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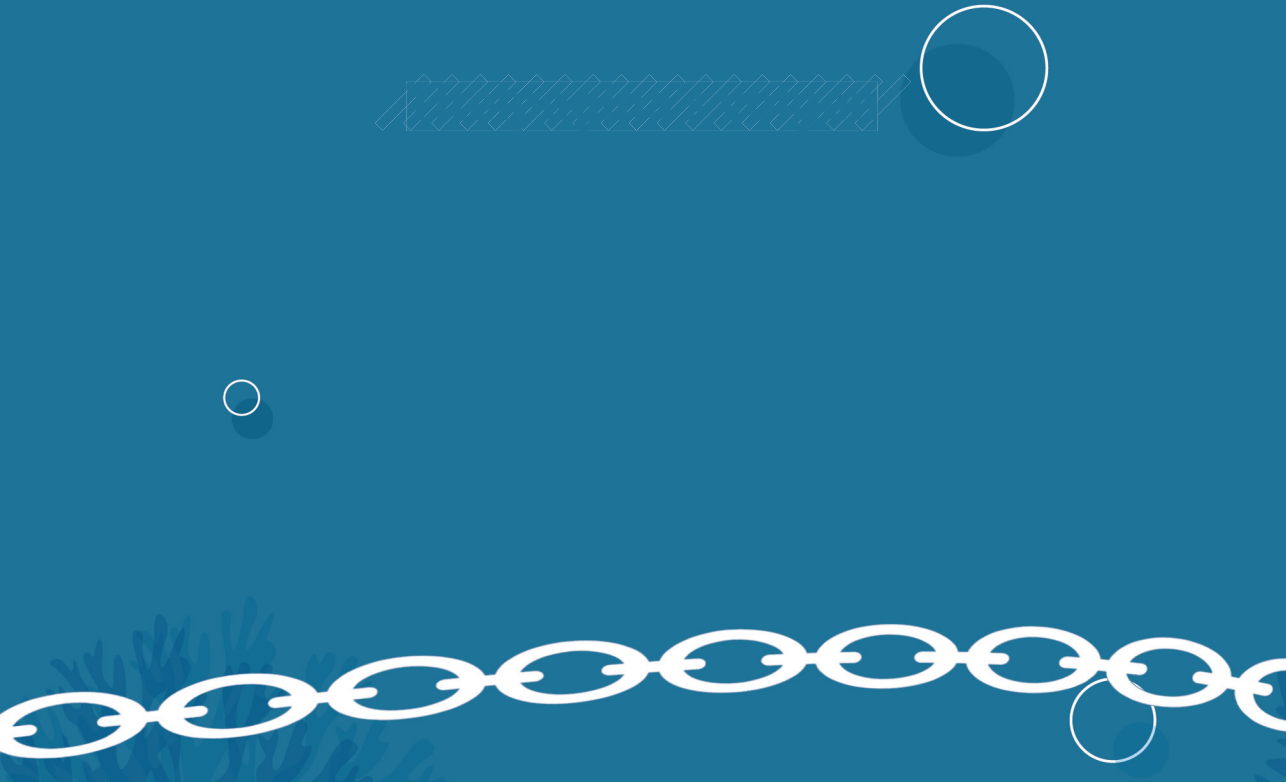
Title: Diversity in disease course of Duchenne and Becker muscular dystrophy

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Forty-Five years of Duchenne muscular dystrophy in the Netherlands



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Abstract

Background

Duchenne muscular dystrophy (DMD) is a progressive muscle disease. No curative therapy is currently available, but in recent decades standards of care have improved. These improvements include the use of corticosteroids and mechanical ventilation.

Objective

To present a detailed population based report of the DMD disease course in the Netherlands (1980-2006) and compare the effect of changes in care by comparing it with an historical Dutch DMD cohort (1961-1974).

Methods

Information about DMD patients was gathered through the Dutch Dystrophinopathy Database using a standardized questionnaire and information from treating physicians.

Results

The study population involved 336 DMD patients (70% of the estimated prevalence), of whom 285 were still alive. Mean age at disease milestones was: diagnosis 4.3 years, wheelchair dependence 9.7 years, scoliosis surgery 14 years, cardiomyopathy (fractional shortening <27%) 15 years, mechanical ventilation 17 years and death 19 years. Within our cohort, corticosteroid use was associated with an increased age of wheelchair dependence from 9.8 to 11.6 years ($p < 0.001$). When comparing the recent cohort to the historical cohort, mean survival improved from 17 to 27 years ($p < 0.001$).

