Original Article

Novel Flow Cytometric Method for the Detection of Podocalyxin-Positive Elements in Urine of Patients with Glomerulonephritides – First Promising Results

(podocyte / podocalyxin / CD10 / glomerulonephritis / flow cytometry)

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Abstract: Glomerulonephritides together create a heterogenic group of supposedly immunologically mediated diseases of glomeruli. They still belong among the most frequent causes of chronic renal failure. Detection of podocytes in urine might serve as an important marker of glomerulonephritides activity. The aim of this study was to develop a novel flow cytometric method for the detection of podocyte fragments and podocytes in urine and assess its possible use in clinical practice. We placed emphasis on the improvement of pre-analytic phase. To suppress the autofluorescence of the background, blocking solutions and magnetic separation were used. An additional surface marker CD10 (nephrilysin) was used together with routinely used podocalyxin (PCX) in order to achieve better identification of podocytes. Based on the surface marker expression, three different element types were identified in the urine samples: PCX+/CD10+ elements (EL) (supposedly podocytes), PCX-/CD10+ EL (supposedly parietal epithelial cells) and PCX+ EL. We examined a total of 36 patients who underwent renal biopsy (non-glomerular nephropathy, MGN, FSGS, IgAN, AAV and MPGN) and 27 healthy controls. Negative results were found in non-glomerular nephropathy and in MGN. In patients with FSGS and IgAN, the levels of urine elements were slightly increased. The highest levels of all elements were found in AAV and MPGN. Our first results suggest that flow cytometric detection may distinguish between glomerular and non-glomerular diseases and that the levels of urine elements might correlate with the degree of glomerular destruction.

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Abbreviations: AAV – ANCA-associated vasculitis, ANCA – anti-neutrophil cytoplasmic antibodies, APC – allophycocyanin, CTRL – healthy control group, FC – flow cytometry, FSGS – focal segmental glomerulosclerosis, GN – glomerulonephritis, IgA – immunoglobulin A, IgAN – IgA nephropathy, IQR – interquartile range, mAb – monoclonal antibody, MGN – membranous glomerulonephritis (nephropathy), MPGN – membranoproliferative glomerulonephritis, PCX – podocalyxin, PCX-/CD10+ EL – podocalyxin-negative/CD10-positive element, PCX+/CD10+ EL – podocalyxin-positive/CD10-positive element, PCX+ EL – podocalyxin-positive element, PDC – podocyte, PE – phycoerythrin, PEC – parietal epithelial cell.

Introduction

Glomerulonephritides together create a heterogenic group of supposedly immunologically mediated diseases of glomeruli. They still belong among the most frequent causes of chronic renal failure. Podocytes (PDCs) are assumed to play the central role in the pathogenesis of some and progression of other glomerulonephritides (Mundel and Shankland, 2002; Kanno et al., 2003; Pavenstadt et al., 2003). Until recently it was believed that podocytes are non-replicating cells which, when damaged, can only either hypertrophy or undergo apoptosis. Recent studies, however, demonstrated that there may be some overlap between podocytes and parietal epithelial cells (PECs), which may partly serve as a source for replacement of damaged podocytes (Smeets et al., 2004, 2011; Bariety et al., 2005, 2006; Achenbach et al., 2008; Appel et al., 2009). Podocytes may detach from the glomerular basement membrane and either intact or as fragments appear in urine; the number of uri-

nary podocytes may be related to the activity of glomerulonephritis or at least to the degree of podocyte damage (Hara et al., 1995, 1998; Kanno et al, 2003; Petermann et al, 2003; Vogelmann et al., 2003; Sakairi et al., 2010). Evaluation of urinary podocytes could serve as a non-invasive marker of the activity of glomerular disease (Yu et al., 2005; Petermann and Floege, 2007).

The aim of our current study was: a) to develop a novel flow cytometric method for the detection of podocyte fragments and podocytes in urine; b) to compare the numbers of podocyte fragments and podocytes in urine between different glomerulonephritides, other renal diseases and healthy individuals; c) to explore a possible link between podocyturia and other markers of glomerular injury such as proteinuria, erythrocyturia and serum creatinine levels.

