Original Article

Development and Validation of an Outpatient Clinical Predictive Score for the Diagnosis of Duchenne Muscular Dystrophy/ Becker Muscular Dystrophy in Children Aged 2–18 Years

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Abstract

Introduction: There is no bedside clinical examination-based prediction score for Duchenne muscular dystrophy/Becker muscular dystrophy (DMD/BMD) in children with neuromuscular diseases (NMDs) presenting with proximal limb-girdle weakness. **Methods:** We compared the details of 200 cases of lower motor neuron type of weakness and had some proximal limb-girdle muscle weakness and divided them into 2 groups: with/without a confirmed diagnosis of DMD/BMD. We determined the predictive factors associated with a diagnosis of DMD/BMD using multivariate binary logistic regression. We assessed our proposed prognostic model using both discrimination and calibration and subsequently used the bootstrap method to successfully validate the model internally. **Results:** A total of 121 patients had DMD/BMD and the rest of the patients had other diagnoses. Male gender, presence of Gower's sign, valley sign, toe walking, calf pseudohypertrophy, and tongue hypertrophy were independent predictors for a confirmed diagnosis of DMD/BMD and included in the final CVT_2MG score (Calf pseudohypertrophy, Valley sign, Toe walking, Tongue hypertrophy, Male gender, and Gower's sign). The final model showed good discrimination (AUC = 87.4% [95% CI: 80.5–92.3%, $P < 0.001$]) and calibration ($P = 0.57$). A score of 6 or above appeared to be the best cutoff for discriminating between the DMD/BMD group and the rest of the group with both sensitivity and specificity of 98%. The interrater reliability was almost perfect between two pediatric neurologists and strong between a pediatric neurologist and a pediatric neurology trainee resident ($k = 0.91$ and 0.87). **Conclusion:** The CVT_2MG score has good sensitivity and specificity in predicting a confirmed diagnosis of DMD/BMD in subsequent tests.

Keywords: Limb-girdle muscle weakness, lower motor disease, muscular dystrophy, neuromuscular disease, proximal weakness

Introduction

Dystrophinopathies are hereditary neuromuscular disorders that follow an X-linked recessive pattern of inheritance and present commonly with either more severe Duchenne muscular dystrophy (DMD) or less severe Becker muscular dystrophy (BMD) phenotype.[1,2] Mutations in the dystrophin gene lead to either absence of dystrophin or structural defects of this protein leading to a lack of functional dystrophin. This, in turn, impairs the structure and function of myofibres.[3] Children with DMD usually become symptomatic between 3 and 6 years of age, with frequent falls while walking, difficulty in getting up from a supine position, difficulty in rising from a squatting position, and difficulty in climbing stairs.[4] The weakness progresses and most of these children become nonambulatory by 12 years of age usually, without any definitive treatment like ataluren or eteplirsen.[5] Although DMD/BMD is the most common cause of hereditary proximal limb-girdle muscular weakness, several other diseases like congenital muscular dystrophies (CMDs), certain limb-girdle muscular dystrophies (LGMDs), spinal muscular atrophy (SMA) type III, Pompe's disease, dermatomyositis, and polymyositis often mimic the clinical presentation of DMD/BMD.[6] Even certain endocrinal illnesses like hypothyroidism can present with calf hypertrophy and proximal weakness.[7] While almost all the CMDs and LGMDs have no definite cure available and only supportive care can be provided to affected subjects, corticosteroids can alter the disease progression rate in patients with dystrophinopathy.[8] Currently, exon-skipping medications are also available for a significant subset of DMD patients.[9] The most common mutation in DMD/BMD patients is usually deletions in some of the 79 exons in the dystrophin gene, which can be identified by techniques such as

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multiplex ligation-dependent probe amplification (MLPA).[10] While screening for DMD/BMD is often considered one of the first-line investigations in male patients with chronic progressive proximal limb-girdle weakness, other causes also need to be considered by clinicians.[11] While the majority of patients show classical signs of calf pseudohypertrophy, Gower's sign, etc., often nonambulatory DMD patients show atrophy of most of the muscles.[12] Gower's sign is often difficult to elicit in nonambulatory patients and accurate history is sometimes not forthcoming, especially in children from poor socioeconomic status.[13] On the other hand, young children in the age group of 3–5 years may present merely with frequent falls, without other classical signs of DMD.[14] Many patients with BMD, who have insidious onset weakness and calf pain starting from adolescence, may not show Gower's sign and may mimic metabolic myopathy or LGMD.[15] Serum creatine kinase (CK) is often raised beyond 10 times the upper limit of normal values in patients with DMD, but patients with sarcoglycanopathy, dysferlinopathy, and certain CMDs often have elevated serum CK in the same range.[16] Nonambulatory DMD cases with atrophy of most of the muscles may also have normal serum CK.[17] Thus, mere elevation of serum CK can't be considered as a sensitive and specific predictor of DMD or BMD. Due to these reasons, a clinical predictive score taking into account patients belonging to wide age group up to 18 years, which has good sensitivity and specificity and can be applied easily in outpatient within few minutes, will be helpful for clinicians.

