Kidney360

Kidney Biopsy Is Required for Nephrotic Syndrome with PLA2R+ and Normal Kidney Function: Pro

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KIDNEY360 1: 887–889, 2020. doi: https://doi.org/10.34067/KID.0003752020

Introduction

It is hard to remember life before phospholipase A2 receptor (PLA2R). The identification by Beck *et al.* (1) in 2009 of the M-type PLA2R as the target antigen in membranous nephropathy (MN) was truly a monumental discovery, and PLA2R antibody testing has revolutionized the management of MN (2). Imagine a conversation 11 years ago about PLA2R antibody testing:

Drs. Beck and Salant: PLA2R antibodies will be present in the majority of patients with MN.
Us: No way!
Drs. Beck and Salant: PLA2R antibodies will be highly specific.
Us: Get outta town!
Drs. Beck and Salant: PLA2R antibody levels will correlate with disease activity and severity
Us: You gotta be kidding me!
Drs. Beck and Salant: Changes in PLA2R antibody levels will predict the future (*i.e.*, they occur prior to changes in proteinuria) and therefore, allow for personalized decisions about clinical management.
Us: We must be dreaming!

Indeed, what is left for this amazing biomarker to prove?

One hurdle that PLA2R antibody testing has yet to clear is to qualify as a diagnostic biomarker—that is, PLA2R antibody testing would replace kidney biopsy to diagnose MN. Over the last decade, multiple studies have described the impressive test characteristics of PLA2R antibody, particularly with regard to specificity, which ranges from 98% to 100%. Some of the most compelling data supporting the PLA2R antibody as a diagnostic biomarker comes from my debate counterparts, Bobart and Fervenza. In order to argue for kidney biopsies in patients with PLA2R antibodies, I must convince the reader that the current evidence is not strong enough to support PLA2R antibody testing as a diagnostic biomarker for MN.

Bobart *et al.* (3) (the Short Version)

This was a retrospective analysis of 838 unique patients who had 1362 PLA2R antibody tests from 2015 to 2018 at three Mayo Clinic locations (Minnesota, Florida, and Arizona). Of the 143 patients with positive PLA2R antibodies, 35 were excluded from analysis after being deemed to have clinical or laboratory features suggesting a secondary cause of MN. For patients with positive PLA2R antibody testing and eGFR>60 ml/min per 1.73 m^2 (n=60), kidney biopsy did not add significant clinical value beyond making the diagnosis of MN. Only two patients had additional biopsy findings (one had a FSGS lesion, and a second also had diabetic kidney disease). The authors concluded that PLA2R antibody testing is a diagnostic biomarker PLA2R for MN for patients with eGFR>60 ml/min per 1.73 m^2, and thus, kidney biopsies are not needed in this population. They also presented algorithms for the use of PLA2R antibody testing in clinical practice.

A Wise Man Taught Me That "the Devil Is in the Details!"

Let us take a closer look at the data in the context of the debate prompt.

What Is Positive PLA2R Antibody Testing?

There are currently two types of PLA2R antibody tests in clinical use. The ELISA is a quantitative assay that uses a recombinant human PLA2R antigen to measure the amount of PLA2R IgG (as well as IgA and IgM) in patients' serum in relative units per milliliter. The indirect immunofluorescence assay (IFA; also called indirect immunofluorescence test) is a semiquantitative assay that uses cells transfected with PLA2R antigen to measure the amount of PLA2R IgG in patients' serum in dilutional titers (<1:10, 1:10, 1:20, 1:40, *etc.*). The reference ranges reported by the United States' two largest clinical laboratories, LabCorp and Quest Diagnostics (which conducts its PLA2R antibody tests at the Mayo Clinic), are listed in Table 1.

