Is There a Role for LAMP-2 Autoantibodies in Patients with Antineutrophil Cytoplasmic Antibody–associated Vasculitis?

To the Editor:

Antineutrophil cytoplasmic antibody (ANCA)–associated vasculitides (AAV) form a group of necrotizing small-vessel vasculitides characterized by the presence of ANCA against either proteinase 3 (PR3) or myeloperoxidase (MPO). ANCA have a key role in the pathogenesis of AAV, inducing excessive activation of neutrophils, which results in injury to small vessels[1]. ANCA can target other neutrophil-derived molecules, among them lysosome-associated membrane glycoprotein 2 (LAMP-2).

LAMP-2 is a glycosylated membrane protein expressed in lysosomes and on the surface of neutrophils and glomerular cells[2]. Antibodies against LAMP-2 were originally detected in cases with active AAV and pauci-immune crescentic glomerulonephritis[3]. Subsequent experimental studies showed that passive immunization with rabbit IgG to recombinant LAMP-2 or active immunization with recombinant FimH (a bacterial adhesion protein present in gram-negative bacteria and sharing 1 epitope of LAMP-2) can induce pauciimmune crescentic glomerulonephritis in rats, thus supporting the pathogenicity of anti–LAMP-2 antibodies[4].

Patients with active AAV defined as a Birmingham Vasculitis Activity Score (BVAS) of ≥ 3 were included in our study. Serum concentration of anti–LAMP-2 antibodies was determined using a commercial ELISA kit according to the instructions of manufacturer (PD-H07441, Puda Scientific). Seropositivity for LAMP-2 antibodies was defined as a value higher than the upper reference limit of the control group[5].

Fifty-nine patients with newly diagnosed or relapsing granulomatosis with polyangiitis (GPA) or microscopic polyangiitis were enrolled. The median BVAS was 16.5 at the time of testing (Table 1). Twenty-three of 35 newly diagnosed patients were sampled 4–6 weeks after initiation of immunosuppressive therapy, whereas 12 were treatment-naive at the time of testing. All patients with relapsing AAV received maintenance therapy. In 28 patients, repeated samples were obtained during stable remission after a median of 16 months. Thirty-six healthy volunteers (9 men and 27 women, average age 55.7 ± 12.4 yrs) comprised the control group. Using data from the control group, the upper reference level of anti–LAMP-2 antibodies was defined as 48.9 ng/ml. The study was approved by the Sechenov First Moscow University Institutional Review Board (IRB approval number 06-104). All patients gave informed consent for participation in our research study.

In patients with AAV, the median concentration of anti–LAMP-2 antibodies was higher than in the control group: 42.1 ng/ml (95% CI 39.7–45.9) and 37.1 ng/ml (95% CI 35.7–39.2), respectively ($p < 0.001$). However, only 10.1% tested positive for these antibodies (Figure 1). Most of them had newly diagnosed AAV with BVAS score of 12 to 26, and were classified as GPA. Of the 6 positive patients, 5 showed only modestly increased LAMP-2 antibodies titers. There was no correlation between the concentration of anti–LAMP-2 antibodies and BVAS score ($r = 0.038$, $p = 0.772$).

All patients with anti–LAMP-2 antibodies were seropositive for PR3-ANCA. Nevertheless, median concentrations of anti–LAMP-2 antibodies were similar in patients with PR3-ANCA and MPO-ANCA [41.4 (35.5, 43.4) and 44.2 (38.3, 46.1), respectively; $p = 0.884$]. Moreover, anti–LAMP-2 antibodies were found in only 1 of 12 patients (8.3%) with a new diagnosis of AAV and no history of any immunosuppressive treatment. At remission, concentration of anti–LAMP-2 antibodies decreased below reference value in all 6 who were seropositive, whereas it increased slightly above the reference value in 1 seronegative patient. The median concentration of LAMP-2 antibodies did not change following achievement of remission (44.0 and 39.8 ng/ml; $p = 0.079$).

Our findings suggest that anti–LAMP-2 antibodies are not useful for clinical evaluation of patients with AAV and play a minor role, if any, in disease pathogenesis. On the contrary, Kain, *et al* detected anti–LAMP-2 antibodies in 80–91% of 64 patients with newly diagnosed AAV from 3 European centers and showed high concordance (80.5%) among the 3 assays (ELISA, Western blot, and an indirect immunofluorescence assay)[6]. Anti–LAMP-2 antibodies rapidly became undetectable after the initiation of immunosuppressive treatment and frequently became detectable again during clinical relapse[6]. In line with our results, Roth, *et al* reported anti–LAMP-2 reactivity in 21% of 329 ANCA-positive patients, in 29% of 104 ANCA-negative patients, and in 16% of 104 patients with a FimH-positive urinary tract infection[7]. Anti–LAMP-2 antibodies titers were usually low, and did not correlate to disease activity scores[7].

Table 1. Demographic and clinical features of 59 patients with AAV.

