



Muscle biopsies in clinical trials for Duchenne muscular dystrophy – Patients' and caregivers' perspective

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Abstract

The number of clinical trials for Duchenne muscular dystrophy is increasing. Many trials require muscle biopsies, which involve an invasive surgical procedure. Little is known about short- and long-term impacts of muscle biopsies as perceived by patients and caregivers. Therefore a survey was held among patients and their caregivers who participated in trials involving muscle biopsies, in seven countries. Seventy-eight responses were received. Analysis revealed that many patients and parents had significant anxiety before the biopsy. The main concern of caregivers was the required general anaesthesia. In most cases biopsies caused pain and temporarily hampered daily activities. The main long-term impact was scarring, although large variation in size was reported. Seventy-nine percent of caregivers were little bothered and 21% were moderately or severely bothered by the scar. Willingness to consider another biopsy in future protocols was higher for open-label studies than for placebo-controlled trials. Caregivers stressed the importance of knowing the results of biopsy analyses; only a minority actually received this information. Recommendations are made on the informed consent procedure regarding risks and consequences of muscle biopsies, and communication of results. Furthermore, efforts should be made to minimise the impact of biopsies through pain management and by considering plastic surgery.

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1. Introduction

Duchenne muscular dystrophy (DMD) is a devastating disorder hallmarked by progressive muscle weakness leading to loss of ambulation in childhood, respiratory insufficiency, cardiomyopathy and early death [1]. Mutations in the *DMD* gene lead to absence of the dystrophin protein that normally links the extracellular matrix to the contractile elements in the sarcolemma, which is thought to protect skeletal muscle

fibres from contraction induced damage [2]. Muscle pathology consists of inflammation, failed muscle regeneration and a progressive replacement of muscle by fibrotic tissue and fat [3]. In order to stop these irreversible processes, a number of therapeutic strategies have been developed that aim to restore some form of dystrophin production or slow down the pathological processes that lead to loss of muscle tissue. These include stop codon read-through compounds that allow the production of low levels of dystrophin in patients with nonsense mutations in dystrophin, various types of antisense oligonucleotides (AON) that allow the production of partially functional dystrophins by restoring the

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disrupted reading frame through exon skipping of pre-mRNA transcripts, and delivery of micro-dystrophins using adeno-associated viral vectors. Other therapeutic approaches aim to increase levels of utrophin, a protein with similar properties as dystrophin, to reduce the levels of myostatin, an inhibitor of muscle growth, in order to increase muscle mass, or to use histone deacetylase (HDAC) inhibitors to simultaneously boost muscle regenerative capacity, and reduce inflammation and fibrosis formation [4].

Over the past 12 years, these compounds have been tested in clinical trials, which involved muscle biopsies of various muscles as defined by the study protocol of that trial [5–18]. While invasive, the biopsies were taken to obtain evidence that the compound was performing as proposed, i.e. showing dystrophin expression for dystrophin restoring approaches, increased utrophin levels for utrophin upregulating approaches, or reducing levels of fibrosis and inflammation for HDAC-inhibitors [16]. Furthermore, in dose escalation studies, biopsies were performed to discover the optimal dose for the therapeutic compounds [6,9,12–14,16,18]. Finally, in 2016 the exon 51 skipping compound eteplirsen received accelerated approval from the Food and Drug Administration (FDA; USA) based on small increases in dystrophin in muscle biopsies obtained from treated patients [19].

Given the invasive nature of muscle biopsies, stakeholders in the DMD field including medical ethical boards throughout the world have struggled with the balance between necessity and burden of muscle biopsies performed in children. A biopsy will cause some kind of pain during the healing process, and will leave a permanent scar. In most cases, general anaesthesia is required for which careful planning is essential in this patient population. The extent of the scar is influenced by the technique used. Needle and conchotome biopsies can be done through relatively small incisions, potentially even smaller than one centimetre, whereas open biopsies require incisions of several centimetres, especially in areas with more subcutaneous fat [20]. The conchotome is a angled scissor-like device that cuts muscle through the opening and closing of a sharp-edged jaw [21]. While smaller biopsies are obviously preferred, sometimes protocols request larger, open biopsies, e.g. when more extensive analyses are required, to obtain spare samples for validation experiments, or to serve as backups in case of complex logistic handling. Although the extent of the burden for patients and families is crucial in the process of deciding whether to participate in a trial involving muscle biopsies, consistent data on short- and long-term effects of these muscle biopsies are lacking [22]. To address this, the aim of the current study was to learn the patients' and caregivers' perspectives on the burden of muscle biopsies obtained in clinical trials. Furthermore, the hope was to identify ways to attenuate the burden of the muscle biopsy procedure for future trials. This effort was initiated by patient representatives and performed in collaboration with academic researchers. Jointly, a questionnaire was constructed, translated, and sent to patients and caregivers who had participated in any relevant clinical trial for DMD between 2007 and 2017.

