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Serum creatinine and urea assays on Atellica® CH and Architect® ci4100: method comparison

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ABSTRACT: Serum creatinine and urea are markers of renal function usually measured in conjunction. This study aims to evaluate the comparability of a new analyzer incorporated to our laboratory, Atellica® with the established analyzer, Architect ® ci 4100 in serum creatinine and urea assays. We ran 110 tests for creatinine and 107 for urea. In both analyzers, serum creatinine assay is based on the Jaffe reaction while urea measurement is based on the Roch-Ramel enzymatic reaction. Linear association between methods was evaluated using Pearson's correlation coefficient. Methods comparability was assessed using Passing-Bablok and Deming linear regression. Differences between analyzers were evaluated using Bland-Altman plot. For serum creatinine, regression equations are Atellica = 0.9721 x Architect - 2.7282 (Passing & Bablok) and Atellica = 0.8884 x Architect + 1.3456 (Deming). The mean difference between the two methods is -11.7 µmol/L as indicated by Bland-Altman plot. For urea, regression lines are expressed as Atellica = 1.0252 x Architect – 0.1609 (Passing-Bablok) and Atellica = 1.1424 x Architect – 0.9532 (Deming). Bland-Altman plot presented a mean difference of -0.1 mmol/L. These results could be described as a very good agreement between the two methods, the two analyzers could be used interchangeably.

Keywords: Method comparison; Urea; Creatinine; Agreement.

1. INTRODUCTION

Creatinine is a product of muscle catabolism. Because of continual production, complete glomerular filtration and no tubular reabsorption, it remains a good marker of renal function. Serum creatinine measurement is used to estimate glomerular filtration rate (GFR) according to the Modification of Diet in Renal Disease (MDRD) study and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Therefore, it allows the diagnosis and stratification of chronic kidney disease (CKD), treatment and monitoring of acute renal failure and adjustment of drug-dose [1]. Serum creatinine concentration will vary with physiologic conditions (gender, age, muscle mass) and analytical methods (enzymatic method is more precise and less susceptible to interferences than Jaffe method) [2]. In adults (ages 18-74 years), serum creatinine averages 64 to 104 µmol/L for men and 49 to 90 µmol/L for women. Urea is an end product of nitrogen metabolism. It is usually measured in conjunction with serum creatinine for the differential diagnosis of prerenal, renal and postrenal hyperuremia. The creatinine/urea ratio is used to distinguish functional and organic renal failure [3].

A new analyzer, Atellica® (Siemens Healthineers, Erlangen, Germany) is incorporated to our laboratory. In this regard, we aim to evaluate its comparability with the established analyzer, Architect ® ci 4100 (Abbott Diagnostics Inc, Park City, IL, USA) in serum creatinine and urea assays.

2. MATERIALS AND METHODS

2.1. General framework

This is a non-interventional, descriptive and comparative study conducted in the Biochemistry Department of Medical Laboratories in the Avicenna Military Hospital of Marrakech. This hospital serves population from the southern region of Morocco.

2.2. Blood samples

Blood analysis for serum creatinine and urea were prescribed by clinicians in medical and surgical departments. We randomly selected samples from the daily routine of our laboratory assays without exclusion criteria for age, gender, underlying diseases or treatment. After centrifugation at 3500 RPM (Revolutions Per Minute) for 10 minutes, serums were divided in two parts and then processed in both analyzers in parallel. Assays were carried out over 8 days within 2 hours after the blood draw. We ran 110 tests for creatinine and 107 for urea.