Conclusion

The current study gives detailed information about the disease course of DMD patients, provides evidence for the positive effect of steroid treatment and mechanical ventilation and supports the use of patient registries as a valuable resource for evaluating improvements in care.

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked progressive muscle disease affecting approximately 1:4700 boys.¹⁴⁷ Symptoms generally appear at the age of 2-4 years, when affected boys have frequent falls and show difficulty in rising from the floor and walking stairs. Weakness slowly progresses, leading to wheelchair dependence, loss of upper extremity function, development of scoliosis and cardiac and ventilatory problems. The main causes of death in DMD patients are respiratory and cardiac failure.

Currently, no curative treatment for DMD is available. However, in the last decades significant improvements have been made in the care for DMD patients, improving both quality of life and life expectancy. These changes include the prescription of corticosteroids, the use of cough support and mechanical ventilation, cardiac medication, and several rehabilitation treatments, like splints, airstacking and training. Over the last decades there has been increasing evidence to support these measures. Steroid trials have shown a significant decrease in rate of disease progression as measured by muscle strength and functional tests.³²⁻³⁴ Several observational studies have further strengthened the evidence for use of corticosteroids by showing a delay in the age at loss of ambulation, decrease in the number of patients needing scoliosis surgery and attenuation of the decline in respiratory function.^{4,35-37,148-150} Use of mechanical ventilation has been shown to significantly improve survival in DMD patients from the late teens to the mid-late thirties.^{5,15,42,151}

Given the increasing amount of drugs in a clinical test phase, in depth information of the natural history of DMD patients has become of increasing interest. In the Netherlands, a database containing extensive information of Dutch Duchenne and Becker muscular dystrophy (BMD) patients was set up in Leiden in 2008, as part of the TREAT-NMD patient registry initiative. As genetic diagnostics for DMD are centralized in Leiden, there is a good overview of the size of the Dutch DMD population. Currently, over 70% of known DMD patients have been registered in the Dutch Dystrophinopathy Database (DDD). Therefore, the Dutch database provides an excellent opportunity to present a study of the natural history of DMD. In the current study, we provide a detailed population-based description of the disease course of DMD in the Netherlands. To further investigate the effect of developments in care for DMD patients, we compare our data to a large historical Dutch DMD cohort.

Patients and methods

Patients

Cohort 1 (1980-2006)

Patients were selected from the DDD. Recruitment was achieved through the Dutch patient organizations 'Duchenne Parent Project' and 'Dutch Association for Neuromuscular Diseases', physicians (paediatricians, (paediatric)neurologists, rehabilitation specialists, cardiologists, geneticists as well as physicians working at the four centres for home mechanical ventilation), internet (www.lumc.nl/duchenne) and an

advertisement in a national glossy. Information about deceased patients was gathered through family members. Written informed consent was provided by all patients or their legal representatives. The study was approved by the medical ethical committee of the Leiden University Medical Center.

Inclusion criteria for the present study were: males born between 1980 and 2006 with a definite diagnosis of DMD, as defined by progressive muscle weakness and

- 1) an out-of-frame deletion or duplication in the *DMD* gene, or
- 2) a small deletion/insertion or point mutation in the *DMD* gene resulting in a premature stop codon, or
- 3) a combination of other mutations in the *DMD* gene (in-frame mutations, splice-site mutations) known to cause a severe clinical phenotype as registered in the Leiden Open Variation Database (LOVD) AND age at wheelchair dependence before the age of 13, or
- 4) absence of dystrophin on immunohistochemistry staining of the muscle biopsy.

The 1980 limit was chosen to facilitate comparison with the 1961-1974 cohort by leaving out an arbitrary number of five years (1975-1979) in between the two cohorts. Patients born after 2006 were excluded since at the time of reviewing the data these patients were too young to provide information about the studied disease milestones.

Cohort 2 (1961-1974)

To evaluate the long term effects of the developments in care, data were compared to those of Dutch DMD patients born between 1961 and 1974 as described previously by Van Essen et al.¹⁵² From this historical cohort only patients with *definite* DMD, as defined by Van Essen et al, were selected.¹⁵³ This definition was based on a scoring system involving data regarding clinical picture, creatine kinase level, electromyogram, muscle biopsy, electrocardiogram and inheritance.