2. Materials and methods

2.1. Questionnaire

The questionnaire was designed in English by four members of the muscle biopsy working group (AJ, AAR, EHN and FM). It consisted of three parts: 1) General characteristics of the patient, which clinical trial they had participated in, the number and location of the biopsies taken as defined in the study protocol, and if general or local anaesthesia had been used; 2) Perception of the child about the biopsy; 3) Perception of the parent/caregiver about the biopsy. It consisted of a combination of multiple choice questions, scales, and open questions. The questionnaire addressed both short-term and long-term impacts, as well as general concerns and recommendations for future trials. It was made clear that responses had to be focused on biopsies performed as part of a clinical trial, and not of any diagnostic procedure. A draft version was reviewed by the United Parent Project Muscular Dystrophy board (UPPMD, now World Duchenne Organization (WDO), see <https://worldduchenne.org/>). The final English version (Appendix 1) was translated in Dutch and Italian. The questionnaire was built as an online version using freely available software from SurveyMonkey® (www.surveymonkey.com).

2.2. Participants

In the Netherlands, a link to the electronic version of the questionnaire was sent to all members of the Dutch Duchenne Parent Project (DPP) as part of a regular newsletter. Families known to the DPP as participants of a clinical trial that had undergone a muscle biopsy as protocol requirement received a personal email containing the same link. Finally, the participants were notified of the possibility to fill out the questionnaire either on paper or electronically by the local principal investigator of the Leiden University Medical Center if they had participated in this centre. In the UK, caregivers and patients were informed of the possibility to complete the survey via emails from charity databases. Furthermore, awareness of the survey was raised by social media. In Italy a personal email containing the link to the electronic version of the survey was sent to caregivers and patients known to Parent Project onlus as participants of a clinical trial that included a muscle biopsy. Furthermore a dedicated announcement containing the electronic link to the survey was published on the organization web site and on social media.

3. Results

Responses were received from 78 children and their caregivers from seven countries (the Netherlands, United Kingdom, Italy, United States, Canada, France and Australia) who reported on 150 biopsies. An overview of all characteristics is given in Tables S1–S6.

Most children had participated in trials for drisapersen, an AON designed to skip exon 51, ezutromid, a compound

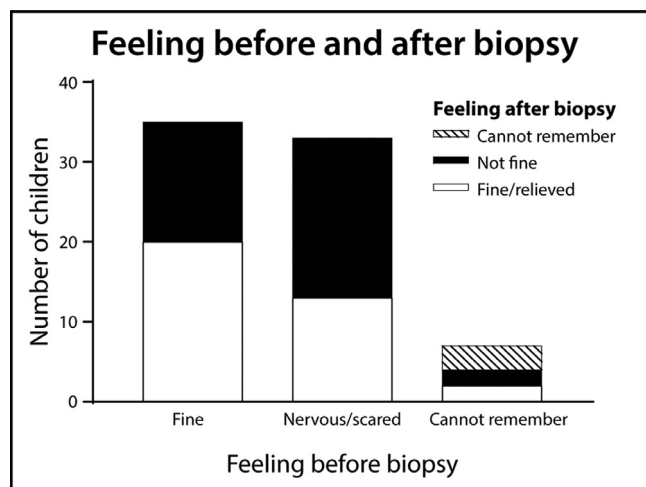


Fig. 1. Feeling of the children before and after the biopsy. Correlation of feeling of the children before and after the biopsy ($n = 75$).

that aims to increase utrophin expression, or the histone deacetylase inhibitor givinostat. Most children had been younger than 10 years old when the first biopsy was taken (range: 5–16 years), and underwent two biopsies in total (range: 1–5 biopsies). Most biopsies were taken from the biceps brachii muscle. For four patients it was unclear from which muscle the biopsy was taken. Their answers have been excluded when results are split by biopsy site. Two patients had biopsies taken from multiple sites. When results are split by muscle, the answers have been taken into account for both muscles. In 83% of the biopsies, general anaesthesia had been used, mainly administered intravenously.

3.1. Short-term impact

Around half of the children ($n = 33$) indicated that they had been nervous or scared before the biopsies, the remaining had felt fine or could not remember (Fig. 1). There was a

correlation between the feeling of the children before and after the biopsies. The majority of those who felt fine before also felt fine afterwards, whereas more children who had been nervous or scared before the procedure did not feel fine after the biopsy. Examples of feelings of children who did not feel fine were pain, tiredness and feeling emotional. In general, children appeared to be less nervous before the biopsy than their caregivers. The largest concern of the parents/caregivers was the anaesthesia applied during the procedure.

In the questionnaire seven children experienced problems with general anaesthesia, and one with local anaesthesia. These problems included nausea, agitation, increased heart rate, and difficulty waking up. One case of rhabdomyolysis resulted in hospitalization.

