Muscle biopsies in children—an evaluation of histopathology and clinical value during a 5-year period

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Abstract
Muscle biopsy is an important diagnostic tool in the investigation of children with neuromuscular disorders. This report presents the experience with paediatric muscle biopsies during a 5-year period at a routine pathology laboratory. A total number of 58 cases were included, and indications, microscopical findings, and final histopathological diagnoses were recorded. A total of 21 biopsies were from females (36%) and 37 biopsies from males (64%); 53% of the cases were from children under 2 years of age. Major pathological findings were found in 30% comprising muscular dystrophy, neurogenic atrophy, and congenital and metabolic disorders, even in cases with vague clinical manifestations. These findings confirm the high diagnostic yield of muscle biopsies, especially as new techniques have been introduced such as immunohistochemistry. Muscle pathology is difficult and emphasizes the importance of this service being undertaken by specialized laboratories with an experienced staff. Microscopical examination of muscle biopsies should be based on adequate clinical information, demonstrating the necessity of close contact between pathologists and referring physicians.

Key words: Biopsy, diagnosis, dystrophy, electron microscopy, immunohistochemistry, myopathy, neuromuscular, paediatrics, pathology, skeletal muscle

Introduction
Investigation of a child with suspected neuromuscular disorder is challenging, and muscle biopsy represents an important diagnostic tool. New techniques, such as immunohistochemistry, have improved the diagnostic value of this procedure (1–4).

Obtaining a muscle biopsy, specimen handling, and microscopic examination are time-consuming and resource-demanding (5). Evaluation of this procedure with special focus on resource management and quality assurance is therefore of great value. Furthermore, the clinicians may have a wish for an insight into this procedure.

The aim of this study was therefore to review and evaluate paediatric muscle biopsies during a 5-year period with focus on indications, histopathology, analyses, diagnoses, and utility value.

Materials and methods
All muscle biopsies in Mid-Norway are examined at the Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway. The hospital serves three counties with a total population of about 650,000 people, of whom approximately one-fourth is under
the age of 18. Muscle biopsies are performed both at
district general hospitals and at the university
hospital. Needle biopsy using local anaesthesia is
the preferred technique; however, sometimes an
open muscle biopsy is carried out under local or
general anaesthesia. Outside the university hospital
the specimen is kept cool on ice, transported to the
pathology department the same day, either by train
or plane. If the muscle biopsy is performed at the
university hospital, it is sent to the laboratory
immediately after removal, kept dry in a test tube.

The computer-based patient data files at the
laboratory were searched for muscle biopsies during
the time period 1998–2002. A total number of 281
biopsies were found, of which 58 cases (21%) were
from patients younger than 19 years of age. These
muscle biopsy reports were reviewed, and the
following data were recorded: age, gender, indica-
tion for biopsy, results of microscopical findings,
histochemistry, enzyme histochemistry, immunohis-
tochemistry, electron microscopy, and histopatho-
logical diagnosis.

The muscle biopsy is prepared for light and
electron microscopy. Both paraffin and frozen sec-
tions are cut routinely; the latter are especially suitable
for enzyme histochemistry and immunohistochem-
istry. General histochemistry comprises haematoxylin/
eosin, Gomory trichrome (detection of ragged-red
fibres and nemaline rods), oil red (detection of lipids),
and periodic acid-Schiff (PAS) (detection of glyco-
gen). Enzyme histochemistry demonstrates endoge-
 nous enzymatic activities in muscle fibres and is used to
analyse different fibre types, detection of subcellular
organelles, structural abnormalities, and specific en-
zymatic defects. Immunohistochemistry is used for
identification of proteins and cellular elements, for
instance to characterize cells in inflammatory myo-
pathies (B cells, T cells, macrophages) and to deter-
mine fibre types, dystrophin, and dystrophin-
associated proteins. Investigation of muscular dystro-
phy sometimes requires Western blotting. Electron
microscopy is frequently performed in paediatric
muscle biopsies, especially when metabolic, mito-
chondrial, and congenital disorders are suspected,
including nemaline myopathy, myotubular myopathy,
and central core disease. In difficult cases biopsies are
sent to the National Competence Centre for Muscle
Disease, University Hospital, Tromsø, Norway, for
consultation.

