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CORRESPONDENCE

Measuring dystrophin—faster is not necessarily better

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

DMD is caused by a deficit of dystrophin protein at the sarcolemma of muscle fibres.[3] Quantification of dystrophin on muscle biopsies is the main diagnostic test for DMD when genetic testing is unavailable. Restoration of dystrophin expression is the aim of several therapeutic approaches such as 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 both before and after treatment is crucial to evaluate the biochemical success of therapeutic interventions for DMD.

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 with spectrin labelling as a normalizing factor. The technique greatly advanced dystrophin quantification owing to its sensitivity, requirement for very little sample, capacity to confirm the localization of the protein at the 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[13,14] 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 records average dystrophin intensity for the whole image. In a manifesting carrier, the clear segregation of positive and negative dystrophin measurements that is evident using the original method[10] is lost with the new 'averaging' method (Figure 1a,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 skewed owing to a few high measurements (Figure 1c).

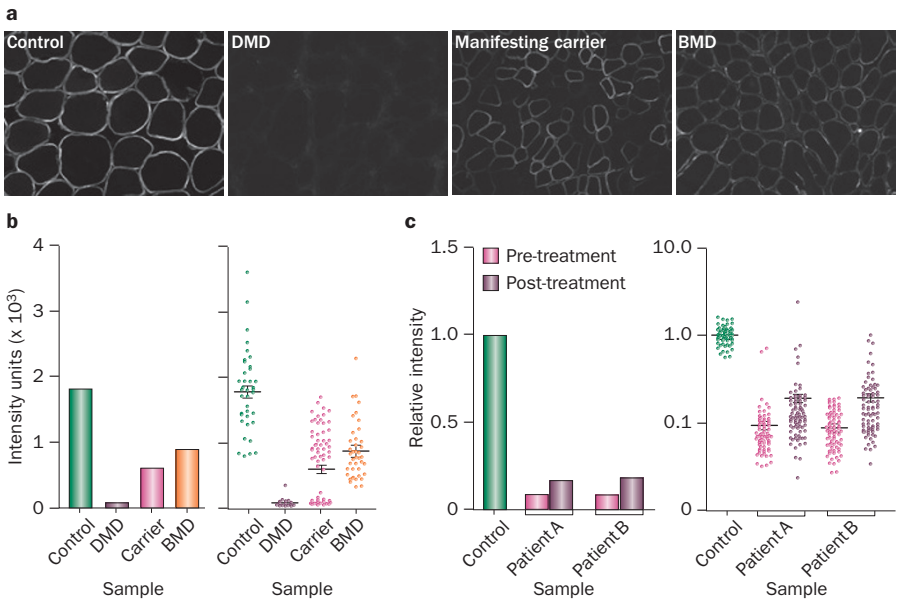

Figure 1 | Comparison of methods for dystrophin quantification. **a** | Transverse cryosections of quadriceps muscle biopsies, immunostained with a dystrophin antibody. **b** | Intensity profiles of images in panel a, captured as an average measurement[2] (left) or with multiple measurements[10] (right). **c** | Two samples from a recent systemic clinical[4] trial analysed using average measurement (left) and multiple measurement (right). Abbreviations: BMD, Becker muscular dystrophy; DMD; Duchenne muscular dystrophy.

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

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Competing interests
The authors declare no competing interests.

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