Serum lysosomal-associated membrane protein-2 levels are increased in small and medium-vessel vasculitis, especially in polyarteritis nodosa

N. Li, B. Zhu, Q. Zhu, M. Heizati, T. Wu, G. Wang, X. Yao, Q. Luo, S. Liu, S. Liu

ABSTRACT

Objective. Lysosomal-associated membrane protein-2 (LAMP-2) is a highly glycosylated type 1 glycoprotein expressed on the membranes of neutrophils, endothelial cells and other cells, which are closely linked to subsets of systemic vasculitis. The aim of this study was to investigate whether serum LAMP-2 can be used as a biomarker in small and medium vessel vasculitis (SMVV).

Methods. Serum samples from 39 patients with SMVV (including ANCA-associated vasculitis (AAV) and polyarteritis nodosa (PAN)) confirmed by angiography and/or biopsy and 78 healthy controls (HC) were collected. Serum LAMP-2 levels were determined by enzyme-linked immunosorbent assay.

Results. Serum LAMP-2 levels in SMVV patients were increased compared with HC (p<0.001). Serum LAMP-2 levels were significantly different between patients with active stage and those with inactive stage (p=0.024). Patients with renal involvement had higher LAMP-2 levels than patients with non-renal involvement at presentation (p=0.022). Furthermore, serum LAMP-2 levels were correlated with Birmingham Vasculitis Activity Score (BVAS), C-reactive protein (CRP), hypersensitive CRP (Hs-CRP), serum creatinine (Scr) and 24-hour proteinuria (all p<0.05). Among SMVV subsets, serum LAMP-2 levels were significantly higher in PAN compared with AAV (p=0.003). In PAN patients, serum LAMP-2 levels were correlated with BVAS and Hs-CRP (all p<0.05).

Conclusion. Serum LAMP-2 levels can reflect the disease activity and renal involvement of SMVV. Furthermore, serum LAMP-2 levels were significantly higher in PAN compared with AAV, and associated with disease activity. LAMP-2 might be a potential biomarker for SMVV, especially in PAN.

Introduction

Small and medium vessel vasculitis (SMVV), including anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and polyarteritis nodosa (PAN) (1), comprise a group of disorders involving blood vessels of small and medium sizes, characterised by inflammatory changes that cause vessel wall injury ranging from necrosis to thrombosis or aneurysm formation, and consequent tissue damage. The SMVV has a broad range of clinical manifestations and lacks the sensitive and/or specific laboratory indicators, especially in PAN. Therefore, the diagnosis and assessment of disease activity of SMVV are very difficult and easily lead to permanent organ damage. Angiography and biopsy are the primary means of diagnosis and assessment of disease activity in SMVV. Nonetheless, the angiographic means and biopsies have imperfect sensitivity due to sampling error and often contain inconclusive findings (2). Furthermore, angiographic means and biopsies are invasive tests, and repeated invasive tests are not feasible. Therefore, the better tests and novel biomarkers are needed for diagnosis and assessment of current disease activity in SMVV.

Lysosomal-associated membrane protein-2 (LAMP-2) is a highly glycosylated transmembrane protein, which contains a C-terminal cytoplasmic tail, a large highly glycosylated luminal domain and a transmembrane domain (3). LAMP-2 is critical for participation in molecular chaperone-mediated autophagy, maintenance of lysosomal membrane integrity and presentation of intracellular antigens (4-6). Recent findings showed that LAMP-2 may play an important role in the pathogenesis of systemic vasculitis (7-11). A study conducted by Kain et al. dis-
played that LAMP-2 was considered as ANCA antigens, expressed on the surface of neutrophils and endothelial cells and circulating autoantibodies to human LAMP-2 (hLAMP-2) can be detected in most patients with AAV (12). Takeuchi et al. reported that LAMP-2 expression on the cell surface of neutrophils was higher in cutaneous polyarteritis nodosa (CPAN) rats than in normal rats, supporting a positive relationship between anti-LAMP-2 antibody and CPAN (13). However, serum LAMP-2 levels have not been evaluated in patients with SMVV, especially in those with PAN. In the present study, serum LAMP-2 levels were measured in patients with SMVV, and correlations of serum LAMP-2 levels with clinical and laboratory variables in SMVV were analysed.