Methods

We developed and validated a clinical predictive score for predicting the diagnosis of DMD/BMD correctly in children of age 2–18 years, between August 2019 and April 2022. We have reported the methods of this study according to the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis reporting guideline.[18]

At first, we scrutinized the initial data for duplicate entries by checking with at least three unique identifiers in the form of the name, age, and Universal Health Identity Number provided at our institute. We included all the consecutive children of 2–18 years of age who presented with lower motor neuron type of weakness, satisfying the criteria for neuromuscular disease (NMDs) in the INCLEN Diagnostic Tool for Neuro-motor Impairment tool and had at least some proximal limb-girdle muscle weakness.[19] We excluded those patients who did not have an established definitive diagnosis and whose caregivers refused consent. We excluded patients younger than 2 years of age, although a significant subset of patients with DMD can have a history of motor developmental delay and typical symptoms of DMD can rarely present in subjects younger than 2 years of age, but almost all subjects with DMD present to clinical practice after 2 years of age in our setting (a tertiary-care center in low-middle income countries [LMIC] like India). At the outset, we decided that we will allow a maximum of 10 variables in the clinical predictive score we intended to develop. With a prespecified subject-to-item ratio of 20, we decided to enroll 200 patients satisfying inclusion criteria after taking approval from institutional ethics committee and an informed consent from the parents.

Then, we divided enrolled patients in our study into two groups, one group, who had a confirmed diagnosis of DMD/BMD (MLPA for deletions in 79 exons of dystrophin gene or dystrophin gene sequencing), and another group, who had other confirmed diagnosis (LGMD, inflammatory myositis, SMA type III, congenital myopathy/muscular dystrophy, Emery–Dreifuss muscular dystrophy, late-onset Pompe's disease, steroid-induced myopathy, myopathy associated with thyroid disease, and chronic inflammatory demyelinating polyradiculoneuropathy [CIDP]). We differentiated DMD and BMD based on the following features clinically: DMD patients usually have an age of onset before 6 years of age, with relatively rapid progression of the disease, and become nonambulatory by 12–15 years of age, without definitive treatment like eteplirsen or ataluren. In doubtful cases, we also utilized mutation characteristics as most patients with DMD harbor protein-truncating or frameshift mutations in the dystrophin gene.

At the outset by reviewing the published literature, two pediatric neurologists (PKP and IKS) made a checklist of possible clinical predictors of DMD/BMD clinically such as the age of onset, gender, Gower's sign, calf pseudohypertrophy, toe walking, pelvic lordotic gait, valley sign, tongue hypertrophy, thenar hypertrophy, hypertrophy of deltoid and infraspinatus, proximal weakness more than distal weakness in limb muscles, more severe lower limb involvement than upper limb involvement, absence of scapular winging, weakness of neck flexor muscles, presence of intellectual disability, autistic features, motor developmental delay, absence of facial, ocular, bulbar involvement, clinical evidence of myocardial dysfunction in advanced cases, more significant hip abductor weakness, loss of ambulation by adolescence (for DMD), and sleep-disordered breathing. Apart from these, we also noted other sociodemographic details like residence, socioeconomic status, age of presentation, and clinical details like the pattern of weakness in the upper limb, lower limb, and other body parts. We also noted the severity of functional limitation of participants using the muscular dystrophy functional rating scale (MDFRS), North Star ambulatory assessment scale, Brooke's and Vigno's functional rating scale for upper and lower limbs timed 10-m walk/run, timed rise from supine (time taken to perform Gower's sign), manual muscle testing (percentage medical research council [MRC2]), and joint range of movement.[20–23] Regarding laboratory parameters, we recorded the abnormalities in electromyography and nerve conduction study (NCS), serum CK level, cardiothoracic ratio in chest X-ray, evidence of left and right ventricular hypertrophy in electrocardiogram or abnormalities in heart rate, PR interval, or QRS complex, abnormalities in echocardiogram (ejection fraction [EF], end-systolic and end-diastolic diameter and volume of left

