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The utility of a portable muscle ultrasound in the assessment of muscle alterations in children with acute lymphoblastic leukaemia

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Abstract

Background During treatment for acute lymphoblastic leukaemia (ALL), children are prone to musculoskeletal deterioration. However, non-invasive tools to measure muscle mass and intramuscular alterations are limited. In this study we explored the feasibility of muscle ultrasound in children with ALL. Additionally, we analysed whether automated ultrasound outcomes of muscle size and intramuscular fat infiltration (IMAT) were associated with appendicular skeletal muscle mass (ASMM), muscle strength and physical performance.

Methods Children with ALL, aged 3–18 years were included during maintenance therapy. Bilateral images of the rectus femoris muscle were captured using a portable linear array transducer connected to a tablet. Subsequently, an automated image annotation software (MuscleSound) was used to estimate cross-sectional area, muscle thickness and IMAT. Feasibility was assessed using acceptance (percentage of children approached who were enrolled), practicality (percentage of children that completed the ultrasound measurement after enrolment) and implementation (percentage of children that had sufficient imaging to be processed and analysed by the software). Assessments of ASMM by bioimpedance analysis, muscle strength using handheld dynamometry and timed physical performance tests were administered at the same visit. Multivariable linear models were estimated to study the associations between muscle ultrasound outcomes and ASMM, strength and physical performance, adjusted for sex, age, body mass index and ALL treatment week.

Results Muscle ultrasound was performed in 60 out of 73 invited patients (76.9%), of which 37 were boys (61.7%), and median age was 6.1 years (range: 3–18.8 years). The acceptance was 98.7%, practicality 77.9% and implementation was 100%. Patients who refused the examination (n = 13) were younger (median: 3.6, range: 3–11.2 years) compared with the 60 examined children (P = 0.0009). In multivariable models, cross-sectional area was associated with ASMM (β = 0.49 Z-score, 95% confidence interval [CI]:0.3,2.4), knee-extension strength (β = 16.9 Newton [N], 95% CI: 4.8, 28.9), walking performance (β = −0.46 s, 95% CI: −0.75, −0.18) and rising from the floor (β = −1.07 s, 95% CI: −1.71, −0.42). Muscle thickness was associated with ASMM (β = 0.14 Z-score, 95% CI: 0.04, 0.24), knee-extension strength (β = 4.73 N, 95% CI: 0.99, 8.47), walking performance (β = −0.13 s, 95% CI: −0.22, −0.04) and rising from the floor (β = −0.28 s, 95% CI: −0.48, −0.08). IMAT was associated with knee-extension strength (β = −6.84 N, 95% CI: −12.26, −1.41), walking performance (β = 0.2 s, 95% CI: 0.08, 0.32) and rising from the floor (β = 0.54 s, 95% CI: 0.27, 0.8). None of the muscle ultrasound outcomes was associated with handgrip strength.

Conclusions Portable muscle ultrasound appears a feasible and useful tool to measure muscle size and intramuscular alterations in children with ALL. Validation studies using magnetic resonance imaging (gold standard) are necessary to confirm accuracy in paediatric populations.

Keywords Acute lymphoblastic leukaemia; Low muscle mass; Muscle ultrasound; Muscle weakness

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Introduction

For children with acute lymphoblastic leukaemia (ALL) in high-income countries advances in treatment strategies have resulted in a 5-year survival rate of >90%.[1] During ALL treatment, children are prone to musculoskeletal side-effects and impairments in physical performance.[2,3] Loss of muscle mass, strength and impaired physical performance can be caused by malnutrition, inflammation, pain, low physical activity and can also be aggravated by different treatment components.[4,5] In particular, dexamethasone, an essential component in the treatment of paediatric ALL, can induce catabolic effects and consequent muscle weakness.[6,7]

Loss of muscle mass, strength and function has been associated with the number and duration of hospital admissions,[8] number of invasive fungal infections,[9] impaired quality of life[2] and even with impaired survival.[10] Although it is unclear if these results indicate causality, they do give rise to further investigation of muscle deterioration in patients with ALL.

