



# Temporal Association Between PLA2R Antibodies and Clinical Outcomes in Primary Membranous Nephropathy

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**Introduction:** Autoantibodies to M-type phospholipase A2 receptor (aPLA2R) are seen in two-thirds of patients with primary membranous nephropathy (PMN) and are associated with disease activity. However, the precise temporal dynamics between the presence and amount of aPLA2R in circulation, as well as the clinical activity, are not known. We evaluated the temporal association between disease activity and serum aPLA2R during and after treatment in PMN.

**Methods:** The study included all patients with PMN and elevated aPLA2R who were started on immunosuppressive therapy for persistent nephrotic syndrome at a single center between December 2014 and December 2015. Serum samples were tested for aPLA2R at baseline and at monthly intervals for 6 months. Clinical details were collected monthly for 9 months. Serological remission was defined as negative aPLA2R in 2 consecutive samples. Clinical remission was defined by standard criteria.

**Results:** A total of 30 patients with PMN were studied. Of these, 28 (93%) had elevated levels at baseline, whereas 2 (7%) became positive after 1 month. The mean age was  $33.2 \pm 1$  (range, 13–52) years. Median baseline aPLA2R titer was 163.41 (range, 70–291.01) RU/ml. A total of 24 patients (80%) achieved serological remission by 6 months. Among all the serological responders, 54% had achieved negative aPLA2R by the end of the first month. Clinical remission was observed in 20 patients (67%). Serological and clinical remission were noted at  $2.7 \pm 1.71$  and  $5.05 \pm 2.64$  months, respectively.

**Conclusion:** In patients with aPLA2R-associated PMN, reduction in circulating aPLA2R precedes clinical remission. Persistence of aPLA2R at the end of therapy is associated with clinical resistance.

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KEYWORDS: membranous nephropathy; PLA2R; proteinuria; serial monitoring

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Beck *et al.* reported autoantibodies to M-type phospholipase A2 receptor (aPLA2R) in 70% of patients with primary membranous nephropathy (PMN).<sup>1</sup> A report by Debiec and Ronco of serological positivity in more than 50% of PMN cases confirmed this finding.<sup>2</sup> Subsequent reports documented seropositivity in 67% to 82% of patients with PMN.<sup>3,4</sup> A minority were found to have no circulating antibody but showed glomerular staining, and vice versa.<sup>5</sup> The current understanding is that more than 80% of PMN is aPLA2R related.<sup>3</sup>

Hoxha *et al.*<sup>6</sup> carried out a prospective observational study in 133 subjects with PMN to evaluate the

association of aPLA2R with disease activity. Antibody levels were monitored quarterly, and the authors concluded that persistence of aPLA2R was an independent risk factor for nonremission, whereas a decrease was associated with reduction in proteinuria. However, the knowledge regarding the closeness of the temporal association between change in aPLA2R titers during therapy and the disease course is not complete. Knowledge of the optimal frequency of monitoring would help in planning of individualized tailoring of immunosuppressive therapy. We examined this relationship in a cohort of patients with PMN and elevated aPLA2R by monitoring aPLA2R level at monthly intervals.

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## MATERIALS AND METHODS

### Study Population

This prospective study was undertaken at the Postgraduate Institute of Medical Education and Research,

Chandigarh, India. Consecutive patients who had PMN and persistent nephrotic state despite 6 months of optimal blockade of the angiotensin pathway and were treated with immunosuppressive therapy were included. Patients with diabetes mellitus, hepatitis B or C, HIV-I/II infection, or past immunosuppressive use were excluded. The Institute Ethics Committee of the Postgraduate Institute of Medical Education & Research, Chandigarh, India, approved the study, and all patients provided written informed consent.

### Follow-up and aPLA2R Detection

Patients were followed up at monthly intervals, and clinical details including proteinuria, serum albumin, and serum creatinine were recorded. Estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease (MDRD) Study equation. Serum samples were collected before starting immunosuppressive treatment and at monthly intervals and were stored at  $-80^{\circ}\text{C}$  until testing. Antibody testing was done by enzyme-linked immunosorbent assay (ELISA; EUROIMMUN AG, Lubeck, Germany) according to the manufacturer's instructions, and titers  $\geq 20$  RU/ml were considered positive.

