Peer Review File

Article information: https://dx.doi.org/10.21037/tp-23-229

Reviewer A

The authors have delineated the utility of Neutrophil-to-Lymphocyte Ratio (NLR) in predicting Vesicoureteral Reflux (VUR) complications during follow-up in a retrospective cohort study conducted at a single center. Despite the presence of several areas for potential enhancement in the study design, the manuscript was composed in a nearly impeccable manner. Given the insufficient elucidation of the utility of NLR, the findings of this investigation appear intriguing and could prove valuable for future inquiry. There are multiple aspects of concern regarding the current investigation.

Major point:

Comment 1. The authors have designated the current study as a "case-control study" in their report. However, in a typical case-control study, the control group should be selected from patients who match the background of the case group. Therefore, the study design should be described as a "retrospective observational cohort study." Furthermore, to explore the risk factors for the development of VUR complications, the authors should use multivariable logistic regression models that assume confounding factors to avoid biased results. The authors should reconsider which background variables are necessary to analyze urinary tract infections and justify their variable selection. These recommendations are also reflected in major comment No. 4.

Following the reviewer's recommendations, we have modified the description of the study design. Regarding the second question, although it is true that we have collected many demographic, perinatal, microbiological, radiological and laboratory variables, in the univariate analysis we could not find differences in demographic factors, perinatal data or microbiological features, as shown in Table 1 and 2. Because of this, we performed a multivariate analysis using ROC curves of the variables in which we found differences in the univariate analysis, such as radiological and laboratory data.

Comment 2. Previous studies have shown the possibility of false negatives in VCUG. How did the authors address this potential issue?

Indeed, with VCUG there is a possibility of false negatives in both diagnosis and detection of VUR resolution. However, these false negatives are usually low grades of VUR (mainly grade I and II), which have a high probability of spontaneous resolution and low risk of complications. We have added this information in the Discussion.

Comment 3. Although the authors collected several variables in the study, they appear to be insufficient. For instance, the use of antibiotics before

admission and a history of hospitalization are thought to be risk factors for infection with resistant bacteria. The authors should reconsider which background variables are necessary to analyze urinary tract infections and justify their variable selection.

In this study we have included demographic, perinatal, microbiological, radiological and laboratory variables that have been associated with the development of APN. Within the clinical data, the use of antibiotics before admission and a history of hospitalization is routinely collected in our institution, but none of the patients included in the study had been recently hospitalized or on antibiotic treatment, because all of them had their first episode of APN in their lives, and only patients with primary VUR were included in the study. The remaining patients with APN and secondary VUR, who are the most frequently hospitalized and on previous antibiotic treatment, were excluded from the study.

Comment 4. The authors only examined laboratory data to identify risk factors for the development of VUR complications. However, demographic factors, perinatal data, microbiological data, and other variables should also be analyzed. I recommend that the authors construct a multivariable model using this data.

As previously explained in point 1, for the identification of risk factors for the development of VUR complication only differential factors were analyzed in the univariate analysis between the spontaneous resolution group and the group that developed complications. As significant differences were only identified in radiological and laboratory data, these were the data on which the multivariate analysis was performed using the ROC curve.

Comment 5. The present study has many limitations and biases because it was a single-center retrospective study. The conclusion is too forceful, implying that all patients with high NLR should undergo surgical correction. Therefore, the authors should revise the conclusion in the abstract.

We agree with the reviewer that the conclusion is too categorical for a retrospective study like ours. We have emphasized the use of "may", as well as added the need for future prospective studies to confirm these results: “Therefore, it should be included in the management algorithm for these patients, although future prospective studies are still required to confirm these results”.

Minor point:

Comment 1. In the abstract, any abbreviations should be spelled out when they are presented for the first time.

We have spelled out the missing abbreviations in the abstract: ROC curve and AUC (area under the curve). All other abbreviations are defined (NLR, VUR, APN).

Comment 2. The number of patients in each group should be indicated in the results section of the abstract.

We have added a sentence including the number of patients in each group: “Spontaneous VUR resolution occurred in 169 patients (group A), while complications development were observed in the remaining 104 patients (group B)”

Comment 3. The statement regarding adherence to the STROBE guidelines should be included in the methods section (L107-108).
We have added statement regarding adherence to the STROBE guidelines in the Methods section.

Comment 4. The group names used (group A and B) were not easily understood. It would be better to name the two groups, such as the "spontaneous resolution group".
We have changed the references of group A to "spontaneous resolution group" (SR group) and of group B to "complications development group" (CD group) in many parts of the Results and Discussion section.

Comment 5. In Figure 1, Group B is labeled as the "Surgical correction group", which is different from the grouping described in the manuscript. It is unclear whether all patients in Group B received surgical correction of the renal system.
Following the reviewer recommendation, we have modified Figure 1 as "development of complications during follow-up", instead of "surgical correction", following the reviewer's recommendation.