The current gold standard for muscle assessment is magnetic resonance imaging (MRI) or computed tomography (CT) which allow for both a quantitative and qualitative assessment.[11] However, these techniques are time-consuming, are relatively invasive as sedation may be required for younger children and a radiologist is needed for interpretation. For the assessment of (appendicular) muscle mass, dual-energy x-ray absorptiometry (DXA) examination is a valid technique, but it does not allow for intramuscular imaging (muscle quality measure).

Due to the risks and consequences of muscle deterioration in children with cancer, an easy-to-use tool for assessing muscle mass and intramuscular quality for timely identification would be beneficial. The ideal imaging tool requires utility in different clinical settings, administration has a low burden and it should not be time-consuming. Ultrasound has easy availability in the clinic, has been used for musculoskeletal tissue research in critically-ill children[12–14] and may therefore offer a promising role in the diagnosis of muscle deterioration in children with ALL. Moreover, muscle ultrasound has the ability to assess intramuscular alterations, such as fat infiltration.[15] This is relevant because intramuscular fat infiltration has been associated with deficits in strength and muscle function,[15] but correct interpretation requires significant knowledge of different structures and echogenicity, which limits usability in clinical practice.

In order to interpret the ultrasound images an automated annotation technology (MuscleSound®, Denver, CO, USA) has been used previously to identify muscle size and intramuscular alterations in ultrasound images in athletes[16,17] and in critically-ill adult patients.[18] This commercially available software provides a standardized method with built-in guidance for assessment of muscle size and intramuscular fat of the rectus femoris muscle.[19,20] Enabling such a standardized non-invasive technique for children with cancer would be of great additional value in understanding and evaluating the process of muscle alterations in children during treatment for cancer. By monitoring muscle quantity and quality, this technique has the potential to improve the diagnosis and monitoring of muscle deterioration in children with cancer, and possibly detect deterioration before it becomes clinically apparent. Thus far, the clinical usefulness of muscle ultrasound in combination with MuscleSound® software in children has not been reported.

This study primarily aimed to explore the feasibility of muscle ultrasound in children during ALL treatment and to investigate whether automated ultrasound outcomes of muscle size and intramuscular fat infiltration were associated with total appendicular skeletal muscle mass and physical performance.

Methods

Study design and patients

This study was performed within the framework of the DexaDays-2 study: a national randomized controlled trial on neurobehavioral side effects of dexamethasone in paediatric ALL patients aged 3–18 years, conducted at the Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands, between 2019 and 2021. The design of this study, including in- and exclusion criteria, has been previously described.[21] In brief, Dutch ALL patients, aged 3–18 years and treated according to the Dutch Childhood Oncology Group ALL-11 protocol medium risk group protocol, were included during maintenance therapy. ALL11 MRG maintenance therapy contained 28 three-week treatment cycles, with a 5-day dexamethasone administration course and vincristine push in every cycle.

All participating patients had a muscle function assessment in the outpatient clinic, on the first day of a 5-day dexamethasone course. The assessment consisted of a muscle ultrasound examination and measurements of muscle mass using bioimpedance analysis, muscle strength and physical performance,[21] carried out by a paediatric physiotherapist (E. V.) or medical physician (A. v. H.) at the Sports and Exercise center of the Princess Máxima Center. Assessments were conducted at children and parents' convenience during the outpatient clinic visit, resulting in assessments being performed at various times of the day. Patients were requested to empty their bladder prior to the bioimpedance assessment.

The study was approved by the Medical Ethics Committee (reference number NL62388.078.174) and all patients and/or parents provided written informed consent to participate.

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Muscle ultrasound imaging procedure and software

Two researchers (E. V. and A. v. H.) involved in this study were trained to capture ultrasound images of the rectus femoris (RF) muscles using a portable linear array transducer (Lumify L12–4, Philips, Amsterdam, the Netherlands) connected to a portable tablet (Samsung Galaxy Table 3, Samsung Electronics Benelux, Hoofddorp, the Netherlands). Bilateral ultrasound measurements were captured using the musculoskeletal preset, with the child in supine position on an examination table with the backrest elevated to approximately 70 degrees. To standardize the ultrasound location, the length of the thighs (from spina iliaca anterior superior to upper edge patella) was determined in standing position, thereafter a mark halfway the thigh was drawn. Ultrasound gel was applied to the marked location and the probe was placed horizontally (short axis). The muscles had to be relaxed during imaging and minimal pressure was applied to the probe. The total muscle ultrasound assessment was generally performed in less than 10 minutes. Images were obtained with the Lumify application (Philips Lumify, usa.philips.com), after which they were uploaded to the MuscleSound® platform (Denver, CO, USA).

