

RESEARCH LETTER

Serum IgA-Fibronectin Concentration and IgA Nephropathy Diagnosis in Adults With Glomerulonephritis: A Diagnostic Test Study*To the Editor:*

Immunoglobulin A (IgA) nephropathy (IgAN) is a common form of primary glomerulonephritis (GN) worldwide, diagnosed using biopsy and direct immunofluorescence (IF) microscopy. In large population-based cohorts in which primary GN is diagnosed clinically, lack of a biopsy diagnosis of IgAN causes considerable challenges for management and prognosis. Finding a serologic test is especially important in places in which tissue diagnosis is not available.^{1,2} In the recent past, a few studies have shown a strong association of serum IgA-fibronectin (IgAFn) complex level with IgAN.^{3,4} However, the studies lacked the robust diagnostic values needed for a dependable biomarker to predict IgAN in the absence of a kidney biopsy diagnosis. Glomerular mesangial area dominant or codominant deposits of IgA are the hallmark of IgAN. However, Fn is associated with the Fn-binding moiety of type V collagen in the mesangial area along with the IgA deposits.⁵ This plausible association between IgAN and IgAFn complex is represented in Fig S1. The objective of this study is to examine the diagnostic association of serum IgAFn levels with biopsy and IF-proven IgAN cases in patients with primary GN.

This was a prospective double-blind study of a cohort of consecutive cases of de novo primary GN in adults in Postgraduate Hospital in Dhaka, capital of Bangladesh (Table S1). Figure 1 summarizes patient recruitment. Serum IgAFn and IF microscopy were done in separate institutions to maintain blindness, simulating a real-world experience (Table S2). Detailed methods, including participant selection, are provided in Item S1. Briefly, serum IgAFn level was measured using enzyme-linked immunosorbent assay using anti-Fn antibody and albumin (count 60 and count 0, respectively), before kidney biopsy and before immunotherapy was started if needed. Because this study was done as a part of routine clinical and laboratory procedures for clinical management and assays were run on residual samples, informed consent was not required based on local regulations.

Extinction values (EVs; value at count 60 minus value at count 0) for the study cases (EVs cases) and the healthy controls (EVc controls) were measured simultaneously.⁶ A cutoff value of EVs cases 3 times or more of EVc controls was considered a diagnostic marker for seropositive IgAN cases (IgAFn positive). Other cases with a cutoff value less than 3 times were defined as seronegative (IgAFn negative; Table S3). The primary outcome of the study was the comparison of serum IgAFn cutoff value of 3 times or greater as a biomarker for IgAN against the gold-standard IF microscopy results. Secondary outcomes included

diagnostic values and odds ratios for IgAFn cutoff value of 3 times or greater, and the clinical correlation of serum IgAFn levels with proteinuria, hematuria, and serum creatinine levels.⁷

We performed a χ^2 test to show the statistical significance of the association between kidney biopsy-positive IgAN and serologic IgAFn-positive cases (Fig S2). Linear regression analysis was performed to explore the clinical correlation of serum IgAFn level with hematuria, proteinuria, and serum creatinine level, respectively (Figs S3-S5). Diagnostic indexes of serum IgAFn cutoff value of 3 times or greater as a diagnostic test were calculated according to a standard formula.⁷ Definitions of true-positive and -negative and false-positive and -negative cases are provided in the Table 1 footnote. Continuous variables were described as mean and standard error with range, and categorical variables were expressed as frequency and proportion. All P values were 2 tailed and statistical significance was set at $P < 0.05$.

Figure S2 shows the statistically significant association of serologic IgAFn-positive cases and serologic IgAFn-negative cases with IF-positive IgAN cases and IF-negative non-IgAN cases, respectively ($\chi^2 = 107.4$; $P < 0.001$). A statistically significant correlation was found between hematuria and serum IgAFn levels in the entire GN cohort ($r = 0.44$; $P < 0.001$) and in IF-positive IgAN cases ($r = 0.9$; $P < 0.001$), as shown in Fig S3.

