Elevated plasma interleukin 21 is associated with higher probability and severity of idiopathic membranous nephropathy

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Abstract

**Objectives:** Interleukin 21 (IL-21) is a receptor participating in innate immunity and correlates with the activation of innate immune cells. We sought to investigate the role of plasma IL-21 in patients with idiopathic membranous nephropathy (IMN).

**Methods:** This was a cross-sectional and case-control study. We analyzed plasma IL-21 in patients with IMN, with other kidney diseases as the diseased controls (DCs), and the healthy controls (HCs), regarding their associations with the risk of having IMN and IMN severity.

**Results:** We enrolled 132, 22, and 38 patients with IMN, DC, and HC, respectively. Plasma IL-21 was significantly higher in those with IMN [9.42 (6.93, 12.60)] and DC [7.84 (2.90, 7.95)] compared with HC [5.60 (2.90, 7.10)] (p < 0.05). Plasma IL-21 was significantly higher in those with IMN stage III [10.36 (6.94, 20.88)] and II [9.75 (7.60, 14.27)] than those with IMN stage I [6.99 (3.91, 9.08)] (p < 0.05). Plasma IL-21 was significantly higher in those with a positive anti-phospholipase A2 receptor antibody (PLA2R) [9.60 (8.27, 12.93)] than those with a negative anti-PLA2R antibody [4.84 (2.90, 11.28)] (p < 0.05). Receiver operator characteristic curve analysis showed that a cutoff value of 7.665 pg/mL distinguished patients with IMN from HC and DC with a sensitivity and specificity of 68.94 and 89.47%, respectively, and the area under the curve was 0.8184. A cutoff value of 7.830 pg/mL identified those with IMN stage II with a sensitivity and specificity of 74.03 and 89.47%, respectively, with an area under the curve of 0.8718 (p < 0.001). Multivariate regression showed that plasma IL-21 was positively correlated with anti-PLA2R and 24 h urine protein, and negatively correlated with total protein and serum albumin.

**Conclusions:** Plasma IL-21 levels increased significantly in patients with IMN. IL-21 may therefore serve as a biomarker for IMN.

**Keywords:** 24 h urine protein; anti-phospholipase A2 receptor antibody; idiopathic membranous nephropathy; interleukin 21.

Introduction

Idiopathic membranous nephropathy (IMN) is an important origin of nephrotic syndrome (NS) and accounts for about 25% of NS in adults, and its prevalence among the spectrum of primary glomerular diseases is rising [1]. IMN exhibits a male predominance [2].

The pathological features of IMN include diffuse immune complex deposition underneath glomerular epithelia’s basement membrane, followed by the diffuse basement membrane thickening [3]. Target antigens have been identified from podocyte membranes, and when such antigens are bound by autoantibodies, immune complexes will form a “spike” under the epithelium processes and damage the glomerular filtration barrier, causing proteinuria [4]. IMN has a slow disease course with one-third of IMN patients having spontaneous remission, another one-third having persistent NS and the remaining one-third having renal function decline leading to end-stage kidney disease [5]. IMN...
Interleukin-21 (IL-21) is an important cytokine among the interleukin family with many functions, and it is mainly produced by follicular helper T cells and T helper 17 cells [8]. IL-21 participates in class transfer and in the production of antibody-secreting cells [9], and is widely involved in immunologic actions and the propagation of inflammatory responses. These actions are mainly mediated by JAK/STAT, MAPK and PI3K pathways [10]. Several targets of IL-21 such as B lymphocyte-induced maturation protein 1, suppressor of cytokine signaling, C-X-C chemokine receptor type 5, and B cell lymphoma 6 contribute to immunological responses [11]. IL-21 may play a role in the development of autoimmune diseases. Furthermore, IL-21 has been recognized for affecting glomerulopathy pathogenesis [12]. Our study therefore aimed to analyze IL-21 levels in IMN patients and their relationship with patients’ clinical features. We will also evaluate clinical implications of IL-21 in the progression, pathological staging, and the diagnosis of IMN, trying to provide potential diagnostic strategies.

Materials and methods

Ethical approval

This study complied with the Helsinki Declaration and was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (BF2020-094-01). All participants signed informed consents before study participation.

Study population

This was a cross-sectional and case-control study, consisting of patients consecutively enrolled with IMN or other kidney diseases, all of whom underwent routine follow-up at the Second Affiliated Hospital of Guangzhou University of Chinese Medicine. Inclusion criteria comprised of the following 2 conditions: (1) IMN was diagnosed based on renal biopsy; and (2) participants should have complete clinical and laboratory data. Exclusion criteria included the following 2 conditions: (1) those with secondary membranous nephropathy associated with hepatitis B or C virus, malignancy, lupus, or drug-related membranous nephropathy; and (2) the receipt of any immunosuppressants or cytotoxic agents within the past 6 months [13].

