Journal of Rheumatology Research
JRR, 2(1): 70-74
www.scitcentral.com

ISSN: 2641-6999

Original Research Article: Open Access

LAMP-2 a Biomarker in Vasculitis: A Case Series of Polyarteritis Nodosa

C Moran-Toro[1], A Fifi-Mah[1,2*], R Tabassum[1] and MJ Fritzler[1-3]

*[*1]Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada*

[2]Department of Medicine, Division of Rheumatology, Calgary, Alberta, Canada

[3]McCaig Institute for Bone & Joint Health, University of Calgary, Alberta, Canada.

Received June 18, 2019; Accepted July 31, 2019; Published January 06, 2020

ABSTRACT

Polyarteritis nodosa (PAN) is a systemic necrotizing vasculitis affecting medium-sized arteries with occasional involvement of small muscular arteries. Unlike small vessel vasculitides, PAN is not associated with anti-neutrophil cytoplasmic antibodies (ANCA). We report the presence of anti-LAMP-2 antibody (lysosome associated membrane protein-2) in 5 patients presenting with biopsy proven PAN. We report five cases of PAN diagnosed between January 2010 and April 2013. Laboratory tests and clinical correlations were studied retrospectively. Serum analysis included c- and p-ANCA and atypical ANCAs including anti-LAMP-2. We compared the autoantibodies titers to control groups of patients with giant cell arteritis (GCA), granulomatosis with polyangiitis (GPA), Takayasu arteritis (TAK) and other small vessel vasculitis (SVV). We observed higher titers of anti-LAMP-2 autoantibodies in the PAN patients in comparison to the other vasculidities. Our study suggests that anti-LAMP-2 autoantibodies represent a novel biomarker of PAN with high disease activity. The role of anti-LAMP-2 antibodies in the pathogenesis and associated clinical phenotype of vasculitis needs further investigation.

Keywords: Polyarteritis nodosa, Necrotizing vasculitis, Anti-neutrophil cytoplasmic antibodies, Atypical ANCA, Lysosome associated membrane protein-2

INTRODUCTION

Polyarteritis nodosa (PAN) is an idiopathic, systemic necrotizing vasculitis primarily involving medium-sized arteries that can affect adults and children alike [1,2]. Anti-neutrophil cytoplasmic antibodies (ANCA), anti-nuclear antibodies (ANA) and other biomarkers in PAN are typically absent. Other laboratory abnormalities, such as an elevated ESR, reflect an acute phase response but are non-specific. Hence, histologic confirmation of PAN as evidenced by vasculitis in medium sized arteries of skin, muscle and/or nerves is usually required to confirm the diagnosis [3]. If biopsies are negative, equivocal or cannot be obtained, angiography of the viscera may be helpful if multiple micro-aneurysms are observed [1].

In the course of our studies where various autoantibodies, particularly atypical ANCAs and other biomarkers were being re-evaluated, we observed an interesting apparent association of autoantibodies to LAMP-2 (lysosome-associated membrane protein) in a small cohort of PAN. This piqued our interest because previous studies had suggested that autoantibodies directed against LAMP-2 have been linked to a subset of primary vasculopathies [4] and ANCA-negative pauci-immune focal necrotizing glomerulonephritis [5].

This report suggests that anti-LAMP-2 autoantibodies may be a useful biomarker for PAN.

PATIENTS AND METHODS

Patient population

Patients included in our study are a subset from an adult cohort of 158 adult vasculitis patients. The cohort included patients diagnosed with GPA (granulomatosis with polyangiitis, giant cell arteritis (GCA), Takayasu arteritis (TAK), Polyarteritis nodosa (PAN) and other small vessel vasculitis (SVV) followed in the Calgary Health Region between 2010 and 2013, all of whom had a diagnosis of vasculitis by a certified rheumatologist. These diagnoses fulfilled ACR criteria for GPA, GCA, TAK and PAN [6] and the Chapel Hill Nomenclature definitions for SVV [7].

