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Validität, Reliabilität und diagnostische Wertigkeit von Zeitfunktionstests bei Kindern mit neuromuskulären Erkrankungen

Dissertation
zum Erwerb des Doktorgrades der Medizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
Lena Ille, geb. Reinhuber
aus
Wangen im Allgäu
2020

Mit Genehmigung der Medizinischen Fakultät
der Universität München

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Tag der mündlichen Prüfung: 12.03.2020

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Validität, Reliabilität und diagnostische Wertigkeit von Zeitfunktionstests bei Kindern mit neuromuskulären Erkrankungen

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INHALTSVERZEICHNIS

1. ABKÜRZUNGEN

CMT	**Charcot-Marie-Tooth Erkrankung**
DMD	**Muskeldystrophie Duchenne**
6-MWT	**6 Minute Walk Test**
2-MWT	**2 Minute Walk Test**
s2LJ	**single 2 Leg Jump**
CRT	**Chair Raising Test**

2. EINLEITUNG

2.1. Neuromuskuläre Erkrankungen

Der Überbegriff „neuromuskuläre Erkrankungen“ steht für eine Vielzahl verschiedener Krankheitsbilder. Diese können grundsätzlich in drei Gruppen unterteilt werden, primär myopathische Krankheitsbilder, primär neurogene Krankheitsbilder sowie Funktionsstörungen der neuromuskulären Übertragung.

Zu den primär myopathischen Krankheitsbildern gehören unter anderem die Muskeldystrophien, darunter die im Kindesalter am häufigsten vorkommende Muskeldystrophie Duchenne (DMD). Des Weiteren gibt es u.a. die genetisch sehr heterogene Gruppe der kongenitalen Myopathien (wobei traditionell kongenitale Myopathien mit Strukturbesonderheiten und kongenitale Muskeldystrophien unterscheiden wurden), metabolische Myopathien, die u.a. mitochondriale Myopathien und den M. Pompe umfassen sowie myotone Erkrankungen und erworbene, z.B. autoimmun vermittelte Myopathien.

Die häufigsten primär neurogenen Krankheitsbilder im Kindesalter sind die Gruppe der hereditären motorischen und sensorischen Neuropathie (Charcot-Marie-Tooth Erkrankungsspektrum, CMT), die spinalen Muskelatrophien, erworbene periphere Nervenläsionen sowie autoimmun vermittelte Neuropathien, wie z.B. das Guillain-Barré-Syndrom und die chronisch inflammatorische demyelinisierende Polyneuropathie.

3.1.1. Muskeldystrophie Duchenne

Erstmals wurde das Krankheitsbild der DMD von G. Duchenne und W. R. Gowers beschrieben (Duchenne, 1868; Gowers, 1879). Es handelt sich um eine progressive, X-chromosomale Erkrankung, welche ca. eines von 3500 männlichen Neugeborenen betrifft (Emery, 2002). Die Erkrankung wird durch Mutationen im Dystrophin-Gen verursacht und führt zum Verlust des Strukturproteins Dystrophin in der Muskelfasermembran (Zubrzycka-Gaarn, et al., 1988). Dieser Proteinverlust äußert sich durch einen fortschreitenden bindegewebig-proliferativen Muskelumbau mit klinisch fortschreitender Muskelschwäche. Charakteristisch ist eine zunächst die Beine betreffende, proximale Schwäche, häufig einhergehend mit einer Pseudohypertrophie der Wadenmuskulatur. Typischerweise fallen dadurch im Kindergartenalter Einschränkungen beim Gehen, beim Treppensteigen und beim Aufstehen vom Boden auf (Boland, Silbert, Groover, Wollan, & Silverstein, 1996; Brooke, et al., 1989). Als typisches klinisches Zeichen der proximalen Schwäche ist bei den betroffenen Kindern das sogenannte „Gowers-Manöver“ zu beobachten, welches das Abstützen der

Hände auf den Oberschenkeln beim Aufstehen beschreibt. Dieses markante Bewegungsmuster wurde nach dem Erstbeschreiber W. R. Gowers 1879 benannt (Gowers, 1879). Im Alter von 10-14 Jahren ist der Krankheitsverlauf zumeist so weit fortgeschritten, dass die meisten an DMD erkrankten Jungen rollstuhlpflichtig sind (McDonald, et al., 1995). Ein motorischer Funktionsverlust der oberen Extremität folgt in den nächsten Jahren.
Zudem entwickeln DMD Patienten typischerweise im jugendlichen Alter eine Schwäche der Atemhilfsmuskulatur und eine fortschreitende Kardiomyopathie (van Westering, Betts, & Wood, 2015). Unter medikamentöser Therapie der Kardiomyopathie und unterstützender Beatmung liegt das mediane Überlebensalter aktuell bei ca. 35 Jahren (Kohler, et al., 2009). Das fehlende Dystrophin hat nicht nur gravierende Auswirkungen auf die Muskelfunktion, auch in der kognitiven Leistung kann sich der Verlust dieses Proteins wiederspiegeln. Bei einem relevanten Anteil an DMD Patienten besteht eine nicht-fortschreitende kognitive Beeinträchtigung (Blake & Kroger, 2000; Bresolin, et al., 1994).

2.1.1.1. Diagnostik

Die Diagnose einer DMD wird zumeist im Alter von ca. 3 - 5 Jahren gestellt. Zu diesem Zeitpunkt unterscheiden sich die Patienten in ihrer motorischen Entwicklung zunehmend von der gleichaltriger Kinder (K. M. Bushby, Hill, & Steele, 1999). Bei Verdacht auf eine DMD kann die Bestimmung der Kreatinkinase einen richtungsweisenden Hinweis geben. Diese ist bereits ab der Geburt stark erhöht (Okinaka, et al., 1961). Bei anhaltend erhöhten Werten sollte eine molekulargenetische Diagnostik zur Bestätigung des Verdachtes durchgeführt werden. Große Duplikationen und Deletionen (in 70% der Fälle krankheitsverursachend) können mit der muliplex ligation-dependent probe amplification nachgewiesen werden (Lalic, et al., 2005). Die häufigsten krankheitsverursachenden Deletions-Hotspots umfassen die Exone 45-55 und 2-19 (White & den Dunnen, 2006). Kann keine Deletion oder Duplikation nachgewiesen werden, ist die Suche nach einer Punktmutation mittels Sequenzierung des Gens indiziert (Flanigan, et al., 2003). Da das Dystrophin-Gen sehr groß ist, sollten methodisch neben der klassischen Sanger-Sequenzierung auch NGS-Panels in Erwägung gezogen werden, um ggf. kosteneffizient bei negativem Ergebnis auch seltenere Formen der Muskeldystrophie differentialdiagnostisch ausschließen zu können (Vill, et al., 2017). Bleibt die molekulargenetische Testung einschließlich differenzialdiagnostischer Überlegungen bei weiter bestehendem klinischen Verdacht ohne Ergebnis, kann in Einzelfällen ergänzend eine Muskelbiopsie erforderlich werden (K. Bushby, et al., 2010), diese ist aber mittlerweile diagnostisch nicht mehr Gold-Standard.

2.1.1.2. Therapie

Neben symptomatischer Therapie wie physiotherapeutischer Maßnahmen, Hilfsmittelversorgung, falls notwendig chirurgisch-orthopädischer Eingriffe, medikamentöser Therapie der Kardiomyopathie und assistierter Beatmung stellen Kortikosteroide die bislang einzige für alle molekulargenetischen Formen der DMD etablierte Therapie dar, die eine kurzfristige Verbesserung oder Stabilisierung der Muskelfunktion und Muskelkraft bewirken kann (Manzur, Kuntzer, Pike, & Swan, 2008). Sofern sie rechtzeitig noch vor Eintreten motorischer Regression, respektive höhergradigen muskulären Umbauvorgängen eingesetzt werden (K. Bushby, et al., 2010; Markham, Kinnett, Wong, Woodrow Benson, & Cripe, 2008), führt ihr Einsatz zu einer Verlängerung der Gehfähigkeit um wenige Jahre. Aktuell wird die Therapie mit 0,75 mg/kg/Tag Prednisolon oder 0,9 mg/kg/Tag Deflazacort empfohlen. Die Nebenwirkungen der Therapie können u.a. Verhaltensauffälligkeiten, Gewichtszunahme, und je nach Therapieschema Kleinwuchs, Osteoporose und cushinoides Äußeres sein (Manzur, et al., 2008). Die Auswirkungen auf Herz- und Lungenfunktion sowie orthopädische Komplikationen sind bislang nicht ausreichend untersucht.

Für Patienten mit Nonsense-Mutationen wurde in Deutschland das orale Medikament Ataluren (Translana ®) zugelassen, welches auf molekulargenetischer Basis das Überlesen eines verfrühten Stop-Codons herbeiführen soll (K. Bushby, et al., 2014).

