Establishment of Galectin-3 time-resolved fluoroimmunoassay and its application in idiopathic membranous nephropathy

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Research Article

Keywords: diagnosis, fluoroimmunoassay, galectin-3, idiopathic membranous glomerulonephritis

Posted Date: September 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-816341/v1

Version of Record: A version of this preprint was published at Journal of Fluorescence on January 13th, 2022. See the published version at https://doi.org/10.1007/s10895-022-02888-4.

Abstract

Objectives

The aim of this study was to establish a time-resolved fluorescent immunoassay (TRFIA) for the detection of serum galactose agglutinin 3 (Gal-3) and apply this method to evaluate the clinical significance of serum Gal-3 in predicting Idiopathic Membranous Nephropathy (IMN) progression.

Methods

The Gal-3-TRFIA was established using the double antibody sandwich method, with the capture antibodies coated on a 96-well microplate and the detection antibodies chelated to Europium (III) (Eu^{3+}). Serum Gal-3 was detected in 81 patients with IMN and 123 healthy controls to further evaluate the value of the Gal-3 in staging of IMN.

Results

The sensitivity of the Gal-3-TRFIA assay was 0.85 ng/mL, and the detection range was 0.85–1000 ng/mL. The Gal-3 intra-batch and inter-batch coefficients of variation were 3.45% and 5.12%, respectively. The correlation coefficient (*R*) between the Gal-3-TRFIA assay and commercially available enzyme-linked immunosorbent assay kits was 0.83. The serum Gal-3 concentration was higher in patients with IMN (65.57 ± 55.90 ng/mL) compared to healthy controls (16.29 ± 9.91 ng/mL, $P < 0.0001$).

Conclusions

In this study, a wide detection range Gal-3-TRFIA assay was developed using lanthanide (Eu^{3+}) chelates for the detection of Gal-3 concentrations in serum. The Gal-3-TRFIA also detected the severity of the IMN course.

Introduction

Galactose agglutinin 3 (Galectin-3, Gal-3), a member of β-galactoside-binding lectin family,[1] is an important regulators of inflammatory responses, and it is expressed in many inflammatory cells, such as macrophages.[2] When the cell is at rest, Gal-3 is stored in the cytoplasm; however, in the event of tissue damage, activated cells secrete Gal-3 via nonclassical pathways.[3] This protein binds to the cell surface and extracellular matrix polysaccharides and influences a variety of physiological and pathological processes, including apoptosis, adhesion, migration, fibrosis,[2] and inflammation.[4, 5] The expression of Gal-3 is detected in many disease states, such as renal disease.[6] Gal-3 is a biomarker associated with the progression of renal functional decline.[7] Elevated serum Gal-3 levels are associated with a rapid

decline in glomerular filtration rate and an increased risk of chronic kidney disease (CKD).[8, 9] However, the role of Gal-3 in MN has not yet been reported.

Membrane nephropathy (MN) is a unique glomerular lesion[10] and is the main cause of adult nephrotic syndrome.[11] Approximately 80% of cases are idiopathic membranous nephropathy (IMN).[10] Approximately 20% of patients with IMN will progress to end-stage renal disease, and approximately 10% of them will die within 5–10 years.[12, 13]

Therefore, we conducted relevant studies to explore the value of Gal-3 in MN. In this study, an effective time-resolved fluoroimmunoassay (TRFIA) (Fig. 1) was established to detect the concentration of Gal-3 in serum with antibodies labeled with the lanthanide element Eu^{3+}, and the fluorescence count of Eu detected was proportional to the amount of Gal-3 present in the binding reaction to achieve the quantification of Gal-3 in serum. This study also explored the diagnostic value of Gal-3 detection in patients with IMN. Moreover, this study was conducted to determine whether Gal-3 was associated with renal function impairment and disease progression in patients with IMN.

Materials And Methods

Reagents and instruments

Two monoclonal antibodies against different epitopes of Gal-3, the capture antibody (10301) and detection antibody (10302), were purchased from Medix (Kauniainen, Finland). The Gal-3 standard (abs 01295) was purchased from Absin Bioscience Inc (Shanghai, China). The enzyme-linked immunosorbent assay (ELISA) kit was purchased from Sino (Beijing, China). Eu^{3+}-N1-(p-isothiocyanatobenzyl)-diethylenetriamine-N1, N2, N3, and N4-tetra acetic acid (DTTA) and enhancement solution were purchased from Zhejiang Boshi Biological Technology Co., Ltd (Zhejiang, China). The Sephadex-G50 column was purchased from Seebio Biotech Co (Shanghai, China). Ninety-six-well plates were purchased from Xiamen Yunpeng Technology Development Co., Ltd (Xiamen, China). The time-resolved immunofluorescence analyzer was purchased from Foshan Da'an Medical Instrument Co., Ltd., and an electric thermostatic incubator was purchased from Shanghai Jinghong Laboratory Instrument Co., Ltd. (Shanghai, China).