### Definitions

Nephrotic syndrome was defined as proteinuria of  $>3.5$  g/d or  $\geq 2.0$  g/d, along with serum albumin  $<2.5$  g/dl.<sup>7</sup> Complete remission was defined as proteinuria  $<500$  mg/d with serum albumin  $\geq 3.5$  g/dl and serum creatinine, and partial remission was defined as proteinuria of 0.5 to 3.5 g/d or a  $<50\%$  decline from baseline with serum albumin  $\geq 3.5$  g/dl and stable serum creatinine.<sup>7</sup> Serological remission was defined as aPLA2R titers of  $<20$  RU/ml in at least 2 sequential samples. We defined the clinico-serological correlation as achievement of clinical remission (complete or partial remission) within a 3-month period following aPLA2R becoming negative.

### Statistical Analysis

Data are expressed as continuous variables, percentages, means and SDs, or medians and interquartile ranges. The Student *t* test was used to compare means for parametric data, and nonparametric data were analyzed using the Mann–Whitney test. The  $\chi^2$  or Fisher exact test examined the association between aPLA2R positivity at the end of 6 and 9 months, respectively. Correlation between the baseline aPLA2R antibody titers, proteinuria, and serum albumin was examined by regression analysis. A *P* value of  $< 0.05$  was considered significant.

Statistical analysis was performed using GraphPad Prism (version 7.0; GraphPad Software, La Jolla, CA).

## RESULTS

Of a total of 40 patients with IMN and persistent nephrotic syndrome seen during this period, 30 had aPLA2R-related PMN (9 female and 21 male patients). All 30 patients had persistent nephrotic syndrome after  $11.9 \pm 4.4$  (range, 6–24) months of optimal angiotensin pathway blockade. The age of the patients was  $33.2 \pm 10$  (range, 13–52) years. The mean proteinuria, serum albumin, and eGFR were  $6.73 \pm 3.93$  (range, 2.43–17.20) g/d,  $2.05 \pm 0.63$  (1.18–3.30) g/dl, and  $99.3 \pm 33.8$  (17.4–149.9) ml/min per  $1.73 \text{ m}^2$ , respectively. Three patients had an eGFR  $< 60$  ml/min per  $1.73 \text{ m}^2$ . Serum aPLA2R was positive at baseline in 28 patients (93.33%), and 2 patients (6.67%) showed a positive serology result in the 1-month sample. The median aPLA2R titer at baseline was  $238.85 \pm 300.56$  (23.65–1568.35) RU/ml. Baseline parameters are provided in Table 1. There was a correlation between baseline aPLA2R and proteinuria ( $r^2 = 0.56$ ,  $P = 0.001$ ) but not with serum albumin ( $r^2 = -0.04$ ,  $P = 0.81$ ). A total of 28 patients (93.33%) received cyclical therapy with cyclophosphamide and steroids (cCTX/GC; i.v. methylprednisolone 1 g/d for 3 consecutive days, followed by oral prednisolone 0.5 mg/kg per day for 27 days in the first, third, and fifth months, and oral cyclophosphamide at 2 mg/kg per day in the second, fourth, and sixth months), and 2 patients (6.67%) were treated with rituximab ( $375 \text{ mg/m}^2$ , followed by doses titrated to CD 19 counts).

Patients were followed up for 9 to 15 months. Details of individual patients are provided in Supplemental Table S1. A total of 24 patients (95.83%) had met the definition of serological remission at 6 months. Of the patients, 20 (66.7%) had achieved clinical remission at

**Table 1.** Baseline parameters of study patients

Parameter	Value
Age, yr	$33.2 \pm 10$ (13–52)
Sex, male:female	21:07
Duration, mo <sup>a</sup>	$11.9 \pm 4.4$ (6–24)
Proteinuria, g/d	$6.73 \pm 3.93$ (2.43–17.20)
Serum albumin, g/dl	$2.05 \pm 0.63$ (1.18–3.30)
Estimated GFR, ml/min per $1.73 \text{ m}^2$	$99.3 \pm 33.8$ (17.4–149.9) <sup>b</sup>
aPLA2R (RU/ml) <sup>c</sup>	$238.85 \pm 300.56$ (23.65–1568.35) Median 163.41 (IQR 70–291.01)

aPLA2R, autoantibodies to M-type phospholipase A2 receptor; GFR, glomerular filtration rate; IQR, interquartile range.