Methods

Patients
We consecutively selected and enrolled in the study 39 SMVV patients diagnosed at Xinjiang People’s Hospital between January 2013 and December 2016 (Fig. 1). All these patients were consulted by the rheumatology doctor and fulfilled the 1990 American College of Rheumatology (ACR) or the 2012 revised International Chapel Hill Consensus Conference (CHCC) classification criteria (14-17).

In brief, criteria for the classification of Churg-Strauss syndrome (CSS) were: 1. Asthma (history of wheezing or diffuse high-pitched rales on expiration); 2. Eosinophilia (eosinophilia>10% on white blood cell differential count); 3. Mononeuropathy or polyneuropathy (development of mononeuropathy, multiple mononeuropathies, or polyneuropathy (i.e. glove/stocking distribution) attributable to a systemic vasculitis); 4. Pulmonary infiltrates, non-fixed (migratory or transitory pulmonary non-fixed infiltrates on radiographs (not pulmonary infiltrates, including fixed infiltrates), attributable to a systemic vasculitis; 5. Paranasal sinus abnormality (history of acute or chronic paranasal sinus pain or tenderness or radiographic opacification of the paranasal sinuses); 6. Extravascular eosinophils (biopsy including artery, arteriole, or venule, showing accumulations of eosinophils in extravascular areas). For purposes of classification, a patient shall be said to have CSS if at least 4 of these 6 criteria are present.

Criteria for the classification of Wegener’s granulomatosis (WG) were: 1. Nasal or oral inflammation (development of painful or painless oral ulcers or purulent or bloody nasal discharge); 2. Abnormal chest radiograph (chest radiograph showing the presence of nodules, fixed infiltrates, or cavities); 3. Urinary sediment (microhaematuria (>5 red blood cells per high power field) or red cell casts in urine sediment); 4. Granulomatous inflammation on biopsy (histologic changes showing granulomatous inflammation within the wall of an artery or in the perivascular or extravascular area (artery or arteriole). For purposes of classification, a patient shall be said to have WG if at least 2 of these 4 criteria are present.

There is no uniform standard for the diagnosis of microscopic polyangiitis (MPA), and must be distinguished from PAN and WG before diagnosis. The following conditions contribute to the diagnosis of MPA: 1. Middle-aged and elderly, and more commonly seen in men; 2. Renal involvement (proteinuria, haematuria and/or acute renal insufficiency); 3. Pulmonary involvement (cough, haemoptysis, dyspnea, pulmonary inflammation and pulmonary renal syndrome); 4. With gastrointestinal, heart, eyes, ears, joints and other organs involved in the performance of the whole body; 5. ANCA positive; 6. Renal and lung biopsy is helpful in diagnosing MPA.

Criteria for the classification of PAN were: 1. Weight loss≥4 kg (loss of 4 kg or more of body weight since illness began, not due to dieting or other factors); 2. Livedo reticularis (Mottled reticular pattern over the skin of portions of the extremities or torso); 3. Testicular pain or tenderness (pain or tenderness of the testicles, not due to infection, trauma, or other causes); 4. Myalgias, weakness, or leg tenderness (diffuse myalgias (excluding shoulder and hip girdle) or weakness of muscles or tenderness of leg muscles); 5. Mononeuropathy or polyneuropathy (development of mononeuropathy, multiple...
mononeuropathies, or polyneuropathy); 6. Diastolic BP>90 mm Hg (development of hypertension with the diastolic BP higher than 90 mm Hg); 7. Elevated BUN or creatinine (elevation of BUN>40 mg/dl or creatinine>1.5 mg/dl, not due to dehydration or obstruction); 8. Hepatitis B virus (presence of hepatitis B surface antigen or antibody in serum); 9. Arteriographic abnormality (arteriogram showing aneurysms or occlusions of the visceral arteries, not due to atherosclerosis, fibromuscular dysplasia, or other noninflammatory causes); 10. Biopsy of the small or medium-sized artery containing PAN (Histologic changes showing the presence of granulocytes or granulocytes and mononuclear). For classification purposes, a patient shall be said to have PAN if at least 3 of these 10 criteria are present (Fig. 2).

Patients with secondary vasculitis, systemic lupus erythematosus, rheumatoid arthritis, malignancy, infection or with any other coexisting renal disease, such as anti-glomerular basement membrane nephritis, IgA nephropathy, diabetic nephropathy, or lupus nephritis were excluded.