Material and Methods

Patients

We examined a cohort of 36 patients indicated to renal biopsy. Nineteen were females and 17 males with a median age of 43 years (interquartile (IQN) range {33; 54} years). Histopathological examination of the renal biopsy sample resulted in the diagnosis of membranous glomerulonephritis (MGN) in five patients, focal segmental glomerulosclerosis (FSGS) in five patients, IgA nephropathy (IgAN) in seven patients, ANCA-associated vasculitis (AAV) in five patients and membranoproliferative glomerulonephritis (MPGN) in one patient. Podocyturia and podocalyxin-positive elements were also examined in 13 individuals with chronic renal insufficiency of non-glomerular origin.

A control age-matched group comprised 27 healthy individuals – healthy control group (CTRL).

Urine samples from patients with glomerular involvement were collected before the renal biopsy.

The study was approved by the Institutional Ethical Committee. The purpose and procedures of the study were explained to participants, who signed informed consent forms.

Laboratory analysis

First morning urine was obtained using the BD Vacutainer (BD Biosciences, San Jose, CA) system for urine collection. The specimen was divided into a preservative tube (BD Biosciences) and a tube without preservative agent. Specimens from preservative tubes were used for detection of the elements, specimens from non-preservative tubes were used for measuring the creatinine level in urine immediately. After collection, specimens in preservative tubes were stored at -20°C.

The presence of podocytes was detected by a flow cytometer (BD FACSCanto, BD Biosciences) within 1-month period after the collection. Urine was thawed at 4 °C and the amount of 2.5 ml was used. After filtering through 50-µm filter (BD, USA), filter rinsing by 500 µl CellWash solution (BD Biosciences) followed. The filtered specimen was centrifuged for 5 min at 600 *g* and 4 °C, then washed once with 2 ml of wash solution – BUF029 (Serotec, Raleigh, NC), diluted 1 : 9 with distilled water and w/w 0.2% Tween 20, and centrifuged once more. The sediment was dissolved in 2 ml of wash solution and incubated for 20 min at 4 °C in the dark, then washed with wash solution and centrifuged for 5 min at 600 *g* and 4 °C. The sediment was resuspended in 50 µl of the second blocking agent – Human Fc Receptor Binding Inhibitor Purified (eBioscience, San Diego, CA), diluted 1 : 9 with CellWash (BD Biosciences). Afterwards, 20 min incubation at 4 °C in the dark followed. After the incubation, monoclonal antibodies conjugated with fluorescent dye – 5 µl, diluted 1 : 6 with wash solution were added. We used anti-CD10 conjugated with allophycocyanin (APC) (clone HI10a, isotype IgG1κ, reactivity human, BD Biosciences) and anti-podocalyxin conjugated with fluorescent dye phycoerythrin (PE) of three clones of Human Podocalyxin: 1 – clone 222328, mouse IgG2A – R&D (R&D Systems, Minneapolis, MN), 2 – clone TRA-1-60, mouse IgMκ – BD, 3 – clone TRA-1-81, mouse IgMκ – BD Biosciences. Processed specimens with added antibodies were incubated for 30 min at 4 °C in the dark, then washed two times with 2 ml of wash solution and once with iMagbuffer diluted with distilled water 1 : 9, BD IMag™ (BD Biosciences) Buffer (10×) for use with BD IMag™ cell separation products. Centrifugation for 5 min at 600 *g* and 4 °C followed.

After supernatant discarding, 15 µl of magnetic monoclonal antibody against fluorescent dye – phycoerythrin (BD IMag™ R-Phycoerythrin Magnetic Particles – DM, clone E31-1459, isotype IgG1κ, BD) and 15 µl of magnetic monoclonal antibody against fluorescent dye – allophycocyanin (APC), BD IMag™ Allophycocyanin (APC) Magnetic Particles – DM, clone E30-221, isotype IgG1, κ, BD was added. Incubation for 30 min in the dark at 4 °C followed. The mixture was dissolved in 2 ml iMagbuffer after that. Three separation cycles on Magnet (BD IMagnet™, Cell Separation) lasting 10, 8 and 8 min each followed. After the third separation, cell suspension was dissolved in 2 ml of iMagbuffer 10× and centrifuged for 5 min at 600 *g* and 4 °C. The sediment was dissolved in 250 µl CellWash. For absolute counting, 25 µl of count beads (AccuCount Fluorescent, 10^6/ml, cat. no.: ACFP-50-5, Spherotech, Inc., Lake Forest, IL) was added. The mixture was stirred using a Vortex machine. Measurement was performed in BD FACSCanto at MEDIUM speed, acquired 50,000 events. We analysed all elements in the range 12–25 µm, defined by calibration beads (Spherotech Inc.). After measuring, the absolute counts were established using the formula for AccuCount Beads and converted to creatinine concentration in urine. Similarly, the isotype control was performed, but fluorescently labelled antibodies were replaced by antibodies for isotype control. Subsequent magnetic separation was performed in the same way as in a normally labelled sample.