As disease controls for IMN, we included patients with non-immunologic chronic glomerular disease (type II diabetes nephropathy, n=6; hypertensive nephropathy, n=3; nephrotic syndrome of non-membranous nephropathy, n=6; chronic nephritis syndrome, n=2; and chronic kidney disease, n=5) [14]. We also included sex-and age-matched healthy subjects with no known autoimmune or inflammatory diseases.

Sample collection and processing

Participants’ blood was collected using vacuum collection tubes (Becton Dickinson Medical Devices Co. Ltd., NJ, USA). All participants were required to fast for at least 8–10 h before their blood was collected. The procedure of blood drawing, sample transportation and centrifugation were performed in accordance with the Clinical & Laboratory Standards Institute guideline EP28-A3c [15]. The recruitment of reference populations was strictly in accordance with the Clinical & Laboratory Standards Institute guideline EP28-A3c. Samples were stored at –80°C until analysis, and none were thawed prior to analysis.

Laboratory testing

All laboratory parameters were assayed routinely in the laboratories of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine during the participants’ most recent clinic visit.

IL-21 levels were assayed with an enzyme-linked immunosorbent assay (ELISA) assay (DY8879-05, R&D, USA) [16]. The polystyrene microwells were precoated with mouse anti-human IL-21 capture antibody and biotinylated goat anti-human IL-21 detection antibody, with the concentrations of IL-21 calculated using a four-parameter logistic regression. Serum anti-phospholipase A2 receptor (PLA2R) IgG was tested using another ELISA kit (EZI0606AF, EUROIMMUN Biotech, Beijing, China) [17]. The polystyrene microwells were precoated with PLA2R antigen and peroxidase labeled anti human IgG (rabbit origin), with the concentrations of PLA2R IgG calculated using a four-parameter logistic regression. A value equal to or greater than 20 RU/ml was regarded positive. An Mk3 enzyme label analyzer was purchased from the Shanghai Somerset Fisher Instruments Co. Ltd.

Twenty-four-hour urine protein (24hUpro), urine creatinine (Ucr), serum urea nitrogen (urea), serum creatinine (Scr), total cholesterol (TC), triglyceride(TG), total protein (TP) and albumin (ALB) were analyzed with the Cobas 8,000 analyzer (Roche Diagnostics, Shanghai, China). Serum IgG, Urine IgG (IgGU), urine microalbumin(mAlb) and urine transferrin (Trf) were measured with the BN ProSeC analyzer (Siemens, Shanghai, China). The estimated glomerular filtration rate (eGFR) was calculated.

Statistical analysis

Statistical analyses were conducted using the SPSS Software version 20.0 and the Prime Prism Software version 5.0. We described continuous variables in each group using the interquartile median (25 and 75 quartiles). We compared between two groups using the t tests for parametric variables and the Wilcoxon rank sum test for the non-parametric variables. We used the Spearman linear correlation analysis
to analyze correlations between non-parametric variables, and calculated the Pearson’s correlation coefficient (Correlations (r)) between parametric variables. Finally, we used logistic regression analyses for receiver operating characteristic (ROC) curve analysis. Two-sided p-values less than 0.05 were considered statistically significant [18].

Results

Clinical characteristics and laboratory profiles

In total, 192 subjects participated in this study, with their plasma samples collected and analyzed for IL-21 and anti-PLA2R IgG levels. Among these subjects, 132, 22, and 38 patients had IMN, diseased controls (DC) and healthy controls (HC), respectively. IMN patients had a mean age of 57 years (range 21–84), with 46 % being women. DC patients had a mean age of 56 years (range 19–75), with 55 % being women. HC patients had a mean age of 46 years (range 22–73), with 50 % being women. We found significantly higher IL-21 levels in IMN patients [9.42 (6.93, 12.60) pg/mL] compared with those in the HC group [5.60 (2.90, 7.10) pg/mL] (p<0.001) and those in the DC group [7.84 (2.90, 7.95) pg/mL] (p=0.020). The demographic and clinical features of each group are summarized in Table 1.

Correlations between IL-21 levels and other laboratory parameters

Using multiple regression analysis, we identified that IL-21 levels were positively correlated with anti-PLA2R and aGFR (correlation coefficients, 0.436 and 0.393, respectively, both p<0.05), and negatively correlated with TP and ALB (correlation coefficients, −0.279 and −0.329, respectively, both p<0.05). Correlation coefficients between plasma IL-21 levels and laboratory parameters are summarized in Table 2 and Figure 1.