Pain after the biopsy was reported by 67 (88%) of the responders, whereas nine (12%) did not (Fig. 2A). The pain was classified as mild to moderate by the majority (88%). Pain classification seemed slightly lower for biopsies taken from the biceps than those obtained from leg muscles (Fig. 2B). The pain control given after biopsies of the biceps was considered good or very good by 83% ($n = 48$). However, for the muscle biopsies taken from the leg, the pain treatment appeared less effective. The majority (89%) considered the treatment of pain sufficient (good or very good; Table 1).

Healing of the wound also had an impact on daily activities according to 51% of the responders (Fig. 3). Children indicated they had, among others, difficulties in walking, showering, writing and were feeling uncomfortable in bed.

3.2. Long-term impact

There was a large difference between the sizes of the scars, ranging from three to 60 mm (median 30 mm). There were no consistent differences in scar sizes between the biopsy sites (Fig. 4A). The majority of children and caregivers indicated that they were not or little bothered by the scars (1–2 on a scale of 1–5) (Fig. 4B). For those who were bothered by their

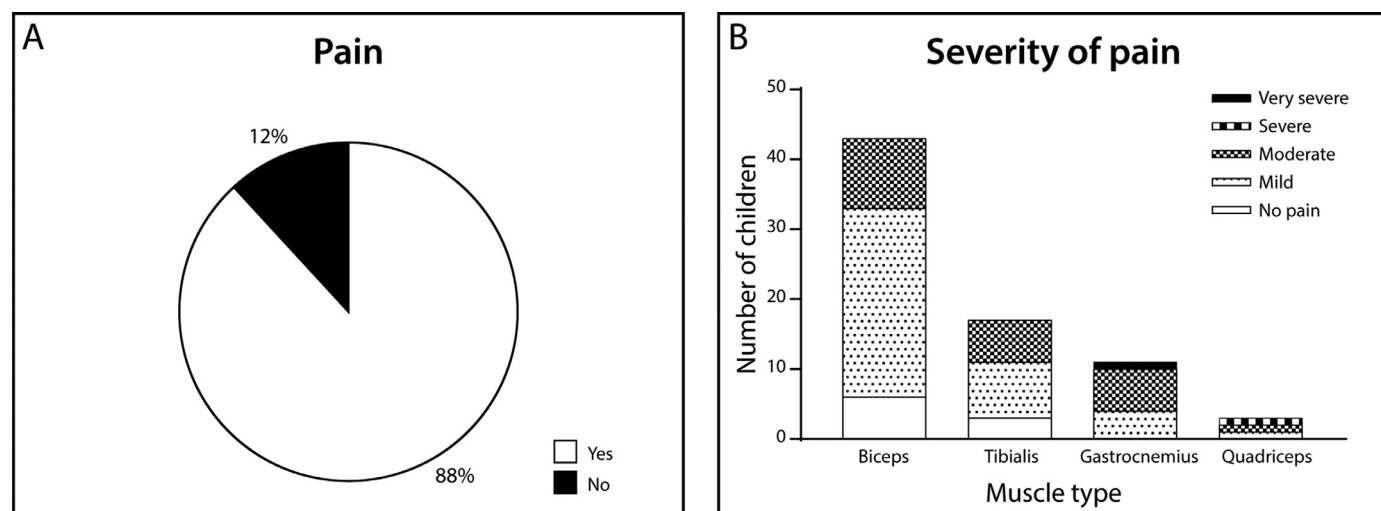


Fig. 2. Pain caused by biopsy ($n = 76$). (A) Pain caused by biopsy. (B) Level of pain.

Table 1
Treatment of pain.

Effectiveness of pain control after biopsy split by biopsy site as considered by parents/caregivers ($n=76$).

Level of pain	Biceps		Tibialis		Gastrocnemius		Quadriceps		Total	
Very good	15	37%	3	19%	1	9%	0	0%	19	27%
Good	23	56%	11	69%	9	82%	1	33%	44	62%
Neither good nor bad	3	7%	2	13%	1	9%	1	33%	7	10%
Bad	0	0%	0	0%	0	0%	0	0%	0	0%
Very bad	0	0%	0	0%	0	0%	1	33%	1	1%

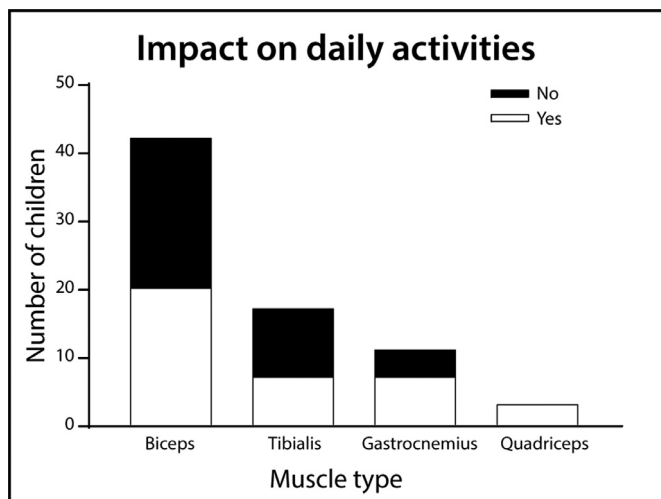


Fig. 3. Impact of biopsy on daily activities ($n=73$).