Comment 6. The number of patients in Figure 1 appears to be incorrect. Although 129 patients were excluded from the original 403, leaving 274 patients, the figure shows 273 patients.
We have modified the mistake in the abstract and in the results section. The patients included in the study were 273 patients with APN and primary VUR.

Comment 7. The cutoff point used in the ROC curve should be clearly indicated in Figure 2.
We have marked the cutoff point with a cross in Figure 2.

Reviewer B

The authors focused on NLR in children diagnosed APN with VUR and evaluated the association between long-term prognosis of VUR and NRL. They demonstrated that NLR may be a predictor of VUR clinical outcome regardless of the grade of VUR.
This paper is very interesting and informative. However, there are some problems in this paper.

Major problems:

Comment 1. The normal values of creatinine in children differ according to gender, age and physique. Shouldn't creatinine-eGFR be evaluated instead of creatinine in Labo data?

We totally agree with the reviewer that creatinine-eGFR should have been evaluated instead of creatinine in laboratory data. However, in our institution this parameter is only available in the routine laboratory and not in the emergency laboratory, so in the samples analysed from our patients, it was not possible to collect them.

Comment 2. Is the observation period after APN the same across patients? I wonder if there aren't any patients assigned to Group B due to short follow-up.

Indeed, being a retrospective study of consecutive cases of patients with VUR and associated APN between 2013-2019, the follow-up time was similar in both groups. We have added this data in Results section.

Comment 3. Did you continue to follow up after you confirmed that the VUR had disappeared?

Has anyone had recurrence of APN after withdrawal antibiotic prophylaxis? VCG might miss low-grade VUR.

Follow-up was performed by reviews in the outpatient clinic every 3 months, with repeat VCUG to monitor the VUR clinical course at 6-monthly intervals. Antibiotic prophylaxis was initiated in all patients diagnosed with VUR after APN episode for at least 6 months, with subsequent withdrawal in those cases where no further episodes of APN occurred and VUR resolved spontaneously on control isotopic cystography.

We have not reported any recurrence of APN after withdrawal of antibiotic prophylaxis. Indeed, VCG might miss low-grade VUR, but these VUR grades are associated with a very low risk of APN, which may explain the absence of recurrences after antibiotic withdrawal.

Comment 4. The hypothesis that progression of residual fibrosis in the urothelium by inflammatory mediators and cytokines perpetuates VUR is of great interest. If it is as hypothetical, the damage to the renal parenchyma due to them should also be correlated. Is there anything that can be compared between groups; for example, blood pressure or urinary β2-microglobulin levels?

Unfortunately, being a retrospective study, we do not have data on blood pressure and urinary β2-microglobulin levels, as they are not routinely collected. However, we agree with the reviewer that urothelial damage should correlate with renal parenchymal damage. This could be the starting point for a future prospective analysis of systemic inflammatory correlation (NLR) with renal damage (urinary β2-microglobulin levels).

Minor problems:

Comment 1. Please check if this sentence is correct.

1) P2 L41-43: Patients were divided into two groups according to VUR evolution after APN: group A (spontaneous resolution) and group B (development of VUR complications during follow-up: new APN or).

We have completed the sentence in the abstract with "or renal function worsening".

2) P2 L50-53: NLR was the parameter with the highest area under the curve (AUC=0.966) for predicting the development of VUR complications (cut-off point=3.41) with a máximum sensitivity of 92.7% and specificity of 91.1% (p.

We have already completed the data of p-value "(specificity of 91.1%(p.<0.001)"

Comment 2. The numbers in the text may not match the numbers in Figure 1.

1) P2 L46: 294 patients with APN and associated primary VUR

2) P6 L181: 274 patients had associated primary VUR

We have modified the mistake in the abstract and in the results section. The patients included in the study were 273 patients with APN and primary VUR.

Comment 3. What is CUMS? Did you spell it out above?

P8 L263: the degree of VUR determined by CUMS

CUMS is the Spanish translation of VCUG (voiding cystourethrography), which was previously defined in the Methods section. We have modified the error in the Discussion section of the manuscript.

Reviewer C

Authors reported that NLR is a simple and cost-effective predictor of clinical outcome of VUR. This research is very interesting and applicable to clinical practice.

I have some questions.

Comment 1. You reported that DMSA were performed during the first 5 days of admission and a new abnormal findings was considerd the complications of VUR "during follow-up". How did you evaluate abromal DMSA findings during the first 5 days of admission?

DMSA scans were performed within the first 5 days to detect acute renal lesions (renal cortical defect), but the diagnosis of VUR was subsequently confirmed by VCUG performed 4 weeks after APN resolution. The performance of DMSA within the first 5 days has been previously described by other authors within the algorithm of management of APN in children (Han SY, Lee IR, Park SJ, Kim JH, Shin JI. Usefulness of neutrophil-lymphocyte ratio in young children with febrile urinary tract infection. Korean J Pediatr. 2016 Mar;59(3):139-44).

