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Title:

Thigh ultrasound monitoring identifies decreases in quadriceps femoris thickness as a frequent observation in critically ill children

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Abstract

Objective: Critically ill adults develop significant muscle wasting, with subsequent worse outcomes. In the pediatric setting, occurrence and effects of muscle wasting are undescribed; this is in part due to a lack of validated, objective methods for assessing muscle wasting. Single measurement of quadriceps femoris (QF) thickness has failed to show consistent reproducibility. We hypothesised that averaging repeated measurements could afford good reproducibility to allow for QF thickness decline detection and monitoring.

Design: Prospective bedside observational study.

Setting: Two pediatric intensive care units (PICU's).

Patients: Mechanically ventilated critically ill children aged 0 to 15 years.

Interventions: Transverse and longitudinal axis measurements of QF anterior thickness were undertaken using bedside ultrasound. The average of 4 measurement values was recorded. The location of measurement was marked for consistency within subsequent measurements by the same or another trained operator, to assess intra- and inter-operator repeatability and reproducibility of the technique. Where feasible, serial measurements were undertaken until the time of extubation in a group of children with prolonged PICU stay (>5 days).

Measurements and Main Results: Seventy-three children were enrolled to assess intra- and inter-operator ultrasound reliability. Their median (25-75interquartiles) age and weight were 30 months (4.5-96) and 10 kg (5-23.5). In the intra-operator repeatability study, mean relative difference in QF muscle thickness was 0.36% +/-2.5 (lower and upper limits of agreement: -4.5/+5.2%). In the inter-operator reproducibility study, intraclass correlation coefficient was 0.998. In the 17 children monitored over their PICU stay, QF thickness significantly decreased at day 5 by 9.8% (p=0.006), and by 13.3% (<0.001) at the last performed measurement.

Conclusions: QF thickness decrease, proposed as a surrogate for muscle mass**,** is an early, frequent and intense phenomenon in PICU. QF ultrasonography is a reliable technique to monitor this process, and in future could help to guide rehabilitation and nutrition interventions.

Introduction

In critically ill patients, intensive care – acquired weakness (ICU-AW) corresponds to an acquired neuro-muscular disease that is potentially reversible. The pathophysiology of ICU-AW is multi-factorial. It involves peripheral nerves alteration (neuropathy) that can lead to muscle wasting. It also involves myopathic features, characterized by muscle alteration and muscle wasting (1,2). First, muscle organ failure (resulting from hypoxia, compromised perfusion, and direct muscle injury) will induce the so called "acute muscle wasting". This leads to muscle turnover metabolism alteration (including increased catabolism and decreased anabolism) and resultant increased muscle breakdown. In addition, disuse atrophy occurs in the comatose bedridden patient, under sedation or neuro-blocking agents. Furthermore, undernutrition is a constant challenge in the care of critical illness. This may be due to fluid restriction, intolerance to enteral feeding and elective suspension of enteral feeds prior to specific procedures or extubation. These are likely to increase muscle wasting from fasting. Finally, in patients who remain ill for extended periods, chronic cachexia also contributes to muscle wasting, as seen in non-critical chronic inflammatory diseases and patients with malignancies (1,3,4).

In the adult setting, ICU-AW has been documented as a frequent, rapid and early phenomenon, especially in multi-organ failure (1). It prolongs ICU- and hospital- stay, as well as post-ICU rehabilitation, resulting in increased health care costs (5,6).

In contrast, Pediatric Intensive Care – acquired weakness (PICU-AW) is not well studied. The medium to long term effects of muscle wasting in children are almost completely undescribed (7–11). This is in part due to a lack of validated, objective methods for screening and assessing muscle wasting in children admitted to PICU (12).

