Morphological indexes: can they predict lupus nephritis outcomes?

Dear Editor-in-chief,

On behalf of the authors responsible for this manuscript I thank you for the possibility of publishing it. Also, we appreciate the reviewers’ comments, which have contributed to the overall improvement of the paper. We tried to answer to all comments/suggestions (please see below).

Please note that we have also included a few changes reflecting some new evidence that has since been published.

Changes to the original manuscript were marked by changing the text color.

Reviewer A:

1) Misconduct: Regarding Authors´s contributions please consider including the first author, David Navarro, contribution.

The following sentence has been added: “DN was involved in all stages of manuscript elaboration.”.

2) Abstract: I suggest the following changes:  
Keywords in Portuguese and English should have the same order.

This has been changed.

Units of measurement should be stated (ex: proteinuria, C3).

This has been added.

Abbreviations should be preceded by the full words (ISN/SPS, LN, eGFR, FUP, EULAR, TFGe)

This has been corrected.

Please consider changing “tendency towards” to “nonsignificantly higher” as p-values are either significant or nonsignificant. The use of terms such as “tendency” may be misleading.

This has been changed.  
  
Please consider changing:  
“Neste estudo retrospectivo, procurámos analisar a aplicabilidade e relevância dos índices morfológicos na predição da resposta à terapêutica e do prognóstico dos doentes do nosso centro cuja biópsia renal documentou nefrite lúpica.”  
To  
““Neste estudo retrospectivo unicêntrico, procurámos analisar a aplicabilidade e relevância dos índices morfológicos na predição da resposta à terapêutica e do prognóstico dos doentes cuja biópsia renal documentou nefrite lúpica.”

This has been changed.  
  
Please consider changing:  
“pior TFGe” to “TFGE mais reduzida”  
“maior proteinuria” to “proteinuria mais reduzida”  
“tendência para maior consumo” to “consumo mais elevado de modo não  
significativo”  
“tanto o índice de Hill quando o score de cronicidade de Austin” to  
“tanto o índice de Hill quanto o score de cronicidade de Austin”

This has been altered as per your suggestions.  
  
3) Introduction:

Please consider changing “…the importance of the different LN morphological indexes” to “…the efficacy of the different LN morphological indexes” as the term “importance” is broad and may be interpreted in different ways.

This has been changed.

4) Methods:

As the definition of complete remission and partial remission is not consensual and different studies use different definitions, I consider that it should be explained how the authors chose their definitions of complete remission and partial remission. If possible it should be referenced. Was it defined using any current guidelines? Was it defined using the definition used in recent LN studies published in high-impact journals? Other?

As the definition for complete and partial remission is not consensual, we have used the definition used in the LUNAR trial, one of the most recent impactful lupus nephritis trial (<https://www.ncbi.nlm.nih.gov/pubmed/22231479>). This had been clarified in the manuscript’s text.  
  
Please consider changing “random protein to creatinine ratio” to “urinary protein to creatinine ratio in a random urine sample”

This has been changed.

Please consider changing “dsDNA” to “anti-dsDNA” as “anti-dsDNA” is the term that is used in the rest of the main text.

This has been changed.

Please consider changing “INS/RPS” to “ISN/RPS” in the fourth paragraph.

This has been changed.  
  
Please consider writing the full words of “CKD-EPI” the first time they appear in the manuscript.

This has been added.  
  
Please consider changing “the chronicity index as our main predictors” to “the chronicity indexes as our main predictors”

This has been changed.  
  
5) Results:   
Please consider mentioning how many kidney biopsies were found to be inadequate due to insufficient number of glomeruli as this is mentioned in the “Materials and Methods” section.

Please consider mentioning in the Methods section the number of glomeruli that you considered as the minimum acceptable number of glomeruli.

This has been added in the Materials and Methods section: “Patients whose kidney biopsies were found to be inadequate due to insufficient number of glomeruli (defined as less than 5) were excluded.”

Please consider changing “while for the remaining” to “while the remaining”.

This has been changed.  
  
Please explain why it was used the cut-off value of <50 ml/min as this is not a cut-off used by KDIGO CKD guidelines. Was it because it is used in LN studies? Other? This needs to be clearly explained and if this cut-off is maintained it should be explained in the methods section.

