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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE IV INTERNATIONAL CAPARICA CONFERENCE ON URINE OMICS AND TRANSLATIONAL NEPHROLOGY 2019 (URINOMICS 2019)

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE IV INTERNATIONAL CAPARICA CONFERENCE ON URINE OMICS AND TRANSLATIONAL NEPHROLOGY 2019 (URINOMICS 2019)

Urinary exosomal miRNAs from chronic kidney disease (CKD) patients: comparison to exosomes from an RPTEC cell culture model system for CKD

Alexander Hüttenhofer[1,3*], Glory Ranches[1], Herbert Schramek[2], Michael Rudnicki[2], Gert Mayer[2]

[1] Division of Genomics and RNomics, Biocenter, Medical University Innsbruck, Austria; [2] Department of Internal Medicine IV, Nephrology and Hypertension, Medical University Innsbruck, Innsbruck, Austria;[3] i-med GenomeSeq Core, Innsbruck, Austria

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ABSTRACT

Tubular inflammation is a hallmark feature of chronic kidney disease (CKD), an extremely prevalent major public health problem. Understanding the molecular mechanisms in response to tubular inflammation may also pave the way to identify sensitive and efficient diagnostic biomarkers necessary for predicting and treating CKD in the future. In this study, we describe exosomal miRNAs in a cell culture system for CKD as potential regulators and/or biomarkers of CKD. To identify exosomal miRNAs, which are regulated in response to tubular inflammation, we employed several cytokines (IL-1β, Oncostatin M and TGF-β1, respectively) to transform renal proximal tubular epithelial cells (RPTECs = CK-) into a wellestablished cell culture model system for CKD (= CK+). The morphology of extracelular vesicles released into the RPTEC culture medium was verified by transmission electron microscopy (TEM) as well as immunoblotting of a tumor susceptibility gene (TSG101), a reported protein marker of exosomes, was performed. By employing a human miRNome PCR panel I, 30 differentially expressed/ abundant ($p<0.05$) miRNAs were identified in exosomes derived from CK+ cells relative to the CK- cells. Among these miRNAs, miR-192-5p, miR- 215-5p, miR-21-5p and miR-23b-5p, respectively, were found to be consistently differentially expressed/ abundant ($p<0.05$) relative to exosomal miRNAs derived from CKcells. Subsequently, these exosomal miRNAs were technically validated and analysed for their release into exosomes. Comparison of the ratio of exosomal miRNAs, relative to the CKD+ or CKD- cells they were derived from, showed an enhanced release ($p<0.05$) of these exosomal miRNAs for CK+ cells when compared to CK- cells. Urinary exosomes are constitutively released from epithelial cells. In order to assess whether differentially expressed exosomal miRNAs from RPTEC cells resembled exosomal miRNAs released into urine, an identical miRNome PCR panel I analysis was employed from urine samples from healthy controls (n=6) and CKD patients (n=6), respectively. In these analyses, urinary derived exosomal miRNA profiling showed 20 differentially expressed/abundant ($p<0.05$) miRNAs in CKD patients relative to healthy controls. Interestingly, eight of these urinary exosomal miRNAs overlapped with the differentially expressed RPTEC exosomal miRNAs, suggesting that these miRNAs may be - at least partly - derived from kidney cells and may reflect changes in miRNA abundance in exosomes in response to tubular inflammation. Our study thus provides novel insights into the regulation of potential biomarkers in tubular inflammation in a cell culture system of CKD,and hence these differentially expressed miRNAs may be employed in elucidating the pathophysiological processes in CKD in the future.

Correspondence: Email - alexander.huettenhofer@i-med.ac.at

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Ultrasensitive biomarker assays using plasmonic films and surface enhanced Raman spectroscopy

Jerry Morrissey

Washington University School of Medicine in St. Louis

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ABSTRACT

Fluorescence-based techniques are the cornerstone of modern biomedical optics, with applications ranging from bioimaging at various scales (organelle to organism) to detection and quantification of a wide variety of biological species of interest. However, the weakness of the fluorescence signal remains a persistent challenge in meeting the ever-increasing demand to image, detect, and quantify biological species with low abundance. Here, we report a simple and universal method based on a flexible and conformal elastomeric film with adsorbed plasmonic nanostructures, which we term a "plasmonic patch," that provides large (up to 100-fold) and uniform fluorescence enhancement on a variety of surfaces through simple transfer of the plasmonic patch to the surface. We demonstrate the applications of the plasmonic patch in improving the sensitivity and limit of detection (by more than 100 times) of fluorescence-based immunoassays implemented in microtiter plates and in microarray format. The novel fluorescence enhancement approach presented here represents a disease, biomarker, and application agnostic ubiquitously applicable fundamental and enabling technology to immediately improve the sensitivity of existing analytical methodologies in an easy-to-handle and cost-effective manner, without changing the original procedures of the existing techniques. Using the power of Surface Enhanced Raman Spectroscopy, capture and detection antibodies of a conventional ELISA kit were modified to measure fg/ml amounts of KIM-1 and cytokine IL-6. This sensitivity allows dilution of samples to eliminate the influence of pH and/or solute concentration that would otherwise affect conventional ELISA results. Also, the sensitivity of the assay allows the use of small samples of urine or plasma from infants, small children or from small animals such as mice with experimental renal diseases. It would be possible to detect preclinical changes in KIM-1 or IL-6 prior to disease manifestation. This assay strategy can be adapted to multiplexing to measure two or more analytes simultaneously in one sample by utilizing different Raman reporter molecules on different detection antibodies, an appropriate mix of capture antibodies on the silicon wafer and monitoring nonoverlapping Raman bands specific for each reporter.