Laboratory parameters between IMN patients with negative and positive anti-PLA2R antibody

We subsequently discovered that IL-21 did not differ significantly between the IMN and the DC groups [19]. We then

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-PLA2R, RU/mL</td>
<td>0.436</td>
<td>0.000</td>
</tr>
<tr>
<td>24hUpro, mg/L</td>
<td>0.393</td>
<td>0.000</td>
</tr>
<tr>
<td>UCr, μmol/L</td>
<td>0.176</td>
<td>0.022</td>
</tr>
<tr>
<td>TP, g/L</td>
<td>−0.279</td>
<td>0.000</td>
</tr>
<tr>
<td>ALB, g/L</td>
<td>−0.329</td>
<td>0.000</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>0.157</td>
<td>0.041</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>0.325</td>
<td>0.000</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.287</td>
<td>0.000</td>
</tr>
<tr>
<td>IgG, mg/L</td>
<td>0.350</td>
<td>0.000</td>
</tr>
<tr>
<td>Trf, mg/L</td>
<td>0.355</td>
<td>0.000</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73m²</td>
<td>−0.118</td>
<td>0.124</td>
</tr>
<tr>
<td>Scr, μmol/L</td>
<td>0.117</td>
<td>0.127</td>
</tr>
</tbody>
</table>

Table 2: Correlations between IL-21 and other laboratory parameters.

Values are presented as [median (P25, P75)], unless otherwise stated. IL-21 and anti-PLA2R (assayed by ELISA), eGFR, estimated glomerular filtration rate. 24hUpro, UCr, mAlb indicators were from urine, others were from plasma.
divided the IMN group into those with negative and positive anti-PLA2R IgG (Table 3).

Plasma IL-21 levels were significantly higher in those with positive anti-PLA2R [9.60 (8.27, 12.93) pg/mL] than those with negative anti-PLA2R IgG (Table 3).

![Figure 1: Correlation analysis between IL-21 and anti-PLA2R IgG, 24hUpro, TP, and ALB.](image)

### Table 3: Comparing laboratory parameters between IMN patients with negative and positive anti-PLA2R antibody.

<table>
<thead>
<tr>
<th>Variables</th>
<th>A2+ (n=106)</th>
<th>A2- (n=26)</th>
<th>p- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-21, pg/mL</td>
<td>9.60 (8.27, 12.93)</td>
<td>4.84 (2.90, 11.28)</td>
<td>0.001</td>
</tr>
<tr>
<td>24hUpro, mg/L</td>
<td>4666.40 (2324.40, 7873.00)</td>
<td>1177.50 (432.00, 3542.00)</td>
<td>0.000</td>
</tr>
<tr>
<td>TP, g/L</td>
<td>53.40 (42.00, 63.70)</td>
<td>60.45 (50.80, 69.70)</td>
<td>0.004</td>
</tr>
<tr>
<td>ALB, mg/L</td>
<td>28.50 (20.40, 36.60)</td>
<td>37.40 (31.00, 42.70)</td>
<td>0.000</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>6.73 (5.12, 8.13)</td>
<td>5.07 (4.81, 5.74)</td>
<td>0.000</td>
</tr>
<tr>
<td>mAlb, mg/L</td>
<td>2075.00 (923.00, 4150.00)</td>
<td>488.00 (62.00, 2389.00)</td>
<td>0.000</td>
</tr>
<tr>
<td>Trf, mg/L</td>
<td>139.00 (63.70, 330.00)</td>
<td>31.65 (9.00, 172.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73m²</td>
<td>82.48 (58.09, 99.89)</td>
<td>73.08 (54.32, 99.11)</td>
<td>0.714</td>
</tr>
<tr>
<td>SCr, μmol/L</td>
<td>81.80 (64.00, 118.00)</td>
<td>76.50 (67.00, 102.00)</td>
<td>0.714</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>6.07 (4.54, 8.08)</td>
<td>6.35 (4.74, 9.40)</td>
<td>0.563</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.83 (1.34, 2.62)</td>
<td>1.86 (1.01, 2.32)</td>
<td>0.248</td>
</tr>
</tbody>
</table>

Values are presented as median (P25, P75), unless otherwise stated. A2+, anti-PLA2R Positive; A2-, anti-PLA2R negative. 24hUpro, mAlb, Trf indicators were from urine, others were from plasma.

### IL-21 levels in IMN patients according to pathological stage

It is plausible that IL-21 can not only help distinguish IMN from DC, but also correlates with disease course [20]. Therefore, we examined the relationship between IL-21 and the pathological stages of IMN. We showed that plasma IL-21 levels in those with IMN stage I were significantly lower than those in patients with IMN stages II and III (p<0.05) (Figure 2).

