Relationship between serum indicators, endothelial injury markers and renal damage in patients with ANCA–associated systemic vasculitis
Wei-Jia Liu*, Jun-Bo Huang

Department of Nephrology, Xiantao First People’s Hospital Affiliated to Yangtze University, Xiantao City, Hubei Province, 433000

ARTICLE INFO
Article history:
Received 21 Jan 2016
Received in revised form 29 Jan 2016
Accepted 25 Jan 2016
Available online 28 Feb 2016

Keywords:
ANCA-associated systemic vasculitis
Autoantibody
Endothelial injury
Renal damage

ABSTRACT
Objective: To study the relationship between serum indicators, endothelial injury markers and renal damage in patients with ANCA-associated systemic vasculitis. Methods: Patients with ANCA-associated systemic vasculitis were selected for study, 30 cases of patients not complicated with renal damage were screened as AAV group and 30 cases of patients complicated with renal damage were screened as renal damage group, and then serum autoantibody contents and endothelial injury marker contents were detected. Results: Serum PR3-ANCA and MPO-ANCA contents of renal damage group were not statistically different from those of AAV group while anti-LAMP-2 antibody and AECA contents were significantly higher than those of AAV group; serum CECs, vWF, ES and VCAM-1 contents of renal damage group were significantly higher than those of AAV group while TM and eNOS contents were lower than those of AAV group; the higher the CKD stage in renal damage group, the more significant the albuminuria, the higher the serum CECs, vWF, ES and VCAM-1 contents and the lower the TM and eNOS contents. Conclusion: Abnormal contents of serum autoantibody anti-LAMP-2 antibody, AECA and endothelial injury markers in patients with ANCA-associated systemic vasculitis are closely related to renal damage and can be used for disease evaluation.

1. Introduction
Antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) is a group of autoimmune diseases involving the systemic small blood vessels, specifically including Wegener granulomatosis (GA), Churg-Strauss syndrome (CSS), granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA)[1,2]. Kidney is the most common impaired organ in ANCA that can show progressive and irreversible renal damage, and the illness needs to be accurately assessed to provide the basis for the diagnosis and treatment of the disease[3,4]. In the following study, the relationship between serum indicators, endothelial injury markers and renal damage in patients with ANCA-associated systemic vasculitis was analyzed.

2. Subjects and methods
2.1. Research subjects
Patients seeing a doctor in our hospital from May 2010 to October 2014 and meeting the diagnostic criteria ofantineutrophil cytoplasmic antibodies-associated vasculitis were collected for study, 30 cases of patients not complicated with renal damage were screened as AAV group and 30 cases of patients complicated with renal damage were screened as renal damage group. AAV group included 20 male cases and 10 female cases who were (54.82±6.82) years old; renal damage group included 19 male cases and 11 female cases who were (55.42±6.34) years old. General information of the two groups were matched and without statistical differences.
2.2. Research methods

2.2.1 Serum sample collection

Blood samples were collected from all patients after diagnosis and before treatment, let stand at room temperature for 10 min and then centrifuged, and upper serum was collected for subsequent test.

2.2.2 Serum sample detection

ELISA kits were used to detect PR3-ANCA, MPO-ANCA, anti-LAMP-2 antibody, AECA, CECs, vWF, ES, VCAM-1, TM and eNOS contents, operation procedure was in strict accordance with the kit instructions, and finally absorbance at 450 nm was read from microplate reader to calculate the contents.

2.3. Statistical methods

SPSS 17.0 software was used to input and analyze data, data between two groups was by t test, data among groups was by variance analysis and differences were considered to be statistically significant at a level of $P<0.05$.

3. Results

3.1. Serum autoantibody

Serum autoantibody PR3-ANCA, MPO-ANCA, anti-LAMP-2 antibody and AECA contents of AAV group and renal damage group were detected and analyzed, and results were as follows: serum PR3-ANCA and MPO-ANCA contents of renal damage group were not statistically different from those of AAV group while anti-LAMP-2 antibody and AECA contents were significantly higher than those of AAV group.