Add-on Plasmonic Patch as a Universal Fluorescence Enhancer Light: Sci. Appl.2018, 7, 29. DOI: 10.1038/s41377-018-0027-8

Correspondence: Email - morrisseyjj@wustl.edu

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Urinary biomarkers of podocyte dysfunction in patients with chronic glomerulonephritis

Natalia Chebotareva*, Irina Bobkova, Vladimir Varshavsky, Olga Li, Lidia Lysenko

Sechenov First Moscow State Medical University (Sechenov University)

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ABSTRACT

The role of podocytes in the mechanisms of proteinuria and chronic glomerulonephritis (CGN) progression is the subject of scientific research recently. Biomarkers of podocyte dysfunction can also provide insight into disease progression, prognosis and monitoring of CGN.

Aim: to estimate the podocyte injury markers in patients with chronic glomerulonephritis (CGN).

Methods: 73 CGN pts were studied: 20 - with inactive CGN (I group), 23 active CGN - with proteinuria (PU) > 1 g/d (II group), 30 - with nephrotic syndrome (NS) (III group), including 11 pts with severe NS (PU more than 10 g/d, hypoalbuminemia < 20 g/L)(IIIb), and 19 pts - with moderate NS (IIIa). 8 healthy subjects were studied as control. Podocyturia (PdcU) was estimated by flow cytometry method. The levels of nephrin, heat shock protein-27 (HSP- 27), VEGF, matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) in urine were assessed by ELISA technique. Using immunohistochemistry with anti- WT-1 monoclonal antibody, glomerular podocytes were marked in patients with active glomerulonephritis. The role of urinary markers in predicting response to immunosuppressive therapy was assessed.

The PdcU, NU and HSP-27 urinary levels were higher in active CGN pts than in control group, in group III - significantly higher than in group II ($p<0.05$) (Tab 1). Podocyturia, NU and urinary HSP-27 level positively correlated with PU ($Rs = 0.27$, $p < 0.05$) and negatively with the level of serum albumin ($Rs = -0.22$, $p = 0.07$) in pts with active CGN. PdcU and urinary HSP-27 levels were higher in severe nephrotic syndrome compared to moderate NS. Correlation between urinary HSP-27, PdcU and NU levels was found. An imbalance between podocyte markers (PdcU, NU), proinflammatory factors (interleukin-6, MMP-2) and selfdefense factor (VEGF, TIMP-2) was observed in pts with severe NS and renal dysfunction. Urinary VEGF was increased in pts with NS, but reduced in pts with renal dysfunction. The glomerular podocyte number correlated with podocyturia and serum creatinine/GFR. 82% pts with low NU and/or PdcU levels (<17ng/ml and 20/μl respectively) had NS remission within 6 months of active immunosuppressive therapy. On the other hand, 67% pts with high NU and/or PdcU levels (>17ng/ml or 20/μl respectively) showed no significant response to immunosuppressive agents, given from 9 month to 2 years. ROC curve analysis demonstrated the potential role of urine markers as predictors of the response to immunosuppressive therapy in CGN.

Nephrinuria, podocyturia and urinary VEGF excretion may be useful noninvasive tests for assessment of podocyte injury and prognosis of CGN.

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Correspondence: Email - natasha_tcheb@mail.ru

Table 1 - Markers of podocyte dysfunction in urine of CGN patients (n=73)

Groups	n	NU, нг/мл	PdcU (pdx+cells/mkl)	VEGF (pg/ml)	HSP-27 ng/ml
Control	8	7.9 [1.7- 9.5]	0[0-0.6]	62.2 [54.85-73.5]	0.73 [0. -0.96]
II	23	9.5 [7.6-13.0] ■	5.45 [2.5- 8.3]*■	73.5[59.4-90.2] ■	0.76 [0.68-1.14]■
IIIa	19	14.8 [10-26.8] *	8.0 [4.2-15.5]*▲	125.2 [94.6-179.7] *	0.94 [0.71-1.73]
IIIb	11	20.45 [16.3-49.0] *▲	36.5[9.0-80.3]*▲	54.65 [38.7-71.5]*▲	1.88 *[0.8-7.2]
I	20	7.25 [5.4-9.8]	5.4[1.0-9.43]*	64.1[53.9-75.6]	0.72 [0.65-0.98]

Median [25th – 75th percentile] p<0,05, * - vs control, ▲- vs group I, ■- vs group III

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Non-invasive urinary biomarker candidates of interstitial cystitis

Jayoung Kim

Cedars-Sinai Medical Center.

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ABSTRACT

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating condition that presents with a constellation of symptoms including bladder pain, urinary urgency, frequency, nocturia, and small voided volumes in the absence of other identifiable etiologies. A lack of objective diagnostic criteria has affected our ability to adequately treat the disease. The goal of this proposed study is to identify/validate sensitive and non-invasive diagnostic biomarkers using urine specimens that stratify IC/PBS patients from healthy subjects. We performed NMR spectroscopy-based metabolomics analysis to search for soluble metabolites that segregate with the diagnosis of IC/PBS. Annotation of the NMR peaks was performed using MeltDB and MetaboloAnalyst software. We were able to annotate several of the discriminant peaks, including the most significant peak, which was identified as tyramine, a neuro-transmodulator related to pain. These results demonstrate our ability to assay for and provisionally identify discrete urine metabolites that are significantly associated with IC/PBS. We believe this will provide novel insights about the etiology of IC/PBS and identify urine metabolites as biomarkers of IC/PBS that have the potential to be employed clinically.