The MuscleSound® algorithm automatically identifies, annotates and calculates cross-sectional area and thickness, as well as intramuscular adipose tissue (IMAT). The estimation of IMAT is based on echo intensity with which a sound wave is reflected from muscle tissue, that is, more fat infiltration produces a brighter image. Subsequently, the software calculates IMAT values for RF using the equations from Young et al., which were developed and validated through comparison of IMAT from T_1-weighted MRI images in adults.[22] For our analyses, IMAT was adjusted for RF muscle cross-sectional area.

Feasibility outcomes

We assessed feasibility using the following domains: acceptance, practicality and implementation. Acceptance was defined as the percentage of children approached who then enrolled in the ultrasound study, practicality was defined as the percentage of children that completed the ultrasound measurement after enrollment, and implementation was defined as the percentage of children from whom processed and analysed images using the MuscleSound software could be obtained. For the muscle ultrasound technique to be deemed feasible, an acceptance rate of at least 80%, a practicality rate of at least 75% (given the need for patients to lie entirely still), and an implementation rate of at least 90% were considered acceptable. These criteria, adapted from previous studies,[23,24] served as benchmarks to determine the feasibility in our study.

Appendicular skeletal muscle mass, muscle strength and physical performance measurements

Skeletal muscle mass was estimated using a multi-frequency segmental bioimpedance analyser (Tanita MC-780, Tanita Corporation, Tokyo, Japan). The sum of the skeletal muscle mass of the extremities (ASMM) was calculated and adjusted for body weight. The Tanita device has shown excellent test–retest reliability.[25] High significant correlations ($R \geq 0.85$) were shown for body composition values in children and between BIA and DXA.[26] As reference data for Dutch children were unavailable, to estimate *Z*-scores, we used age- and sex-specific mean and standard deviation values from a UK population (5–18 years), acquired using the same Tanita software.[27] Due to lack of bioimpedance reference values of 3- to 4-year-old children in the UK cohort, we used sex- and age-specific expected values of ASMM, derived by a dual-energy X-ray absorptiometry prediction equation in Canadian children.[28]

Two different measures of muscle strength with the handheld dynamometry (HHD) were performed. Handgrip strength was assessed using a Jamar HHD (Sammons Preston, Bolingbrook, IL, USA). Handgrip dynamometry has shown good validity (intraclass correlation coefficients [ICCs] 0.73–0.91) with high reproducibility and has excellent test–retest reliability in children (ICCs 0.91–0.93).[29,30] The mean score of three repeats for the dominant hand was used and compared with population-based age and sex-specific reference values.[31]

Knee-extension strength was measured with the eccentric break-technique protocol using the MicroFET-2 HHD (Hoggan Health Industries, Salt Lake City, UT, USA). This method has shown good validity against Cybex (gold standard) (ICC 0.88, 95% CI: 0.72–0.95), intra-reliability (ICC 0.9, 95% CI 0.65–0.97) and inter-reliability (ICC 0.84, 95% CI 0.48–0.96).[32] Measures were carried out bilaterally and the mean of three repeats was calculated and was used for analyses, as reliable normative values are lacking.

Physical performance was assessed with the timed up and go test (TUG). The children started the test seated on a chair and were asked to stand up, walk 3 m, turn around, walk back and sit down again as quickly as possible. The average time in seconds of three trials was considered as the test result.[33] The TUG has shown excellent test–retest reliability (ICCs 0.80–0.98) and inter-observer reliability (ICCs 0.86–0.99) in the paediatric population,[34] and the measurement was found to be feasible in children with leukaemia.[35] We also used the 'Time to Rise from the Floor test' (TRF).[36] Children were asked to sit on the floor in cross-legged position and were asked to rise as fast as they could. The average time of two TRF trials in seconds was calculated.