scars (scores 4 and 5), the reasons varied and were mainly aesthetic. However, four responders indicated that their scar(s) were sometimes itchy or sensitive to touch even several years after the biopsy. A difference was observed between feelings of the children and the caregivers' perception on how their child feels; of the eight children who felt 'bothered', only two of their caregivers reported similarly. Scar healing was considered to be not satisfactory by 35%, but there was no clear correlation between the biopsy site and how this healing was reported (Fig. 4C). Caregivers' descriptions of scars that had not healed well, indicated that they were large, prominent, wide, or that discoloration of the skin had occurred. Pain was not reported as a long-term symptom. Examples of scars are shown in Fig. 4D-F.

3.3. Quality of the information provided before and after the biopsies

Caregivers had been provided with information on the clinical trial beforehand. Furthermore, most caregivers had a discussion with a clinician about their concerns and had been told about the risks of the biopsy and anaesthesia (Fig. 5A). Most caregivers (80%) were satisfied with the information given in the informed consent (Fig. 5B). However, some felt that the documents did not have sufficient information, and that the risks of the procedure and scarring were minimized. Twenty-six percent of the caregivers indicated they had not

been informed beforehand what the approximate size of the incision would be, whereas 7% of those who were informed felt that the information was not accurate.

Around three quarter of the caregivers received advice on how to take care of the wound, although with considerable variations in the instructions. Many were advised to keep the wound dry and clean. Some others were told to apply antibiotic cream or avoid direct sunlight. Several also received advice on what to do in case of an infection (i.e. seek medical advice).

Nearly all caregivers indicated that being informed about the results of the trial was very important to them. By contrast, only 26% had actually received such information (Fig. 6A). In general, caregivers considered it important to know the overall results of the trial as well as the biopsy results of their own child (Fig. 6B). Not receiving this information resulted in feelings of frustration and irritation, but sometimes also anxiety, because they felt that something had been deliberately hidden to them.

3.4. Recommendations for future trials

When asked whether they would participate in another clinical trial that would require one or more biopsies, 45% of the children responded positively, while 55% answered no or was not sure (Fig. 7). Of the caregivers, 78% of caregivers responded positively, while 22% said no. Opinions of patients and caregivers often matched. Of the 34 children who would take part in such a trial, 33 caregivers agreed as well. Of the 58 caregivers who would allow their child to have another biopsy, less than 10% of their children would not want to participate. Furthermore, the majority of the children (66%) would not discourage a friend from participating in this type of trial. Children did not report a clear preference for a biopsy site (data not shown). Finally, caregivers found trial biopsies far more acceptable as part of an open-label trial than for a placebo-controlled trial (Fig. 8).

4. Discussion

Over the last years a significant progress has been made in the development of therapies for DMD. Whereas five years ago only a handful of trials was ongoing, currently over 50 compounds are or have been tested in clinical trials. In many of these trials one or more muscle biopsies are used to determine restoration of protein expression or to show