Es gibt bislang keine kurative Therapie der DMD, daher werden fortlaufend im Rahmen klinischer pharmakologischer Studien die Effekte neuer Medikamente untersucht (Beytia Mde, Vry, & Kirschner, 2012; Le Guiner, et al., 2017; Lourbakos, et al., 2017; McNally & Wyatt, 2017; Nakamura, 2017), (Crone & Mah, 2018).
Um den Effekt dieser neuen Therapien evaluieren zu können sind geeignete klinische Tests notwendig. Aktuell wird der 6 Minuten Gehtest (6 Minute Walk Test, 6MWT) in den meisten Studien zur primären Outcome-Messung eingesetzt.

2.1.2. Hereditäre motorische und sensorische Neuropathie

Die hereditäre motorische und sensorische Neuropathien (syn. Charcot-Marie-Tooth-Erkrankungen, CMT) stellen die Gruppe der häufigsten vererbten neurologischen Erkrankungen dar und weisen eine Prävalenz von ca. 1:2.500 auf (Dyck & Lambert, 1968; Skre, 1974). Die Gruppe der CMT ist klinisch und genetisch sehr heterogen. Der Erbgang kann autosomal dominant (AD), autosomal rezessiv (AR) oder X-chromosomal gebundenen sein (Saporta, et al., 2011). Es wurden bis jetzt mehr als 80 Gene entdeckt, welche mit der CMT assoziiert sind (Rossor, Polke, Houlden, & Reilly, 2013), (Rudnik-Schoneborn, et al., 2016) die Anzahl ist stetig steigend. Mit Hilfe elektrophysiologischer und histopathologischer

Kriterien wurden Initial drei Hauptgruppen der CMT unterschieden: die primär demyelinisierende Form (CMT1, CMT4), die primär axonale Form (CMT2) und die spinale Form. Letztere wird auch als distale hereditäre motorische Neuropathie oder als distale spinale Muskelatrophie bezeichnet. Zunehmend werden auch demyelinisierend-axonale Mischformen beschrieben, welche im Zusammenhang mit übergeordneten Störungen beispielsweise im mitochondrialen Stoffwechsel der Nervenzelle auftreten.

Die Mehrheit der CMT Patienten ist von einer demyelinisierenden Form betroffen, welche durch eine Nervenleitgeschwindigkeit von weniger als 38 m/s charakterisiert ist (Harding & Thomas, 1980a). Die häufigste Untergruppe ist die CMT Typ 1a mit einer Prävalenz von ca. 1:2.500. Die zugehörige Mutation ist die 1,5-Mb-CMT1a-Tandem Duplikation auf dem Chromosom 17p11.2-p12, diese schließt das *PMP22*-Gen ein (Patel, et al., 1992; Raeymaekers, et al., 1991).

Klinisch charakteristisch bei der CMT sind erloschene Eigenreflexe, symmetrische, vornehmlich distale Paresen (Harding & Thomas, 1980b; Reilly, 2007). Durch die Paresen der Unterschenkel- und Fußmuskulatur kommt es im Laufe der Erkrankung häufig zur Ausbildung von Hohlfüßen mit Krallenzehen, charakteristischen „Storchenbeinen“ und Fußheberschwäche (Dyck, Karnes, & Lambert, 1989).

2.1.2.1. Diagnostik

Für die Diagnosestellung der CMT ist neben Anamnese und körperlicher Untersuchung die elektrophysiologische Diagnostik richtungsweisend. Sie bilden die Grundlage für den molekulargenetischen Zielbereich (Saporta, et al., 2011). Die molekulargenetische Zuordnung ist für Aussagen zu Prognose und Wiederholungsrisiko sowie zur Vermeidung invasiver Untersuchungen wie Nervenbiopsie oder Liquordiagnostik essenziell, wenngleich sie bislang noch nicht in allen CMT-Fällen gelingt.

2.1.2.2. Therapie

In der Versorgung der CMT-Patienten nimmt die symptomatische Therapie mit physiotherapeutischen Maßnahmen (Schenone, Nobbio, Monti Bragadin, Ursino, & Grandis, 2011) und Hilfsmittelversorgung den vornehmlichen Stellenwert ein. Bei stark ausgeprägten Deformitäten der Füße sind auch orthopädiechirurgische Eingriffe in Betracht zu ziehen.

Eine kausale Therapie gibt es aktuell noch für keinen CMT-Typ. Jedoch ist bei der durch eine Duplikation des *PMP22* Gens verursachten CMT1a, die Reduzierung der *PMP22* Expression ein denkbarer therapeutischer Ansatz. Eine pharmazeutische Zusammensetzung von D-Sorbitol, Baclofen und Naltrexon befindet sich aktuell in der klinischen Studienphase. (Pharnext I-L-M, France. PLEO-CMT STUDY: An international pivotal Phase 3 Study of PXT3003 or the treatment of Charcot-Marie-Thooth disease type 1a).

2.2. Zielsetzung der Arbeit

Durch die stetige Entwicklung neuer medikamentöser Therapieansätze bei neuromuskulären Erkrankungen, und deren Erprobung in klinischen Studien, gewinnt die Quantifizierung der Muskelkraft und muskulärer Leistungsfähigkeit bei den Kindern immer weiter an Bedeutung.
Entsprechend besteht die Notwendigkeit geeigneter klinische Messinstrumente zur Evaluierung des Krankheitsverlaufes und der Effektivität neuer therapeutischer Targets. Aktuell wird der 6MWT als primärer Outcome-Parameter sowohl in den meisten DMD-Studien als auch bei den CMT-Studien genutzt. Bei der DMD stellt allerdings häufig die mangelnde Kooperation der Patienten ein einschränkendes Problem dar. Die Kinder sind häufig aufgrund von mentaler Beeinträchtigung oder Verhaltensauffälligkeiten nicht in der Lage, die Testungen adäquat durchzuführen.
Bislang kommen neben dem 6MWT klassische Zeitfunktionstest zum Einsatz bspw. die Zeitmessung beim Aufstehen vom Boden aus Rückenlage, bei der Bewältigung von vier Treppenstufen und bei einer 10 Meter-Rennstrecke.
Im Bereich der CMT sind zudem seit kurzem neuere validierte klinische Skalen zur Beurteilung der Krankheitsschwere verfügbar (Burns, et al., 2012). Diese wurden jedoch noch nicht bei allen CMT-Formen, insbesondere der seltenen Subtypen eingesetzt.

Im Leistungssportbereich und der Geriatrie wurde in den letzten Jahren die sogenannte Mechanographie, eine Methode zur Messung von Bodenreaktionskräften, etabliert. Sie ermöglicht eine effiziente Quantifizierung von Kraft, Leistung und Bewegungsabläufen im ambulanten Setting. Auch in der Pädiatrie wird die Mechanographie nun zunehmend eingesetzt (Duran, et al., 2017; Hockett, et al., 2013).

Im Rahmen dieses Promotionsprojektes wurden zwei Studien durchgeführt, die sich mit dem Problem der Quantifizierung motorischer Leistungen von Kindern mit neuromuskulären Erkrankungen beschäftigten.

Zum einen wurde eine Gruppe von 23 pädiatrischen CMT-Patienten mit der Frage untersucht, ob eine ausreichende Korrelation zwischen der mittels Mechanographie ermittelten Leistung und den bislang etablierten Zeitfunktionstests besteht und ob entsprechend die Mechanographie als Outcome-Parameter zur Evaluierung von pädiatrischen CMT Patienten geeignet ist.

Zum anderen wurde eine Gruppe von 13 gehfähigen DMD-Patienten mit der Frage untersucht, ob eine zeitliche Verkürzung der Testzeit des 6MWT auf 2 Minuten eine

gleichwertige Aussage über den funktionellen Status der Kinder ergibt, und ob Validität und Reliabilität vergleichbar sind. Es wurde zudem die Schrittgeschwindigkeit analysiert, um Aussagen über Ausbelastung und eventuell pathologische Ermüdbarkeit zu erhalten.

Eigenanteil

Der geleistete Eigenanteil an diesen Studien belief sich auf die physikalische Testung der Patientenkohorte. Die promovierende Doktorandin führte die Mechanographie-Tests, den 6MWT mit minütlicher Zeitnahme und die Zeitfunktionstestungen durch.

Alle Patienten wurden regelmäßig im sozialpädiatrischen Zentrum des Dr. von Haunerschen Kinderspitals der Ludwig-Maximilians-Universität München betreut. Die Tests wurden im Rahmen der regelmäßigen Routineuntersuchungen durchgeführt.

Die Durchführung des 6MWT erfolgte gemäß den American Thoracic Society (ATS) Empfehlungen. Der 6MWT wurde ohne Pulsoximetrie durchgeführt (Laboratories, 2002).

Die Strecke des 6MWT wurde in Metern gemessen, zusätzlich wurden die zurückgelegten Meter in Minuten-Intervallen dokumentiert.
Die Kinder mit CMT absolvierten neben dem 6MWT und den TFT auf der Mechanographieplatte einen „Single Two Leg Jump“ und einen „Chair Raising Test“.