We used mean scores of the measurements of muscle strength and physical performance to assess functional muscle endurance. By calculating the average of multiple repetitions, we were able to capture variations in performance that may not have been apparent with a single peak measure-

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ment. We ensured a rest period of maximum 30 s between attempts to allow partial recovery without fully resting the muscles.

Statistical analyses

Height, weight and body mass index (BMI) values were compared with Dutch national sex- and age-specific reference values,[37,38] and *Z*-scores were calculated. All data were expressed as means along with standard deviations for normally distributed variables or median and interquartile ranges (IQRs) for skewed distributions and number (per cent) for categorical variables. Feasibility was explored by determining acceptance, practicality and implementation in young (non-sedated) children. Multivariable linear regression models were estimated to study the associations between muscle ultrasound outcomes of RF cross-sectional area and thickness, and outcome measures of ASMM, strength and physical performance. The mean results of the right and left RF together were used in analysis. To investigate the association between IMAT and muscle function, multivariable linear models were estimated to determine the associations between IMAT, and the outcomes of muscle strength and physical performance. In the models for which we had an outcome in *Z*-scores available (ASMM and handgrip strength), we adjusted for BMI and ALL treatment week as possible covariates. In the models for knee-extension strength (Newton) and physical performance (seconds), we also adjusted for sex and age (years). All analyses were performed in Rstudio environment Version 1.4.1106 for Windows.

Results

Recruitment and patient characteristics

As the ultrasound device became available after the onset of the DexaDays-2 study only 78 of the 105 patients included in the DexaDays-2 study, could be offered muscle ultrasound examination. Muscle ultrasound was eventually performed in 60/78 patients (76.9%) between March 2019 and March 2021 (Figure 1). Thirty-seven were boys (61.7%), with a median age of 6.1 years (range: 3–18.8 years), mean height was −0.61 SDS (SD: 0.98), mean weight was 0.52 SDS (SD: 1.1) and mean BMI was 1.1 SDS (SD: 1.0) (Table 1).

Feasibility

Acceptance

The percentage of children approached who enrolled in the study was 98.7%. Only one parental couple refused the ultrasound examination as well as the other physical measurements because it was considered too burdensome.

Practicality

The percentage of children that completed the ultrasound measurement after enrolment was 77.9%. Twelve patients refused the examination (15.6%), during four examinations we had a device malfunction (broken cable) (5.2%) and one measurement could not be performed because of a logistic issue (1.3%). The 12 patients who declined the ultrasound assessment were similar regarding sex, but younger compared with the 60 children who completed the ultrasound examination (median: 3.6, range: 3.0–11.2 years, $P = 0.0009$). Of the 78 enrolled patients, 17 were 3-year-olds, nine of them refused the ultrasound examination (52.9%) at the time of assessment.

Implementation

We were able to make good-quality images of the RF muscle in all children across all age groups, the percentage of processed and analysed images (i.e. number of children) using the MuscleSound software was therefore 100%.

Muscle ultrasound, appendicular skeletal muscle mass, muscle strength and physical performance outcomes

The MuscleSound-derived estimates of cross-sectional area and IMAT, as well as ASMM, strength and physical performance outcomes are depicted in Table 2.

Median RF muscle thickness was 12.5 mm (range: 5.5, 26.0), cross-sectional area was 3.08 cm^2 (range: 1.2, 7.1) and IMAT was 4.6% adjusted for cross-sectional area (range: 1.5, 11.0).

Mean ASMM was 25.3% (IQR: 22.1, 27.8%). Compared with normative values, the mean standardized deviation score (SDS) was −0.41 (IQR: −1.36, 0.22). The mean SDS of the dominant hand was 0.01 (IQR: −0.58, 0.58). Median knee-extension strength of both legs was 105.7 Newton (range: 33.1, 400.8). The knee-extension strength measurement was performed in fewer children ($n = 42$) due to limited understanding and/or ability to perform the assignment. Median TUG time was 5.3 s (range: 3.7, 7.9 s) and median TRF time was 2.7 s (range: 1.3, 13.6 s).

Associations between muscle ultrasound outcomes and appendicular skeletal muscle mass, muscle strength and physical performance

The multivariable linear models are depicted in Table 3.