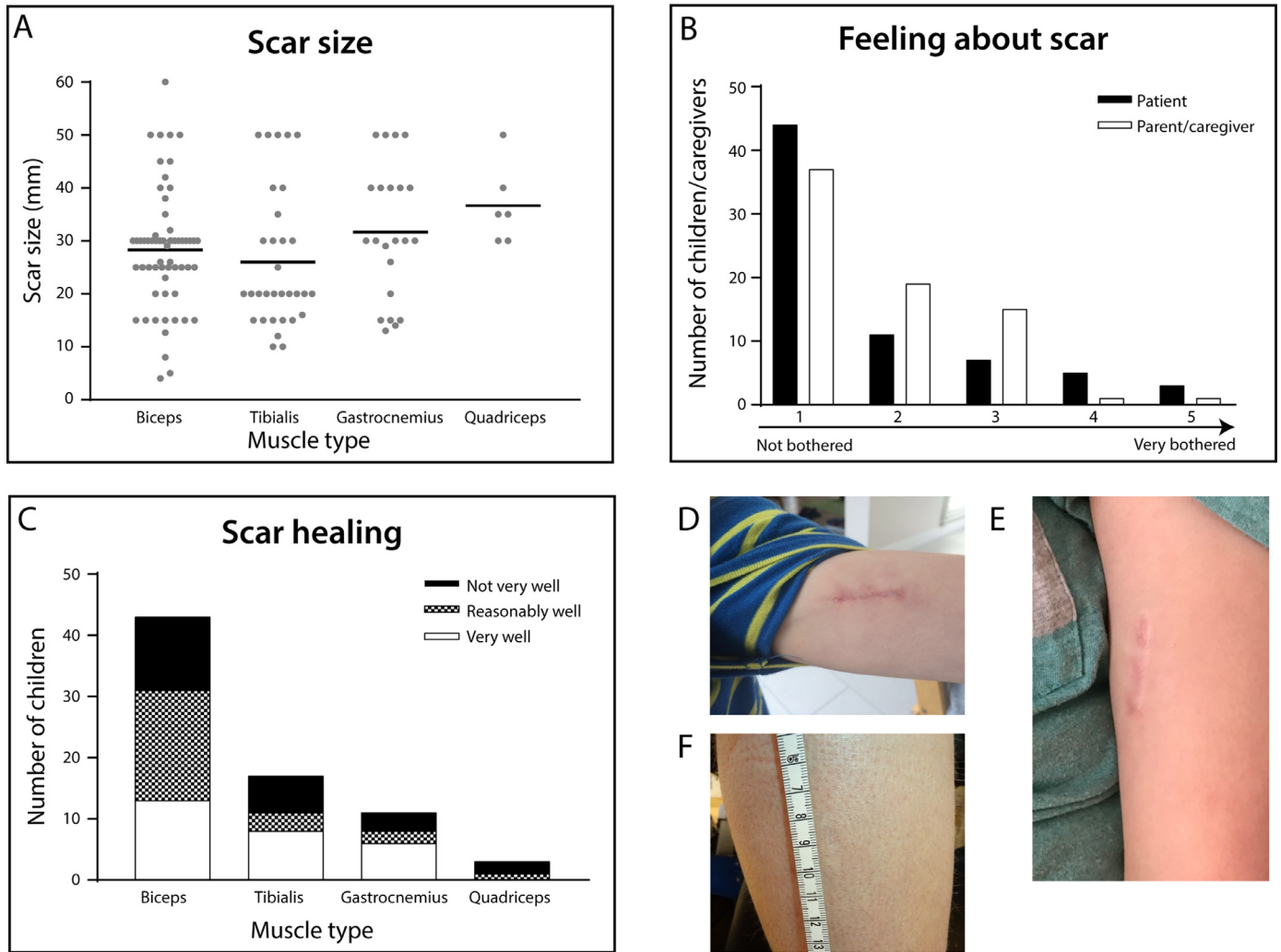


Fig. 4. Scar. (A) Scar sizes per muscle: biceps ($n=60$), tibialis ($n=32$), gastrocnemius ($n=22$) and quadriceps ($n=6$). Some patients had more than one biopsy taken. (B) Perception of the scars by children ($n=70$) and their parents/caregivers ($n=73$). (C) Correlation between the biopsy site and scar healing ($n=74$). (D-F) Examples of scars showing two open biopsies from the biceps (D and E) and one conchotome biopsy taken from the tibialis anterior muscle (F).

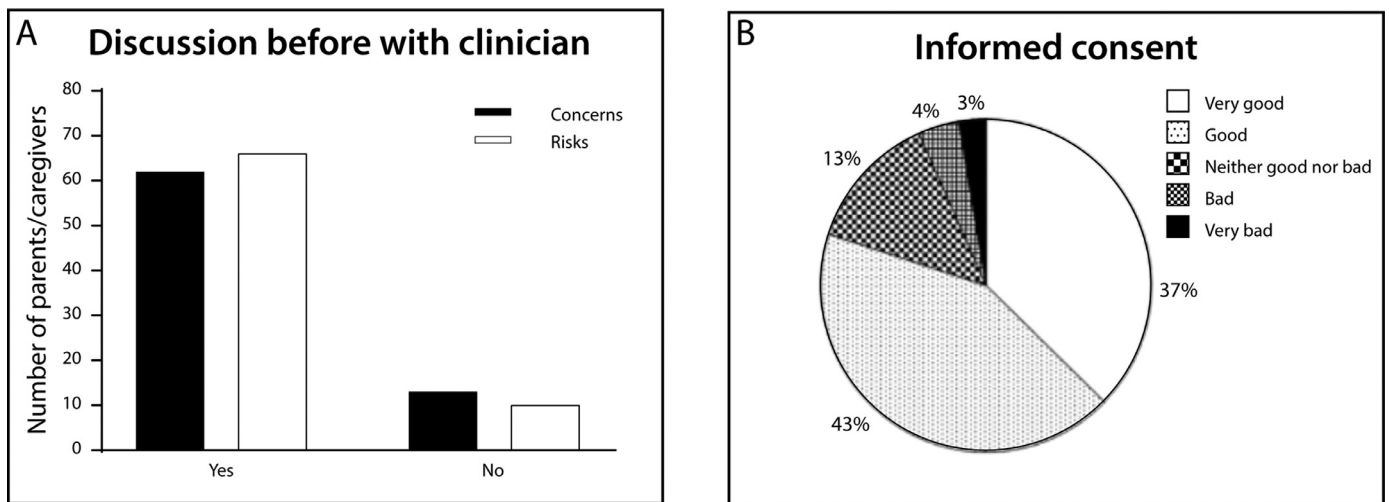


Fig. 5. Information before biopsy. (A) Number of parents/caregivers who discussed their concerns ($n=65$) and/or the risks ($n=76$) with a clinician before the biopsy. (B) Opinion of the parents/caregivers about the clarity of the risks/benefits in the informed consent ($n=75$).

