Myopathies of Unknown Etiology (Western Blot)

April 2013
1 GENERAL INFORMATION

1.1 Submitting Company, Institution or Organization
McGill University Health Centre

1.2 Application Submitted: June 29, 2012

1.3 Notice Issued: April 2, 2013

Note:
This notice is based on the scientific and commercial information (submitted by the requestor(s) and on a complementary review of the literature) according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY AND LICENCE(S)

2.1 Name of the Technology
Western blot (WB) analysis for calpain-3, dysferlin and dystrophin deficiency for a differential diagnosis of myopathy of unknown etiology.

2.2 Brief Description of the Technology
WB reveals the presence or absence of a specific protein within a complex mixture (a muscle biopsy, in this case). The proteins are first purified and separated by molecular weight using denaturing polyacrylamide gel electrophoresis (SDS-PAGE). They are then transferred and bound to a membrane by blotting. The protein of interest is detected with a specific antibody directed against it. The abundance of the protein and its subfragments, if any, can be assessed semiquantitatively (see Figure 1).

Figure 1. Western Blot Analysis of Dystrophin (Adapted from Vainzof and Zatz, 2003).

2.3 Company or Developer: Dr. John Richardson, a pathologist at MUHC.

2.4 Licence(s)

2.5 Patent, if Applicable

2.6 Approval status (Health Canada, FDA): Does not apply.

2.7 Weighted Value: 209.05.
3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patients

Individuals with myopathy of unknown etiology.

3.2 Targeted Diseases

Myopathy is a disorder of the muscle fibres with an inflammatory, endocrine, toxic, infectious or genetic origin. In fact, more than 600 hereditary defects are related to the progressive atrophy of skeletal muscles (muscular dystrophy MD) ([http://www.musclegenetable.fr](http://www.musclegenetable.fr)). Duchenne muscular dystrophy is the most common form, affecting one in 3,500 boys. It is characterized by a deficiency in dystrophin, encoded by the DMD gene located on the X chromosome (Pichavant et al., 2011; Arahata et al., 1989). Calpain-3 is a cytosolic protease whose aberrant autolytic activity is responsible for the recessive form of limb girdle muscular dystrophy or LGMD2A (Richard et al., 1995). A deficiency in dysferlin is responsible for LGMD2B and Miyoshi myopathy (Ilia et al., 2001; Liu et al., 1998). Table 1 summarizes the main protein abnormalities related to MD.

3.3 Number of Patients to Be Tested: Eighteen Tests/Year.

3.4 Medical Specialties Involved (and Other Professionals, if Applicable)

Genetics and neuropathology.

3.5 Testing Procedure: Muscle Biopsy.

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

Complementary to a terminal diagnosis of a dystrophic disorder.

4.2 Brief Description of the Current Technological Context

Immunohistochemistry (IHC) and WB are complementary techniques used to assess proteins expression involved in MD. The immunoreactivity of muscle tissue should be tested with several antibodies simultaneously and the results interpreted in the context of multiple proteins (Anderson and Davison, 1999). The gold standard remains genetic analysis, but testing several genes simultaneously is a lengthy and expensive process. Additionally, the association between a mutation and a pathology is not always direct (Barresi, 2011). A diagnostic algorithm for LGMD2A is presented as an example and demonstrates the importance of the various tests required to identify a specific disease (see Appendix).

4.3 Brief Description of the Advantages Cited for the New Technology

Pathological and histochemical analysis of a muscle biopsy provides information on the severity and stage of the disease, the presence of mitochondrial abnormalities and inflammation, and helps characterize certain congenital myopathies (Navarro et al., 2009). In most cases, IHC must be conducted with several monoclonal antibodies to make the correct diagnosis. In these situations, WB can act as a complementary test (Laval and Bushby, 2004). Changes in expression are sometimes more subtle and IHC alone can lead to uncertain diagnosis; WB is essential in these cases (Teijeira et al., 1998). WB has major
diagnostic value for most recessive diseases where showing a lack of labelling for a particular protein cannot directly pinpoint the gene in question (Barresi, 2011).