Characteristics	Values
Females, n (%)	37 (62.7)
Average age, yrs, mean, ± SD	52.5 ± 14.3
Diagnosis, n (%)	
GPA	41 (69.5)
MPA	18 (30.5)
Newly diagnosed AAV, n (%)	35 (59.3)
Visceral disease, n (%)	
Pulmonary	29 (49.1)
Renal	37 (62.7)
ENT	38 (64.4)
Eyes	14 (23.7)
ANCA, n (%)	
PR3-ANCA	36 (61.0)
MPO-ANCA	13 (22.0)
Unspecified	4 (6.8)
Negative	6 (10.2)
Previous immunosuppression, n (%)	47 (79.7)
Glucocorticoids*	47
Induction therapy**	23
Maintenance therapy***	24
Laboratory, median (95% CI)	
Serum creatinine, mmol/l	105.2 (95.7–200.7)
ESR, mm/h	55.0 (38.0–64.0)
CRP, mg/l	10.2 (9.8–36.7)
Median BVAS (95% CI)	16.5 (13.6–16.9)

* Median dose of prednisone was 12 mg (13–22). ** Induction therapy: rituximab (n = 4), cyclophosphamide (n = 20), methotrexate (n = 3), or mycophenolate mofetil (MMF; n = 1). *** Maintenance therapy: glucocorticoids only (n = 12), azathioprine (n = 8), methotrexate (n = 2), and MMF (n = 2). GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; ANCA: antineutrophil cytoplasmic antibody; AAV: ANCA-associated vasculitis; PR3: proteinase 3; MPO: myeloperoxidase; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BVAS: Birmingham Vasculitis Activity Score.

Methodologic issues may be responsible for the conflicting results of our and previous studies[8]. Kain and Rees suggested that the existing controversies will be resolved once robust "clinical grade" assays have been developed[2]. Thus far, no such efforts have been undertaken. The results published by Roth and colleagues and ours clearly argue against a role of LAMP-2 in AAV[7]. The fate of non-reproduced published findings is well established, with confirmation in below 50% of the scientific reports[9]. This number may be even more pronounced, because reports of positive findings are published more often and more quickly compared to negative data[10].

Our study has some limitations. The number of patients was relatively small. However, we do not expect that our findings would have been different with a larger sample size given the very low prevalence of anti–LAMP-2 reactivity.

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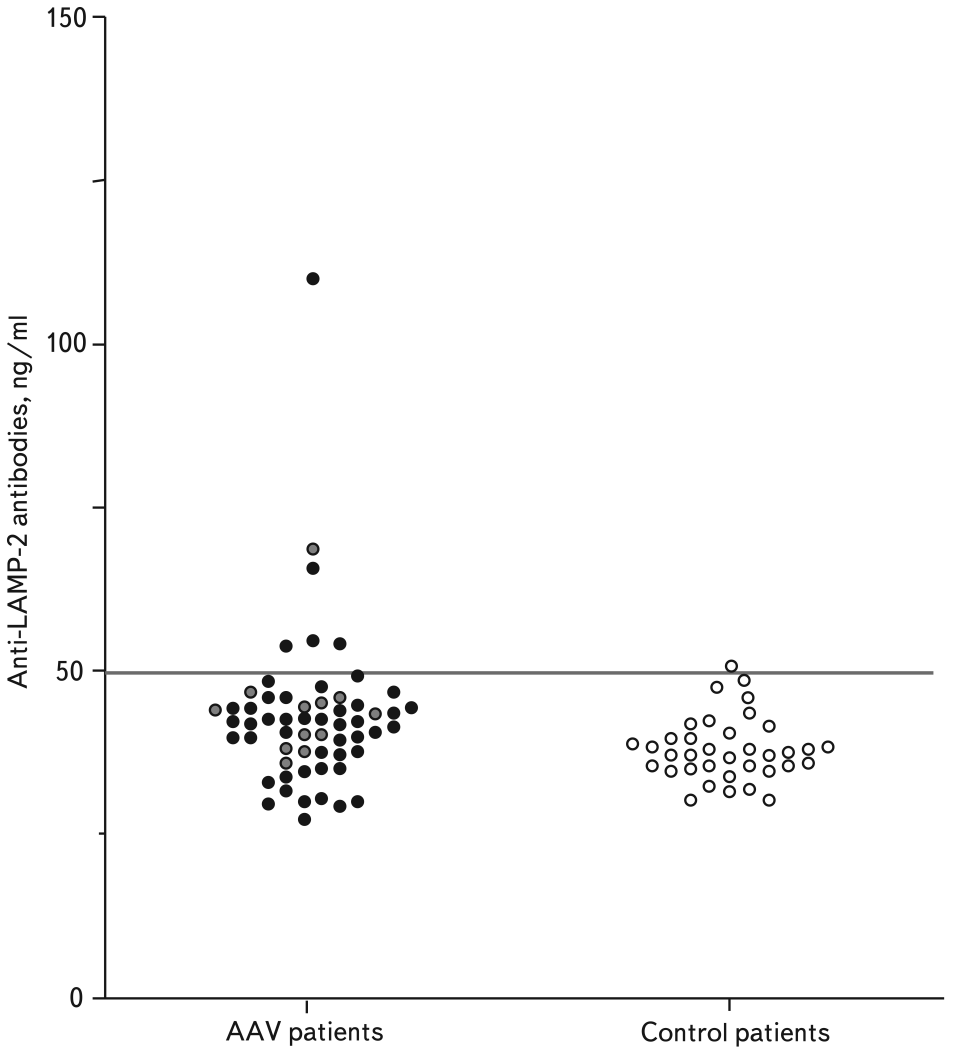

Figure 1. Concentration of anti–LAMP-2 antibodies in patients with AAV and in the control group. Treatment-naive patients marked in gray. LAMP-2: lysosome-associated membrane glycoprotein 2; AAV: antineutrophil cytoplasmic antibody–associated vasculitides.

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