

## Random spot urine protein/creatinine ratio: a reliable method for monitoring lupus nephritis?

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### Abstract

**Background.** Lupus nephritis (LN) is a common and severe manifestation of systemic lupus erythematosus (SLE) that can lead to end-stage renal disease. According to the Kidney Disease Outcomes Global Improving clinical Guidelines for Glomerulonephritis, spot urine protein/creatinine (P/C) ratio should be used for monitoring LN. However, some reports write that the random spot urine P/C ratio is unreliable in monitoring proteinuria in SLE glomerulonephritis patients. The aim of this study was to evaluate the agreement of these two assay methods.

**Methods.** The prospective observational study was performed. Fifty-three paired (total 106) spot and 24-h urine collections were evaluated. Statistical analysis: SPSS 20.0.

**Results.** Paired samples t-test did not reveal significant differences between the two-paired assay methods (spot P/C ratio versus 24-h proteinuria and 24-h P/C ratio) and a statistically significant correlation was observed between them: Pearson's coefficient of 0.847 ( $P < 0.001$ ) and 0.863 ( $P < 0.001$ ), respectively. However, after stratifying by degrees of proteinuria, a poor correlation was found in the range of  $< 500$  mg/day and only 26.6% of 24-h P/C ratio was explained by the spot P/C ratio. Adding to this, for proteinuria range between 500 and 1000 mg/day, there was no correlation (Pearson's  $-0.098$ ;  $P > 0.05$ ). In fact, only 1% of 24-h measurements could be explained by the spot P/C ratio.

**Conclusions.** Our study demonstrated a good correlation between 24-h proteinuria and random P/C ratio among patients with LN. However, this correlation was poor for proteinuria under 500 mg/day and did not exist in a range between 500 and 1000 mg/day. This finding is of greater importance because this range is quite common in patients with LN remission. Until further clarification, to the best of our knowledge, we maintain reluctant to completely substitute the 24-h collection by the P/C ratio especially when a renal flare is suspected, or before any change in therapy.

**Keywords:** glomerulonephritis; lupus nephritis; proteinuria

### Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder affecting multiple organs. The reported incidence of kidney disease in SLE is ~38% and 40–60% at the time lupus is diagnosed [1–3]. Lupus nephritis (LN) is a common and severe manifestation of SLE that can lead to end-stage renal disease (ESRD) and death [4]. Based on a retrospective cohort from the UK, 19% of Caucasians and 62% of blacks with LN progressed to ESRD [5].

To improve the efficacy and decrease the adverse effects of immunosuppression, biomarkers of renal disease [first glomerulonephritis (GN) and renal flares] are used to predict the course and activity of LN and adjust therapy appropriately.

A significant has been demonstrated a significant correlation between response to aggressive immunosuppressive treatment and reduction or remission in proteinuria

and outcomes in LN [4, 6–10]. There is less agreement regarding the best method to determine urinary proteins.

The *gold standard* to assess proteinuria is the protein content of accurately collected 24-h urine [11, 12]. However, errors in urine collection are frequent, compromising the accuracy of the method [13–15]. In an effort to simplify proteinuria assessment and minimize false results, Ginsberg *et al.* [16] reported that the P/C ratio of a single-void ('spot') specimen was highly correlated with 24-h proteinuria. Since then, numerous studies have confirmed Ginsberg's work [17, 18] and the Kidney Disease Outcome Quality Initiative (K-DOQI) now recommends (Level A recommendation) replacing 24-h proteinuria testing with the P/C ratio of random spot collections [12, 14]. According to the European League Against Rheumatism [19] and Renal Disease Subcommittee of the American College of Rheumatology (ACR) [20], a random spot urine protein/creatinine (P/C) ratio provides information on the

presence and prognosis of renal involvement [19, 21–23] and for this reason, should be used for monitoring LN. This recommendation is supported by Kidney Disease Outcomes Global Improving (KDIGO) clinical practice Guidelines for Glomerulonephritis, recently published in June 2012 [24].