Mean estimated cross-sectional area of the RF muscles was significantly associated with total ASMM, knee-extension

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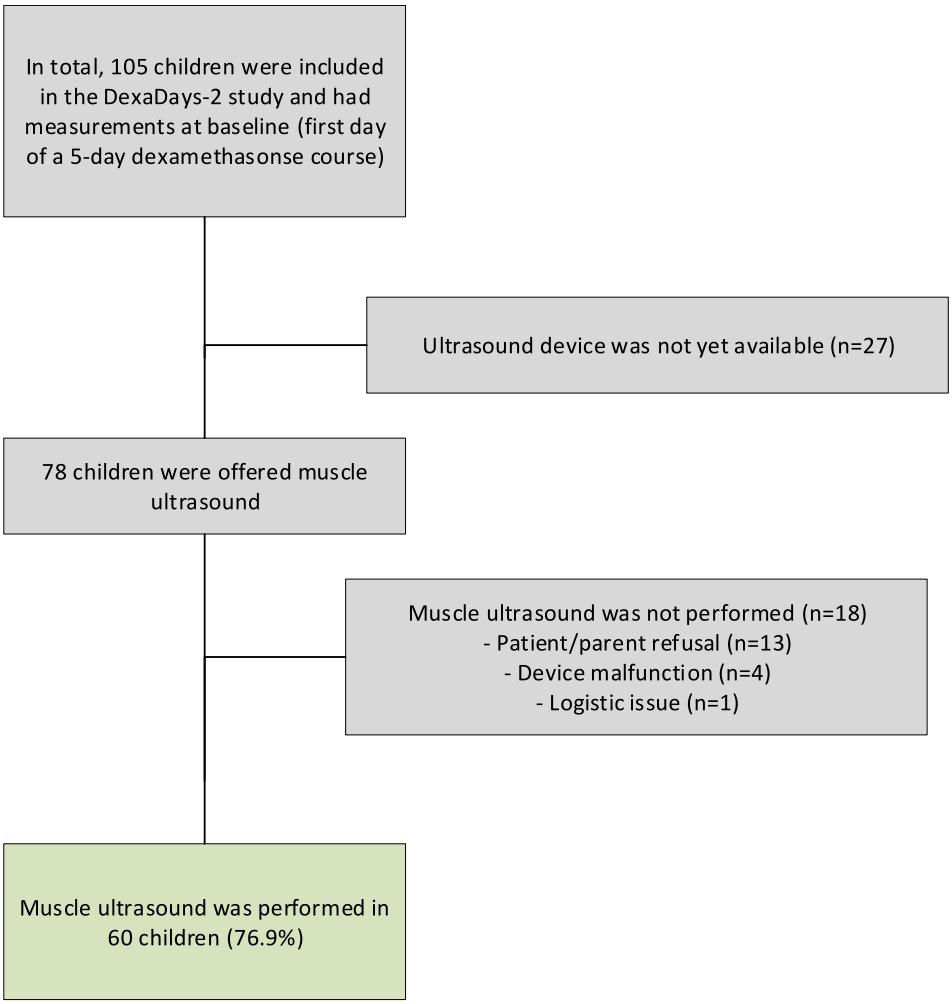

Figure 1 Flow diagram of patients included in this study.

strength and physical performance. On average, one cm^2 increase was associated with 0.49 SDS (95% CI: 0.3–2.4) increase in ASMM, and with 16.9 Newton (95% CI: 4.8, 28.9) increase in knee-extension strength. One cm^2 increase in RF cross-sectional area was associated with −0.46 s (95% CI: −0.75, −0.18) faster TUG performance, as well as −1.07 s (95% CI: −1.71, −0.42) faster TRF.

Mean estimated RF thickness was significantly associated with total ASMM, knee-extension strength and physical performance. On average, 1-mm increase in thickness was associated with 0.14 SDS (95% CI: 0.04, 0.24) increase in ASMM, and with 4.73 Newton (95% CI: 0.99, 8.47) increase in knee-extension strength. One millimetre increase in RF thickness was also associated with −0.13 s (95% CI: −0.22, −0.04) faster TUG performance, as well as −0.28 s (95% CI: −0.48, −0.08) faster TRF.