Samples

Serum samples were collected from 81 patients with IMN diagnosed by pathology and from 123 healthy subjects in the Department of Nephrology, Wuxi People's Hospital Affiliated to Nanjing Medical University. All serum samples were collected using a separation gel-sampling tuble. After centrifugation, the samples were stored in a refrigerator at -20°C and returned to room temperature before detection. Informed consent was obtained from all participants. The research project was approved by the Wuxi People's Hospital, Affiliated to Nanjing Medical University.

Buffers

The buffers included a coating buffer (50 mM Na_2CO_3-$NaHCO_3$, pH 9.6), analysis buffer (50 mmol/L Tris-HCl, containing 0.01% Tween-20, 20 μM diethylenetriamine pentaacetate, 0.9% NaCl, 0.2% bovine serum albumin (BSA), and 0.05% NaN_3, pH 7.8), labeling buffer (50 mmol/L Na_2CO_3-$NaHCO_3$, pH 9.0), blocking buffer (50 mmol/L Tris-HCl, containing 0.9% NaCl, 1% BSA, and 0.05% NaN_3, pH 7.8), washing solution (50 mmol/L Tris-HCl, containing 0.9% NaCl, 0.05% proclin-300, pH7.8) and elution buffer (50 mmol/L Tris-HCl, containing 0.2% BSA, 0.05% Proclin-300, pH 7.8). Gal-3 standard (1000 ng/mL) was diluted with analysis buffer to final concentrations of 0, 12.5, 25, 50, 100, and 1000 ng/mL.

Immobilization of monoclonal antibodies

Gal-3 antibody (10301, capture antibody) was diluted to 3 μg/mL in coating buffer at pH 9.6. Then, 100 μL of the diluted antibody was added to each well and incubated overnight at 4°C. The plates were washed with washing solution, and 150 μL of blocking buffer containing 1% BSA was added to each well. The coated antibody were blocked at room temperature for 2 h, dried thoroughly, and stored at − 20°C.

Labeling monoclonal antibodies with Eu^{3+}

Anti-Gal-3 antibody labeling was performed according to the manufacturer's instructions. A total of 0.3 mg anti-Gal-3 antibody was added to the Ultracel-50K ultrafiltration tube and the labeling buffer was replaced by multiple centrifugation steps. The antibody was mixed with 60 μg Eu^{3+}-DTTA chelate and incubated overnight at 28°C. The labeling solution was injected onto a Sephadex-G50 column chromatography balanced with 0.2% BSA eluent, and the fluorescence count of the collected proteins was detected by time-resolved immunofluorescence analyzer. The first elution peak was collected, and the labeled antibodies were packaged and stored at -20°C.

Gal-3-TRFIA assay procedure

A two-step method was used for detection to avoid the influence of ethylenediaminetetraacetic acid in the serum. Standard or serum samples (50 μL) were added to 96-well microplates. Subsequently, 50 μL of the analysis buffer was added to each well. The samples were then incubated at 37°C for 2 h and washed with washing solution twice. Thereafter, 100 μL diluted Eu^{3+}-anti-Gal-3 antibody was added to each well, and the plate was incubated at 37°C for 2 h. After washing with washing solution six times, 100 μL enhancement solution was added to each well. After shaking for 3 min, the fluorescence count of each well was measured using a time-resolved immunofluorescence analyzer. Finally, the Gal-3 concentration in serum samples was calculated according to the standard curve of the Gal-3-TRFIA.

Optimization of the experimental reaction conditions

Selection of the coated antibody concentration

The Gal-3 coated antibody was diluted to different concentrations (0.375, 0.7, 1.5, 3, and 6 μg/mL). After coating and blocking, Gal-3 standards and the Eu^{3+}-anti-Gal-3 antibody were added to obtain the optimal concentration of Gal-3 coated antibody.

Selection of the dilution ratio of labeled antibody

Eu^{3+}-anti-Gal-3 was diluted at ratios of 1:25, 1:50, 1:100, 1:200, 1:400 and 1:800. Different dilution ratios of Eu^{3+}-anti-Gal-3 were tested with 100 ng/mL Gal-3 standard or analytical buffer to determine the fluorescence counts or background counts, respectively. The optimal dilution ratio of labeled antibodies was selected when both high fluorescence counts and low background counts were obtained.

Evaluation of the Gal-3-TRFIA method

Sensitivity and linearity

The Gal-3 standard curve was determined by plotting the corresponding fluorescence counts across a serial of concentrations (0, 12.5, 25, 50, 100, and 1000 ng/mL) of the Gal-3 standard and analyzing it logit-log linear regression fitting. Based on the standard curve, the assay sensitivity was determined by the concentrations of Gal-3 which corresponded to the fluorescence of 10 wells of zero calibrators (mean + 2SD).

