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Cardiorespiratory progression over 5 years and role of corticosteroids in DMD: a single site retrospective longitudinal study

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Cardiorespiratory progression over 5 years and role of corticosteroids in DMD: a single site retrospective longitudinal study

Running title: Corticosteroids in DMD cardiorespiratory progression

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Disclosure

Dr. Trucco, Dr. Tay, Dr. Ridout, Dr. Maresh, Dr. Munot, Dr. Sarkozy, Dr. Robb, Dr. Quinlivan, Ms. Riley, Prof. Burch, Dr. Fenton, Dr. Wallis, Dr. Chan, Dr. Abel and Dr. Manzur report no disclosures.

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Abbreviations list

CS Corticosteroids

DMD Duchenne muscular dystrophy

FVC%P Forced Vital Capacity percentage of predicted

FVC Absolute Forced Vital Capacity

LVFS % Left Ventricular Fractional Shortening

LoA Loss of ambulation

ABSTRACT

Background. Duchenne muscular dystrophy (DMD) boys treated with corticosteroids (CS) have prolonged survival and respiratory function when compared to CS-naïve.

Research question The differential impact of frequently used corticosteroids and their regimens on long-term (>5 years) cardiorespiratory progression in DMD children is unknown

Study Design and Methods. Retrospective longitudinal study including DMD children followed at Dubowitz Neuromuscular Centre (Great Ormond Street Hospital London), May 2000-June 2017. Patients enrolled in any interventional clinical trials were excluded.

We collected patients' anthropometrics, respiratory (forced vital capacity, FVC% predicted and absolute FVC, non-invasive ventilation requirement, NIV) and cardiac (left ventricular shortening function, LVFS%) function. CS-naïve patients had never received CS. CS-treated took either deflazacort or prednisolone, daily or intermittently (10 days on/10 days off) for >1 month. Average longitudinal models were fitted for yearly respiratory (FVC%P) and cardiac (LVFS%) progression. A time-to-event analysis to FVC%P<50%, NIV start and cardiomyopathy (LVFS<28%) was performed in CS-treated (daily and intermittent) vs CS-naïve patients.

Results There were 270 patients, mean age at baseline 6.2 ($\pm$2.3) years. Median follow-up 5.6 ($\pm$ 3.5) years. At baseline, 263 were ambulant. Sixty-six were CS-daily, 182 CS-intermittent >60% treatment, 22 CS-naïve.

Yearly FVC%P declined similarly from 9 years (5.9% and 6.9%/year, p=0.27) in CS-daily and CS-intermittent. CS-daily declined from a higher FVC%P than CS-intermittent (p<0.05) and both reached FVC%P<50% and NIV requirement at similar age, >2 years later than CS-treated.

LVFS% declined by 0.53%/year in CS-treated irrespective of CS regimen, significantly slower (p<0.01) than CS-naïve progressing by 1.17%/year. Age at cardiomyopathy was 16.6 in CS-treated (p<0.05) irrespective of regimen and 13.9 years in CS-naïve.

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Interpretation. CS irrespective of their regimen significantly improved respiratory function

and delayed NIV requirement and cardiomyopathy.

Clinical trial registration. N/A

INTRODUCTION

Cardiorespiratory complications have a major impact on survival of Duchenne muscular dystrophy (DMD) patients. Along with anticipatory cardiorespiratory care [1] [2], long-term corticosteroids (CS) [3] have prolonged DMD patients' survival [4] [5] and delayed cardiomyopathy [6-8]. While the rate of progression of cardiomyopathy in steroids naïve patients is known, the extent of protection provided by steroids is debated, and the difference of the 2 regimens unknown.

The role of steroids on DMD respiratory function is also an unresolved issue. Previous studies reported a similar respiratory decline in CS-treated and CS-naïve patients (*NCT01027884*) [9] [10] and in patients treated with different steroid treatments[11], others showed that CS-treated DMD boys aged 7-18 years maintained higher percentage-predicted forced vital capacity (FVC%P) than age-matched CS-naïve boys [12] [13] and reached FVC<50% predicted and absolute FVC<1L later [14].

CS regimens most commonly used are daily and intermittent (most patients on 10 days on/10 days off; in the past also 10 days on/20 off). The intermittent regimen was proposed to limit the severity of chronic CS-related side effects.

We hypothesized that the two mostly used CS, deflazacort and prednisolone, administered intermittently or daily, would differentially affect the cardiorespiratory progression (FVC%P and left ventricular shortening fraction, LVFS%) and the age to meaningful cardiorespiratory endpoint (FVC<1 L, NIV requirement and LVFS%<28%) in a large UK paediatric cohort of DMD. We additionally hypothesized that the cardiorespiratory progression and the age at cardiorespiratory endpoint would be different in CS-treated and CS-naïve patients.