Healthy controls (HC) were recruited from the Center for Medical examination of the People’s Hospital of Xinjiang after excluding those with evidence of any acute or active chronic infection as well as diseases that cause vascular damage, such as hypertension, diabetes and renal disease by clinical examination, and eventually 78 HC were included.

Data collection and measurements
All the information of clinical data came from the patient’s medical records during hospitalisation (including demographics, clinical, biologic, imaging, and biopsy findings).

The following clinical manifestations were recorded: general symptoms (fever, weakness, astasia, and weight loss); myalgias and arthralgias; peripheral neuropathy (mononeuropathy multiplex, or polyneuropathy); central nervous system involvement; urologic and renal involvement (orchitis, dialysis, peripheral limb oedema, and recent-onset or severe hypertension); cutaneous symptoms (nodules, purpura, erythra, and livedo); alimentary manifestations (nausea, vomiting abdominal pain, haemorrhage, pancreatitis, peritonitis); cardiovascular involvement (pectoralgia, cardiomyopathy, pericarditis, and ischaemic); ophthalmologic involvement (retinal vasculitis/exudates, visual impairment, conjunctivitis, keratitis, and uveitis); pulmonary involvement (cough, haemoptysis, dyspnea, pleural effusion, and lung infiltrates). Biologic parameters: blood cell counts; renal parameters (proteinuria, haematuria, 24-hour proteinuria, and serum creatinine (Scr); erythrocyte sedimentation rate (ESR); C-reactive protein (CRP); hypersensitive C-reactive protein (hs-CRP); and the ANCA testing by indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA).

Imaging examination: the angiographies results as abnormal when showing the blood vessels was sparse, irregular stenoses and/or microaneurysms; chest x-ray showed that nodules, infiltrating lesions and/or cavity. The results were determined by two radiologists. Biopsy findings: inflammatory cell infiltration was present in small- and medium-vessel and/or formation of the crescent; immunofluorescence demonstrated that no or little immune complex deposition in the mesangial area, vascular loops or small vascular walls. The results were determined by two pathologists.

Definitions of disease activity
Disease activity was assessed in accordance with the third version of Birmingham Vasculitis Activity Score
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Table I. Demographic and laboratory features of patients with small and medium vessel vasculitis and healthy controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SMVV (n=39)</th>
<th>HC (n=78)</th>
<th>p-value</th>
<th>AAV (n=15)</th>
<th>PAN (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic features</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (years)</td>
<td>39.36 ± 9.64</td>
<td>39.08 ± 13.93</td>
<td>0.899</td>
<td>41.33 ± 9.62</td>
<td>38.13 ± 9.65</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>16 (41.0)</td>
<td>34 (43.6)</td>
<td>0.792</td>
<td>9 (60.0)</td>
<td>6 (25.0)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>162.87 ± 25.55</td>
<td>124.32 ± 12.13</td>
<td>&lt;0.001</td>
<td>158.80 ± 23.60</td>
<td>165.42 ± 26.87</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>105.90 ± 18.19</td>
<td>77.54 ± 8.14</td>
<td>&lt;0.001</td>
<td>100.47 ± 21.59</td>
<td>109.29 ± 15.23</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>20.00 ± 13.99</td>
<td>9.63 ± 4.71</td>
<td>0.001</td>
<td>24.67 ± 15.94</td>
<td>17.08 ± 12.07</td>
</tr>
<tr>
<td>Scr (μmol/L)</td>
<td>112.13 ± 47.07</td>
<td>73.43 ± 16.17</td>
<td>&lt;0.001</td>
<td>116.39 ± 60.55</td>
<td>109.46 ± 37.55</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>8.62 ± 8.88</td>
<td>2.94 (0.91–10.31)</td>
<td>0.001</td>
<td>5.33 (3.33–11.57)</td>
<td>11 (4.8)</td>
</tr>
<tr>
<td>Proteinuria (+), n (%)</td>
<td>17 (41.0)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>5 (33.3)</td>
<td>11 (45.8)</td>
</tr>
<tr>
<td>Haematuria (+), n (%)</td>
<td>6 (15.4)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>4 (26.7)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Proteinuria (+), n (%)</td>
<td>24-hour proteinuria (g)</td>
<td>0.20 (0.07–0.55)</td>
<td>0.001</td>
<td>0.11 (0.05–0.36)</td>
<td>0.28 (0.12–0.76)</td>
</tr>
<tr>
<td>LAMP-2 (ug/L)</td>
<td>0.52 (0.28–2.88)</td>
<td>0.19 (0.10–0.28)</td>
<td>&lt;0.001</td>
<td>0.29 (0.15–0.81)</td>
<td>0.70 (0.50–3.00)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as median and interquartile range or as mean ± standard deviation.