3.2. Serum endothelial injury marker molecules

Serum CECs, vWF, ES and VCAM-1 contents of renal damage group were significantly higher than those of AAV group. Analysis of serum endothelial injury marker molecule contents within observation group with different CKD stages was as follows: the higher the CKD stage, the higher the serum CECs, vWF, ES and VCAM-1 contents; analysis of serum endothelial injury marker molecule contents within observation group with different conditions of albuminuria was as follows: serum CECs, vWF, ES and VCAM-1 contents of patients with macroalbuminuria were significantly higher than those of patients with microalbuminuria.

3.3. Serum endothelial protection molecules

Serum TM and eNOS contents of renal damage group were significantly lower than those of AAV group. Analysis of serum endothelial protection molecule contents within observation group with different CKD stages was as follows: the higher the CKD stage, the lower the serum TM and eNOS contents; analysis of serum endothelial protection molecule contents within observation group with different conditions of albuminuria was as follows: serum TM and eNOS contents of patients with macroalbuminuria were significantly lower than those of patients with microalbuminuria.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>PR3-ANCA (μg/L)</th>
<th>MPO-ANCA (μg/L)</th>
<th>Anti-LAMP-2 autoantibody (μg/L)</th>
<th>AECA (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>30</td>
<td>17.78±2.05</td>
<td>47.67±5.84</td>
<td>29.42±3.52</td>
<td>33.15±3.86</td>
</tr>
<tr>
<td>Renal damage</td>
<td>30</td>
<td>18.04±1.89</td>
<td>48.14±5.39</td>
<td>13.52±1.63</td>
<td>13.78±1.68</td>
</tr>
<tr>
<td>$T$</td>
<td></td>
<td>0.748</td>
<td>0.339</td>
<td>12.844</td>
<td>17.594</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CECs (pcs/mL)</th>
<th>vWF (%)</th>
<th>ES (ng/mL)</th>
<th>VCAM-1 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>30</td>
<td>20.34±2.94</td>
<td>134.57±15.32</td>
<td>54.85±7.52</td>
<td>1.48±0.18</td>
</tr>
<tr>
<td>Renal damage</td>
<td>30</td>
<td>38.52±4.49</td>
<td>213.54±22.54</td>
<td>84.56±9.33</td>
<td>2.92±0.33</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>CKD stage</th>
<th>Conditions of albuminuria</th>
<th>CECs (pcs/mL)</th>
<th>vWF (%)</th>
<th>ES (ng/mL)</th>
<th>VCAM-1 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD1 stage</td>
<td>Microalbuminuria</td>
<td>25.86±2.85</td>
<td>33.42±4.18</td>
<td>41.62±4.95</td>
<td>47.39±5.29</td>
</tr>
<tr>
<td>CKD2 stage</td>
<td></td>
<td>33.42±4.18</td>
<td>41.62±4.95</td>
<td>47.39±5.29</td>
<td>26.85±3.21</td>
</tr>
<tr>
<td>CKD3 stage</td>
<td></td>
<td>41.62±4.95</td>
<td>47.39±5.29</td>
<td>26.85±3.21</td>
<td>51.33±5.69</td>
</tr>
<tr>
<td>CKD4 stage</td>
<td></td>
<td>47.39±5.29</td>
<td>26.85±3.21</td>
<td>51.33±5.69</td>
<td></td>
</tr>
<tr>
<td>Statistics</td>
<td></td>
<td>$F=9.191, P&lt;0.05$</td>
<td>$T=9.558, P&lt;0.05$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vWF (%)</td>
<td></td>
<td>163.58±17.69</td>
<td>194.52±21.48</td>
<td>240.39±27.94</td>
<td>293.17±33.56</td>
</tr>
<tr>
<td>Statistics</td>
<td></td>
<td>$p=8.783, P&lt;0.05$</td>
<td>$T=7.485, P&lt;0.05$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES (ng/mL)</td>
<td></td>
<td>63.35±7.27</td>
<td>81.49±9.33</td>
<td>88.33±9.85</td>
<td>114.23±14.28</td>
</tr>
<tr>
<td>Statistics</td>
<td></td>
<td>$p=7.183, P&lt;0.05$</td>
<td>$T=6.584, P&lt;0.05$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCAM-1 (μg/mL)</td>
<td></td>
<td>1.77±0.20</td>
<td>2.48±0.31</td>
<td>3.52±0.48</td>
<td>4.29±0.53</td>
</tr>
<tr>
<td>Statistics</td>
<td></td>
<td>$F=15.382, P&lt;0.05$</td>
<td>$T=6.585, P&lt;0.05$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
activity of AA V and the involvement of kidney is essential and can
prognosis is poor. In clinical practice, accurate judgment of extent of
specific diagnostic index for early disease, treatment is difficult and
of renal damage caused by AA V is rapid, but due to the lack of
autoantibody can destroy the corresponding target cells and cause
membrane of endothelial cells and neutrophils, and anti-LAMP-2
(AECA) in AA V patients. LAMP-2 is mainly expressed in the cell
protein 2 (LAMP-2) antibody and anti-endothelial cell antibodies
abnormal autoantibodies such as lysosome-associated membrane
damage to the kidney function
vessels and blood capillary of the kidney, and will causes progressive
above pathological changes are most likely to involve the small blood
edema as well as endothelial cell denudation and damage
monocyte infiltration, early characterized by vascular endothelial
fibrinoid necrosis of small vessel wall as well as lymphocyte and
organ injury. The pathological characteristics of AA V are segmental
of small vessel disease, which is mostly complicated with multiple
organ injury. The pathological characteristics of AAV are segmental
fibrinoid necrosis of small vessel wall as well as lymphocyte and
monocyte infiltration, early characterized by vascular endothelial
dema as well as endothelial cell denudation and damage[5]. The
above pathological changes are most likely to involve the small blood
vessels and blood capillary of the kidney, and will causes progressive
damage to the kidney function[6]. In clinical practice, the progression
of renal damage caused by AAV is rapid, but due to the lack of
specific diagnostic index for early disease, treatment is difficult and
prognosis is poor. In clinical practice, accurate judgment of extent of
activity of AAV and the involvement of kidney is essential and can
provide reference and basis for the establishment of treatment[7,8].
However, the molecular mechanisms of renal damage caused by
AAV have not been fully elucidated at present, and related molecules
participating in renal damage caused by AAV are still not clear. It is
certainly not favorable for the early detection and diagnosis of AAV
with renal damage as well as the assessment of disease severity.
AAV is essentially a kind of autoimmune disease, and autoantibody
ANCA plays a crucial role in the occurrence and development of the
disease. PR3-ANCA and MPO-ANCA are common types
of ANCA that identify proteinase 3 (PR3) and myeloperoxidase
(MPO) respectively. AAV patients have abnormal autoimmune
function, and there may be a variety of autoantibodies in the body
at the same time[9,10]. Related research has confirmed that there are
abnormal autoantibodies such as lysosome-associated membrane
protein 2 (LAMP-2) antibody and anti-endothelial cell antibodies
(AECA) in AAV patients. LAMP-2 is mainly expressed in the cell
membrane of endothelial cells and neutrophils, and anti-LAMP-2
autoantibody can destroy the corresponding target cells and cause
viscera function damage[11]; AECA antibodies directly target at
endothelial cells and cause endothelial injury[12]. Detection of serum
autoantibody contents of AAV patients and AAV patients with renal
damage showed that serum PR3-ANCA and MPO-ANCA contents
of renal damage group were not statistically different from those
of AAV group while anti-LAMP-2 antibody and AECA contents
were significantly higher than those of AAV group. It indicated that
abnormal anti-LAMP-2 antibody and AECA contents were closely
related to the renal injury caused by AAV.