Precision

The precision of the method was evaluated using two Gal-3 standards. Two Gal-3 standards with low (12.5 ng/mL) and high (100 ng/mL) Gal-3 concentrations were selected. Meanwhile, 10 independent experiments were conducted on each of the three samples to obtain intra-batch precision. The inter-batch precision was repeated three times with ten independent experiments each time.

Specificity

Specificity was assessed using 1000 ng/mL Kidney injury molecule 1 (KIM-1) and 1000 ng/mL T cell immunoglobulin-3 (TIM-3) as a potential interferers. The ratio of the actual measured KIM-1/TIM-3 concentration to the theoretical concentration was calculated as the specificity.

Recovery rate

The Gal-3 standard at a concentration of 1000 ng/mL was mixed with serum sample with low concentrations of Gal-3 in a ratio of 1:9. The ratio of the actual measured Gal-3 concentration to the theoretical concentration of the mixed serum was calculated as the recovery rate using the following formula: recovery (%) = (measured concentration/theoretical concentration) × 100. The ratio of each measurement to the theoretical value was then calculated.

Correlation between Gal-3-TRFIA and ELISA

Gal-3 serum levels were measured using the established Gal-3-TRFIA in 28 patients with IMN and compared to those detected using commercially available ELISA kits, serums whose Gal-3 concentration exceeded the ELISA kit's range were diluted and remeasured.

Clinical application of Gal-3-TRFIA

Gal-3 serum levels in 81 patients with IMN and 123 healthy controls were measured using the established Gal-3-TRFIA. The patients' urea, uric acid, urine protein,serum albumin, serum creatinine, and glucose levels were obtained from database of the hospital.

Statistical analysis

The data are presented as the mean ± SD. Median and quartile ranges were used if the data distribution was skewed. Results were analyzed using SPSS (version 24.0, Chicago, IL, USA), and charts were plotted using PRISM 7.00 (San Diego, CA, USA) and Origin 2018 (Origin Lab, USA). The Pearson correlation coefficient (R) or Spearman correlation coefficient (ρ) was used to evaluate whether the Gal-3 concentration was correlated with each parameter. A Mann-Whitney U-test was used to analyze whether a significant difference existed between the two groups.

Results

Optimization selection of the optimal reaction conditions for the Gal-3-TRFIA

Selection of the coated antibody concentration

Diluted anti-Gal-3 antibodies at 0.375, 0.75, 1.5, 3, and 6 μg/mL were coated on 96-well microplates. After blocking, the Gal-3 standard and Eu^{3+}-anti-Gal-3 antibodies were added to the plate. The counts per second (CPS) increased with an increase in the coated antibody concentration and leveled off when the coated concentration was 3 μg/mL (Fig. 2A). Therefore, the optimal concentration of the coated antibody was determined to be 3 μg/mL.

Selection of the labeled antibody dilution ratio

Eu^{3+}-anti-Gal-3 at 1:100 was considered the optimal because the background is low and the Gal-3 binding rate is high (Fig. 2B).

Assay sensitivity

Linear results are shown in Fig. 3A. The fluorescence count of 10 wells of 0 ng/mL standard was substituted into the standard curve, and the mean + 2 SD was calculated to obtain a sensitivity of 0.85 ng/mL. The Gal-3 detection range was 0.85–1000 ng/mL. The correlation coefficient of the Gal-3 standard curve was 0.996, indicating a good linear relationship between Gal-3 concentration and fluorescence count.

Assay precision and specificity

Precision was expressed as the coefficient of variation (CV%). The Gal-3 intra-and inter-batch coefficient of variation was 3.45% and 5.12%, respectively. All CV values were less than 10%, indicating that the

precision of this experiment was acceptable. Studies of the specificity showed that the cross-reactivity with KIM-1 and Tim-3 were less than 1%, which would not affect the experimental results.

Assay recovery rate

The original concentration of the serum sample was 26.59 ng/mL. After the addition of the Gal-3 standard, the three measured concentrations of Gal-3 were 132.90, 124.79, and 127.70 ng/mL. The average recovery rate was 104%.

Correlation between Gal-3-TRFIA and ELISA

The established Gal-3-TRFIA and ELISA methods were used to simultaneously detect the serum Gal-3 concentration in patients. Linear regression analysis showed that the results of the two tests were well correlated (Fig. 3B, R = 0.83), indicating that the Gal-3-TRFIA detection method could be used for clinical detection of Gal-3 in human serum. The maximum detection limit of the TRFIA was 1000 ng/mL, which was higher than that of the ELISA (100 ng/mL).

Clinical application

Eighty-one serum samples from patients with IMN and 123 serum samples from healthy controls were assayed using Gal-3-TRFIA methods to evaluate its clinical applicability. Serum Gal-3 concentration of patients with IMN was 65.57 ± 55.90 ng/mL, which was significantly higher than that of healthy controls (16.29 ± 9.91 ng/mL, P < 0.0001) (Fig. 4A). Receiver operating characteristic (ROC) curve analysis was performed based on the serum Gal-3 concentrations of patients with IMN and healthy controls (Fig. 4B). The area under ROC was 0.811 ($P < 0.0001$), indicating that Gal-3 was useful in the differentiation of IMN from healthy controls.

