Reviewer's report

Title: Validation of a newly proposed histopathological classification in Japanese patients with anti-neutrophil cytoplasmic antibody-associated glomerulonephritis

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Reviewer: Arda Goceroglu

Reviewer's report:

The authors of this manuscript recognize the importance to validate the histopathologic classification of ANCA-associated glomerulonephritis in different cohorts. In their manuscript Iwakiri and colleagues validated this classification in a Japanese cohort. In addition they performed immunohistochemical staining. For both they investigated the predictive value for the development of ESRD during follow up. This study provides important information on the usefulness of this classification in a Japanese cohort regarding the prediction of ESRD. This kind of information is needed and hopefully will lead to classification refinements in the future.

The manuscript improved significantly. Before publication, this article needs some detailed refinements, which are suggested below.

- Major Compulsory Revisions

- Minor Essential Revisions

Abstract

1) Results: ‘Multivariate analysis identified the new classification system as a prognostic factor for ESRD (p = 0.0686, crescentic, mixed and sclerotic vs. focal, hazard ratio (HR) [95% confidence interval, CI]; 2.99 [0.61–22.7], 5.04 [1.11–36.4] and 9.93 [1.53–85.7], respectively).’ Change to ‘In the multivariate analysis, the classification system tended to be a prognostic factor for ESRD (p = 0.0686, crescentic, mixed and sclerotic vs. focal, hazard ratio (HR) [95% confidence interval, CI]; 2.99 [0.61–22.7], 5.04 [1.11–36.4] and 9.93 [1.53–85.7], respectively).’

2) Results: Regarding the previous comment. The authors should check (for the whole manuscript) whether it is correct that the overall p-value of the classification (p=0.0686) is not significant, while the difference between mixed vs. focal and sclerotic vs. focal seems to be significant (95%CI does not include the 1). If it is correct, this could be mentioned in the discussion. Regarding the conclusion the authors should rely on the overall p-value (so the classification tended to be associated with ESRD), because the 95%CI are quite wide.

3) Conclusion: ‘The new histopathological classification correlated with eGFR at 1 year and tended to be associated with ESRD in our Japanese cohort with ANCA-associated glomerulonephritis.’ Change to ‘The new histopathological
classification was associated with eGFR at 1 year and tended to be associated with ESRD in our Japanese cohort with ANCA-associated glomerulonephritis.'

Methods ‘Patient parameters and outcomes’

4) ‘The development of ESRD during follow-up was defined as a primary endpoint, while estimated glomerular filtration rate (eGFR) at 1 year was defined as a secondary endpoint. ESRD was defined as requiring permanent renal replacement therapy.’ Change into ‘The development of ESRD during follow-up was the primary outcome. ESRD was defined as requiring permanent renal replacement therapy. Estimated glomerular filtration rate (eGFR) at 1 year was the secondary outcome.’

Methods ‘Immunohistochemical analysis’

5) ‘Cytoplasmic staining was positive for #-SMA, CD68 and cytokeratin, while nuclear staining was positive for WT1’ change into ‘A cell was considered positive for a-SMA, CD68 and/or cytokeratin in case of cytoplasmic staining. A cell was considered positive for WT1 in case of nuclear staining.’

6) ‘The definition of a normal glomerulus was a glomerulus without collapse, crescent formation, adhesion, or segmental/global glomerulosclerosis.’ Was a glomerulus with focal glomerulosclerosis considered as normal? In case of ‘yes’ this sentence is correct. ‘In case of ‘no’, please add focal to segmental/global.

Results ‘Clinical and histopathological studies’

7) There is a small discrepancy between the text and table 1 regarding eGFR (11.6 and 11.5)

Results ‘Immunohistochemical study’

8) ‘The mean incidence of #-SMA-positive glomeruli per normal glomeruli was 82.9%.’ Change into ‘The mean proportion of #-SMA-positive glomeruli per normal glomeruli was 82.9%.’