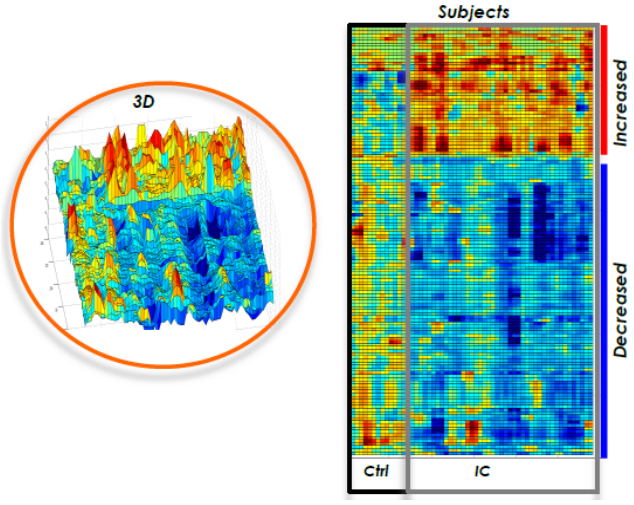

Figure 1 - Identification of NMR Peaks Perturbed in Specimens from IC Patients

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Correspondence: Email - jayoung.kim@csmc.edu

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A prospective study on prevention of contrast – induced nephropathy in Croatia

Ana Vujaklija Brajković[1]*, Marija Križić[2], Jakša Babel[1], Mia Rora[1], Radovan Radonić[1], Ivan Gornik[3]

1 Department of Intensive Care Medicine, University Hospital Centre Zagreb, Zagreb; 2 Department of Oncology, University Hospital Centre Zagreb, Zagreb; 3 Department of Emergency Medicine, University Hospital Centre Zagreb, Zagreb

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ABSTRACT

To explore the protective role of hydration, urine alkalization (Na bicarbonate) and high doses of antioxidant (N-acetylcysteine) in the prevention of CIN. Material and methods: In a prospective, randomized, single-blinded study patients were divided into three groups: 1) peroral hydration, 2) Na bicarbonate infusion and 3) Nacetylcysteine (NAC) plus NaHCO3 infusion. Serum creatinine (SCr), blood urea nitrogen (BUN), and neutrophil gelatinase-associated lipocalin (NGAL) were measured before and 48 hours after the angiography. Mehran score was calculated for each patient. Results: The study included 106 patients. Groups were comparable regarding the baseline characteristics. According to Mehran risk score 70 % of patients had a low risk, 24% medium and 6% high risk score for development of CIN. After the procedure renal function was preserved in all patients regardless of the Mehran risk score (Table 1.). The follow up was completed for 73 patients (68 %). Twenty-two patients (32 %) developed chronic kidney disease. Chronic kidney disease developed in patients with positive history of diabetes and in patients who had higher initial Mehran score. Conclusion: The study showed that patients with preserved renal function are not prone to CIN. Regardless of the protocol used, no case of CIN was observed. Our results indicate that adequate hydration is a key component in maintaining the renal function. Higher Mehran score might be useful in predicting the development of chronic kidney disease.

Table 1 - Laboratory values at the baseline and after the procedure

Variable	All patients (N= 106)	Controls (N= 37)		NaHCO3 (N= 40)		NaHCO3+NAC (N= 29)	
Baseline							
Serum creatinine (µmol/mL)	101(88-117.25)	97 (89.5-114.25)	a	102(88.5-117.5)	a	104(86.5-130)	a
BUN (mmol/L)	6.35(5.1-8.0)	6.6(5.5-7.7)	ab	5.7(4.9-7.3)	a	7.6(6.0-9.7)	b
Creatinine clearance (ml/min)	71.8(54.9-92.5)	69(55.9-90.1)	a	65.1(52.8-91.1)	a	80.3(68.1-97.6)	a
NGAL (ng/mL)	8.8 (5.5-16.7)	6.9(4.1-14.0)	a	8.4(4.9-13.5)	a	13.7(8.5-28.4)	b
Post-procedural							
Serum creatinine (µmol/mL)	103(87.0-121.5)	100(90.0-112.5)	a	106(90.0-123.0)	a	99.0(83.0-127.8)	a
BUN (mmol/L)	5.8 (4.9-7.6)	6.4(5.-8.8)	a	5.4(4.9-6.5)	b	5.9(4.3-7.6)	ab
Creatinine clearance (ml/min)	74.7(55.3-97.6)	75.0(60.5-105-2)	a	75.5(51.6-93.7)	a	72.1(58.9-94.5)	a
NGAL (ng/mL)	11.4(5.4-19.9)	10.6(4.6-19.8)	a	10.8(4.7-16.1)	a	14.0(8.8-33.8)	a
Difference							
Serum creatinine (µmol/mL)	0.0(-5.0 – 8.0)	1.0(-3.5 – 8.0)	a	1.5(-4.0 – 9.0)	a	-2.5 (-14 – 2.0)	b
BUN (mmol/L)	0.2(-0.7 – 1.3)	0.0(-0.8 – 0.7)	a	0.1(-0.9 – 0.85)	a	1.9(0.43–2.9)	b
Creatinine clearance (ml/min)	-2.0(-19.6–17.3)	-7.2(-20.0 –26.4)	a	-0.75(-17.9–15.5)	a	2.1 (-21.0 – 16.3)	a
NGAL (ng/mL)	0.6(-1.27 – 4.3)	1.4 (-0.2 – 3.6)	a	-0.2 (-1.4 – 3-4)	a	1.3 (2.3 – 6.9)	a

BUN blood urea nitrogen, NGAL neutrophil gelatinase-associated lipocalin

Correspondence: Email - avujaklija@gmail.com

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Hypertension in adult polycystic kidney disease: a narrative review

Sarah Mian[1]*, Yogesh Acharya[2], Ranjan Dahal[2]

1 Avalon University School of Medicine, Curacao, Netherlands Antilles; 2 Saint Peter's University Hospital, New Jersey, USA.

