Human *Bartonella* Infective Endocarditis is Associated with High Frequency of Antiproteinase 3 Antibodies

To the Editor:

Bartonella henselae and *B. quintana* are the 2 *Bartonella* species most commonly involved in human disease and are associated with the formation of vasoproliferative tumors[1]. Chronic infections are bacteremia (particularly affecting homeless patients), endocarditis, bacillary angiomatosis, and liver peliosis[2]. Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides are a group of diseases characterized by necrotizing vasculitis of small vessels and associated with autoantibodies against neutrophil constituents such as myeloperoxidase (MPO) and proteinase 3 (PR3)[3]. Granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MP), and Churg-Strauss syndrome (CSS) are all forms of vasculitis associated with anti-PR3. Several chronic infectious diseases (mycobacterial infections, chronic hepatitis C virus infection, and amoebic liver abscess) have been reported to be associated with a positive ANCA by immunofluorescence, but the specificity was not always defined by ELISA-testing specificity[4]. A few cases of subacute endocarditis have been reported to be associated with ANCA positivity, mainly anti-PR3.

A 40-year-old homeless man was admitted for aortic endocarditis due to *Bartonella*, associated with high anti-PR3 antibody titers (110 IU/l, normal < 20 IU). Retrospectively, we looked for ANCA positivity (indirect immunofluorescence using ethanol-fixed fresh normal neutrophils) and anti-MPO and anti-PR3 antibodies in sera from 46 consecutive cases of infective endocarditis: 22 scored as gram-positive definite endocarditis and 25 were diagnosed as having *Bartonella* endocarditis on the basis of serology[5] and/or sequencing of the 16S rDNA from heart valves[6] (Table 1). The indirect immunofluorescence detection method revealed cytoplasmic ANCA positivity in 20 of our 47 patients (46 plus our case). Five of these positive cases were in the gram-positive endocarditis group (22.7%) and 15 in the *Bartonella* endocarditis group (60%; Table 2). The ELISA detection method for anti-PR3 antibodies scored positive for 1 (4.5%) and 10 (40%) patients, respectively, in the gram-positive endocarditis group and in the *Bartonella* endocarditis group ($p = 0.0053$). One patient was positive for both anti-PR3 and anti-MPO antibodies in the *Bartonella* endocarditis group. *Staphylococcus aureus* was the pathogen implicated in the positivity of anti-PR3 antibodies in the gram-positive endocarditis group. *Bartonella* serology testing was negative in all sera of the gram-positive endocarditis group. In cases of *Bartonella* endocarditis, anti-PR3 antibodies are probably more a marker than an effector of vascular inflammation.

ANCA-associated vasculitides are characterized by necrotizing inflammation of the walls of the small arteries, venules, arterioles, and capillaries. PR3 ANCA are positive in about 80% of patients with GPA and 35% of patients with MP or CSS. The prevalence of PR3-ANCA in patients with GPA depends greatly on the vasculitis disease activity and the extent of vasculitis. In our case, anti-PR3 positivity was only associated with the active phase of the infection, and the titer fell below the threshold of detection after the endocarditis was cured. This frequent association of anti-PR3 or anti-MPO antibodies with vasculitides has led to the assumption that ANCA are directly involved in the pathogenesis of these diseases. Less is known about the pathogenesis of ANCA-associated vasculitides (AAV), and particularly that of anti-PR3-ANCA vasculitides[7]. *In vitro* experiments have demonstrated activation of neutrophils by

Table 1. Indirect immunofluorescence and specificity of ANCA testing in the 25 *Bartonella* endocarditis group.