The correlation analysis of serum Gal-3 level with PLA2R-Ab revealed no significant correlation between Gal-3 and PLA2R-Ab (Fig. 4C). Patients with pathologic staging records were singled out and were divided into two stages according to the criteria by Ehrenreich et al. Increases in serum Gal-3 concentration were related to an increase in the degree of pathological damage in patients (Table 1).

Table 1. Distribution of serum Gal-3 concentration as a function of IMN stage

	I	II	*P*
Number of participants (n, %)	17 (53.1)	15 (46.9)	
Gal-3 concentration (ng/mL, mean ± SD)	94.51 ± 48.8	117.6 ± 52.4	0.286

Abbreviations: Gal-3, galectin-3; IMN, idiopathic membranous nephropathy; SD, standard deviation; n, number.

Discussion

Gal-3 is a cytoplasmic protein synthesized in the cytoplasm.[14] Gal-3 is also released into the blood or urine from damaged cells and inflammatory cells under pathological conditions.[8, 15] Many different blood assays have been used in Gal-3 clinical practice, with ELISA being the most commonly used.[16] However, some defects are observed in ELISA, such as unstable compounds labeled by the enzyme and narrow detection range. Gal-3 concentrations fluctuate widely in patients and are beyond the range of ELISA detection. Therefore, it is necessary to find a method with a wider detection range to detect Gal-3 concentration in patients. TRFIA is widely used in many fields, such as clinical research.[17] The unique fluorescence properties of lanthanide chelates provide TRFIA with many advantages, such as a narrow emission peak and high quantum yield.[18] In addition, TRFIA has a wider detection range and less impact on the activity of the marker compared to ELISA; thus, it is suitable for the establishment of a wide detection range method for Gal-3.

In this study, the Eu^{3+}-Gal-3 antibody was used as a tracer in the Gal-3-TRFIA to detect Gal-3 in the serum. The Gal-3-TRFIA assay showed a clear linear relationship between the concentration of the analyte and the fluorescence count over a wide range of detection. The detection range of the Gal-3-TRFIA assay was 0.85–1000 ng/mL. The Gal-3 intra- and inter-batch coefficients of variation were 3.45% and 5.12%, respectively. The correlation coefficient (*R*) between the Gal-3-TRFIA assay and commercially available enzyme-linked immunosorbent assay kits was 0.83. KIM-1[19, 20]and TIM-3[21] were also found to be elevated in a variety of nephropathy and were selected as potential interferers for the test, which showed no effect on the measured Gal-3 concentration. Serum Gal-3 levels are negatively correlated with renal function.[22] However, the role of Gal-3 in IMN has not been reported yet. Therefore, the established Gal-3-TRFIA method was used to detect the levels of Gal-3 in patients with IMN and normal subjects. The results of the study showed that Gal-3 concentrations were higher in the serum of patients with IMN.

Idiopathic membranous nephropathy is the most common cause of nephrotic syndrome.[10] Early diagnosis of IMN mainly depends on renal biopsy.[10] Given that the M-type phospholipase A2 receptor (PLA2R) is now widely accepted as the primary target for IMN, [23, 24] detection of anti-PLA2R antibodies in the blood and PLA2R antigen in renal biopsy is a definitive diagnosis.[25] PLA2R targets IMN with a positive rate of 70–80%.[10] But other indicators are expected to achieve better assessment of disease progression. Therefore, we studied the value of Gal-3 in IMN and found that Gal-3 was associated with the disease progression of IMN, and the concentration of Gal-3 was correlated with the degree of kidney injury.

However, in our results, no significant correlation was found between Gal-3 and PLA2R-Ab levels. Different production mechanisms may be responsible for the uncorrelation between Gal-3 and PLA2R-Ab concentrations. PLA2R as a specific intrinsic podocyte antigen bind to its corresponding autoantibodies and leads to the formation of glomeruli subepithelial immune complex deposits in IMN.[26] Initial PLA2R-Ab production tends to precede proteinuria by weeks/months, and the disappearance of PLA2R-Ab antibodies also precedes clinical remission by weeks/months.[27] However, Gal-3 is widely involved in renal inflammation and fibrosis, and it is readily secreted from inflammatory cells and injured cells.[2] It is

speculated that Gal-3 concentration will gradually increase with the severity of the disease after the occurrence of IMN. The results also confirmed that the concentration of Gal-3 increased with the stage of IMN.

To our knowledge, this is the first prospective study to examine the relationship between Gal-3 and IMN progression. Serum Gal-3 concentration gradually increased with the progression of IMN staging. This is of certain significance to judge the disease course of patients with IMN.

