Progranulin and Insulin-like Growth Factor Binding Protein-2 as Biomarkers of Disease Activity and Pathological Changes in Lupus Nephritis

Progranulin und Insulin-ähnliches Wachstumsfaktor-bindendes Protein-2 als Biomarker für Krankheitsaktivität und pathologische Veränderungen bei Lupusnephritis

Authors
Samar H. Goma1, Marwa Mahmoud Abdelaziz1, Eman H. El-Hakeim1, Mona H. El Zohri2, Sohair K. Sayed3

Affiliations
1 Department of Rheumatology, Rehabilitation, and Physical Medicine, Assiut University Hospital, Assiut, Egypt
2 Department of Internal Medicine, Rheumatology Unit, Assiut University Hospital, Assiut, Egypt
3 Department of Clinical Pathology, Assiut University Hospital, Assiut, Egypt

Key words
progranulin (PGRN), insulin-like growth factor binding protein (IGFBP-2), lupus nephritis, pathology

Schlüsselwörter
progranulin (PGRN), Insulin-ähnliches Wachstumsfaktorbindungsprotein (IGFBP-2), lupus nephritis, Pathologie

Bibliography
DOI https://doi.org/10.1055/s-0043-121629
Published online: 27.11.2017
Akt Rheumatol 2019; 44: 121–127
© Georg Thieme Verlag KG Stuttgart · New York
ISSN 0341-051X

Correspondence
Dr. Samar H. Goma
Rheumatology and Rehabilitation Department Assiut University Hospital
algalaa street
Assiut 71515
Egypt
Tel.: +20/106/1828 586, Fax: +20/882/310 888
samarhassanein2010@yahoo.com

ABSTRACT

Background Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease, characterised by the production of auto-antibodies and the formation of immune complexes due to the polyclonal activation of T and B lymphocytes, which results in tissue and organ damage. During inflammation, neutrophils and macrophages release serine proteases to cleave progranulin (PGRN) into granulin, which exerts its pro-inflammatory effects that counteract the anti-inflammatory effects of intact PGRN. It is suggested that insulin-like growth factor binding protein-2 (IGFBP-2) is a dependable biomarker of renal deterioration but it is still unclear if it has high sensitivity and specificity for discriminating SLE-caused kidney disease from other-cause kidney disease.

This study aimed to investigate the diagnostic value of PGRN and ILGFBP-2 in patients with lupus nephritis (LN) and the correlation of these biomarkers with disease activity and renal biopsy pathology.

Patients and methods Patients with SLE (n = 25) and chronic kidney disease (CKD) (n = 25), and age- and sex-matched controls (n = 25) were enrolled in the study. Serum PGRN and ILGFBP-2 levels were measured for each group.

Results Disease duration was 4.78 ± 4.26 years in the SLE patients. The mean SLE Disease Activity Index score was 15.04 ± 7.54. All renal biopsy results were class 2, 3, and 5 with a percentage of 32, 24, and 44 % respectively. PGRN and ILGFBP-2 were significantly higher in SLE patients (p < 0.001 all) than in the CKD and control groups. All patients with high levels of biomarkers showed higher values of SLE disease activity. No significant difference was noted between active and inactive LN or classes of renal biopsy with PGRN and ILGFBP-2.

Conclusion PGRN and ILGFBP-2 are significantly elevated in SLE compared to CKD and the general population and were associated with the SLE Disease Activity Index but not with active LN or classes of renal biopsy.

**Patienten und Methoden** In die Studie wurden Patienten mit SLE (n = 25) und chronischer Nierenerkrankung (CKD) (n = 25), Kontroll- und Geschlechtskontrollen (n = 25) eingeschlossen.
The second group (n = 25), who were patients with chronic kidney disease (CKD) due to causes other than LN, were enrolled as disease controls.

The third group (n = 25) was the control group including age- and sex-matched healthy volunteers.

Informed written consent was obtained from all participants before their participation in the study.

Disease activity
Global disease activity was assessed using the SLE Disease Activity Index (SLEDAI) [18]. In this study, we considered a score of 0 as no activity, 1–5 mild activity, 6–10 moderate activity, 11–19 high activity, and >20 very high activity [19].

The renal SLEDAI (SLEDAI-R) score (range 0–16) represents the sum of the renal items of the SLEDAI 2000. If present, each of the four SLEDAI-R items receives a score of 4: proteinuria > 0.5 g/day, haematuria and pyuria (both > 5 cells/high-power field), and cellular casts. In this study, we considered SLEDAI-R = 0 as inactive LN and SLEDAI-R > 0 as active LN [20].