2.3. Analytical procedures

2.3.1. Creatinine assay

Both analyzers use the Jaffe reaction in serum creatinine measurement. In an alkaline medium, creatinine interacts with picric acid to form a reddish-yellow creatinine-picrate complex. The rate of complex formation is measured spectrophotometrically and is directly proportional to creatinine concentration:

$$\text{Creatinine} + \text{Picric acid} \xrightarrow{OH^-} \text{Creatinine-Picrate}$$

The Atellica CH 930 creatinine reagent is actually based on a modified version of the original Jaffe method using rate blanking and intercept correction. Rate blanking is used to minimize bilirubin interference. Also, because nonspecific serum/plasma protein interactions with this reagent have been found to produce a positive bias of approximately 0.3 mg/dL (26.5 µmol/L), serum/plasma measurements are automatically corrected by subtracting 0.3 mg/dL (26.5 µmol/L) from each result. Parameters applied for serum creatinine assay on both analyzers are summarized in Table 1.

2.3.2. Urea assay

Urea assay is based on the Roch-Ramel enzymatic reaction in the two analyzers. Urea is hydrolyzed by urease to produce ammonia and carbon dioxide. The ammonia reacts with α-Ketoglutarate and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase to yield glutamate and oxidized nicotinamide adenine dinucleotide (NAD). The oxidation of NADH to NAD is measured spectrophotometrically at 340/410 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\ \text{Ammonia} + CO_2$$

$$\text{Ammonia} + \text{NADH} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{Glutamate Dehydrogenase}} \text{Glutamate} + \text{NAD} + H_2O$$

2.4. Calibration and quality control

For both instruments, calibration of methods was performed when changing lot numbers of reagent packs, at the end of the lot calibration interval, when quality control results do not fall within the assigned limits and after major maintenance. Internal quality controls were executed each day using two levels (normal and low or high) of the appropriate quality control material of known analyte concentration. Violations of Westgard rules were detected and corrected.

Table 1. Analyzers parameters for serum creatinine and urea assays.

	Creatinine		**Urea**	
	Architect ci4100	**Atellica CH 930**	**Architect ci4100**	**Atellica CH 930**
Principle	Jaffe reaction	Modified Jaffe reaction	Roch-Ramel enzymatic reaction	
Reagent onboard stability	5 days	17 days	25 days	90 days
Sample volume	9.6 µL	24 µL	2 µL	7 µL
Limit of detection	4.5 µmol/L	7 µmol/L	0.25 mmol/L	0.7 mmol/L
Reference interval in adults	Males: 63.6 - 110.5 µmol/L Females: 50.4 - 98.1 µmol/L	Males: 62-115 µmol/L Females: 49-90 µmol/L	Males : 3.2-7.4 mmol/L Females: 2.5-6.7 mmol/L	3.2 - 8.2 mmol/L
Total imprecision	≤ 6 %	≤ 8 %	≤ 4.5 %	≤ 3.4 %
Test duration	21 - 26 minutes	8 minutes	24 – 28 minutes	7 minutes
Interferences	Bilirubin, Hemoglobin, Lipemia, Ascorbate, Glucose, Proteins	Bilirubin, Hemoglobin, Lipemia	Bilirubin, Hemoglobin, Lipemia	Bilirubin, Hemoglobin, Lipemia

2.5. Statistical analysis

Statistical analysis was performed using MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium). Linear association between methods was evaluated using Pearson's correlation coefficient (r) with P-value. Methods comparability was assessed using Passing-Bablok and Deming linear regression procedures. Differences between analyzers were evaluated using Bland-Altman plot. Quantitative variables are expressed as means ± standard deviation (SD) and qualitative variables as percentages. The confidence interval (CI) is computed to illustrate the precision of our analysis and interpretation.

2.6. Evaluation of the clinical impact

To highlight the clinical utility of Atellica® CH analyzer on serum creatinine assay, we evaluated the renal function of our patients according to the MDRD study equation for calculating GFR. Concerning urea, we used acceptance limit as identified by CLIA in 2019 to judge whether the mean bias found in Bland-Altman plot analysis is clinically acceptable or not.