Table 1. Basic clinical data and measured parameters (all described in mean and median of values) in healthy controls, patients with non-glomerular disease and with GN. Results are expressed as arbitrary units/μmol of urine creatinine.

Diagnosis	N	Age	S-Crea mmol/l	S-Alb g/l	EryU /ul	PRU g/24h	Clearance by MDRD ml/s	PCX- /CD10+ EL	PCX+ /CD10+ EL	PCX+ EL
Healthy c. Mean	**27**	42	<105	>35	<7	<0.15	>1.5	***0.28***	***0.32***	***2.27***
Median		42	<105	>35	<7	<0.15	>1.5	***0.15***	***0.24***	***2.09***
Non-GN Mean	**13**	43	177	32	18	2.83	0.98	***0.69***	***0.46***	***2.16***
Median		42	131	31	11	1.70	0.93	***0.22***	***0.39***	***0.97***
All-GN Mean	**23**	44	287	31,9	29	4.94	0.69	***6.43***	***8.98***	***20.25***
Median		41	216	36	17	2.30	0.52	***4.32***	***3.65***	***14.10***
Subgroups of glomerulonephritides										
FSGS Mean	**5**	42	170	21	9	7.94	0.78	***8.60***	***3.96***	***16.33***
Median		41	171	21	9	8.20	0.65	***8.45***	***3.65***	***16.66***
MGN Mean	**5**	54	99	19	0	12.37	1.35	***1.33***	***0.61***	***3.91***
Median		61	81	19	0	11.20	1.60	***1.33***	***0.66***	***3.56***
IgAN Mean	**7**	46	195	40	39	1.33	0.83	***3.24***	***3.58***	***6.99***
Median		48	216	39	23	1.23	0.52	***3.84***	***3.09***	***6.90***
AAV Mean	**5**	39	>600	42	59	0.70	<0.25	***8.67***	***14.83***	***30.38***
Median		37	>600	43	51	0.50	<0.25	***4.04***	***12.64***	***29.88***
MPGN	**1**	43	334	25	20	6.90	0.31	***15.60***	***56.90***	***104.55***

Legend: S-Crea – serum creatinine, S-Alb – albuminaemia, EryU – erythrocyturia, PRU g/24h – proteinuria for 24 h, MDRD (modification of diet in renal disease) – renal clearance calculation formula, Healthy c. – healthy controls, Non-GN – non-glomerular renal diseases, All-GN – group of all glomerulonephritides.

Statistical analysis

The results are given in the arbitrary units/μmol of urine creatinine, and are expressed as a median and an interquartile range {IQR}.

Statistically significant difference between the cohorts was tested with nonparametric tests (Kruskal-Wallis and Mann-Whitney). The Spearman rank test was used to identify the correlation between variables. The STATISTICA data analysis software system version 9.0 (StatSoft, Inc., OK) was used for calculations. For all statistical tests, the selected significance level was 5 % ($P \leq 0.05$).

Results

We identified three different populations of podocalyxin-positive elements. We called them according to the expressed signs podocalyxin (PCX) and CD10:

1) PCX+ elements (EL) – elements with podocalyxin expression only – podocalyxin-positive elements, supposedly equivalents to podocyte fragments;
2) PCX-/CD10+ EL – elements with CD10 expression only – podocalyxin-negative and CD10-positive elements, supposedly parietal epithelial cells (PECs) or an overlap of PECs/PDCs;
3) PCX+/CD10+ EL – elements expressing both podocalyxin and CD10 marker: podocalyxin-positive and CD10-positive elements, supposedly podocytes.

The results are summarized in Table 1 and Figs. 1–4. They were negative in healthy controls (Fig. 5), non-glomerular lesions and also membranous nephropathy. All elements were found in FSGS, with PCX-/CD10+ EL predominating. The distribution of element types was homogenous in IgAN and the total number of elements was similar to FSGS. In AAV, PCX+/CD10+ EL dominated and the total number of elements was higher than in FSGS and IgAN. The total number of elements was highest in MPGN (Fig. 6), where PCX+/CD10+ EL a PCX+ EL predominated while PCX-/CD10+ EL were very rare.