METHODS

Study design

Retrospective study of paediatric DMD patients (aged <18years) followed at the Dubowitz Neuromuscular Centre (Great Ormond Street Hospital, London) from May 2000 to June 2017. We included patients whose parents consented to the North Star database. UK national Ethics Committee and Institutional Review Board approved the North Star UK Network for data collection and the conduct of research studies within the Network.

Patients enrolled in any interventional clinical trials were excluded. Patients in the Heart Protection Trial ("A double-blind randomised multi-centre, placebo-controlled trial of combined ACE-inhibitor and beta-blocker therapy in preventing the development of cardiomyopathy" *EudraCT2007-005932-10*)[15] were further excluded from the cardiac analyses (eFigure 1).

Patients Characteristics and Genotyping Information

All information was collected from medical records. The first visit recorded for each patient at the enrolment of the study was defined "baseline". Clinical visits were carried out six montly from five years of age onwards. Lung function was performed at every visit, while echocardiogram yearly. Height was assessed standing for ambulant, or calculated from arm span in non-ambulant patients. Ambulatory status was recorded at each visit. Loss of ambulation (LoA) was the inability walking independently for 10 meters. Scoliosis was defined as a Cobb angle $>20°^1$ from spine x-ray . Age of scoliosis surgery was collected. None of the patient enrolled were on ventilator support (NIV) at baseline until the time to the primary respiratory endpoint. No patient were on any cardiac medication at the baseline visit. Dystrophin (*DMD*) gene mutations were analysed by multiplex ligation-dependent probe amplification, polymerase chain reaction or direct sequencing. We stratified patients based on

their lack of dystrophin isoforms. Dp427, produced in skeletal and cardiac muscle, is affected by all mutations. The shorter isoforms are produced by promoters spread along *DMD* gene. Patients carrying mutations in exons 1 to 79, 30 to 79, 45 to 79, 56 to 79 and 63 to 79 respectively lack Dp427, Dp260, Dp140, and Dp116 and Dp71. Dp116 is expressed in cardiac muscle and peripheral nerve [16-18] and Dp71 in lung, skeletal and cardiac muscle besides brain and kidney [19-22]. Cardiorespiratory progression was analysed in patients lacking Dp71 and Dp116 [17] and in patients amenable to exon 44, 45, 51 or 53 skipping [23-25].

CS regimens

CS-naïve patients had never received CS therapy. CS-treated patients took either daily or intermittent CS (10 days on/10 days off) for >1 month. CS consisted of prednisolone 0.9 mg/kg or deflazacort 0.75mg/kg. The CS dose was collected throughout the study period for all visits. There was a slight difference in the management of CS throughout the study. CS dose was adjusted for weight and tapered down when patients reduced their ability to walk up to a minimum dose of prednisolone 0.3 mg/kg and deflazacort 0.4 mg/kg. The boys who had mixed steroids or regimens were defined "switchers". For them we explored two CS and regimens definitions to compare daily vs intermittent. As per previous work by Ricotti et al. we have defined, for each patient, either patients' treatment at study baseline or the majority CS regimen they were treated with. We have considered for each patient the total duration of the observation and considered the regimen he was treated with for ≥60% observation time[26]. Results were similar and we have presented the most clinically relevant majority treatment, defined in the manuscript "CS-daily" and "CS-intermittent". Patients' treatment were labelled "Deflazacort" or "Prednisolone" based on the majority CS. Patients whose CS information was missing were called "not known". They were excluded from the CS regimens comparison. For patients who stopped CS during the study, only data prior to stopping was included.

Respiratory status outcomes

Spirometry was performed in seated position according to ERS/ATS guidelines [27]. Absolute FVC in litre (L) was collected and FVC%P calculated according to reference data [28].

We considered the age when FVC%P<50% as the main respiratory endpoint [1] and the age to absolute FVC<1L and requirement of non-invasive ventilation (NIV) as secondary endpoints. Absolute FVC< 1 L is known to predict nocturnal hypoventilation [29][30].

The yearly progression of FVC%P and FVC and the time to clinically meaningful respiratory endpoints were compared between CS regimens and between CS-treated and CS-naïve.

Cardiac status outcomes

The Left Ventricular Fractional Shortening (LVFS%) was used for the cardiac progression analysis. LVFS% was defined as the change in diameter of the left ventricle between the contracted and relaxed states [31]. LVFS% was used as more easily available and less prone to inter scorer variability than Simpson Left Ventricular Ejection Fraction (LVEF%) in patients such as DMD with a poor echogenic window[32].

We considered as main cardiac endpoint the onset of cardiomyopathy, defined as LVFS<28%. This threshold has been previously considered as clinically meaningful in several studies focused on cardiac function in DMD and other muscular dystrophies [7][32].