Conclusions

A reliable immunoassay with a wide detection range was established for Gal-3. Serum Gal-3 concentrations in patients with IMN were higher than those in healthy controls. This study demonstrated the value of Gal-3 in the judge the disease course of patients with IMN.

Declarations

Funding:

Funding was provided by the Social Development Fund of Zhejiang Province (No. LGF20H200008), Key Research and Development Project of Zhejiang Province (No. 2020C03066), the Scientific Research Projects of Jiangsu Provincial Health Commission (LGY201801), The precision medicine key project of Wuxi health commission (J202001), Opening Project of Zhejiang Provincial Preponderant and Characteristic Subject of Key University (Traditional Chinese Pharmacology)Zhejiang Chinese Medical University (No. ZYAOXYB2019004).

Conflicts of interest

Not applicable.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Each of the authors has contributed to the article. Xiaomei Yu and lingli Chen were in charge of doing the experiment and completing the study. Xue Yang, Xiaobing Liu, Yueming Liu and Bo Lin were responsible for providing serum samples and clinical data. Xiumei Zhou, Yigang Wang, Pengguo Xia, Yuan Qin, liang

Wang, Zhigang Hu and Biao Huang made many suggestions on the conception and revision of the article, and guided the experiment for many times. The writing of the article is mainly done by Xiaomei Yu.

Ethical approval:

The study was approved by the institutional research ethics committee of Jiangsu Institute of Nuclear Medicine. All enrolled subjects provided their written informed consent for study participation, and all methods were performed in accordance with the relevant guidelines and regulations. The research project was approved by Wuxi People's Hospital Affiliated to Nanjing Medical University. Ethic number is ky12016001.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Patients signed informed consent regarding publishing their data and photographs.

References

1. Barondes SH, Castronovo V, Cooper DN et al (1994) Galectins: A family of animal beta-galactoside-binding lectins[J]. Cell 76(4):597–598
2. Hara A, Niwa M, Noguchi K et al. Galectin-3 as a next-generation biomarker for detecting early stage of various diseases[J]. Biomolecules, 2020, 10(3)
3. Davidson PJ, Li SY, Lohse AG et al (2006) Transport of galectin-3 between the nucleus and cytoplasm. I. Conditions and signals for nuclear import[J]. Glycobiology 16(7):602–611
4. Ochieng J, Fridman R, Nangia-Makker P et al (1994) Galectin-3 is a novel substrate for human matrix metalloproteinases-2 and – 9[J]. Biochemistry 33(47):14109–14114
5. Yang RY, Hsu DK, Liu FT (1996) Expression of galectin-3 modulates t-cell growth and apoptosis[J]. Proc Natl Acad Sci U S A 93(13):6737–6742
6. Tan KCB, Cheung CL, Lee ACH et al (2018) Galectin-3 is independently associated with progression of nephropathy in type 2 diabetes mellitus[J]. Diabetologia 61(5):1212–1219
7. Iacoviello M, Di Serio F, Rizzo C et al (2019) Association between high gal-3 serum levels and worsening of renal function in chronic heart failure outpatients[J]. Biomark Med 13(9):707–713
8. Dong R, Zhang M, Hu Q et al (2018) Galectin-3 as a novel biomarker for disease diagnosis and a target for therapy (review)[J]. Int J Mol Med 41(2):599–614
9. Alam ML, Katz R, Bellovich KA et al (2019) Soluble st2 and galectin-3 and progression of ckd[J]. Kidney Int Rep 4(1):103–111
10. Couser WG (2017) Primary membranous nephropathy[J]. Clin J Am Soc Nephrol 12(6):983–997

