**The Transpire Study**

**A prospective observational study to explore the relationships between nuTRition, protein intake ANd muScle mass loss during and after Pediatric Intensive caRE: the TRANSPIRE Study**

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**Glossary of Abbreviations**

|  |  |
| --- | --- |
| AE | Adverse Event |
| AW | Acquired Weakness |
| CI | Chief Investigator |
| CRP | C Reactive Protein |
| CRF | Clinical Report Form |
| ECMO | Extra Corporeal membrane Oxygenation |
| EN | Enteral Nutrition |
| EPR | Electronic Patient Record |
| ESPNIC | European Society for Pediatric and Neonatal Intensive Care |
| FSS | Functional Status Score |
| GCP | Good Clinical Practice |
| HRA | Health Research Authority |
| ISRCTN | International Standard Randomised Controlled Trial Number |
| NHS R&D | National Health Service Research & Development |
| MFM 20/ 32 | Motor Function Measurements scores |
| NIHR-HTA | National Institute of Health Research – Heath Technology Assessment |
| NRES | National Research Ethics Service |
| PN | Parenteral Nutrition |
| PCCS | Paediatric Critical Care Society |
| PCCS-SG | Paediatric Critical Care Society – Study group |
| PELOD | Paediatric Level of Organ Dysfunction score |
| PedsQL | Pediatric Quality of life Score |
| PERMIT | Paediatric Early Rehabilitation/Mobilisation during Intensive Care |
| PICANet | Paediatric Intensive Care Audit and Research Network |
| PICU | Paediatric Intensive care Unit |
| PIM3 | Paediatric Index of Mortality Score 3 |
| PPI | Patient and public Involvement |
| REC | Research Ethics Committee |
| SAE | Serious Adverse Event |
| SMG/T | Study Management Group/Team |
| SOP | Standard Operating Procedure |
| STROBE | Strengthening the Reporting of Observational Studies in Epidemiology |
| SAG | Study Advisory Group |
| TBI | Traumatic Brain Injury |
| UK | United Kingdom |
| WAZ | Weight for Age score |

**STUDY Summary**

|  |  |
| --- | --- |
| **TITLE** | A prospective observational study to explore the relationships between nuTRition, protein intake ANd muScle mass loss during and after Pediatric Intensive caRE: the TRANSPIRE Study |
| **DESIGN** | A prospective observational single centre study to determine the association between muscle mass loss and function, nutrition and protein intake during and after pediatric critical illness. |
| **AIMS** | To examine the relationships between muscle mass loss (measured via non-invasive ultrasound of the muscles) with nutritional intake and inflammatory markers during and after critical illness using standard, readily available bedside equipment.  **Primary:**   1. To assess the relationship and determine any correlation between PICU-muscle wasting (assessed on ultrasound) and protein intake during critical illness (from admission to PICU discharge)   **Secondary:**   1. To assess the relationship and determine any correlation between PICU-muscle wasting and energy intake during critical illness (from admission to PICU discharge) 2. To assess the relationship and determine any correlation between PICU-muscle wasting and nitrogen balance during critical illness (from admission to PICU discharge) 3. To describe the relationship between PICU-muscle wasting and inflammatory markers (CRP) that relate to the severity of illness, over the PICU stay 4. To assess and further describe muscle wasting and function changes during PICU admission and from PICU discharge to 3 months after PICU discharge 5. To describe and further quantify risk factors for quantify PICU-muscle wasting 6. To examine and quantify PICU-muscle wasting and muscle function and its impact on short term (Length of ventilation, PICU Length of stay, Hospital length of stay) and longer-term outcomes (Functional physical state (FSS), muscle function recovery (Bayley’s motor score, MFM 20 or MFM 32) and Quality of Life (PedsQL) at 3 months after PICU discharge) |
| **POPULATION** | 50 Mechanically ventilated children in a single PICU |
| **ELIGIBILITY** | **INCLUSION CRITERIA**:  Invasively ventilated children (Term neonate -16 years) expected to stay >48 hours in PICU receiving some form of nutrition (enteral and/or parenteral) whose parents consent to the study. Children included will be normal neurologically, if of walking age, will be independently ambulatory pre-admission, with no previous PICU admission within the last 5 years and with no known neuromuscular disease.  **EXCLUSION CRITERIA:**  Children will be excluded if they are extubated (not on a breathing machine) at PICU admission, they are expected to stay a short time, are not expected to survive, are undergoing palliative care, have an existing or acute muscle disease or we are unable to perform muscle ultrasound or where no follow up possible (out of region referrals).  **OUTCOME MEASURES**  1. Muscle mass assessed via ultrasound.  2. Muscle function assessment via age-validated scoring tools  3. Protein and energy outcomes  4. Inflammatory biomarkers (CRP)  5. Paediatric Quality of life and functional status scores |
|  |
| **duration** | Follow-up for three months after PICU discharge. |