1. PCX+EL, PCX-/CD10+ EL and PCX+/CD10+ EL in the urine of patients with different glomerulonephritides, other renal diseases and healthy individuals

The amount of urinary PCX-/CD10+ EL was significantly higher in patients with glomerulonephritides (6.26 {1.55; 8.45}) compared to patients with other renal diseases (0.22 {0.08; 1.00}) and healthy individuals (0.13 {0.05; 0.23}), see Fig. 1A.

Similarly, the number of urinary PCX+/CD10+ EL was significantly higher in patients with glomerulonephritides (5.10 {2.13; 11.29}) compared to other groups (0.39 {0.21; 0.60} and 0.24 {0.18; 0.37}, respectively), see Fig. 1B.

Urinary PCX+ EL were also significantly more frequent in patients with glomerulonephritides (17.50 {11.90; 24.09}) compared to patients with other renal diseases (0.97{0.60; 1.90}) and to healthy individuals (1.71 {0.77; 3.25}), see Fig. 1C.

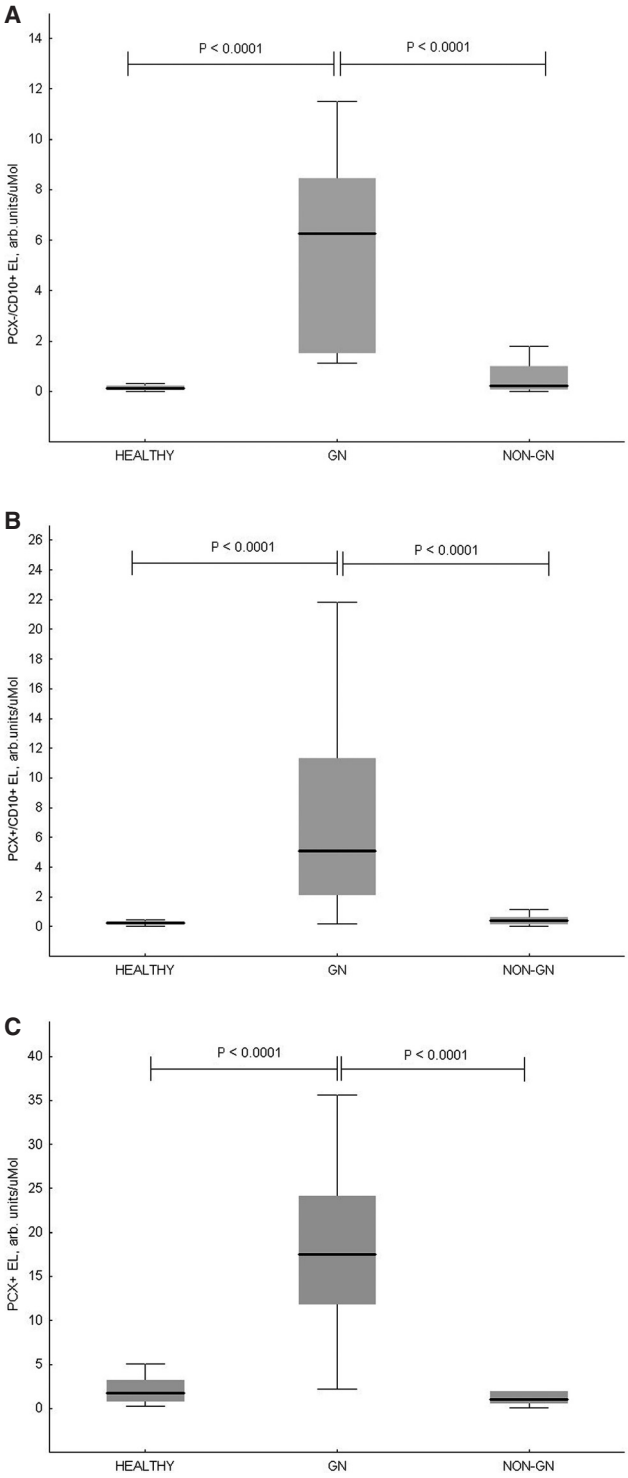

Fig. 1. A – PCX+ EL, B – PCX-/CD10+ EL (supposedly PEC), and C – PCX+/CD10+ EL (supposedly PDC) in urine of patients with different glomerulonephritides, other renal diseases and in healthy individuals. Healthy – healthy controls; GN – glomerulonephritides; NON-GN – other renal diseases. The box-plot represents lower and upper quartiles, the horizontal line represents median, the whiskers represent sample minimum and maximum.

