Pathology (June 2016) **48(4)**, pp. 363–366

IMMUNOPATHOLOGY

Diagnostic performance of a commercial immunoblot assay for myositis antibody testing

CHRIS BUNDELL[1,2], ARADA ROJANA-UDOMSART[3,4], FRANK MASTAGLIA[5], PETER HOLLINGSWORTH[1,2] AND ANDREW MCLEAN-TOOKE[1]

[1]*Department of Clinical Immunology, PathWest Laboratory Medicine WA, Queen Elizabeth II Medical Centre,* [2]*School of Pathology and Laboratory Medicine, and* [3]*Western Australian Neuroscience Research Institute, University of Western Australia, Perth, WA, Australia;* [4]*Department of Medicine, Yala Hospital, Yala, Thailand; and* [5]*Institute for Immunology and Infectious Diseases, Murdoch University, Perth, WA, Australia*

Summary

The objective of this study was to establish a population based reference range for a commercial immunoblot assay detecting myositis specific autoantibodies (MSAs) and myositis associated autoantibodies (MAAs), and to assess the diagnostic performance of this reference range against the manufacturer's recommended ranges in a myositis patient cohort.

A total of 124 patients from a myositis cohort and 197 healthy controls were serologically assessed using a commercial immunoblot containing eleven autoantigens (Jo-1, EJ, OJ, PL7, PL12, Mi-2, SRP, Ku, PMScl75, PMScl100 and Ro52) according to the manufacturer's instructions.

Use of the manufacturer's reference ranges resulted in detection of MSAs in 19.4% of myositis patients and 9.1% of controls; MAAs were detected in 41.1% of myositis patients and 14.2% of controls. Reference values derived from the healthy control population resulted in significant differences in cut-off values for some autoantibodies, particularly Ro52 and PMScl75. Use of local reference ranges reduced detection of MSAs to 16.9% of myositis patients and 3% of healthy controls, with MAAs 23.4% of patients and 2% of healthy controls.

Application of population based reference ranges resulted in significant differences in detection of MSAs and MAAs compared to the manufacturer's recommended ranges. Cut-off levels should be assessed to ensure suitability for the population tested.

Key words: Autoantibodies; polymyositis; dermatomyositis; myositis; diagnosis; immunoblot.

Received 25 June, revised 26 October 2015, accepted 24 January 2016
Available online 22 April 2016

INTRODUCTION

Idiopathic inflammatory myopathies (IIM) are the largest group of acquired myopathies in both children and adults. The most common IIMs are dermatomyositis, polymyositis, sporadic inclusion body myositis and the more recently recognised necrotising autoimmune myopathy. They are primarily characterised by inflammatory cell infiltration (with the exception of necrotising autoimmune myopathy in which the inflammatory infiltrate is not prominent), muscle fibre necrosis and regeneration which leads to varying degrees of muscle weakness. In inclusion body myositis the autoimmune features co-exist with degenerative features, similar to those seen in Alzheimer's disease.[1,2] Inflammatory myopathies may occur alone or in association with systemic connective tissue diseases, which can be categorised as overlap myositis (OM).

A number of autoantibodies directed against nuclear and cytoplasmic elements are found in patients with inflammatory myopathies. These antibodies have been classified into two groups, based upon their diagnostic specificity: myositis specific antibodies (MSAs) which are found specifically in patients with IIMs, and myositis associated antibodies (MAAs) which may be found in myositis or overlap syndromes but are not specific for myositis.[3,4] These antibodies correlate with the clinical manifestations of disease and therefore are important in assisting in the diagnosis, prediction of prognosis and complications, as well as choice of therapy.[5,6] The number of MSA antibodies which can be assessed in the diagnostic laboratory has increased over recent years to include antibodies directed against 5 amino acyl tRNA synthetases (EJ, OJ, PL12, PL7, Jo-1), signal recognition particle (SRP), Mi-2 and two epitopes of the PMScl molecule (PMScl75 and 100). Furthermore, the presence of an MSA is reported to be almost mutually exclusive.[7,8]

The aim of this study was to determine the reference range for a commercial immunoblot assay for detection of MSA and MAA using a local healthy control population and assess the performance of these ranges against a group of clinically well characterised myositis patients.

MATERIAL AND METHODS

Serum samples were collected from 124 biopsy proven cases of IIM[9,10] which consisted of the following groups: 27 dermatomyositis patients, 11 polymyositis patients, 51 inclusion body myositis patients, 10 necrotising autoimmune myopathy patients and 25 patients with overlap myositis. All myositis patients were recruited from the Myositis Clinic at the Western Australian Neuroscience Research Institute, Sir Charles Gairdner Hospital, Perth. Serum samples from 197 healthy controls, collected as part of the Busselton Community Health Study,[11] were made available by The Busselton

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: http://dx.doi.org/10.1016/j.pathol.2016.03.012

Population Medical Research Foundation for testing. The mean age was 60 years for the myositis patients (range 15–87) and 50 years for the healthy controls (range 18–88). The commercial immunoblot Euroline Myositis Profile 3 (Euroimmun, Germany) consisting of a membrane strip with autoantigens Jo-1, EJ, OJ, PL7, PL12, Mi-2, SRP, Ku, PMScl75, PMScl100 and Ro52, was performed according to the manufacturer's instructions. Band intensity was reported relative to a grey scale intensity measured on a Canon LiDE 90 Scanner (Canon, Japan) using Line Scan scanning software (Euroimmun). Ethical approval for this study was granted by the Sir Charles Gairdner Hospital Human Research Ethics Committee.