In the study by Bobart *et al.* (3), testing for PLA2R antibodies was performed by ELISA (in relative units per milliliter) and IFA (resulted only as positive or negative), and positive ELISA results were defined as any result >2 RU/ml. As the authors acknowledge, this is not the reference range provided by Euroimmun, the ELISA manufacturer, and it is not the reference range listed by any clinical laboratory. Moreover, although combination testing with ELISA and IFA is

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Table 1. Phospholipase A2 receptor antibody tests and reference ranges offered by LabCorp and Quest Diagnostics

Clinical Laboratory	ELISA Reference Range	Immunofluorescence Assay Reference Range
LabCorp	Negative <14.0 RU/ml Borderline =14–19.9 RU/ml Positive >19.9 RU/ml	N/A
Quest Diagnostics *via* Mayo Clinic/Euroimmun	Negative <14 RU/ml Borderline >14 or =14–<20 RU/ml Positive >20 or =20 RU/ml	Negative

Reference ranges were obtained from https://www.labcorp.com/tests/141330/phospholipase-a2-receptor-autoantibodies-igg and https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/64327 (accessed June 11, 2020). N/A, test not available.

performed in some laboratories, this is not currently the standard of care for clinical practice. Indeed, LabCorp only offers ELISA testing for PLA2R antibody and does not offer IFA testing.

Moreover, for patients with ELISA results between 2 and 20 RU/ml and a negative IFA, more than half (46 of 80=58%) had MN on kidney biopsy, and of these patients, almost half of those tested with PLA2R staining on kidney biopsy (five of 11) were PLA2R positive. These data show that further study is needed to determine optimum ELISA reference ranges and optimum use of combination testing for PLA2R antibodies.

What Is the Population of Interest?

Defining the population of interest is critical for a few reasons. First, the study population will affect the positive predictive value of PLA2R antibody testing—that is, the probability that a patient with a positive PLA2R antibody screening test will truly have the disease on kidney biopsy. The positive predictive value is the most important characteristic of PLA2R antibody testing for use as a diagnostic biomarker, and in the context of this debate, the study population is patients with nephrotic syndrome and normal kidney function. In the study by Bobart *et al.* (3), patients with positive PLA2R antibodies tended to have nephrotic syndrome (median proteinuria =8.0 g, median serum albumin =2.7 g/dl), but the study does not describe the prevalence of nephrotic syndrome in the screening population or the prevalence of MN in the overall population of PLA2R antibody–tested patients. There are no studies to date that have assessed the positive predictive value of PLA2R antibody testing for diagnosing MN only in patients with nephrotic syndrome and normal kidney function.

Additionally, increasing test sensitivity (as occurs when lowering the ELISA positive range to >2 RU/ml) will sacrifice specificity and positive predictive value. As an example, one recent study examined PLA2R antibody testing by ELISA in patients with diabetes who underwent kidney biopsy, finding that the positive predictive values were 100%, 100%, 96.55%, and 73.47% with ELISA cutoff values of ≥40, ≥20, ≥14, and ≥2 RU/ml, respectively (4). Moreover, in the study by Bobart *et al.* (3), 92% of PLA2R antibody–positive patients were white, so further studies are needed to determine the generalizability of these findings to use in other racial groups.

As noted above, 35 patients were excluded for having "secondary causes of MN," and the kidney biopsy diagnoses of these patients are not described in the manuscript. The exclusion of these patients, which account for more than one quarter of PLA2R antibody–positive patients in the study, could significantly affect the calculated test characteristics (sensitivity, specificity, and positive and negative predictive values) of PLA2R antibody testing. Furthermore, the exclusion of these patients is particularly problematic in the context of this debate, which does not specify that the hypothetical patient in question has been excluded from having "secondary" causes of MN. Indeed, one could argue that establishing PLA2R antibody testing as a diagnostic biomarker would most affect the care of patients who have

Table 2. Data needed to validate phospholipase A2 receptor antibody testing as a diagnostic biomarker for membranous nephropathy

Timing of Phospholipase A2 Receptor Antibody Testing	Samples Collected Prior to Kidney Biopsy (Prospective)
PLA2R antibody test characteristics	Further study of combination testing with ELISA and IFA in larger and more diverse patient populations with nephrotic syndrome Further study of reference ranges for ELISA Determine positive and negative predictive values in the population of interest (*i.e.*, nephrotic syndrome)
Improve generalizability	Further study of PLA2R antibody testing in larger, more diverse populations with additional medical problems, including those traditionally associated with "secondary" membranous nephropathy
Algorithm for clinical use of PLA2R antibody testing	Requires validation in other prospective cohorts of patients with nephrotic syndrome

PLA2R, phospholipase A2 receptor; IFA, immunofluorescence assay.

medical conditions or laboratory values that excluded them from analysis in the study by Bobart *et al.* (3).