Patients	ANCA			Serum titers (normal < 50 IU)		
	IIF	ELISA (normal < 20 IU)				
		MPO	PR3	*B. henselae*	*B. quintana*	16S RNA PCR (cardiac valve)
Our patient	Cytoplasmic +	2	107	800	100	negative
Serum 1	—	3	2	400	< 25	ND
Serum 2	—	3	3	1600	1600	ND
Serum 3	Cytoplasmic +	3	3	800	800	ND
Serum 4	Cytoplasmic +	2	2	100	200	ND
Serum 5	—	1	1	400	400	ND
Serum 6	—	1	0	100	200	ND
Serum 7	—	1	0	200	200	ND
Serum 8	—	1	0	50	200	ND
Serum 9	Cytoplasmic++	2	3	1600	1600	ND
Serum 10	—	2	3	400	400	*B. henselae*
Serum 11	—	1	0	200	200	*B. quintana*
Serum 12	Cytoplasmic+	1	37	200	200	ND
Serum 13	Cytoplasmic+	1	93	800	800	*B. quintana*
Serum 14	Cytoplasmic+	46	129	3200	800	*B. quintana*
Serum 15	Cytoplasmic++	19	186	1600	800	*B. quintana*
Serum 16	Cytoplasmic+	3	> 200	1600	3200	*B. quintana*
Serum 17	—	1	0	200	200	ND
Serum 18	Cytoplasmic+	2	2	800	800	ND
Serum 19	Cytoplasmic+	2	75	800	800	ND
Serum 20	Cytoplasmic+	1	90	400	400	*B. quintana*
Serum 21	—	6	2	200	200	ND
Serum 22	Cytoplasmic++	3	> 200	1600	1600	*B. quintana*
Serum 23	Cytoplasmic+	2	10	800	200	ND
Serum 24	Cytoplasmic+	2	29	200	800	negative

Cytoplasmic + refers to a positive fluorescence (titers about 40 IU/ml), and cytoplasmic ++ refers to a high positive fluorescence (titers about 160 IU/ml). ANCA: antineutrophil cytoplasmic antibodies; IIF: indirect immunofluorescence; MPO: myeloperoxidase; PR3: proteinase 3; ND: not determined.

Table 2. Indirect immunofluorescence and specificity of ANCA testing in the 22 gram-positive endocarditis group.

Endocarditis Pathogen	IIF	ANCA ELISA (normal < 20 IU)	
		MPO	PR3
Staphylococcus aureus	—	3	1
S. aureus	Cytoplasmic+	3	2
S. aureus	—	5	1
S. aureus	—	3	1
Staphylococcus epidermidis	—	3	1
S. aureus	—	3	1
S. aureus	Cytoplasmic+	3	2
S. aureus	—	4	3
S. aureus	—	4	3
S. aureus	Cytoplasmic+	9	28
S. aureus	—	3	2
Streptococcus sanguinis	—	3	2
Streptococcus mitis	—	2	1
S. mitis	Cytoplasmic+	2	1
Streptococcus gordonii	—	4	5
Streptococcus dysgalactiae	—	3	1
S. sanguinis	—	3	2
S. mitis	—	3	1
Enterococcus faecalis	—	3	2
E. faecalis	—	3	2
E. faecalis	—	2	3
E. faecalis	Cytoplasmic+	3	5

Cytoplasmic + refers to a positive fluorescence (titers about 40 IU/ml), and cytoplasmic ++ refers to a high positive fluorescence (titers about 160 IU/ml). . ANCA: antineutrophil cytoplasmic antibodies; IIF: indirect immunofluorescence; MPO: myeloperoxidase; PR3: proteinase 3.

PR3-ANCA, with oxidative bursts and the release of lytic enzymes including elastase and PR3. The role of microbes has also been suggested. Chronic nasal carriage of *S. aureus* is associated with a higher relapse rate, and prophylactic treatment with cotrimoxazole can prevent relapse in cases of GPA[8].

Endocarditis associated with ANCA remains very rare: 18 reported cases, mostly due to gram-positive endocarditis and with an anti-PR3 specificity. *Bartonella* endocarditis shares 2 characteristics with PR3-AAV: they are chronic diseases involving endothelial cells. The extent of endothelium involvement is larger in cases of *Bartonella* (diffuse endothelial cells of blood vessels) than in subacute bacterial endocarditis (endocardium of the cardiac valves). The *Bartonella* species implicated in human endocarditis have a specific tropism for endothelial cells and enhance vascular neoproliferation by induction of the proliferation of endothelial cells, inhibition of their apoptosis, or production of vascular endothelial growth factor by infected macrophages[9].

Bartonella infection of endothelium results in the release of chemoattractants, mostly interleukin 8, resulting in recruitment of circulating polymorphonuclear leukocytes (PMN) and monocytes/macrophages to the site of infection[9,10]. Further, the apoptotic behavior of isolated human neutrophils is delayed by the presence of *B. quintana* lipopolysaccharide[10]. Therefore, it is possible that during delayed PMN apoptosis induced by *Bartonella*, PR3 remains in contact with the host immune system longer and stimulates increased production of anti-PR3 antibodies.

PR3-AAV and *Bartonella* endocarditis exhibit high prevalences of anti-PR3 antibodies positivity.

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J Rheumatol 2014;41:2; doi:10.3899/jrheum.130150