11. Akiyama S, Imai E, Maruyama S. Immunology of membranous nephropathy[J]. F1000Res, 2019, 8
12. Sim JJ, Bhandari SK, Batech M et al (2018) End-stage renal disease and mortality outcomes across different glomerulonephropathies in a large diverse us population[J]. Mayo Clin Proc 93(2):167–178
13. Rozenberg I, Kotliroff A, Zahavi T et al (2018) Outcome of idiopathic membranous nephropathy: A retrospective study[J]. Isr Med Assoc J 20(3):186–189
14. Hughes RC (1999) Secretion of the galectin family of mammalian carbohydrate-binding proteins[J]. Biochim Biophys Acta 1473(1):172–185
15. Wang C, Zhou X, Ma L et al (2019) Galectin-3 may serve as a marker for poor prognosis in colorectal cancer: A meta-analysis[J]. Pathol Res Pract 215(10):152612
16. Salvatore S, Luca L, Alessandra M et al (2018) Galectin-3: One molecule for an alphabet of diseases, from a to z[J]. Int J Mol Sci 19(2):379-
17. Huang B, Xiao H, Zhang X et al (2006) Ultrasensitive detection of pepsinogen i and pepsinogen ii by a time-resolved fluoroimmunoassay and its preliminary clinical applications[J]. Anal Chim Acta 571(1):74–78
18. Aceti A, Pennica A, Celestino D et al (1988) A new serological technique, time-resolved fluoroimmunoassay (trfia), for the immunological diagnosis of urinary schistosomiasis[J]. Trans R Soc Trop Med Hyg 82(3):445–447
19. Bakal U, Sarac M, Tartar T et al (2019) A study of the utility of novel non-invasive urinary and serum biomarkers of blunt kidney injury in a rat model: Ngal, kim-1, and il-18[J]. Cent Eur J Immunol 44(3):219–225
20. Shahbaz SK, Pourrezagholi F, Barabadi M et al. High expression of tim-3 and kim-1 in blood and urine of renal allograft rejection patients[J]. Transpl Immunol, 2017, 43–44: 11–20
21. Chen M, Wang L, Wang Y et al (2020) Soluble tim3 detection by time-resolved fluorescence immunoassay and its application in membranous nephropathy[J]. J Clin Lab Anal 34(6):e23248
22. Drechsler C, Delgado G, Wanner C et al (2015) Galectin-3, renal function, and clinical outcomes: Results from the luric and 4d studies[J]. J Am Soc Nephrol 26(9):2213–2221
23. Cattran DC, Brenchley PE (2017) Membranous nephropathy: Integrating basic science into improved clinical management[J]. Kidney Int 91(3):566–574
24. De Vriese AS, Glassock RJ, Nath KA et al (2017) A proposal for a serology-based approach to membranous nephropathy[J]. J Am Soc Nephrol 28(2):421–430
25. Hoxha E, von Haxthausen F, Wiech T et al (2017) Membranous nephropathy-one morphologic pattern with different diseases[J]. Pflugers Arch 469(7–8):989–996
26. Liu WB, Gao C, Dai HR et al. Immunological pathogenesis of membranous nephropathy: Focus on pla2r1 and its role[J]. Frontiers in Immunology, 2019, 10
27. Francis JM, Beck LH Jr, Salant DJ (2016) Membranous nephropathy: A journey from bench to bedside[J]. Am J Kidney Dis 68(1):138–147