**Study team**:

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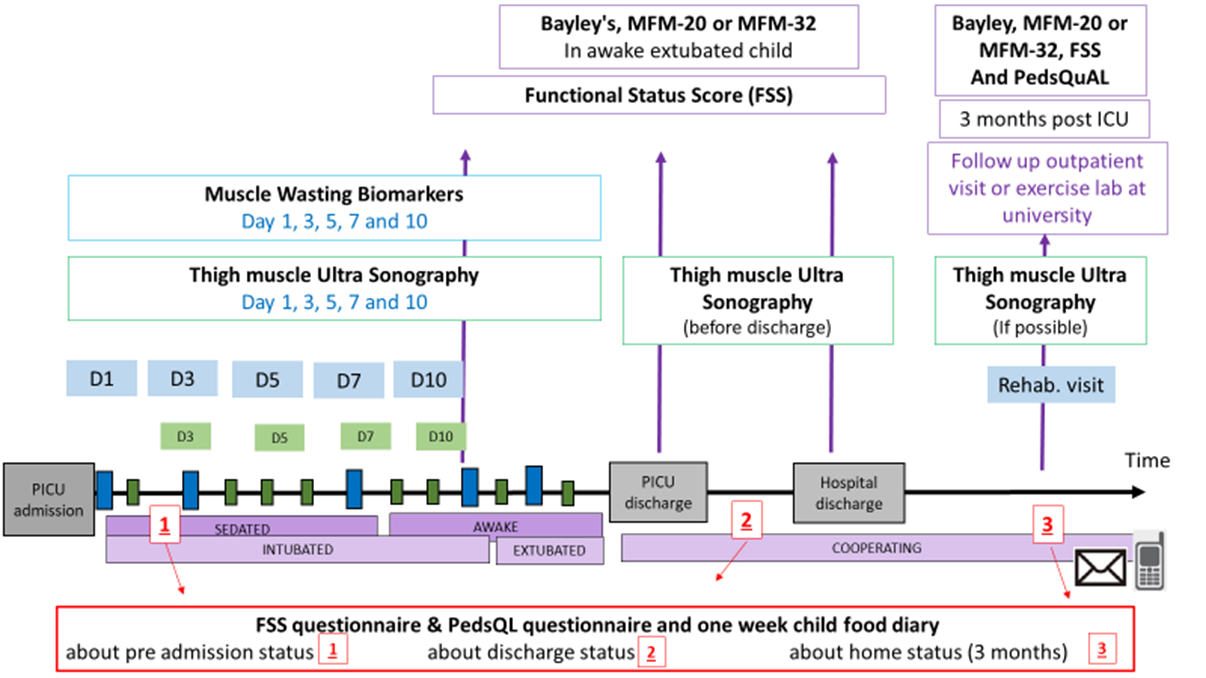
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PPI: Archie Veale BSc (ex PICU patient of Alder Hey PICU now 22 years old) and Lewis Veale (Father of Archie).

**Expert collaborator**: Carole Vuillerot MD PhD Paediatric Rehabilitation specialist, Lyon France

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**Figure 1 Diagram of TRANSPIRE Study**



**1 INTRODUCTION**

## PLAIN ENGLISH SUMMARY OF STUDY

Around 20 000 children are admitted to intensive care each year in the UK and Ireland and around 13 000 of these stay more than 3 daysin intensive care, with some children staying a lot longer. As children’s intensive care in the UK has improved in the last decade, almost all children now survive critical illness, but for many, their recovery is prolonged both physically and psychologically. Children on the breathing machine in intensive care lose a lot of weight and muscle very quickly, and this slows down their recovery and can lead to longer stays both in the intensive care and in hospital.

In adults in intensive care, research has shown that some of this muscle loss may be able to be lessened by giving them a higher protein feed combined with early rehabilitation in intensive care, but in children we still do not know if this weight and muscle loss is modifiable by and related to the nutrition and the amount of protein they receive. This is what we want to find out, as children are not the same as adults, and frequently respond in different ways to adults. Furthermore, children admitted to ICU are quite different both in their age range and underlying conditions to those of adults.