Corresponding author Aurore Fifi-Mah, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada, Tel: 403 956 2493; E-mail: avfifima@ucalgary.ca

Citation: Moran-Toro C, Fifi-Mah A, Tabassum R & Fritzler MJ. (2020) LAMP-2 a Biomarker in Vasculitis: A Case Series of Polyarteritis Nodosa. J Rheumatol Res, 2(1): 70-74.

The study was approved by the Conjoint Health Ethics Review Board and each patient provided signed informed consent. Accordingly, this study was carried out in compliance with the Helsinki Declaration of 1975 for human studies as revised in 2013. SVV included Henoch-Schonlein purpura, microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis, cryoglobulinemic vasculitis and leukocytoclastic vasculitis. Due to the small numbers we included them together as one comparator group.

Data collection

Each patient's clinical reports, consultation letters, electronic medical records were retrospectively reviewed and biologic, histopathological, radiologic findings and treatments, from the time of first symptoms and during follow-up were obtained. The diagnosis of PAN was based on clinical presentation, histopathology and CT angiogram. Using retrospective data collection, each of the organ system was assessed based on rheumatologist evaluation.

Antibody profile and luminex assay

An addressable laser bead immunoassay (ALBIA) was developed using protocols as previously described [8]. We tested various cytoplasmic target proteins including LAMP-2, early endosome antigen, Golgi reassembly and stacking protein - 1(GRASP-1) and mRNA Processing-body antigens GW182 and GE-1 **(Table 1)**. A desired volume of beads (MicroPlex® Microspheres, Luminex Corp., Austin, TX, USA) was pipetted into micro centrifuge tubes (USA Scientific Inc. Ocala, FL, USA) and centrifuged at 14,000 rpm for 1-2 min. The supernatant was carefully decanted, the desired amount of activation buffer was added and the beads were re-suspended by gentle sonication and vortexing. Diluted EDC (50 mg/ml) and Sulfo-NHS (50 mg/ml) were added and the beads, sonicated and vortexed again followed by a 20 min incubation in the dark at room temperature. While the beads were incubating, human recombinant LAMP-2 protein samples (R&D Systems, Minneapolis, MN, USA) were diluted in coupling buffer (PBS, pH 7.4). After incubation, beads were washed by centrifugation at 14,000 RPM for 1-2 min followed by addition of the optimal amount of protein to the beads. The coupling reaction was conducted by overnight incubation at 4°C on a rotator followed by washes with PBS-TBN (PBS, 0.1% BSA, 0.02% Tween-20, 0.05% azide, pH 7.4) and then storage at 4°C in PBS-TBN buffer in the dark until use. For 96 well plates, 2 µl of coupled beads, 35 µl of HRP sample diluent (Inova Diagnostics Inc.) and 5 µl of diluted serum (1:100) were added to each microtiter well. The plates were covered and incubated with agitation at 600 RPM for 30 min at room temperature. This was followed by addition of 40 µl of phycoerythrin-conjugated secondary antibody (goat anti-human IgG, diluted at 1:50) and additional incubation for another 30 min at room temperature in the dark. Antibody reactivity to LAMP-2 was analyzed by using a Luminex-100 plate reader (Luminex Corp.) and assay results expressed as median fluorescence units (MFU). MFU cut-offs were established to be 3 standard deviations above age-matched normal and irrelevant disease controls.

Table 1. Atypical ANCA in vasculitis.

Biomarker †	PAN (n=5)	GCA (n=11)	GPA (n=23)	TAK (n=11)	SVV (n=14)
GW182	1 (20)*	1 (9)	0	1 (9)	1 (7)
Elastase	0	0	0	0	0
GE-1	0	0	0	0	0
EEA-1	0	0	1 (4)	0	0
LAMP 2	3 (60)	0	0	2 (18)	0
GRASP 1	0	0	0	0	0

Values in brackets represent percentages

Abbreviations: *EEA-1: Early Endosome Antigen 1; GCA: Giant Cell Arteritis; GE-1: Enhancer of mRNA Decapping Protein 4; GPA: Granulomatosis with Polyangiitis; GRASP1: Golgi Reassembly and Stacking Protein 1; GW182: Trinucleotide Repeat-Containing Gene 6A Protein Isoform 3; LAMP-2: Lysosomal Associated Protein 2: PAN: Polyarteritis Nodosa; SVV: Small Vessel Vasculitis; TAK: Takayasu Arteritis*