Die Testungen erfolgten nach standardisierten Bedingungen und wurden immer in der gleichen Reihenfolge durchgeführt. Zwischen den einzelnen Subtests wurde den Kindern ausreichend Zeit zur Erholung gegeben.
Zur Messung des Single Two Leg Jump und des Chair Raising Test wurde die Leonardo Mechanograph ground reaction force platform (GRFP;Novotec Medical GmbH, Pforzheim) verwendet. Diese besteht aus einer Kraftmessplatte, welche computerisiert Bodenreaktionskräfte analysiert. Die gesamten Messungen wurden durch die Leonardo Mechanography Software Version 4.3 analysiert.

Beim Single Two Leg Jump wurden die Kinder angewiesen, so hoch wie möglich zu springen und auf dem Vorfuß zu landen.

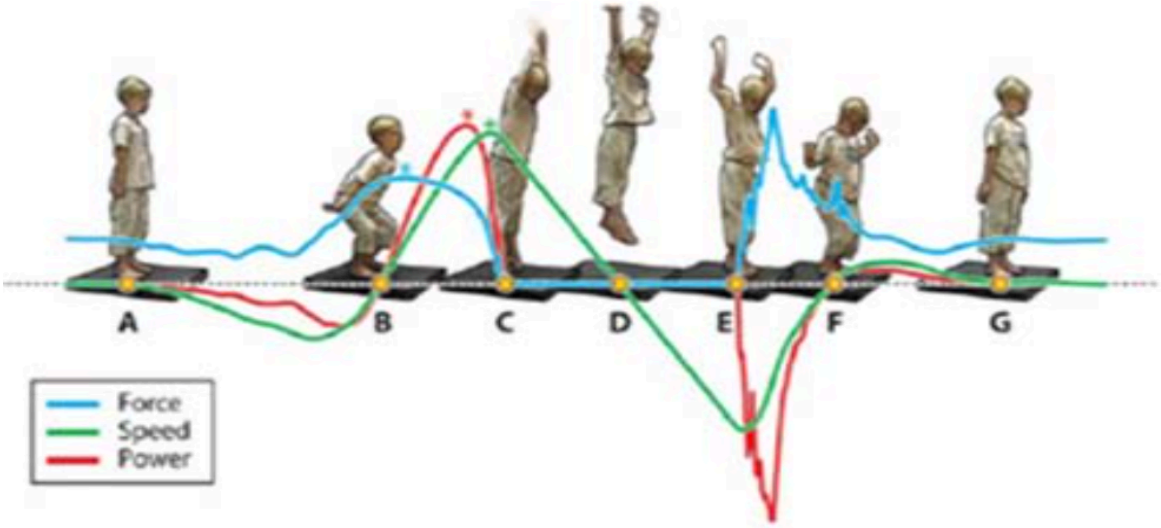


Abbildung 1: Der Single Two Leg Jump ist ein *Countermovement jump* welcher als Vertikalsprung mit Ausholen der Arme durchgeführt wird. A: Ausgangspunkt. B: Tiefster Punkt des Sprungs. C: Absprung. D: Höchster Punkt des Sprungs. E: Erste Berührung der Landephase. F: Tiefster Punkt der Landephase. G: Endpunkt. Die blaue Linie zeichnet die Kraft auf, die grüne Linie die Geschwindigkeit und die rote Linie die Leistung (Veilleux & Rauch, 2010).

Für den Chair Raising Test wurde eine Bank auf der Plattform befestigt, die Höhe der Bank wurde individuell auf einen 90 Grad Winkel im Knie des Patienten eingestellt. Die Kinder wurden angehalten, zur Testung die Arme vor dem Brustkorb zu verschränken um eine Mithilfe der oberen Extremität auszuschließen. Die Anweisung lautete, so schnell wie möglich aufzustehen, bis die Beine gestreckt sind und sich dann wieder zu setzen.

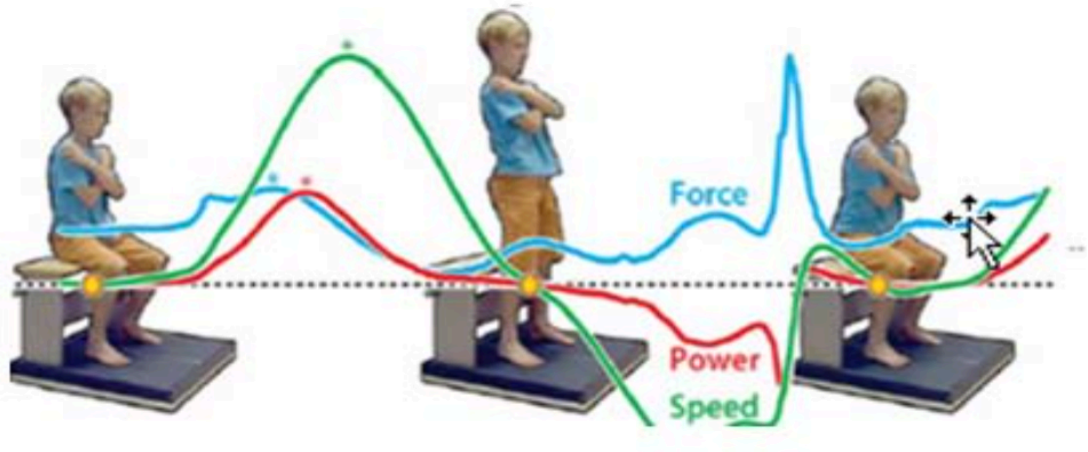


Abbildung 2: Chair Raising Test. A: Ausgangspunkt. B: Höchster Punkt der Bewegung. C: Endpunkt. Die grüne Linie steht für die Geschwindigkeit, die rote Linie für die Leistung und die blaue Linie für die Kraft. (Veilleux & Rauch, 2010).

Sowohl für den Single Two Leg Jump als auch den Chair Raising Test wurde die Leistung P_{max}rel in W/kg gemessen. Alle Messergebnisse des Systems wurden händisch einzeln auf technisch korrekte Durchführung überprüft.

3. ZUSAMMENFASSUNG

3.1. Deutsch

Zu den häufigsten Formen der neuromuskulären Erkrankungen (NME) zählen die Muskeldystrophie Duchenne (DMD) und die hereditäre motorische und sensorische Neuropathie (CMT). Um sowohl die natural history, als auch den Erfolg neuer Therapien beurteilen zu können, sind aussagekräftige klinische Tests nötig. In der vorliegenden kumulativen Doktorarbeit, basierend auf zwei Publikationen, wurde je eine Patientengruppe hinsichtlich neuer physikalischer Tests bezüglich ihrer Reliablilität bzw. ihrer Validität im Sinne von Korrelation zu etablierten Verfahren geprüft.

In der ersten Studie („*Six-minute walk test versus two minute walk test in children with Duchenne muscular dystrophy: Is more time more information?“)*, wurde in einer Kohorte von 13 DMD Patienten Gehstrecke und Schrittgeschwindigkeit in Minuten-Intervallen einer 6 Minuten-Gehstrecke mit einer 2 Minuten-Gehstrecke verglichen, es wurde zur Messung der Reliabilität je ein kurzfristiger Wiederholungstest durchgeführt.

Zusammenfassend konnte in dieser Studie gezeigt werden, dass die Schrittgeschwindigkeit nach einer Minute bis zum Schluss konstant blieb und sich somit bei der Muskeldystrophie Duchenne innerhalb von 6 Minuten keine pathologische Erschöpfung im Sinne von „Fatigue“ einstellte. Die Reliabilität und Validität sowohl der 6 Minuten-Gehstrecke als auch der 2 Minuten-Gehstrecke waren gut. Es bestand kein relevanter Unterschied zwischen beiden Tests. Somit gelangten wir zu dem Ergebnis, dass der 6MWT im Vergleich zum 2MWT keine essentiellen zusätzlichen Informationen liefert. Eine Verkürzung ist somit grundsätzlich denkbar.

In der zweiten Studie (“*Jumping Mechanography as a Complementary Testing Tool for Motor Function in Children with Hereditary Motor and Sensory Neuropathy“)* wurden in einer Kohorte von 23 pädiatrischen CMT-Patienten die Ergebnisse aus dem 6MWT und etablierten Zeitfunktionstests mit den Ergebnissen der Mechanographie, verglichen. Wir konnten bei guten bis sehr guten Korrelationskoeffizienten zeigen, dass sich die Mechanographie als weiteres Messinstrument gut zur Beurteilung von pädiatrischen CMT Patienten eignet. Insbesondere der Single Two Leg Jump, für den der Einsatz von proximaler und distaler Muskultur nötig ist, erscheint bei weniger schwer betroffenen Kindern den anderen Tests aufgrund der möglichen Quantifizierung und dem Vergleich zu alters- und geschlechtskorrigierter Normwerte überlegen.

In beiden Studien konnte die Anwendbarkeit und Zuverlässigkeit neuer klinischer Test bei neuromuskulären Erkrankungen gezeigt werden. Beide Verfahren (2MWT und

Mechanographie) sind bei pädiatrischen Patienten bislang wenig erforscht. Weitere Studien zur Überprüfung und Bestätigung unserer Ergebnisse sind sinnvoll.

3.2. English

In order to assess both the natural history and the effectivity of new therapies, suitable clinical tests are mandatory. In the present cumulative thesis, which is based on two publications, patients groups with both diseases were tested for new physical tests with regard to their reliability and validity in terms of correlation to established methods. In the first study *("Six-minute walk test versus two minute walk test in children with Duchenne muscular dystrophy: Is more time more information*?"), a 6 minute- and a 2 minute walking distance as well as walking speed were compared in repeated testings of 13 DMD patients. This study showed that walking speed remained constant after one minute until the end and thus there was no pathological fatigue within 6 minutes. Reliability and validity of both distances were good. Thus we came to the conclusion that the 6MWT does not provide essential additional information compared to the 2MWT. Thus a shortening is basically conceivable.

In the second study ("*Jumping Mechanography as a Complementary Testing Tool for Motor Function in Children with Hereditary Motor and Sensory Neuropathy*"), the results of the 6MWT and established time function tests were compared with the results of mechanography tests in a cohort of 23 pediatric CMT patients. We were able to show good or very good correlation coefficients, thus mechanography seems to be a suitable additional measurement tool for the assessment of pediatric CMT patients. Especially the “Single Two Leg Jump”, which requires the use of proximal and distal muscles, appears superior to other tests in less severely affected children due to the possibility of quantification and comparison to age- and gender-corrected norm values.

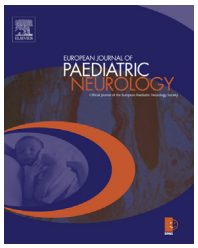
Both studies showed the applicability and reliability of new clinical outcome tools in pediatric neuromuscular conditions. The new methods (2MWT and mechanography) have so far only been sporadically studied in pediatric patients. Further studies to verify and confirm our results are required.

4. PUBLIKATIONEN

4.1. Vill, K., Ille, L., Schroeder, S. A., Blaschek, A., & Muller-Felber, W. (2015). Six-minute walk test versus two-minute walk test in children with Duchenne muscular dystrophy: Is more time more information? *Eur J Paediatr Neurol, 19*, 640-646.

ELSEVIER

Official Journal of the European Paediatric Neurology Society



Original article

Six-minute walk test versus two-minute walk test in children with Duchenne muscular dystrophy: Is more time more information?

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ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form
12 June 2015
Accepted 10 August 2015

Keywords:
Duchenne muscular dystrophy
Outcome measures
Six minute walk test
Two minute walk test
Test–retest reliability

ABSTRACT

Background/purpose: The six minute walk test is a widely accepted primary outcome parameter in most studies in Duchenne muscular dystrophy (DMD). To compare information obtained by the six minute walk distance (6MWD) test and the two minute walk distance (2MWD) in patients with DMD, a cohort of 13 voluntary DMD boys did a repeated six minute walking test.
Methods: Patients had to be ambulatory with a physical disability according to Levels 1–3 on the Vignos-Scale for lower extremity. Measurements were taken at one minute intervals. Reliability was measured by intraclass correlation.
Results: Test–retest reliability for 6MWD and 2MWD in two different age classes was very good for both subgroups. Test–retest-reliability was lower in patients with more advanced disability in both tests.

Walking speed remained completely stable from time points 1–6 minutes in the whole study patient collective, which indicates that physical exhaustion is not reached after six minutes even in more disabled patients.
Conclusion: Thus the 6MWD in DMD patients does not give additional information as compared to a 2MWD.

1. Introduction

Duchenne muscular dystrophy (DMD) is a devastating x-linked muscular disorder affecting one in 3500 newborn boys.[1] The disease is caused by mutations in the dystrophin gene, leading to a loss of the dystrophin protein in muscle cells,[2] resulting in progressive weakness.

Ambulation becomes progressively difficult in early childhood with difficulties to climb stairs and to get up from the

Abbreviations: DMD, Duchenne muscular dystrophy; ICC, intraclass correlation coefficient; 2MWT, Two minute walk test; 6MWT, six minute walk test.

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http://dx.doi.org/10.1016/j.ejpn.2015.08.002

Table 1 – Patients and characteristics.

Pat. No.	Age at examination (y; m)	No. of walks absolved	Time between walks (days)	2MWD	6MWD	Falls/Rests/ Peculiarities	Vignos u.e.	Vignos l.e	Walton Gardner Medwin Scale	Steroid therapy[b] from age (y; m)	School type and Neurocognitive problems	Genetics *(DMD-Gene)*
1	8; 4	2	21	75/94	251/274	–	1	3	3	6; 2	[f]; elective mutism at school	Duplication Exon 17[a]
2	9; 5	2	8	99/102	284/308	–	1	3	3	5; 9	[g]; behavioural problems	Deletion Exons 45–50[a]
3	10; 4	2	23	90/83	267/250	–	1	3	3	6; 10	[f]; elective mutism in public	Deletion Exons 49–52[a]
4	10; 1	3	19/29	93/92/95	261/263/267	–	1	3	3	6; 9	[e]	Deletion Exons 49–50[a]
5	10; 1	2	15	154/153	452/454	–	1	1	1	6; 2	[e]	Deletion Exon 45[a]
6	10; 2	2	21	66/78	205/232	–	1	3	3	9; 7	[g]	[c]
7	11; 1	2	23	100/93	269/255	Low motivation	1	3	3	8; 5	[g]	c.101411C > T (p.Arg3381Stop)
8	10; 1	2	7	70/91	204/235	–	1	3	3	9; 7	[h]; behavioural problems	Duplication Exons 56–61[a]
9	7; 4	2	20	75/77	231/249	Fall at 4:15	1	2	3	6; 0	[g]	c.10033C > T (p.Arg3345Stop)
10	9; 0	2	3	77/84	221/215	Rests at 3:11; 5:23	1	2	3	5; 0	[f]	Deletion Exons 49–50[a]
11	8; 0	2	21	89/83	253/243	–	1	3	3	7; 4	[g]	c.5026 G > T (p.Glu1676Stop)
12	5; 6	4	36/42	127/126; 123/125	393/391; 345/375	–	1	1	0	5; 5	[d]	Deletion Exons 45–50[a]
13	7; 11	3	14/28	134/118/121	400/370/350	–	1	2	3	5; 10	[f]	Deletion Exons 46–51[a]

u.e. = upper extremity; l.e. = lower extremity.

[a] Out of frame.

[b] Treatment with 0.75 mg/kg bodyweight; therapy given over 10 days followed by a 10 day-break.

[c] No evidence of a pathogenic mutation in DMD gene in patient 6, diagnosis was confirmed by muscle biopsy.

[d] Normal playschool without special support.

[e] Normal school without special support.

[f] Normal school with integration assistant.

[g] Special school for children with learning difficulties.

[h] Conductive support centre and special school for children with learning difficulties.

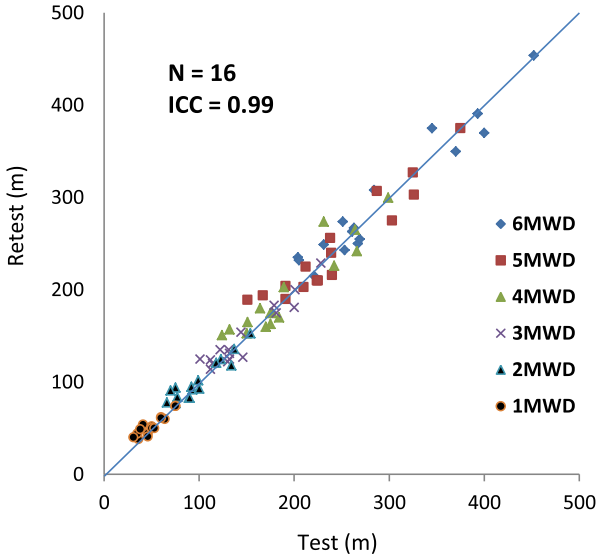

Fig. 1 – *Reliability*. 1–6 min walk distance (MWD) at test (x-axis) and retest (y-axis) for all test–retest pairs at time points 1,2,3,4,5 and 6 min. The overall reliability measured by intraclass correlation (ICC) is excellent with 0.99, visually confirmed by the linear mapping and the low scattering of the point cloud.

floor.[3,4] By the age of 10–14 years, most boys with DMD become wheelchair dependent.[5]

Under supportive therapy of cardiomyopathy and assisted ventilation, the actual median age of survival is approximately 35 years.[6] So far the only established medical treatment in DMD are corticosteroids, resulting in a slight prolongation of ambulation.

Although there is still no cure for DMD, several potential new drugs are currently under investigation.[7,8] On a molecular genetic basis Ataluren is proposed to overread premature termination codons. Exon-skipping affects RNA splicing leading to a shortened but partially functioning dystrophin molecule. The phosphodiesterase inhibitor Tadalafil is intended to correct the disturbed NO-synthesis by stimulating the NO-cGMP pathway.

In order to evaluate the effect of new therapeutic targets, suitable clinical instruments are mandatory.

Currently the six minute walk test (6MWT), which initially was used for the monitoring of adult patients with cardiopulmonary diseases, is choosen as a primary outcome parameter in most studies.[9,10] There are reference values for the 6MWT in healthy children and adolescents available from the age of 5 years on.[11]

Nevertheless behavioural problems in a considerable number of DMD patients make the 6MWT a demanding task for patients and physiotherapists. Weak but cooperative patients are at risk to fall due to exhaustion. To overcome these problems reducing the walking time might be a possible way. The 2MWT has been shown to be better tolerated than the 6MWT in older persons in geriatric rehabilitation.[12] For the use in pediatric patients only scarce information is available.[13]

The purpose of this study was

- to figure out if prolonged ambulation up to six minutes leads to a fatigue of the muscle resulting in a decline of the speed.
- to investigate if findings at shorter time intervals are as reliable as the results of the 6MWD to determine the functional status of the DMD patients.

For this reason the walking distance and the walking speed were calculated at intervals of 1 min up to 6 min. The results for the 2-min distance are selectively presented, because the 2MWT is increasingly discussed and favorized as an alternative to the 6MWT.

2. Materials and methods

A cohort of thirteen ambulatory boys (age 5 and 11 years) with genetically proven DMD was investigated. Clinical status, treatment, type of school the boys visit, neurocognitive particularities and genetic data are listed in Table 1.

Tests were repeated in each patient between two and four times with test–retest-intervals between three and 42 days (mean 21 days) to be sure that no relevant change of the clinical status due to worsening of the disease was reasonable. Patients with intercurrent problems affecting the ability to walk (like intermittent trauma) had to be excluded.

The testing followed standardized procedures according to the ATS recommendations for 6MWT without pulse oximetry.[14] As developed in other studies with DMD patients,[9] a physiotherapist walked behind the patients to provide encouragement and to assist in the event of falling. The walking distance was assessed in meters.

Distance was measured at one minute intervals. All patients were examined by trained physiotherapists experienced in trials with neuromuscular disorders for many years. To minimize the impact of the physiotherapist on the outcome, the tests were repeated by the same physiotherapist.

Statistical analysis was done using SPSS Statistics 22 (IBM, Armink, New York). Test–retest reliability was measured using intraclass correlation analysis (ICC) by calculating Cronbach's Alpha. For visualisation of the means of the walking distance after each minute and 95% confidence intervals are presented.

The study was approved by the local ethics committee of the Ludwig Maximilians University of Munich (internal No: UE 141-14).

3. Results

All patients were able to repeat the test. There was one inadvertent fall during the test in a seven year old patient (vignos lower extremity scale grade 2) at minute 4′15″ and two rests necessary in a nine year old patient (vignos lower extremity scale grade 2).

The overall reliability measured by intraclass correlation (ICC) including all test–retest pairs from 1 to 6 min was 0.99, visually confirmed by the linear mapping and the low scattering of the point cloud (Fig. 1).

The test–retest reliability, regarding the whole test group at all time points was minimum 0.94 after one minute and maximum 0.98 after six minutes. Regarding the test–retest reliability at time points 1,2,3,4,5 and 6 min in different age classes group 1 contained 5–8 year old patients (n = 7 test/retest pairs); group 2 contained 9–11 year old patients (n = 9 test/retest pairs), ICCs were still good for both subgroups. Regarding test–retest-reliability in subgroups according to physical disability, intraclass correlation coefficient was lower for DMD boys with high disability (Vignos Scale Lower Extremity Level 3). In this group of patients the ICC for the 2MWT was lower with 0.61 and became higher with longer duration of the test up to 0.84 for the 6MWT (Fig. 3).

The walking speed was highest in the first minute and then remained stable during prolonged exercise, as can be seen by a straight line crossing all distance means. This was true for the whole test group in test and retest including patients with more severe handicap (level 3 on the Vignos lower extremity scale) (Fig. 2).

4. Discussion

The six minute walk Test (6MWT) is a well established outcome measure in adults in a variety of diseases. It has been shown to be accurate, reproducible, feasible and generally well tolerated.[14] It is increasingly used as a primary outcome measure in pediatric studies.[15] A recent multicenter study in 116 DMD boys[16] showed that amongst various clinical scales the 6MWT was the most reliable test.[17] In a study in healthy children all 19 five-year old probands could perform the test according to the protocol.[11]

Nevertheless compliance and motivation are a problem especially in boys with DMD. Since most of the studies are done in young boys with significant weakness, choosing the 6MWT as a primary outcome parameter limits the number of suitable candidates for clinical studies. Mental retardation and behavioural problems which are common in Duchenne[18] further aggravate this problem. In elder patients with severe

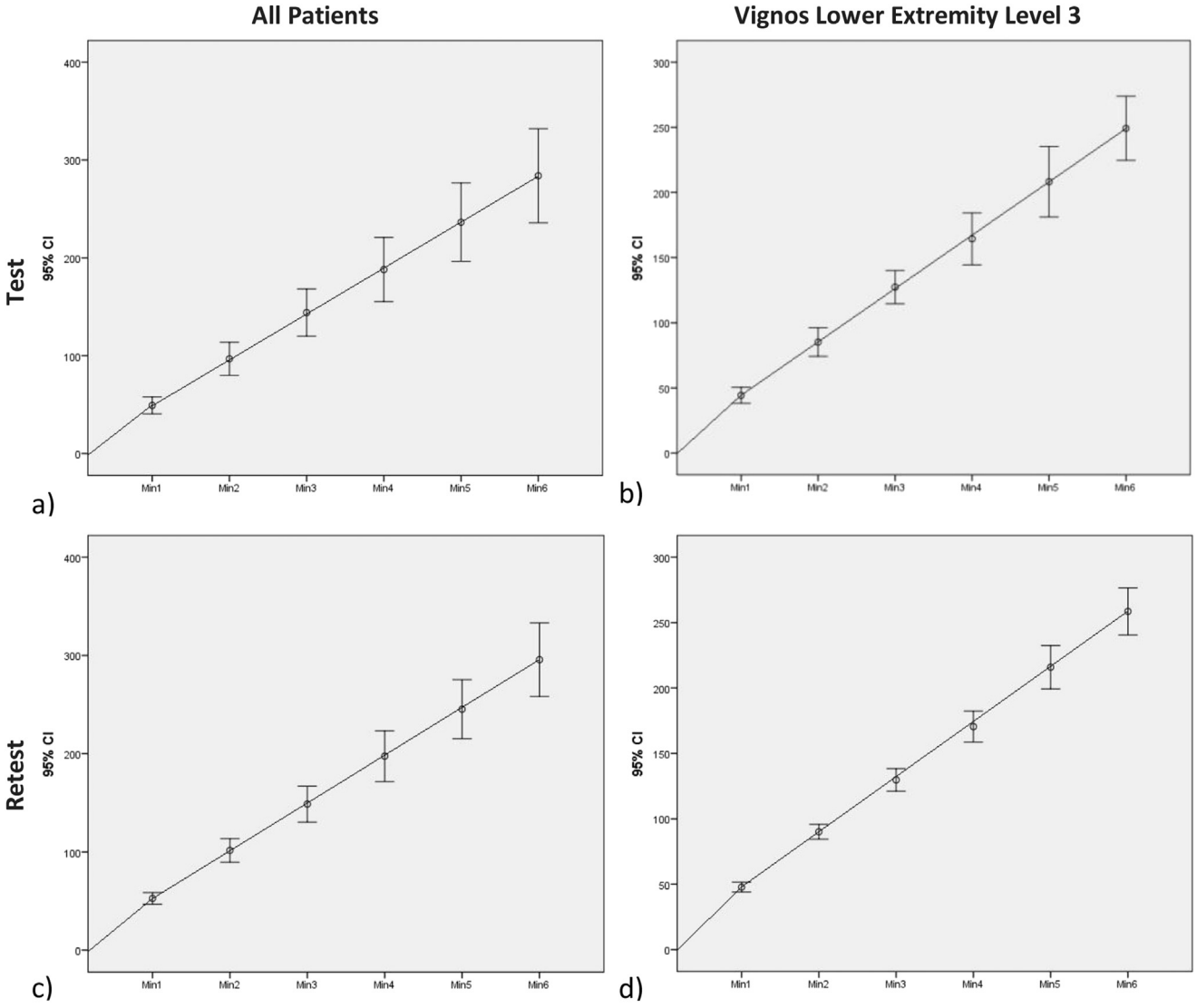

Fig. 2 – *Walking speed*. Means of absolved distance after 1–6 min and their 95% confidence intervals for all patients (a/c) and the vignos lower extremity level 3-subgroup (b/d). X-axis: Minutes, y-axis: Absolved distance in meters.

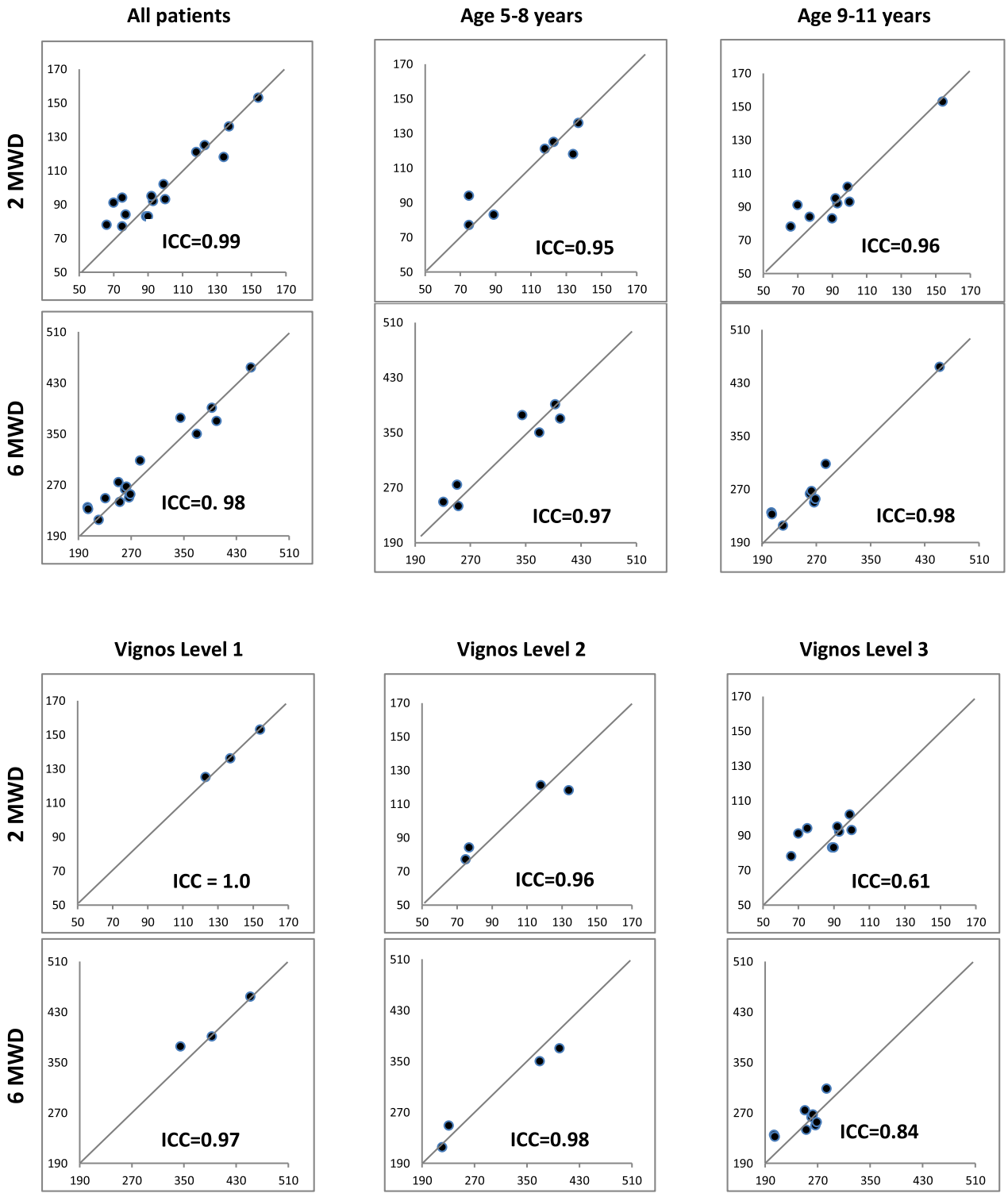

Fig. 3 – *Plotting of 2MWD and 6MWD*. Test–retest reliability for all patients and for subcollectives considering age and Vignos level (lower extremity), respectively, for 2MWD and 6MWD. X-axis = test (baseline); y-axis = retest (screen). ICC = Intraclass correlation coefficient (Cronbach's alpha).

proximal weakness falling during the 6MWT occurs in up to 7%[19] making the 6MWT potentially dangerous in this group of patients. In our study one fall occurred after 4 min during 32 performed walks (3%), suggesting that longer times might increase the risk of falling.

A recent systematic review has provided evidence for the reliability and validity and of the 2MWT in adults, which varied across different patient groups. However, the authors claimed that little is known about the usefulness of the 2MWT especially in paediatric population.

Our results show that test–retest reliability, measured by intraclass correlation analysis, is very strong for both the 6MWD and the 2MWD inside one test, regarding the whole patient group of boys between 5 and 11 years of age and a physical disability between grade 1–3 at the Vignos' scale lower extremity scale.

Increased physical disability as shown by a vignos scale grade 3 might have an influence on the test–retest reliability. In this group the results after six minutes showed good reliability and after two minutes moderate reliability (ICC = 0.84 versus ICC = 0.61, Fig. 2).

According to the "ATS Statement: Guidelines for the Six-Minute Walk Test", the patients choose their own, comfortable velocity of walking. Running (or if running is not possible, maximum speed) is not demanded. Interestingly, we found that the walking speed after one minute remained very constant up to six minutes. Speed during the first minute is little faster (Fig. 2). This was observed in both test and retest, so a learning effect does not have to be assumed. It holds true even for the weaker patients (vignos 3 subgroup) up to six minutes. This finding indicates clearly that physical exhaustion is not be reached within six minutes even in more disabled patients. This is a strong argument that the information from the test at two minutes concerning muscular endurance is not different from that at six minutes since maximal effort is not reached in both kinds of tests. So the question is, if prolonging the test from two to six minutes gives additional information.

Shortening the test would lower the risk of falling and increase motivation, which is a severe problem especially in higher disabled patients with duchenne muscular dystrophy. Furthermore, patients with lower motor performance might be included in clinical studies by using the 2MWT instead of the 6MWT.

4.1. *Study limitations*

Additional studies will be required to confirm our findings. Our setting does not allow to evaluate the sensitivity for the 2MWD to detect changes over time. This assumption can only theoretically be extrapolated from the data.

5. Conclusion

We found that test–retest reliability is very strong for both the 6MWD and the 2MWD at all time points, regarding all results. High physical disability had a negative influence on the test–retest reliability. The constant walking speed indicates that physical exhaustion is not reached within six minutes even in more disabled patients and that the 6MWT in patients with Duchenne does not give additional information compared to a 2MWT.

Conflict of interest

None declared.

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* contributed equally

Jumping Mechanography as a Complementary Testing Tool for Motor Function in Children with Hereditary Motor and Sensory Neuropathy

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Abstract*:*

Objective: To compare Mechanography, measuring force in jumping and arising, with the six minute walk test and time function tests in pediatric patients with hereditary motor and sensory neuropathies.

Method: A cohort of 23 patients performed the six minute walk test and time function tests (time to run ten meters, to arise from a supine position, to climb four stairs), as well as the chair rising test and the single two legged jump on a Mechanography ground reaction force platform. Results were correlated calculating linear regression.

Results: Correlation revealed high or moderate correlation between Mechanography and the six minute walk and the time function tests: S2LJ/6MWT=0.64; CRT/6MWT=0.52; S2LJ/arising from floor=0.63; CRT/arising from floor=0.67; S2LJ/10-meter run=0.74; CRT/10-meter run=0.66; S2LJ/climb 4 stairs=0.56; CRT/climb 4 stairs=0.47.

Interpretation: Jumping Mechanography is a good additional tool for the assessment of pediatric patients with CMT and might be used for primary outcome measures. It was not feasible in more advanced stages of the disease. In less disabled children, the single two legged jump, which quantifies force generated by proximal and distal muscles, might be superior to other tests.

What this paper adds:

- Mechanography adds further quantitative information about the neuromuscular system.
- We demonstrate good correlation with established time function tests in pediatric CMT patients.
- Mechanography seems to be a good additional tool for primary outcome measures.

Keywords:

Jumping Mechanography

Hereditary motor and sensory neuropathy

Outcome measures

Chair rising test

Single two legged jump

Abbreviations:

Charcot Marie Tooth (CMT)

Hereditary motor and sensory neuropathy (HMSN)

Six Minute Walk Test (6MWT)

Chair rising test (CRT)

Single two legged jump (S2LJ)

Introduction

Hereditary motor and sensory neuropathies (HMSN, syn. Charcot-Marie-Tooth disease, CMT) are a group of inherited neuropathies with a broad range of phenotypes, inheritance patterns and causative genes. They range among the most common inherited neurological diseases with a prevalence of 1 in 2500 individuals. The number of disease causing genes identified in CMT has expanded rapidly over the past few decades. More than 60 CMT-associated genes have now been discovered (1). The natural course of various forms of CMT remains poorly understood; in the last years there is effort to get more precise information. In 2013, a large natural history study (2) was performed, including 423 pediatric patients via cross-sectional analysis. Currently, a multicenter prospective natural history study over a 3-year period including pediatric and adult patients is performed in Germany (3).

At the moment there is no causal treatment for any type of CMT. In CMT1a, which is caused by a duplication of the *PMP22* gene, reduction of PMP22 expression might be a treatment. Ascorbic acid, which acts in this way did not show a clinical effect (4). Currently other drugs like a pharmaceutical composition comprising D-Sorbitol, Baclofen and Naltrexone are tested in CMT1a (5).

In order to evaluate both, the natural history of the disease and the effect of new therapeutic targets, suitable clinical instruments are mandatory. Validated clinical instruments for measuring disease severity have become available only recently and have not yet been employed in many of the rare CMT subtypes (6). The six minute walk test (6MWT), which initially was used for the monitoring of adult patients with cardiopulmonary diseases, is chosen as a primary outcome parameter in most studies for outcome measures and natural history in neuromuscular conditions in children. There are reference values for

the 6MWT in healthy children and adolescents available from the age of 5 years on. Classical time function testing such as time to walk or run 10 meters, to climb 4 steps of stairs, or to rise from a supine position are usual endpoints too (7).

Measuring ground reaction forces adds further quantitative information about the function of the neuromuscular system. Portable ground reaction force plates collect dynamic information as a subject jumps or arises. Maximum power relative to body weight is calculated from the ground reaction force counter movement or rise, which is used as an indicator of motor function, primarily in sports (8) (9). It was found to be an easy, safe and reliable tool for the investigation of lower limb musculoskeletal function (10) (11). Reproducibility was reported to be good (12) (10) (13); reference values have been assessed on several cohorts each including more than 300 children and adolescents for grip force, one leg whole body stiffness and multiple one leg hopping (14) for counter movement jumps and chair rising tests (15) as well as the single two legged jump (non-restricted counter movement jump) (16). The influence of short term repetition of the measurements on the analysis results was considered to be small as compared to the effects of training or therapy (17). Mechanography is increasingly used in a medical context (18) (19) (20).

Rittweger et al reported in 2007 a strong correlation of Mechanography with other time function assessment indices including the “timed up & go test”, the “chair rising test”, and walking speed (12).

It was shown, that from the age of 12 years on, there is a significant gender dependent difference in the age-depending normative values (21). When assessing the effects of ageing on the measurements in Mechanography, a high correlation between age and maximum power relative to body weight (P_{max}rel) in both males and females (associated with body mass) was reported. Based on two studies (22) (16), an assessment index called the Esslinger

Fitness Index (EFI) was developed, which is calculated as a percentage of the standard value of motor function for each age and gender (9) (19).

In neuromuscular conditions, Mechanography has so far not been used as a tool for measuring motor function.

The purpose of this study was to compare Mechanography, measuring force in jumping and arising (the single two legged jump test and the chair rising test) with established time function tests in a cohort of pediatric CMT patients.

Method

Participants:

A cohort of 23 ambulatory patients (patient's age at examination 4.33- 17.8 years, mean 11.7 years, median 13.1 years, participating females n=9, males n=14), was retrospectively analysed. All patients were regularly seen in our pediatric neuromuscular center at the Dr. v. Hauner Children's Hospital, Ludwig-Maximilian University of Munich, Germany.

Inclusion criteria were a neurophysiological and / or genetic proof of a hereditary motor and sensory polyneuropathy and the physical and intellectual ability to perform all tests correctly. Patient's clinical status as shown by the Vignos-Scale for lower extremity, their gender, their age at examination and their genetic data are provided in Table 1.

All tests were done as part of the routine monitoring of CMT patients in our outpatient clinic on a single day.

Six patients performed the testing twice, four patients three times. In total, 37 exams are included in the study, in 6 of them only the "single two legged jump" (S2LJ) without the "chair rising test" (CRT) was done.

Measurements: All patients were examined by trained physiotherapists with longstanding experience in clinical trials in the field of neuromuscular disorders. All children performed the six minute walk test (6MWT), time function tests and jumping Mechanography.

The time function testing followed standardized conditions. The measurements were performed always in the same order. Sufficient resting time between the single items was given. Patients were advised to climb four stairs, to run ten meters and to arise from a supine position as fast as possible. Results were assessed in seconds. For the stair climbing test, a standardised four-step-model was used, adjusted as required for the "North star test". The six minute walk test was performed according to the ATS recommendations for 6MWT without pulse oximetry. A physiotherapist walked behind the patients to provide encouragement and to assist in the case of falling. The six minute walking distance was assessed in meters.

For Mechanography, the system Leonardo Mechanograph® GRFP (Novotec Medical GmbH, Pforzheim, Germany) was used. It consists of a split ground reaction force platform (GRFP) connected to a computer. The force was analysed using the Leonardo Mechanography software Version 4.3.

Every patient absolved the "single two legged jump" (S2LJ) test consisting of three consecutive jumps. The platform was zeroed before each patient entered and the patient's body weight was measured before and after each jump automatically. For jumping, the patients were advised to jump as high as possible and to try to land on their forefeet. For the "chair rising test" (CRT), a bench was installed on the platform which serves as a seat with a sitting height that produces a 90° angle in the patient's knees. For measuring, the patient was advised to stand up as fast as possible until the legs are straight, and to sit down again.

The arms had to be crossed over the chest (Supplemental Figure 1). P_{max}rel (SI unit is W/kg) was measured from CRT and from S2LJ.

All results acquired by the software have been manually reviewed and correctly elaborated by the computer scientist of the company (co-author R.R.).

Statistical analysis:

Analysis was performed using Excel (Microsoft, Redmond, Washington, USA). Results from Mechanography were independently correlated with the results from the time function tests and the six minute walk and analysed using linear regression.

The study was approved by the local ethics committee of the Ludwig Maximillian's University of Munich (internal N^{o}: UE 141-14).

Results:

No inadvertent falls occurred during the testing of the single items in all patients.

There was significant correlation between both Mechanography tests and the time function tests and the 6MWT:

Correlation between the six minute walk test (6MWT) and the single two legged jump (S2LJ) was high with a correlation coefficient of 0.64. 6MWT and chair rising test (CRT) correlated moderately with a coefficient of 0.52. Correlation of both the CRT and the S2LJ with arising from the floor was high with a coefficient of 0.67 and 0.63, respectively. Comparison between the CRT / the S2LJ and the ten meter run showed highest correlation of 0.66 and 0.74, respectively. Correlation with the time needed to climb four stairs was lowest but still moderate with 0.56 for the S2LJ and 0.47 for the CRT. All results are visualized in Figure 1.

Since both Mechanography tests revealed comparable results in correlation with the different time function tests, we checked for intra-correlation between the results of the CRT and the S2LJ, which revealed the expected high correlation with a coefficient of 0.77.

Discussion:

We investigated a cohort of pediatric patients with hereditary motor and sensory neuropathy and compared results from time function testing and the six minute walk test with two Mechanography tests, the chair rising test (CRT) and the single two legged jump (S2LJ). The six minute walk test is so far the best established outcome measure in children with neuromuscular disorders (23). It has been shown to be accurate, reproducible, feasible and generally well tolerated (7). Nevertheless, walking distance is only one parameter in assessment of motor function, disability and strength. In CMT1a, potential new drugs are currently under investigation. In order to evaluate the effect of new therapeutic targets, suitable clinical testing instruments for physical ability are mandatory.

For this reason other time function tests and more complex assessment scales have been established or are under evaluation for suitability. The CMT Neuropathy Score (CMTNS), a 36 point composite score, is the most established score to assess clinical outcome in adults. In children, among others (24) the CMT Pediatric Scale as an outcome measure of disability was published 2012 (25). It establishes raw scores for each individual with CMT via eleven items, including sensory testing, fine motor skill testing and gross motor function testing via gait (foot drop, difficulty heel walking), long jump and 6 minute walk test, indicating to be a relatively precise, but very time- and resource consuming test.

So far there are no studies about the usefulness of Mechanography in pediatric patients with neuromuscular disorders. Our results show that the CRT and the S2LJ correlate well with the

10-meter run and the time to arise from a supine position. These entire tests require a short term activation of the motor system. There is a moderate correlation with the 6MWT. These findings are in line with the well-known clinical observation that patients with CMT perform much better in longer term endurance exercises than in short term activation like short distance running.

The only result with a low correlation was the time to climb four stairs compared with the chair rising test. Our data show that all patients in our cohort needed very little time to climb stairs. The only exceptions were very small children who were unable to use the rails. This indicates that stair climbing in contrast to the S2LJ does not reflect the functional status of the lower extremities in this CMT population.

Our results demonstrate that jumping Mechanography is a useful tool in assessing motor function in patients with hereditary motor and sensory neuropathy and that it might be helpful as well in other neuromuscular conditions. It can be used in neuromuscular patients able to perform a jump with two legs and to stand up from a chair without using their hands, describing levels 1 and 2 on the Vignos-Scale for lower extremity. It seems to be very useful especially in this group of patients with lower grades of disability since exact force is quantified and intra-individual decline is measured concisely. Especially the single two leg jump acquires information from both proximal and distal musculature.

Since there is already some normative data available in children (22) (16) and a tool (the Esslinger Fitness Index), which is calculated as a percentage of the standard value of motor function for each age and gender, statements defining comparison with healthy children will as well be possible.

Conclusion:

The Leonardo Mechanography seems to be a good additional tool for the assessment of (pediatric) CMT patients. There was a good correlation between clinical tests (6MWT and time function tests) and Mechanography (CRT and S2LJ) in children and adolescents with CMT. It seems to be a good additional tool - quantifying strength - and should be considered for primary outcome measures in clinical trials.

Especially in less disabled children, the single tow legged jump, which quantifies force and power generated by both proximal and distal muscles in a single test item, might be superior to other tests. Additional studies will be required to confirm our findings.

Table 1: Patient's characteristics. y = years, mo = months, l.e. = lower extremity.

*Patients 9 and 10 are identical twins. The deletion results in functional haploinsufficiency, the phenotype is an overlap between CMT1 and HNPP.

Supplemental Figure 1: Visual instruction for the single two legged jump (a) and chair rising test (b) as formerly provided on the Novotec homepage.

Figure 1: Correlation of CRT (a) and S2LJ (b) with 6MWT and time function testing

Correlation, calculated by linear regression. X-axis = Mechanography; y-axis =Time Function Tests. R= correlation coefficient.

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Table 1:

Pat Nr	Gender	Mutation	Age at test 1 (y;mo)	Age at test 2 (y;mo)	Age at test 3 (y;mo)	Vignos Scale l.e.
1	m	duplication of *PMP22*	4;9	5;3		2
2	m	duplication of *PMP22*	6;2			1
3	m	duplication of *PMP22*	5;9	6;11		1
4	w	duplication of *PMP22*	9;3			1
5	m	duplication of PMP22	12;7			1
6	w	duplication of *PMP22*	16;5			1
7	m	duplication of *PMP22*	17;9			1
8	m	duplication of *PMP22*	17;11			1
9*	m	*PMP22* gene: c.407_418del12 (heterozygous)	16;7	17;6		1
10*	m	*PMP22* gene: c.407_418del12 (heterozygous)	16;7	17;6		1
11	m	duplication of *PMP22*	7;6			1
12	w	*SBF2*: c.2678T>C (homozygous)	13;6	13;7	14;6	1
13	m	*SBF2*: c.2678T>C (homozygous)	15;7	15;8	16;7	1
14	m	*SH3TC2*: c.1550delG (A518fs) / c.2860C>T (R954X)	13;4	14;10		1
15	w	*NDRG1*: c.442C>T (p.Arg148Ter)	4;4	4;8	5;2	1

Table 1: Patients and characteristics. y = years, mo = months, l.e. = lower extremity.
*Patients 9 and 10 are identical twins. The deletion results in fuctional haploinsufficiency, the phenotype is an overlap between CMT1 and HNPP.

Figure 1:

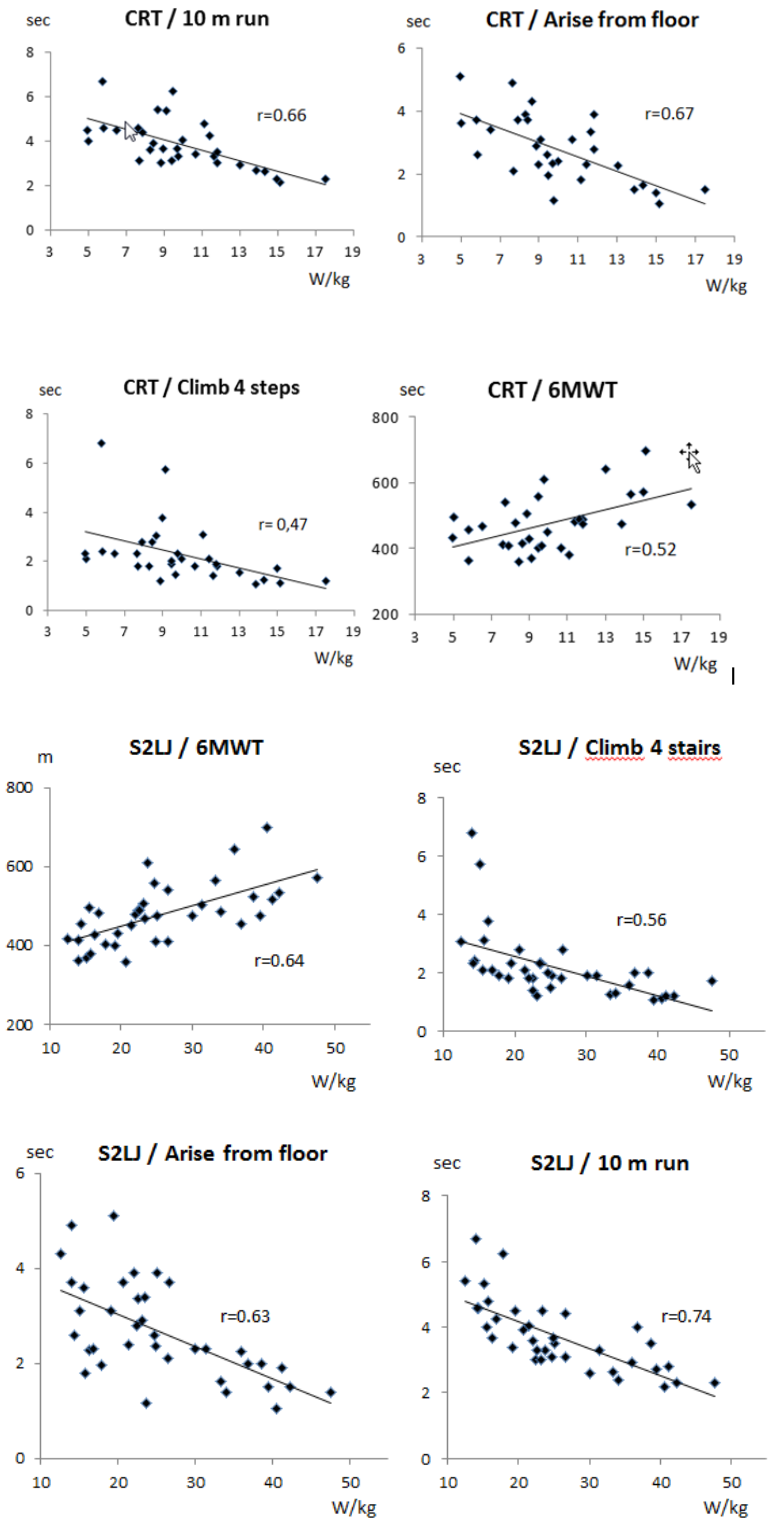

Figure 1: *Correlation of CRT and S2LJ with other time function testing*
Correlation, calculated by linear regression. X-axis = mechanography; y-axis =TFT.
R= correlation coefficient.

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6. DANKSAGUNG

Zuerst möchte ich mich bei Herrn Prof. Dr. med. Müller-Felber bedanken für die freundliche Überlassung des Themas und die Möglichkeit alle Testungen im SPZ des Haunerschen Kinderspitals durchführen zu können.

Mein ganz besonderer Dank gilt meiner Betreuerin Frau PD Dr. med. Katharina Vill, ohne sie wäre diese Arbeit nicht möglich gewesen. Sie stand mir über den gesamten Zeitraum meines Promotionsvorhabens mit viel Geduld und großer Hilfsbereitschaft zur Seite.

Zudem möchte ich mich ganz herzlich bei dem Team der Physiotherapeuten des Haunerschen Kinderspitals bedanken, insbesondere bei Frau Therese Well.

Ebenso mochte ich mich bei meinen Eltern Rita & Klaus Reinhuber bedanken, welche mir immer zur Seite stehen und durch diese mir das Studium der Humanmedizin erst möglich wurde.

Mein größter Dank gilt meiner Familie, meinem Mann Sebastian und meinem Sohn Moritz, welche mich über die gesamte Zeit meiner Promotion unterstützten und bekräftigten.