Results

In this series of 58 paediatric muscle biopsies no
major artefacts were observed after dispatching or
processing that might have prevented an adequate
microscopical evaluation.

Our pathology department receives annually
about 75 muscle biopsies, of which paediatric ones
comprise 10–15 specimens. The female:male ratio
was 0.6. More than half of the patients (53%) were
below 2 years of age, 17 patients (30%) between 3
and 9 years, and 10 patients (17%) in the age-group
10–18 years.

Indications for obtaining muscle biopsies were
recorded according to a modification of Heffner and
Schochet (6). Corresponding histopathological diag-
oses were listed as well. Out of 58 cases, 22 (38%) 
underwent a muscle biopsy intervention due to
different muscle symptoms (pain, cramps, stiffness,
and paraesthesia), and 16 of them (73%) revealed
normal skeletal muscle or unspecific changes. The
remaining 6 cases revealed specific entities including
neurogenic atrophy (2 cases), muscular dystrophy
(not otherwise specified) (2 cases), mitochondrial
myopathy (1 case), and congenital myopathy (central
core disease) (1 case). Weakness or hypotonia were
indications in 11 out of 58 cases (19%), of which
8 cases demonstrated no pathology or unspecific
changes. In the remaining three cases congenital
disorders were detected (one congenital myopathy
and two cases with nemaline myopathy). Metabolic
disease was suspected in 10 cases (10/58, 17%); all
showed normal skeletal muscle or unspecific
changes. Questions of muscular dystrophy were
raised in 9 cases (9/58, 16%), and dystrophic changes
were seen microscopically in 4 cases (Duchenne
muscular dystrophy (3 cases) or Becker muscular
dystrophy (1 case)), mitochondrial myopathy (1
case), and neurogenic atrophy (1 case). Three cases
with elevated serum creatine kinase values displayed
no histopathological changes. In two cases with
suspected inflammatory myopathy, one disclosed
dermatomyositis and one connective tissue disease.

Normal skeletal muscle was found in about half
of the cases (28/58, 48%). Unspecific or chronic
myopathic changes, defined by Cumming et al. (7)
as atrophy, hypertrophy, internal nuclei, necrosis,
regeneration, fibrosis, and splitting, were encoun-
tered in 13 cases (22%). Four out of six cases with
dystrophic changes were found to represent Duch-
enne or Becker type due to lack of or reduced
immunostaining for dystrophin; it was not possible
to classify the two remaining cases. Neurogenic
atrophy was seen in three cases, one was Werdnig-
Hoffmann disease and one with neuroaxonal dystro-
phy. There were two cases with myositis consistent
with dermatomyositis and one case with muscular
involvement as a facet of connective tissue disorder.
Ragged-red fibres were found in two cases consistent
with mitochondrial myopathy. Four cases turned out to be congenital muscular disorders: nemaline myopathies (2 cases), central core disease (1 case), and one could not be classified. Table I shows cases with major pathological findings.

Auxiliary techniques such as immunohistochemistry were frequently requested (74%) in order to achieve a more distinct diagnosis, as illustrated by lack of dystrophin expression (Figure 1). Electron microscopy was performed in 24 out of 58 biopsies (41%) and demonstrated important ultrastructural pathology in 8 cases showing abnormal mitochondria, deposits, or nemaline rods (Figure 2). In 23 cases (40%) the centre of competence was consulted, for the most when muscular dystrophy was suspected. In such cases extended immunohistochemical analyses were performed with a battery of antibodies against dystrophin-associated proteins, often supplemented with protein blotting. Sometimes genetic analyses were recommended, especially in patients under investigation for mitochondrial disorders and muscular dystrophies.