Diagnostic values of a serum IgAFn cutoff value of 3 times or greater as a diagnostic test for prediction of IgAN cases are shown in Table 1. Serum IgAFn cutoff values of 3 times or greater had 83% sensitivity with 90% specificity and 87% accuracy for the diagnosis of IgAN, with a positive predictive value of 77% and negative predictive value of 93% (odds ratio, 43.3%; 95% CI, 6.3-299.3; $P < 0.001$). We used AUROC (area under receiver operating characteristic) curve analysis to determine the appropriate criterion-value of EVs to get the true-positive and true-negative rates with statistical significance, and maximum diagnostic values (Item S1; Tables S4 and S5; Figs S6-S9).

These results confirm previous studies on serum IgAFn complex levels in primary GN and also fulfill the primary aim of our study in predicting IgAN with statistical significance and validating its diagnostic values.⁵⁻⁷

The present study has several limitations. First, the study was performed at a single center with a small cohort of consecutive cases of clinically diagnosed de novo primary GN in adults. The overall number of de novo GN cases was 41, and of these, 12 were IgAN and 29 were non-IgAN, as shown on kidney biopsy with IF microscopy. Therefore, we cannot confer generalizability to a larger cohort. However, this is a real-world experience in which the blood test for the serum IgAFn assay was done before kidney biopsy and immunosuppressive medications. Hence, the findings are unbiased and could be applicable for other cohorts of primary GN. Second, the study was blinded to the clinicians, pathologist, and immunologists and therefore lacked their clinical contribution to the study

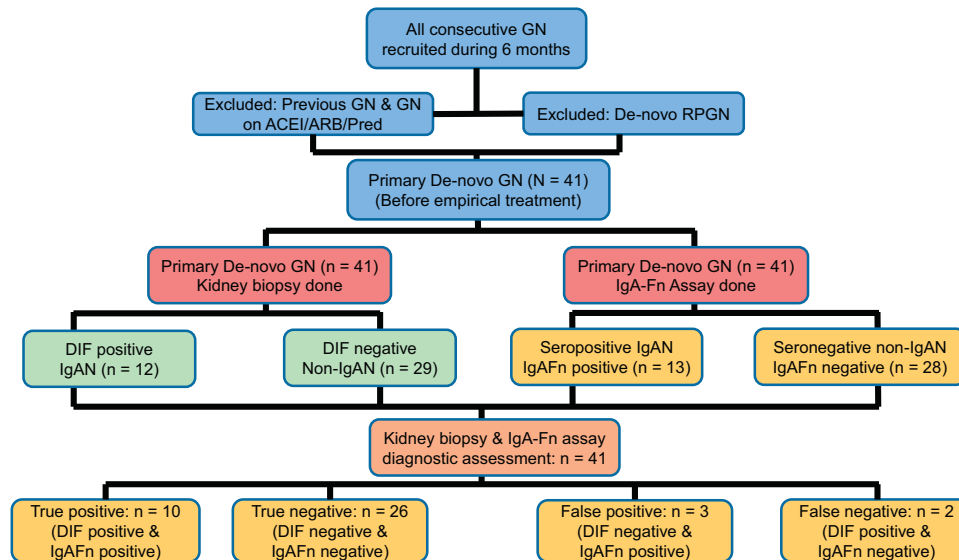


Figure 1. Standards for Reporting Diagnostic Accuracy Studies (STARD) diagram shows recruitment of glomerulonephritis (GN) cases, selection of primary de novo GN before the start of any empirical treatment, and classification after kidney biopsy and serum immunoglobulin A-fibronectin (IgAFn) assay. Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; DIF, direct immunofluorescence; IgAN, immunoglobulin A nephropathy; Pred, prednisone; RPGN, rapidly progressive glomerulonephritis.

team. Third, because of the blinded nature of relevant data collection and recording, the clinical courses of the patients were not charted. Therefore, we could not assess the prognostic attributes of serum IgAFn complex levels to the