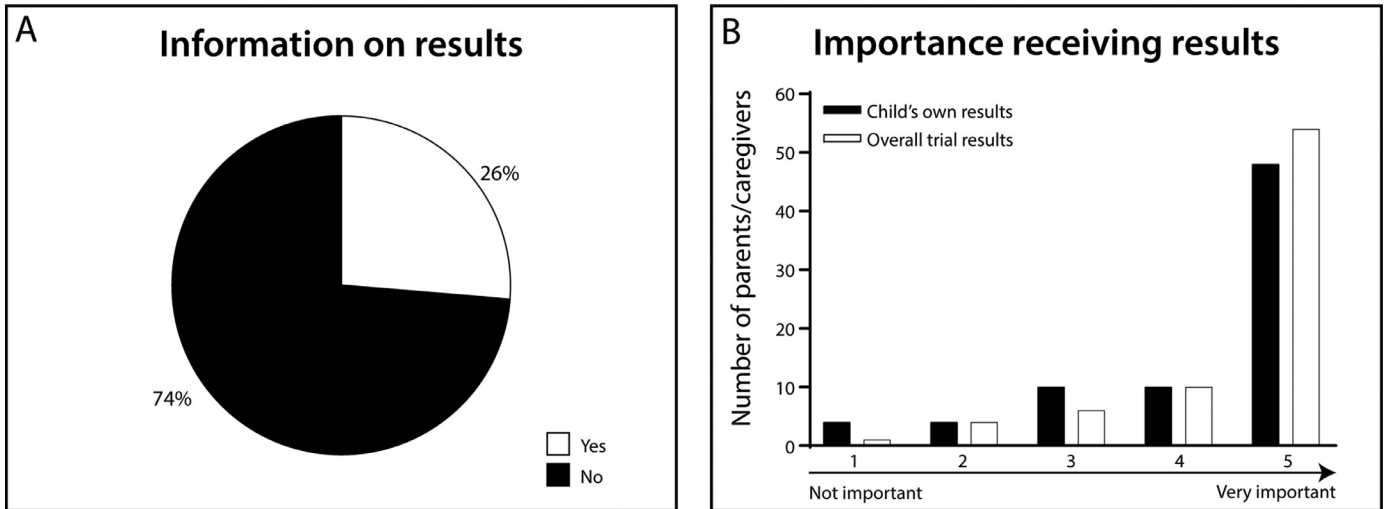


Fig. 6. Communication about results. (A) Number of parents/caregivers that did or did not receive the results of their child or the overall trial ($n=76$). (B) Importance of receiving trial results ($n=75$) and/or results of their child's own biopsy ($n=76$).

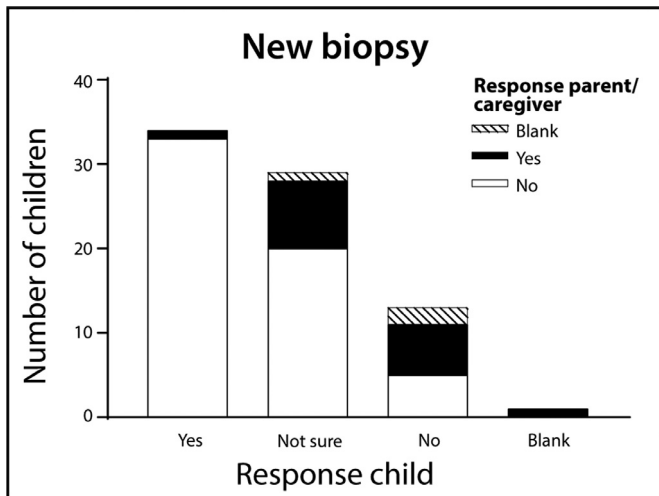


Fig. 7. New biopsy. Number of children ($n=76$) who would or would not agree with a new biopsy versus their parents/caregivers ($n=73$).