**Discussion**

This report deals with the experience with paediatric muscle biopsies in the daily routine at a general pathology department during a 5-year period.

All muscle biopsies taken in Mid-Norway are analysed at the Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim, Norway, and close contact has evolved between pathologist and referring physicians providing relevant clinical information for optimal diagnostic histopathology. Thus, one consultant pathologist is dedicated to this service since muscle pathology has
become highly specialized. In fact, collaboration with centre of expertise is requisite, and several biopsies are sent to The National Competence Centre for Muscle Disease at the University Hospital in Tromsø, Norway, for consultation. This laboratory is equipped with experienced scientific and medical staff that provides more sophisticated and extensive services and investigations, including advanced immunohistochemistry with a large panel of antibodies, as well as Western blotting and molecular genetic analyses.

Histopathological changes in muscular disorders are often unspecific, and clinical information is the prerequisite of making good diagnoses. In each case, however, the pathologist should try to identify a diagnostic category, such as normal, unspecified, dystrophic, inflammatory, etc., since the biopsy report serves as therapeutic guideline for the clinician; even exclusion of a diagnosis is important (5).

Indication for performing muscle biopsy is in general wide, and in the present study the most common cause was various muscular symptoms and signs, including pain, cramps, stiffness, paraesthesia, weakness, and hypotonia (57%). The high number of cases under investigation for metabolic (17%) and hereditary disorders (including muscular dystrophy) (17%) was not unexpected. Among the first group normal muscle tissue or chronic myopathic changes were found, whereas in the latter specific pathology was recorded in 6 cases (Duchenne muscular dystrophy (3 cases), Becker muscular dystrophy (1 case), neurogenic atrophy (1 case), mitochondrial myopathy (1 case)). Thus, the clinical yield of muscular biopsy is fruitful as this procedure may unveil specific disorders despite sparse clinical manifestations.

Since several biopsies (41/58, 71%) displayed normal skeletal muscle tissue or unspecific changes, one may ask whether the indications are too liberal. Performing a muscle biopsy is painful, and full anaesthesia may be necessary. Accordingly, the physician should consider the indications for muscle biopsy carefully. On the other hand, investigation of children with neuromuscular disease is challenging, and this study has clearly confirmed the clinical value of a muscle biopsy, defending a liberal attitude to this procedure. Needle biopsy is nowadays the preferred method as it is less time-consuming, more cost-effective, and encumbered with fewer complications than an open biopsy. Disadvantages are small and often traumatized tissue samples, although we did not experience troublesome artefacts in this series of muscle biopsies.

Supplementary analyses, such as immunohistochemistry, histochemistry, and electron microscopy, have resulted in identification of new pathological structural changes (1–4, 9), clearly illustrated by ultrastructural findings in cases of congenital and metabolic myopathies, and immunostaining in cases of muscular dystrophies and inflammatory myopathies. In this manner the utility value of a muscle
biopsy has been greatly improved, providing more accurate and specific diagnoses. Nevertheless, advances in molecular genetic analyses have led to identification of diverse gene defects. For this reason, in patients with a family history or with a phenotype suggesting a well characterized hereditary disorder, available genetic tests on a blood sample or of any other tissue should be considered (4,5).

In conclusion, muscle biopsy is invaluable in the investigation of children with neuromuscular disorders, especially by implementing auxiliary techniques such as immunohistochemistry and electron microscopy. Histopathological examination is, however, time-consuming and requires experienced personnel, so co-operation with a skills centre is necessary. Further, close liaison between pathologist and clinicians is essential, and histopathological findings should only be interpreted in the light of clinical manifestations and laboratory findings. Finally, a study like this represents a useful evaluation of this medical procedure and is important in the quality assurance routine of the pathology department.

Acknowledgements
The authors especially thank Dr Christina Vogt Isaksen for excellent commentaries and critical reading of the manuscript.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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