We performed a ROC analysis to evaluate the role of plasma IL-21 in distinguishing patients with IMN from DC ones and in distinguishing between different IMN pathological stages [21]. We found that a cutoff level of 7.665 pg/mL had a sensitivity and specificity of 68.94 and 89.47 %, respectively, for distinguishing between patients with IMN and other kidney diseases, with an area under the curve of 0.8184 (95% confidence interval 0.756–0.881, p<0.001).
Figure 2: IL-21 levels in IMN patients according to their pathological stage. *p<0.05, **p<0.01, p >0.05 as ns.

Figure 3: ROC curves of using plasma IL-21 to distinguish between patients with IMN and DC.

Figure 4: ROC curves of using plasma IL-21 levels to distinguish between patients with IMN stages I, II, and III.

Discussion

In this study, we found that there was no statistical difference regarding age between the IMN patients, DCs and HCs. There were, however, significant differences regarding gender between IMN patients and HCs.

Previously, 24hUpro and serum anti-PLA2R were the mainstay for predicting disease progression among IMN patients [22]. However, these two parameters potentially lag behind the occurrence of kidney damage [23]. In this study, through examining the associations between plasma IL-21 and key clinical parameters, we aimed to elucidate whether IL-21 has a potential role in assisting the diagnosis of IMN. Indeed, we found that IL-21 levels were higher in IMN patients compared to those in the DC and the HC groups [24]. Although IMN patients’ anti-PLA2R IgG and 24hUpro levels were higher than those of the HC group, no statistical difference was observed between those of the IMN and the DC groups. These findings are consistent with those reported by Liu W et al. [25].

We then found that plasma IL-21 and 24hUpro levels in those with positive anti-PLA2R were significantly higher than those in the negative anti-PLA2R group. In addition, TP and ALB levels in those with positive anti-PLA2R were lower than those in the negative anti-PLA2R group. Prior studies reported that IL-21 exhibited a close correlation with anti-PLA2R [26], and these findings in combination further support the fact that IL-21 could serve as a new biomarker for IMN.

Anti-PLA2R antibody has been established as a diagnostic indicator for IMN [27], but we did not find significant differences regarding IL-21 levels between IMN patients and DCs. In addition, there was a study suggesting that the correlation between IL-21 and anti-PLA2R was high [28]. We therefore divided IMN patients into those with negative and positive anti-PLA2R for further analyses, and found that IMN patients with positive anti-PLA2R had more severe kidney damage than those with negative anti-PLA2R. This finding may help explain why we did not identify differences in IL-21 levels between IMN patients and DCs [29]. We presumed that IMN patients with negative anti-PLA2R had lower plasma IL-21 levels, and indeed those with positive anti-PLA2R had 2-fold higher IL-21 levels than those with negative anti-PLA2R. This phenomenon may indirectly support the correlation between IL-21 and anti-PLA2R, as reported by others [30]. Therefore, we believe that plasma IL-21 level may be an indicator of IMN disease course.

We further revealed that IL-21 could not only distinguish IMN from DC, but also predict IMN severity [31]. Plasma IL-21 levels differed significantly between those with stage I and
those with stages II/III, but they did not differ between those with stage II and stage III. It is suggested that IL-21 is more valuable for the early diagnosis of IMN [32], and the detection of IL-21 helps assess IMN severity at the very early stage of disease [33]. From this perspective, IL-21 may be used to detect early IMN progression.

Our ROC curves showed that IL-21 worked fairly during the differential diagnosis of IMN from other kidney diseases, and also assisted in identifying the pathological staging of IMN. IMN is a primary glomerulopathy whose severity depends on the extent of glomerular damage [34], and podocytes are the main targets injured immunologically by the anti-PLA2R antibody [35]. Depending on the injury extent, affected podocytes may undergo apoptosis or assume serial adaptive changes, leading to foot process effacement and the detachment of podocytic proteins [36]. This finding explains why IMN patients have higher IL-21 levels than other patients. Therefore, plasma IL-21 may serve as an early biomarker in patients with IMN. Identifying IL-21 as a potential IMN marker and for disease progression assists critically in IMN management.

During the past decade, the mechanism by which IL-21 affected our immune system has been extensively studied. More and more studies have identified a critical role of IL-21 in the pathogenesis, initiation, and progression of multiple autoimmune diseases [37]. More studies are needed to elucidate the underlying mechanisms of IMN and even other kidney diseases.

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Competing interests: The authors declared no potential conflict of interest with respect to the research, authorship and publication of this article.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This study was in compliance with the Helsinki Declaration and was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine. All participants had signed an informed consent before the participation of this study. Ethics number: 2020-094-01.

References