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited renal disorder that impacts approximately 12 million worldwide. It is characterized by bilateral kidney enlargement and cystic growth. Hypertension (HTN) is a focal point in the management of ADPKD and is linked to a faster progression to end stage renal disease. Current novel therapies have proven to reduce the progression of renal damage. The ideal goal is to minimize risk through preventative studies and pharmacology to further increase life expectancy and quality. The purpose of this article is to highlight the importance of blood pressure management in ADPKD and review current literature to determine the most effective preventative pharmacotherapy.

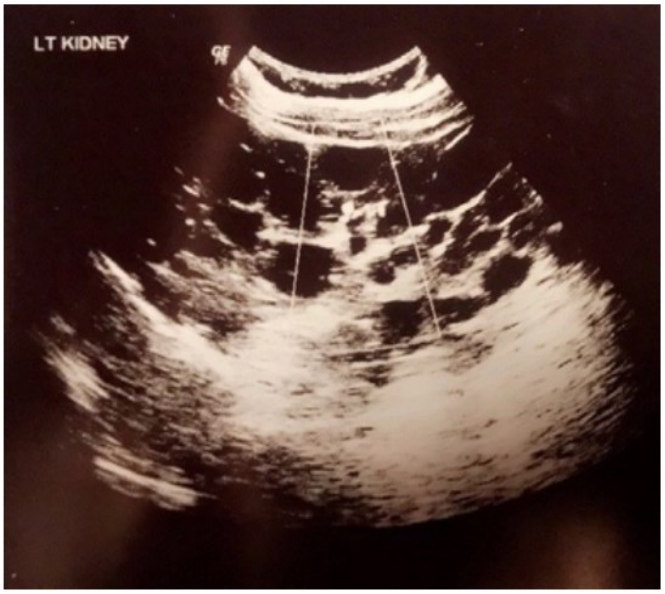

Figure 1 - Ultrasound image showing multiple cysts in kidney.

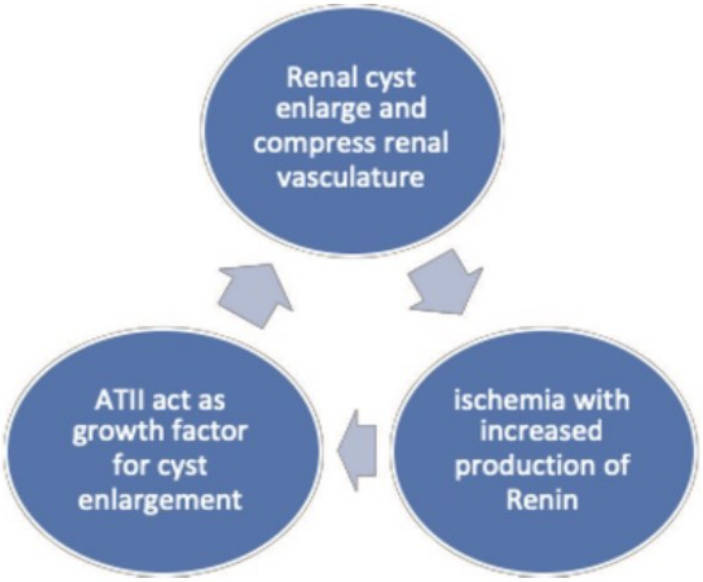

Figure 2 - Picture showing vicious cycle ofc yst expansion and subsequent activation of RAAS.

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Correspondence: Email - sarahamidmian@gmail.com

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Bioactive lipids in human renal cell lesion and repair: Liquid Chromatography - High Resolution Mass Spectrometry (LC-HRMS) as a tool to investigate new lesion/repair biomarkers.

Marcelo Einicker-Lamas

Laboratório de Biomembranas, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil.

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ABSTRACT

In spite of the great development in the medical procedures and pharmacology, there has been little progress in the treatmentof renal failure, which leads to an increasing number of acute and chronic renal patients worldwide. Cell therapy-based protocols as well as nanomedicine initiatives had emerged as potential alternatives. Our group is trying to merge these concepts to achieve an efficient and feasible protocol to minimize renal injury, exploring the versatility of bioactive lipids signaling within the kidney using UHPLC-Q Orbitrap high resolution MS (LC-HRMS), in order to identify early markers for kidney injury as well as renoprotective molecules. Bioactive lipids are an interesting class of mediators that are either important in injury progression or tissue repair. It is well known that some cellular processes are closely regulated by lipid mediators such as diacylglycerol(DAG), phosphatidic acid (PA), lysophosphatidicacid (LPA) and those from the sphingolipid rheostat: ceramide (Cer), sphingosine and sphingosine-1-phosphate(S1P). For example, DAG is associated with phospholipase C activation during injury/repair; LPA, through LPA1 receptor, is specially associated to the progression of renal fibrosis; while Cer released after activation of sphingomyelinases can be an indicative of tissue damage. In contrast, S1P formation is associated with cell survival and proliferation. Previous results from our group, had demonstrated that the Plasma Membrane Ca^{2+}- ATPase was placed and active in caveolar microdomains [1], being the assembly of these membrane microdomains closely related to the cholesterol amount in the plasma membrane. Therefore, impairment in the cholesterol synthesis would disturb lipid rafts formation and consequently, inhibit the ion transport cited above. These observations led us to postulate that kidney injury would affect the cholesterol content and the bioactive lipids profile, which would disrupt the physiological regulatory network responsible for ions and other solutes homeostasis. Using two different models of renal injury – undernutrition and unilateral ureteral obstruction (UUO) - we had demonstrated significant alterations in the lipid profile from the injured kidney when compared to the control ones [2, 3]. Kidney homogenates from undernourished rats had 25% lower cholesterol content, which disturb membrane microdomains, affecting Ca^{2+} homeostasis and the enzymes responsible for the synthesis of important lipid mediators. We observed a decrease in phosphatidylinositol (4)-phosphate (8.8 ± 0.9 vs. 3.6 ± 0.7 $pmol.mg^{-1}.mim^{-1}$), and an increase in phosphatidic acid (2.2 ± 0.8 vs. 3.8 ± 1.3 $pmol.mg^{-1}.mim^{-1}$). We also observed higher amount of Cer in the kidney tissue from undernourished rats (18.7 ± 1.4 vs. 21.7 ± 1.5 $fmol.mg^{-1}.min^{-1}$) indicating an ongoing renal lesion. On the other hand, using UUO and an attempt to ameliorate kidney injury using stem cells, we had observed that the stem cell treated kidneys presented significative higher amounts of S1P (12%) and lower amounts of Cer (76%), which are lipidic markers for repair and lesion, respectively. More recently, we started a metabolomics study of the conditioned medium and human kidney proximal tubule cells (HK-2) submitted to ischemia–reperfusion condition via ATP depletion in vitro by UHPLC-Q Orbitrap high resolution MS. Our goal is to find alterations in the bioactive lipids production in the injured cells in order to add these lipids as biomarkers for kidney lesion or repair.