RESULTS

Myositis immunoblot results

The results according to band intensity for each MSA (Jo-1, EJ, OJ, PL7, PL12, Mi2 and SRP) and MAA (Ku, PMScl75/100, Ro52) in the healthy controls are shown in Fig. 1. Using these data, band intensity cut-offs at the 95th and 99th percentiles were calculated (Table 1). The performance of the assay using the 99th percentile from the healthy controls was then compared against the recommended manufacturer's cut-off of band intensity >10 (Tables 2 and 3).

Using the manufacturer's recommended cut-offs of a band intensity of 10, MSAs were found in 24 (19.4%) of the patients with myositis and 18 (9.1%) of the healthy controls. Using the 99th percentile cut-off reduced the number of MSAs detected in the myositis patients from 24 to 21 (sensitivity 19.4 to 16.9%, respectively) and in healthy controls from 18 to six (specificity 90.9% to 95.9%, respectively). When the 99th percentile cut-off was applied in the myositis group, two weak anti SRP results (band intensity 12 and 21) in the necrotising autoimmune myopathy and OM groups, two anti Jo-1 results (band intensity 18 and 16) in the inclusion body myositis and dermatomyositis groups, and an anti-Mi-2 result (band intensity 12) in the OM group became negative. In addition an anti-OJ result (band intensity 8) became positive in the inclusion body myositis group. In comparison, when the 99th percentile cut-off was applied to the healthy controls, six of seven anti SRP antibody results, four of five anti Jo-1 antibody results, three of three anti PL-7 and one of two anti-Mi2 and anti-EJ results became negative. One additional healthy control became anti-OJ positive. There were two myositis patients with two concurrent MSAs (1 inclusion body myositis with PL7 and Mi2; 1 dermatomyositis with EJ and Jo-1) using the manufacturer's cut-off, but only one patient using the 99th percentile cut-offs (1 inclusion body myositis with PL7 and Mi2). There were two healthy control patients with multiple MSAs (1 EJ and PL7; 1 SRP and Jo-1), one of whom became completely negative and another had a single MSA (SRP) after application of the 99th percentile cut-offs.

Again using the manufacturer's recommended cut-offs, MAAs were found in 51 (41.1%) of patients with myositis and 28 (14.2%) of the healthy controls. Application of the 99th percentile cut-off reduced the number of MAAs detected in the myositis patients from 51 to 29 (sensitivity 41.1% to 23.4%, respectively) and in healthy controls from 28 to four (specificity 85.8% to 98.0%). In the myositis group when the 99th percentile cut-off was applied 15 of 17 (88.2%) anti-PMScl75, seven of 11 (63.6%) anti-PMScl100, and 16 of 40 (40%) anti-Ro52 results became negative. In the healthy control group when the 99th percentile cut-off was applied, two of three (66.3%) anti-Ku, nine of 11 (81.8%) anti-PMScl75, three of four (75%) anti-PMScl100, and 11 of 12 (91.7%) anti-Ro52 results became negative.

Table 1 95th and 99th percentile values for band intensity on the immunoblot from the healthy control population

	Band intensity	
	95th percentile	99th percentile
MSA		
Jo-1	6	19
EJ	3	14
OJ	2	5
PL7	3	14
PL12	3	10
Mi-2	5	13
SRP	5	23
MAA		
Ku	7	30
PMScl75	11	79
PMScl100	3	25
Ro52	17	62

MAA, myositis associated antibodies; MSA, myositis specific antibodies.

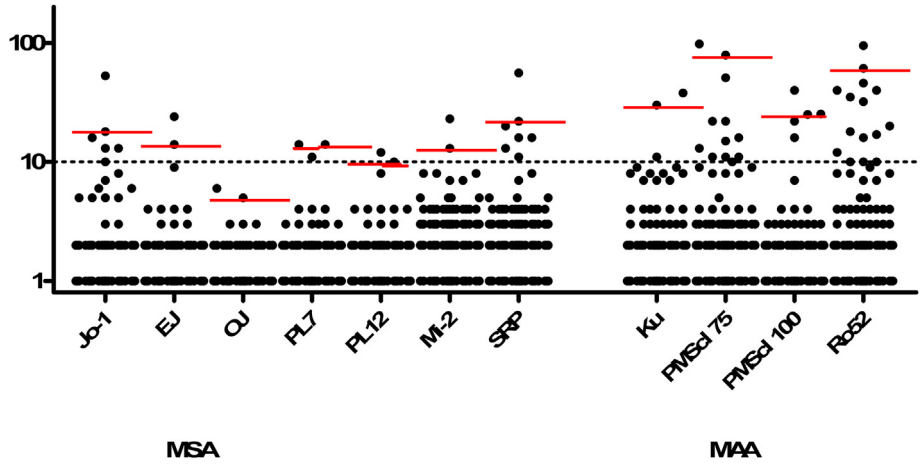

Fig. 1 Band intensity on immunoblot for individual myositis specific antibodies (MSA) and myositis associated antibodies (MAA) in healthy controls. Dotted line represents the cut-off level according to the manufacturer. Bars represent 99th percentile.