It should also be clarified that if PLA2R antibody testing eventually qualifies as a diagnostic biomarker in patients with nephrotic syndrome and normal kidney function, this would not grant it diagnostic biomarker status outside of this setting: for example, in patients with diabetes and kidney disease and/or in patients with subnephrotic proteinuria. This is also important to highlight because a study from the National Institutes of Health found that patients often have circulating PLA2R antibodies for months to years prior to biopsy diagnosis of MN, and aspects of the nephrotic syndrome phenotype (as represented by hypoalbuminemia) do not always correlate with PLA2R antibody level (5). Finally, PLA2R testing used as a diagnostic biomarker would ideally occur prior to kidney biopsy, which was not the case in the study by Bobart *et al.* (3), where PLA2R antibody testing was performed after kidney biopsy in 95% (92 of 97) of patients.

Primary versus Secondary MN

The exclusion of conditions that are related to "secondary" MN also deserves mention. The authors note that "in patients with ELISA>20 RU/ml and eGFR>60, and negative results of a full laboratory and radiologic evaluation for secondary causes, were found to have MN on kidney biopsy" (3). The exact scope of a "full laboratory and radiologic evaluation" for secondary workup has not been clearly established on the basis of high-quality evidence (6) and is also not described in the paper by Bobart *et al.* (3). The recommendations of the 2012 Kidney Disease Improving Global Outcomes Clinical Practice Guidelines for GN are also vague, recommending that physicians "perform appropriate investigations to exclude secondary causes in all cases of biopsy-proven MN (Evidence Level: Not Graded)" (7). Indeed, the discovery of PLA2R has forced our field to rethink the classification of primary versus secondary disease. For example, in study by Bobart *et al.* (3), 21 of the 35 PLA2R-positive patients who were excluded for potential secondary causes of MN were excluded due to having malignancy or suggestion of malignancy ($n=2$ positive chest X-ray/lung cancer, $n=4$ positive prostate-specific antigen/prostate, $n=6$ paraproteinemia, $n=9$ other malignancy). In contrast to thrombospondin type 1 domain–containing 7A-positive MN (8–10), no case of PLA2R-positive MN has been definitively linked to cancer, thus calling into question the *a priori* exclusion of such cases in determining optimal characteristics for PLA2R antibody testing.

The Burden of Proof

Data from the last decade have solidified how special PLA2R antibody testing is for the diagnosis and management of patients with MN and may speak to its ability to replace kidney biopsy in the majority of patients with MN. Indeed, I believe that data published in the next decade will confirm PLA2R antibody testing as a diagnostic biomarker. However, the evidence clearly shows that we are not there yet! The nephrology community must demand the necessary additional studies to determine best practice for using PLA2R antibody testing to replace kidney biopsies in patients with MN (Table 2). Until these data are available, kidney biopsy is required for patients with nephrotic syndrome, normal kidney function, and positive PLA2R antibody testing.

Disclosures

J. Hogan has a patent to WO2019213434A1 pending.

Funding

None.

Acknowledgments

The content of this article reflects the personal experience and views of the author(s) and should not be considered medical advice or recommendation. The content does not reflect the views or opinions of the American Society of Nephrology (ASN) or *Kidney360*. Responsibility for the information and views expressed herein lies entirely with the author(s).

Dr. Jonathan J. Hogan reports personal fees from Alexion, Complexa, Dimerix, Goldfinch Bio, GSK, LTD, Retrophin, UpToDate.com, and Zyversa, and grants from Alexion (Achillion), Boehringer Ingelheim, BMS, Complexa, Gilead, Omeros, Regeneron, and Retrophin, outside the submitted work.

Author Contributions

J. Hogan wrote original draft and reviewed and edited the manuscript.

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Received: June 12, 2020 **Accepted:** July 13, 2020

See related debate, "Kidney Biopsy Is Required for Nephrotic Syndrome with PLA2R1 and Normal Kidney Function: The Con View" and commentary, "Kidney Biopsy Is Required for Nephrotic Syndrome with PLA2R1 and Normal Kidney Function: Commentary" on pages 890–893 and 894–896, respectively.