A transcription mistake was made while writing the manuscript. The used cut-off value was 45 ml/min, corresponding to CKD stage 3b. This was corrected in the appropriate sections.

Please confirm if “median of 11 glomeruli (7.75 – 14.25)” is correct as is it might be confusing to consider a fourth of a glomerulus.

We have slightly changed the sentence for clarity: “Regarding histology, sample quality was adequate, the minimum number of glomeruli per sample was 5 and we had a maximum of 30 glomeruli, with a median of 11 glomeruli (7.75-14.25).”  
  
Please consider using always either “,” or “.” in the manuscript when separating the unit from the decimal digit.

This issue was corrected, and all decimal digits are now separated with “.”  
  
Please consider changing “…we observe 15 complete and 15 partial remissions and 4 patients…” to “…we observe 15 complete, 15 partial remissions, and 4 patients…”

This has been changed.  
  
Please consider changing “…tendency towards…” to “…nonsignificant…”.

This has been changed.  
  
6) Discussion:   
  
Please consider rephrasing the sentence “…shed some light in this respect…” as I believe it may be adaptation from portuguese. A possible alternative might be “…shed some light on this subject…”

This has been changed.  
  
7) Conclusions:   
  
Please consider hedging the last sentence (ex: “provide a useful” to “may provide a useful”).

This has been changed.  
  
8) References:   
In reference 9 there are three question marks. Please consider reevaluating if they are needed. Please consider removing the “[Internet]” and “Available from:…” sections of different references

References have been corrected.  
  
9) Tables / Figures:   
Please consider expanding the Tables legends as Tables should be self-explanatory.

We added table legends for improved understanding.

Please mention that “Activity Index” and “Chronicity Index” both pertain to Austin Index.

This has been added.

Please consider including in the Table legends the meaning of the abbreviation (eGFR).

This has been added.

Please consider mentioning in Table 2 if these are univariate or multivariate models. Please check if p values are the same in the text and in the table 2 (although I am not certain, p-value of eGFR using chronicity index appears as 0.02 in the text and as 0.002 in the Table 2).

We corrected the p-values. Table 2 represents an univariate analysis. This information was added to the table legend.  
  
Please consider changing Hill Biopsy Index to the last column in Table 1 and Table 2 as it has been developed after Austin Index.

This has been changed.  
  
10) Acknowledgments:   
Please consider changing “The authors declared no potential conflicts…” to “The authors declare no potential conflicts…”

This has been corrected.  
  
------------------------------------------------------  
  
------------------------------------------------------  
Revisor B:  
  
1) Métodos:   
Os scores não devem estar descritos conceptualmente nos metidos mas sim na Introdução.

Não concordamos com a sugestão, pelo que não modificámos a estrutura da Metodologia. Acreditamos que os métodos são o coração de um artigo original e que será nessa secção que deverão estar descritas as diferentes formas de avaliação das biopsias renais estudadas (neste caso os diferentes índices morfológicos). Colocá-los na Introdução iria não só torná-la bastante mais extensa, mas também, iria alterar a forma de como achamos que uma introdução de um artigo original deve ser feita: dar a visão geral de um problema (o LES e a NL), expôr o problema específico e terminar com a questão fulcral do artigo.

2) Resultados:   
Os resultados costumam ser apresentados com média +/- desvio padrão.

Os resultados são apresentados como médias se a amostra tiver uma distribuição normal. Não foi o caso da nossa amostra e das diferentes variáveis, pelo que apresentámos os dados em medianas com variação interquartil.  
  
O grau de Hipertensão arterial deve ser citado ou mencionado ou descrito em termos numéricos

Concordamos com a sugestão. Acrescentámos a definição de hipertensão arterial usada.  
  
Penso ser relevante descrever a causa de morte do outro doente

Acrescentámos a causa de morte do doente em causa.  
  
3) Tabelas / Figuras:

Penso que a tabela 2 pode ter uma legenda que descreva sucintamente os Indexes apresentados.

Concordamos com a sugestão e acrescentámos uma legenda à tabela.