Various cytoplasmic target proteins including mRNA P-body antigens GW182 and GE-1; Elastase; EEA-1; LAMP2 and GRASP-1 produce atypical ANCA patterns

STATISTICAL ANALYSIS

Statistical analysis was used to compare titers of the antibodies and compared across all entities of vasculitis. We used non-parametric analysis (Kruskal Wallis) to compare medians and IQR (Inter Quartile Range) between groups.

RESULTS

This study included a total of 64 patients with vasculitis (PAN, GCA, GPA, TAK and SVV) who had serologic profiles for atypical ANCAs **(Table 2)**. Of these patients 58% were female; the median age was 57 years. Among the

cohort, 5 patients were identified as PAN with all patients having negative c- and p-ANCA serology (PR3 and MPO). All of the PAN patients were female and the median age was 45 years. Among the PAN patients 3/5 (60%) had high titer anti-LAMP-2 antibodies with MFU ranging from 46 to 4691. The median anti-LAMP-2 MFU in the PAN group was 1217 (198-1729) which was higher than the other vasculitides (129-317), p=0.0078. By comparison, the frequency of anti-LAMP-2 antibodies in 14 GCA sera was 0.0%, in 23 GPA was 0.0% and in 11 TAK was 18%. Of the 3 PAN patients with high titer anti-LAMP-2 antibodies, the titers correlated with disease activity based on the evaluation by a rheumatologist. Out of these 3 patients, 2 patients' LAMP-2 antibody titers became negative after initiation of immunosuppressive therapy including high dose steroids and disease modifying drugs. The third patient with the highest LAMP-2 antibody titers had severe multi-system involvement and they remained elevated as the disease progressed despite high dose steroids and eventually succumbed to the disease. The two patients with inactive disease or a milder form of the disease involving skin, mononeuritis multiplex or musculoskeletal manifestations had negative anti-LAMP-2 antibody values. All our patients were found to be negative for concomitant infections and none had a history of illicit drug use. Of the 5 PAN patients, retrospective review showed that Hepatitis B status was available for 4/5 patients. Of these 4 patients, 1 tested positive for Hepatitis B and 3 tested negative for hepatitis B serology.

Table 2. Patient demographic and clinical characteristics.

Characteristics	PAN (n=5)	GCA (n=11)	GPA (n=23)	TAK (n=11)	SVV (n=14)
Age (median)	45	78	56	47	48
Sex (M/F) (% F)	0/5 (100)	3/8 (73)	15/8 (35)	2/9 (82)	7/7 (50)
Clinical Features					
Skin Manifestations	4 (80)*	0 (0)	7 (30)	0 (0)	9 (64)
ENT Symptoms	0 (0)	0 (0)	14 (60)	0 (0)	1 (7)
Respiratory Symptoms	1 (20)	0 (0)	6 (26)	0 (0)	3 (21)
Gastrointestinal Symptoms	2 (40)	0 (0)	1 (4)	0 (0)	4 (29)
Neurological Symptoms	1 (20)	8 (73)	2 (9)	4 (36)	2 (14)
Musculoskeletal Symptoms	3 (60)	2 (18)	8 (35)	2 (18)	6(43)
Renal Involvement	1 (20)	0 (0)	7 (30)	0 (0)	1 (7)

Values in brackets represent percentages
Abbreviations: *GCA: Giant Cell Arteritis; GPA: Granulomatosis Polyangiitis; ENT: Ear, Nose and Throat; PAN: Polyartertis Nodosa; SVV: Other Small Vessel Vasculitides; TAK: Takayasu Arteritis*

DISCUSSION

The diagnosis of vasculitides is often complex and delayed due to overlap of clinical patterns and vague or protean symptoms; requiring invasive and/or costly investigations. The use of molecular biomarker arrays has changed our diagnostic approach and understanding of ANCA associated vasculitis. It is in this setting that we commenced our studies of systemic vasculitis in search of biomarkers that might have hitherto been overlooked. A variety of other proteins can be targeted by autoantibodies to cellular components, and some of these antibodies directed to LAMP-2, elastase, GW 182, GE-1, EEA-1, GRASP 1 can produce atypical ANCA-staining patterns [9,10] and many of these are also reported to be associated with a variety of autoimmune diseases. Anti-LAMP-2 antibodies have been described to be associated with various diseases including glomerulonephritis and ANCA vasculitis [11-13].