ventricle), abnormalities in pulmonary function tests (in forced vital capacity [FVC] (percent predicted for height and height) and peak expiratory flow rate [PEFR]), evidence of osteopenia/impaired bone health, abnormalities in genetic testing, including details of mutation, abnormalities in muscle magnetic resonance imaging, and dystrophin status in muscle biopsy whenever performed. Treatment details including corticosteroid dose and duration, side effects and tolerance, and other medications used were also noted. For diagnosing and managing NMDs, we followed standard recommendations. Being a resource-constrained setting, our center was unable to provide medications like eteplirsen and ataluren for DMD.[24,25] As the majority of dystrophinopathy patients in our study were of DMD phenotype, we noted the age of loss of ambulation or becoming wheelchair dependent, although this was not relevant for dystrophinopathy manifesting carriers or patients with BMD phenotype. The predesigned structured questionnaire was first filled by a pediatric neurologist and the pieces of information in which they were considered for multivariate analysis and calculating the prediction score. Another pediatric neurologist and pediatric neurology trainee also filled that questionnaire, which was later used to estimate the prediction score by two separate observers to determine interrater reliability only, but not for other statistical analysis purposes.

Statistical analysis

Statistical analysis was performed using the software "Statistical Package for Social Sciences" version 29 (SPSS-29, IBM, Chicago, USA). Continuous variables were presented either as mean/standard deviation or median/interquartile range (IQR), depending on whether the variable is normally distributed or not (Z score of skewness within ±3.29). Categorical variables were presented as frequency (in percentage) with a 95% confidence interval (CI). Initially, all the possible predictors listed above were subjected to univariate analysis to shortlist the predictors associated with a confirmed diagnosis of DMD/BMD. While a Chi-square test/Fisher exact test was chosen for categorical variables, an independent sample t-test or Mann–Whitney U test was used for continuous variables in this univariate analysis, and those with P values less than 0.05 were considered to be significant predictors. These were subsequently subjected to multivariate binary logistic regression analysis to determine independent predictors.

We decided if the number of independent predictors exceeded the maximum number of candidate predictors allowed for our sample population, keeping in mind the events per variable rule (1 candidate predictor per 20 outcomes), then we begin with a full model, but afterward, we applied a stepwise backward elimination procedure based on log-likelihood ratio test for removal. Once we succeeded in developing a final model, we dichotomized the continuous variables in the model using a cutoff point driven by a univariate classification tree procedure. Again, we checked for refitting of the model using these new dichotomized variables. We used both discriminations as well as calibration for assessing the performance of this multivariate model. The area under the curve (AUC) of the receiver operating characteristic curve (ROC) was determined for assessment of discrimination, whereas the calibration was assessed using the calibration plots as well as the Hosmer and Lemeshow test.[26] Internal validation of the prognostic model was done by using the bootstrap procedure, including random sampling from the source population.[27] In our case, we created 1000 samples of the same sample characteristics as the study sample size using the replacement method. Then, we calculated bias-corrected and accelerated (BCA) values along with 95% CI for all the regression coefficients (β). Thereafter, we rounded off the regression coefficients of the final model to derive the final prognostic score which can be easily used by the clinicians. Then, we again evaluated the performance of this bedside prognostic score using the AUC of the ROC curve. Interrater reliability of the score was established by estimating kappa statistics.

Results

We included a total of 200 patients (10.1 ± 3.7 years, 84% boys) satisfying the inclusion criteria, out of which 121 patients were suffering from dystrophinopathy (104—DMD, 17—BMD). The diagnosis of the rest 79 patients has been described in Supplementary Table 1. Sarcoglycanopathy (12), calpainopathy (10), inflammatory myositis (13), and SMA type III (9) were the most predominant diagnosis among the nondystrophinopathy group. As described in the existing literature, patients with BMD had a later age of onset (beyond 6 years), remained ambulatory in mid-to-late adolescence, and had a slower rate of progression of the illness. Out of the 121 patients with dystrophinopathy, 96 had a deletion of single or multiple exons, 3 had duplications altering the reading frame, 19 had point mutation (nonsense mutations), and 1 patient each had a large deletion in the Xp21 region (6 mb) encompassing dystrophin gene (also had autistic features) and splice site mutation in the dystrophin gene. Among the 96 patients with dystrophin gene deletion, clustering was observed in two regions: exon 45–54 region (61%) and near the 5′-end region in exons 3–22 (23%). Similarly, the 5′-end region was found to be duplicated in all three patients with duplication in the dystrophin gene. However, no such clustering was observed in patients with nonsense mutations.

When we tried to compare the genotype and phenotype of DMD/BMD patients, we found that all patients with out-of-frame mutations showed a DMD phenotype. However, 7/24 (29%) patients with in-frame mutations also showed DMD phenotype. However, when we tried to correlate between the location of mutation with the presence of various clinical features, age of onset, and severity of functional limitation, those with very large deletions and deletions in the later part of the dystrophin gene had a trend toward the occurrence of more frequent intellectual disability. However, the difference did not reach the point of statistical significance ($P = 0.19$). We could not observe any other genotype–phenotype correlation.