The yearly progression of LVFS% and the time to cardiomyopathy were compared between CS regimens and between CS-treated and CS-naïve.

We recorded the use and the age at start of ACE-inhibitors and β-blockers. They were started by the cardiology team based on patients' cardiac function and clinical symptoms (See Supplementary).

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Statistical analysis

Characteristics of the sample are presented as mean (SD), median (range or interquartile range) for skewed data and frequency (percentage) for categorical data.

For LVFS% and FVC%P we describe the longitudinal trajectories and estimate the mean annual change using mixed effects regression models, accounting for the longitudinal data and age at baseline. Models were fitted including patient as a random effect and CS regimen (intermittent or daily) and treatment (deflazacort or prednisolone) as fixed effects, using an unstructured correlation matrix. For FVC%P we considered the decline after the age of 9 years onwards, as respiratory capacity continues to increase until up to this age. We compared rates of decline between steroid regimens in a separate set of models according to patients' amenability to exon- 44, 45, 51 and 53 skipping, using appropriate interaction terms. Results are presented as mean annual change, or difference in mean annual change between subgroups, with 95% confidence intervals.

Using Kaplan Meier analysis we estimated the median age at which clinically meaningful endpoint occurred: loss of ambulation, scoliosis, NIV, cardiomyopathy (LVFS<28%), FVC%P <50% and FVC <1L. We used Cox regression analysis to investigate whether the average age at which these events occurred varied according to majority steroid and regimen through the inclusion of an interaction term and hazards ratios with 95% confidence intervals are presented. We compared the estimated age at respiratory and cardiomyopathy endpoint by Dp71 and Dp116 isoform-deficiency. The proportional hazards assumption was checked for all Cox models, by inspection of log-log plots and formal testing of Schoenfeld residuals. We present estimated median time to event only where this assumption was unclear.

All analyses were conducted in Stata v15 with significance level of $p < 0.05$.

RESULTS

Study population

There were 270 patients, with a mean of 8 visits per patient. Mean age at baseline visit was 6.2 (± 2.3) years, mean follow-up 5.6 (± 3.5) years. Seventy-seven boys (29%) transitioned to adult care, 36 (13%) were lost to follow-up. Seven boys (2%) died, mean age 16.5 (± 3.8) years, 1 for cardiomyopathy, 1 after general anesthesia, no information for 5. At the time of death, 3 patients had stopped CS and 4 were still CS-treated (2 CS-daily, 2 CS-intermittent) (Table 1).

At baseline visit, 263 boys (97%) were ambulant, mean age 6.0 (±2.1) years. Seven (3%) were non-ambulant, mean age 11.5 (±2.9) years. At last assessment, 140 (52%) patients were ambulant. Median (IQR) age at LoA was 12.1 (4.5) years in the whole population, 12.5 (5.7) years in CS-daily, 12.0 (4) years in CS-intermittent, 10.5 (2.1) years in CS-naïve patients. CS-naïve lost ambulation at similar age of CS-daily (p= 0.09) and CS-intermittent (p= 0.34). Fifty-seven patients (21%) had scoliosis. Five had scoliosis already at baseline, 52 developed scoliosis throughout the study. The median age of scoliosis was 17.1 years in the whole population, 17.1 years in CS-treated, 13.9 years in CS-naïve (p=0.18) (Table 2 and eFigure2).

CS duration and regimens

Sixty-six of 270 (24%) patients were on CS-daily, 182 (67%) on CS-intermittent, 22 (8%) CS-naïve. In the cardiac cohort, 52 of 229 (23%) patients were on CS-daily, 156 (68%) on CS-intermittent, 21 (9%) CS-naïve.

Thirty-seven boys (14%) stopped CS, median age (IQR) 10.1 (5) years. Five were CS-daily prior to stopping and their reasons were unavailable, 32 were CS-intermittent. One stopped due to behavioral issues, one to weight gain and one to blood pressure increase, information was missing for the remainder. In the cardiac cohort, 33 patients stopped CS.

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Two-hundred four of 270 (75%) were on prednisolone, 36 (13%) were on deflazacort for ≥60% of treatment. Twenty-five switched compound, all from prednisolone to deflazacort.

Respiratory status

Progression of FVC%P and FVC

FVC%P slowly increased with age then started declining linearly from age 9 years. In the whole population the yearly decline was by 6.1%/year, 95%CI (5.6, 6.6). CS-daily had the fastest FVC%P decline of 6.9% per year, 95% CI (-7.7, -6.0). These patients progressed by an extra 1% per year than those on majority intermittent CS. There was no difference between regimens (p=0.27) (Figure 1a).