However, in the subnephrotic range, the random spot urine P/C ratio appears to be only weakly related to 24-h proteinuria [17]. There have been numerous reports on the use of the random spot urine P/C ratio to estimate 24-h proteinuria in non-SLE CKD [17, 18] but only few relating to SLE GN patients have been published. Leung *et al.* [25] reported a good correlation and limits of agreement between the spot urine P/C ratio and 24-h urine total protein across a wide range of proteinuria and recommended using the spot urine P/C ratio in screening and monitoring significant proteinuria in patients with LN. In opposition to this statement, Birmingham *et al.* concluded from two studies that the random spot urine P/C ratio is unreliable in monitoring proteinuria changes in individual SLE GN patients and is also unreliable as a screening test. They found that this method underestimates and overestimates the results with about equal frequency. In conclusion, they recommended collecting 24-h urines that are at least 50% complete based on their creatinine content [15, 26].

Since there is a paucity of data regarding the utility of the urine P/C ratio in monitoring proteinuria in patients with LN [27, 28], the aim of our study is to evaluate the agreement of the urine P/C ratio in untimed specimens with proteinuria measured by 24-h urinary collection in patients with LN and try to ensure the best choice.

## Methods

A prospective observational study was performed between October 2012 and January 2013. A total of 27 patients over 18 years old with SLE (GN/non-GN) were included and proteinuria was determined in 53 paired spot and intended 24-h urine collections (total 106) as part of routine monitoring of their disease activities. All the patients were recruited on an outpatient setting but some were during an in-patient stay in the Nephrology Department of Hospital dos Covões.

The study of 24-h sample and spot P/C ratio was undertaken simultaneously. Patients were instructed to collect untimed spot urine in the morning of the appointment day (the second urination of the day), after discarding the first urine of the day which was included in the 24-h urine sample.

To determine the accuracy of the 24-h collections, the following ratio was calculated: [measured creatinine content (M)/expected creatinine content (E)] using the Cockcroft–Gault (C-G) equation, where  $E = (140 - \text{age}) \times \text{weight (kg)} \times 0.2 \times (0.85, \text{ if female})$  [26]. An M/E ratio of 0.75–1.20 was deemed a complete 24-h collection.

The P/C ratio was calculated, for both samples, dividing the urinary proteinuria (mg) by creatinine (g). Glomerular filtration rates (eGFR) were calculated by the modification of diet in renal disease (MDRD) equation and only included those with GFR 90–120 mL/min.

Statistical analysis was performed by SPSS 20.0 Statistical Analysis (SPSS, Inc., Chicago, IL, USA). In the univariate analyses, data were presented by mean, median, mode, standard deviation, minimum and maximum for scale variables. To analyse whether or not the measurements

were different under the two methods, we performed a paired *t*-test. The strength of the correlation was determined by calculating Pearson's correlation coefficient. The linear regression model was used to find out whether the spot P/C ratio can predict 24-h P/C ratio and 24-h proteinuria simple measurement. Two-sided  $P < 0.05$  was considered as statistically significant.

## Results

We analysed 27 European Caucasian patients (70% female gender) with SLE, with or without active LN. Their age ranged from 22 to 64 years with an average of  $38 \pm 13$  years and weight from 50 to 102 kg with an average of  $66 \pm 14$  kg. eGFR assessed by MDRD had an average of  $112.4 \pm 15.8$  mL/min.

The accuracy of the 24-h collections was assessed by the ratio measured creatinine content (M)/expected creatinine content (E) described in the section above and only the samples with a value between 0.75 and 1.20 were included (53 paired samples). The average of M was  $1.235 \pm 0.265$  g/day and E was  $1.233 \pm 0.281$  g/day. Their ratio M/E ranged from 0.81 to 1.20.

It was confirmed that the 24-h proteinuria simple measurements and the 24-h P/C ratio were highly correlated, without any significant differences through a paired *t*-test and linear regression (Pearson's 0.991;  $P: 0.000$ ;  $R^2 98.2\%$ ). The basic characteristics of the three methods are presented in Table 1.

The paired samples *t*-test did not reveal significant differences between the two-paired assay methods (spot P/C ratio versus 24-h proteinuria and 24-h P/C ratio) and a statistically significant correlation was observed between them: Pearson's coefficient of 0.847 ( $P < 0.001$ ) and 0.863 ( $P < 0.001$ ), respectively. Linear regression showed that 71.7% of 24-h proteinuria and 74.6% of 24-h P/C ratio were explained by the spot P/C ratio (Figure 1).

