Electromyography and muscle biopsy in paediatric neuromuscular disorders – evaluation of current practice and literature review

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HIGHLIGHTS

- In the detection of myopathies in children, a concise EMG protocol is as accurate as a muscle biopsy.
- In children under the age of two years, the accuracy of EMG is superior to previous studies.
- Sedation is not required for a successful EMG recording in children at all ages.

ABSTRACT

The conduct and interpretation of electromyography in children is considered difficult and therefore often avoided. We assessed the diagnostic accuracy of the paediatric electromyography protocol used in our tertiary reference centre and compared it to muscle biopsy results and clinical diagnosis. Electromyography was performed in unsedated children with suspected neuromuscular diseases between January 2010 and September 2017 and was analysed quantitatively. Muscle pathology was classified into seven groups based on existing histopathology reports. The clinical diagnosis, including myopathic, neurogenic and non-neuromuscular categories was used as the gold standard.

171 children between the age of 12 days to 17.4 years were included in the analysis. 41 children (24%) were under the age of two years at the time of electromyography. 98 (57%) children were diagnosed with a myopathic disorder, 18 (11%) with a neurogenic disease and 55 (32%) as not having a primary neuromuscular disorder.

In detecting myopathic disease, electromyography performed as well as muscle biopsy (sensitivity 87.8% for electromyography vs 84.5% for muscle biopsy; specificity 75.7% vs 86.4%). This also applied to children under the age of two years (sensitivity 81.8% vs 86.4%). Quantitative analysis of a limited electromyography protocol performed in unsedated children is a very valuable diagnostic tool.

Keywords:

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1. Introduction

The diagnostic approach to neuromuscular diseases in children is often challenging. Medical history, clinical examination, laboratory tests, electromyography (EMG) and muscle biopsy are well known components of the functional classification of neuromuscular diseases whilst imaging and genetic testing are increasingly gaining importance [1-3]. These tests have individual advantages and limitations, and are used in a complementary fashion to efficiently diagnose patients with neuromuscular diseases. EMG plays a pivotal role in differentiating between neurogenic and myopathic diseases and disorders of neuromuscular transmission, in localising lesions in the peripheral nervous system, in grading severity, in monitoring disease progress and in excluding conditions without neuromuscular involvement. However, whilst in adults EMG is widely used and has well-defined parameters, EMG in children is considered to be difficult and thus is often avoided. This is due to the greater technical challenges, the smaller number of children being examined, age-dependent changes in muscle fibre dimensions and thus interpretation of EMG results, and the different spectrum of diseases that have to be considered.

Muscle biopsy has historically been considered to be the gold standard in the diagnosis of muscle disease, a position which is being superseded by molecular genetics. Whilst biopsies are more invasive than EMG, imaging and genetic testing, they permit the precise characterisation of muscle disorders through histological, biochemical, immunocytochemical and ultrastructural analyses. There are however a number of challenges in accurate pathological characterisation of disease

including overlapping morphological signatures across different diseases, absence of specific pathological features, and the small sample size available for assessment.

Multiple studies on the accuracy and utility of EMG and muscle biopsy in paediatric neuromuscular diseases have been performed over the last decades [4-14]. Different EMG protocols have been evaluated in a range of paediatric populations and compared against muscle biopsy and/or clinical diagnosis. However, the diagnostic approach to neuromuscular disorders has evolved. In patients with recognisable phenotypes such as Duchenne muscular dystrophy or spinal muscular atrophy, molecular genetic testing without preceding EMG and muscle biopsy have become the clinical standard [8, 11]. In our experience, children referred for EMG and muscle biopsy are now younger and and have more heterogeneous phenotypes, making it necessary to recognise early and subtle changes.

In our tertiary reference centre, referring clinicians often use EMG results to guide the direction of further investigation, which may include muscle biopsy. The accuracy of EMG findings is thus pivotal to the diagnostic pathway. Here, we assessed the diagnostic accuracy of the concise paediatric EMG protocol used over recent years in our institution and compared it to muscle biopsy results and the final clinical diagnosis.

2. Methods

2.1 Patients

The records of children under 18 years of age who had undergone an EMG of tibialis anterior and a muscle biopsy between January 2010 and September 2017 were screened retrospectively. Inclusion criteria were a definite or probable diagnosis of a myopathy or a neurogenic disorder, or a conclusion that there was no neuromuscular disorder as assessed by a paediatric neuromuscular specialist. The study was performed at Great Ormond Street Hospital, United Kingdom.