We incorporated various sociodemographic, clinical variables, commonly performed diagnostic parameters, and objective assessment measures for disease severity and functional limitation in the univariate analysis to determine predictors for a diagnosis of DMD/BMD, after performing confirmatory tests like dystrophin gene MLPA and dystrophin gene sequencing. Among sociodemographic variables [Supplementary Table 2], in univariate analysis, the age at onset and age at loss of ambulation were significantly lower in patients with dystrophinopathy (probably because the proportion of BMD patients was less in our cohort) ($P < 0.0001$ for both) and the proportion of boys and proportion with loss of ambulation at the time of inclusion in the study were higher in DMD/BMD group ($P < 0.0001$ for both). Among clinical features [Table 1], the variables which were significantly associated with the diagnosis of DMD/BMD in univariate analysis were probable X-linked recessive pattern of inheritance, the presence of Gower's sign, calf pseudohypertrophy, tongue hypertrophy, hypertrophy of deltoid, hypertrophy of infraspinatus, more severe involvement of hip abductor compared to hip adductor, intellectual disability (intelligence quotient [IQ] <70), autistic features, absence of any delay in motor milestones, absence of scapular winging, absence of fever/sign of systemic inflammation clinically, presence of valley sign, presence of toe walking, presence of neck flexor weakness, absence of involvement of extraocular muscles, facial muscles, bulbar muscles, and presence of sleep-disordered breathing. Among all these variables, we carefully selected those variables for which, the effect size for the difference between the two groups was large and $P < 0.0001$. Among commonly performed diagnostic tests or laboratory tests to assess severity, presence of FVC <80% of expected for age and height, PEFR <80% of expected, serum CK >10 times of upper limit of normal, serum CRP <6 mg/dl, and EF <55% in echocardiogram was found to be more prevalent in DMD/BMD group ($P < 0.0001$), but we mainly intended to develop a prediction score based on history/clinical examination findings. So, we did not include these variables in subsequent multivariate logistic regression.

Among objective assessment measures of disease severity and functional limitation measures, North Star ambulatory

Table 1: Comparison of clinical and diagnostic variables in DMD/BMD and non-DMD/BMD groups

Variable	DMD/BMD group ($n=121$)	Non-DMD/BMD group ($n=79$)	*P*
Probable X-linked recessive pattern of inheritance	12	0	0.003
Gower's sign	89/89 (rest were unable to perform)	9	<0.0001
Calf pain	78	25	0.0004
Calf pseudohypertrophy	96	12	<0.0001
Tongue hypertrophy	68	0	<0.0001
Hypertrophy of deltoid	56	3	<0.0001
Hypertrophy of infraspinatus	49	2	<0.0001
Proximal weakness more than distal weakness	121	79	–
Lower limb more severely affected than upper limb	121	71	0.0029
Hip abductor more severely affected than hip adductor	106	27	<0.0001
Intellectual disability (intelligence quotient <70)	37	5	<0.0001
Autistic features	15	0	0.003
Motor developmental delay	19	34	<0.001
Scapular winging	0	23	<0.001
Fever/Sign of systemic inflammation clinically	0	13	<0.001
Valley sign	76	0	<0.0001
Pelvic lordotic posture	86	54	0.76
Waddling gait	54/54	73	0.41
Toe walking	53	22	<0.0001
Neck flexor weakness	114	48	<0.0001
Weakness of extraocular muscles	0	11	<0.0001
Weakness of facial muscles	6	15	0.002
Weakness of bulbar muscles	0	10	<0.0001
Sleep-disordered breathing	39	15	0.051
Hyporeflexia at knee joint	107	73	0.47
Hyporeflexia at ankle joint	104	61	0.31
Clinical evidence of myocardial dysfunction	7	1	0.15
Forced vital capacity <80% of expected for age and height	56	23	0.018
PEFR <80% of expected	54	22	0.017
Serum creatine kinase >10 times upper limit	105	21	<0.0001
Serum C-reactive protein >6 mg/dl	0	13	<0.0001
Ejection fraction <55% in echocardiogram	21	1	<0.0001

DMD/BMD: Duchenne muscular dystrophy/Becker *muscular dystrophy*

assessment scale and timed function tests like 10-m walk/run velocity (m/s), four stairs climb time (s), rise from supine time (s), 6-min walking distance (m), Brooke's functional scale, and Vigno's functional scale for lower limb were all found to be more significantly affected in DMD/BMD group, as compared to rest of the sample population [Table 2]. However, a significant subset of patients with DMD was either nonambulatory or able to walk a few steps only or unable to get up from a supine position unsupported and hence was not able to complete one or other objective assessment measures. Moreover, performing these objective assessments often requires several logistic prerequisites, which are difficult to be met in resource constraint settings by general physicians. So, we did not include them in the subsequent multivariate binary logistic regression analysis.