<sup>a</sup>Months of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers received.

<sup>b</sup>Three patients had eGFR of  $< 60$  ml/min per  $1.73 \text{ m}^2$ .

<sup>c</sup>Two patients had aPLA2R during follow-up.

Data are mean with range in parentheses unless otherwise indicated.

the end of the follow-up, whereas 10 (33.3%) had a resistant disease. Negative aPLA2R at 6 months was associated with achievement of clinical remission by 9 months ( $P < 0.001$ ). aPLA2R titer at the end of 6 months correlated with proteinuria at the sixth and ninth months (6 months:  $r^2 = 0.389$ ,  $P = 0.03$ ; 9 months:  $r^2 = 0.46$ ,  $P = 0.01$ ). Figure 1 depicts the serial trend in aPLA2R, proteinuria, and serum albumin.

Four patients with negative aPLA2R at the sixth month continued to have nephrotic range proteinuria on follow-up. However, all 4 patients had  $> 50\%$  reduction in proteinuria and improvement in serum albumin (Supplemental Table S1: patients 8, 13, 20, and 24). aPLA2R titers were repeated in these 4 patients between 9 and 12 months. One of these patients (patient 24) maintained serological remission at the ninth month and went on to achieve partial remission by 15 months, whereas the other 3 patients experienced a rebound in the aPLA2R titer and were in a persistent nephrotic state (Supplemental Figure S1). All the other patients with serological remission at 6 months were in remission at 12 months, and all 6 patients with positive aPLA2R at 6 months continued to have nephrotic range proteinuria (Supplemental Table S1).

Among the responders, serological remission preceded clinical remission by  $2.35 \pm 0.93$  months ( $2.7 \pm 1.71$  [range, 1–6] and  $5.05 \pm 2.64$  [range, 2–9] months for serological and clinical remission, respectively). An examination of temporal trends showed that the decline in titers was noted as early as at the end of the first month in  $>50\%$  of cases, but the initial fall was not uniformly sustained. With continued therapy, however, there was a progressive increase in the response rate. Among serological responders, 54.1%, 37.5%, 62.5%, 79.1%, and 95.6% achieved negative aPLA2R at 1, 2, 3, 4, and 5 months, respectively.

There was no difference in the baseline aPLA2R titer among patients who did or did not show serological or clinical remission (Figures 2 and 3). Patients with

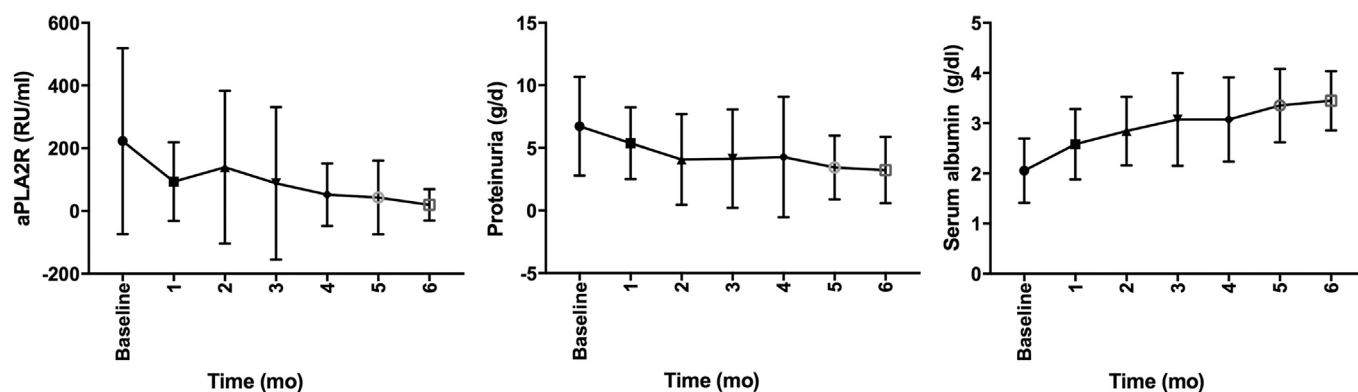
clinical remission had a significant reduction in their aPLA2R titers at all time points compared to those who did not experience remission (Figure 3).