2.2 Audit registration.

The project was registered by the Clinical Governance and Safety Team, Great Ormond Street NHS Foundation Registration Number: 1856

2.3 Materials

All studies were acquired on a Medtronic KeyPoint EMG machine (Natus Medical, Pleasanton, CA, USA). For nerve conduction studies, reusable hand-held felt pad stimulating electrodes (bi-polar stimulation electrode (part number STIM1571), Unimed, Farnham, UK) were used with an inter-electrode distances of 10mm (under 2 years) or 26mm (over 2 years). Single-use, self-gelled ECG electrodes were used for surface recordings (part number NF-10-SC/12; Ambu A/S, Ballerup, Denmark). EMG recordings were performed using single-use 30G concentric needle electrodes (Ambu Neuroline, part number 74025-30/25; Ambu A/S, Ballerup, Denmark). EMG signals were band-pass filtered from 2Hz to 10kHz and stored for offline analysis. EMG data were analysed using the proprietary automatic and manual motor unit potential (MUP) detection and interference pattern (IP) analysis programmes built into the KeyPoint.Net software.

2.4 Procedure

2.4.1 Electromyography

Parental consent and patient assent were obtained following detailed explanation of the reasons for the investigation. All EMG studies were performed in the Department of Clinical Neurophysiology with the majority of the studies performed by the senior author (MCP); some studies were performed by other neurophysiologists under his supervision. Studies were conducted in the conscious, un-sedated state.

All studies followed the departmental protocol for investigation of generalised weakness [15]. As a minimum, this included assessment of one sensory nerve and one motor nerve in the leg and EMG of tibialis anterior. If the lower limb nerve conduction studies were abnormal, median or ulnar sensory studies (orthodromic, palm to wrist) were performed with motor nerve conduction studies of the ulnar nerve recording from ADM. For EMG analysis at least 20 MUPs were analysed

quantitatively, but fewer MUPs were evaluated in the presence of very pronounced abnormalities or where the recording was too short.

2.4.2 EMG data analysis and conclusion

EMG analysis was performed offline after the departure of the patient. When using either the manual or the automatic MUP analysis program at least three identical repetitions of a MUP were required to confirm the MUP was unique. Non-identical MUPs were discarded. Duration measurement was performed at a sensitivity of 100μV per cm. Automatic placement of cursors was accepted unless clearly inaccurate. IP analysis (including turns/amplitude and number of short segments/amplitude measurement) was initially not performed in every case but was included routinely after 2014. Finally, a qualitative assessment of the interference pattern was made.

All quantitative and qualitative data were taken into consideration when formulating the conclusion of the EMG. In general, neurogenic EMGs showed a combination of large amplitude, long duration high firing units seen in a reduced interference pattern, while myopathic EMGs had a predominance of short duration low amplitude units recruiting early in a full interference pattern.

2.4.3 Muscle biopsy

Muscle biopsies were performed locally using a needle technique under general anaesthesia; the quadriceps muscle was sampled in the majority of cases. Where muscle biopsy had already been performed by the referring institution, the biopsy specimens were transferred for a second opinion by the local pathology service. Based on a retrospective review of pathology reports, muscle pathology was categorised into seven groups: (1) non-diagnostic or end-stage, (2) neurogenic, (3) myopathic, (4) ambiguous –mixed features precluding definite categorisation of the underlying disease process, (5) minimal change – implying subtle morphological departure from normality, (6) histologically normal and (7) type II/fast fibre atrophy.

2.4.4 Clinical Diagnosis

The clinical diagnosis was established from the digital patient records. A definite diagnosis of a neurogenic or a myopathic disorder required the relevant pathogenic genetic mutation to have been detected. A probable diagnosis was made when a multidisciplinary team of paediatric neuromuscular specialists considered the diagnosis highly likely, taking into account, in varying combinations, a typical clinical presentation, muscle biopsy, EMG and other paraclinical tests including laboratory parameters and imaging, where available. If there was no indication of a primary neuromuscular disorder and children had been discharged from the neuromuscular clinics, they were labelled as not having a primary neuromuscular disorder. Cases were excluded if a diagnosis was not found yet or if further diagnostic results were pending.