Renal pathology
Renal biopsies were reviewed and classified by an experienced renal pathologist, using the 2004 International Society of Nephrology/Renal Pathological Society (ISN/RPS) classification [21]: class I (minimal mesangial LN), class II (mesangial proliferative LN), class III (focal LN), class IV (diffuse LN), class V (membranous LN), and class VI (advanced sclerosing LN).

Laboratory Investigation
7 ml of whole blood was collected from each subject into plain tubes without any additives. After 20 min of incubation at room temperature, the tubes were centrifuged for 10 min at 1000 G. The supernatant was carefully separated, aliquoted, and stored at −80 °C until use. In order to avoid protein degradation from multiple freeze-thaw cycles, each aliquot was retrieved and thawed only once for assays in this study.

Kidney function test, liver function test, and lipid profile were performed with an auto-analyser Cobas c 311 (Roche/Hitachi Cobas c systems, Roche Diagnostics GmbH, Germany). Complete urine analysis was performed using a 10-parameter reagent strip (Parsvalor, ORGENTEC Diagnostika). C-reactive protein was detected using the latex agglutination test kit (Biotec Laboratories Ltd, UK). Antinuclear antibodies (ANAs) were determined using the indirect immunofluorescence technique on Hep2 cells (DiaSorine, HEP2 cell line substrate). Anti-dsDNA IgG autoantibody detection was performed using Alegra® (ORGENTEC Diagnostika), C-reactive protein was detected using the latex agglutination test kit (Biotec Laboratories Ltd, UK).

Measurement of serum PGRN and IGFBP-2 levels
Serum levels of PGRN and IGFBP-2 were measured using the human PGRN ELISA kit (Cat. no.: E-EL-H1578) and human IGFBP-2 ELISA kit (Cat. no.: E-EL-H0446), respectively, from Elabscience Biotechnology Co. Ltd., according to the manufacturer’s manual. The optical density at 450 nm wavelength was measured using a microplate reader Stat Fax® 2100. PGRN and IGFBP-2 concentration were then calculated according to the standard curve. Serum PGRN levels are expressed as pg/ml and serum ILGFBP-2 levels are expressed as ng/ml.

Statistical analysis
The data were tested for normality, using the Anderson-Darling test, and for variances in homogeneity prior to further statistical analysis. Categorical variables are described as number and percentage (N, %), while continuous variables are described as mean and standard deviation (mean, SD). The chi-square and Fisher’s exact tests were used to compare categorical variables. Continuous variables were compared using the unpaired t-test and ANOVA. Pearson’s correlation was used to assess the correlation between continuous variables. The receiver operating characteristics curve was used to predict cut off values and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and...
accuracy. A 2-tailed p < 0.05 was considered statistically significant. All analyses were performed with IBM SPSS 20.0.

Results

Table 1 presents the clinical data for SLE patients, who had a disease duration of 4.78 ± 4.26 years. The percentage of patients with photosensitivity was 72%, malar rash 76%, discoid rash 24%, oral ulceration 72%, arthralgia 96%, arthritis 40%, pleuritis 20%, pericarditis 12%, seizures 12%. All the SLE patients were ANA positive, while 92% were anti-dsDNA positive. The white blood cell count was 5.68 ± 2.15 10^9/L, platelets 262.12 ± 97.95 10^9/L, hemoglobin 11.1 ± 1.9 g/dl, and erythrocyte sedimentation rate 37.52 ± 24.4 mm/h. Proteinuria was noted in 52% of patients and the 24-hour protein was 1214.52 ± 1167.9 mg/day. The mean SLE-DAI score was 15.04 ± 7.54. All renal biopsy results were class 2, 3, and 5 with a percentage of 32, 24, and 44%, respectively.

The mean PGRN level was 2558.92 ± 1170.77 pg/ml for the SLE group, 1814.6 ± 330.28 pg/ml in the CKD group, and 1052 ± 276 pg/ml for the healthy controls, while the mean IGFBP-2 level was 26.44 ± 11.55 ng/ml, 6.14 ± 2.25 ng/ml, and 3.3 ± 1.7 ng/ml in the SLE, CKD, and control groups, respectively. Serum PGRN and IGFBP-2 levels showed a significant elevation in the SLE group in comparison with the CKD and control groups (p < 0.001). PGRN was significantly higher in the CKD group than in the control group (P < 0.001). There was no significant difference between the CKD and control group regarding the serum level of IGFBP-2 (Figs 1 and 2).

