List of Excel supplementary tables:

Supplementary Table 1: MRM method.

Q1 and Q3: m/z values used for selection of respectively parent and fragment ions; CE: collision energy; DP: declustering potential; RT: retention time.

Supplementary Table 2: Triplicate test of MRM method.

Triplicate aliquots from a unique healthy urinary sample from the cohort of the verification study were processed in parallel (10µg total protein concentrated on SDS-PAGE, in-gel digestion and peptide extraction) and submitted to LC-MRM analysis using the MRM method described in Sup table 1.

Sheet1: raw data as exported from the QuaSAR module under Skyline.

Sheet2: results from the AuDIT module, showing the pvalue based on a t-test on the hypothesis that the relative ratios between transitions for the analyte are different from the relative ratios of the heavy standard. A transition is considered as bad (« status ») if the p-value is less than the threshold of 10-5. All transitions monitored were flagged as « good » based on this test. The CV of the PAR (analyte/heavy standard) is also calculated between triplicate injections, and the quality of the measurement is also indicated based on this CV (« CV.status », threshold set at 20%). The « final.call » column indicates wether a transition was flagged as « bad » based on either one or the other of these two tests.

Sheet3: table showing the values measured for each transition across triplicate injections (column headers correspond to triplicate values obtained for the analyte, the internal standard, and the PAR).

Sheet4: Boxplots obtained for the CVs across the triplicate experiments (shown in sup data 2) for all transitions or only those qualified after AuDIT analysis.

Supplementary Table 3: Response curves QuaSAR on AQUA peptides.

The isotopically labeled AQUA peptides were spiked in increasing amounts in a pooled urine sample from healthy donors (9 concentrations resulting in the range: 0,05; 0,1; 0,5; 1; 10; 20; 50; 100; 250; 500 fmol of peptide on column, labeled as conditions A,B,C,D,E,F,G,H,I,J in QuaSAR) and 3 transitions were monitored for each peptide. Results were processed with the QuaSAR software to generate response curves (raw peak area of the heavy peptide against spiked amount in fmol) for each transition.

Sheet 1: raw data as exported from QuaSAR.

Sheet2: Determination of the LOD and LLOQ using the method implemented in QuaSAR, expressed as a peak area value.

Sheet3: Equation of the response curve calculated by the software (slope and y-intercept) was used for each transition to convert the LOD and LLOQ from peak area values to peptide amount (fmol on column) and to concentration values (fmol per µg of urinary proteins).

Sheet4: the transition showing the best LOD was selected.

Supplementary Table 4: MRM clinical study.

Sheet1: raw data exported from QuaSAR, showing for each of the monitored transition the peak area ratio ("area.ratio") between analyte peak area ("area") and heavy internal standard ("IS,area"), the estimated amount in fmol of the analyte on column ("quantite.estimate") calculated from the peak area ratio and the spiked amount on column (fmol) of heavy standard ("IS.conc"). The "concentration.estimate" (fmol/µg) was calculated by dividing the estimated amount of analyte on column by the total weight (µg) of urinary protein injected.

Sheet2: sub-table after selection of the best transition for each monitored peptide: transitions tagged as "bad" based on the calculated p-value with AuDIT were first eliminated (14 transitions), then for each peptide the transition showing either the best LOD in the calibration curve experiments (for the 9 monitored together with an AQUA heavy standard), or the best CV in the preliminary triplicate test experiment (remaining peptides monitored with a PEPotec partially purified standard) was selected. Colour code: green, Healthy group; yellow, Mild group; brown, Severe-Bladder group.

Sheet 3 and 4: summary tables showing the estimated amounts of the candidate proteins, based on the different peptides monitored, respectively in fmol (amount on column) or in fmol/µg (concentration per weight of urinary protein), with column headers representing the different patient analyzed.

Supplementary Table 5: Primers designed for RT-PCR analysis.

Supplementary Table 6: List of identified and quantified proteins and their peptides in the quantitative label-free LC-MS/MS analysis of the urinary proteome of UPJ obstruction patients.

The MFPaQ software reevaluated raw spectra after realignment of all LC-MS runs, using a predicted RT value and a time tolerance window to extract XIC for the total number of peptide ions identified across all samples. Based on this signal cross-assignment procedure, the protein quantification performed on the 60 raw files simultaneously allowed the identification and quantification of 970 unique urinary proteins.

***Sheet 1*:** List of proteins identified and quantified from the 60 LC-MS/MS experiments (4 groups: Healthy, Mild, Severe-Bladder, Severe-pelvis, 5 urinary samples per group, 3 LC-MS/MS replicates measurements per sample). For each protein, AC indicates the accession number in International Protein Index (IPI) database. If several proteins match the same set of peptides, only one member of the protein group is indicated and details of protein groups are shown in the column "other ACs". MW is the molecular weight. The column #QPep indicates the number of peptides quantified for the 60 samples. The Mascot score is indicated for the 60 samples analyzed (3 LC-MS/MS replicates of samples A, B, C, D, E from the Healthy group, 3 LC-MS/MS replicates of samples A, B, C, D, E from the Mild group, 3 LC-MS/MS replicates of samples A, B, C, D, E from the Severe-Bladder group, and 3 LC-MS/MS replicates of samples A, B, C, D, E from the Severe-Pelvis group).

***Sheet 2*:** List of identified and quantified peptides from the 60 LC-MS/MS experiments (4 groups: Healthy, Mild, Severe-Bladder, Severe-Pelvis, 5 urinary samples per group, 3 LC-MS/MS replicates measurements per sample). Data corresponding to each peptide including the international protein index (IPI) accession number, protein score, protein description, peptide sequence, modification site,

theoretical mass, specificity and charge are shown. For each sample, m/z value, peptide score, retention time (RT) and measured XIC Area value are indicated. Columns in green correspond to the Healthy group with samples A, B, C, D and E and LC-MS/MS replicates 1, 2 and 3 for each sample. Columns in purple correspond to the Mild group with samples A, B, C, D and E and LC-MS/MS replicates 1, 2 and 3 for each sample. Columns in red correspond to the Severe-Bladder group with samples A, B, C, D and E and LC-MS/MS replicates 1, 2 and 3 for each sample. Columns in grey correspond to the Severe-pelvis group with samples A, B, C, D and E and LC-MS/MS replicates 1, 2 and 3 for each sample.

Supplementary Table 7: List of proteins subjected to statistical analysis after correction for missing values and normalization against a reference run for the Healthy/Severe-Pelvis, Healthy/Severe-Bladder and Healthy/Mild comparisons (Sheets 1, 2 and 3 respectively). The differences between paired samples were assessed by Student's *t* test and corresponding p-values (p value *t* test) were adjusted for multiple testing using the Benjamini-Hochberg procedure (pvalue BH). Proteins were ranked by increased p value *t* test and all proteins with a significant p value *t* test ($p<0.05$) were listed under a dark line. For each group compared, the median normalized area of the 5 samples is indicated for each protein. Ratio and fold changes were calculated using these values. For the statistical analysis, normalized area were log transformed (using natural logs). For information only, log transformed value of the median normalized area is indicated for each group. An "indicator" of the comparison between the 2 groups is also shown.

Supplementary Table 8: Annotated MS/MS spectra of single peptide based protein identifications.