3. RESULTS

The lowest and highest value of serum creatinine assay were: 42.07 and 1221.84 µmol/L by Architect® ci4100, 30 and 1089 µmol/L by Atellica® CH 930. The arithmetic mean ± SD was 117.31 ± 164.44 µmol/L for Architect® ci4100 and 105.57 ± 146.54 µmol/L for Atellica® CH 930. The Pearson's correlation coefficient was too close to one ($r = 0.9964$, $P < 0.0001$, 95% CI 0.9947 to 0.9975) indicating an excellent linear relationship between the two methods. Outcomes regarding method comparison are displayed in Table 2 and Figure 1 (A and B).

Table 2. Results of Passing-Bablok and Deming regression analysis for serum creatinine.

	Passing-Bablok	Deming
Regression equation	Atellica = 0.9721 x Architect - 2.7282	Atellica = 0.8884 x Architect + 1.3456
Systematic differences Intercept A 95% CI	- 2.7282 - 8.5636 to 1.9746	1.3456 - 0.9783 to 3.6695
Proportional differences Slope B 95% CI	0.9721 0.9044 to 1.0567	0.8884 0.8816 to 0.8952
Random differences RSD ± 1.96 RDS Interval	14.0624 - 27.5623 to 27.5623	NA
Linear model validity Cusum test for linearity	No significant deviation from linearity (p = 0.09)	NA

CI: Confidence Interval; RSD: Residual Standard Deviation; NA: Not Applied.