Estimated IMAT adjusted for area was significantly associated with functional muscle outcomes, that is, knee-extension strength and physical performance. On average, one percentage increase in IMAT was associated with −6.84 Newton (95% CI: −12.26, −1.41) knee-extension strength, 0.2 s (95% CI: 0.08, 0.32) slower TUG performance, as well as 0.54 s (95% CI: 0.27, 0.8) slower TRF (increased fat infiltration associated with poorer muscle function).

RF cross-sectional area, thickness and IMAT were not associated with handgrip strength. Scatterplots are shown in Figure S1.

Discussion

In this exploratory study, we found that muscle ultrasound of the RF muscle was feasible in three-quarters of the number of children we measured during ALL treatment. Moreover, our findings indicate that employing an automated annotation technique for assessing RF muscle size may serve as a

Table 1 Patient characteristics

	N	%	
Sex			
Boy	37	61.7	
Girl	23	38.3	
Type of ALL			
Precursor B-ALL	52	86.7	
T-ALL	7	11.7	
BPDCN	1	1.6	
	Median	IQR	Range
Age, years	6.1	4.5–9.6	3–18.8
Height			
(cm)	116.8	105.4–141.1	89.3–193
SDS[a]	−0.61	0.98	−2.9 – 1.6
Weight			
(kg)	23.3	19.2–38.7	12.9–112.8
SDS[a]	0.52	1.1	−1.8 – 4.4
Body mass index			
(kg/m^2)	17.3	15.9–19.2	13.8–35.2
SDS[a]	1.1	1.0	−1.0 – 3.9
Maintenance treatment week	34	22–42	16–68

[a]SDS values are mean with standard deviation.
ALL, acute lymphoblastic leukaemia; BPDCN, blastic plasmacytoid dendritic cell neoplasm; IQR, interquartile range; SD, standard deviation; SDS, standard deviation score.

valuable indicator for detecting muscle alterations. Notably, this study is the first to estimate IMAT using ultrasound in children with ALL, revealing significant associations with muscle strength and physical performance outcomes.

Our pre-defined feasibility criteria of acceptance, practicality and implementation confirmed the feasibility of the ultrasound technique in the majority of patients with ALL. We observed a high acceptance rate, with 98.7% of children approached enrolling in the study. Practicality was somewhat lower, with 77.9% of children completing the ultrasound measurement after enrollment, but still exceeding our predefined rate of 75%. Notably, the practicality rate, specifically in the context of children lying still, was impacted by refusal and anxiety among the youngest age group, particularly the three-year-olds, where the refusal rate reached 50%. It is of importance to note that among the 12 children who declined the ultrasound examination, nine were 3-year-olds. This finding suggests that muscle ultrasound may not be feasible as an easily repeatable measurement in younger children. However, in school-aged children who can be better instructed to remain still, muscle ultrasound is highly feasible. Of the patients that completed the ultrasound as-

Table 2 Results of the muscle ultrasound, muscle mass, muscle strength and physical performance assessments

	N	Median	IQR	Range
Ultrasound outcomes (MuscleSound)				
Thickness, mm	60			
Rectus femoris left		13	10.1–15	5–23
Rectus femoris right		12	11–14.5	6–29
Cross-sectional area, cm^2	60			
Rectus femoris left		3.1	2.5–3.8	1.1–6.3
Rectus femoris right		3.0	2.5–3.8	1.3–7.9
IMAT, percentage adjusted for area	60			
Rectus femoris left		4.6	3.7–6.3	1.6–12
Rectus femoris right		4.4	3.8–6	1.4–11.8
Bio-electrical impedance analysis				
Appendicular skeletal muscle mass	57			
Kilogram		6.5	4.7, 12.2	2.5–31.5
Percentage		25.5[a]	22.9, 29.3	14.6–68.6
Z-score		−0.6[a]	−1.24, 0.06	−2.5 – 2.7
Muscle strength				
Handgrip strength	54			
Left hand, kilograms		10	7.9–14.3	4–45.8
Right hand, kilograms		10.5	8.1–16.3	4.5–53.7
Dominant hand, *Z*-score[a]		0	0.9	−2 – 2.4
Knee extension strength	42			
Left leg, Newton		106.4	73.3–170.2	31.8–408.7
Right leg, Newton		104.9	81.5–181.2	34.4–393
Physical performance				
Timed up and go test	59			
Time, s		5.3	4.6–6.1	3.7–7.9
Time to rise from the floor	60			
Time, s		2.7	2–4.2	1.3–13.6

Missing values are explained by impaired cooperativeness or limited understanding to perform the measurement.
IQR, interquartile range; IMAT, intramuscular adipose tissue.
[a]Presented as mean value.