4.4 Cost of the Technology and Options
No information provided by the requestor.

5 EVIDENCE
5.1 Clinical Relevance (Utility and Validity) and Analytical Validity

5.1.1 Other Tests Replaced
Does not apply.

5.1.2 Diagnostic Value

Table 1: Main Protein Abnormalities Related to Muscular Dystrophies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene, Transmission</th>
<th>Protein Analysis Using Western Blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duchenne Muscular Dystrophy (DMD)</td>
<td>DMD, X-linked</td>
<td>Dystrophin is completely absent or markedly reduced.</td>
</tr>
<tr>
<td>Becker’s Muscular Dystrophy (BMD)</td>
<td>DMD, X-linked</td>
<td>Lower dystrophin molecular weight, lower quantity or completely absent.</td>
</tr>
<tr>
<td>DMD/BMD Carriers</td>
<td>DMD, X-linked; germinal mosaicism</td>
<td>Patchy reduction in dystrophin.</td>
</tr>
<tr>
<td>LGMD 2A</td>
<td>CAPN3, autosomal recessive chromosome 15q</td>
<td>The bands corresponding to calpain-3 can be reduced in varying degrees.</td>
</tr>
<tr>
<td>LGMD 2B/Miyoshi myopathy</td>
<td>DYSF, autosomal recessive chromosome 2p</td>
<td>Dysferlin is absent or markedly reduced.</td>
</tr>
</tbody>
</table>

Pathak et al. (2010) published a study that meant to investigate the frequency of LGMD2A in the Indian population. Histopathological and protein data (WB and IHC) were tested in 171 cases of clinically suspected LGMD, muscular dystrophy and unclassified myopathy. Western blot identified 36 and 39 patients, respectively, with a complete and partial deficiency in calpain-3.

Fanin et al. (2009) published the results of qcalpain-3 quantitation in 519 muscle biopsies from patients with unclassified myopathy or LGMD. Additionally, functional assay of calpain-3 autolytic activity was conducted in 108 cases of LGMD with normal protein quantity. Genotyping revealed 94 cases of LGMD2A with 66 different mutations of the CAPN3 gene. The authors showed that a deficiency in protein quantity and autolytic activity predicted LGMD2A in 80% and 88% of cases, respectively.

In 2006, Moore et al. published a study in which the objective was to determine the distribution of patients, referred to a national centre with a diagnosis of LGMD, across known subtypes of the disease. Muscle biopsies were performed in order to conduct histopathological and immunodiagnostic testing, and these results, together with individual clinical characteristics, directed genetic testing. Of all the patients tested, 23 were excluded.
for a diagnosis other than LGMD. Of the 289 other patients, 266 had sufficient biopsy material for complete microscopic analysis and 121 for WB with anti-calpain-3 and anti-dysferlin. The combined analyses indicated calpainopathy in 12% of cases (n = 31), dysferlinopathy in 18% of cases (n = 47), sarcoglycanopathy in 15% of cases (n = 40), dystroglycanopathy in 15% of cases (n = 40), and caveolinopathy in 1.5% of cases (n = 4). In a few cases (9%; n = 24), there was a reduction in expression of more than one protein. A specific genotype was determined for 83 patients.

In 2003, Tagawa et al. published a study in which IHC and WB were used to assess a prior clinicopathological diagnosis of unclassified LGMD (N = 53) or Miyoshi myopathy (N = 28), two forms of dysferlinopathy. A deficiency in dysferlin was found by IHC and WB in 31 patients, or 19% of putative cases of LGMD and 75% of cases of MM (see Table 2). Genetic analysis was performed for these 31 patients only. A mutation of the DYSF gene was found in 15 of the 31.

Table 2: Diagnostic Value of Protein Analysis by Western Blot (Tagawa et al., 2003)

<table>
<thead>
<tr>
<th>Clinical Diagnosis (N)</th>
<th>Protein Quantity by WB*</th>
<th>IHC Staining†</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD (53)</td>
<td>Deficiency: 10 (19%)</td>
<td>Negative: 9; Faint: 1</td>
</tr>
<tr>
<td></td>
<td>Normal: 43 (81%)</td>
<td>Normal: 16</td>
</tr>
<tr>
<td>MM (28)</td>
<td>Deficiency: 21 (75%)</td>
<td>Negative: 14; Faint: 5; Abnormal: 2</td>
</tr>
<tr>
<td></td>
<td>Normal: 7 (25%)</td>
<td>Negative: 1; Variable: 6</td>
</tr>
<tr>
<td>Controls (26)‡</td>
<td>Normal: 26 (100%)</td>
<td>Normal: 13; Abnormal: 13</td>
</tr>
</tbody>
</table>

Abbreviations: IHC = immunohistochemistry; LGMD = Limb Girdle Muscular Dystrophy; MM = Miyoshi Myopathy; N = number of patients; WB = Western blot.

*With densitometry, deficiency is defined as a dysferlin quantity that is < 30%.
† Normal: clear membrane staining, like normal muscle; Negative: absence of staining; Faint: normal faint staining ; Abnormal: accumulation of staining in the cytoplasm without membranes or normal staining in a positive/negative mosaic pattern.
‡Control patients are patients with neuromuscular disorders other than dysferlinopathy (DMD, BMD, inflammatory myopathies, mitochondrial myopathies, congenital myopathies, and neuropathies all diagnosed clinically and by IHC).