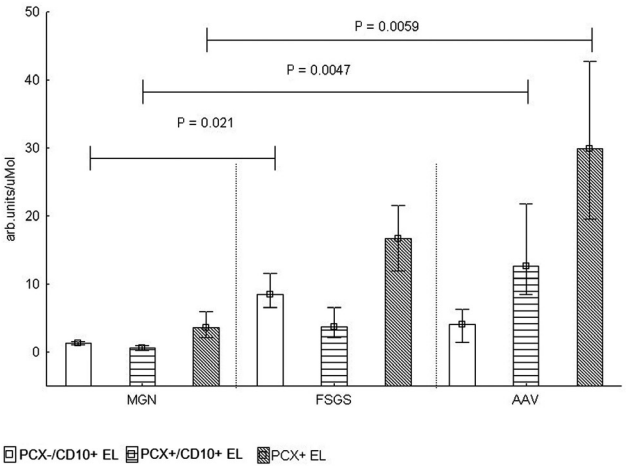

Fig. 2. PCX-/CD10+ EL (supposedly PEC), PCX+/CD10+ EL (supposedly PDC) and PCX+ EL in urine of patients with different glomerulonephritides.

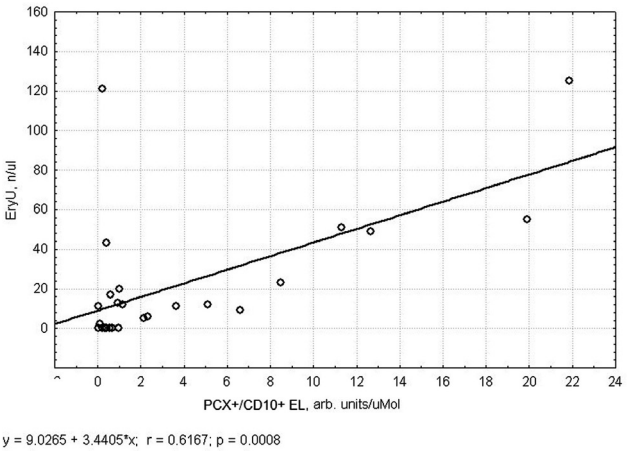

Fig. 3. PCX+/CD10+ EL and erythrocyturia (EryU) in patients with different renal diseases. PDC (PCX+/CD10+ EL). N = 36.

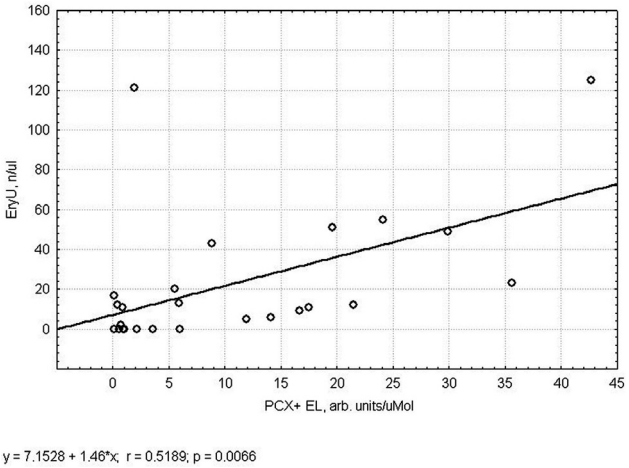

Fig. 4. PCX+ EL and erythrocyturia (EryU) in patients with different renal diseases. N= 36.