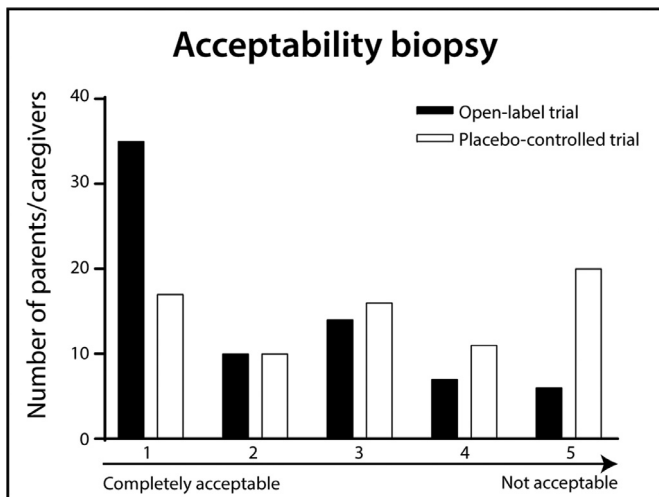


Fig. 8. Acceptability biopsy. Acceptability of taking a biopsy for an open-label versus a placebo-controlled trial.

improvement in muscle quality via histological studies. Since muscle biopsies are invasive, we here intended to investigate the patient and caregiver perspective on their short-term and long-term impacts.

4.1. The biopsy procedure

First, the procedure itself poses risks, mainly due to the use of anaesthesia, which requires special precautions in DMD boys compared to healthy individuals. This relates to age and the respiratory pathology, with risks including perioperative respiratory and cardiac complications, but also rhabdomyolysis [23–25]. Our study included a relatively young population as most trials had been performed in ambulant boys of 5 years and older. Nonetheless, 10% of children suffered from complications, with one patient having to be hospitalized due to severe rhabdomyolysis. Wound infection is considered a general, although small, risk in any surgical procedure, but this complication was not reported. It is important to communicate the risks well to the caregivers when explaining the clinical trial, and to ensure that the anaesthetic team is aware of the risks in DMD.

Another important point is the stress the biopsy procedure poses to the children and their family. Around half of them had been anxious before the biopsy and had not felt well afterwards, although some also said they had felt relieved the procedure was over. This underscores the important role of professional educational and psychological support for children in paediatric care, and its importance to be considered in the decision to perform biopsies in clinical trials.

4.2. Impact of the biopsy

The muscle biopsy had short-term and long-term impacts on patients and caregivers. On the short-term, most patients experienced pain, which was quite severe in some cases, while

in fact good pain management should avoid (most of) the pain. Here, good communication to the caregivers is again important to raise awareness of this.

Healing of the wound can have a negative impact on daily life. Some boys were unable to go to school and had problems with, for example, showering. From the questionnaire, we were unable to define the extent of this period or to provide a consistent explanation for the variability in the responses here. No clear relationship with the location of the biopsy was reported. The large variability in clinical advice on wound management is striking and it would be interesting to further study the effect of these protocols and if these may be optimized.

In our study, all patients had a visible scar, although a large variation in the length and healing of the scar was observed, and only a subset of patients and caregivers was bothered by it. It became clear that the likelihood of a permanent scar was not always pointed out in the informed consent. Some consent forms understated or even neglected the risk of scarring. As an example one of the participants responded: “*Risks of scarring was stated to be very low however, the biopsy site scars are significant*” and “*The consent document put the risk of scarring, pain and suffering at approx. 20%. It should have been 100%*.” We feel that combining estimated risks of different symptoms does not provide the most adequate type of information, and that information leaflets should explain that scarring and some level of pain will occur after any muscle biopsy. These risks should be pointed out clearly in the informed consent and should be communicated by the clinician who obtains informed consent to set realistic expectations, but also encourage the family to seek for pain management after the procedure. Despite the fact that all patients had a scar, and this bothered a subset of parents and patients, the majority of parents found the benefits of participating in the clinical trial to outweigh the scarring, as they indicated their willingness to be involved in future clinical trials involving a muscle biopsy. Nonetheless, scarring is an important factor too to take into account in the medical-ethical evaluation of such trials. The questionnaire did not include questions if, for example, the location of biopsies from the biceps was chosen to minimize visibility or if treatment with plastic surgery had been offered to patients anywhere in the follow-up. Despite these limitations, we feel that more attention could be paid on how to minimize the cosmetic impact of the scar.

4.3. Informed consent

For many participants understanding of the informed consent is problematic. The consent forms tend to be long, and contain complex medical and legal language. This potentially results in parents not reading or understanding the whole informed consent documents or missing important points. Possibilities to improve the informed consent are to add a simple extract with the main points or use a video format. In addition, sufficient time should be taken to

discuss the procedure, consequences and expectations of the participants.

4.4. Role of biopsies in drug research

Biopsies currently play an important role in therapy development for DMD. Early phase clinical trials often are open-label and biopsies are required to show proof-of-mechanism of test compounds or to find the optimal dose. As such the biopsy analysis is used as a pharmacodynamics biomarker [26]. However, in later phase clinical trials, a placebo group is added and the focus is on showing that longer term treatment with a compound is safe and leads to clinically meaningful effects, in DMD this would be a slower disease progression.

