Kidney biopsy is required for nephrotic syndrome with PLA2R+ and normal kidney function: COMMENTARY

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Almost seven decades have elapsed since the paradigm-shifting description of percutaneous aspiration kidney biopsy was first published by Iverson and Brun (1,2). There is no doubt that this procedure has impacted profoundly on our understanding of kidney disease in general, and it has contributed greatly to improving medical care specifically in the field of glomerular diseases. But like all such advances, over time, new findings can alter the way in which the procedure is used and viewed. The concept of a potential “liquid biopsy” has emerged as an alternative to “tissue biopsy” with the development of sophisticated urinary proteomics and serum biomarkers of specific disease entities (3,4). Nevertheless, kidney biopsy continues to be the “gold standard” for diagnosis of underlying conditions when the clinical features are insufficiently distinct to provide a diagnosis and when additional information is needed to better guide prognosis and management. While percutaneous kidney biopsy is generally regarded as safe when performed under ideal conditions by an experienced operator, major but usually non-life threatening complications can ensue in a small percentage of cases (5). Thus, the decision to pursue a strategy of kidney biopsy, is governed by the risk of the procedure compared to the likelihood that the tissue will provide information that cannot otherwise be obtained non-invasively and that will make a difference in the management of the patient going forward. When the tissue is
examined thoroughly by light, immunofluorescence, and electron microscopy, including special staining methods, kidney biopsies can often deliver a specific diagnosis and useful therapeutic guidance. These considerations form the crux of the proposition advanced in this Debate, highlighted by the PRO (6) and CON (7) responses. *Essentially*, the Debate asks whether positive results of testing for serum-auto-antibodies to PLA2R antigen in a well-defined subset of adult patients presenting with nephrotic syndrome and normal kidney function will determine a diagnosis of a lesion of membranous nephropathy with such precision that the need for a kidney biopsy is obviated in a way that does not sacrifice vital information for aiding clinical therapeutics and prognostication. This is a challenging question and both the PRO and CON positions have elucidated crucial issues involved in its analysis. The central reference point is the study published by Bobart, et al in 2019 (8).

This study was a retrospective and single center analysis of the kidney biopsy findings in 132 patients with a serum level of $\geq 2$RU/ml of anti-PLA2R antibody by a commercial Enzyme-linked immunosorbent assay (ELISA, Euroimmun) confirmed by a positive indirect immunofluorescence assay (IFA, Euroimmun) on the same sample. Most, but not all, of the patients in this cohort had overt Nephrotic syndrome. Importantly, 97 of these cases had no clinical evidence of a
secondary cause by evaluation of anti-nuclear antibodies, anti-double stranded DNA antibodies. Hepatitis serology, chest X-rays, age-appropriate cancer screening, medication history and monoclonal antibody testing. The presence of diabetes mellitus was not a specific reason for exclusion from this cohort. Of these 97 subjects, 60 (62%) had a “normal” eGFR arbitrarily defined as >60ml/min/1.73m2, not adapted to the age of the subjects (which had an interquartile range of 44-60 years). An eGFR of <45ml/min/1.73m2 was present in 22/97 subjects. The median proteinuria was 8.0gms per day and the median serum albumin level was 2.7gms/dL (by the bromcresol green method). The crucial finding was that among the 60 patients with a “normal” eGFR, 100% had a lesion of Membranous Nephropathy (MN). One case had superimposed diabetic nephropathy and one case had a superimposed lesion of focal and segmental glomerulosclerosis. No features of secondary MN were found in any of the biopsies. PLA2R antigen staining of glomeruli was pursued in 52 of these 97 cases, and was positive in 51 (98%). Thus, the diagnosis of presumed Primary (PLA2R mediated) MN could be obtained with 100% specificity (no false positives) in patients meeting the definition of a positive anti-PLA2R antibody used in this study (a combination of ELISA and IFA test results), who had normal kidney function and no evidence for a secondary form of MN. Also, in this cohort, the
ability to stain glomeruli for PLA2R antigen added little to diagnostic accuracy. These findings meet criteria for a “liquid biopsy”. According to the authors, the findings in kidney biopsy in this cohort provided no additional information useful for prognostication or therapeutic decision making, but no follow-up is provided for verification of this statement. On the other hand, Kidney biopsy in the 37/97 (38%) patients meeting all of the definitions but having reduced eGFR, additional diagnostic information was uncovered in 5 patients. As this subgroup is not the subject of this debate, they need no further discussion. In the CON discussion the authors (7), add another 42 cases meeting the definitions described above (bringing the total studied between January 2015- and April 2020 to a total of 139 cases). The finding of MN was 42/42 (100%, no false positives), confirming the earlier published studies. These findings are quite similar to those using the Western blot technique for identifying anti-PLA2R antibodies described in the classic and seminal description of PLA2R-mediated MN by Beck, et al in 2009 (9).