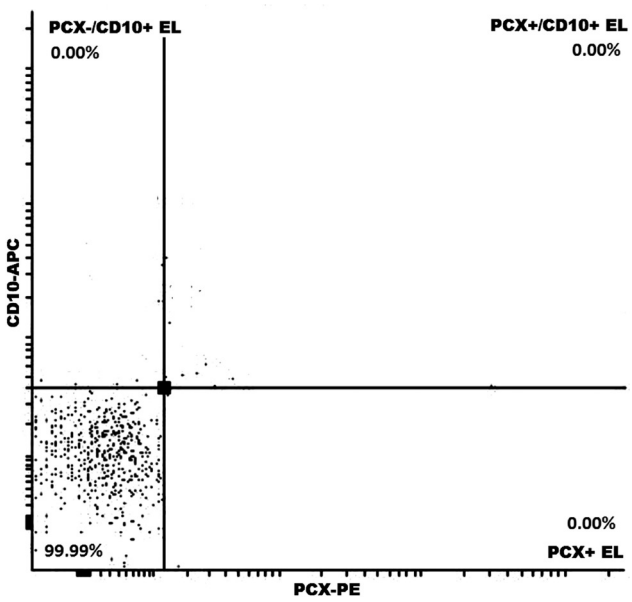

Fig. 5. Dot-plot for the analysis of PCX+ EL, PCX-/CD10+ EL and PCX+/CD10+ EL in a 28-year old healthy male. PCX-/CD10+ EL displayed in the upper left quadrant, PCX+/CD10+ EL in the upper right quadrant, PCX+ EL in the lower right quadrant and "negative" elements (expressing neither PCX nor CD10) in the lower left quadrant. We did not find any PCX+ EL, CD10+/PCX+ EL, CD10+/PCX- EL.

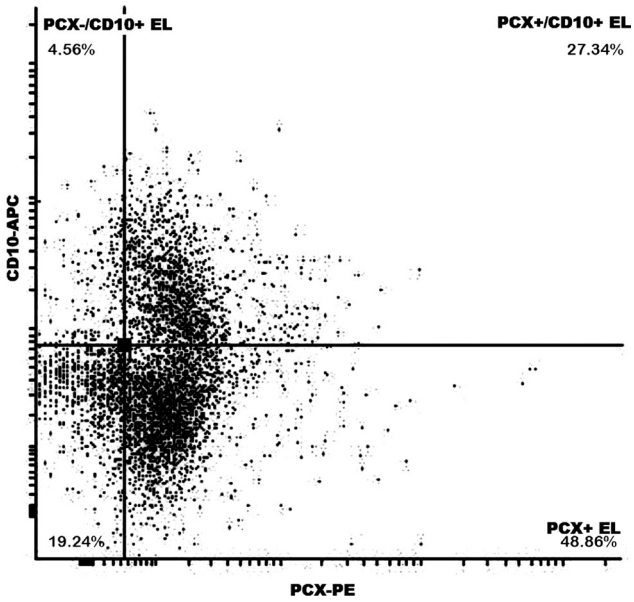

Fig. 6. Dot-plot for the analysis of PCX+ EL, PCX-/CD10+ EL and PCX+/CD10+ EL in a 43-year old patient with membranoproliferative GN. PCX-/CD10+ EL displayed in the upper left quadrant, PCX+/CD10+ EL in the upper right quadrant, PCX+ EL in the lower right quadrant and "negative" elements (expressing neither PCX nor CD10) in the lower left quadrant. We found positivity in all the following elements (PCX+ EL = 48.86 % = 104.55 arb. units/μmol creatinine, CD10+/PCX+ EL = 27.34 % = 56.90 arb. units/μmol creatinine, CD10+/PCX- EL = 4.56 % = 15.60 arb. units/μmol creatinine).

Urine PCX+ EL, PCX-/CD10+ EL and PCX+/CD10+ EL were not dependent on the gender and age of the examined individuals.

2. PCX-/CD10+ EL, PCX+/CD10+ EL and PCX+ EL in the urine of patients with different glomerulonephritides

Our GN groups were small so that it was not possible to make a general conclusion. However, AAV patients (N = 5) showed the highest values of PCX+/CD10+ EL and PCX+ EL compared to MGN (N = 7) and FSGS (N = 7), see Fig. 2.

3. Relation of PCX+ EL, PCX-/CD10+ EL and PCX+/CD10+ EL in urine to proteinuria, erythrocyturia and serum creatinine level in patients with glomerulonephritides and other renal diseases

The amount of PCX-/CD10+ EL in urine did not correlate with serum creatinine ($r = 0.12$, $P = 0.60$), proteinuria ($r = 0.016$, $P = 0.99$) and erythrocyturia ($r = 0.18$, $P = 0.38$).

Similarly, no correlation was found in PCX+/CD10+ EL between serum creatinine ($r = 0.05$, $P = 0.84$) and proteinuria ($r = 0.31$, $P = 0.12$). However, the number of urine PCX+/CD10+ was in strong positive correlation with erythrocyturia, see Fig. 3.

