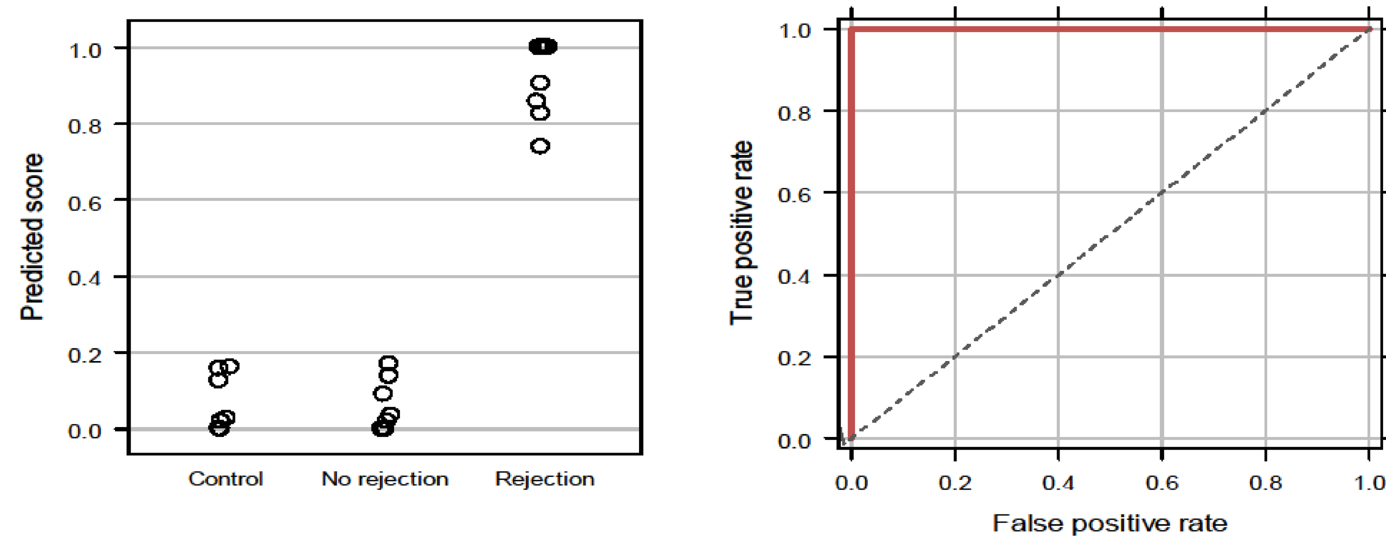


**Fig1:** NGAL levels distinguish TCMR from other causes of KT failure. **Fig2:** gene profiling of urinary EV in patients with TCMR, other rejection types, non rejection-associated KT failure and healthy controls. **Fig3:** example of nanoparticle analysis; TCRM (left), normal histology (right) **Fig4:** logistic regression analysis of EV mRNA accurately detects TCMR (AUC=1)

## METHODS

Urine samples of 65 KT admitted to hospital for graft biopsy were collected to evaluate EV concentration and size by nanoparticle tracking analysis (Nanosight, UK). Plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) was also evaluated (Alere, San Diego, CA). In 20/65 patients (10 stable function, 10 biopsy-proven TCMR) and 10 healthy controls, mRNA profiling of urine EV was performed by collection devices (Hitachi Chemical Research Center, Inc., CA) followed by poly(A)$^+$ RNA purification and RT-qPCR for kidney-specific mRNA. To predict transplant rejection from urinary EV, mRNA data, logistic regression analysis was conducted. All the possible combinations of up to 4 genes were screened. For each gene combination, the area under the curve (AUC) was calculated through 5 repeats of 5-fold cross validation. The combinations of genes with top 500 AUC were selected for larger-scale calculation by 100 repeats of 10-fold cross validation. Identified genes were then analyzed by a systematic literature screening via the web platform ProteinQuest (Biodigital Valley, Aosta, Italy) aimed to find associated miRNA.

## RESULTS

Quantitative Nanosight analysis showed an increase of EV concentration (2.19E9$\pm$1.5E8 vs 1.29E9$\pm$1.6E8 particles/ml $p<0.05$) and a size decrease (93.1$\pm$32 vs 104$\pm$22nm; p=0.21) in TCMR not observed in KT with stable function and in non KT controls ($p<0.05$). NGAL levels were significantly higher in TCMR than in other KT patients subjected to biopsy (517$\pm$155 vs 271$\pm$92 ng/ml; $p<0.0002$). Urinary EV gene profiling showed that kidney-related mRNA (*SLC12A2*, *SLC12A1,* SLC6A19, *AQP2, UMOD*) and other nonspecific mRNA (*ACTB, ALB*) were significantly decreased in TCMR ($p<0.001$). By logistic regression analysis of quantitative mRNA expression, we then selected the gene combination with the largest AUC (AUC = 1; ALB + AQP2 + SLC12A2 + SLC6A19) to distinguish TCMR from other causes of graft functional impairment in respect to controls. This preliminary analysis partially confirmed previous published data about gene profiling of TCMR in KT biopsy[2]. By ProteinQuest, we found 5 miRNA (miR-10a, miR-142, miR-192, miR-30a and miR-let7c) associated with published data on TCMR and with mRNA found to be down-regulated in our TCMR patients. In addition, our preliminary data suggested an increase of the identified miRNA within EV. Taken together, these results suggest that the variation of RNA profiling within urinary EV may be an early diagnostic indicator of the possible de-differentiation of tubular cells during TCMR.

## CONCLUSIONS

In conclusion, the evaluation of urinary EV concentration, size and mRNA profiling may allow early, accurate and noninvasive TCMR diagnosis. Concomitant analysis of pNGAL and in particular of EV-associated miRNA may further improve the diagnostic accuracy.

## REFERENCES:

1. Heyne, N. *et al.* Urinary neutrophil gelatinase-associated lipocalin accurately detects acute allograft rejection among other causes of acute kidney injury in renal allograft recipients. *Transplantation* 93, 1252–7 (2012).
2. Einecke, G. *et al.* Loss of solute carriers in T cell-mediated rejection in mouse and human kidneys: an active epithelial injury-repair response. *Am. J. Transplant* 10, 2241–51 (2010).

51ERA-EDTA 615--MP
N) Renal Transplantation. N1) Transplantation basic science and immune-tolerance of allogenic and xenogenic transplants.
Sergio Dellepiane
DOI: 10.3252/pso.eu.51era.2014
Poster presented at:

PosterSessionOnline.com