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Correspondence: Email - einicker@biof.ufrj.br

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE IV INTERNATIONAL CAPARICA CONFERENCE ON URINE OMICS AND TRANSLATIONAL NEPHROLOGY 2019 (URINOMICS 2019)

Metabolomics study by liquid chromatography - high resolution mass spectrometry (LC-HRMS) to investigate ischemia/ATP depletion injury in Human kidney renal cells: Searching for lesion/repair biomarkers and bioactive lipids

Gloria M. R. Soares Grelle[1,2], Rafael R. H. F. Valverde[1], Rafael Garrett[2], Marcelo Einicker-Lamas[1*]

[1] Laboratório de Biomembranas, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil;
[2] Metabolomics Laboratory (LabMeta-LADETEC), Institute of Chemistry, Federal University of Rio de Janeiro

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ABSTRACT

Acute renal disease can be triggered by n different causes, such as ischemia. During ischemic injury, the proximal tubule cels are affected, thus impairing kidney function, which led different groups to develop and improve protocols that mimic ischemic injury in vitro to a better understanding of the molecular and cellular events triggered. However, the use of in vitro protocols still raises doubts about the similarity of that lesion provoked in cells with those observed in vivo. Our objective was to analyze and quantify through LC-HRMS, metabolites in the conditioned medium from cultured renal cells either in the control or in the presence of antimycin A, an ATP depletion agent. We aimed to accurately detect different metabolites directed related to ATP depletion injury, such as inosine, xantine and uric acid, as well as, bioactive lipids that would be related either to lesion or repair processes, such as ceramide (Cer), sphingosine-1 phosphate (S1P), and lysophosphatidic acid (LPA). The conditioned medium from human proximal tubule cells (HK-2 strain) cultured both in the control condition and treated with Antimicin A were collected and a metabolomic study using LC-HRMS - UHPLC-Q Orbitrap was performed. Our results clearly confirm the ATP-depletion lesion, as we could detected metabolites that are classically related to the ischemic process, such as: inosine, xanthine, hypoxanthine and uric acid; besides ceramide, a sphingolipid with a direct correlation with apoptosis. S1P and LPA, which are bioactive lipids known to play a role in kidney protection will be also analyzed in the antimycin A-treated cells, as an increase in their content may indicate that these cells are increasing the production of such bioactive lipids in order to face the ATP-depletion injury. We cannot rule out the possibility to find other molecular species of bioactive lipids that would be altered in the ATP depleted cells. Among then, we are particularly interested in the endocannabinoids. Further experiments are on the way to quantify the lesion metabolites, as well as a lipidomic analysis either from the conditioned medium or from the human proximal tubule cells in the experimental conditions described above. In conclusion, LC-HRMS provided evidences to validate the protocol for renal cell injury. In addition, metabolomics and lipidomics can be applied to the prospection of bioactive lipids - that might play a crucial role both in the progress of renal diseases as well as in cellular and molecular events that occur within the renal tissue recovery and / or protection.

Correspondence: Email - einicker@biof.ufrj.br

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Paracetamol sulfonation to investigate sulfotransferase activity in man

Natália Marto[1,2*], Judit Morello[3], Alexandra MM Antunes[3], Emília C Monteiro[1], Sofia A Pereira[1]

[1] CEDOC - Chronic Diseases Research Centre, NOVA Medical School|Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal; [2] Department of Internal Medicine, Hospital da Luz, Lisboa, Portugal; [3] Instituto Superior Técnico, Universidade de Lisboa.

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ABSTRACT

Cytosolic sulfotransferases (SULT) are important enzymes in Phase II metabolism of several drugs.[1] These enzymes display wide interindividual variability, which is bound to affect efficacy and adverse reactions for drugs metabolized by SULT and underlie drug interactions relevant in clinical practice.[2]

To evaluate intraindividual variability of SULT activity with a phenotyping method that uses paracetamol as probe substrate.