In recent years, clinical scholars are devoted to exploring AAV
with renal damage-related molecular mechanisms and markers.
Endothelial injury is an important pathological feature of AAV
and also the important link causing the glomerular capillary
damage[13,14]. Circulating endothelial cells (CECs) are the products
falling off from endothelial cells into bloodstream after endothelial
injury, and can directly reflect the degree of endothelial cell damage.
In addition to CECs, there are a variety of endothelial injury marker
molecules in serum, such as von Willebrand factor (vWF), E-selectin
(ES) and vascular cell adhesion molecule 1 (VCAM-1). vWF is the
product after endothelial injury, exposure of collagen and platelet
activation, and can accelerate platelet adhesion and aggregation and
increase endothelial injury. Both ES and VCAM-1 are adhesion
molecules that mainly mediate adhesion between endothelial cells
and inflammatory cells, promote inflammatory cells infiltration
within vessel wall tissue and increase endothelial cell damage[15].
Analysis of above serum endothelial injury marker contents
showed that serum CECs, vWF, ES and VCAM-1 contents of renal
damage group were significantly higher than those of AAV group
and the higher the CKD stage, the more significant the albuminuria,
and the higher the serum CECs, vWF, ES and VCAM-1 contents.
In addition to the above endothelial injury marker molecules,
there is protection mechanism of endothelial function in the body
itself. Thrombomodulin(TM) and endothelial nitric oxide synthase
(eNOS) in vascular endothelial cells are two types of molecules with
endothelial function protection effect, TM and eNOS expression