SMVV: small and medium vessel vasculitis; SBP: systolic blood pressure; DBP: diastolic blood pressure; ESR: erythrocyte sedimentation rate; Scr: serum creatinine; WBC: white blood cell; HB: haemoglobin; PLT: platelet; ANCA: antineutrophil cytoplasmic antibody; CRP: C-reactive protein; Hs-CRP: hypersensitive C-reactive protein; LAMP-2: lysosomal-associated membrane protein-2; HC: healthy control; AAV: ANCA-associated vasculitis; PAN: polyarteritis nodosa.

Table II. Clinical features of patients with small and medium vessel vasculitis.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>SMVV (n=39)</th>
<th>AAV (n=15)</th>
<th>PAN (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache, n (%)</td>
<td>27 (69.2)</td>
<td>9 (60.0)</td>
<td>18 (75.0)</td>
</tr>
<tr>
<td>General symptoms, n (%)</td>
<td>23 (59.0)</td>
<td>9 (60.0)</td>
<td>14 (58.3)</td>
</tr>
<tr>
<td>Nervous systems, n (%)</td>
<td>3 (7.7)</td>
<td>2 (13.3)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Renal systems, n (%)</td>
<td>25 (64.1)</td>
<td>8 (53.3)</td>
<td>17 (70.8)</td>
</tr>
<tr>
<td>Cutaneous, n (%)</td>
<td>2 (2.6)</td>
<td>0 (0)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Arthritis/joint pain, n (%)</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Eye, n (%)</td>
<td>11 (28.2)</td>
<td>5 (33.3)</td>
<td>6 (25.0)</td>
</tr>
<tr>
<td>Ear nose throat, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pulmonary system, n (%)</td>
<td>8 (20.5)</td>
<td>8 (53.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cardiovascular system, n (%)</td>
<td>5 (12.8)</td>
<td>3 (20.0)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Active stage, n (%)</td>
<td>30 (76.9)</td>
<td>11 (73.3)</td>
<td>17 (70.8)</td>
</tr>
<tr>
<td>BVAS</td>
<td>11.87 ± 8.13</td>
<td>12.67 ± 10.66</td>
<td>11.42 ± 6.30</td>
</tr>
</tbody>
</table>

Continuous variables are presented as median and interquartile range or as mean ± standard deviation.

SMVV: small and medium vessel vasculitis; AAV: antineutrophil cytoplasmic antibody-associated vasculitis; PAN: polyarteritis nodosa; BVAS: Birmingham Vasculitis Activity Score.

(BVAS) (18). Patients with BVAS ≥1 were considered at an active stage, and patients with a BVAS score=0 were considered to have the inactive stage.

Definitions of renal injury

Renal injury was defined as the presence of any haematuria and/or proteinuria and/or serum creatinine increased. Haematuria was defined as more than 5 red blood cells per high-power field in urine sediment. Proteinuria was defined as more than 1+ in urine routine and/or 24-hour urine collection containing more than 150 mg of proteins. Serum creatinine increased was defined as male serum creatinine>104 μmol/L or female serum creatinine>84 μmol/L.

Measurement of LAMP-2 in human serum samples

Venous blood was collected into serum tubes and allowed to clot at room temperature before being centrifuged at 3000 r/min for 10 minutes at 4°C. Serum was removed and stored at -80°C until assayed. Serum LAMP-2 levels were determined by ELISA (Uscn Life Science Inc, Wuhan, China) following the manufacturer’s instructions by the trained staff blinded to subject conditions. Serum samples were diluted 1:500. Results of serum LAMP-2 levels were expressed in micrograms per millilitre.