Population and Methods: This study was approved by the Portuguese National Ethics Committee. A population of 36 healthy adults (12 men and 24 women, 12 on oral contraceptives) received 1 g of oral paracetamol on three different occasions. Paracetamol (P) and its metabolites (paracetamol sulfate - PS, paracetamol glucuronate - PG, paracetamol cysteine-S-conjugate - PC and paracetamol mercapturate - PM) were measured in urine using liquid chromatography-high resolution mass spectrometry. SULT activity was measured as the ratio between PS and the sum P+PS+PG (paracetamol sulfonation index – PSI). Differences between groups were tested using ANOVA. Mean PSI was 0.36±0.08. The frequency histogram revealed a normal distribution of PSI in the studied population (Figure 1). Mean intraindividual coefficient of variation of PSI was 15%. There were no significant differences in PSI between men and women, irrespective of oral contraceptive intake.

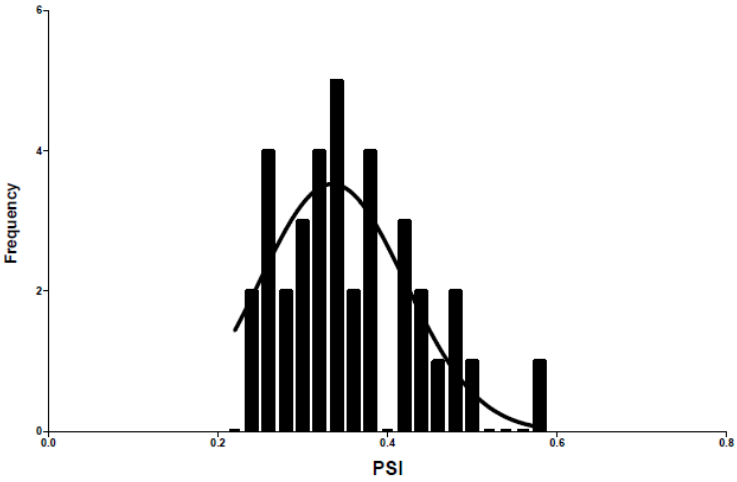

Figure 1 - Frequency histogram plotted as PSI vs. number of individuals.

The use of PSI provides the first evidence of measurements of SULT activity in man. The low intraindividual variability encountered in PSI across genders stimulates further studies envisaging its application in therapeutic drug monitoring and identification of high/poor SULT metabolizers.

Acknowledgments:

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Correspondence: Email - nfmarto@hospitaldaluz.pt

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AKI prediction using NGAL biomarker. A critical point of view on the renal biomarker interpretation

Giovanni Introcaso*, Maria Luisa Biondi

Unit of Laboratory Medicine, Centro Cardiologico Monzino, IRCCS, Milan, Italy

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ABSTRACT

In the last years it's well understood that the acute kidney injury (AKI) is increasingly frequent as severe complication after cardiac surgery. An expert opinion suggested to use a biomarker based tubular damage score to identify the cardiac surgery-associated acute kidney injury (CSA-AKI)1. Our work hypothesized that the neutrophil gelatinase-associated lipocalin (NGAL) could be a reliable biomarker to detect early renal impairment. Hence, we conducted a study on 69 patients undergoing cardiac surgery selected by at least two AKI risk factors. Study design provided the NGAL assay by two plasma samples: before surgery (pre NGAL) at general anesthesia induction and within 4 hours from the patient arrival in Intensive Care Unit (ICU) (pos tNGAL), to obtain timely information on acute tubular stress. Serum creatinine (SCrea) was measured every day and after 10-18 hours after surgery to monitor the renal function. According to KDIGO guidelines for AKI definition, a clinical diagnosis was made and patients were divided into AKI group (N= 24) and NO AKI group (N= 45). NGAL interpretation was made considering clinically significant an increase with a second test (post NGAL)≥ 100 ng/mL. Likewise, slight SCrea increases were interpreted in ICU using the reference change value (RCV). Results showed NGAL increases statistically significant only in the AKI group (p< 0,001) (Figure 1).

The better diagnostic outcomes were obtained combining the NGAL increases to the SCrea increases at 10-18 hours post-surgery: sensitivity= 86%, specificity= 70%, NPV= 96%. The combination of NGAL as a tubular damage biomarker to the SCrea as functional marker may be a worthy and efficient strategy to predict AKI in an adult population. In fact, our results may represent a step forward to schedule protocolized biochemical measurements for an early AKI detection then to guide a possible renal replacement therapy.

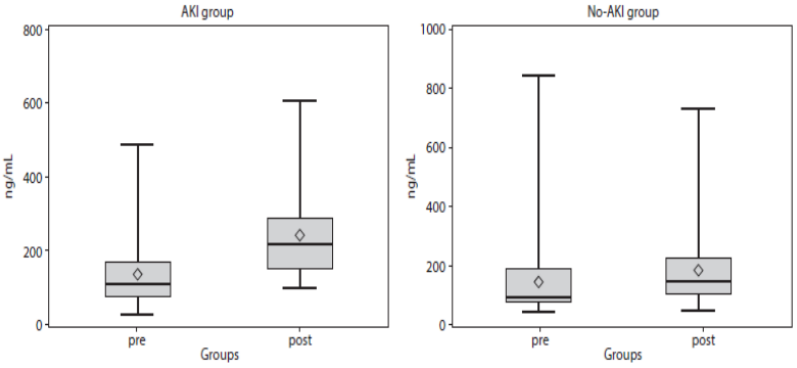

Figure 1 - Comparison between AKI and NO AKI groups related to NGAL test pre and postsurgery, continuous variables expressed as mean±SD, evaluated through a t-test for independent samples

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The authors acknowledge the ICU and anesthesiologists staff for the efficient collaboration. A special thank to Dr. Erminio Sisillo as study inspirer and for his fundamental contribution to our work.