After stratifying by degrees of proteinuria, the methods still were not significantly different (paired *t*-test with  $P > 0.05$ ). However, according to Table 2, for proteinuria range  $< 500$  mg/24-h the correlation was poor (Pearson's 0.471), and 26.6% of 24-h P/C ratio and 22.2% of 24-h proteinuria were explained by the spot P/C ratio ( $R^2$  of the linear regression model). Adding to this, for proteinuria range between 500 and 1000 mg/24-h there was no correlation (Pearson's  $-0.098$  for 24-h P/C ratio and  $-0.106$  for 24-h proteinuria, both with  $P > 0.05$ ). In fact, according to linear regression (Table 2 and Figure 2), only 1% of both 24-h measurements could be explained by the spot P/C ratio.

When stratifying according to the background of LN in two groups (no LN and LN in remission or in activity), we did not observe differences between correlations calculated for each subgroup. We did not separate LN according to its activity because we only had three patients with

**Table 1.** Samples characteristics

	24-h proteinuria (mg)	24-h P/C ratio (mg/g)	Spot P/C ratio (mg/g)
Mean	662.56	490.78	509.05
Standard deviation	1397.77	820.76	690.66
Minimum	18.00	14.99	27.00
Maximum	9650.10	5597.18	3949.00

active LN, and we did not stratify proteinuria for a nephrotic range (>3000 mg/day) because we only had one patient making a statistical analysis impossible.

**Discussion**

The adequate detection and quantification of proteinuria is of great importance in the management of patients with kidney disease.

There have been numerous reports on the use of the random spot urine P/C ratio to estimate 24-h proteinuria in non-SLE CKD [29–37]. However, few papers have been published regarding SLE patients [38, 39]. Furthermore, according to Hebert [26], the random spot urine P/C ratio is unreliable for estimating 24-h proteinuria in individual SLE patients. In contrast our study demonstrated a good correlation between 24-h proteinuria (simple measurement and 24-h P/C ratio) and random P/C ratio among patients with LN, which is in accordance with prior cross-sectional, observational studies performed with other subgroups (pregnant women, kidney transplanted patients and patients with diabetic and non-diabetic nephropathy) [31, 33–37].

Yet, previous studies suggested that this correlation varies in accordance with different levels of proteinuria

[31–33, 39] and it is worse (or there is none) for proteinuria >3500 mg. In our population, we only observed one patient with proteinuria in the nephrotic range due to an inaugural LN whose results differed greatly between the P/C ratio and 24-h collection, in agreement with previous data.

Adding these results, when we stratified by degrees of proteinuria, the correlation between 24-h proteinuria and 24-h P/C ratio with spot P/C ratio was poor for values under 500 mg/day and not observed between 500 and 1000 mg/day. The random spot urine P/C ratio underestimated and overestimated the 24-h measurements. It only explained 22–26% of the results in the first range (<500 mg/day) and merely 1% in the second range (500–1000 mg/day). This finding is of greater importance and concern because this range is quite common in patients with LN in remission, in whom it is essential to monitoring and detecting renal flares early. Finding answers for this lack of relationship, we must point out that SLE GN patients are overwhelmingly female, generally smaller than non-SLE CKD (lower urine creatinine excretion rates) and more likely to manifest inflammation, which lowers serum albumin by increasing catabolism [38]. In addition, the daily variation in protein excretion [32] adding to minor changes in the patients usual routine (nocturia, diet—salt or protein intake, medication timing, exercise, posture, body temperature [11, 15, 17]) can importantly influence the protein or creatinine content of the shorter collection.

On the other hand, the collection of 24-h urine is complicated, and it is not always performed correctly. Hence, the simplification of the collection and subsequent calculation of the ratio could be easier to perform and results in lower healthcare costs.