<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum endothelial protection molecule contents of AAV group and renal damage group.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TM (ng/mL)</th>
<th>eNOS (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>30</td>
<td>14.37±1.64</td>
<td>28.44±2.96</td>
</tr>
<tr>
<td>Renal damage</td>
<td>30</td>
<td>8.37±0.91</td>
<td>18.45±2.15</td>
</tr>
<tr>
<td>T</td>
<td>7.585</td>
<td>8.137</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of different CKD stages and conditions of albuminuria on serum endothelial protection molecule contents in patients with renal damage.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CKD stage</th>
<th>Conditions of albuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD1 stage</td>
<td>CKD2 stage</td>
</tr>
<tr>
<td>TM (ng/mL)</td>
<td>12.41±1.41</td>
</tr>
<tr>
<td>eNOS (ng/mL)</td>
<td>22.54±2.65</td>
</tr>
</tbody>
</table>

4. Discussion

Antineutrophil cytoplasmic antibodies-associated vasculitis (AAV)
is autoantibody ANCA-induced immune inflammation and necrosis
of small vessel disease, which is mostly complicated with multiple
organ injury. The pathological characteristics of AAV are segmental
fibrinoid necrosis of small vessel wall as well as lymphocyte and
monocyte infiltration, early characterized by vascular endothelial
edema as well as endothelial cell denudation and damage[5]. The
above pathological changes are most likely to involve the small blood
vessels and blood capillary of the kidney, and will causes progressive
damage to the kidney function[6]. In clinical practice, the progression
of renal damage caused by AAV is rapid, but due to the lack of
specific diagnostic index for early disease, treatment is difficult and
prognosis is poor. In clinical practice, accurate judgment of extent of
activity of AAV and the involvement of kidney is essential and can
provide reference and basis for the establishment of treatment[7,8].
However, the molecular mechanisms of renal damage caused by
AAV have not been fully elucidated at present, and related molecules
participating in renal damage caused by AAV are still not clear. It is
certainly not favorable for the early detection and diagnosis of AAV
with renal damage as well as the assessment of disease severity.
AAV is essentially a kind of autoimmune disease, and autoantibody
ANCA plays a crucial role in the occurrence and development of the
disease. PR3-ANCA and MPO-ANCA are common types
of ANCA that identify proteinase 3 (PR3) and myeloperoxidase
(MPO) respectively. AAV patients have abnormal autoimmune
function, and there may be a variety of autoantibodies in the body
at the same time[9,10]. Related research has confirmed that there are
abnormal autoantibodies such as lysosome-associated membrane
protein 2 (LAMP-2) antibody and anti-endothelial cell antibodies
(AECA) in AAV patients. LAMP-2 is mainly expressed in the cell
membrane of endothelial cells and neutrophils, and anti-LAMP-2
autoantibody can destroy the corresponding target cells and cause
deletion are important pathways causing endothelial injury[16]. eNOS and TM have the function of resisting thrombosis, alleviating inflammation and reducing cell apoptosis, and local immune inflammatory response can inhibit the generation of eNOS and TM, causing vasospasm and endothelial injury. Analysis of above serum endothelial injury molecule contents showed that serum TM and eNOS contents of renal damage group were significantly lower than those of AAV group and the higher the CKD stage, the more significant the albuminuria, and the lower the serum TM and eNOS contents. Analysis of above endothelial injury marker molecule and endothelial protection molecule contents showed that impaired endothelial function was associated with the occurrence and development of renal function injury in AAV patients, and detection of serum endothelial injury marker molecule and endothelial protection molecule contents could provide reference for disease evaluation.

To sum up, abnormal contents of serum autoantibody anti-LAMP-2 antibody, AECA and endothelial injury markers in patients with ANCA-associated systemic vasculitis are closely related to renal damage and can be used for disease evaluation.

References