Medical history

Information on disease course was provided by patients and their caretakers through a standardized questionnaire. Self-reported information was gathered about height and weight, education, age at diagnosis, age at loss of ambulation, occurrence of scoliosis surgery and age at surgery, age at start of mechanical ventilation, use of corticosteroids and cardiac medication and age at death. Z-scores for height and BMI were calculated using standards for Dutch boys as published by Fredriks et al.^{154,155}

Cardiac analysis

Written reports on echocardiographic studies were retrieved from (paediatric)cardiologists concerned with the care of DMD patients. Our analysis was focussed on the left ventricular fractional shortening (FS) as this provides an important measurement of the existence and severity of a cardiomyopathy. Additionally the FS was considered relatively robust, given the fact that patients were seen in different centres over the years. Cardiomyopathy was defined as a $FS \leq 27\%$.¹⁵⁶

DNA analysis

Information about mutational analysis was retrieved from the department of Clinical Genetics of Leiden University Medical Center, where all analyses on Dutch patients had been performed. DNA analysis was performed as previously described by Van den Bergen et al and Almomani et al.^{157,158}

Statistical analysis

Mean/median age at reaching the different disease milestones was estimated using a Kaplan Meier Survival Analysis. Differences in patient characteristics between steroid treated and steroid naïve patients were calculated using the Students T-test. Differences in ages at wheelchair dependence between different sites of mutation as well as between steroid users versus non-steroid users were calculated with a Kaplan Meier Survival Analysis using the Log Rank Test. The correlations between birth year and age at diagnosis, between the age at reaching the different disease milestones and between the age at first cardiac examination and Fractional Shortening were calculated using Pearson's Correlation Coefficient. Significance level for all analysis was set at 0.05. Analyses were performed using SPSS Statistics version 20.

Results

Patients

The DDD contains 462 patients with a clinical diagnosis of DMD, of whom 377 have a DNA-confirmed diagnosis. Three-hundred thirty-six patients met our inclusion criteria. Number of patients per year of birth ranged from 7 (1984) to 24 (1996) (Figure 1). Forty-one patients had died at the time of the study, at a mean age of 19 years (± 5.6). Mean age of patients that were still alive was 15 years (± 6.6). The standard-

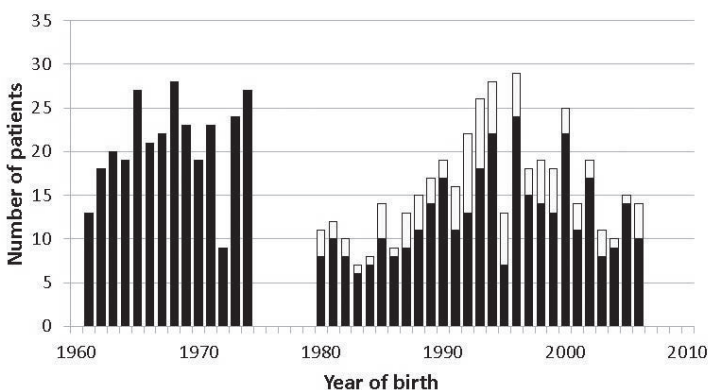


Figure 1. Study population.

Included DMD patients categorized by year of birth for both the historical (1961-1974; n=293) and the present cohort (1980-2006; n=336). The white part of the columns represents DNA-confirmed DMD patients not participating in the present study. For the historical cohort, these data were not available.

Table 1. Characteristics of the 1980-2006 study population for all patients together, as well as separately for steroid-treated and steroid-naïve patients.

	All patients (n=336)		Steroid-treated patients (n=165)		Steroid-naïve patients (n=150)		<i>p-value</i>
	<i>n</i>	<i>Mean (SD)</i>	<i>n</i>	<i>Mean (SD)</i>	<i>n</i>	<i>Mean (SD)</i>	
Year of birth	336	1994 (7.1)	165	1998 (4.3)	150	1990 (6.6)	<0.001
Age (years)	295	15 (6.6)	165	12 (4.3)	124	20 (6.6)	<0.001
Height (z-score)	224	-0.75 (1.4)	140	-0.53 (1.3)	83	-1.12 (1.5)	
BMI (z-score)	201	0.92 (1.5)	140	1.2 (1.4)	60	0.37 (1.6)	
Age at death (years)	41	19 (5.6)	0	-	26	19 (5.9)	
		<i>Percentage</i>		<i>Percentage</i>		<i>Percentage</i>	
Chronic Pain	241	23	143	19	97	29	
Fractures	256	33	150	25	105	45	
Use of laxatives	256	27	149	22	106	36	
PEG tube	241	23	148	1	103	11	

Of 21 patients steroid use was unknown.

ized height corrected for age of patients still alive was below the average of the general Dutch male population (z-score -0.75) for both steroid users and non-users, while BMI was above average (z-score 0.92). See Table 1 for patient characteristics.