## DISCUSSION

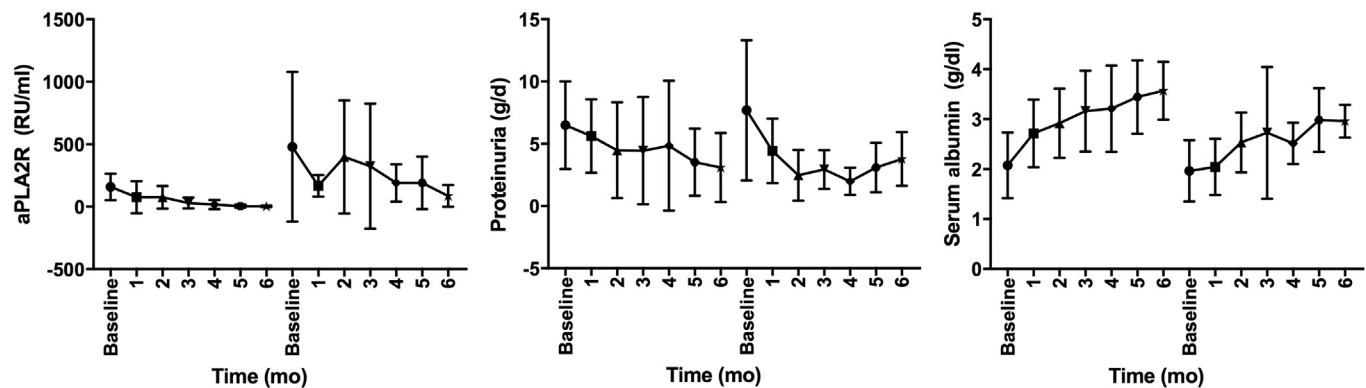
We examined the temporal relationship between the serological and clinical response to immunosuppressive treatment in this population of PLA2R-related PMN patients who were predominantly treated with cCTX/GC, and observed that, based on our study findings, reduction in aPLA2R starts after the first month and that the serological remission precedes clinical remission by 2.3 months. This knowledge is critical for developing management algorithms that target aPLA2R as treatment surrogate to minimize exposure to potentially toxic immunosuppressive therapy. Another notable finding was that a single negative value was not always indicative of a persistent negativity; hence, if treatment decisions were to be made on the basis of serology, it would be prudent to confirm it on at least 1 more occasion.

We examined the serological response in the first 6 months, because that is the duration of treatment for the cCTX/GC regimen, and if tailoring is needed it would apply in that period. There was indeed an association of the sixth-month aPLA2R with clinical outcome at 9 months. This finding was also valid in subjects who did not have detectable aPLA2R at baseline but showed elevations later in the course, consistent with the “kidney as a sink” hypothesis.<sup>5,8</sup>

cCTX/GC is recommended as the firstline agent in the management of PMN. Ruggenti *et al.* concluded that a 6-month depletion of aPLA2R independently and strongly predicted clinical remission in rituximab-treated PMN patients.<sup>9</sup> In the present study, we show that serological response becomes evident in almost half of the cCTX/GC-treated patients as early as the end of first month. Serological remission was observed in  $\sim 75\%$  and  $>95\%$  of patients 4



**Figure 1.** Serum autoantibodies to M-type phospholipase A2 receptor (aPLA2R), proteinuria, and serum albumin at various time points.