Defining and validating accurate tools to assess PICU-muscle wasting is therefore essential in order to enable an accurate description of its incidence in the clinical setting. Furthermore, this will aid understanding of the underlying biological processes and functional implications, whilst also helping

to develop future therapeutic approaches to prevent and minimize PICU-muscle wasting (with the inclusion of feeding regimens, pharmaconutrition, early rehabilitation, etc). Classic methods for the assessment of muscle mass changes are neither accurate nor validated in the critically ill child (13). Firstly, bioimpedance analysis (BIA) of body composition is impacted by major fluid shifts and is not validated in critically ill infants and young children. In addition, other methods such as DEXA or CT imaging are challenging to perform in an unstable child because of the requirement for specific equipment (13). Magnetic resonance imaging (MRI), considered the gold standard for the assessment of body composition, is also technically difficult to perform in the PICU setting and is not practical for use as a daily bedside muscle mass assessment tool (13).

We aimed in this study to assess PICU muscle wasting over PICU stay, based on quadriceps femoris (QF) muscle thickness repeated measurements, as a surrogate for changes in muscle mass. Muscle mass assessment using anterior thigh ultrasonography has been shown to be reliable in adults, but single measurements of quadriceps femoris muscle thickness have thus far failed to be reproducible in children (12). We hypothesized that by designing a detailed measurement protocol using anterior thigh ultrasonography, coupled with the use of multiple measurements of QF muscle thickness across different axes, we could improve the accuracy and reproducibility of the technique. Thus allowing for the accurate assessment and serial monitoring of PICU muscle wasting over the course of critical illness.

Material and methods

We conducted a prospective observational study across two PICU's (Lyon, France and Cambridge, United Kingdom) between November 2015 and April 2016. Intra- and inter-operator repeatability and reproducibility of QF muscle thickness measurement using anterior thigh ultrasonography were

assessed. Within each aspect of the study (intra- and inter- operator repeatability and reproducibility) we intended to enrol 35 PICU children aged between 0 to 15 years, with the aim of reliably detecting a 5% change in QF thickness. A sample size of 35 critically ill children was calculated based on previous literature and expected measurement variability within patients (12,14). The study was approved by each of the institutional research ethics committees (France ANSM: 2015-A01158-41; UK: 13/LO/0974).

Measurement of QF thickness:

Ultrasonographic examination was performed using B-mode ultrasonoraphy with either the Vivid S6 (GE Healthcare, Little Chalfont United Kingdom) or the SonoSite EDGE (Fujifilm sonosite, Bothel, USA), in Lyon and Cambridge respectively. We used a linear transducer which frequency was adapted according to the accumulative thickness of thigh muscle and fat, ranging from 9 to 13 Hz. The measurements were performed strictly perpendicularly to the skin plane in order to limit oblique scanning related measurement errors, and with an excessive amount of gel (to ensure no direct transducer-skin contact, thus avoiding any inadvertent compression of the thigh by the operator). QF muscle is composed of 4 heads, 2 of which (rectus femoris and vastus intermedius muscles) are located anteriorly. QF thickness was defined as the sum of the anterior thickness of these two heads (see figure 1).

Measurements were obtained whilst recruited children were either sedated or fully cooperative in order to obtain muscle relaxation, therefore avoiding the confounding effects of muscle contraction on measured QF thickness. In order to obtain the most accurate QF thickness and maintain consistency for all children, each patient was positioned in a supine position and measurements of only one leg were taken. On each occasion, the leg was fully extended and positioned in neutral rotation whilst external compression of the muscle was avoided to limit artefactual deformation of the muscle shape (this included removal of positioning aids and pillows). To overcome the progressive increase in thickness of the anterior QF muscle, we used a measuring tape to identify the

widest portion of the thigh and recorded the distance of this point from the superior tip of the patella (identified to be the most accessible and consistent landmark in this population). Using an indelible marker, we then marked this point to ensure that all subsequent measurements were taken at exactly the same location.

Operators: Within the intra-operator repeatability study which was conducted in Lyon, France, one single reference operator (FV) performed all measurement sets twice consecutively for each patient (procedure detailed below); paired results were then compared. The reference operator had previously been trained by a pediatric radiologist, outside of this study.

Prior to the commencement of the inter-operator reproducibility study, all operators (DY, UP, MJ, NP, FC, FB) were firstly trained outside of the study by the reference operator. The additional operator's involvement in the study was deemed suitable once the reference operator had supervised 4 accurate measurement sessions.