The PRO position in this debate taken by Jonathan Hogan (6) contains three main elements, specifically: 1) it is premature to make a change in practice based on a single retrospective study from one institution; 2) the optimum criteria for a “positive” anti-PLA2R antibody test are not well understood; 3) to what population of patients does the paradigm of “liquid biopsy” apply? . The outline of
data needed to further validate the Bobart, et al paradigm/algorithm is quite reasonable.

The original authors fully agree with statement #1- “Further validation in a prospective study is warranted to determine whether PLA2R antibody testing be used as a non-invasive diagnostic test to guide therapy” concludes the Abstract portion of the paper (8). Further, they postulate that the evidence provided might help in biopsy decision making in patients with a high risk of complications (e.g. coagulation defects, inability to cooperate with the requirements of the procedure, such as breath-holding) or situations where the procedure might be relatively contra-indicated (e.g. single kidney). Clearly, the presence of abnormal kidney function will nearly always justify a kidney biopsy even with a positive anti-PLA2R antibody testing. Whether the definition of abnormal kidney function needs to be age-adapted is a question for future studies.

The optimum criteria for defining a positive PLA2R antibody test has been clearly identified by the original paper of Bobart et al (8). *Both the ELISA and the IFA tests are required*, preferably performed on the same sample of serum. A negative IFA does not require a confirmatory ELISA, but a positive IFA test,
requires confirmation by an ELISA ≥ 2 RU/ml (see the Algorithm in Bobart, et al) (8).

False positive ELISA assays (ELISA >20RU/ml with a non- MN lesion by kidney biopsy) have been described (largely anecdotally) but not systematically studied. This phenomenon seems to be more common in patients with Diabetic Nephropathy, for unexplained reasons. The IFA is usually negative in such circumstances, and thus would not qualify as a positive test, according to the Bobart et al criteria (7,8). At the 2020 ERA-EDTA Virtual meeting an Abstract was presented by Mok and Choo (10) describing 31 patients with non-MN lesions on kidney biopsy who had positive ELISA anti-PLA2R tests- 26 of whom had levels <50RU/ml and 5 of whom had levels >50RU/ml (ranging from 56 to 168RU/ml). All of the subjects were Chinese or Indian. No IFA results were reported so this study cannot be interpreted in relationship to the Bobart, et al criteria (7, 8). However, this single study validates the need to study additional ancestral populations, beyond those included in the original Mayo Clinic study (8). The results described in the positive anti-PLA2R patients in the Bobart, et al study (8) presenting with Nephrotic Syndrome and potential secondary causes of MN, or Systemic diseases such as Diabetes, or an eGFR <60ml/min/1.73m² demand that
a kidney biopsy be undertaken as clearly stated in the CON position (7,8). And I agree.

Although the data provided in the Bobart, et cohort study (8) are very compelling concerning the utility of anti-PLA2R serology (both ELISA and IFA) for non-invasive diagnosis of suspected MN in patients presenting with nephrotic syndrome in the absence of reduced renal function and evidence of a secondary cause, an unresolved question remains. Does the absence of morphological data concerning variations in the MN lesion impair or constrain therapeutic decision making and/or prognostication? We can be re-assured by the authors claims that the answer is NO to this question, at least in the cohort described (7,8), but more detailed and longer term follow-up would help to assuage doubts. At the present time, serial determinations of anti-PLA2R serology (mainly ELISA) quantitative urinary protein excretion and serum creatinine levels seem to be sufficient for management purposes.

After due consideration of both the PRO and CON positions, my own conclusions are more supportive of the CON position, so long as the definition of PLA2R sero-positivity conforms to the Bobart, et al criteria (8), the eGFR is normal (for age) and secondary causes seem unlikely based on a standardized evaluation.
However, in concurrence with the PRO position and the authors of the original paper, this new proposed paradigm needs independent confirmation by a prospective study involving individual subjects of diverse ancestry.

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R Glassock: Conceptualization; Data curation; Formal analysis; Writing - original draft; Writing - review and editing
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