PCX+ EL have also shown significant positive correlation with erythrocyturia, see Fig. 4.

Discussion

Our results affirm our hypothesis that flow cytometric detection may distinguish between glomerular and non-glomerular diseases and that the levels of urine elements might correlate with the degree of glomerular destruction.

In our previous work we have identified so-called podocalyxin-positive elements PCX+ EL using flow cytometry (Habara et al., 2008). Although the original cytological method (Hara et al., 1995) with certain modifications is still considered the gold standard, it is not suitable for routine use because of the necessity of highly qualified laboratory technician, who evaluates each specimen subjectively and analyses only a small amount of elements. However, flow cytometry (FC) provides a relatively objective assessment of a large number of elements in a few seconds. Routine use of FC is limited by autofluorescent background of the urine. To facilitate podocyte identification, we have selected two markers: PCX and CD10, which are supposed to be expressed by both parietal cells and podocytes. The advantage of both markers is that they are expressed on the cell surface. To increase the sensitivity of the method to podocalyxin an-

tigen we used a mixture of three different mAb with the same fluorochrome but focused on different epitopes. Phycoerythrin was used as a fluorochrome. Monoclonal antibodies against CD10 were labelled with APC. The problem with autofluorescence of the background was solved using magnetic antibodies against fluorochromes. Magnetic monoclonal antibodies were bound on the fluorochrome of the primary monoclonal antibody (in our case the first type on PE and the second type on APC). All elements bound to magnetic antibodies were then separated using a magnet. Thus, the result was a suspension of elements with only antigens bound to the primary antibody. This procedure greatly reduced the influence of the background, which was controlled by isotype controls.

Due to the conflicting opinions on the expression of CD10 and PCX on PECs and PDCs in the literature (Smeets et al., 2004; Bariety et al., 2006; Achenbach et al., 2008; Appel et al., 2009), we finally abandoned the claim that we identified PEC, PDC and PCX+ EL. The identified populations were named based on the specific antigen expression, i.e. PCX+ EL, PCX-/CD10+ EL and PCX+/CD10+ EL. The first results cannot definitely say which population is the most suitable for monitoring. In our opinion, simultaneous monitoring of multiple parameters in one step could be more beneficial.

Our results are in keeping with some other studies. In the study by Hara et al. (1998), podocyturia was proved by a cytological method in patients with FSGS and proliferative forms of GN (MPGN, IgAN, LN) and correlated with both proteinuria and erythrocyturia. Similar findings were presented in the study by Vogelmann et al. (2003); the detection was performed by cultivation and viable podocytes were found in urine of healthy controls as well; these authors also proved that much greater excretion of podocytes into urine was present in proliferative forms of glomerulonephritides. A cultivation method was also presented by a team from Germany (Achenbach et al., 2008). In the study by Kanno et al. (2003), the global podocalyxin mass was quantified by the ELISA method, with comparable results. Experiments with RT-PCR methods were also performed (Sakairi et al., 2010). According to a review article (Peterman and Floege, 2007), the most attractive method for the future is FC; cytological methods have well-known limitations, cultivation methods depend on the quality of urine specimen, they are very sensitive to bacterial contamination and require immediate processing of urine. RT-PCR methods are also rather sensitive to bacterial contamination.

In conclusion, we can summarize that we developed a novel flow cytometric method for the detection of podocyte fragments and podocytes in urine and assessed its possible use in clinical practice. We improved the pre-analytical phase using the preservation agent. To identify or highlight the "podocytes" we added anti-CD10 antibody. Autofluorescence of the background was eliminated using magnetic separation. Quantification was performed by transferring to the absolute values of the standard FACS method expressed in relation to one micromole of creatinine in urine. We proved that healthy controls and patients with "non-glomerular" renal diseases with positive urinary sediment excreted minimal amounts of all examined elements. The excretion of podocalyxin-positive elements was much more significant in patients with AAV with crescents compared to patients with FSGS; this fact corresponds to the theory that the elements could reflect the activity of the glomerular disease resulting in the acute loss of podocytes into the urine. These promising results are to be confirmed in larger groups of patients with specific glomerular disease and during longer follow-up. It is also to be demonstrated whether this "podocyturia" may serve as a marker of response to the immunosuppressive treatment.

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References

Achenbach, J., Mengel, M., Tossidou, I., Peters, I., Park, J. K., Haubitz, M., Schiffer, M. (2008) Parietal epithelia cells in the urine as a marker of disease activity in glomerular diseases. *Nephrol. Dial. Transplant.* **23**, 3138-3145.