One of our main interest is a group of atypical ANCA (aANCA) usually characterized by neutrophil cytoplasmic immunofluorescent staining but a negative anti-PR3 and anti-MPO antibody test. Our studies have been facilitated by using multiplex arrays on the Luminex platform that allows a rapid, high throughput assay that is referred to addressable laser bead immunoassay (ALBIA) [8].

Three distinct ANCA staining patterns have been described: cytoplasmic-ANCA (c-ANCA), perinuclear-ANCA (p-ANCA) and atypical ANCA (aANCA) [11,12]. C-ANCA is classically associated with GPA and p-ANCA with eosinophilic granulomatosis with polyangiitis (EGPA) and microscopic polyangiitis (MPA). A-ANCA is used to identify immunofluorescence staining patterns that do not conform to the typical c-ANCA or p-ANCA patterns and react with a variety of cellular targets seen in a wide variety of conditions. The specific antigen target of c-ANCA is

typically proteinase 3 (PR-3) and for p-ANCA is myeloperoxidase (MPO). Together these clinical entities have been referred to as ANCA associated vasculitis (AAV) [13].

The 2 classical antigens of PR3 and MPO are clearly not the only ones. For example, the lysosomal membrane protein LAMP-2 which has a related protein in renal glomerular endothelial cells has been demonstrated as yet another target of ANCAs [14] and a link to the pathogenesis of renal disease [4].

In the present study, we found significantly elevated levels and a high frequency of anti-LAMP-2 antibodies in a group of PAN patients that was distinct from that observed in other vasculitides. In the PAN group with active disease, 60% of the patients had positive anti-LAMP-2 antibodies. We also noted elevated titers that were associated with high disease activity. Our results are interesting in the context of a recently published study by Li et al. [15] who reported that serum LAMP-2 protein levels reflected both disease activity and renal involvement of SVV and were significantly higher in PAN compared with AAV. Our observations suggest that in PAN there may be anti-LAMP-2 antibody excess and that circulating LAMP-2 immune complexes may be an important feature of PAN. Future studies should focus on this aspect of the disease. Interestingly, other studies also reported that anti-LAMP-2 autoantibodies fluctuated in concert with disease activity in a variety of vasculitides [4,13,14,16]. The association of anti-LAMP-2 autoantibodies with disease activity and their decline after initiation of immunosuppression treatment suggests that they are also involved in the pathogenesis of disease.

The role of ANCAs as causative and/or pathogenic agents in vasculitis has been debated [16,17]. It is thought that PAN may be triggered by viral infections, particularly hepatitis B virus, but remains idiopathic in most cases [2]. There has been a decreased incidence of PAN paralleling increased protection against hepatitis B from immunization [18]. LAMP-2 protein which has some sequence similarity to bacterial proteins is shedding some light toward pathophysiology of systemic vasculitis [15,19].

CONCLUSION

Our study suggests that anti-LAMP-2 autoantibodies represent a novel biomarker of PAN with high disease activity. Studies of larger, multi-center cohorts are needed to validate this association.

ACKNOWLEDGEMENT

These studies were supported by funds from Mitogen Advanced Diagnostics Laboratory, Calgary, Alberta, Canada. The authors acknowledge the technical support of Meifeng Zhang and Haiyan Hou at Mitogen Advanced Diagnostics Laboratory, University of Calgary.