Within the inter-operator reproducibility study, conducted in both Lyon and Cambridge, the reference operator performed the first set of measurements, which was immediately followed by a second set of measurements completed by another operator. This procedure was repeated for all patients to ensure consistency was maintained throughout. In both intra- and inter-operator studies, the second set of measurements was done blind to the results of the first set.

Procedure: Each operator performed a set of 4 measurements of QF muscle anterior thickness. First a transverse measurement was performed (transducer placed perpendicularly to thigh axis), then a longitudinal one (transducer placed parallel to thigh axis); both measurements were repeated once. QF thickness was measured vertically on the image, from the outer cortex of the femur to the internal border of the QF fascia, as shown on figure 1. Measurements of reference and additional operators were compared. Averages of 2 measurements (obtained in the same angle and in different angles) and of the 4 measurements were then calculated in order to account for operator-related

error, and compared to averages of the measurement set performed by the second operator (following the same protocol).

Further to the inter- and intra- operator repeatability studies, we also designed a QF measurement protocol which would enable the accurate detection of a 5% change in QF muscle thickness during the course of a patient stay on PICU. We used a prospective observational study of 17 children enrolled between April and May 2016 from one PICU (Cambridge, UK). These patients were prospectively recruited under the inclusion criteria previously described: children admitted to PICU aged between 0 to 15 years, fully sedated or cooperative. Due to the short-longitudinal nature of this observational study, patients were only recruited if it was anticipated that their stay on PICU would be longer than 4 days. QF thickness monitoring was discontinued when children were no longer sufficiently sedated or cooperative for accurate measurement to take place. Patients presenting with a known history of neuromuscular disease were excluded from the study. Wherever possible, QF muscle thickness was measured on a daily basis, following the above described protocol. Muscle wasting would be considered if QF thickness decreased by more than 5% over PICU stay (which would correspond to the limit of our ultrasound measurement protocol reliability). Our first endpoints aimed to compare admission values, named time-point-a (TP-a) and obtained within the first 24 hours after PICU admission, to time-point-day-5 (TP-b) values (obtained between day-4 and day-5), and to time-point-c (TP-c) values which corresponded to the final measurement considered accurate according to our protocol (namely when the patient was fully sedated or cooperative). We also compared indexed values of QF thickness measurement to admission body weight.

Certain characteristics were recorded for all patients, including age, gender, admission weight, height, body mass index z-score (WHO reference), and primary diagnosis. Potential risks factors for muscle wasting were also recorded, exploring each feature of muscle wasting physiopathology: disuse atrophy (length of ventilation, mechanical ventilation duration, use of sedation and neuro-

blocking agents), starvation (energy-protein intake deficits (15)), acute muscle wasting (PELOD2 score, highest CRP, length of PICU stay), and cachexia (underlying disease). No standardized physical therapy intervention was conducted for all patients during the study, but rather each patient is assessed and treated individually. Study protocol was approved by the respective UK and French Institutional Review Boards, who waived the need for informed consent.

Statistical analysis: Pearson correlation coefficient was used to identify associated factors to QF thickness. Intra-operator repeatability was assessed using the Bland-Altman method. The bias was quantified as the mean relative difference between two repeated measurements carried out by the same operator. The lower and upper limits of variability were calculated as the values at 2 standard deviations below the mean relative difference and at 2 standard deviations over the mean relative difference respectively. The analysis was carried out for each of the 4 measurements (2 longitudinal and 2 transverse), but also for the mean of 2 measurements (longitudinal-longitudinal, transverse-transverse, or longitudinal-transverse) and the mean of 4 measurements. The intra-operator repeatability was quantified by the coefficient of variation with its 95% confidence interval (CI). The inter-operator reproducibility of the mean of the 4 measurements was quantified using the intra-class correlation coefficient. A random-intercept linear model was used to estimate the inter-patient variance and the intra-patient variance corresponding to the inter-operator variance. The intra-class correlation coefficient was obtained as the ratio of the inter-patient variance on the total variance (inter plus intra-patient). The Bland-Altman method was also used to quantify the reproducibility between the reference operator and each of the six fully trained operators. Muscle wasting longitudinal study: the paired Wilcoxon test was used to compare TP-a values to TP-b and TP-c values respectively. Pearson correlation coefficient was used to estimate and test the link between the cumulative energy or protein deficit and the QF thickness difference between two times. A linear mixed model with a random effect on intercept and slope was used to quantify the change of QF

thickness over time adjusted on age, gender, BMI z-score, PELOD2 score and highest CRP value. All data analysis was carried out using the R software, version 3.1.3.