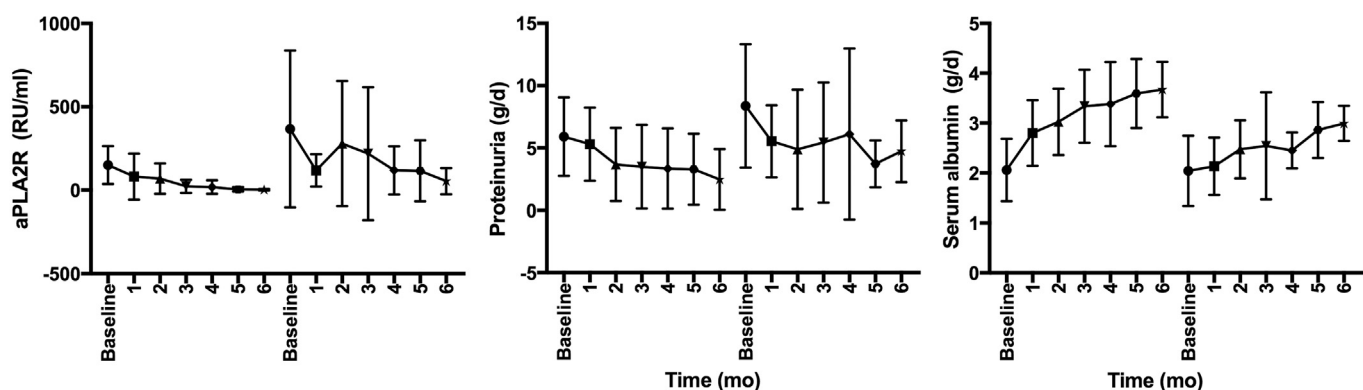
**Figure 2.** (Left) Serum autoantibodies to M-type phospholipase A2 receptor (aPLA2R), (center) proteinuria, and (right) serum albumin at various time points in serological responders (left) and resistant patients (right).

and 5 months after starting treatment, respectively. An interesting observation was that negative aPLA2R was achieved in 54% of the patients at end of the first month. Past studies have reported significant reduction in aPLA2R 3 months after starting therapy.<sup>6</sup> Hoxha *et al.* reported 81% and 39% reduction at 3 months in aPLA2R titer and proteinuria, respectively. Bech *et al.* evaluated aPLA2R in 48 patients with PMN. Although the baseline aPLA2R titer did not predict response, at the end of therapy, titers predicted long-term outcomes.<sup>10</sup> Ruggenenti *et al.* reported that lower aPLA2R titer at baseline and complete antibody depletion at 6 months were predictive of remission.<sup>10</sup> Medrano *et al.* analyzed the relationship between quarterly aPLA2R estimation and clinical response in patients treated with immunosuppressive agents, and concluded that dynamics was useful in predicting clinical response.<sup>11,12</sup> However, since the initial response may be variable, we suggest starting monitoring for aPLA2R-targeted treatment by the end of the second month, and ensuring at least 2 negative values before contemplating reduction of therapy (Figure 4).

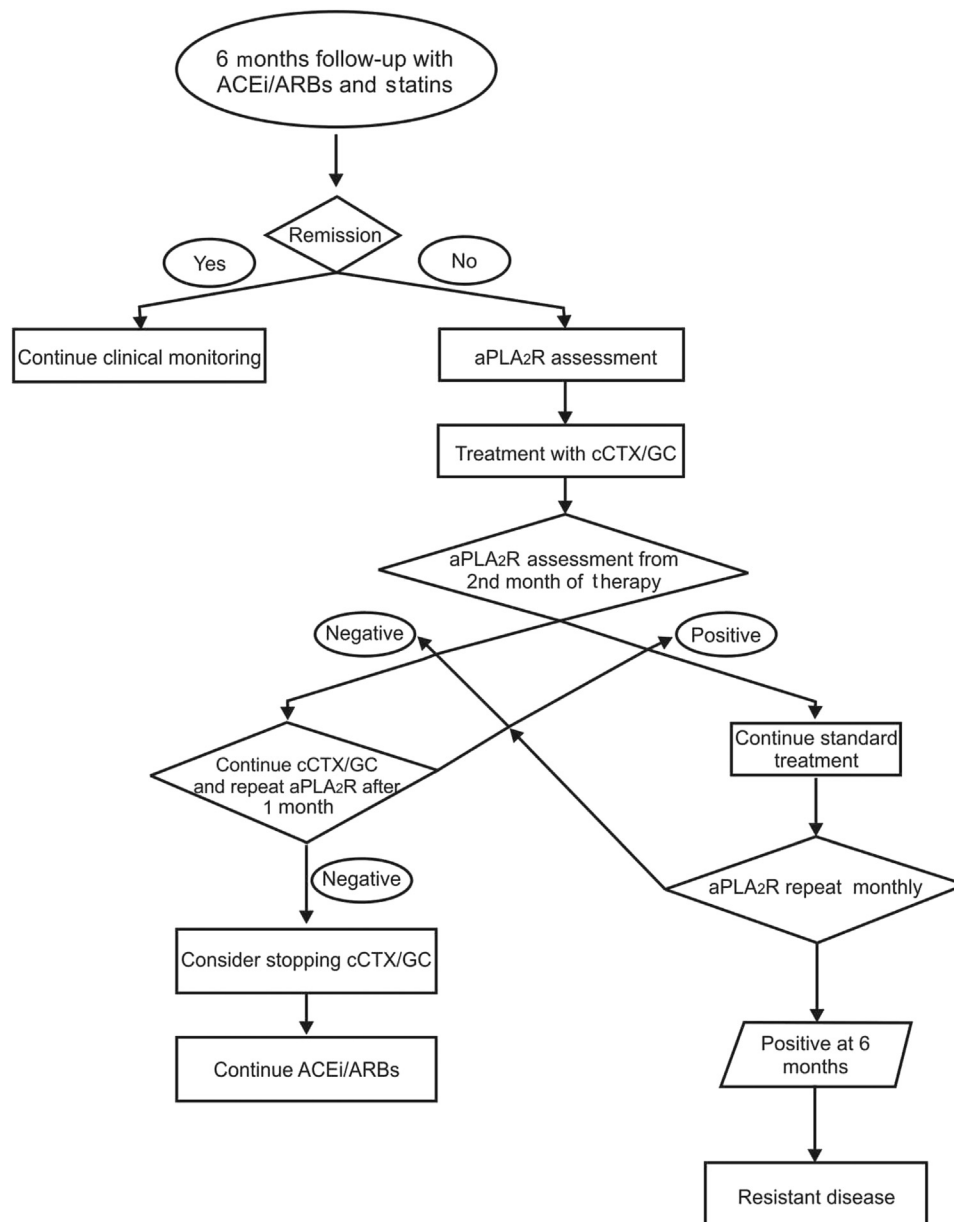
The reduction in aPLA2R was not sequential in all the patients, and many patients had a transient rise after an initial fall, which may be explained by the titer being decided by both production and tissue saturation.<sup>3</sup> In a minority of cases (13%), serological remission was not followed by clinical remission.