Table 1. Diagnostic Values of Serum IgAFn Cutoff Value ≥ 3 Test as a Serologic Investigation for Prediction of IgAN in Primary Glomerulonephritis

Diagnostic Value	Formula	Value	95% CI
Sensitivity	TP/(TP+FN)	83.33%	51.59%-97.91%
Specificity	TN/(FP+TN)	89.66%	72.65%-97.81%
Positive likelihood ratio	Sensitivity/(1 – specificity)	8.06	2.68-24.22
Negative likelihood ratio	(1 – sensitivity)/specificity	0.19	0.05-0.66
Disease prevalence	(TP+FN)/(n)	29.27% ^a	16.13%-45.57%
Positive predictive value	TP/(TP+FP)	76.92%	52.57%-90.93%
Negative predictive value	TN/(FN+TN)	92.86%	78.45%-97.89%
Accuracy	(TP+TN)/(n)	87.80%	73.80%-95.92%

Odds ratio, 43.3; 95% CI, 6.3-299.3; $P < 0.0001$.⁷

Abbreviations: FN, false negative (IF positive and IgAFn negative); FP, false positive (IF negative and IgAFn positive); IF, immunofluorescence; IgAFn, immunoglobulin A-fibronectin; IgAN, immunoglobulin A nephropathy; n, total number of cases; TN, true negative (IF negative and IgAFn negative); TP, true positive (IF positive and IgAFn positive).

^aThe prevalence of IgAN in the local population is not exactly known because kidney biopsy is not available in non-hospital-based centers and when patients are not referred to central hospitals.

cases of GN. Finally, we have no follow-up data regarding the attenuation of serum IgAFn levels in the course of treatment by the clinical teams because it was not included in the study design.

In conclusion, our study suggests that serum IgAFn (IgAFn cutoff value ≥ 3) level has strong diagnostic values with 83% sensitivity, 90% specificity, and 87% accuracy for predicting IgAN. Although the results are interesting, given the relatively low N, these results are most useful for designing and powering a larger multicenter study.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1: IgA system and development of IgA nephropathy (IgAN) with IgAFn involvement.

Figure S2: Statistical association between biopsy-confirmed and direct immunofluorescence (IF)-positive IgAN and IF-negative non-IgAN cases with serologic IgAFn-positive and IgAFn-negative cases.

Figure S3: Clinical correlation of hematuria with levels of serum IgAFn complex in whole cohort, IgAN cases, and serologic IgAFn-positive cases.

Figure S4: Clinical correlation of total urinary protein excretion with serum IgAFn complex levels in whole cohort, IgAN cases, and serologic IgAFn-positive cases.

Figure S5: Clinical correlation of serum creatinine with serum IgAFn complex levels in whole cohort, IgAN cases, and serologic IgAFn-positive cases.

Figure S6: Distribution of serologic diagnostic test of serum IgAFn levels with trend view in the cumulated cases of IgAN.

Figure S7: Distribution of stratified true- and false-positive and -negative IgAN cases according to serum IgAFn criterion level of 234 as derived from AUROC.

Figure S8: Area under receiver operating characteristics (AUROC) curve shows the distribution of true-positive and true-negative rates of biopsy-confirmed IgAN and non-IgAN against the serologic diagnostic test of serum IgAFn levels.

Figure S9: Serum IgAFn levels and corresponding true-positive and true-negative rate.

Item S1: Supplementary Methods

Table S1: Patients' demographics, clinical presentation, laboratory parameters, histologic types, and serologic IgAFn patterns

Table S2: Level of serum IgAFn complex in IgAN and non-IgAN cases

Table S3: Numbers of true-positive, false-positive, true-negative, and false-negative cases for IF-positive IgAN with respect to serologic IgAFn patterns

Table S4: Statistical significance of area under the curve

Table S5: Serum IgAFn levels and corresponding true-positive rate (sensitivity) and false-positive rate (1 – specificity)

ARTICLE INFORMATION

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Author's Contributions: Study design and conduct and statistical analysis: SMS. SMS contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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