Figures

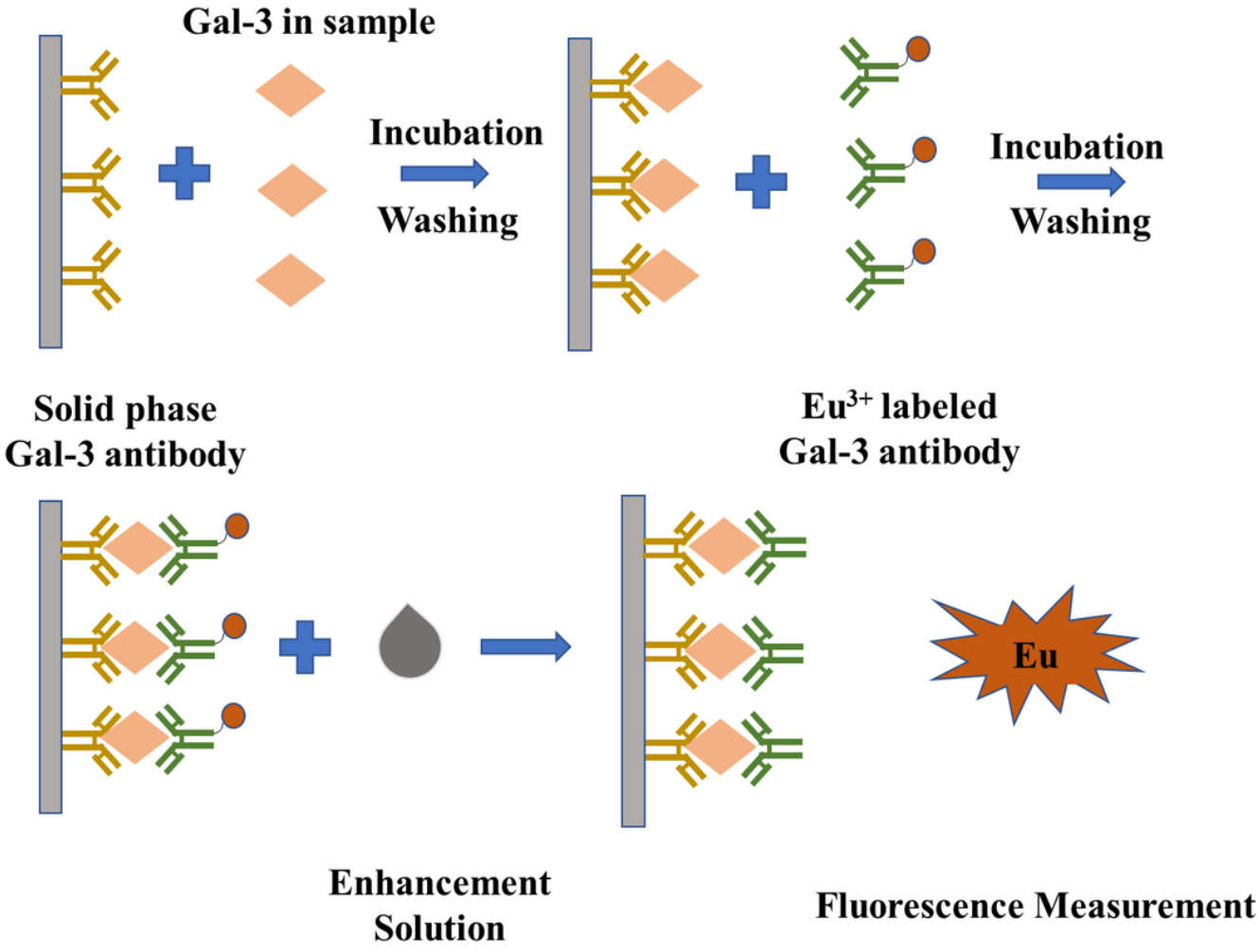

Figure 1

Schematic diagram of the Gal-3-TRFIA

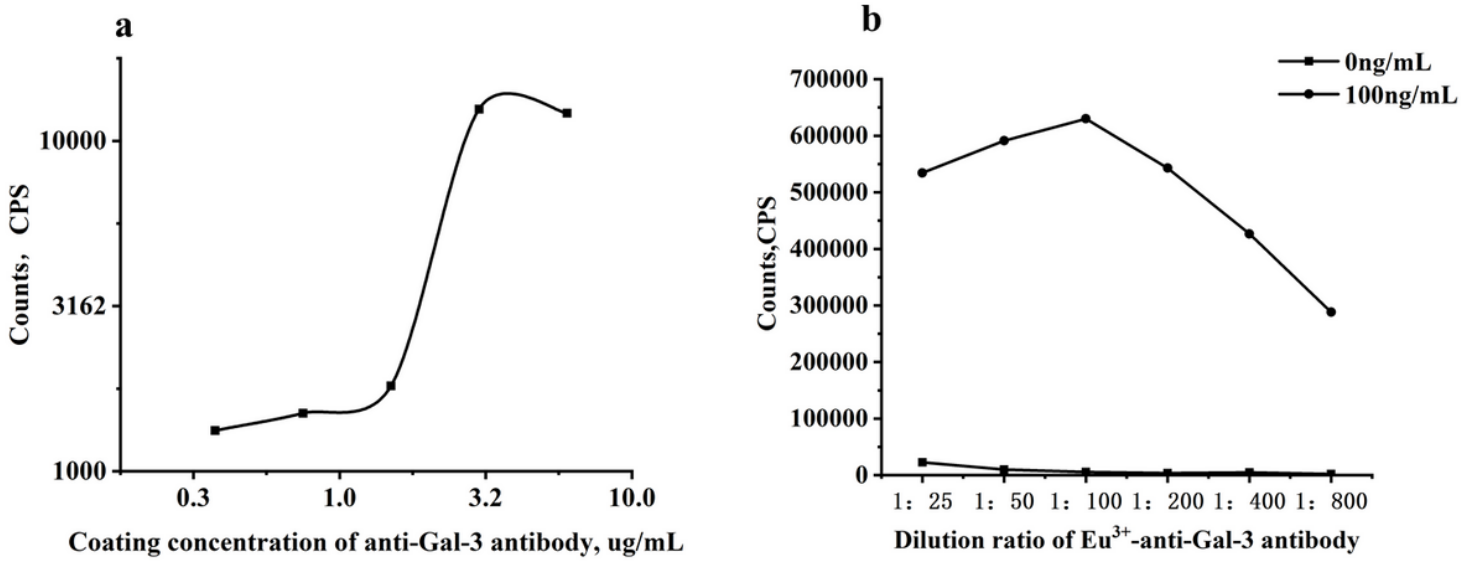

Figure 2

Optimization of the Gal-3 antibody concentration for microplate coating (a). Optimal dilution ratios of the Eu3+-anti-Gal-3 (b). CPS, counts per second.