Disease course

In our cohort the mean age at diagnosis was 4.3 years (± 2.2). Two-hundred-fifteen patients had become wheelchair dependent at a mean age of 9.7 years (± 1.8). When including the still ambulant patients, the median estimated time to wheelchair dependence was 10.0 (± 0.16) years. Information about scoliosis surgery was present for 315 patients, of whom 102 patients (32%) had scoliosis surgery, at a mean age of 14 years (± 2.1). Mechanical ventilation (day and/or night) was initiated in 93 patients at a mean age of 17 years (± 3.7), of whom 17 patients had a tracheostomy, while 31 patients used a mouthpiece and/or (oro)nasal mask. Of the other 43 patients the form of mechanical home ventilation was not known. The median age of survival without mechanical ventilation was 20 years (± 0.53). Patients who had scoliosis surgery used mechanical ventilation at a significantly younger age than patients who did not have scoliosis surgery (18 versus 23 years; $P < 0.001$). This difference remained significant when excluding steroid-treated patients. Age at wheelchair dependence predicted the age at scoliosis surgery and age at start of ventilatory support: patients who lost ambulation at a younger age also had scoliosis surgery at a younger age ($R 0.54 P 0.0003$) and used ventilatory support earlier ($R 0.57 P < 0.0001$). This correlation remained significant when correcting for steroid use ($P 0.001$ and $P < 0.001$ resp). The mean age at death of the 41 deceased patients was 19 years (± 5.6), whereas the estimated mean age of survival was 27 years (± 0.49). Causes of death were not provided. Figure 2 shows the range of ages at which all disease milestones were reached.

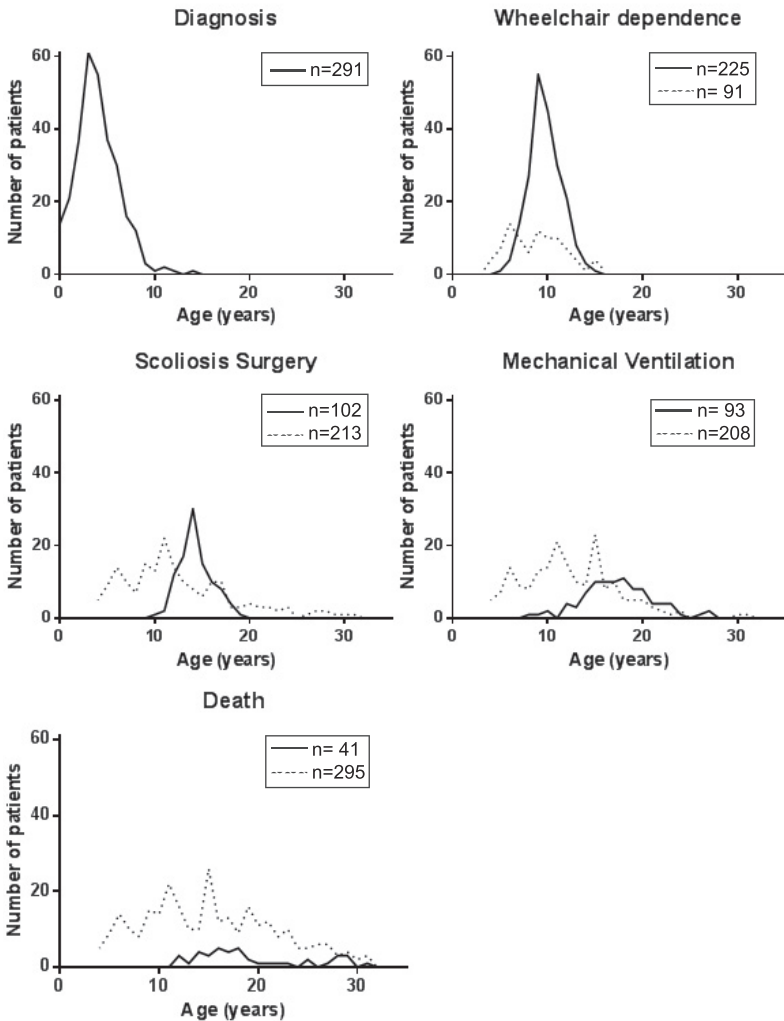


Figure 2. Disease milestones in DMD.

Age at which disease milestones are reached for the 1980-2006 cohort. The continuous lines represent the age at which patients reached the specific milestone, while the dotted lines represent the current ages of patients who have not yet reached the milestone.

