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Measuring dystrophin—faster is not necessarily better

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A Research Highlight in the February issue (A novel imaging method to quantify low levels of dystrophin in Duchenne muscular dystrophy. *Nat. Rev. Neurol.* **8**, 120; 2012)[1] presented findings on a new method for rapid dystrophin quantification in Duchenne muscular dystrophy (DMD)[2]. Although of interest, we believe that caution is required in the interpretation of dystrophin measurements obtained using this new technique.

DMD is caused by the deficit of dystrophin protein at sarcolemma of muscle fibres[3]. Quantification of dystrophin on muscle biopsies is the main diagnostic test for DMD when genetic testing is unavailable. Several therapeutic approaches in DMD aim to restore dystrophin expression: redirection of splicing with antisense oligonucleotides;[4, 5] gene therapy[6]; stem cell therapy;[7] and nonsense mutation read-through.[8, 9] Precise quantification of dystrophin protein in muscle biopsies taken before and after treatment is crucial to evaluate the biochemical success of the therapeutic intervention.

Until recently, counting dystrophin positive fibres or western blotting were the only quantitative methods available, but researchers preparing for the first trials developed a method to sensitively quantify dystrophin and other associated proteins in the muscle fibre sarcolemma using only two muscle sections per antibody.[10] The method uses intensity measurements from fluorescently labelled dystrophin antibodies and spectrin labelling as a normalising factor. The technique greatly advanced dystrophin quantification owing to its sensitivity, requirement for very little sample, capacity to confirm the correct localization of the protein at sarcolemma, and accessibility to most pathology laboratories. Despite being labour intensive, this method has been used in the analysis of several clinical trials,[4, 5] and in human[11-13] and mouse[14, 15] studies.

Aided by a new spectrin antibody that enabled immunostaining for dystrophin and spectrin on the same section, researchers have been able to automate this technique[2], which should accelerate the analysis of muscle biopsies in ongoing clinical trials. Despite the unequivocal advance that this method entails, one should note the potential drawbacks. One important aspect is that the original method involves collection of up to 40 data points per section, each corresponding to a muscle fibre, whereas the recent modification involves collecting an average dystrophin intensity of the whole image. For averages or multiple measurements in sections from a manifesting carrier, a clear segregation of measurements is immediately evident using the original method[10], which is lost with the new method (Figure 1a and b).[2] Similarly, two patients from a recent clinical trial have almost identical levels of dystrophin when assessed using the new

method, whereas the original method shows that the average of one sample is increased due to a few high measurements (Figure 1c).

Some patients have a few intensely dystrophin-positive fibres, whereas others have more dimly dystrophin-positive fibres, which accounts for the variability in dystrophin expression in these trials[4]. To guarantee the optimal evaluation of the response to treatment, it is vital that the maximum amount of information is collected from the very small samples available. As image capture methods do not differ,[2, 10] only a slight modification of the method to include several measurements per image would suffice.

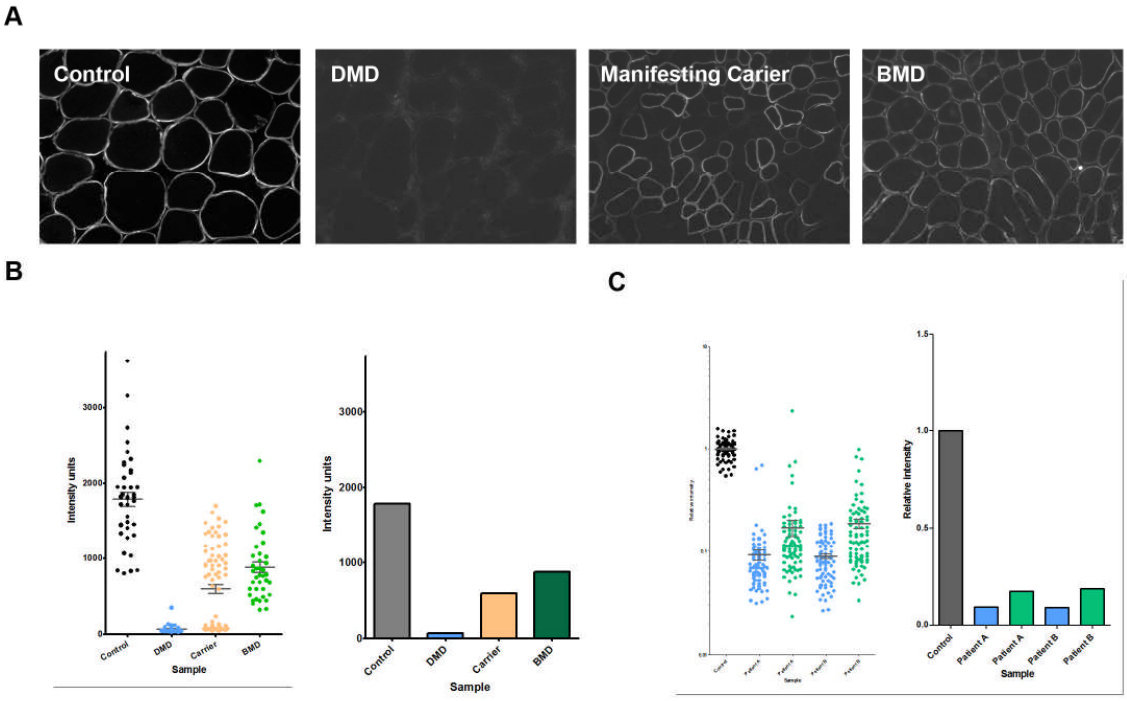

Figure 1 Comparison of methods for dystrophin quantification. a. Transverse cryosections of quadriceps muscle biopsies, immunostained with a dystrophin antibody. b. Intensity profiles of images in a, captured as an average measurement[2] (left) or with multiple measurements[10] (right). c. Two samples from a recent systemic clinical[4] trial analysed using average measurement (left) and multiple measurement (right) Pre-treatment in blue, post treatment in green.

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Competing interests

The authors declare no competing interests

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