Data on absolute FVC progression according to CS treatment is shown in the Supplementary. In the whole population, the mean age at peak FVC%P before declining was 9.7 (±3.4) years. It was similar between regimens and in CS-treated vs CS-naïve. Conversely, the peak FVC%P value before the decline was significantly higher in patients on CS-daily (90.8%) than CS-intermittent (83.9%, p<0.01). The FVC%P, being affected by patients' height, was significantly higher in CS-daily than CS-intermittent unlike the absolute FVC. Since patients on CS-daily experience a more severe height restriction (up to 1.8 cm per year [33]) than CS-intermittent [26], their FVC%P may be artifactually higher.

CS-naïve had a FVC%P decline of 4.7%/year, 95%CI (2.8, 6.6), not different than CS-treated (p=0.15) but CS-treated peaked up to a significantly higher FVC%P than CS-naïve (68.9%, p<0.01).

Age at respiratory endpoints

Fifty-two patients fell to FVC%P<50%. Twelve were CS-daily, 34 CS-intermittent, 6 CS-naïve. Median age at FVC%P<50% was similar (p=0.86) between regimens (16.1 years CS-daily in and 16.3 years in CS-intermittent). The median age at FVC%P<50% was

significantly lower (p=0.04) in those treated with deflazacort compared to prednisolone (15.4 vs 16.8 years) HR 2.3, 95%CI(1.03, 5.31) (Figure 2 and eFigure 3a).

Absolute FVC fell below 1 L in 11 patients (4%), 2 of 66 (3%) were CS-daily, 6 of 182 (3%) were CS-intermittent and 3 of 22 (14%) were CS-naïve. In CS-daily and CS-intermittent FVC fell below 1L after 18 years.

Twenty of 270 (7%) required NIV. Five of 66 were CS-daily, 12 of 182 CS-intermittent and 3 of 22 were CS-naïve. Less than 25% patients on any CS regimen required NIV by 18 years (Figure 3).

CS-naïve reached FVC%P <50% at a median age of 13.2 years and FVC <1L at median age of 17years, significantly earlier (p<0.01 and p<0.05) than CS-treated. CS-naïve required NIV at a median age of 15.7 years, earlier than CS-treated.

Cardiac status

Progression of LVFS%

Two-hundred twenty-nine patients were included. The yearly decline of LVFS% was 0.67%/year, 95%CI (0.55, 0.79) p<0.001 in the whole population adjusted for age at baseline. Cardiac function decline was not different between CS regimens (p=0.59) (Figure 1b).

LVFS% yearly decline was 1.17%/year, 95%CI (0.79, 1.55) in CS-naïve and 0.53%/year 95%CI (0.40, 0.67) in CS-treated (p<0.01).

Age at cardiomyopathy

Sixty (22%) patients had cardiomyopathy (LVFS%<28%), six had it already at baseline, 54 developed it during the study. Ten were CS-daily, 41 CS-intermittent, 9 CS-naïve. Median age at cardiomyopathy was 16.6 years in CS-treated and was similar between regimens (p=0.45). The median age at cardiomyopathy for patients on prednisolone was 16.6 years. Less than 25% patients on deflazacort had cardiomyopathy by 18 years of age, HR 0.74, 95%CI(0.27, 2.08). Age was not different (p=0.57) according to CS treatment (Figure 4 and

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eFigure 3b). CS-naïve developed cardiomyopathy at 13.9 years of age HR 2.2, 95% CI (1.1, 4.6), earlier (p<0.05) than CS-treated. See Supplementary for further details on cardiac medications.

Genotype/phenotype correlation

Children amenable to exon 44 skipping had a slower respiratory decline (4.5% per year) than patients not amenable to skip of exon 44 (p<0.05). Respiratory decline was not different in patients amenable to skip 45, 51 and 53 compared to the remaining patients. There was no difference in decline of cardiac function according to amenability to skip of any exon.

Eighteen (7%) and 28 (10%) patients had mutations causing Dp71 and Dp116 shorter dystrophin isoform deficiency. FVC%P <50%, absolute FVC <1L, age at NIV and cardiomyopathy were similar in patients lacking Dp71 and Dp116 isoforms compared to the children expressing them.

DISCUSSION

Corticosteroids are the current standard mutation-independent treatment for DMD. The impact of CS regimen and compound on long-term cardiorespiratory function is unknown.