Education

Data about education was present for 247 patients (74%). One-hundred-fifteen patients (47%) attended a regular elementary school, while 78 patients (32%) attended a school for chronically ill children. Fifty-four patients attended a school for children with behavioural difficulties or attended multiple types of schools consecutively. There was a difference in educational level at secondary school when comparing our DMD cohort with the general Dutch population (Practical education 16% versus 9%, Lower general

Table 2. Educational level of the 1980-2006 DMD cohort.

Educational level	n	%
Elementary school		
1. Regular elementary school	115	46
2. School for chronically ill children	78	32
3. School for children with behavioural difficulties	7	3
4. Multiple schools (1&2 or 1&3)	47	19
Secondary school (pt>12yrs)		
1. Practical education	18	16
2. Lower general secondary education	48	43
3. Higher general secondary education	12	11
4. Pre-university education	7	6
5. Other	4	4
6. None	24	21
Professional education (pt>18yrs)		
1. Vocational education	15	29
2. University of Applied Sciences	1	2
3. University	1	2
4. None	34	67

secondary education 43 versus 24% and Higher general secondary education/Pre-University education 41 versus 17%; data provided by Statline, Statistics Netherlands, www.cbs.nl). Twenty-four patients older than twelve years (21%), did not attend any secondary school. Of the 51 patients over 18 years of age of whom data about education was available only 17 (31%) followed vocational/college/university training. See Table 2 for all data about education.

Cardiac analysis

Data regarding fractional shortening was present for 133 patients, of which 97 patients had multiple measurements (range 2-20). Median age at first analysis was 8 years (range 3-26). There was a significant negative correlation between age at first examination and FS ($R = -0.64$ $P < 0.0001$), indicating the progressive nature of cardiac involvement in DMD (Figure 3A). Survival analysis showed an estimated mean time to cardiomyopathy ($FS \leq 27$) of 16.4 years (Figure 3B). Data concerning cardiac medication was present for 269 patients. Of those 49 (17%) used cardiac medication: 41 (84%) an ACE-inhibitor, 18 (37%) a beta-blocker, 10 (20%) a diuretic and 4 patients (8%) an angiotensin-II-antagonist. Only three patients used oral anticoagulation and only two patients an anti-arrhythmic agent. Median age of patients on cardiac medication was 18.5 years (range 5-29).

Corticosteroids

One-hundred-sixty-five patients (49%) used steroids at some point, of whom 46 (14%) had ceased to use steroids at the time of the study. In 21 patients steroid use was unknown. The mean age at start of steroid use was 6.5 years, while mean age at which patients ceased steroid use was 9.5 years. The steroid

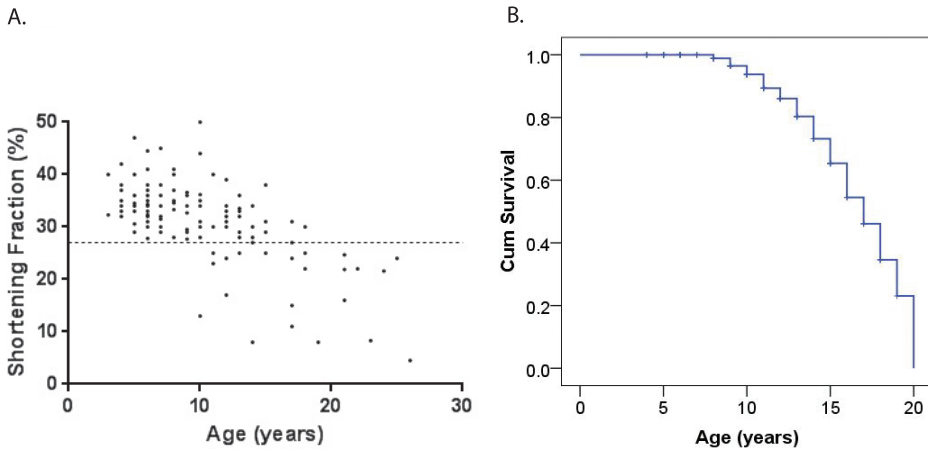


Figure 3. Echocardiographic examination of DMD patients in the 1980-2006 cohort. A. Fractional Shortening of patients at first echocardiographic examination (n=133). The dotted line represents a fractional shortening of 27%, often used as the cut-off value to diagnose a cardiomyopathy. B. Survival analysis for cardiomyopathy, as defined by a fractional shortening equal to or less than 27% (n=108).

treated patients were significantly younger than the steroid naïve patients (mean age 12 versus 20 years; $P < 0.001$) (Table 1). Figure 4 represents the percentage of steroid users per birth year. To investigate the possible effect of steroids on age at wheelchair dependence patients were divided into two groups: 1) patients who did not use steroids prior to wheelchair dependence (n=175) 2) patients who started using steroids at least two years before losing ambulation and continued steroids for at least one year (n=99). Patients who had used steroids for less than one year or who started less than two years before loss of ambulation, as well as patients who could not be classified (n=62) were excluded.