Statistical analysis

Data were expressed as mean ± standard deviation (SD; for data that were normally distributed) or median and interquartile range (IQR; for data that were not normally distributed). Differences of quantitative parameters between groups were assessed using the t-test (for data that were normally distributed) or the non-parametric test (for data that were not normally distributed). Correlation between numerical data was calculated using Spearman’s or Pearson’s correlation coefficient. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of LAMP-2. Analyses were performed using SPSS software v. 20.0 (IBM Corp, Armonk, NY USA) and graphs were built using Graph Pad Prism v. 5.0 (GraphPad Software, La Jolla, CA, USA). p-values <0.05 were considered to be statistically significant.

Ethics approval and consent to participate

All the participants in the current study

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had signed the consent form before participation. The study protocol was approved by the ethics committee of the People’s Hospital of Xinjiang Uyghur Autonomous Region.

Results

Baseline features of SMVV patients and healthy controls

In this study, of the 39 patients, 23 were male and 16 were female, and of the 78 HC, 44 were male and 34 were female. The mean age of patients with SMVV and of the HC was 39.36 and 39.08 years, respectively. There was no significant difference between the two groups with respect to demographic characteristics (Table I). 15 SMVV patients were diagnosed as AAV and 24 patients were diagnosed as PAN. Among 39 SMVV patients, 30 patients with SMVV had in the active stage and 9 patients with SMVV in the inactive stage. 25 patients with SMVV had renal involvement, the other 14 patients were selected for without renal involvement. Furthermore, among 30 SMVV patients with the active stage, 25 patients had renal involvement and 5 patients without renal involvement. Clinical and laboratory features of the 39 patients and 78 HC included in the study were presented in Table I and Table II.

Measurement of serum LAMP-2 levels in patients with SMVV and HC

Serum LAMP-2 levels in patients with SMVV were significantly higher than in HC (0.52 versus 0.19 ug/ml, p<0.001) (Fig. 3A). Serum LAMP-2 levels were significantly higher in SMVV patients with active stage than in HC and SMVV patients with inactive stage (0.60 vs. 0.19 ug/ml, p<0.001 and 0.60 vs. 0.27 ug/ml, p=0.024, respectively). However, no significant differences in serum LAMP-2 levels were observed between SMVV patients with inactive stage and HC (0.27 vs. 0.19 ug/ml, p=0.120) (Fig. 3B). Patients with renal involvement and non-renal involvement had increased LAMP-2 levels compared with HC (0.62 vs. 0.19 ug/ml, p<0.001 and 0.27 vs. 0.19 ug/ml, p=0.015, respectively). Serum LAMP-2 was significantly higher in patients with SMVV and with renal involvement than in those with non-renal involvement patients (0.62 vs. 0.27 ug/ml, p=0.022) (Fig. 3C). Among subsets of SMVV, serum LAMP-2 levels were significantly higher in AAV and PAN than in HC (for AAV: 0.29 vs. 0.19 ug/ml, p=0.008 and for PAN: 0.70 vs. 0.19 ug/ml, p<0.001). More interestingly, serum LAMP-2 was significantly higher in patients with PAN than in those with AAV (0.70 vs. 0.29 ug/ml, p=0.003) (Fig. 3D). Within the group of 25 SMVV patients with renal involvement, serum LAMP-2 levels were slightly but not significantly higher in PAN patients with renal involvement than in AAV patients with renal involvement (0.76 vs. 0.49 ug/ml, p=0.145). However, among 30 SMVV patients with active stage disease, no difference could be found in serum LAMP-2 levels between the 25 patients with renal involvement and the 5 patients with non-renal involvement (0.62 vs. 0.46 ug/ml, p=0.254).

Correlations of serum LAMP-2 levels with clinical and laboratory variables

As LAMP-2 might be a marker of certain disease activity in SMVV, we evaluated whether serum LAMP-2 levels were associated with clinical and laboratory variables in SMVV patients. Analysing data of the total group of patients, we observed a significant positive correlation between LAMP-2 levels and BVAS (r=0.47, p=0.003) (Fig. 4A), CRP (r=0.46, p=0.010) and Hs-CRP (r=0.67, p<0.001) (Fig. 4B and 4C). Furthermore, we assessed whether serum LAMP-2 was related to renal involvement. In the 39 patients, a significant positive correlation was observed between serum LAMP-2 levels and Scr (r=0.33, p=0.028) (Fig. 4D), 24-hour proteinuria (r=0.43, p=0.012) (Fig. 4E). In PAN patients, serum LAMP-2 are in significant positive correlation with BVAS and Hs-CRP (r=0.54, p=0.007; r=0.66, p<0.001).