Table 2  Sensitivity, specificity, and cut-off of PGRN and IGFBP-2 in the 3 groups vs. each other.

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE vs. CKD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILGFBP-2</td>
<td>1.0</td>
<td>&gt;12.5</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>PGRN</td>
<td>0.718</td>
<td>&gt;2077.5</td>
<td>56.0</td>
<td>84.0</td>
<td>77.8</td>
<td>65.6</td>
<td>71.2</td>
</tr>
<tr>
<td>SLE vs. control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILGFBP-2</td>
<td>1.0</td>
<td>&gt;6</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>PGRN</td>
<td>0.99</td>
<td>&gt;1594</td>
<td>92.0</td>
<td>100.0</td>
<td>100.0</td>
<td>92.6</td>
<td>94.2</td>
</tr>
<tr>
<td>CKD vs. control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILGFBP-2</td>
<td>0.844</td>
<td>&gt;4</td>
<td>84.0</td>
<td>68.0</td>
<td>72.4</td>
<td>81.0</td>
<td>76.3</td>
</tr>
<tr>
<td>PGRN</td>
<td>0.971</td>
<td>&gt;1336</td>
<td>100.0</td>
<td>88.0</td>
<td>89.3</td>
<td>100.0</td>
<td>94.0</td>
</tr>
</tbody>
</table>


Fig. 1  Levels of PGRN in SLE, CKD and control groups. PGRN: progranulin, SLE: systemic lupus erythematosus, CKD: chronic kidney disease.

Fig. 2  Levels of ILGFBP-2 in SLE, CKD, and control groups. ILGFBP-2: insulin like growth factor binding protein-2, SLE: systemic lupus erythematosus, CKD: chronic kidney disease.
Table 2 shows the cut off, sensitivity, and specificity of PGRN and ILGFBP-2 in the 3 studied groups. This study showed that all SLE patients had moderate, high, or very high disease activity with a positive significant difference between these levels of disease activity and the levels of PGRN and ILGFBP-2 (p < 0.002 and < 0.006, respectively) (Table 3).

Figs 3 and 4 show that there was a significant positive correlation (p < 0.001) between PGRN, ILGFBP-2, and SLEDAI. Arthritis, urea, creatinine, creatinine clearance, 24-hour protein, haemoglobin and anti-dsDNA were uncorrelated with both markers, except for the correlation of arthritis with ILGFBP-2 (p < 0.025). Moreover, in this study, we found no significant difference between active and inactive LN or classes of renal biopsy with respect to PGRN and ILGFBP-2.

**Discussion**

SLE is a potentially fatal disease with the deposition of immune complexes and inflammation leading to severe tissue damage [1]. In spite of contemporary treatment using immunosuppressive drugs for LN, results are unsatisfactory regarding disease activity with remission and drug intolerance [9, 23]. Thus, new biomarkers to identify early renal involvement in SLE patients are being sought [24–31]. An ideal biomarker should detect disease activity and renal involvement and damage as early as possible to enable prompt treatment and minimise organ damage [32].

In LN, glomerular immune complexes, at their site of deposition on the kidneys, are considered the main mediators of renal involvement. The progression of LN, leading to renal failure, is due to renal infiltration by macrophages, dendritic cells, and T cells [33].

We included a CKD group to evaluate if elevated serum levels of PGRN and IGFBP-2 were involved in the pathogenesis of LN which differs from the pathogenesis of CKD. Our results showed that both markers were elevated in CKD compared to the control group, but were more elevated in LN patients.

Our results showed a significant difference between the 3 groups with respect to PGRN and IGFBP-2, except for IGFBP-2, where no significant difference was noted between the CKD and control groups. These findings agreed with the findings of some studies on PGRN [14, 34]. Tanaka et al [13] also found higher levels of PGRN in SLE patients than in controls. For IGFBP-2, our findings were in consonance with those of Ding et al [10]. Mok et al found

---

**Table 3** Levels of PGRN and ILGFBP-2 in relation to SLEDAI score.

<table>
<thead>
<tr>
<th>SLEDAI score</th>
<th>PGRN pg/ml</th>
<th>ILGFBP-2 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>1945.6 ± 300</td>
<td>26.8 ± 11.7</td>
</tr>
<tr>
<td>High</td>
<td>2072.1 ± 545.5</td>
<td>20.1 ± 5</td>
</tr>
<tr>
<td>Very high</td>
<td>4269.2 ± 1106.8</td>
<td>37.6 ± 12.6</td>
</tr>
</tbody>
</table>