So far, the comparison between deflazacort and prednisolone on respiratory function in 60 DMD (age 5-24 years) found no differences in yearly progression of FVC%P according to treatment [11]. Other studies have instead focused on the impact of CS on delaying respiratory deficiency than no treatment, yet providing controversial results. The respiratory decline in DMD seems in fact affected by variables only partially addressed by CS, as age, ambulation and additional co-morbidities (poor swallowing, ineffective cough) affecting intrinsically the lung. In the placebo arm of DELOS trial, non-ambulant CS-naïve DMD (n=33, mean age 15 years) had a similar FVC%P decline >8% over one year as those on previous CS [9 34]. In 91 non-ambulant DMD men (mean age 16.8 years) respiratory function declined at a similar rate in CS-treated and CS-naïve [10]. In younger DMD, instead, CS positively acted on lung function by reaching higher peak FVC%P than CS naïve before the onset of respiratory decline. In 397 DMD aged 7 to >20 years the FVC%P remained significantly higher in boys CS-treated than CS-naïve at all ages. We can postulate that CS positive effect on diaphragmatic function led to greater lung function [35]. While in the age range 7-10 years FVC%P declined slower in CS-treated than CS-naïve (0.69% vs 5.9%), FVC%P yearly progression was similar in boys aged 10-18 years (5.44% vs 6.06%)[14]. All these results suggested that CS delay the onset of respiratory decline and the achievement of respiratory milestones (FVC<1 L) but do not slow down its progression once decline is started [4 14].

Because the standard of care for DMD have changed in the last years and virtually no CS-naïve exist anymore, the main aim of our work was to identify the impact of different CS regimens and compounds on yearly FVC%P progression. In our population, CS-daily treated DMD reached a peak FVC%P 10% higher than CS-intermittent and 22% higher than CS-

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naïve predicted before the decline (eFigure4). CS-daily and CS-intermittent led to a similar extent of delay in FVC%P <50% and in NIV requirement of respectively 3 years and >2 years compared to CS-naïve. In addition, our results showed that patients on deflazacort reached FVC%P <50% over 1 year earlier than those on prednisolone (15.4 vs 16.8 years). Previous report on ambulation and timed motor outcomes, carried out over a shorter period and/or on younger patients, found a more preserved function in DMD treated with deflazacort [36-38]. Our data suggests that deflazacort is effective on respiratory function in short/medium term but that its long-term efficacy might be inferior compared to prednisolone in the late stages, probably as a result of the more severe growth restriction induced by this drug.

The existing studies on cardiac function in DMD have demonstrated the beneficial role of daily CS over no treatment. A cross-sectional study demonstrated significantly higher LVFS% in CS-treated (n=48) vs age-matched CS-naïve DMD boys (n=63)[8]. Daily CS significantly reduced LVFS% decline over 5 years in CS-treated (n=14) vs CS-naïve (n=23) DMD boys[7]. CS duration delayed cardiomyopathy in DMD by 4% per year (n=462) [6]. Finally, daily CS treatment was associated with fewer heart failure-related deaths (0% vs 22%) and a slower LVFS% decline (-0.32% vs -0.65%) in 63 CS-treated vs 23 CS-naïve DMD [5]. CS duration was associated with a lower age-related fibrosis at cardiac MRI [39]. In 174 DMD boys cardiomyopathy was associated with age and clinical stage but not with CS treatment [40]. Our long-term data on a wide DMD population confirms the cardio-protective effect of CS[41] adding that CS, particularly daily, delayed the onset of cardiomyopathy through slowing down cardiac decline. To the best of our knowledge our findings showed for the first time that the cardio-protective effect was longer-lasting in CS-daily versus CS-intermittent patients and that, in contrast with respiratory data, patients on deflazacort developed cardiomyopathy later than those on prednisolone (>18 vs 16.6 years). DMD boys

CS-naïve developed cardiomyopathy significantly younger than CS-treated (13.9 vs 16.6 years).

These results support the administration of CS after LoA in DMD. However, the side effects caused by the prolonged use of CS reported by other studies within UK North Star Network should not be underestimated. Daily CS had stronger effect than intermittent on ambulation, but negatively affected behaviour, growth and BMI [26]. Similarly, DMD on CS-daily (deflazacort) had a significantly higher bone fractures rate than CS-intermittent. Of note, vertebral fractures further affect height [42].

Long-term cardiorespiratory trajectories according to amenability to exon skipping have implications for ongoing trials and will help the design of future studies. Patients amenable to exon 44 skip have better walking distance and slower decline than others [24] and lose ambulation later [23] [25]. We demonstrate for the first time that these patients also have a slower respiratory function decline ($p<0.05$). There were no significant differences in time to cardiomyopathy or respiratory failure in boys lacking Dp71 (6%) or Dp116 (9%) compared to those expressing them. We had hypothesized that a Dp71deficiency could have a protective role in dystrophic heart; and previous studies suggested a protective role of Dp116 deficiency. The overexpression of AAV mediated-Dp71 worsened *mdx* mouse phenotype by competing with utrophin in its binding to dystrophin-associated protein complex [43]. The previously reported protective role of Dp116 deficiency on heart function in 181 DMD boys was not supported by our results on a wider cohort [17].

In our population 10.6% CS-daily, 24.2% CS-intermittent and 27.3% CS-naïve developed scoliosis. The small numbers of events did not allow a time-to-event analysis. The percentage of scoliosis in our CS-naïve is lower than previously reported [44] due to our more stringent definition. The majority of patients CS-naïve were enrolled in the first five years of the study.