2.5 Statistical methods

The diagnostic performance of EMG and biopsy in detecting neuromuscular disorders was assessed using thes ensitivity, specificity, and positive and negative likelihood ratios as compared to the above gold standard [16]. Confidence intervals for the positive and negative likelihood ratios were calculated using the log method, and confidence intervals for sensitivity and specificity were calculated using the Clopper and Pearson method [17]. The pretest probabilities for myopathic, neurogenic and normal studies were calculated from the distribution of diagnoses in the cohort of 171 patients. All calculations were performed in Excel (Microsoft, Redmond, WA, USA).

3. Results

We identified 235 children within the study period, of which 171 children (mean age 6.8 years, 54 girls, 117 boys) fulfilled the inclusion criteria. In the majority of cases (n=123, 72%) EMG preceded the muscle biopsy. 41 children (24%) were under the age of two years at the time of EMG. Using the clinical criteria for diagnosis described, 98 (57%) children were diagnosed with a

myopathic disorder, 18 (11%) with a neurogenic disease and 55 (32%) as not having a primary neuromuscular disorder. Genetic confirmation was available in 33/171 patients (19%).

Within the myopathic group there were 48 (49%) congenital myopathies, 14 (14%) muscular dystrophies, 4 (4%) inflammatory myopathies, 3 (3%) mitochondrial myopathies and 29 (30%) cases with an unspecified myopathy. 23 cases (24%) showed pathogenic mutations in the following genes: *COL6A1* (1), *COL6A2* (2), *DMD* (3), *SEPN1* (1), *RYR 1* (2), *TTN* (1), *FKTN* (1), *NEB* (1), *FHL1* (1), *PIEZO2* (1), *CAV3* (1), *MTM1* (2), *TPM2* (1), *MYH7* (1), *DNM2* (1) and *STAC3* (2), *ZC4H2* (1) (Supplementary Table 1) with EMG accurately predicting a myopathic condition in 20 (87%). The three cases in which EMG did not suggest a myopathic condition included two girls with Bethlem myopathy (*COL6A2*) aged 3.4 and 11.7 years, both of whom showed neurogenic EMG changes, and a 1.7 year old girl with *RYR1*-related congenital myopathy, who had a normal EMG. In the same cohort muscle biopsy was positive for myopathy in 21 (84%). In the remainder two showed minimal non-diagnostic histological change, a 9 year old boy with a *STAC3* mutation and a 11.2 year old boy with Becker muscular dystrophy, while results were ambiguous in another 9.1 year old boy with *MYH7* mutation. In the final case, the muscle biopsy in a 4 month old boy with a mutation in *PIEZO2* was normal. The accuracy of EMG and muscle biopsy overall for myopathy is shown in Table 1.

Given a pre-test probability of 57% for a myopathic condition, the post-test probability for either EMG or muscle biopsy detecting a myopathy is about 80%. If either EMG or muscle biopsy do not indicate a myopathy, the post-test probability of a myopathy falls below 2% (Fig. 1A).

In the subgroup of children under the age of two years, 22/41 patients (53.7%) were diagnosed as having a myopathy. In this subgroup, the EMG sensitivity was 81.8% (95% CI 59.7% to 94.8%), specificity 68.4% (43.4% to 87.4%). Biopsy sensitivity was 86.4% (65.1% to 97.1%), specificity 84.2% (60.4% to 96.6%).

In the group of 18 patients with neurogenic disorders, four had pathogenic mutations in the following genes: *SMN1* (2), *DYNC1H1* (1) and *PLA2G6* (1) (Supplementary Table 1). EMG

misdiagnosed one of these, a 17 year old boy with a homozygous deletion of exon 7 of the *SMN1* gene, as a myopathy. Muscle biopsy showed ambiguous results with combined features of myopathic and neurogenic changes. Ambiguous histological results on muscle biopsy were also found in a 2.6 years old boy with a pathogenic *DYNC1H1* mutation.

The sensitivity, specificity, positive and negative likelihood ratios for EMG and muscle biopsy predicting neurogenic disorders are shown in Table 1. With a very low pre-test probability of 11% in our cohort, post-test probability for EMG predicting neurogenic disorders is between 70% and 80%, for muscle biopsy between 80% and 90%. When an EMG result did not predict a neurogenic condition, the post-test probability of that diagnosis is 0.5%. For the muscle biopsy the same analysis gave the post-test probability of a neurogenic disorder between 5% and 10% (Fig. 1B).