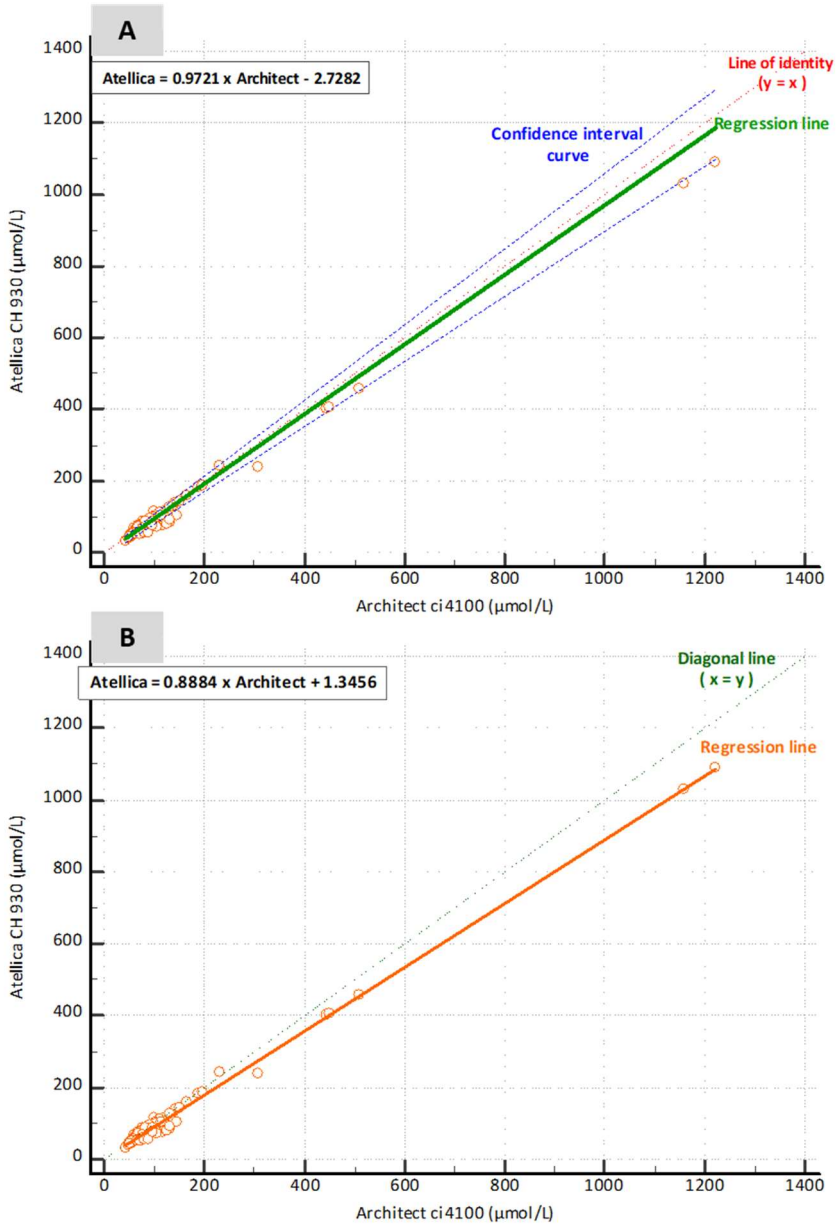

Figure 1. A: Passing-Bablok regression graph for serum creatinine, B: Deming regression graph for serum creatinine.

Table 3. Results of Passing-Bablok and Deming regression analysis for urea.

	Passing-Bablok	Deming
Regression equation	Atellica = 1.0252 x Architect – 0.1609	Atellica = 1.1424 x Architect – 0.9532
Systematic differences Intercept A 95% CI	- 0.1609 - 0.4721 to 0.08269	-0.9532 - 3.0028 to 1.0964
Proportional differences Slope B 95% CI	1.0252 -2.9424 to 2.9424	1.1424 0.8147 to 1.4701
Random differences RSD ± 1.96 RDS Interval	1.5012 - 2.9424 to 2.9424	NA
Linear model validity Cusum test for linearity	No significant deviation from linearity (p = 0.19)	NA

CI: Confidence Interval; RSD: Residual Standard Deviation; NA: Not Applied.