Table 3 Multivariable linear models: The association of muscle ultrasound outcomes with appendicular skeletal muscle mass, knee extension strength and physical performance

	Appendicular skeletal muscle mass (*Z*-score)		
	β	SE	*P*-value
Intercept	−1.47		
RF cross-sectional area, cm^2	**0.49**	**0.16**	**0.003**
Body mass index, *Z*-score	−0.04	0.18	0.8
ALL maintenance week	−0.01	0.01	0.62
Intercept	−1.73		
RF thickness, mm	**0.14**	**0.05**	**0.007**
Body mass index, *Z*-score	−0.09	0.19	0.63
ALL Maintenance week	−0.01	0.01	0.72
	Knee extension strength (Newton)		
	β	SE	*P*-value
Intercept	−63		
RF cross-sectional area, cm^2	**16.9**	**5.93**	**0.007**
Sex (male vs. female)	−4.48	11.2	0.69
Age, years	14.7	1.64	<0.0001
Body mass index, *Z*-score	7.55	5.38	0.17
ALL maintenance week	−0.08	0.46	0.86
Intercept	−71.5		
Thickness, mm	**4.73**	**1.84**	**0.01**
Sex (male vs. female)	−5.44	11.4	0.64
Age, years	14.9	1.67	<0.0001
Body mass index, *Z*-score	5.6	5.8	0.34
ALL maintenance week	−0.01	0.46	0.98
Intercept	5.69		
RF IMAT index, %/cm^2	**−6.84**	**2.67**	**0.015**
Sex (male vs. female)	−0.97	11.7	0.93
Age, years	16.7	1.33	<0.0001
Body mass index, *Z*-score	12.96	5.24	0.019
ALL maintenance week	−0.15	0.48	0.75
	Timed up and go test (s)		
	β	SE	p-value
Intercept	6.72		
RF cross-sectional area, cm^2	**−0.46**	**0.14**	**0.002**
Sex (male vs. female)	0.43	0.25	0.09
Age, years	−0.03	0.04	0.44
Body mass index, *Z*-score	0.27	0.12	0.029
ALL maintenance week	−0.01	0.01	0.55
Intercept	7.02		
Thickness, mm	**−0.13**	**0.04**	**0.004**
Sex (male vs. female)	0.48	0.25	0.06
Age, years	−0.04	0.04	0.29
Body mass index, *Z*-score	0.31	0.13	0.019
ALL maintenance week	−0.01	0.01	0.44
Intercept	4.68		
RF IMAT index, %/cm^2	**0.2**	**0.06**	**0.001**
Sex (male vs. female)	0.37	0.25	0.14
Age, years	−0.08	0.03	0.005
Body mass index, *Z*-score	0.14	0.12	0.23
ALL maintenance week	−0.002	0.01	0.82
	Time to rise from the floor (s)		
	β	SE	p-value
Intercept	4.06		
RF cross-sectional area, cm^2	**−1.07**	**0.32**	**0.002**
Sex (male versus female)	−0.06	0.56	0.91
Age, years	0.32	0.09	0.0005
Body mass index, *Z*-score	0.72	0.28	0.01
ALL maintenance week	−0/02	0.02	0.43
Intercept	4.61		
Thickness, mm	**−0.28**	**0.1**	**0.007**
Sex (male vs. female)	0.07	0.57	0.9

(*Continues*)

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Table 3 (continued)

	Time to rise from the floor (s)		
	β	SE	p-value
Age, years	0.28	0.09	0.002
Body mass index, *Z*-score	0.78	0.3	0.01
ALL Maintenance week	−0.02	0.02	0.32
Intercept	−1.16		
RF IMAT index, %/cm^2	**0.54**	**0.13**	**0.0002**
Sex (male vs. female)	−0.25	0.55	0.64
Age, years	0.2	0.06	0.002
Body mass index, *Z*-score	0.41	0.26	0.12
ALL Maintenance week	−0.006	0.02	0.78

B, Betacoefficient; IMAT, intramuscular adipose tissue; RF, rectus femoris muscle; SE, standard error.

sessment, we were able to obtain good-quality images of the RF muscle in all children measured across all age groups, which confirms that the usage of the software is feasible with an implementation of 100%.