REFERENCES

1. Hernandez-Rodriguez J, Alba MA, Prieto-Gonzalez S, Cid MC (2014) Diagnosis and classification of polyarteritis nodosa. J Autoimmun 48-49: 84-89.
2. Kawakami T (2012) A review of pediatric vasculitis with a focus on juvenile polyarteritis nodosa. Am J Clin Dermatol 13: 389-398.
3. Strunk DA, Schmidt-Pogoda C, Beuker LS, Milles C, Korsukewitz SG, et al. (2019) Biomarkers in vasculitides of the nervous system. Front Neurol 10: 591.
4. Bosch X, Mirapeix E (2009) Vasculitis syndromes: LAMP-2 illuminates pathogenesis of ANCA glomerulonephritis. Nat Rev Nephrol 5: 247-249.
5. Peschel A, Basu N, Benharkou A, Brandes R, Brown M, et al. (2014) Autoantibodies to hLAMP-2 in ANCA-negative pauci-immune focal necrotizing GN. J Am Soc Nephrol 25: 455-463.
6. Hunder GG, Arend WP, Bloch DA, Calabrese LH, Fauci AS, et al. (1990) The American College of Rheumatology 1990 criteria for the classification of vasculitis introduction. Arthritis Rheum 33: 1065-1067.
7. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, et al. (2013) 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 65: 1-11.
8. Amlani A, Choi MY, Tarnopolsky M, Brady L, Clarke AE, et al. (2019) Anti-NT5c1A autoantibodies as biomarkers in inclusion body myositis. Front Immunol 10: 745.
9. Selak S, Woodman RC, Fritzler MJ (2000) Autoantibodies to early endosome antigen (EEA1) produces a staining pattern resembling cytoplasmic anti-neutrophil cytoplasmic antibodies (C-ANCA). Clin Exp Immunol 122: 493-498.
10. Stinton LM, Eystathioy T, Selak S, Chan EKL, Fritzler MJ (2004) Autoantibodies to protein transport and messenger RNA processing pathways: Endosomes, lysosomes, Golgi complex, proteasomes, assemblyosomes, exosomes and GW Bodies. Clin Immunol 110: 30-44.
11. Wiik AS (2010) Autoantibodies in ANCA-associated vasculitis. Rheum Dis Clin North Am 36: 479-489.
12. Fervenza FC, Specks U (2013) Vasculitis: Refining phenotypes in ANCA-associated vasculitis. Nat Rev Nephrol 9: 6-8.
13. Kain R, Matsui K, Exner M, Binder S, Schaffner G, et al. (1995) A novel class of auto-antigens of anti-neutrophil cytoplasmic antibodies in necrotizing and

crescentic glomerulonephritis: The lysosomal membrane glycoprotein h-LAMP-2 in neutrophil granulocytes and a related membrane protein in glomerular endothelial cells. J Exp Med 181: 585-597.

14. Takeuchi S, Kimura S, Soma Y, Waki M, Yamaguchi M, et al. (2013) Lysosomal-associated membrane protein-2 plays an important role in the pathogenesis of primary cutaneous vasculitis. Rheumatology (Oxford) 52: 1592-1598.
15. Li N, Zhu B, Zhu Q (2019) Serum lysosomal-associated membrane protein-2 levels are increased in small and medium-vessel vasculitis, especially in polyarteritis nodosa. Clin Exp Rheumatol 117: 79-85.
16. Jennette JC, Falk RJ (2008) New insight into the pathogenesis of vasculitis associated with anti-neutrophil cytoplasmic autoantibodies. Curr Opin Rheumatol 20: 55-60.
17. Jennette JC, Falk RJ (2014) Pathogenesis of anti-neutrophil cytoplasmic autoantibody-mediated disease. Nat Rev Rheumatol 10: 463-473.
18. Guillevin L, Mahr A, Callard P, Godmer P, Pagnoux C, et al. (2005) Hepatitis B virus-associated polyarteritis nodosa: Clinical characteristics, outcome and impact of treatment in 115 patients. Medicine (Baltimore) 84: 313-322.
19. Kain R, Exner M, Brandes R, Ziebermayer R, Cunningham D, et al. (2008) Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. Nat Med 14: 1088-1096.