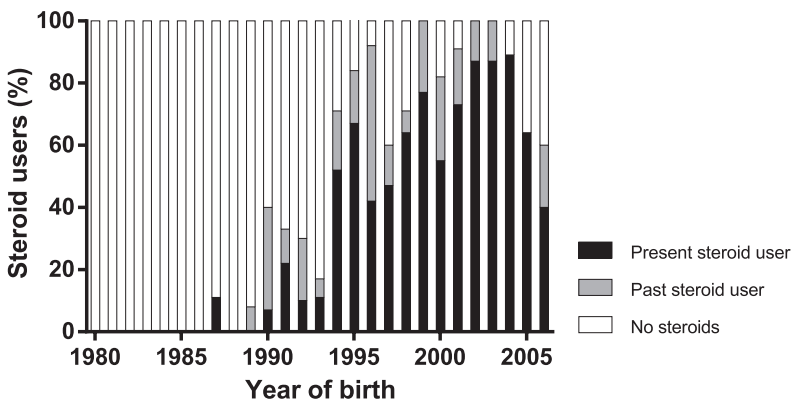


Figure 4. Percentage of steroid users among DMD patients per year of birth. The light columns represent patients who previously used steroids, but have ceased to do so, the dark columns represent patients currently using steroids.

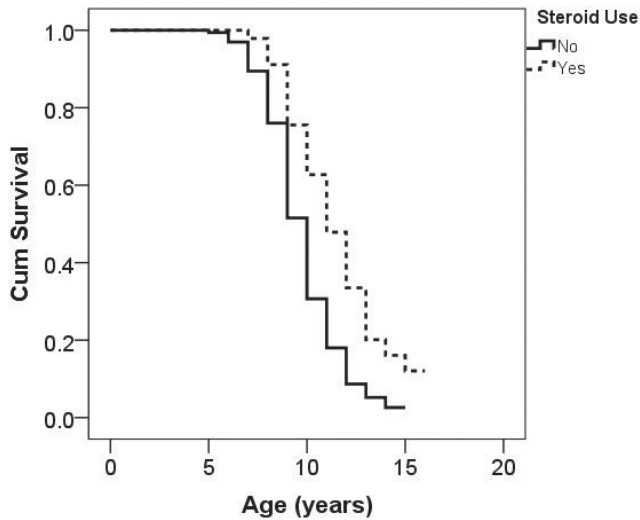


Figure 5. Age at wheelchair dependence.

Kaplan Meier Survival Curve for age at wheelchair dependence for steroid-treated (dotted line) and non-treated (black line) patients in the 1980-2006 cohort.

The limits were chosen to ensure that steroid use had been long enough to influence ambulation. The effect of steroid use on the other disease milestones could not be analysed, as too few patients in the steroid treated group had reached these milestones (scoliosis surgery 14/122, mechanical ventilation 9/120, death 0/124).

As expected, use of steroids had a positive effect on ambulation: median age at wheelchair dependence increased from 10 years to 11 years ($P < 0.001$) in steroid treated compared to steroid-naïve patients (Figure 5).

Mutation analysis

A deletion of one or several exons was present in 212 patients, of whom 204 had an out-of-frame deletion. The eight patients with an in-frame deletion presented with a severe phenotype consistent with the inclusion criteria. A duplication was found in 42 patients, a premature stopcodon in 49 and splice-site mutations in 18 patients. Of 12 patients the exact mutation was not known. Three patients were diagnosed on the basis of the absence of dystrophin staining in a muscle biopsy.

Most mutations ($n=243$) were located in the central rod domain (exons 8-61), while 21 mutations involved only the actin binding site (exons 2-8). Thirty-three mutations involved both the actin binding site and the central rod domain. Mutations at the 3'-end of the *DMD* gene were rare: only 4 patients presented with a mutation in the cysteine-rich domain (exons 63-69) and 3 patients with a mutation in the carboxy-terminal domain (exons 70-79). In one patient the mutation involved both these regions (an exon 61-76 deletion).