Our results showed that RF cross-sectional area and thickness were related to ASMM *Z*-scores. This finding cautiously supports the hypothesis that muscle ultrasound may be a valid method for estimating skeletal muscle mass in the extremities of children with ALL, which would be a simple and convenient alternative to current methods that require visits to the radiology department and an expert opinion on the results. Assessment of ASMM is of importance because serious reductions of ASMM with incomplete recovery have been observed in children with ALL, and the degree of loss has been associated with the burden of illness.[8] ASMM is crucial for neurodevelopment and metabolic health[39] and a fundamental component of sarcopenia and frailty.[40] However, further validation against gold standard measures by DXA or MRI needs to reveal if muscle ultrasound can be used as a true indicator for ASMM.

Intramuscular adipose tissue adjusted for muscle area (IMAT) was associated with the functional outcomes: knee-extension strength, TUG and TRF. This revealed that higher fat infiltration in the RF muscle was related to decreased force generation and in slower performance in walking and turning, as well as rising from the floor. In previous studies, IMAT was inversely related to impaired muscle functionality (running/jumping) and increased disabilities in children and adolescents.[15,41] Moreover, recent results in adult cancer patients showed that IMAT accumulates during cancer treatment and is an important factor in the development of exercise intolerance.[42] Therefore, the availability of automatically estimated IMAT would be an advance in muscle deterioration assessment, to detect alteration maybe even before it becomes clinically apparent, and will thus support our clinical decision making.

We hypothesized that decreased size and quality of the RF muscle in children with ALL may exemplify general muscle health, and we appear to have unravelled a part of this theory with our findings. However, somewhat surprisingly none of the muscle ultrasound outcomes was associated with handgrip strength. In healthy children hand grip strength is an indicator for general muscle strength.[43] However, this may not apply to children with ALL, in view of the high-dose corticosteroids (as well as other chemotherapeutic agents) which are an essential part of the treatment and are known to induce proximal muscle weakness rather than distal muscle weakness.

Although our results are promising for future automated annotation muscle ultrasound technique, caution with regard to interpretation is warranted because of limitations of the study. First, due to the cross-sectional nature of the study, our ability to establish causal relationships is limited. Second, we did not have ASMM data available measured by MRI, which limited us in validating the accurateness of MuscleSound® muscle size and IMAT calculations against gold standard measures. Third, because of lack of normative values for children, we are not able to interpret the MuscleSound® values in our cohort. To put our results in context, future studies should investigate whether the associations identified between ultrasound and other outcome measures are similar in a group of typically developing children. Additionally, future longitudinal studies should explore the potential of using ultrasound as a tool for monitoring changes over treatment course and its role in earlier identification of muscle deterioration.

Nevertheless, muscle ultrasound is feasible and may become an important addition to the diagnostic assessment for muscle deterioration in children with cancer. This convenient portable technique allowing rapid and non-invasive real-time monitoring is a sizeable reduction of burden in physically vulnerable children with cancer with a high risk of muscle wasting. Furthermore, this tool may help in distinguishing muscle impairments from lack of cooperation or cognitive inability in immobilized patients.

In conclusion, muscle ultrasound in combination with automated annotation seems a promising instrument, allowing quick and non-invasive diagnosis of muscle deterioration at the bedside if necessary. However, validation

studies using gold standard assessments are necessary to further determine the accuracy and validity in paediatric populations.

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Conflict of interest

Jeroen Molinger has an ancillary function as science committee consultant for MuscleSound®. His role in this study was to train the ultrasound technique to the researchers and to support in interpreting the raw results. He was not involved in data collection or data analyses. The other authors declare no conflict of interest.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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