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Correspondence: Email - giovanni.introcaso@ccfm.it

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE IV INTERNATIONAL CAPARICA CONFERENCE ON URINE OMICS AND TRANSLATIONAL NEPHROLOGY 2019 (URINOMICS 2019)

Diagnosis of Fluorosis and its Recovery; Fluorosis linked Renal failure

N. K. Mondal

Fluorosis Foundation of India, B -1, Saransh, 34 – I. P. Extension, New Delhi – 110092, India; Camping and working in Nagaur, Rajasthan, India

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Extended Abstract

Background: Fluorosis is a public health problem, caused by ingestion of excess fluoride (F) from a variety of sources. Doctors and Clinicians face difficulties in suspecting and diagnosing Fluorosis correctly as the symptoms often overlap with other diseases. The objective of this study is to highlight how to suspect Fluorosis in Out Patient Clinics by retrieving the history and diagnosing the disease through confirmative tests and practice of dietary interventions for recovery. The protocol is well defined and field tested for diagnosis of Fluorosis at an early stage.

Material and Methods: After collecting the patient's history, a battery of tests namely F levels in body fluids (urine & serum) and drinking water are investigated by using Ion Selective Electrode (ISE) potentiometry method besides forearm X-ray radiograph are taken to assess interosseous membrane calcification. Haemoglobin (Hb) is also checked for monitoring purpose. F in all 3 samples tested may be higher compared to normal range with presence of ligamental calcification and this confirmsthe diagnosis of Fluorosis. In patients of Fluorosis, they are advised to practice 2 corrective measures namely diet editing anddiet counselling in daily life for recovery. In the former, all sources of F ingestion and use are withdrawn to stop further progression of the disease whereas in the latter, the patient is encouraged to consume a diet rich in essential nutrients, antioxidants and micronutrients through fruits, vegetables and dairy products for repair and maintenance of the damaged body parts. The patients are monitored at intervals to assess F in body fluids and Hb level. Reduction in F levels and rise in Hb have a direct relationship with disappearance of health complaints and subsequent recovery.

Results & discussion: With the history retrieved and the results obtained, a correct diagnosis of Fluorosis is arrived at. For a better understanding of management-cum-monitoring of the patients based on the source of F entry, patients are classified under 3 categories that is summarized in Table 1. Patients were diagnosed Fluorosis with high F in body fluids along with normal or high F level in drinking water besides interosseous membrane calcification in the forearm. The patient was therefore explained the importance and significance of practicing of interventions which focused on elimination F and promotion of nutrients through dietary sources. The main source(s) of F entry identified are (i) drinking of untreated ground water; (ii) consuming F contaminated food / snacks / beverages laced with black rock salt (157 ppm F); (iii) using fluoridated toothpaste The duration of recovery varies from patient to patient depending upon their body physiology and compliance of interventions. It is observed that recovery is faster with nutritive diet than pharmaceutical products. While testing urine and serum fluoride, there is a possibility that results may provide a lead suggestive of renal failure.

Conclusion: This communication provides an overview on manifestations of Fluorosis, diagnostic tests, results, differential diagnosis, interventions practiced, monitoring and recovery from the disease. The report highlights that kidney failure may occur due to fluoride toxicity and reveals that it is a Fluorosis linked disorder.

Key words: Fluorosis, Diagnosis, Interventions, Monitoring, Recovery, Case studies, Renal failure.

Correspondence: Email - nisithkm@gmail.com

Table 1 - Comparison Patients classified into 3 categories and interpretation of the test results

	F level in			X-ray radio-graph showed IMC*	Additional Test	Interpretation
	Urine	Serum	Water			
Category 1	↑	↑	↑	√	-	Fluorosis confirmed; Source of F entry either contaminated drinking water / food items / other sources rich in F.
Category 2	↑	↑	↓	√	-	Fluorosis confirmed; Source of F entry through consumption of food / beverages / other sources rich in F but not drinking water.
Category 3	↓	↑	↑	√	**KFT	Fluorosis with Renal failure confirmed; Source of F entry either contaminated drinking water/ food items / other sources rich in F.

Normal Range of F: (Urine = 0.1 - 1.0 mg/L); (Serum = 0.02 - 0.05 mg/L); (Drinking Water: Permissible limit for F = Up to 1.0 mg/L, less the better as per BIS, 2012).

*IMC - Interosseous Membrane Calcification ** KFT – Kidney Function Test

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First-void urine for detection of cancer biomarkers

Quinten Van Avondt*, Stephanie Jordaens, Arya Mehta, Danielle Pasmans, Koen Beyers, Vanessa Vankerckhoven.

Novosanis NV, Wijnegem, Belgium

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Extended Abstract

The predicted global cancer burden is expected to increase significantly – By 2040, 29.5 million new cancer cases are estimated, compared to 18.1 million cases reported worldwide in 2018 [1]. Early detection of cancer can greatly increase chances of survival and improve overall quality of life of a patient. Cancer biomarkers* can be found in tissues as well as bodily fluids and can be used to detect the disease early as well as monitor disease progression [2]. An ideal biomarker should only be detectable in case of disease and thus have a high sensitivity and specificity. Additionally, biomarker levels can help understand the severity of disease through predictive and prognostic values [3]. A high number of potentially informative cancer biomarkers have been found, based on detection of DNA, RNA, proteins and metabolites. This has been made possible through the ability to sequence the entire human genome as well as advances in key technologies such as high throughput DNA sequencing, microarrays, and mass spectrometry [2].