Results

Intra- and inter-operator repeatability and reproducibility studies.

In total, 73 patients were enrolled; 37 children were included in the intra-operator repeatability study, and 36 other children in the inter-operator reproducibility study. The overall median (25-75 inter-quartiles (IQ)) age and weight were 13 (3-98) months and 9.8 (4.6-23.5) kg respectively, and 30 (41%) were girls (table1). The main reasons for PICU admission were respiratory failure, sepsis, trauma and post-operative care. The overall median (25-75 IQ) QF thickness was 1.71 (1.40-2.26) cm. QF thickness was positively correlated ($p<0.001$) with age (r=0.69 IC95%:0.55-0.79), weight (r=0.80; IC95%:0.70-0.87), and height (r=0.77; IC95%:0.66-0.85) (see figure 2). Seven operators participated to the inter-operator reproducibility study (5 pediatric intensivists and 2 pediatric physical therapists). The comparison of any single measurement or 2-measurement sets (longitudinal, transverse, or mixed) failed to be reproducible (the coefficient of variation of the 4 measurements within a set of 4-measurements was high in children less than 40kg, as shown in figure 3a). Four-measurement sets comparison are detailed below.

Intra-operator repeatability study: the mean (+/- standard deviation) difference and relative difference between the two means of 4-measurements sets were 0.012 cm (+/-0.49) and 0.36% (+/-2.5) respectively, with a lower limit and upper limit estimated at -4.5 and +5.2 % respectively, as shown in figure 3b. The coefficient of variation was 2.5% (95% CI: [2.0; 3.2]). **Inter-operator reproducibility study**: the intraclass correlation coefficient was 0.998 (close to 1). The mean (+/- standard deviation) difference between the two means of 4-measurement sets performed by the reference operator and the first operator was 0.006 cm (+/-0.49). Their mean relative difference was

0.5% (+/-2.5), with a lower limit and upper limit estimated at -5.4% and +4.5% respectively. Similar results were found for other operators.

QF thickness decrease in acute critical illness. As we had found satisfying intra- and inter- operator repeatability and reproducibility, QF thickness decrease was considered accurate and used as a surrogate to muscle wasting. Seventeen children were enrolled from Cambridge PICU in the muscle wasting monitoring study that followed. Their median (25-75 IQ) age and weight were 47 (5-126) months and 20 (7.8-29.6) kg respectively, and 2 (11.7%) were girls (table2). Respiratory failure, sepsis and brain injury were the most common admission diagnoses. The median (25-75 IQ) PICU length of stay of 10 (7-13.5) days allowed for a median of 5 (4-6) measurements per patient. The reference operator performed 84% of the 104 QF measurement sets, and 16% were performed by 3 different fully trained operators who had previously taken part in the inter- operator reproducibility study.

The median (25-75 IQ) QF thickness was 2.25 (1.72-2.79) cm at admission (TP-a). At TP-b (day 5), QF thickness measurement showed a significant (p=0.008) decrease of -9.8% (-13.3 - +0.0). At TP-c (last measurement performed), QF thickness had shown further significant (p<0.001) decrease by -13.3% (-23.6 - -8.9) as illustrated in figure 4. When considering TP-b and TP-c respectively, 12 (71%) and 15 (88%) children had more than 5% QF thickness decrease, whilst 7 (41%) and 10 (59%) had more than 10% QF thickness decrease, and 3 (18%) and 6 (35%) more than 20% QF thickness decrease, as shown in figure 5. Additionally, after indexing QF thickness values to admission body weight, we also found a significant decrease between TP-a and TP-b (p=0.01) and between TP-a and TP-c (p=0.0005).