In the subgroup of children under the age of two years, 5 patients (12.2%) were diagnosed with a neurogenic disorder. The EMG sensitivity was 100% (46.2% to 100%), specificity 94.4% (81.3% to 99.3%). Biopsy sensitivity was 40% (5.3% to 85.3%), specificity 100% (90.3% to 100%).

Finally, 48 patients were found to not have a primary neuromuscular disorder. In six cases, pathogenic non-neuromuscular related mutations were revealed. These were: *ATP13A2* causing parkinsonism with dystonia, *MECP2* leading to Rett syndrome, a *NRXN 1* mutation associated with Pitt-Hopkins-like syndrome 2, a pathogenic mutation in the *TWIST* gene leading to Saethre-Chotzen syndrome, a *PHOX2B* mutation causing congenital central hypoventilation syndrome and a mutation in *PEX16* causing a peroxisomal biogenesis disorder (Supplementary Table 1). Variable findings were found on muscle biopsy and EMG (Table 1). With a pre-test probability of 32%, the post-test probability when EMG predicted that there was no neuromuscular condition (normal EMG result) was about 80%. For muscle biopsy the post-test probability was between 60% and 70%. If EMG is not normal the post-test probability to still have no neuromuscular disorder lies between 20% and 30%, for muscle biopsy the post-test probability is around 30%.

3. Discussion

The study has evaluated the accuracy of EMG and muscle biopsy in a cohort of children with suspected neuromuscular disorders seen in a single centre over a period of over seven years and compared sensitivity, specificity, positive and negative likelihood ratios of the two tests. An important finding is that the EMG protocol used in our department with quantitative analysis of usually only one muscle performed in unsedated children with suspected neuromuscular disorders is equivalent in making a diagnosis of myopathic disorders to muscle biopsy. This is relevant for children of all ages including the very young under the age of two years. In children, neuromuscular disorders tend to be generalised and focal findings are unusual, and these results confirm that it is sufficient to examine one muscle provided this is done comprehensively. Offline quantitative analysis of stored EMG traces, performed after the departure of the patient, constitutes an important aspect of the approach. Indeed, the accuracy of our EMG results in detecting myopathies was comparable to that of EMG protocols examining at least two muscles [6, 8, 11, 18] (Table 2).

Looking in more detail at other studies, Gosh et al. [8] analysed the accuracy of an extensive EMG protocol including semiquantitative EMG of four or more muscles. In patients under the age of ten years, conscious sedation was used as standard. In 72 patients where a diagnosis of a myopathy - as defined by genetic testing, diagnostic biopsy or pathognomonic EMG - was made, the reported EMG sensitivity of 91.43% and specificity of 67.57% were very similar to the values we reported here. Other studies using quantitative assessment [6, 11] described comparable EMG sensitivity. The fact that the study by Rabie et al. [18] found an EMG sensitivity of 36.4% with qualitative analysis of four muscles strengthens the belief that a thorough examination of one or two muscles is superior to several muscles being examined qualitatively. The sensitivity of muscle biopsy is reduced compared with the one study shown [18].

41 patients (24%) of all children in our cohort were below the age of two years at the time of EMG. Subgroup analysis of 22 children under the age of two years diagnosed with a myopathy revealed a EMG sensitivity of 81.8% and a biopsy sensitivity of 86.4%. Likely due to the more demanding test conditions, EMG sensitivity in this age group is slightly below the one found in the entire

myopathic cohort. However, these results still clearly highlight that it is possible to accurately detect myopathic disorders with a concise EMG protocol and quantitative analysis in unsedated children at all ages. Analysing others' work in children under the age of two years with neuromuscular disorders [5, 7, 12, 13], concordance rates for myopathic disorders were often low, between 10% and 56% even though multiple muscles were examined, further supporting the use of quantitative analysis on a single muscle as being clearly superior (Table 3).