5.2 Clinical Validity

<table>
<thead>
<tr>
<th>Component</th>
<th>Presence</th>
<th>Absence</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive predictive value (PPV)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Likelihood ratio (LR)</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ROC Curve</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Saenz et al. (2005) presented a multi-centre clinical study in which the objective was to analyze clinical, molecular and biochemical data for a cohort of 314 patients, 282 of whom presented an LGMD phenotype. Of those, clinical data were deemed sufficient to suspect or rule out calpainopathy (181 and 32 patients, respectively). Uncertainty remained for 19 other patients. As a diagnostic tool, the authors calculated that the sensitivity and specificity of WB alone were 52.5% and 87.8%, respectively. When clinical phenotype and biochemical information were combined, the PPV for calpainopathy was 91%. However, when one of the tests was missing, the value varied from 78.3% to 73.7%, depending on the information available. When both tests were negative, the probability that the patient would be confirmed as LGMD2A (CAPN3 mutation) was 12.2.
Doriguzzi et al. investigated dystrophin expression in 201 muscle biopsies using IHC and WB. Combined use of both techniques detected 100% of cases of dysferlinopathies (Doriguzzi et al., 1997).

5.3 Analytical (Technical) Validity

<table>
<thead>
<tr>
<th>Component</th>
<th>Presence</th>
<th>Absence</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Reproducibility</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Analytical sensitivity</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Analytical specificity</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Matrix effect</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Concordance</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Correlation</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gallardo et al. (2011) presented a clinical study in which the objective was to verify the correlation between the dysferlin expression level in a muscle biopsy and a sample of peripheral blood monocytes using WB and IHC. The case series included 21 control patients with myopathies with no DYSF gene mutation and 17 patients with dysferlinopathy confirmed by the presence of two mutations in DYSF. Using WB, a good correlation in dysferlin expression levels between monocytes and muscle was noted in the 21 control cases (114.3 ± 17.8% versus 105 ± 16.3%, respectively) and in the 17 dysferlinopathy cases (0.1 ± 0.2% versus 1.1 ± 4.3%, respectively). As for using IHC alone on the muscle biopsies, 6 of the 17 dysferlinopathy cases and 13 of the 21 control cases showed dysferlin staining that was often abnormally localized.

5.4 Recommendations for Listing in Other Jurisdictions

European Federation of Neurological Societies (2007): Quantitative protein analysis by WB can be a useful supplementary technique to clarify primary and secondary protein abnormalities (Norwood et al., 2007).

DMD Care Considerations Working Group (2010): Key criteria for diagnosing DMD must be based on immunohistochemical and WB analyses of muscle biopsy and must be interpreted by an experienced neuromuscular pathologist (Bushby et al., 2010).

6 ANTICIPATED OUTCOMES OF INTRODUCING THIS TEST I

6.1 Impact on human and material resources: No study was found.

6.2 Economic Consequences of Adding the Test to the Quebec Health and Social Services System

No cost-effectiveness study was found.

6.3 Main Organizational, Ethical and Other (Social, Legal, and Political Issues)

A multiplex approach allows several antibodies to be tested at the same time and therefore maximizes biopsy tissue (Anderson and Davison, 1999). Use of an antibody directed against a control muscle protein (myosin, for example) ensures that there are no interpretation errors due to a sample-to-sample protein load variation (Barresi, 2011).
## Myopathies of Unknown Etiology (Western Blot)

### Status of the diagnostic technology
- Established
- Innovative
- Experimental *(for research only)*
- Replacement for technology: ______________, which is becoming obsolete

### INESSS recommendation
- Include in the Index
- Do not include in the Index
- Reassess *
  - Not enough information for a recommendation: incomplete application form, no article submitted, little evidence regarding validity.
  - No participation in external controls.

### INESSS decision regarding any required work
- Draw connection with listing of drugs, if it is a companion test
- Produce an optimal use manual
- Produce indicators, if close monitoring is required
- None

* Additional information is required for the committee to take a position and issue a recommendation. Experts have been contacted and new information could be available for March.
REFERENCES


APPENDIX

Suspected LGMD2A

<table>
<thead>
<tr>
<th>Clinical assessment</th>
<th>CK levels</th>
<th>Muscle imaging</th>
<th>Muscle histopathology</th>
<th>Muscle protein testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>(family history, age at onset, pattern of muscle impairment, scapular winging, etc)</td>
<td>(from 5-30 times normal)</td>
<td>(wasting of posterior compartment of the leg)</td>
<td>(regeneration, degeneration, lobulated fibers)</td>
<td>(calpain-3 immunoblotting)</td>
</tr>
</tbody>
</table>

Genetic analysis
(CAPN3 gene sequencing)

Genetic counseling
(heterozygote and prenatal diagnosis)

Clinical management
(physical therapy, surveillance of respiratory insufficiency and body weight)