A more pro-active indication to spinal surgery and the availability of new techniques in recent years may explain why in our cohort none of CS-naïve underwent surgery by 18 years of age.

Our long-term real world data are novel and were collected over > 5 years in a single centre within the North Star UK database. The only ongoing randomised trial, the FOR-DMD [45] will address the question of which regimen is more effective. Patients were enrolled at 4-7 years of age. They could still be too young to reach cardiorespiratory endpoint.

The main limitations of this study are its retrospective and monocentric design. The imbalance in cohorts sample size, with a lower numbers of CS-naïve boys, might have affected our results. We have included assessments only conducted in a single tertiary site by the same highly skilled operators to limit the risk of bias, which is however inevitable. The collection of long-term data over 17 years was potentially affected by changes in the standard of care in DMD. In the most recent 7 years of study CS were stopped after LoA in 14% vs 42% in the previous 7 years. When we ran sensitivity analyses adjusting for date of visit in the mixed models this had minimal, non-significant impact on estimated coefficients of interest, therefore we presented results for models without this factor. The use of the CS regimen administered over the majority of the study to minimise the weight of switchers, has previously proven effective[26]. The use of arm span used as surrogate of height after LoA could have affected the FVC%P at the time point of switch. However, the results on FVC absolute matched with those on FVC%P. The use of cardiac medications could have potentially influenced the cardiac progression, but >90% patients started cardiac medication after the diagnosis of cardiomyopathy.

INTERPRETATION

Our data confirm the long-term beneficial effect of corticosteroids on respiratory and cardiac function in 270 DMD, irrespective of regimen. CS-daily treated DMD reached a significantly higher FVC%P than CS-intermittent before decline but a similar yearly FVC%P decline. There was no difference in the age at clinically meaningful respiratory thresholds (FVC%P <50% and NIV requirement) according to CS-regimen. DMD on CS-daily and CS-intermittent had a similar rate of cardiac decline that resulted in a delayed onset of cardiomyopathy (2.7 years) compared to CS-naïve.

Further work is needed to evaluate the differential role of CS in older non-ambulant patients, particularly in view of the evidence for their positive effects on cardiac function.

Guarantor statement: Prof Francesco Muntoni takes responsibility for the content of the manuscript, including the data and analysis.

Disclosure

Dr. Trucco, Dr. Tay, Dr. Ridout, Dr. Maresh, Dr. Munot, Dr. Sarkozy, Dr. Robb, Dr. Quinlivan, Ms. Riley, Prof. Burch, Dr. Fenton, Dr. Wallis, Dr. Chan, Dr. Abel and Dr. Manzur report no disclosures or financial relationships to declare.

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Name	Location	Role	Contribution
Federica Trucco, MD	Dubowitz Neuromuscular Centre, UCL, London University of Genoa	Author	Design and conceptualized study; analyzed the data; drafted the manuscript for intellectual content
Joana Domingos, MD	Dubowitz Neuromuscular Centre, UCL, London	Author	Major role in the acquisition of data, design of the study
Chee Geap Tay, MD	University of Malaya	Author	Major role in the acquisition of data, design of the study
Deborah Ridout, PhD	UCL, London	Author	Design and conceptualized study; analyzed the data;
Kate Maresh, MD	Dubowitz Neuromuscular Centre, UCL, London	Author	Interpreted the data; revised the manuscript for intellectual content
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TABLE

Table 1. Clinical and genetic features of study population (N=270) and cardiac cohort (N=229).

	Total population		Cardiac cohort[d]	
	N=270 (%)	Mean age (SD)	N=229 (%)	Mean age (SD)
Age diagnosis	255	4.5 (2.3)	216	4.4 (2.4)
Age first visit	270	6.2 (2.3)	229	6.2 (2.3)
Age last visit	270	12.1 (4.0)	229	11.9 (4.2)
Age of starting CS	248	6.2 (1.7)	208	6.3 (1.8)
CS[a] regimen (≥60% treatment)				
- Daily	66 (25)	5.8 (1.4)	52 (23)	5.8 (1.5)
- Intermittent[b]	182 (67)	6.4 (1.8)	156 (68)	6.4 (1.8)
- Naïve	22 (8)		21 (9)	
Deflazacort (≥60% treatment)	36 (12.3)		43 (17.1)	
Prednisolone (≥60% treatment)	204 (69.6)		166 (65.9)	
CS regimen and compound (n=240)				
- Daily Deflazacort	14 (4.8)		14 (5.6)	
- Intermittent Deflazacort	22 (7.5)		29 (11.5)	
- Daily prednisolone	50 (17.1)		38 (15.1)	
- Intermittent prednisolone	154 (52.6)		127 (50.4)	
Stopped steroids	38 (13)		33 (13)	
- Daily	5		2	
- Intermittent	32		30	
- Not Known	1		1	
Steroid switchers				
- Daily to Intermittent	0			
- Intermittent to daily	39 (12.5)	8.9 (2.2)		
Amenable to exon skipping				