Comparison with cohort 1961-1974

Data were present for 293 DMD patients born between 1961 and 1974. The median birth year was 1968. Number of patients per year ranged from 9 (1972) to 28 (1968) (Figure 1). Ninety-eight patients had died at the time of data collection in 1998.

Mean age at diagnosis for the 1961-1974 cohort was 5.3 years (± 2.2). There was a significant correlation between year of birth and age at diagnosis ($R -0.32 P < 0.001$). Data about wheelchair use were present for 248 patients, of whom 232 were wheelchair dependent. The mean age at loss of ambulation for these patients was 8.9 years (± 2.7). When comparing age at wheelchair dependence between the two cohorts, patients born between 1980 and 2006 lost ambulation at a significantly later age than those born between 1961 and 1974 (survival-analysis 10.4 versus 9.6 years $P < 0.001$) (Figure 6A). Interestingly, this difference remained significant when excluding steroid treated patients (9.8 versus 9.6 years $P 0.046$). No comparison for steroid treated patients was possible, since none of the patients in the 1961-1974 cohort had used steroids.

The mean age at death of the 98 deceased DMD patients in the 1961-1974 cohort was 16 years (± 2.9). Mean survival was significantly higher in the 1980-2006 cohort than in the 1961-1974 cohort (27.3 versus 17.7 years $P < 0.001$) (Figure 6B).

No information about scoliosis surgery, mechanical ventilation or education was present for this cohort.

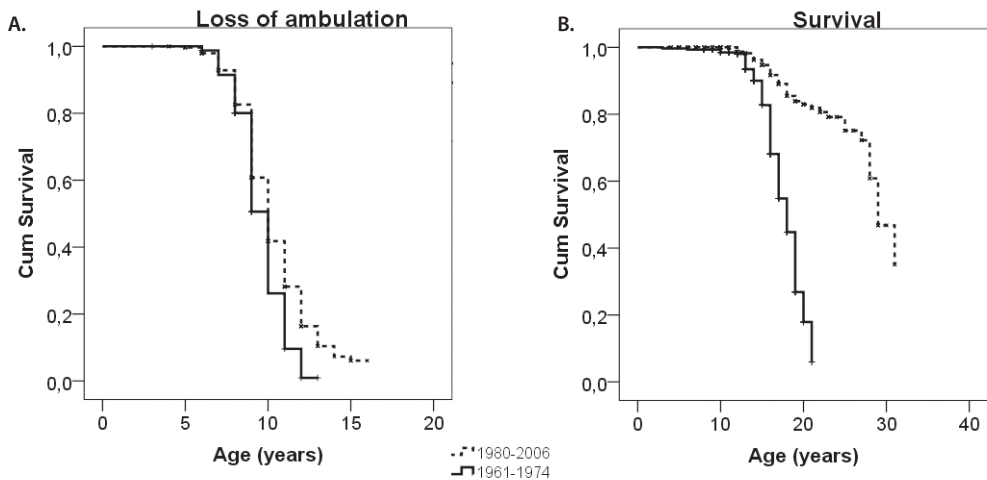


Figure 6. Comparison of disease course between the 1961-1974 and 1980-2006 birth cohorts. Kaplan Meier Survival Curve of age at loss of ambulation (A.) and age at death (B.) for all patients from both cohorts.

Discussion

In the current study we present detailed clinical information about a large Dutch DMD cohort and compare this information with an historical Dutch DMD cohort. Several studies regarding disease course in DMD have been published, but often cover only a small part of all patients in a country or region.

Our data present a cohort from one and the same country including more than 70% of all Dutch DMD patients.

Comparison of the present cohort with the historical data revealed several improvements in diagnosis and care. First of all, mean age at diagnosis decreased from 5.3 years historically to 4.3 years. This age is in accordance with previous (smaller) studies, showing mean ages at diagnosis between 3.2 and 4.8 years of age.^{9,159-161} Interestingly, most of this decrease occurred before the introduction of standard DNA-diagnostics for DMD in the Netherlands, illustrating the power of clinical diagnosis of DMD and the possible increasing awareness of the diagnosis. Although diagnosis is made at a younger age, there is still time to gain in diagnosing DMD, both to provide good care for the patient in question as well as to counsel the parents regarding further family planning. With the development of therapies, early diagnosis will become increasingly important.