The mean change of QF thickness over time was estimated at -0.05 cm per day (95% CI: [-0.07; -0.03]; p<0.001). The mean QF thickness increased significantly with age (0.013 cm per supplementary month; 95% CI: [0.01; 0.015]; p<0.001) and BMI z-score (0.02 cm for an increase of 0.1 unit of z-score 95% CI: [0.01; 0.03]; p=0.004). It decreased significantly with the highest value of CRP (-0.002 cm for an increase of 1 unit of CRP; 95% CI: [-0.003; -0.001]; p=0.004). Gender and

Pelod2 score were not significantly linked to the mean QF thickness (p=0.34 and 0.91 respectively). No factor was significantly linked to QF thickness change over time. Correlation between cumulative energy or protein deficit and QF thickness decrease was not statistically significant.

Discussion

In a short-longitudinal study, monitoring of QF thickness over PICU stay showed an early, statistically significant, and clinically important decrease, concerning the vast majority of children of all admission weight ranges. This was made possible by the development of a reliable QF thickness measurement protocol, which demonstrated both sufficient intra- and inter- operator repeatability and reproducibility, using average scores for repeated ultrasound measurements in different axes.

Fivez et al. previously failed to design a measurement protocol offering sufficient intra-operator reliability in children on PICU (12). They were unable to accurately detect a change in QF thickness of less than 30% which unfortunately is not beneficial in clinical practice. Indeed, early and precise recognition of muscle mass change is mandatory for future epidemiological and treatment studies. Based on our pilot study, in conjunction with adult literature, a technique which allows for accurate detection of a 5% change in muscle mass is recommended (1). One explanation for the negative results seen in Fivez et al. study (12) may be that 8 of 30 patients were sedated but not intubated. Though the authors state that their patients were adequately sedated for the exam, subtle contractions of muscles in sedated but moving patients may transiently increase muscle thickness measurements. Similarly, McLeod et al. found high coefficient of variation whilst measuring different muscle groups in preterm babies (14). The operator related risk of error may be diminished using the average of subsequently repeated measurements, according to a detailed measurement protocol.

The measurement of the area of rectus femoris or vastus intermedius has been proposed as another way to estimate muscle mass. During our pilot training, we faced difficulties in accurately capturing

the entire cross-section of these muscles within one single ultrasound image, especially in infants and children with low level of fat mass. As a result, this technique was not selected for the study.

Ultrasonography is a core skill within the PICU intensivist's daily practice and care. Additionally, the learning curve for both experienced and non-experienced team members was quite rapid. This is a non-invasive technique, available at the bedside, which requires only a few minutes, in comparison to other muscle mass assessment techniques like CT imaging or MRI. This method may therefore be utilized in future research and eventually enable muscle mass assessment integration into the systematic recommended nutritional status assessment in PICU.

We did not compare QF thickness ultrasonographic measurement to MRI measurement which is the gold standard. In fact, MRI is technically difficult to perform in a critically ill child. Our primary outcome was to develop a technique that would allow for the accurate measurement and reliable monitoring of change in muscle thickness rather than examine absolute value measurement of muscle thickness (12). Similarly, anthropometric measurements, including thigh circumference and weight for example, were not compared to ultrasonographic measurements as they may be sensitive to fluid shift and fat mass change within this clinical setting. No healthy control group was recruited as full cooperation of infants and toddlers, which is essential for good reliability of the technique, was not possible. QF was chosen over other muscle groups because of its large thickness and ease of accessibility, allowing for more accurate assessment (14). However, it remains an indirect estimation of the overall muscle mass. In adults, muscle echogenicity shifts over intensive care stay (using greyscale assessment software) has been correlated to muscle wasting and outcomes (16), and has been proposed to assess and monitor muscle quality. It was also correlated to histological necrosis findings (17). No such data is available in the pediatric setting as it remains difficult to obtain parental consent for muscle biopsies. Extrapolation of adult results should be tested and their utility in muscle quality monitoring should also be further investigated.

The degree of muscle mass change over the course of a PICU admission can be easily and reliably estimated using ultrasound measurement of QF thickness. Implementation of this method may allow for the early detection and monitoring of muscle wasting over PICU stay in sedated or cooperative children.

PICU-acquired weakness is however, a type of reversible neuro-muscular disease and its assessment should comply with the neuro-muscular assessment recommendations. A holistic overview of muscle function in relation to patient activity and participation, as described by the disability creation process (DCP) model or the WHO International Classification of Functioning (18,19), is required rather than simply assessing for degree of muscle wasting in isolation. This would result in assessment of muscle wasting and PICU-acquired weakness with an overall approach, focussing not only on muscle mass, but also on muscle strength and muscle function, as well as on its consequences on daily life. This will allow for further investigation of the correlation between early ultrasonography recognition of muscle wasting during PICU stay and its functional outcome. This may additionally enable researchers to examine the extent to which adjuncts such as nutritional optimization and rehabilitation can minimize muscle wasting and its consequences.

Our PICU muscle mass monitoring study demonstrates that muscle mass decline is an early and extended phenomenon that occurs in most critically ill children. In critically ill adults, Parry et al. (16) similarly found a large decrease (30%) of thigh muscle thickness, while Puthucheary et al. (1) found that rectus femoris cross sectional area decreased significantly at day 7 of ICU stay (−12.5% [95% CI, −35.4% to 24.1%]; P = .002), and continued to decrease at day 10 (−17.7% [95% CI, −25.9% to 8.1%]; P < .001). However, muscle wasting is not synonymous with muscle weakness, as shown by the higher incidence of ICU muscle weakness or ICU-AW, ranging from 25 to 100% in various studies. This also emphasizes the fact that validation of accurate assessment tools and standardization of diagnostic protocols are mandatory in this field. Field-Ridley et al. recently published a large analysis of PICU-AW (7), using the data collected in a US register (Virtual PICU System, a clinical database

with nationally participating PICUs). Incidence of critical illness myopathy was 0.02%, based on the international classification of diseases coding. PICU-AW independent risk factors were identified (including age, respiratory and infectious primary diagnosis, mechanical ventilation, renal replacement therapy and extra-corporal life support). In this study, PICU-AW was also associated with longer length of stay and an increased need for rehabilitation following discharge from PICU. However, this study was limited by the voluntary report of PICU-AW into the register without any clear overarching PICU-AW definition. This may have led to an underestimation of the extent of PICU-AW. Furthermore, the risk factors that were identified should ideally be analysed within the spectrum of other confounding factors, such as cumulative energy and protein deficit, cumulative doses of neuro-blocking agents and sedative drugs, for example. The extent of inflammation may also impact muscle metabolism shift in critical illness. In 2003, Banwell et al. reported an incidence of PICU-AW of 1.7%, based on prospective neurological examination of 830 children, assessing muscle weakness (8). Electromyography and muscle biopsy performed on 7 and 3 patients respectively, confirmed myopathy features, in a majority of them. Banwell et al. identified length of stay and post transplantation admission as risk factors for muscle weakness. In addition, the children in their sample were found to have prolonged muscle weakness three months after discharge.

Our intra- and inter-operator reliability study enrolled a large number of children with an extended age range, and a wide variety of clinical conditions, thus optimizing potential for wide extrapolation of results into other PICUs. When considering clinical application, the results identified in the current research, together with those of Fivez et al. (12), emphasize the importance of adherence to a strict protocol which includes multiple measurements, and excludes non-cooperative children. This will limit per se its use in non-cooperative children especially in the youngest with withdrawn sedation. However, our technique should be able to be used in the early hypermetabolic phase.

Our research findings suggest that ultrasonography used for the assessment of QF muscle thickness has good intra-rater reliability. The findings also support the hypothesis that this technique has good

inter-rater reproducibility amongst pediatric intensivists and Physical Therapists following appropriate training. In this study, only the reference operator received initial training from a pediatric radiologist, however he was then able to train additional operators in the clinical setting where ultrasonography belongs to intensivists' daily practice; as replication of the technique was good, this approach of dissemination may be implemented in future research and clinical practice.

The power of the muscle mass monitoring study did not allow for independent risk factor recognition. In consequence, it does not yet allow the identification of a specific feature of interest that would particularly induce muscle wasting (such as disuse atrophy, fasting, severity of the disease, and/or inflammation). Future studies should seek to identify these risk-factors, and to correlate the degree of muscle wasting with various outcomes including length and cost of stay, mechanical ventilation weaning, muscle function, rehabilitation needs and quality of life.