---

**Table 4**

<table>
<thead>
<tr>
<th>SLEDAI score</th>
<th>PGRN pg/ml</th>
<th>ILGFBP-2 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>1945.6 ± 300</td>
<td>26.8 ± 11.7</td>
</tr>
<tr>
<td>High</td>
<td>2072.1 ± 545.5</td>
<td>20.1 ± 5</td>
</tr>
<tr>
<td>Very high</td>
<td>4269.2 ± 1106.8</td>
<td>37.6 ± 12.6</td>
</tr>
</tbody>
</table>


---

**Fig. 3** Correlation between SLEDAI and progranulin. SLEDAI systemic lupus erythematosus disease activity index.

**Fig. 4** Correlation between SLEDAI and ILGFBP-2. ILGFBP-2 insulin like growth factor binding protein-2, SLEDAI systemic lupus erythematosus disease activity index.
that IGFBP-2 was significantly elevated in patients with active SLE than in patients with inactive SLE and controls; in this study, SLE-DAI ≥ 6 was considered active SLE [32].

Several studies have reported that the pathway of inflammatory and immune signalling are related to the pathogenesis of LN in which the TLR cascade has an important role [35]. DNA with nucleosomal DNA and immune complexes stimulate TLR-9 on plasmacytoid dendritic cells [9, 36]. Authors found that PGRN was involved in TLR-mediated stimulation in the pathogenesis of LN which is considered a part of disease activity [12–14].

There was a significant increase in PGRN and IGFBP-2 with an increase in the SLEDAI score (p < 0.002 and p < 0.006 respectively). Moreover, there was no significant difference between PGRN and IGFBP-2 and classes of renal biopsy. For PGRN, our results were consistent with those of Tanaka et al [13] and Wu et al [14]. For IGFBP-2, our results agreed with those of Ding et al [10].

During inflammation, the proteolytic product of PGRN activates the delivery of nuclear auto-antigens to TLR-9 at the endolysosomal compartments. In addition, it helps the interaction between TLR-9 and these auto-antigens, which increases the inflammatory response [12]. That is why PGRN might reflect disease activity in LN.

Our results showed that there was no significant difference between active and inactive LN regarding PGRN and IGFBP-2. Moreover, there was no correlation between arthritis, urea, creatinine, creatinine clearance, 24-hour protein, haemoglobin, and anti-dsDNA, and both markers, except for the correlation of arthritis with IGFBP-2; the reason for these resultare unclear. However, Tanaka et al [13] and Wu et al [14] found a significantly higher serum level of PGRN in active LN compared to inactive LN and non-LN controls, while Ding et al [10] noted a similar pattern with IGFBP-2. Wu et al [14] also found no correlation between PGRN and serum creatinine and 24-hour protein in urine. Ding et al [10] found a correlation between IGFBP-2 and serum creatinine levels. The bioactivity of IGFs is regulated by IGFBPs of which IGFBP-3 is the most abundant form in human plasma, followed by IGFBP-2. IGFBP-1 is the major anabolic factor in human serum and synovial fluid with respect to the proteoglycan synthesis of chondrocytes exposed to these fluids. In arthritis, however, chondrocytes seem to be unresponsive to IGFBP-1, a fact that may be related to the modulation of IGFBP-1 bioactivity by increased levels of IGFBPs [37].

A report showed that IGFBP-2 was elevated in nephrotic syndrome in paediatric patients [38], and in type 2 diabetes [39] as a predictor of the deterioration of renal functions. PGRN plays an important role in early embryogenesis [40], wound healing [41], tumorigenesis [42], and maintenance of neuronal survival [43].

There were some limitations in our study. First, the sample size was relatively small. Secondly, all our patients were from a single institution and of the same ethnic group. Several follow-up studies are needed to estimate levels of PGRN and IGFBP-2 in all SLE patients with and without LN. Moreover, studies could compare these biomarkers with the chronicity index in LN patients.

Conclusion

The present study demonstrated that the serum levels of PGRN and IGFBP-2 were elevated in LN patients and correlated with disease activity but did not correlate with active and inactive LN or with classes of renal biopsy.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**References**

[34] Qiu F, Song L, Ding F et al. Expression level of the growth factor progranulin is related with development of systemic lupus erythematosus. Diagnostic Pathology 2013; 8: 88