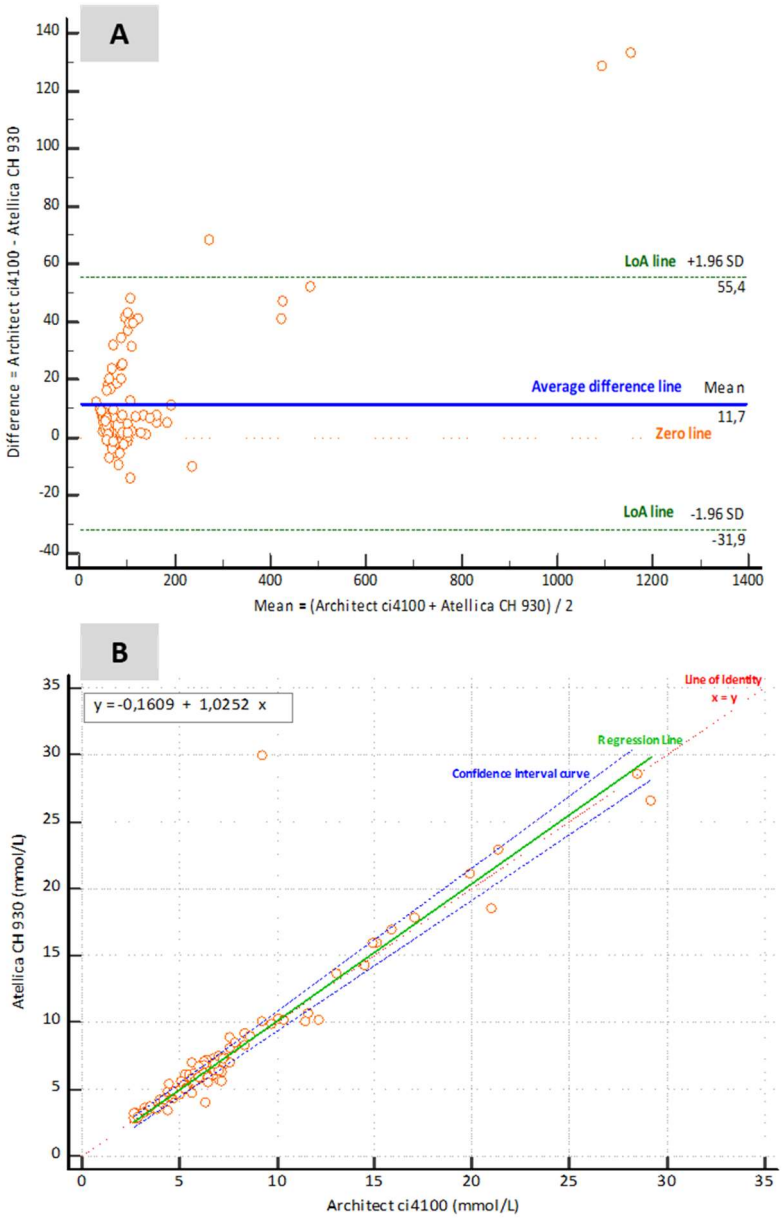

Figure 2. A: Bland-Altman scatterplot for serum creatinine, B: Passing-Bablok regression graph for urea.

Bland-Altman plot analysis (Figure 2A) showed that the difference between the two methods ranges from -31.9 to 55.4 µmol/L with a mean of 11.7 µmol/L (95% CI 7.53 to 15.95). According to MDRD Study equation, 17 patients have renal insufficiency using Atellica test results versus 16 patients using those of Architect.

Urea test results ranged from 2.80 to 29.9 mmol/L for Architect and from 2.67 to 29.21 for Atellica. The arithmetic mean ± SD was 7.63 ± 5.12 mmol/L for Architect and 7.51 ± 4.6 mmol/L for Atellica. Association between the two analyzers was high and linear as indicated by the Pearson's correlation coefficient (r = 0.95, P < 0.0001, 95% CI 0.9312 to 0.9675). Figures 2B and 3A show the regression lines between Architect and Atellica using Passing-Bablok and Deming analytical methods. Specifications related to regression equations are mentioned in Table 3. Bland-Altman plot analysis presented a mean difference of -0.1 mmol/L (95% CI -0,5263 to 0,2916) and the limits of agreement (LoA) were -4.2995 and 4.0647, as shown in Figure 3B. The maximum allowed difference between methods Δ is higher than the upper LoA and −Δ is less than the lower LoA. These results could be described as a very good agreement between the two methods.