Comment 2. You should assess the risk factors for VUR complications with multivariate analysis, for example using some of the key risk factors listed in Table 5. It should be possible to assess whether NLR is an independent factor.
For the identification of risk factors for the development of VUR complication only differential factors were analyzed in the univariate analysis between the spontaneous resolution group and the group that developed complications. As significant differences were only identified in radiological and laboratory data, these were the data on which the multivariate analysis was performed using the ROC curve.

Comment 3. NLR is a convenient marker, but easily fluctuates. The timing of blood sampling after fever should be different for each individual patient. You need to add a limitation section.
Indeed, the timing of blood sampling after fever was probably different for each individual patient. The fever peak of >39 °C is a risk factor for developing APN, which was reported in all our patients. The time course of fever was not collected, although it may influence the systemic inflammatory response, so we have added it in the limitations section of the manuscript.

Comment 4. We also expect further reports on NLR in febrile patients without VUR, although they were excluded in this study.
To the best of our knowledge, there is only one study analysing the role of NLR in patients with febrile UTI both with and without VUR showing that NLR can be used as a diagnostic marker of APN with DMSA defect (Han SY, Lee IR, Park SJ, Kim JH, Shin JI. Usefulness of neutrophil-lymphocyte ratio in young children with febrile urinary tract infection. Korean J Pediatr. 2016 Mar;59(3):139-44.), which we have included in the references. However, we have not found reports on NLR in febrile patients without VUR.

Reviewer D

Comment 1. In general
a. Since serum NLR is expected to vary depending on the duration of the fever, it would be helpful to describe the duration of the fever prior to visiting the ED
Indeed, the timing of blood sampling after fever was probably different for each individual patient, although this data was not recorded in this study. The fever peak of >39 °C is a well-known risk factor for developing APN, which was reported in all our patients. The time course of fever was not collected, although it may influence the systemic inflammatory response, so we have added it in the limitations section of the manuscript.

Comment 2. 1st abbreviation must be written in full words.
a. Line 263: CUMS.

CUMS is the Spanish translation of VCUG (voiding cystourethrography), which was previously defined in the Methods section. We have modified the error in the Discussion section of the manuscript.

b. Line 315: NLI

This is also an error, as we actually meant to write NLR (neutrophil-to-lymphocyte ratio), which is also previously defined in the Methods. We have corrected this typo.

Comment 3. Abstract

a. Line 43: Complete the sentences after " new APN or)

We have completed the sentence in the abstract with "or worsening of renal function".

b. Line 53: complete the data of p-value (specificity of 91.1%(p.)

We have already completed the data of p-value "(specificity of 91.1%(p.<0.001)"

Comment 4. Methods

a. Line 149: specify or describe "the pregnancy control" (Do you want to describe it as antenatal care or planned pregnancy?)

Indeed, by pregnancy control we refer to antenatal care. We have changed the term both in methods and in Table 1 of the manuscript.

b. I think it would be better to describe the diagnosis of prenatal VUR in Table 3. If you meant it as prenatal ureterohydronephrosis (in line 200), please list it.

Indeed, prenatal diagnosis of VUR was made by detection of ureterohydronephrosis on prenatal ultrasound. In cases where urinary dilatation was still present after birth, VCUG was performed to confirm the diagnosis of VUR. We have described the prenatal diagnosis of VUR in Table 3, following the reviewer's recommendations.

c. Line 154: the Emergency Department → the emergency department

We have changed the term in the Methods section.

d. Table 3: summation of VUR is 169 in group A and 104 in group B which matched with the total count in groups A and B. It would be better the method of describing the grade of VUR in the patient with bilateral VUR, in the method section.

In cases of bilateral VUR, in case of grade discordance between both kidneys, the higher grade was considered, as it was the one that determined the evolution of the patient (spontaneous resolution or development of complications). This explains why the sum of patients in the VUR grade section in Table 3 coincides with the total number of patients in each group.

e. Figure 1: Group B is the patient with primary VUR and development of

complications during follow-up, however, you describe it as surgical correction in Figure 1. It would be better to change figure 1.
We have modified Figure 1 as "development of complications during follow-up", instead of "surgical correction", following the reviewer's recommendation.

Comment 5. Results
a. Line 183 and Table 1 and 4: It would be better to delete "Q1-Q3" because IQR means Q1-Q3 and you already described it in line 166.
We have changed the term both in the Results section and in Table 1 and 4.

b. Line 186: Female gender was the most frequent in both groups. → Female gender was frequent in both groups.
We have modified the sentence considering the reviewer's suggestion.

c. Line 187: in Table 1, there was only 1 item of prenatal feature, therefore it would be better to switch it with "perinatal".
We have changed the term both in the Results section and in Table 1.

d. Line 193: the differences being statistically significant → it would be better to delete it.
We have removed that part of the sentence.

e. Table 4: Line platelet: 380,000 is not a significant digit, therefore it is better to describe it as 380 and 10^3/uL
We have changed the unit of platelets to 10^3/uL, following the reviewer's recommendation.