------------------------------------------------------  
  
------------------------------------------------------  
Revisor C:  
  
1) In the Materials and Methods’ section it is indicated that data for continuous variables is presented as “mean +/- standard deviation values, when normally distributed, or as median (interquartile range) otherwise”. This choice made the presentation of results rather confusing, because one (almost) never knows when authors are writing about means or medians and corresponding ranges calculated using standard deviations or inter-quartile ranges. I would suggest medians and inter-quartile ranges only, as with this choice the symmetry of the corresponding data would snow immediately. If this is not the choice, then it should be clearly stated whether medians or means are being used in each case. 

We accept your suggestion and all continuous variables will be presented as medians. and inter-quartile ranges. We changed the sentence to: “Data are presented as frequencies for categorical variables; continuous variables are presented as median (interquartile range).”

In this section you refer that both Austin and Hill scores describe findings in a semi-quantitative fashion and I am not sure what does that mean. Does that make the score not to be quantitative? (I believe in the statistical analysis they are treated as such).

No. This term is usually used in the histomorphology field to refer to such 0 to 3 quantification. For clarity, we deleted the term “semi”.

2) Results:   
There must be an error in table 1, for the Nephrotic syndrome presentation data divided into the LN classes – the corresponding percentages, 0%, 25%, 63% and 31% sum up to 119%!

The percentages are correct. The reason for this is that Class V can exist in isolation or co-exist with other classes.  
  
P-values should always be referred to the test they correspond to, what does not always happen in the text (as for example in tables 1 and 2). This is even more problematic when they correspond to different types of tests.

This information has been added.

Table 1: The analysis of the proportions between renal characteristics and LN classes was performed using Fisher exact test; the analysis of the variance of the different indexes was performed using one-way ANOVA.

Table 2: All correlations were made using Sperman correlation in a univariate analysis.

It is mentioned in the Materials and Methods’ section that:  
1)    One-way ANOVA was used for comparing between the different indications for kidney biopsy, the laboratory data and the morphologic data obtained after the application of the different morphological indexes;   
2)    Chi-square test of independence was performed when comparing the different indications for kidney biopsy and the ISN/RPS LN classes;  
3)    Associations between the different LN classes and clinical and laboratory variables were performed using qui-square test or Fisher’s exact test and t-test or Wilcoxon, depending on normality;  
4)     Associations between Austin and Hill indexes and laboratory variables were performed investigated using Spearman or Pearson test, depending on normality.  
5)    A model of multivariate analysis using all subjects was performed fitted, and defining the chronicity index as the main predictors and eGFR as the main outcome (There are lots of different models for multivariate analysis… is it possible that you were wanting to say multiple linear regression model – the response variable is normally distributed and the mean response variable is explained by a set of covariates?).  
  
What I see afterwards in the Results’ section is the following:  
1)    I am not sure about where are the results for ANOVA. Do they correspond to the p-values of the last 3 columns of table 1? These results are not commented nor presented in this section;

That’s the case. We added that information in Table 1.

2)    I’m guessing that results shown in columns 3-6 of Table 1 are the results of the chi-square tests of independence, for comparing the different indications for kidney biopsy and the ISN/RPS LN classes – although that is not said in the text;

That’s correct. We added that information to the legend in table 1.

3)    Ok;  
4)    Ok;  
5)    The results presented about the so called multivariate model, which I fail to understand which model it is, are insufficient to understand it. Are the confounders relevant?

We performed a linear regression between the Chronicity index and eGFR at the end of follow-up. We add to the model age and gender and proteinuria (older patients may have less renal function, women tend to have lower GFR and proteinuria is associated with rapid decline of renal function) we used those variables as potential confounders. Doing a univariate analysis between Chronicity index and eGFR at the end of FUP, the p-value is the same (p=0.002).  
  
3) I would think that the two first paragraphs of discussion could have been presented in introduction.

We acknowledge your suggestion, but think those two paragraphs are important for a coherent discussion of the results we found.  
  
4) In page 7, line 14, where it reads “As shown in table 1, we observe a correlation…” it would be more adequate to write “As shown in table 1, we observe an association…”.

This has been changed.  
  
5) In page 7, line -5, where you write about “…observe a correlation between the renal outcomes and the indexes…” maybe you mean that “…observe a significant correlation between the renal outcomes and the indexes…”  (there is always a correlation between numerical quantities; what might happen is that the correlation might be/might not be  
significant).

This has been corrected.

Once again, we acknowledge and kindly thank the reviewers for their observations and hope the manuscript has been improved by these changes.

Best regards,