Drug approval is generally not based on biomarker analysis but on functional effects. The approval of eteplirsen by the FDA exceptionally was based on dystrophin analysis in biopsies. However, this approval is conditional. FDA requested that Sarepta provides additional data to confirm functional effects of the drug by 2021. If Sarepta fails to provide the requested confirmatory data, the eteplirsen approval will be revoked. By contrast, the European Medicines Agency (EMA) did not approve eteplirsen based on the same data, since functional data was lacking [27]. Nevertheless this position of FDA on dystrophin as a surrogate biomarker, which has a likely possibility to predict clinical benefit, has set a precedent for several other companies involved in developing other related compounds.

We queried the parents about the acceptance of muscle biopsies in clinical trials. Parents indicated they considered it more justifiable to obtain a biopsy in an open-label trial, where it is certain their child is treated with the active compound, than in a trial in which the child may be receiving placebo. In the latter case, it was more often seen as undesirable to perform such an invasive procedure, more so because the main goal of a placebo-controlled trial is to show functional benefits of the treatments. In fact, confirmation of drug action should already have been established in early phase trials and therefore should not be needed in follow-up pivotal trials in larger patients groups.

4.5. Communication of trial results

Another important aspect for increasing the acceptability of biopsies is the communication about the results of the trial. In many cases parents did not receive such results. From our questionnaire it became clear that most caregivers found it very important to have the results of the trial both in general and of their own son in particular. Although it is appreciated that, especially in small phase 1 and 2 trials, such individual information can be difficult to interpret, careful communication prior to inclusion in the trial is warranted to set realistic expectations about what information they will, and what information they will not receive. In addition, we recommend companies to send out regular newsletters to the trial centres/patients organisations participating in the trial, so

they can update their patients on the progress of the trial. Caregivers prefer to receive regular updates on the status of the trial and the biopsy analysis so they know they have not missed information.

4.6. Future

Considering the invasiveness of the procedure and the usefulness of the results in the approval process, there is a need for less invasive methods to determine efficacy of study drugs. There are studies showing that dystrophin restoration can be measured in smooth muscle layers of the skin [28,29]. As such in the future it may be possible to take skin biopsies rather than muscle biopsies. However, the disadvantage of this approach is that it is possible that the distribution of the therapeutic compound varies between skin and skeletal muscle, which could result in both false positive and false negative conclusions.

Currently, open biopsies are used in most clinical trials. Since the techniques to quantify dystrophin are improving [30], it may be possible to use needle biopsies in the future that obtain smaller amounts of muscle tissue and induce smaller scars. In the immediate future however, it is anticipated that open biopsies are unavoidable in early phase clinical trials for DMD. Although this questionnaire does not allow a direct comparison, biopsies taken from the biceps may be better tolerated than those obtained from other muscles, causing less pain, less impact on daily activities, and better healing of the scar. It should, however, be taken into account that more patients who underwent a biceps biopsy received general instead of local anaesthesia. This may have influenced the level of pain. Furthermore, the low number of patients in the study that received a biopsy from another muscle than the biceps, prevents definite conclusions. Standardising the location, techniques and processing of the tissue, would also be essential for comparison of results between trials.

4.7. Limitations to our study

There are several limitations to our study. Firstly, the study was performed retrospectively. For some participants the biopsy was taken ten years ago, which may make it hard to remember the circumstances at that time. Secondly, some patients have had more than one biopsy. Even though the questionnaire was aimed at the first biopsy, it may have been difficult to separate the feelings and effects of the different biopsies. In addition, the acceptance of further biopsies may also change by the usefulness and results obtained from previous biopsies.

The study was performed in several countries. Thereby responses may be influenced by cultural differences and hampers the uniform interpretation of the answers. Only in the Netherlands, the coverage was around 100%, whereas in other countries it was (much) lower, causing a possible bias.

Finally, some of the subgroups were very small. Therefore it was not possible to perform statistical analysis. Moreover it is difficult to capture individual variation in statistics and

there were several open questions, leaving room for multiple interpretations.

5. Future recommendations

Based on our study we propose several recommendations for future studies. Firstly, sponsors should be very careful when considering to include one or more biopsies in their clinical trial. For early phase trials a biopsy to confirm mechanism of action of a drug may be unavoidable, while for later phase placebo-controlled trials biopsies are considered unethical by part of the caregivers.

Secondly, optimal communication is crucial to explain to caregiver and patients what to expect. Expectation management is crucial. Caregivers need to understand about the risks of the biopsy (anaesthesia-related) and the consequences (scarring). Sponsors should also think ahead of time on which information (aggregated and individual) to communicate to the participants and how to inform participants regularly on updates. Clinicians need to communicate to caregivers that generally the pain can be managed well after the biopsy and to seek medical advice in case patients are in moderate or severe pain.

Thirdly, aesthetic aspects of the scars could benefit from more thorough selection of the location of the biopsy and the consultation of cosmetic surgeons in the longer follow-up.

Finally, caregivers and patients are keen that the biopsy is used optimally. Ideally the informed consent should allow to share data with others to do research or additional analysis when the clinical trial-related analyses have been completed.

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Supplementary materials

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