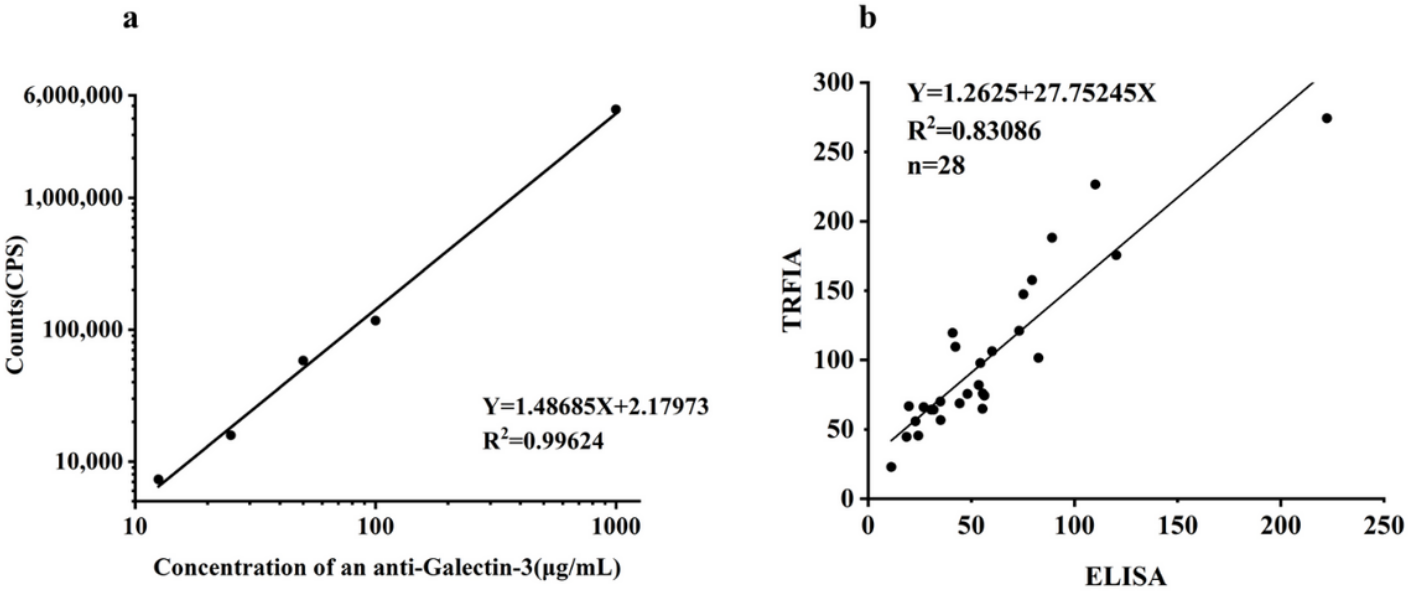

Figure 3

Standard curves for Gal-3 by double antibody sandwich TRFIA at different concentrations (a). Correlation of Gal-3 concentration results between ELISA and the established TRFIA (b).

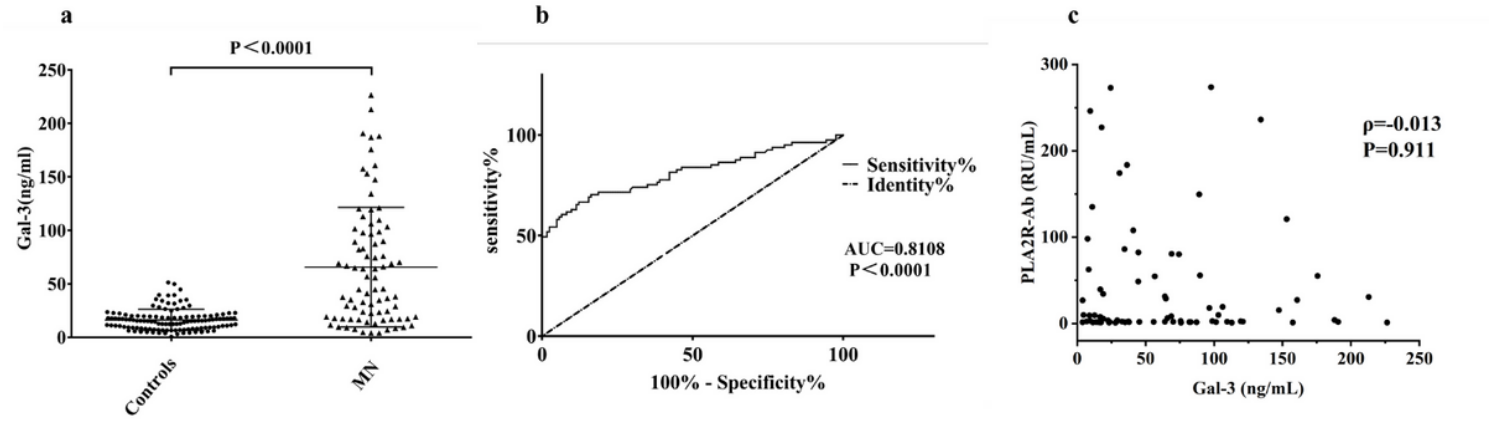

Figure 4

Gal-3 concentration in patients with MN (n = 81) and normal control (n = 123, P <0.0001); Mann-Whitney U-test was used to analyze the P value; P < 0.05 was considered significant (a). ROC curve of controls and patients with IMN (b). Correlation of the Gal-3 level with PLA2R (c).