Discussion ‘Immunohistochemical findings’

9) Another limitation that should be mentioned by the authors is that the association between the histopathologic classification system and eGFR at 1 year is only based on an univariate analyses. Multivariate analysis with eGFR at 1 year as outcome was not performed.

Conclusion

10) ‘The histopathological classification system proposed by Berden et al. [8] is a simple method, which is associated with eGFR at 1 year and tends to be correlated with the incidence of ESRD in Japanese patients with ANCA-associated glomerulonephritis in our cohort.’ Change to ‘The histopathological classification system proposed by Berden et al. [8] is a simple method, which is associated with eGFR at 1 year and tends to be associated with the incidence of ESRD in Japanese patients with ANCA-associated glomerulonephritis in our cohort.’
Abstract
1) Results: ‘Kaplan-Meier renal survival analysis was similar to that of the new classification system.’ Change to ‘Kaplan-Meier renal survival analysis was similar to that of the new classification system proposal.’

Methods ‘Renal histopathology’
5) ‘For immunofluorescence analysis, sections were incubated with antibodies raised against IgG, IgA, IgM, C3, C4, C1q, and fibrinogen, to exclude other renal diseases.’ Change into ‘For immunofluorescence analysis, sections were incubated with antibodies against IgG, IgA, IgM, C3, C4, C1q, and fibrinogen, to exclude other renal diseases.’

6) ‘Samples with >50% normal glomeruli were classified as focal, those with #50% cellular crescentic glomeruli were classified as crescentic, and those with #50% globally sclerotic glomeruli were classified as sclerotic. The other cases, those with #50% normal, cellular crescentic, and globally sclerotic glomeruli were classified as mixed.’ Change into ‘Samples with #50% normal glomeruli were classified as focal, those with #50% cellular crescentic glomeruli were classified as crescentic, and those with #50% globally sclerotic glomeruli were classified as sclerotic. The other cases, those with <50% normal, cellular crescentic, and globally sclerotic glomeruli were classified as mixed.’

Methods ‘Immunohistochemical analysis’
7) ‘Of the 50 cases in either the crescentic or mixed classes, 16 cases did not
have sufficient tissue remaining for immunohistochemical analysis.' Change into 'Of the 50 cases in either the crescentic or mixed class, 16 cases did not have sufficient tissue remaining for immunohistochemical analysis.'

8) ‘Reproducibility was assessed by blinded replicate counting of immunopositive cells performed by two observers;’ change into ‘Reproducibility was assessed by blinded replicate counting of immunopositive cells performed by the two observers;’

Results ‘Clinical and histopathological studies’

9) Sometimes the authors use ‘versus’ and sometime they use ‘vs’. My suggestion is to use ‘versus (vs.)’ the first time and after that only ‘vs.’

10) ‘Although 11 patients were negative for both proteins’ change into ‘Although 11 patients were negative for both antibodies’

Results ‘Immunohistochemical study’

11) ‘We performed immunohistochemical staining for #SMA, WT1, CD68, and cytokeratin on samples from 34 patients with crescentic and mixed classes.’ Change into ‘We performed immunohistochemical staining for #SMA, WT1, CD68, and cytokeratin on samples from 34 patients with crescentic or mixed class.’

Discussion ‘Clinical and histopathological findings’

12) ‘However, studies investigating the clinical and histopathological predictors of renal outcome have provided different results with some overlap, such as in terms of the predictive values of normal glomeruli, glomerulosclerosis and eGFR at diagnosis. [3,4,15-18].’ Change to ‘However, studies investigating the clinical and histopathological predictors of renal outcome have provided different results with some overlap, in terms of the predictive values of normal glomeruli, glomerulosclerosis and eGFR at diagnosis. [3,4,15-18].’

13) ‘infectious events’ change into ‘events of infection.

Table 1 and table 2

14) Subscription: ‘means’ change into ‘mean’.

Whole manuscript

15) The authors use the plural form of class (classes) quite often in their manuscript, although this is not necessary in most cases. The authors could reconsider the use of ‘classes’ in most cases (and use ‘class’ instead).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests