Renal function in children and adolescents with Duchenne muscular dystrophy

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Abstract

Improved life expectancy and the need for robust tools to monitor renal safety of emerging new therapies have fueled the interest in renal function in Duchenne muscular dystrophy (DMD) patients. We aimed to establish a methodology to accurately assess their renal function. Twenty DMD patients (5–22 years) were included in this prospective study. After obtaining medical history, all patients underwent a clinical examination, 24-hour ambulatory blood pressure monitoring, ultrasound of the kidneys, direct GFR measurement (51Cr-EDTA, mGFR), complete blood and urine analysis. Seventeen of 20 patients were treated with corticosteroids and 5/20 with angiotensin converting enzyme inhibitor (lisinopril). No patient suffered from urinary tract infections or other renal diseases. Hypertension (systolic or diastolic blood pressure >95th percentile) was found in 9/20 patients. Inverse correlation between mGFR and age was observed (R² = 0.45, p = 0.001). Serum creatinine based estimated GFR (eGFR) equations overestimated mGFR up to 300%. eGFR based on cystatin C Filler equation was closest to the mGFR (median eGFR (IQR) of 129.5 (39.7) mL/min/1.73 m²). Our study demonstrates a high prevalence of hyperfiltration and hypertension in children and adolescents with DMD. Because the majority of hypertensive patients were under corticosteroid treatment, the iatrogenic cause of hypertension cannot be excluded. Serum or urine creatinine measurements are of no value to evaluate renal function in DMD patients due to the reduced skeletal muscle mass.

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Keywords: Duchenne muscular dystrophy; Kidney function; GFR; Hyperfiltration; Blood pressure

1. Introduction

Duchenne muscular dystrophy (DMD, MIM 310200) is an X-linked recessive muscle disorder affecting around 1 in 3500 to 6000 newborn boys [1]. An absent or reduced expression of the dystrophin protein, caused by mutations (mainly deletions) in the dystrophin gene, results in progressive muscle degeneration [2]. Without treatment patients rarely survive beyond their teens as the disease also causes cardiorespiratory failure. In recent decades, life expectancy of patients with DMD has increased, due to improved respiratory, cardiac and orthopedic treatment and potentially also due to long-term corticosteroid treatment [3–6]. Emerging therapies, targeting dystrophin restoration, muscle growth or pathophysiological events downstream from the dystrophin deficiency have moved into clinical development. Among these, RNA modulating approaches such as antisense mediated exon skipping and nonsense codon suppression aim to restore the production of partially functional or full length dystrophin protein [7]. A recent phase 1-2a study of local intramuscular administration of the antisense oligonucleotide PRO051 showed a modest improvement in the 6-minute walk test after 12 weeks of extended treatment, but variable proteinuria was an adverse event in all patients [8]. However, in general, little is known about renal function in DMD patients. A Japanese cause-of-death analysis reported death caused by renal failure in 14% of
286 DMD patients [9]. The same group demonstrated increased plasma levels of cystatin C (CysC) in more than 30% of these patients over the age of 30 [10].

Improved life expectancy and the need for robust tools to monitor renal safety of emerging new therapies have fueled the interest to evaluate renal function in DMD patients. Methodologically, estimating glomerular filtration rate (GFR) and urinary excretion of proteins and other electrolytes in this patient population are seriously hampered by the uselessness of creatinine, due to reduced skeletal muscle mass. Therefore, we evaluated other accurate and validated creatinine-independent methods for monitoring GFR and urinary excretions. In this regard, CysC, which is independent of muscle mass and hydration, was suggested as a valuable alternative for calculating estimated GFR (eGFR) in these patients [11,12].

In this study we aimed to establish a methodology for studying renal function in patients with DMD and, using this methodology, to describe their renal function in detail.

2. Patients and methods

2.1. Patients: medical history and clinical examination

Twenty DMD patients, aged between 5 and 22 year, with proven mutations in the dystrophin gene attending the neuromuscular reference center at the University Hospitals Leuven, Belgium, were enrolled in this cross-sectional prospective study. Medical history, personal or family history of urinary tract infections or other renal diseases, cardiac shortening fraction, corticosteroid regimen, treatment with angiotensin converting enzyme inhibitors (ACEi), age of diagnosis and ambulatory status were recorded. During a general clinical examination, length, weight and body mass index (BMI) were determined. Twelve non-ambulatory patients were weighed with a hoist and their height was predicted from their ulnar length, measured with a Harpenden anthropometer in a sitting position, based on the formula of Gauld et al. [13].

2.2. Blood and urine analyses

The blood tests included complete blood count, electrolytes, total serum protein, creatine kinase, 1,25-dihydroxy vitamin D, serum creatinine (Scr), urea and CysC. The enzymatic assay of Roche was used to determine Scr. CysC was measured using nephelometry (BN II Nephelometer). A 24-hour urine specimen was used to measure total protein, alfa-1 microglobulin, creatinine, electrolytes and glucose.

2.3. Ambulatory blood pressure monitoring (ABPM)

An oscillometric device (Mobil-O-Graph NG) was attached to the non-dominant arm with an appropriate cuff. Measurements were performed every 15 minutes during daytime and every 30 minutes during the night and lasted for 24 hours. Except for one participant, treatment with ACEi lisinopril was temporarily stopped 7 days before ABPM. We used the reference values of Wühl et al. [14] defining hypertension as a blood pressure (BP) >95th percentile. We defined a non-dipping BP profile as a nocturnal decrease <10% of daytime BP [15].

2.4. Renal ultrasound

Ultrasound of the urinary bladder and kidneys was performed using a Philips iU22 ultrasound system. Bipolar diameters were measured and compared with the reference values according to age [16] and length [17]. We defined nephromegaly as a kidney length > mean + 2 standard deviations for length. We also considered kidney length according to reference values for age.

2.5. Measured GFR (mGFR)

After administering a single bolus injection of $^{51}$Cr-EDTA, eight blood samples were taken at 15, 30, 45, 60, 120, 180, 240 and 300 minutes after injection. Data were bi-exponentially fitted followed by correction for body surface area (BSA), according to the formula of Du Bois and Du Bois [18]. Hyperfiltration was defined as mGFR >150 mL/min/1.73 m². This value is based on reference values of Pottel et al. [19]: a median GFR of 107.3 mL/min/1.73 m² with a standard deviation of 21.5 mL/min/1.73 m² leads to a 97.5th percentile of 150 mL/min/1.73 m².

2.6. Estimated GFR (eGFR)

Estimated GFR values were calculated using four Scr-based equations (Schwartz [20], Flanders Metadata [19], simple height-independent [21], Q(height) [22]), three CysC-based equations (Larsson [23], Filler [24], Zappitelli [25]) and two Scr/CysC-based equations (Zappitelli [25], Bouvet [26]). Only formulas based on enzymatic Scr and/or nephelometry were included. The different formulas are shown in Table 1.

2.7. Statistics

SPSS 20 and GraphPad Prism 6.0 (La Jolla, CA, USA) were used for statistical analyses. The median and interquartile range (IQR) were calculated for all the variables. Associations between categorical variables were evaluated using the Pearson Chi-Square test. For continuous variables, Spearman’s rank test was used. P-values are considered significant at the 5% significance level but should be considered as explorative, therefore there is no correction for multiple testing. There was no a-priori power analysis (or sample size calculation) as there was no pre-set hypothesis for this study. Consequently, recruitment of patients in the DMD population for this invasive direct mGFR measurement was limited to 20 for ethical reasons.

2.8. Ethical approval

The study was approved by the Institutional Ethical Board of UZ Leuven. Consent forms were signed by parents of participants ≤17 years of age or subjects ≥18 years old. Assent forms were signed by participants 5–17 years old.

3. Results

The clinical characteristics of the patients are presented in Table 2. The median age was 15.5 years. Sixteen of the 20 patients were treated with daily corticosteroids (deflazacort or...
prednisone), 1 patient was treated with intermittent deflazacort (one day on/one day off) and 5/20 patients were treated with ACEi lisinopril. No patient had a personal or family history of urinary tract infections or other renal diseases.

3.1. ABPM

Nine of 20 subjects had an elevated BP (BP >95) (of which there were 8 patients treated with steroids) and 13/20 subjects had a non-dipping BP profile (of which there were 10 patients treated with steroids) (Table 2).

3.2. Blood and urinalysis

The blood samples showed no important electrolyte abnormalities. Protein to creatinine ratio was elevated in 17/18 patients (not determined in two patients), while all patients had normal 24-hour urinary protein excretion. Twenty-four-hour urinary sodium excretion was between 2 and 3, between 1 and 2 and below 1 mmol/kg/day for 11/19, 4/19 and 4/19 subjects respectively (not determined in one patient). Renal tubular reabsorption of phosphate (TmP/GFR) was normal in all subjects. There were no subjects with glycosuria or low molecular weight proteinuria. Hypercalciuria (>4 mg/kg/day) was present in three patients.

3.3. Renal ultrasound

When compared to reference values for length of the patients, 2/20 patients showed bilateral nephromegaly and 4/20 subjects had unilateral nephromegaly. When reference values

### Table 2

Clinical and biochemical characteristics of the DMD patients.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age at diagnosis</th>
<th>Age at evaluation</th>
<th>Scr (mg/dL)</th>
<th>Cys C (mg/L)</th>
<th>U protein (g/g creat)</th>
<th>U protein (mg/24 hr)</th>
<th>SD (Right, Left)</th>
<th>Day HT y/n</th>
<th>Night HT y/n</th>
<th>Non-dipping y/n</th>
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</table>

Median (IQR)

1.8 (2.9) 15.5 (4.3) 0.13 (0.07) 0.75 (0.22) 0.25 (0.11) 0.07 (0.05) 130.4 (29.1)

Scr, serum creatinine; Cys C, cystatin C; HT, systolic and/or diastolic blood pressure >95; non-dipping, systolic and/or diastolic non-dipping blood pressure profile; y/n, yes/no; ACEi, angiotensin-converting enzyme inhibitor; d/p, deflazacort/prednisone.

* 6 mg of deflazacort is equivalent to 5 mg prednisone, "one day on/one day off" [28].
for age were used, the majority of the patients had normal (n = 17) or decreased (<2SD) kidney size (n = 2), one patient showed unilateral increased kidney size (>2SD). No structural renal anomalies were observed.

3.4. GFR measurements

The eGFR values obtained by different Scr and/or CysC based formulas and mGFR by 51Cr-EDTA are shown in Table 3. Median (IQR) mGFR measured by 51Cr-EDTA was 130.4 (29.1) mL/min/1.73 m². One patient had a slightly decreased mGFR of 85.7 mL/min/1.73 m². Five patients had mGFR >150 mL/min/1.73 m², indicating hyperfiltration. Median eGFR calculated using Scr was 438.2, 439.1, 177.9 and 353.8 mL/min/1.73 m² for Schwartz, Flanders Metadata, Simple height-independent and Q(height) equations respectively. Median eGFR was 142.2, 129.5 and 108.9 mL/min/1.73 m² using the CysC-based Larsson, Filler and Zappitelli eGFR formulas, respectively. For the Scr/CysC combined eGFR formulas, median eGFR was 155.9 and 169.5 mL/min/1.73 m² for Zappitelli and Bouvet, respectively.

Overall the CysC-based Filler equation gave the best performance compared to the true mGFR, with a bias of 10.9 mL/min/1.73 m² and eGFR values within 30% of mGFR in 83% (15/18) of patients and within 10% in 56% (10/18) (Figure 1). The median (IQR) creatinine clearance calculated using 24-hour urine collection was 174.6 (61.6) mL/min/1.73 m². A progressive decline in mGFR with age of the DMD patients was observed (R² = 0.45, p = 0.001; Figure 2).

There was no correlation between cardiac shortening fraction, wheel chair dependency, BMI or steroid use and mGFR, the presence of hypertension or non-dipping BP profile (data not shown).

4. Discussion

During the last decades, dramatically improved clinical care resulted in prolonged life expectancy of patients with numerous, previously lethal genetic diseases of childhood allowing them to survive into adulthood. These exciting advances changed the clinical history of these patients and revealed novel disease- or therapy-related problems including renal dysfunction [30,31]. Muscular dystrophies are among the most common single gene disorders with DMD being the most frequent form affecting 1 of 3500 to 6000 newborn males. Average life expectancy in DMD patients has shifted from adolescence in the 70s of last century to third and fourth decade.

Table 3

<table>
<thead>
<tr>
<th>Prediction performance of eGFR equations.</th>
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<td><strong>Scr based eGFR</strong></td>
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<tr>
<td>Schwartz equation (n = 20)</td>
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<tr>
<td>FM equation (n = 20)</td>
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<tr>
<td>Simple equation (n = 20)</td>
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<tr>
<td>Q(height) equation (n = 20)</td>
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<tr>
<td><strong>CysC based eGFR</strong></td>
</tr>
<tr>
<td>Larsson equation (n = 18)</td>
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<tr>
<td>Filler equation (n = 18)</td>
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<td>Zappitelli equation (n = 18)</td>
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<tr>
<td><strong>Scr/CysC based eGFR</strong></td>
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<tr>
<td>Zappitelli equation (n = 18)</td>
</tr>
<tr>
<td>Bouvet equation (n = 18)</td>
</tr>
<tr>
<td>Median mGFR (IQR) (n = 20)</td>
</tr>
</tbody>
</table>

eGFR and mGFR are expressed in mL/min/1.73 m². Bias is calculated as mGFR-eGFR.

Fig. 1. Relation between mGFR (51Cr-EDTA) and eGFR calculated by Cystatin C-based Filler equation.

Fig. 2. mGFR (51Cr-EDTA) as a function of age in DMD patients. The black lines represent the 10th (81 mL/min/1.73 m²), 50th (107.3 mL/min/1.73 m²) and 90th (135 mL/min/1.73 m²) percentiles according to Piepsz [29] and Pottel [19]. The dotted lines represent the 2.5th (65 mL/min/1.73 m²) and 97.5th (150 mL/min/1.73 m²) percentiles. Hyperfiltration is defined as mGFR > 150 mL/min/1.73 m² (=97.5th percentile.).
of life, with some patients surviving now even in their forties [3,4,32], mainly because of improved supportive treatment of cardiac, respiratory and orthopedic complications [5,6]. As a reference center for DMD we were alarmed by few recent publications suggesting that renal function might be compromised by this disorder [8–10]. Moreover, the emergence of new therapeutic strategies moving into clinical development has highlighted the need for safe and robust tools to monitor renal safety of new compounds. This stimulated us to perform a systematic analysis of renal function in 20 genetically identified DMD patients to describe the natural history of the kidney function in DMD. Furthermore, this study aimed at providing better insight in the different eGFR equations currently used to determine renal function and their applicability for monitoring renal function in DMD.

As it could be expected, our study convincingly demonstrated that serum and urine creatinine measurements are of no value in DMD patients because of the reduced muscle mass. All equations using Scr for calculating eGFR showed a significant overestimation up to 300% compared to mGFR by 51Cr-EDTA clearance, considered as gold standard. Twenty-four hours creatinine clearance was on average 1.3 times higher compared to 51Cr-EDTA clearance and the variability was twice as high. Urinary protein excretion expressed per gram creatinine was slightly above the reference range in the majority of patients, while it was normal when 24-hour protein excretion was considered. Together these results indicate that creatinine should not be measured in DMD for estimating kidney function.

In contrast, CysC might be a promising marker to be used for this purpose as it is independent of muscle mass [33–35]. CysC is a 13.3 Da non-glycosylated basic protein produced by all nucleated cells. Because of its low molecular weight, more than 90% of CysC is filtered in the glomeruli followed by an almost complete degradation by tubular cells [35]. High dose steroids are known to increase CysC production, whereas low and medium doses seem to have no influence [36]. As no clear cut-off steroid dose has been determined in this respect, it remained uncertain to which extent the steroid doses administered in 17/20 of our DMD patients could affect their CysC levels. For the whole group CysC-based eGFR obtained by Filler equation most closely corresponded to the mGFR, however, for the individual patients the difference between mGFR and eGFR was substantial in many cases. Further studies in larger cohorts are required to evaluate whether Filler equation might be of clinical value in DMD patients.

An interesting and rather unexpected observation of our study was the presence of glomerular hyperfiltration found in 25% of DMD patients. Moreover, 19 of 20 patients had mGFR values above the median reference GFR of 107.3 ml/min/1.73 m² [19]. Glomerular hyperfiltration has been associated with various diseases including diabetes mellitus, polycystic kidney disease, secondary focal segmental glomerulosclerosis, obesity, sleep apnea, cancer and blood disorders [37]. In patients with diabetes, hyperfiltration was reported to be a significant risk factor for developing macro- or microalbuminuria [38]. In patients with pre-hypertension, subpopulations with hyperfiltration were supposed to be at increased risk for subsequent kidney damage [39]. In our study, with an exception of one patient (age 16) having mGFR of 85 ml/min/1.73 m², no other patients with decreased mGFR were found, however, it is tempting to speculate that hyperfiltration might also lead to renal dysfunction in DMD patients in their late twenties or thirties as a progressive decline in GFR was observed in our small cohort with increasing age.

Hyperfiltration may also occur during an episode of acute pyelonephritis in both normal and abnormal kidney [29]. In our study population, none of the subjects with an elevated GFR had a urinary tract infection in the prior history or at the moment of the scintigraphy, making this latter cause unlikely.

Harrap et al. reported that in healthy young adults (16–24 years) with elevated blood pressure, glomerular hyperfiltration correlated with high plasma renin [40]. An activated renin angiotensin aldosterone system (RAAS) acts as a possible underlying pathophysiological mechanism due to efferent arteriolar vasoconstriction. Although plasma renin activity and aldosterone levels were not measured in this study, rather low renal sodium excretion might indicate RAAS activation in some of our patients. Of note ACEi therapy was discontinued prior to inclusion in 4/5 patients.

Another observation supporting RAAS activation was the elevated BP and the presence of a non-dipping BP profile found in more than 50% of the patients. The prevalence of hypertension (47%) in our patients treated with corticosteroids was higher compared to the previously reported larger cohort of DMD patients who were on a daily prednisone regimen for 4 years (22%) [41]. The discordance between ours and the latter study might be due to the use of ABPM in our patients, which is known to be a more reliable method for BP evaluation compared to conventional ambulant measurements [42]. Consequently, corticosteroid use should be considered as a potential risk factor for hypertension in the DMD population and the impact of long-term chronic steroid treatment on renal function in DMD should be further investigated. At present, a non-dipping BP profile in adults is a proven risk factor for target organ damage and increases cardiovascular risk in both hypertensive and normotensive patients [15]. Although no correlation between hypertension or non-dipping BP profile and cardiac shortening fraction could be observed, rigorous follow-up of BP is of particular importance in DMD patients because of their compromised cardiac function.

It remains an intriguing question whether mutations in the dystrophin protein as such can underlie (are responsible for) kidney damage rather than secondary causes such as RAAS activation or the presence of hypertension. The expression of non-muscular isoforms of dystrophin demonstrated in the macular densa, mesangial and endothelial cells of the kidney supports this possibility [43,44]. Based on our observation in this small patient cohort, further assessment of kidney function and morphology in animal models of DMD is an exciting area for future research and might shed more insight in the underlying pathophysiology of renal dysfunction in DMD patients.
5. Conclusions

This cross-sectional prospective study convincingly demonstrated that serum and urine creatinine measurements are of no value for the evaluation of kidney function in DMD patients. Direct measurement of GFR using $^{51}$Cr-EDTA should be used instead. Serum CysC-based equations might be an alternative to evaluate GFR but their utility should be further studied in larger patient cohorts. The presence of hyperfiltration or a non-dipping blood pressure profile found in more than 50% of the patients indicates a subtle kidney damage warranting a rigorous follow-up of kidney function in patients with DMD surviving into adulthood.

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References