In neurogenic disorders sensitivity for EMG diagnosis is comparable with other studies [5, 7, 11-13, 18]. Only a few studies [7, 12, 18] compared muscle biopsy sensitivity and our results were inferior (Table 2). Since the purpose of this study was to compare the accuracy of EMG and muscle biopsy in detecting disease in the same cohort, patients were required to have EMG and muscle biopsy as part of their clinical investigations. This introduced recruitment bias as it would be unnecessary to perform muscle biopsy in patients with obvious neurogenic clinical features and EMG results. Hence, the neurogenic cases seen were often those with unusual and difficult presentations. Several factors precluded confident distinction between a neurogenic and myopathic process, particularly in needle biopsies from a proximal muscle such as the quadriceps (standard biopsy site in our series). These include large swathes of neurogenic fibre type grouping mimicking myopathic slow fibre predominance/uniformity, non-specific fibre size variation (pre-pathological SMA) in some cases of severe SMA I, and a constellation of pseudomyopathic architectural changes including unevenness of staining, moth-eaten fibres, mini-cores and larger cores, internal nuclei, split fibres, whorled fibres and fibro-fatty infiltration, seen in milder 5q-SMA I and SMA-LED [19, 20]. This fact as well as the small number of patients can explain the reduced biopsy sensitivity for a neurogenic diagnosis in this series.

At the time of data analysis a genetic diagnosis had been achieved in 33 of 171 patients (19%). This low proportion might reflect that genetically well described neuromuscular disorders in children are diagnosed in primary and secondary neuromuscular centres. Ccases with an existing genetic diagnosis are not referred on to a tertiary centre for further diagnostic testing. Additionally, genetic

results from large research based genome studies are still pending in many patients. Nevertheless, it highlights the sustained importance of EMG and muscle biopsy in the diagnoses of neuromuscular disorders.

Likelihood ratios are an intuitive way to apply statistical results to individual patients irrespective of the prevalence of the disease [21, 22]. When comparing the positive and negative likelihood ratios of EMG and muscle biopsy in detecting the presence or absence of myopathic or neurogenic disorders, it becomes evident that the tests provide similar utility for the clinician in making a diagnosis of myopathy (Table 1, Fig. 1). These results emphasise that a concise protocol including nerve conduction studies and EMG of a single muscle in unsedated children offers a diagnostic accuracy for myopathic disorders that is equivalent to that of a muscle biopsy. Nevertheless, whilst EMG can predict myopathic conditions with similar accuracy, muscle biopsy has the advantage of permitting the detection of specific histological findings to further guide focused genetic testing. In the children in whom no neuromuscular disease was diagnosed both EMG and muscle biopsy were less accurate than when there was neuromuscular disorder. This is probably attributable to the patient population referred to a tertiary neuromuscular reference centre to rule out a neuromuscular disorder, often with complex neurological symptomatology.

A strength of our study is that electrophysiologists, pathologists and clinicians all worked independently and did not alter the documented reports. EMG and muscle biopsy conclusions were considered as correct when they clearly indicated a myopathic or neurogenic condition or if they found normal results. Non-conclusive minimal change, ambiguous results and mixed patterns when reported in the muscle biopsy or EMG were regarded as false negatives for the purposes of calculating sensitivity and specificity. Additionally the cohort did not include myopathic cases which were so clinically obvious such as to prompt focused genetic screening possibly without either EMG and muscle biopsy. If such cases were included, it would be anticipated that the sensitivity of both EMG and muscle biopsy would be lower. It is a measure of the strength of our findings that this did not occur.

In conclusion, focused EMG in children of all ages is an indispensable diagnostic tool guiding neuromuscular paediatricians in their decision-making and helping them to arrive at a prompt diagnosis. It can be achieved without anaesthesia and is as accurate in determining myopathic conditions as a muscle biopsy. EMG still remains the more accurate test when determining neurogenic abnormality. EMG and muscle biopsy complement each other and therefore myopathic EMG findings should promptly lead to muscle biopsy and focused genetic screening.

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Authors' contributions

MCP designed the study, acquired the data and drafted the manuscript. PH participated in the study design study and drafted the manuscript. AM and FM reviewed clinical records and contributed to the manuscript. RP participated in the study design, reviewed muscle biopsies and drafted the manuscript. RS worked on database and computer management. SJ performed statistical analyses and critically revised the manuscript. PS and CS produced pathology reports and contributed to the manuscript. All authors read and approved the final manuscript.

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