When finally these variables were subjected to multivariate binary logistic regression for the development of a prognostic model, age of onset <6 years, male gender, presence of Gower's sign, presence of valley sign, toe walking, calf pseudohypertrophy, and tongue hypertrophy were independent predictors for a confirmed diagnosis of DMD/BMD on subsequent testing. However, we subsequently excluded age of onset from our prediction model, as we supposed that although the age of onset seems to be independent of other factors, the prevalence of BMD in our study was relatively low (only 8%). Probably, due to this fact, it was found to be an independent predictor for DMD/BMD.

For predicting DMD/BMD, we retested the model by excluding the variable of the age of onset and all the variables were found to be predictors of poor functional outcomes. The final model incorporating all these six variables showed good discrimination in differentiating patients with and without DMD/BMD (AUC = 87.4%; 95% CI = 80.5–92.3%, $P < 0.001$). Hosmer and Lemeshow test showed good calibration of the final model (concordance between observed and predicted possibilities) ($P = 0.57$). In the internal validation also, the final model held good, as suggested by the narrow BCA 95% CIs of all the six predictor variables. All the six predictors which were found to have a significant correlation with the confirmed diagnosis of DMD/BMD continued to have a significant association, even after the bootstrap procedure [Table 3].

The beta regression coefficients of all six predictors were multiplied by two so that we could round them off easily to the nearest integers. Then, we developed a final model containing our clinical predictive score, as shown in Table 4. We provided the acronym of CVT_2MG score to this score we were developing, where each letter denoted Calf pseudohypertrophy, Valley sign, Toe walking, Tongue hypertrophy, Male gender, and Gower's sign. The final score was calculated for each patient by adding up the scores assigned to each individual item, determined on the basis of their beta coefficients. The final score ranged between 0 and 10 and the higher the score, the more was the probability of a confirmed diagnosis of DMD/BMD. We also plotted a nomogram and ROC curve [Figure 1] showing sensitivity and one specificity at various cutoff points in the score. While the predicted probability for a confirmed diagnosis of DMD/BMD was 0% at scores 0–4, it was 100% for scores 7–10 [Supplementary Table 3]. Overall, the bedside score had good discrimination capacity with AUC under the ROC being 99.7% (95% CI: 92.5–99.9%, $P < 0.01$). A score of 6 or above appeared to be the best cutoff for discriminating between the DMD/BMD group and the rest of the group with both sensitivity and specificity of 98%. Overall, the score performed better than any of the individual categories or a combination of calf pseudohypertrophy and Gower's sign in predicting the confirmed diagnosis of DMD/BMD. The median time taken for applying the score was 6 min (IQR—3–11 min). The interrater reliability, measured by kappa statistics, was almost perfect between two pediatric neurologists and strong between a pediatric neurologist and a pediatric neurology trainee resident (kappa coefficient 0.91 and 0.87, respectively). We could not observe any specific trend between CVT_2MG score and location of mutation on dystrophin gene. However, the CVT_2MG score of BMD patients was lower than DMD patients ($P = 0.03$). A score of 8 or more was able to discriminate between DMD and BMD patients with 70% specificity and 72% sensitivity [Supplementary Table 4]. When we analyzed the performance of CVT_2MG score in subgroups of ages 2–5 years, 5–10 years, and 10–18 years, there was no significant difference between the AUC of ROCs plotted for individual subgroups, suggesting age group had no significant impact on the performance of CVT_2MG score ($P = 0.23$).

Table 2: Comparison of objective functional assessment variables in DMD/BMD and non-DMD/BMD groups

Variable	DMD/BMD group ($n=121$)	Non-DMD/BMD group ($n=79$)	*P*
North Star Ambulatory Assessment scale	18.2±4.9	23.9±5.3	<0.0001
Timed function tests			
Ten-m walk/run velocity (m/s)	1.67±0.48	1.93±0.47	<0.0001
Four-stairs climb time (s)	4.9±2.3	3.7±1.8	
Rise from supine time (s)	7.2±3.5	5.1±2.3	
Six-min walking distance (m)	289.43±36.27	365.43±49.87	
Brooke's functional scale	2.4±0.6	1.2±0.3	
Vigno's functional scale for lower limb	4.3±2.7	3.1±1.9	

DMD/BMD: Duchenne muscular dystrophy/Becker *muscular dystrophy*

Table 3: Regression coefficients with 95% confidence interval after Bootstrap internal validation for the prognostic model