Exon 44	20 (7.4)		16 (7.0)	
Exon 45	23 (8.5)		21 (9.2)	
Exon 51	29 (10.7)		24 (10.5)	
Exon 53	21 (7.8)		20 (8.7)	
Mutations leading to lack of Dys Isoforms				
Dp427	270 (100)		229 (100)	
Dp116	28 (10)		27 (18)	
Dp71	18 (7)		18 (8)	

[a]CS:Corticosteroids.

[b]Intermittent regimen: 10 days on/10 days off steroids.

[d]Cardiac cohort: patients in the Heart Protection Trial ("A double-blind randomised multi-centre, placebo-controlled trial of combined ACE-inhibitor and beta-blocker therapy in preventing the development of cardiomyopathy in genetically characterised males with DMD without echo-detectable left ventricular dysfunction") were excluded from the overall population for cardiac progression analyses.

Table 2. Ambulatory status and scoliosis of study population

	N=270 (%)	Mean age (SD)
Ambulatory status		
Ambulant at baseline	263 (97.4)	6.0 (2.1)
Not ambulant at baseline	7 (2.6)	11.5 (2.9)
		Median age at LoA[a](IQR)
Not ambulant at last follow-up	128/268 (47.8)	12.1 (10.0, 14.5)
- Daily	28/65 (43.1)	12.5 (10.0, 15.7)
- Intermittent	88/181 (48.6)	12.0 (10.0, 14.0)
- Naïve	12/22 (54.6)	10.5 (9.1, 11.2)
Scoliosis		
Scoliosis at baseline	4/269 (1.5)	13.1 (1.0)
		Median (IQR)
Scoliosis	57 (21.1)	17.1 (13.7, *)
- Daily	7/66 (10.6)	-
- Intermittent	44/182 (24.2)	15.5 (13.5,*)
- Naïve	6/21 (28.6)	13.9 (12.7, *)
Scoliosis surgery	16/269 (5.9)	
- Daily	1/66 (1.5)	
- Intermittent	15/182 (8.2)	
- Naïve	0/21	

*Median age to events was calculated estimated by Cox regression. * not possible to estimate*

Corticosteroid treatment: regimen used for ≥60% total CS treatment duration.

[a]LoA: loss of ambulation.

FIGURES LEGEND

Figure 1. Slopes of annual respiratory and cardiac progression according to CS regimen

a. FVC% predicted decline in CS-daily, CS-intermittent, CS-naïve DMD patients Linear population average model of respiratory function progression expressed as FVC% predicted according to CS regimen after the age of nine years. In the whole population FVC% predicted declined linearly by 6.1% per year, 95% CI (- 6.6, - 5.6). FVC% predicted declined by 4.7% per year, 95% CI (- 6.6, -2.8) in CS-naïve. There were no differences in the yearly rate of decline with between CS-naïve and CS-treated patients (p=0.15).

b. LVFS% decline in CS-daily, CS-intermittent, CS-naïve DMD patients. Linear population average model of cardiac function progression expressed as Left Ventricular Shortening Fraction (LVFS %) according to CS regimen. In the whole population LVFS % declined by 0.67%, 95% CI (0.55, 0.79) per year. CS-naïve boys had a LVFS % decline of 1.17%/year, 95%CI (-1.55, -0.79). Patients on any CS progressed by 0.53%/year, 95% CI (-0.67, -0.40), slower than CS-naïve patients (p<0.01). There was no difference in daily and intermittently treated patients (p=0.59)

Figure 2. Time to respiratory failure defined as FVC% predicted < 50% according to CS regimen and compound

a. Time to reach FVC% predicted <50% according to regimen. Median age at FVC% predicted <50% was 13.2 years in CS-naïve patients. It was lower than CS-daily (16.1 years, p<0.01) and intermittent (16.3 years, p=0.001). Age at FVC% predicted <50% was similar between the two CS regimens (p=0.86).

b. Time to reach FVC% predicted <50% according to steroid compound. The median age at FVC% predicted <50% was significantly lower (p=0.04) in those treated with deflazacort compared to prednisolone (15.4 vs 16.8 years) HR 2.3, 95%CI(1.03, 5.31)

Figure 3. Time to respiratory clinically meaningful endpoints, absolute FVC < 1 litre and non-invasive ventilation (NIV) requirement according to CS regimen

a. Time to reach absolute FVC < 1L. Eleven of 270 patients (4%) had absolute FVC <1 litre. Two of 66 (3%) were CS-daily, 6 of 182 (3%) CS-intermittent and 3 of 22 (14%) were CS-naïve. CS-naïve patients reached absolute FVC<1 L at a median age of 17 years, earlier than those on CS-daily (p=0.04) and CS-intermittent (p=0.01) who fell below 1L after 18 years.