ROC curve analysis was used to identify optimal cut-off values of serum LAMP-2

The best cut-off value of serum LAMP-2 levels was investigated by calculating ROC curves. For the diagnosis of SMVV and PAN, the best cut-off point was at 0.36 and 0.40 ug/ml. The sensitivity and specificity were calculated as 71.8%, 87.5% and 87.2%, 88.5%, respectively. To distinguishing SMVV patients with active stage and the result showed that the 0.50 ug/ml was identi-
fied as the best cut-off value for serum LAMP-2 levels, resulting in a sensitivity of 66.7% and a specificity of 77.8% for SMVV. Further, ROC analysis of patients with SMVV subgroup showed that the best LAMP-2 cut-off value for differentiating PAN from SMVV is 0.52 ug/ml with 75.0% sensitivity and 73.3% specificity. All estimated values was shown in Table III.

**Discussion**

SMVV is a kind of autoimmune diseases with insidious onset, multiple organ and systemic damage. The clinical diagnosis and disease activity evaluation for SMVV is a challenge. Because of the laboratory, imaging, and other tests in common use have limited ability to help the clinician diagnose and differentiate the disease. Therefore, there is need for the search of reliable biomarkers that would help to clinical diagnosis and evaluation of disease activity for SMVV. It has been reported that antibodies against LAMP-2 occur in AAV and CPAN patients. Until now, to our knowledge, no reports are available on serum LAMP-2 levels in SMVV and on whether relation to disease activity and renal involvement exists. We report here for the first time evaluated serum LAMP-2 levels in patients with SMVV.

In the present study, serum LAMP-2 levels in SMVV patients (including AAV and PAN) were examined by ELISA. We found that serum LAMP-2 levels were significantly higher in patients with SMVV than in HC. The serum levels of LAMP-2 in patients with active stage of SMVV were significantly higher than those with inactive stage. Further analysis showed that serum LAMP-2 levels were correlated with BVAS, CRP and Hs-CRP levels. These results might indicated that LAMP-2 levels could reflect the disease activity of SMVV. ROC analysis showed that a cut-off value of 0.50 ug/ml in LAMP-2 levels would help to discriminate between active and inactive stage. Kain et al. indicated that circulating autoantibodies to hLAMP-2 can be detected in most patients with ANCA-associated vasculitis, and possibly related to the disease activity (19). Roth et al. conclude that antibodies that react with LAMP-2 may exist at very low titres in a minority of patients with AAV (20). The causes of the difference between the two results were analysed, and the differences between the study may be case selection and the detection methods (21).

Peschel et al. have identified autoantibodies to hLAMP-2 that bind native glomerular, suggesting play an important role of pathogenesis in ANCA-negative pauci-immune focal necrotising GN (22). Therefore, we investigated whether serum LAMP-2 were related to renal involvement in SMVV. Our result revealed that se-
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rum LAMP-2 levels were increased in SMVV patients with renal involvement as compared to non-renal involvement patients, and could observe a positive correlation between serum LAMP-2 levels and Scr, 24-hour proteinuria. All these data indicated that LAMP-2 was not only used as an indicator of the disease activity of SMVV, but also might be an important molecule involved in renal pathology in SMVV. Among the SMVV subsets, PAN is a primary systemic necrotising vasculitis predominantly targeting medium-sized arteries defined as the main visceral arteries and their branches (23). There have been few studies of biomarkers in PAN, and no sensitive and specific markers were found (24). In this study, serum LAMP-2 levels were significantly higher in PAN patients than in AAV patients, and the ROC analysis showed that a cut-off value of 0.52 µg/ml in LAMP-2 levels would help to differentiate PAN from SMVV. Furthermore, a positive correlation was found between serum LAMP-2 levels and BVAS, Hs-CRP. Based on these findings, we propose that the serum LAMP-2 levels might reflect the disease activity of PAN. Our study did have some limitations as well. Since our case group was from the Hypertension Centre, there is a possibility of selective bias. In addition, our results may be better if the study was validated with an independent cohort. Moreover, serum LAMP-2 levels were associated with disease subsets in SMVV. Serum LAMP-2 was significantly higher in PAN than in AAV, and potentially a useful biomarker for SMVV, especially in PAN.

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