Bootstrap final model			
Variable	***B*-coefficient**	**Bootstrap (BCA 95% confidence interval)**	***P***
Male gender	1.19	0.23–2.07	0.003
Presence of Gower's sign	1.06	0.52–1.76	0.004
Toe walking	0.57	0.14–0.96	0.02
Calf pseudohypertrophy	1.12	0.49–1.87	0.006
Tongue hypertrophy	0.47	0.18–1.43	0.01
Valley sign	0.89	0.41–1.45	0.005
Constant	5.27	3.21–7.58	

BCA: Bias corrected and accelerated

Table 4: Clinical predictive score CVT_2MG from the final model

Variable	Score
Gender	
Male	2
Female	0
Gower's sign	
Present	2
Absent	0
Toe walking	
Present	1
Absent	0
Calf pseudohypertrophy	
Present	2
Absent	0
Tongue hypertrophy	
Present	1
Absent	0
Valley sign	
Present	2
Absent	0
Maximum score	10
Minimum score	0

CVT_2MG: Calf pseudohypertrophy, Valley sign, Toe walking, Tongue hypertrophy, Male gender, and Gower's sign

Among various other diagnostic subgroups, which came closer to dystrophinopathy in the CVT_2MG scoring was the sarcoglycanopathy subgroup (11 females, 1 male). But most of them were female and did not have valley sign or tongue hypertrophy and all of them had hip abduction sign during eliciting the Gower's maneuver (not the classical Gower's sign). The average CVT_2MG score in the sarcoglycanopathy group (3.7 ± 1.5) was lower than the dystrophinopathy group (8.1 ± 1.9), but one patient in the sarcoglycanopathy group had a score of 5 and one had a score of 6.

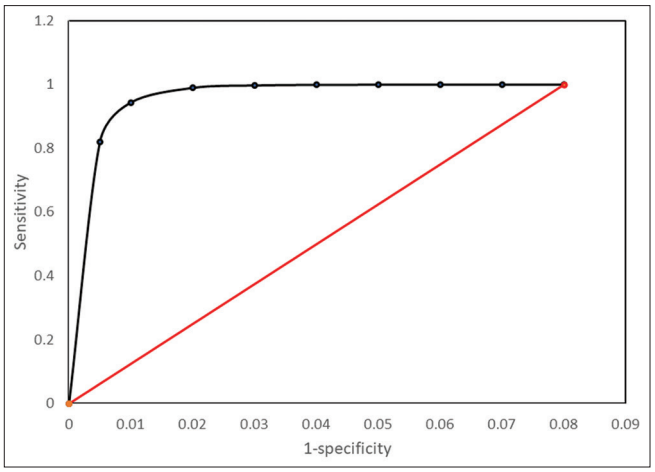

Figure 1: Area under receiver operating characteristic curve showing performance of CVT_2MG score at various cutoff points

Discussion

We developed a simple bedside clinical predictive score which can be used in children with insidiously progressive proximal limb-girdle weakness to predict the confirmed diagnosis of DMD/BMD, with acceptable sensitivity and specificity. This CVT_2MG score is better than serum CK or any of the individual clinical signs in predicting the confirmed diagnosis of DMD/BMD.

While valley sign and tongue hypertrophy are not as widely described as Gower's sign and calf pseudohypertrophy for DMD patients, when present, they indicate the probability of dystrophinopathy in patients with LGMD. Hence, we included these two clinical indicators in our predictive score. Even a combination of calf pseudohypertrophy and Gower's sign were not able to predict all cases of DMD and performed poorly as compared to the CVT_2MG score.

Although we considered a score of 6 or above to be the best indicator of DMD/BMD, even a score of 5 has 100% sensitivity and 97% specificity. Future studies with larger sample size and multicentric study design, with a more diverse study population, will probably determine whether the cutoff of 5 or 6 is more accurate. However, for differentiating between DMD and BMD, this score only had modest efficacy. Future studies need to develop separate prognostic models for predicting DMD alone. However, some patients have the age of onset around 6 years and often do not strictly follow DMD or BMD phenotype. Even some patients with DMD may not lose ambulation beyond 12–13 years, especially after treatment with eteplirsen or ataluren.[24] While BMD patients show the presence of some dystrophin staining in muscle biopsy, patients with DMD phenotype usually show almost absence of dystrophin staining on muscle biopsy, except for reverent fibers.[28] Currently, muscle biopsy is usually not indicated in all DMD/BMD patients, as the diagnosis can be confirmed in patients

with clinical suspicion easily by genetic testing. Still, muscle biopsy plays an important role in establishing the diagnosis of dystrophinopathy, particularly in patients with later symptom onset, comorbidities, or normal DMD genetic testing results.[28] Rarely, patients with DMD/BMD harbor deep intronic mutation, difficult to detect with currently available genetic tests commercially. In such cases, muscle biopsy helps in establishing a diagnosis. Treatment of DMD and BMD differs in certain expects, but corticosteroids are indicated in both of them.[8] Future studies need to explore whether modification of CVT_2MG score by adding or removing a few variables like age of onset will be able to predict DMD and BMD accurately and also discriminate between them. Such a score will help clinicians in predicting the future prognosis, in combination with findings from dystrophin gene testing and muscle biopsy.