b. Time to Non-Invasive ventilation (NIV) requirement. Twenty of 270 (7%) required NIV. Five of 66 (8%) were CS-daily, 12 of 182 (7%) CS-intermittent and 3 of 22 (14%) were CS-naïve. CS-naïve boys required NIV at a median age of 15.7 years, while less than 25% of patients on any CS regimen required NIV at 18 years of age.
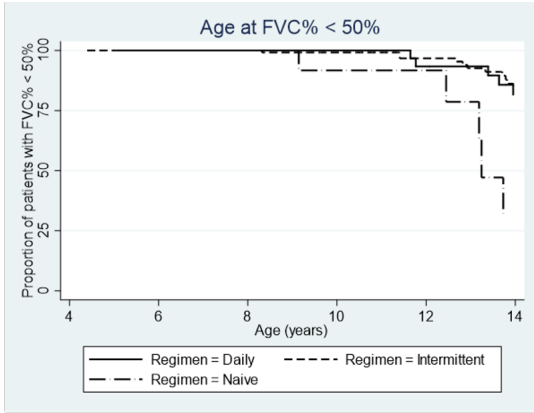
Figure 4. Time to cardiomyopathy defined as left ventricular shortening fraction (LVFS%) < 28% according to CS regimen and compound

a. Age at onset of cardiomyopathy according to regimen. Median age was 13.9 years in CS-naïve and 16.6 years in CS-treated boys (p<0.05). There were no differences in age at cardiomyopathy between CS regimens (p=0.45).

b. Age at onset of cardiomyopathy according to steroid compound. The median age at FVC% predicted <50% was not different according to CS treatment (p=0.57). The median age at cardiomyopathy for patients on prednisolone was 16.6 years. Less than 25% patients on deflazacort had cardiomyopathy by 18 years of age, HR 0.74, 95%CI (0.27, 2.08).
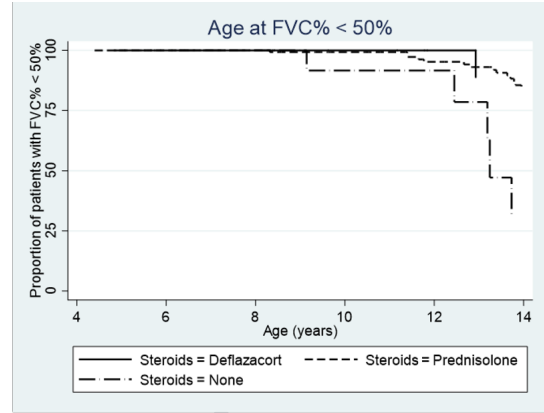
Patients who started ACE-Inhibitors (4/82) and Beta-blockers (3/37) prior to the onset of cardiomyopathy were included.

a.

FVC% decline by steroid regime

b.

Fractional shortening decline by steroid regime
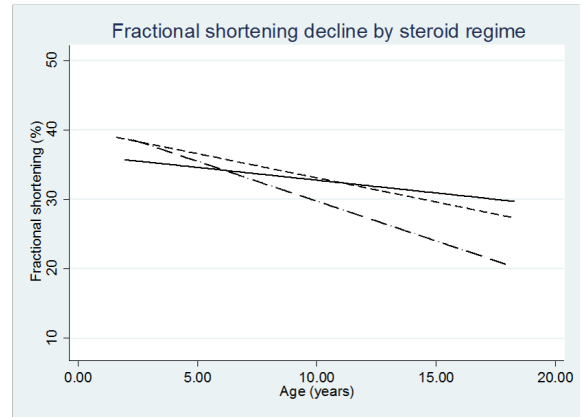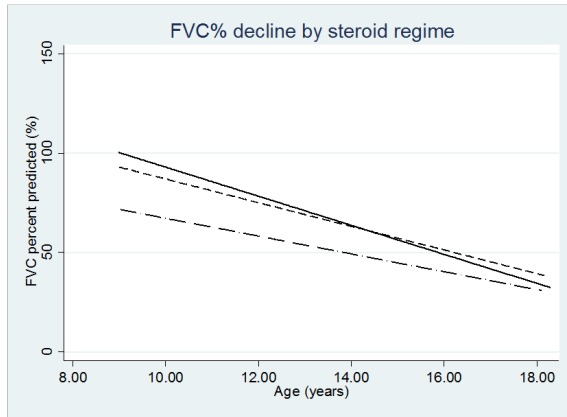
Regimen = Daily   Regimen = Intermittent
Regimen = Naiive

a.

b.

a.

b.

a.

b.