Hydration surely impacts on muscle mass and compromises the muscle wasting assessment accuracy of our technique. We did not perform muscle biopsies to distinguish the implication in QF thickness variations, of muscle wasting itself and of muscle hydration. Assessment of body fluid overload is also challenging in the critically ill child: weight is a poor indicator in this setting, as it is influenced by both fluid shifts and nutritional status changes, and accuracy of fluid balance between inputs and outputs is questionable. The extend of the impact of fluid overload may be balanced by the combination of both muscular cell dehydration and muscular extracellular overhydration, as described by Gamrin et al. (20,21) and Haussinger et al. (22). In consequence, moderate fluid shifts, that are frequent in critically ill children, may not alter QF measurements significantly, as stated by Puthucheary et al. (23). However, the use of QF thickness as a surrogate of muscle wasting may not be perfectly accurate.

Accuracy of the technique may be challenged by the magnitude of change of QF thickness in neonates or infants, which is expected to be small. Average of two measurements did not show sufficient repeatability nor reproducibility in our study. We may hypothesize that averaging more

than 2 measurements performed in the same angle (longitudinal or transverse) would have allowed for sufficient repeatability and reproducibility and should be tested in a future study. Averaging measurements of the same structure from different angle is not commonly proposed in the literature. However, in our study the combination of transverse and longitudinal measurements was the only successful technique which allowed for good repeatability and reproducibility.

Finally, QF thickness ultrasound measurement should also be validated against the gold standard measurement which is MRI.

To conclude, multiplane ultrasonography enables QF thickness assessment and monitoring (as a potential surrogate to muscle wasting) in the critically ill child. QF thickness decrease is an early intense phenomenon that occurs in the majority of critically ill children. Further research is required in order to better understand PICU muscle wasting, to define early biomarkers, and to assess its impact on long term functional outcomes.

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Figure Legends

Figure 1

Thigh ultrasonography in transverse and longitudinal views in two different patients (0: layer of gel, no contact between the transducer and the skin is accepted, to confirm the absence of pressure of the transducer on the thigh; 1: skin; 2: fat; 3: quadriceps femoris fascia; 4: rectus femoris head of

quadriceps femoris; 5: vastus intermedius head of quadriceps femoris; 6: outer cortex of the femur; 7: femur). On the transverse view, the femur is easily identified as a hyper-echogenic semi-circle, which has to be in the centre of the image; quadriceps femoris thickness is measured vertically from the upper border of femur cortex, to the under border of quadriceps femoris fascia. On the longitudinal view, the femur is identified as a roughly horizontal hyper-echogenic row; the same measurement technique is followed, the centre of the image corresponding to the centre of the transducer that has to be located on the adequate marked skin location. The transducer has to be strictly perpendicular to the skin.

Figure 2

Average Quadriceps Femoris (QF) thickness according to patients' body weight (intra- and inter-operator reproducibility study). Red circles and blue dots represent measurements performed by the reference operator and other operators respectively

Figure 3a and 3b

Figure 3a: Coefficient of variation of quadriceps femoris thickness measurement, within a set of 4 measurements, according to patient's weight. Figure 3b: Bland-Altman plots: Intra-operator repeatability of quadriceps femoris measurement (sets of 4 measurements)

Figure 4

Quadriceps femoris thickness (in cm) monitoring at admission (TP-a), day5 (TP-b) and at last measurement performed (TP-c). Data are presented in cm +/- 2 standard errors (SE).

Figure 5

Monitoring over PICU stay of Quadriceps femoris thickness (cm), assessed by thigh ultrasonography. Each of 17 lines represents one of the 17 children enrolled in the PICU muscle wasting longitudinal study. It is noticeable that the decrease of muscle thickness over stay is almost constant but not linear and subject to fluctuations. These fluctuations are related to errors of measurement (within

the 5% change reliability of the technique) and eventually to patients' specific conditions (e.g. severe overhydration). TP-a: admission; TP-b: day 5; TP-c: last measurement performed.