The CVT_2MG score was only able to predict the diagnosis of dystrophinopathy, but we did not explore whether it has also any prognostic value or not. Across all age groups, it was effective in predicting the diagnosis of dystrophinopathy. However, young patients, patients with advanced disease, and BMD phenotype had a trend toward a lower score. Probably, the patients with advanced disease and loss of ambulation had a lower score, as their Gower's sign could not be tested.[13] Calf pseudohypertrophy and tongue hypertrophy also tend to be less prominent in nonambulatory patients with significant muscle atrophy. Toe walking in dystrophinopathy is mainly due to the relatively greater weakening of the dorsiflexors of the foot as compared with the plantar flexors. Toe walking also develops to compensate for the weakening quadriceps muscle.[29] Once a patient becomes nonambulatory, toe walking is difficult to check. Several other CMDs and LGMDs like calpainopathy-affected patients also demonstrate toe walking. Still, all nonambulatory DMD cases managed to score at least 5 on the CVT_2MG score.

Historically caregivers of many nonambulatory patients reported Gower's sign and toe walking, but still, we scored them 0 for the corresponding variables. Future studies need to determine whether to administer any score for these variables when they can't be tested due to non-ambulation, as scoring based on historical data may increase the accuracy of the scoring or may even increase the false positivity rate. Our study included patients up to 18 years of age, but its utility for patients with DMD >18 years of age need to be explored in future studies. The score needs to be adopted for the same purpose by including more or excluding some score items. Our sample population did not include any manifest carrier female of dystrophinopathy. Usually, they follow a relatively milder course, resembling the BMD phenotype. This CVT_2MG score needs to be tested in these manifest carriers also, whether it holds good for them. Two patients in the sarcoglycanopathy group scored 5 or more. We scored the variable "presence of Gower's sign" in them as zero, as they did not have the classical Gower's sign, but rather the hip abduction sign, first described by Khadilkar *et al*.[30]

Differential and selective muscle involvement is an important feature of DMD and other LGMDs. While hip abductors tend to be weaker than hip adductors in DMD and most other LGMDs, resulting in the typical waddling gait. However, in sarcoglycanopathies, the adductor and flexor group of muscles of the pelvic girdle are more severely affected as compared to the abductors of the hip which tend to be stronger.[2,4–6] The sign demonstrated in this study arises out of this selective muscular involvement in sarcoglycanopathies. Khadilkar *et al*.[30] have shown that this hip abductor sign has a sensitivity and specificity of 76% and 98%, respectively. While during the early years of illness, the disparity in the weakness of hip adductors and abductors may not be sufficient to produce this sign, usually this sign is present in patients between 3 and 10 years from the onset of illness.

Valley sign is also due to selective muscle involvement in DMD, with pseudohypertrophy of DMD and infraspinatus muscles and atrophy of anterior and posterior axillary folds. In the original study by Pradhan *et al*.,[31] this sign was present even in those patients when the calves had failed to enlarge in the early stages of the disease or had resolved to normal bulk during the late stage. Hence, this is one of the most specific clinical signs of DMD and we used this as one of the variables in our score.

Although X-linked recessive pattern of inheritance was more frequently found in the dystrophinopathy group, but it was not significantly associated with diagnosis of DMD/BMD in further analysis. It could be probably due to the fact that male gender and X-linked recessive inheritance are interrelated and male gender was more strongly associated with diagnosis of DMD/BMD, as compared to X-linked recessive inheritance. We also included two patients with acquired etiology like CIDP, because our inclusion criteria were clinical examination based and did not include NCS abnormalities. Moreover, we are proposing this clinical criterion to be used by neurologists as well as pediatricians and general physicians, who might not be expert in accurately interpreting NCS abnormalities. Sometimes, CIDP patients can present with proximal predominant weakness in bilateral lower limbs lasting for months together, although sensory symptoms and fluctuating course is more common in these patients as compared to dystrophinopathy.