Appel, D., Kershaw, D. B., Smeets, B., Yuan, G., Fuss, A., Frye, B., Moeller, M. J. (2009) Recruitment of podocytes from glomerular parietal epithelial cells. *J. Am. Soc. Nephrol.* **20**, 333-343.

Bariety, J., Bruneval, P., Meyrier, A., Jacquot, C. (2005) Podocytopathie and transdifferentiation of the podocytes in human glomerulonephritis. An immunomorphological study. *Bull. Acad. Natl. Med.* **189**, 535-545.

Bariety, J., Mandet, C., Hill, G. S., Bruneval, P. (2006) Parietal podocytes in normal human glomeruli. *J. Am. Soc. Nephrol.* **17**, 2770-2780.

Habara, P., Marečková, H., Sopková, Z., Malíčkova, K., Živorová, D., Zima, T., Tesař, V. (2008) A novel method for the estimation of podocyte injury: podocalyxin-positive elements in urine. *Folia Biol. (Praha)* **54**, 162-167.

Hara, M., Yamamoto, T., Yanagihara, T., Takada, T., Itoh, M., Adachi, Y., Kihara, I. (1995) Urinary excretion of podocalyxin indicates glomerular epithelial cell injuries in glomerulonephritis. *Nephron* **69**, 397-403.

Hara, M., Yanagihara, T., Takada, T., Itoh, M., Matsuno, M., Yamamoto, T., Kihara, I. (1998) Urinary excretion of podocytes reflects disease activity in children with glomerulonephritis. *Am. J. Nephrol.* **18**, 35-41.

Kanno, K., Kawachi, H., Uchida, Y., Hara, M., Shimizu, F., Uchiyama, M. (2003) Urinary sediment podocalyxin in children with glomerular diseases. *Nephron Clin. Pract.* **95**, 91-99.

Mundel, P., Shankland, S. J. (2002) Podocyte biology and response to injury. *J. Am. Soc. Nephrol.* **13**, 3005-3015.

Pavenstadt, H., Kriz, W., Kretzler, M. (2003) Cell biology of the glomerular podocyte. *Physiol. Rev.* **83**, 253-307.

Petermann, A. T., Krofft, R., Blonski, M., Hiromura, K., Vaughn, M., Pichler, R., Griffin, S., Wada, T., Pippin, J., Durvasula, R., Shankland, S. J. (2003) Podocytes that detach in experimental membranous nephropathy are viable. *Kidney Int.* **64**, 1222-1231.

Petermann, A., Floege, J. (2007) Podocyte damage resulting in podocyturia: a potential diagnostic marker to assess glomerular disease activity. *Nephron Clin. Pract.* **106**, 61-66.

Sakairi, T., Abe, Y., Kajiyama, H., Bartlett, L. D., Howard, L. V., Jat, P. S., Kopp, J. B. (2010) Conditionally immortalized human podocyte cell lines established from urine. *Am. J. Physiol. Renal Physiol.* **298**, 557-567.

Smeets, B., Te Loeke, N. A., Dijkman, H. B., Steenbergen, M. L., Lensen, J. F., Begieneman, M. P., van Kuppevelt, T. H., Wetzels, J. F. M., Steenbergen, E. J. (2004) The parietal epithelial cell: a key player in the pathogenesis of focal segmental glomerulosclerosis in Thy-1.1 transgenic mice. *J. Am. Soc. Nephrol.* **15**, 928-939.

Smeets, B., Kuppe, C., Sicking, E. M., Fuss, A., Jirak, P., van Kuppevelt, T. H., Moeller, M. J. (2011) Parietal epithelial cells participate in the formation of sclerotic lesions in focal segmental glomerulosclerosis. *J. Am. Soc. Nephrol.* **22**, 1262-1274.

Vogelmann, S. U., Nelson, W. J., Myers, B. D., Lemley, K. V. (2003) Urinary excretion of viable podocytes in health and renal disease. *Am. J. Physiol. Renal Physiol.* **285**, 40-48.

Yu, D., Petermann, A., Kunter, U., Rong, S., Shankland, S. J., Floege, J. (2005) Urinary podocyte loss is a more specific marker of ongoing glomerular damage than proteinuria. *J. Am. Soc. Nephrol.* **16**, 1733-1741.