Based on the evidence gathered from the present study, we propose an algorithm to manage PLA2R related PMN (Figure 4) with cCTX/GC, which might allow reduction of exposure to steroids and cyclophosphamide. The proposed algorithm needs to be prospectively validated. De Vriese *et al.*,<sup>13</sup> in their algorithm for managing PMN, suggest stopping immunosuppressive therapy in patients with >90% reduction in aPLA2R titer. However, going by the results of the present study, the presence of any titer >20 RU/ml was associated with resistant disease, irrespective of the degree of reduction, and even patients with a 57% to 94% reduction in aPLA2R titer (but >20 RU/ml) had persistent nephrotic syndrome.

The present study is limited by a small number of patients who were treated predominantly with cCTX/GC, younger age, and short duration of follow-up.



**Figure 3.** (Right) Serum autoantibodies to M-type phospholipase A2 receptor (aPLA2R), (center) proteinuria, and (right) serum albumin at various time points in patients with clinical remission (left) and resistance (right).



**Figure 4.** Management algorithm for serum autoantibodies to M-type phospholipase A2 receptor (aPLA2R)-related primary membranous nephropathy. ACEi/ARBs, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers; cCTX/GC, cyclical therapy with cyclophosphamide and steroids.

Furthermore, monitoring of aPLA2R titers beyond 6 months in all patients would have allowed us to establish the ability of serological relapse in predicting clinical relapse. Nevertheless, the study is hypothesis generating and highlights the need to better understand the relationship between aPLA2R and clinical response. This approach may not be relevant in situations in which other immunosuppressive agents, such as calcineurin inhibitors or rituximab, are used.

To conclude, reduction in aPLA2R precedes clinical remission, and persistence of antibodies at the end of

treatment is associated with resistant disease in patients with aPLA2R-related PMN. Hence, the objective of treatment should be to deplete and maintain antibodies below the pathological limit.

## DISCLOSURE

All the authors declared no competing interests.

## ACKNOWLEDGMENTS

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## SUPPLEMENTARY MATERIAL

**Table S1.** Clinical details of the study cases.

**Table S2.** Serological details of the study cases.

**Figure S1.** Clinical and serological details of patients with no clinico-serological correlation.

Supplementary material is linked to the online version of the paper at [www.kireports.org](http://www.kireports.org).

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