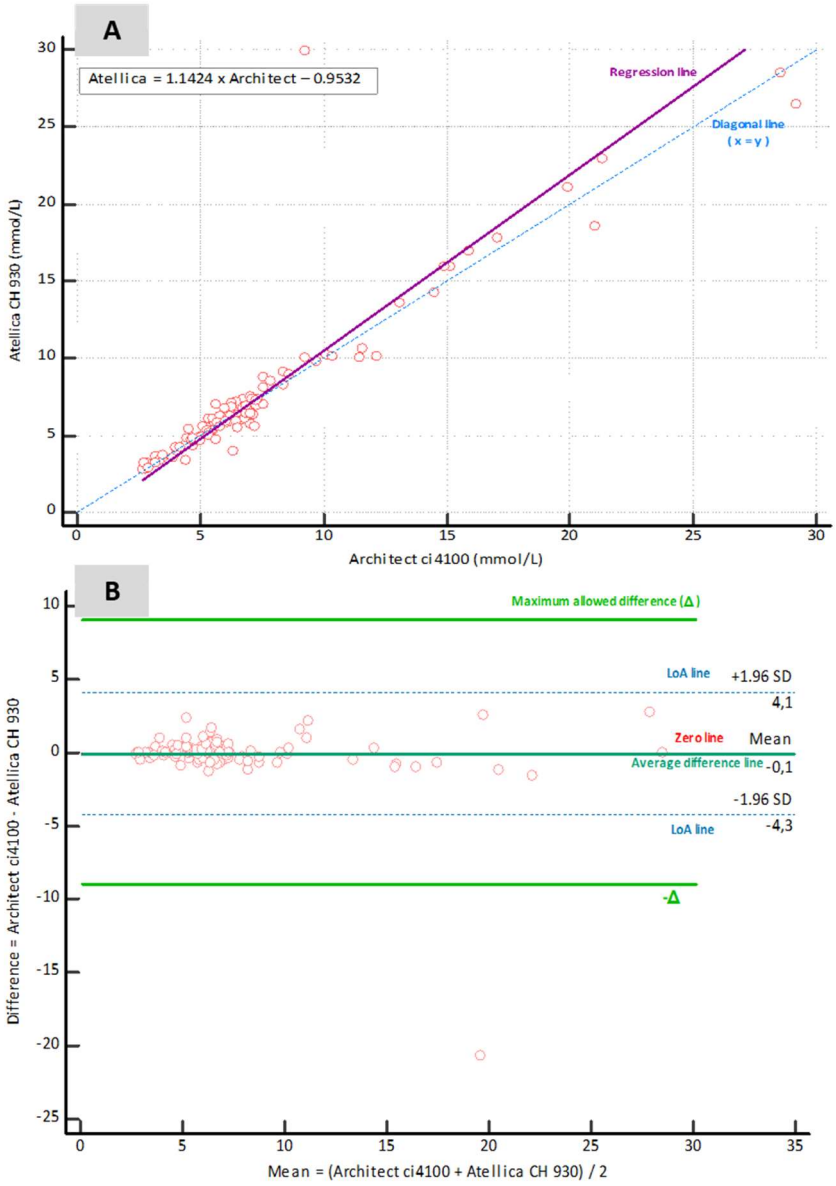

Figure 3. A: Deming regression graph for urea, B: Bland-Altman scatterplot for urea.

4. DISCUSSION

Laboratory tests are crucial in the prevention, diagnosis, prognosis, treatment and in the monitoring of diseases. Therefore, providing accurate, reliable, and timely testing results is a requirement for quality in medical laboratories [4]. Quality is applied throughout the operational processes, i.e., the pre-examination, examination, and post-examination processes. ISO 15189 standard emphasizes method verification as part of continuous improvement of quality. It requires the laboratories to ascertain that launching of a new instrumentation do not have impact on the quality of examination results [5]. Validation and verification of the new instrument includes evaluation of its performance, robustness, precision and its comparability with a reference or an established method following the respective Clinical and Laboratory Standards Institute (CLSI) protocols [6,7]. In case of significant discrepancies between the two methods, the COFRAC - SH GTA 04 (French accreditation committee - Human Health Accreditation Technical Guide) specifies that causes should be appraised, reference intervals adapted and prescribing clinicians informed [8].

In this study, we evaluated a new serum creatinine and urea assays using the Atellica® CH 930 analyzer by comparing it to the established method of Architect® ci4100. The correlation coefficient (r) is a number between 1 et -1, it expresses the degree that two variables change in the same (positive correlation) or in the opposite direction (negative correlation) [9]. It was approximately equal to one and statistically significant in either serum creatinine or urea study, and thus the two methods are positively correlated. This intends that creatinine and urea values on Atellica® CH 930 increase correspondingly with their values on Architect® ci4100. Correlation coefficient is calculated to assess association but not comparability between methods [10]. Therefore, judging acceptability and the overall analytical performance of creatinine and urea assays on Atellica® CH 930 requires using other statistical analysis such as Bland-Altman plot, Passing-Bablok and Deming regression procedures.

Passing-Bablok regression is a statistical procedure to estimate agreement, systematic and random differences between two analytical methods. It is valid only when the relationship between the two laboratory methods is linear which can be evaluated by a cusum test [11]. In both serum creatinine and urea studies, the cusum test had a P-value > 0.05 indicating a linear relationship between the two measurements and therefore the Passing-Bablok method is applicable. For serum creatinine as for urea, the 0 value is in the CI of intercept (creatinine [- 8.5636 to 1.9746], urea [- 0.4721 to 0.08269]), and 1 value is in the CI of slope (creatinine [0.9044 to 1.0567], urea [0.9044 to 1.0567]), then the two methods are comparable within the investigated concentration range.

