Anti-PLA₂R antibodies clinical effectiveness in monitoring idiopathic membranous nephropathy patients

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Introduction

Idiopathic membranous nephropathy (IMN), a once unknown cause of membranous nephropathy (MN), is now considered an organ-specific autoimmune disease by the discovery of autoantibodies targeting the M-type phospholipase A2 receptor (PLA₂R) on renal podocytes. 20-25% of MN cases are caused by various underlying disorders, and these types of MN cases are referred to as secondary MN. It is of great clinical importance to discriminate between IMN and secondary MN as the therapeutic strategies are completely different. Studies have demonstrated anti-PLA₂R levels correlating with proteinuria levels, however, their relationship is not perfect as anti-PLA₂R levels proceed proteinuria levels due to the time it takes for injured podocytes to heal, see figure 1.

Euroimmun has produced an indirect immunofluorescence (IIF) transfected cell line assay, which was the first diagnostic commercial assay for the detection of anti-PLA₂R autoantibodies (predominantly IgG₄) and subsequently developed a quantitative ELISA assay. Both of these methods provide a rapid diagnosis for IMN by replacement of the renal biopsy. However, the quantitative ELISA assay enables the monitoring of patients disease activity, which in turn can improve patient prognosis and efficient therapy administration.

Method

A total of 87 samples were tested from a cohort of 29 biopsy proven IMN patients who supplied serial samples from 2009 to 2013. A comparison study was initial performed between Euroimmun’s anti-PLA₂R IIF assay and their ELISA assay. The ELISA assay performance was verified by assessing its assay precision, linearity and assay interference. The ELISA’s MN disease correlation was assessed by comparing five patients anti-PLA₂R levels with their urinary protein to creatinine ratio (UPCR).

Results

Comparison of IIF and ELISA results: A good concordance of 98% was achieved, with a kappa value of 0.968.

ELISA assay precision: Mean intra-assay CV% was 4.3% and mean inter-assay CV% was 12.5%.

ELISA assay linearity: Assay linearity was observed between the range of 14 RU/ml-300 RU/ml. The top end of the assay appeared to be less linear.

ELISA assay interference: One sample with a high IgG₁ and IgG₄ value at 9.18 and 6.04 RU/ml respectively gave a low positive ELISA result of 22.2 RU/ml. Other samples with elevated IgG₁ levels tested negative, indicating the IgG₄ result potentially contributed to the positive result. Assay specificity was 98.4%.

Comparison and correlation of anti-PLA₂R ELISA and UPCR levels: Figures 2 to 6 display dates the five patient samples were taken with their anti-PLA₂R and UPCR levels. Anti-PLA₂R ELISA interpretation: <14 RU/ml = negative, 14-20 RU/ml = borderline positive, >20 RU/ml = positive. UPCR interpretation: <100 = mild, 100-300 = significant, >300 = nephrotic range. Figure 7 demonstrates no significant correlation between the anti-PLA₂R and UPCR levels between the five patients, p-value = 0.32.

Discussion

The anti-PLA₂R ELISA Euroimmun assay has demonstrated to be comparable to the IIF assay, reproducible with a best linearity range between 14 - 300 RU/ml. IgG₄ subclasses potentially may interfere with the assay, however, the effect was minimal by producing a very low positive anti-PLA₂R result, furthermore it is not known if this patient had IMN.

Anti-PLA₂R levels in most cases mirrored and proceeded a decline in UPCR levels but do not precisely reflect renal disease activity, as clearly shown in figures 3 and 5. When anti-PLA₂R levels had subsided, renal impairment was still apparent due to UPCR levels remaining in the nephrotic range. This finding is further demonstrated in figure 7, which shows a non-significant correlation between anti-PLA₂R and UPCR levels (p-value = 0.32).

Conclusion

Implementing the anti-PLA₂R Euroimmun ELISA assay for routine testing has greatly assisted in efficiently diagnosing and monitoring the immunological activity in IMN patients.

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References