There are several limitations in our study. The absence of adult dystrophinopathy cases and only a few cases in the <5 years and >14-year age group, could have introduced some selection bias into our study population. The non-DMD group was also heterogeneous and the number of patients in individual subgroups like sarcoglycanopathy and calpainopathy were less. Even the number of patients with BMD was only 17, so it is difficult to conclude firmly that this predictive tool holds good for cohorts with pure BMD phenotype. Future studies need to explore the discriminative ability of this CVT_2MG score between DMD/BMD and other individual muscular dystrophies. This score was not found

to have excellent psychometric properties to differentiate between DMD and BMD. This score can't be used to predict the nature and location of the mutation in the dystrophin gene neither does it can predict the rate of progression. The interrater reliability between a primary care physician and pediatric neurologist also needs to be tested in future studies. The persons not aware of the Gower's sign and valley sign won't be able to apply this score accurately. Our study only included dystrophin mutation confirmed cases, thus it might have missed those cases with deep intronic mutations missed in dystrophin gene sequencing or whole-exome sequencing. Still, the CVT_2MG score developed and internally validated in the current study is a novel attempt at developing a diagnostic scoring for DMD/BMD cases. This score will help a clinician in predicting dystrophinopathy in a child/adolescent coming with proximal limb-girdle type of LMN weakness with reasonable accuracy and investigate accordingly. As the laboratory diagnostics for dystrophinopathy are more easily available as compared to other muscular dystrophies in LMIC, this simple bedside predictive tool can save a lot of time and resources.

Conclusion

CVT_2MG score developed and internally validated in our study has good sensitivity and specificity in predicting a confirmed diagnosis of DMD/BMD in subsequent tests. However, it needs to be externally validated in different populations.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Final confirmed diagnosis of study participants

Diagnosis	Number of patients ($n=200$)
DMD	104 (57%)
BMD	17 (8.5%)
Sarcoglycanopathy (LGMD 2C–2F)	12 (6%)
Polymyositis/dermatomyositis	13 (6.5%)
SMA III	9 (4.5%)
Late-onset Pompe disease	1 (0.5%)
Caveolinopathy (LGMD 1C)	2 (1%)
Calpainopathy (LGMD 2A)	10 (5%)
Dysferlinopathy (LGMD 2B)	3 (1.5%)
Laminopathy (LGMD 1B)	1 (0.5%)
Titinopathy (LGMD 2J)	1 (0.5%)
LGMD 2N-POMT2 mutation	1 (0.5%)
Collagen VI-related myopathy	8 (4%)
Other congenital myopathies	6 (3%)
Merosin negative muscular dystrophy	7 (3.5%)
Congenital muscular dystrophy–dystroglycanopathy	2 (1%)
Rigid spine muscular dystrophy (SERPN1-related myopathy)	2 (1%)
CIDP	2 (1%)

DMD: Duchenne muscular dystrophy, BMD: Becker muscular dystrophy, LGMD: limb girdle muscular dystrophy, SMA: spinal muscular atrophy, CIDP: chronic inflammatory demyelinating polyneuropathy

Supplementary Table 2: Comparison of sociodemographic variables of DMD/BMD and non-DMD/BMD group

Variable	DMD/BMD group ($n=121$)	Non-DMD/BMD group ($n=79$)	*P*
Age at presentation (years)	8.1±2.4	12.4±3.6	<0.0001
Gender			
Males	121	47	<0.0001
Females	0	32	
Residence			
Urban	12	7	0.89
Rural	109	72	
Socioeconomic status			
Upper	1	0	0.47
Middle	64	44	
Lower	56	35	
Age at onset (years)	4.5±2.1	9.4±4.6	
Patients with loss of independent ambulation	67	6	<0.0001
Age at loss of ambulation	12.4±3.6	15.1±4.8	<0.0001

DMD/BMD: Duchenne muscular dystrophy/Becker muscular dystrophy

Supplementary Table 3: Performance of CVT_2MG score in predicting a confirmed diagnosis of DMD/BMD

Total score	Number of patients	Number of patients with confirmed diagnosis of DMD/BMD (%)	Predicted probability of confirmed diagnosis of DMD/BMD (%)	Sensitivity (%)	Specificity (%)
0–1	23	0	0	–	–
2	25	0	0	–	–
3	17	0	0	–	–
4	11	0	0	–	–
5	14	12	85	100	97
6	15	14	93	98	98
7	16	16	100	96	100
8	21	21	100	96	100
9	23	23	100	95	100
10	35	35	100	92	100
Total	200	121			

DMD/BMD: Duchenne muscular dystrophy/Becker muscular dystrophy

Supplementary Table 4: Performance of CVT_2MG score in discriminating between DMD and BMD

Total score	Number of patients with confirmed diagnosis of DMD/BMD	Number of patients with confirmed diagnosis of DMD	Predicted probability of confirmed diagnosis of DMD (%)	Sensitivity (%)	Specificity (%)
5	12	8	66	100	0
6	14	10	71	96	23
7	16	12	75	83	47
8	21	19	90	72	70
9	23	21	91	53	82
10	35	34	97	32	94
Total	121	104			

DMD/BMD: Duchenne muscular dystrophy/Becker muscular dystrophy