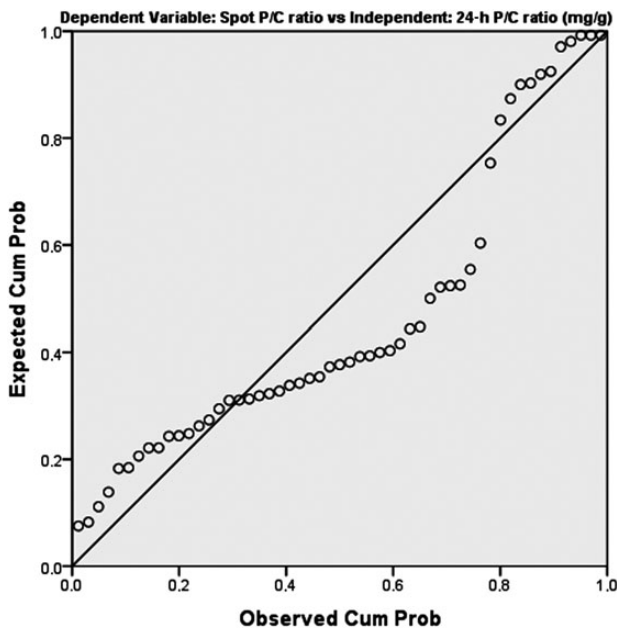


Fig. 1. Normal P–P plot of regression standardized residual for 24-h P/C ratio versus spot P/C ratio.

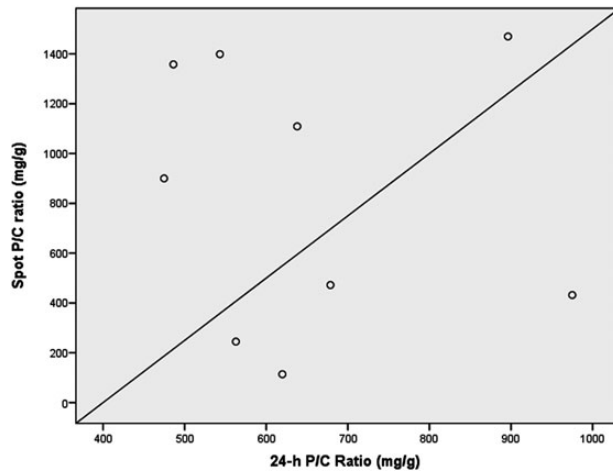


Fig. 2. Scatter plot of spot P/C ratio and 24-h P/C ratio for proteinuria range 500–999.99 mg/day.

Table 2. Statistics analysis sorted by class of proteinuria (correlation and regression)

		Spot P/C ratio (mg/g)		
		Pearson's coefficient	Sig. (one-tailed)	R <sup>2</sup>
Proteinuria (<500 mg)	24-h P/C ratio (mg/g)	0.516	0.000	0.266
	24-h proteinuria (mg)	0.471	0.003	0.222
Proteinuria (500–999.99 mg)	24-h P/C ratio (mg/g)	–0.098	0.401	0.010
	24-h proteinuria (mg)	–0.106	0.359	0.011
Proteinuria (>1000 mg)	24-h P/C ratio (mg/g)	0.942	0.008	0.887
	24-h proteinuria (mg)	0.917	0.005	0.841

In conclusion, it is important to consider that 24-h proteinuria remains as the gold standard to diagnose proteinuric diseases, and it is the most used parameter concerning glomerular diseases.

Nevertheless, isolated collection of the random urine sample to determine the P/C ratio has some advantages concerning facility, reliability, accuracy and diagnostic speed; it could also be used as the preferential marker in subgroups of subjects with more difficulty to properly collect urine in 24-h, such as children, elderly patients and those with intellectual disabilities, or when the collection is incompatible with the professional activities of the patient, in the case of refusal to do this examination or at the suspicion of the lack of adherence [40]. Further studies are warranted to evaluate if urine collection periods that are longer than spot collections but shorter than intended 24-h collections may also reliably estimate 24-h proteinuria.

One limitation to our study is the number of patients.

Until further clarification, to the best of our knowledge, we maintain reluctant to completely substitute the 24-h collection by P/C ratio especially when (according to other activity parameters) a renal flare is suspected, as well as before any change in therapy.

*Conflict of interest statement.* None declared

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