Editorial

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The standardization of the urine albumin assays: no longer deferrable

DOI 10.1515/cclm-2015-0831

The diagnosis and monitoring of chronic renal diseases (CKD) require the evaluation of the presence of a proteinuria in conjunction with the estimation of the glomerular filtration rate [1]. The recognized parameter to evaluate the urine protein excretion is the albumin to creatinine ratio (ACR) and the available guidelines recommend to use for ACR definite decisional levels for clinical decision making [1]. It is well known that the adoption of universal cut-off values requires that the analyte is measured with adequate accuracy (precision and trueness) or the usage of these values will be impracticable. It is rather questionable that this is the case for the urine albumin and creatinine measurements. A candidate reference method and reference material are currently under development, but not yet available [2].

In this issue of the Journal, a study by Jacobson et al. reports about the state-of-the-art of the ACR measurement in 24 accredited laboratories in Canada and in the USA [3]. Twelve pools of normal human urine were augmented with purified human albumin to generate a series of samples covering the range of clinical interest for ACR (<3; 3–30; >30 mg/mmol); the albumin concentrations in these pools were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [2] and ranged between 7.8 and 334.8 mg/L. These pools were sent to the participating laboratories together with 10 urine samples from single donors covering a wider range of albumin concentrations (9.9–1261 mg/L). The laboratories were asked to measure all the samples using their routine analytical systems that included a range of instruments and reagents from representative manufactures (Abbott, Siemens, Beckman, Ortho and Roche). The correlation by linear regression between the albumin values obtained by LC-MS/MS and the median values from the 24 laboratories was excellent ($R^2 = 0.997$, a slope of 0.88 and an intercept of –5.6 mg/L) with no significant difference between the pools and the patient samples, attesting the commutability of the pools. The results could thus be comparable to those obtained by laboratories in clinical samples during their routine practice. The median bias was negative for all samples and decreased as albumin concentration increased, ranging from –19.6% for albumin concentration <100 mg/L to –12.6% for albumin concentration >300 mg/L. A large variability exists among laboratories with some laboratories reporting a positive bias while other are reporting a negative one in the same sample. Another recent study comparing routine measurements in human urine samples to the LC-MS/MS reference method reported very close results [4]: some routine procedures show small biases, while most procedures exhibit large biases (–35% to 34%) that were not constant trough the concentration range. The results of these two different studies that used commutable samples are very similar to those observed in external quality assessment programs (EQAs), confirming the problems affecting the albumin measurement in routine practice. A large variability of biases across different analytical procedures with largest biases linked to lowest albumin concentration is reported in the 2014 EQA for urine albumin provided by the Center of Biomedical Research, Department of Laboratory Medicine, Hospital-University, Padua, Italy (250 participants, unpublished data). The bias to the consensus mean ranged between +18% and –11% across the analytical systems considered. While the vast majority of systems show a negative bias, some of them show relevant positive biases. For some procedures however the bias was very small <5%. The large variability of bias observed in the same sample reported by both studies and observed in the Italian EQA program could be attributable to different specificity to albumin fragments of the antibodies used in the different immunoassays. However, in the study by Jacobson the phenomenon was reported not only in the human urine samples but also in the pools augmented with purified albumin where the contribution of the albumin fragments is very limited. A more likely explanation could therefore be linked to the calibration procedures of the different analytical systems or to the assignment of the value to the commercial calibrators. The variability of bias over the measuring range clearly indicates that many manufacturer should improve their

calibration strategies as part of their contribution to the standardization process. Trueness is the predominant issue for urine albumin determination; some analytical systems show also inadequate precision [3, 4] in particular at low albumin concentration, reaching an impressive CV of 30% in samples with albumin <10 mg/L [3]. The Italian EQA data for precision are not so worrying, (CV <10% for all the participants laboratories in the 2014 program) but it should be noted that the lowest albumin concentration in the samples used for the scheme was 30 mg/L. The combined trueness and precision performances reported in the two studies and observed in the Italian EQA program seriously hampers the clinical use of the test. It is actually very reasonable to transfer the results obtained in these two independent studies that used commutable materials and obtained comparable results to the albumin measurements performed every day in clinical laboratories during their routine practice.

While the accuracy of the ACR measurements depends on the standardization of two analytes (albumin and creatinine), Jacobson et al. [3] produced a convincing and brilliant demonstration that the efforts should be mainly directed towards urine albumin. After establishing an arbitrary total error goal of 15%, they observed that only 10% of laboratories met the goal; the correction for the albumin calibration bias allowed an impressive 84% of laboratories to reach the goal. This percentage was increased to 86% (a very limited increase) when the correction for creatinine calibration bias was applied [3]. As the standardization process for a given analyte is costly in terms of resources and time, this is a clear message about the priority to be established.

At the moment, an IFCC working group (WG-SAU) operating in strict cooperation with the National Kidney Disease Education Program (NKDEP) and involving manufacturers, is actively working on the project of standardization of urine albumin assay. The status of the project was well described a couple of years ago [2]; some improvements have been achieved since then. The current status of the project includes progresses in the preparation of the reference method and the reference materials, as emerged during the more recent workshop of the WG-SAU (available at: http://nkdep.nih.gov/about-nkdep/working-groups/laboratory-working-group/meeting-summaries/02052015-UA-workshop.shtml). At the moment the LC-IDMS method developed at the Mayo Clinic Renal Reference Laboratory is under validation at the National Institute for Standards and technology (NIST) before being submitted to the Joint Committee for Traceability in Laboratory Medicine (JCTLM) for listing; some discrepancies observed in individual patient urines are still to be resolved. Regarding the reference materials, the NIST SRM 2925 containing pure albumin intended for calibration of LC-IDMS is now available as it is the NIST 3667 containing creatinine in frozen human urine intended for calibration of routine measurement procedures. The commutability assessment of the NIST SRM 3666 containing albumin in frozen human urine intended for calibration of routine measurements procedures is at the moment under investigation and it is expected to be terminated in the first part of next year.

The performance goals have not been developed both for urine albumin and creatinine. The approaches to establish the goals have been recently defined at the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and summarized in a Consensus Statement [5]. The topic has been considered so relevant for our profession that an entire issue of this journal (2015, issue no 6) has been dedicated to the proceedings of the conference. Out of the three proposed models to set the analytical performances specifications, the most suitable for urine albumin at the moment is probably the one based on the state-of-the-art. The biological variability of the urine albumin excretion is very high and varies between the studies being dependent on the choice of people included in the study (i.e. the disease status) and the sampling frequency [6, 7]. Setting the analytical performances on the basis of the data on biological variability would have resulted in goals much higher than those obtained on the basis of the state-of-the-art. A modeling approach based on the effect of analytical performances on clinical outcomes may be considered, but more robust studies are needed for this purpose.

This complicated work requires the contribution of different roles and competencies: NIST, NKDEP, manufactures, researchers, not forgetting clinical laboratories that should be definitely involved in the process by applying correct analytical procedures, and by checking constantly the performances of their routine methods participating in specific EQAs and critically evaluating and discussing the results obtained.

While the scientific community is actively working on urine albumin standardization, Jacobson's et al. study is a great contribution to this important issue and gives an appropriate spur to all the people involved.

The clinical relevance of the evaluation of the albumin excretion in CKD definitely calls for an urgent improvement of the analytical performances of the test.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Financial support: None declared.

Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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