Deming linear regression is performed to provide further evidence of agreement between two methods. It includes analytical variability of both methods (Coefficient of Variation), assumes that errors are independent and normally distributed and that both methods prone to errors [12]. In serum creatinine study, Deming regression equation showed that 95% CI for the intercept contains the 0 value, which means the absence of constant errors between the two methods. Otherwise, 95% CI for the slope exclude the value 1 indicating the presence of a proportional error. It would be necessary to investigate whether this proportional error could affect the clinical interpretation of results. However, in urea study, the Deming regression analysis supports Passing-Bablok findings and reveals an intercept (- 3.0028 to 1.0964) not statically significant from 0 and a slope (0.8147 to 1.4701) not statically significant from 1. Hence, no constant neither proportional error are present and the two methods are considered to be in agreement.

Compared with the Deming regression, the Passing-Bablok procedure could be more appropriate for comparing methods, since it is robust against outliers and does not assume that imprecision have to be normally

distributed or constant over the data range [10]. Payne proclaimed that the Passing-Bablok regression procedure is likely to be more accurate than Deming's method in the case of proportional bias [12].

The graphical approach of Bland-Altman plot is used to assess differences between two quantitative measurement pairs [13]. In this work, we used the original graph where the differences are plotted against the averages of the two measurements. The resulting scatter diagram from serum creatinine study showed that 97.27 % of the data points are lying within limits of agreement (LoA), which are defined as the mean difference ± 1.96 SD [14]. For urea study, 99.06% of differences are within ± 1.96 SD of the mean difference, which indicates almost ideal performance.

Coming to clinical evaluation, discrepancies for detecting renal insufficiency were low, as only one patient was in stage 3A of CKD using Atellica value to calculate GFR, but was in stage 2 using Architect value. Moreover, a mean bias of 11.7 µmol/L is obviously acceptable for serum creatinine levels and will not lead to serious complications in patients with CKD.

Like urea is no longer used to assess kidney function, we settled for using CLIA 2019 pre-defined clinical agreement limit to evaluate the clinical acceptance of our results. This limit was set at 9% [15]. As exposed in figure 3B, LoA do not exceed the maximum allowed difference between methods. Thus, the difference between the two methods is not clinically important.

Regarding parameters applied for serum creatinine and urea assays in both analyzers, Atellica® is almost four times faster than Architect®. Though, Architect® can be the instrument of choice to analyze small volume samples, especially in patients with hard-to-find veins. Architect® is also more reliable than Atellica in detecting small amounts of serum creatinine and urea. Yet, this does not influence the comparability between the two instruments, since for both analytes higher values are the ones to have clinical impact. There is no interest in comparing reference intervals provided by the two manufacturers, since it is up to our laboratory to establish its own reference ranges depending on the population served and the analyzer employed.

In this study, we were able to compare high values of serum creatinine and urea between the two instruments since they were included in our sample. Yet, some analytical performances such as repeatability (within-run) and total precision (within-lab) couldn't be assessed which constitutes a limit of our work. This type of experiments of method verification and validation gives our laboratory the opportunity to be engaged in a quality policy and establish a procedure for accreditation.

5. CONCLUSION

The novel serum creatinine and urea assays on Atellica® CH analyzer showed acceptable clinical utility in patients. Therefore, proportional bias found in the Deming regression related to serum creatinine study could be ignored and the two methods can be used interchangeably. Incorporation of Atellica® CH analyzer in Avicenna Military Hospital of Marrakech would further allow monitoring patients with CKD and those under hemodialysis in the southern region of Morocco.

Authors' contributions: HZ: corresponding author, conception and design, development of methodology, acquisition of data, analysis and interpretation of data, writing, review and/or revision of the manuscript, administrative, technical, or material support, study supervision. SN: acquisition of data, analysis and interpretation of data, writing, review and/or revision of the manuscript. IM: acquisition of data, analysis and interpretation of data. SC: administrative, technical, or material support, study supervision. AB: conception and design, development of methodology, acquisition of data, analysis and interpretation of data, review and/or revision of the manuscript, administrative, technical, or material support, study supervision. All authors discussed the results and contributed to the final manuscript.

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