A tissue biopsy is the traditional approach used to diagnose many cancers. Moreover, biopsies are essential to achieve the objectives of precision oncology and allow for targeted therapies based on the genetic profile of the disease. However, obtaining a tissue sample is not always feasible and the process can be invasive, painful, expensive, time-intensive, difficult and requires the intervention of a clinician [4-6]. In addition, due to intratumor heterogeneity, in some instances, the entire tumor landscape may not be reflected by a tissue biopsy. As a result, researchers are continuously exploring alternative methods to detect cancer types. The use of minimally invasive procedures such as liquid biopsies and detection of circulating tumor markers in body fluids is gaining interest [6]. Circulating molecules such as cell-free DNA, circulating tumor cells, circulating RNAs, proteins, peptides and exosomes can provide a global view of primary and metastatic tumors. Circulating molecules can be detected in various biological fluids, including cerebrospinal fluid, plasma, saliva, seminal plasma, serum and urine. Liquid biopsies have several advantages - they allow (repeated) sampling, providing a personalized snapshot of a disease at successive time points. Additionally, they can offer a solution to tumor heterogeneity, and better reflect the genetic profile of all tumor subclones, as opposed to tissue biopsies which are obtained from one tumor region. Liquid biopsies are also associated with significantly less morbidity and can prevent complications associated with traditional biopsies [6].

In general, liquid biopsy is associated with blood, which uses either serum or plasma as a sample type. However, blood as a liquid biopsy has several limitations that have hampered its development as a clinically useful biomarker test. Blood has a relatively high and complex protein repertoire. Furthermore, components of the blood matrix can interfere with biomarker measurements. The invasive nature of blood tests also limits access to repeated measurements and poses a risk of infection for both the patient and caregivers, along with the additional costs of minimizing this risk [7].

Urine has been proposed as an alternative biofluid for detecting and monitoring treatment of urological and systemic cancers. Urine is easily accessible, non-invasive, available in larger quantities and suited for home collection [7]. Moreover, the collection of urine is not limited by the health status of a patient8 and does not entail any risk of transmission of blood-borne pathogens9. In addition, urine testing enables cost-efficient rapid and serial sampling, allowing for patient monitoring as well as for reproducibility assessment of assays [7,8]. In terms of analysis, the isolation of DNA from urine is in theory easier than bbod, due to the low protein content after filtration in the kidney [7,10]. Several studies have shown that the use of urine as a liquid biopsy for cancer detection and monitoring is promising due to the ease of sampling and high acceptability compared to blood

Correspondence: Email - quinten.vanavondt@novosanis.com

and tissue [6,11-13]. Urine cell free tumor DNA has proven to be of value in biomarker studies of bladder, kidney and prostate cancer, but surprisingly also in breast, colon and lung cancer [12, 14-16].

However, just as in the case of blood, urine sampling comes with challenges. Collection, transport and storage bring preanalytical variation to the diagnostic process. For example, the amount and proportions of biomarkers in urine might vary according to time of collection and environmental aspects

such as diet. Moreover, urine volume and fraction are preanalytical variables that influence biomarker levels. More specifically, the urine flow breaks up in two distinct fractions. First void urine (FVU) refers to the initial flush of urine, typically the first 10 to 30 mL, in contrast to midstream urine which is considered a sterile sample [17]. FVU contains a higher concentration of biomarkers than other fractions of urine. Many of these preanalytical variables can and should be minimized to improve assay performance. Volumetric and standardized collection is virtually impossible with a urine cup because interrupting urine flow immediately after starting urination is challenging. As a result, sample dilution is likely to occur, and assay sensitivity will be compromised. Additionally, use of a urine cup can be awkward, messy and inconvenient for the user.

To decrease the variation in collected volumes of FVU and to ensure immediate mixing of the sample with a preservative, a self-sampling device has been developed, Colli-Pee®, which allows for standardized and volumetric collection of FVU [11,17]. Colli-Pee® is a user-friendly method to capture FVU improving sample collection for downstream analysis, improving diagnostic sensitivity [17,18].

Several diagnostic assays have been developed and/or validated on FVU in the field of oncology. In the case of HPV (Human Papilloma Virus), the primary cause of cervical cancer, testing has traditionally been performed on cervicovaginal samples. As there is a high correlation between urinary and cervical HPV DNA and as it has been shown that FVU contains significantly more human DNA and HPV DNA than the subsequent fractions, the role of urinary detection of HPV in cervical cancer screening is being investigated [11,17]. Leeman et al. showed that FVU samples appear to be suitable for detection of moderate to severe precancerous cervical lesions through HPV testing with high analytical and clinical sensitivity. FVU samples demonstrated high concordance with HPV detected in clinician-taken smears and brush-based self-samples [19]. Moreover, a recent study has investigated the potential of urine-based DNA methylation testing in cervical cancer triage [20]. The drawback of hrHPV (high risk HPV) screening is that it doesn't allow differentiation between a transient productive infection and a persistent transforming infection, which lowers the specificity of the test. Testing of hrHPV-positive women for hypermethylated genes offers an objective triage tool for the detection of CIN3 and cervical cancer [20-22].

With regard to prostate cancer, blood-based PSA testing, characterized by low clinical specificity, is being complemented by novel molecular tests using FVU as a sample. Theodorescu et al. found that FVU testing identified patients with prostate cancer with 91% sensitivity and 69% specificity whereas midstream urine was uninformative [16]. Tests developed by diagnostic companies such as MDxHealth (SelectMDx™), ExosomeDx (IntelliScore®) or Diagnolita measure specific RNA markers for prostate cancer. These tests all enable discrimination between patients with high grade tumors versus patients with low-grade or benign disease at biopsy, reducing over-detection and over treatment of indolent disease.

In conclusion, FVU is a non-invasive sample that is rich in disease biomarkers offering huge potential for infectious disease and cancer biomarker testing. Several diagnostic assays are commercially available or on the verge of launch in the clinical practice. Moreover, research on urine-based liquid biopsy will flourish in the coming years.

Acknowledgments:

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