Secondly, we noticed a prolonged survival in the second cohort. Dutch patients born in the 1960s hardly ever reached the age of 20 years (15%), while this improved to 89% for patients born in the 1980s. The mean survival has improved with 10 years. This change is mostly due to the introduction of mechanical ventilation in the late 1980s, which in two large studies have shown to increase survival with 6 to 20 years.^{5,7} We were unable to investigate the effect of steroid use on survival, as steroids were only prescribed routinely in the Netherlands since 2005. Previous smaller studies, however, showed a clear positive effect of steroid use on age at start of mechanical ventilation.^{4,34,36,37,148-150} It would be of great interest to confirm these results in a large population based study.

Next, when comparing our cohort to the historical cohort, there is a significant later age at wheelchair dependence. This difference is mostly due to the introduction of steroids. In our cohort there was a significant effect of steroid use on ambulation, retaining ambulation for an average of nearly two years. Interestingly, when excluding all steroid treated patients, a small (2-3 months) but significant difference in age at wheelchair dependence still remained. This might represent the effect of improvements in care, like the use of more advanced walking aids, prevention by splints, more consistent use of physiotherapy and Achilles tendon lengthening.

In our cohort, survival analysis of age at wheelchair dependence showed a mean age of 10.4 years. This is in concordance with a previous report by Magri et al, but somewhat higher than other reports.^{5,7,10,14,162} This difference might be explained by different percentages of steroid users between these cohorts.

As previously reported, age at wheelchair dependence was positively correlated with age at scoliosis surgery and start of mechanical ventilation.^{6,12,162} This indicates that the disease course of some patients is more severe. Alternatively, it could be that there is a protective effect of maintenance of ambulation on disease progression.

In our cohort, 32% of patients had scoliosis surgery, at a mean age of 14 years (46% when excluding patients <12 years). When comparing different cohort studies in DMD, these percentages vary greatly, ranging from 4 to 77%.^{6,8,12,13} There are several factors that could contribute to these differences. Firstly, indications for scoliosis surgery vary between different countries and also have changed in the last decades. Furthermore, not having scoliosis surgery does not necessarily mean a milder scoliosis, as in

some cases there are contra-indications for surgery (most importantly a decreased pulmonary function and the presence of a cardiomyopathy).⁹ Lastly, several studies have shown steroid use to reduce the number of scoliosis surgeries.^{4,9,35,36,148} As the percentage of steroid users might vary between the different studies, this could influence the total number of surgeries within the cohorts.

In our DMD cohort, patients who had scoliosis surgery were in need of mechanical ventilation at a significantly younger age than patients who did not have surgery. Several previous studies found a trend showing scoliosis surgery being correlated with a faster decline of Forced Vital Capacity (FVC) or a younger age at start of mechanical ventilation.^{6,12,163} Again, this could be explained by the possibility that scoliosis surgery is one of the markers of a more progressive disease.

Cardiac analysis showed presence of a cardiomyopathy in 35% of patients. The estimated mean time to cardiomyopathy was 16.4 years. Unfortunately, due to heterogeneity of our steroid data, we were unable to investigate the possible effect of steroids on the development of cardiomyopathy. Previous reports, however, have shown a positive effect of steroids on cardiac function.^{36-38,164}

In our cohort, steroid use was associated with an increase in BMI, but no reduced growth, when compared to steroid naïve patients. Most Dutch patients on steroids (>80%; data not shown) use a 10-days-on-10-days-off regimen.¹⁶⁵ Previous studies have shown that this regimen has less deleterious effects on growth and weight than the daily regimen.^{39,40} Our study confirms the limited effect of this regimen on growth.

Our study has several limitations. Firstly, our data represent about 70% of the Dutch DMD population. Although this is a large percentage, there still is the possibility of a selection bias. Most importantly, there could be an underrepresentation of patients with a severe disease course, who might have died at an early age. However, through the involvement of patient organisations, media and specific information for surviving relatives of deceased patients, we have spent much effort into including these patients as well.

A second limitation of our study is the strict use of inclusion criteria for the diagnosis of DMD. Especially in the 1980s, when DNA-diagnostics were not available, diagnosis of DMD was based on clinical characteristics, combined with high CK-levels and an X-linked inheritance. Therefore, it could be that some patients were erroneously excluded from the study. Lastly, only younger patients in our cohort have been treated with steroid. Therefore, the effect of steroid use on disease milestones like mechanical ventilation and age at death could not be compared between the two cohorts.

In conclusion, our study presents important data about the current disease course of the Dutch population of DMD patients, supporting the positive effect of steroid use on delay of disease progression and of mechanical ventilation on survival. Interestingly, we also found a discrete positive effect of improvements in standards of care on age at wheelchair dependence. These findings emphasize that databases like the DDD are of great importance, not only as a resource for future trials, but also to evaluate the effect of developments in care and treatment.

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