To do this, we want to look at the children’s muscles (by ultrasound, so using gel and running a probe over the muscle, which does not hurt at all) when they first come to intensive care, every few days, when they leave intensive care, when they leave the hospital and 3 months later. We will then see what happens to their muscles and how strong their muscles are, as well how much nutrition and protein they got in intensive care to see if they are related. We will look at one of their usual daily blood tests in addition to collecting information about their age and weight, why they came to intensive care and other important things that might impact on their muscles. We will do this in one intensive care unit, Alder Hey Children’s NHS Trust in Liverpool. This PICU is big and admits around 1200 children every year for lots of different reasons.

We have involved one of our past patients, who is now 21 years old and who spent around 3 months in our intensive care a few years ago. He and his family say that this (weight loss and weak muscles) was a real problem for him and his recovery, and he believes it is a really important area to investigate.

**1.2 Rationale for study**

Around 20 000 children are admitted to Paediatric Intensive Care Units (PICU) each year in the UK and Ireland and around 13 000 of these stay > 3 days 1. The acuity of PICU patients is increasing with the use of advanced life-saving therapies such as extra corporeal membrane oxygenation (ECMO) and a small subset of children staying for prolonged periods of time.  Despite this, the survival rate for paediatric critical illness is high at >96%2 (much greater than adult intensive care) but their recovery and physical morbidity can be prolonged, so interventions to improve their recovery are vital to improve both child and family outcomes. Muscle wasting is dramatic in critically ill patients (both children and adults) and related to worsened outcomes 3-12. This muscle wasting delays the patient’s rehabilitation and prolongs their recovery, due to reduced functional capacity associated with this loss in muscle mass. Therefore, it is important to know whether muscle wasting in critically ill children is influenced by illness severity and can be modified by interventions such as nutrition (and more specifically protein) delivered during critical illness and whether this muscle mass loss persists in the longer term (after PICU and hospital discharge).

With significantly higher survival rates in children after critical illness (than those of adults), with even greater potential gains in life productivity, these effects are even more important to quantify, with the aim of improving the child’s functional recovery to maximize their physical recovery. Furthermore, the importance of this issue for patients and families has been demonstrated by a recent UK PICU research prioritization exercise where families/parents ranked research into longer term outcomes highly important, even more so than the healthcare professionals 13. Yet currently no researchers have investigated this relationship in children and tried to determine the relationship between muscle loss to nutrition and protein intake during and after critical illness. Determining such relationships is the first step before being able to develop an intervention to target this problem. This topic has been highlighted as a priority research area for paediatric critical care nutrition by clinicians internationally.14

**1.3 Review of existing evidence**

In critically ill adults, ICU-Acquired Weakness (AW) is a frequent, rapid and early phenomenon, especially in multi-organ failure patients 8,9. It leads to prolonged ICU and hospital- stay, prolongs rehabilitation and increases health care costs 9. In the most recent study of 78 critically ill adults, they reported that muscle mass reduced significantly in three muscle groups, from a starting muscle thickness 8.5 cm (SD: 3.2 cm) to end muscle thickness, 6.8 cm (SD: 2.2 cm) over the first 14 days of critical illness, with mean difference -1.67 cm (95%CI: -2.3 to -1 cm), p<0.000112. A strong association was observed between blood concentration of C-Reactive Protein (CRP) and the percentage of muscle thickness loss at day 14 (r = -0.66, p = 0.017). They also noted a continuous muscle mass loss and negative nitrogen balance (indicating malnutrition) over 14 days of critical illness, with catabolism (protein breakdown) remaining a dominant metabolic feature 12.

In the paediatric setting, Paediatric Intensive Care – acquired weakness is not well studied, with limited data regarding the magnitude of the acquired weakness, while the medium to long term magnitudes and effects of skeletal muscle wasting in children are currently completely undescribed3-7,10,11.  One of the earliest studies of muscle weakness in critically ill children 11found that in PICU survivors, significant muscle weakness persisted for 3 to 12 months following ICU discharge.  Others have examined specific muscle weakness of the diaphragm in critically ill children and found a reduction in diaphragm muscle thickness and evidence of diaphragm atrophy in 56 ventilated children 13.  Most recently, Johnson et al. prospectively studied 34 children to determine the incidence, severity and risk factors for muscle atrophy in critically ill children 3. This muscle mass loss is important for several reasons, but most importantly the ability of the child to use their respiratory muscles (mainly the diaphragm) to breathe effectively and enable them to come of the ventilator (breathing machine). Severe muscle weakness will delay this considerably. They found diaphragm muscle thickness decreased 11.1% (95%CI, -19.7% to -2.52%) between the first two assessments or 2.2%/day. Quadriceps thickness decreased 8.62% (95%CI, -15.7% to -1.54%) or 1.5%/day. Biceps (-1.71%; 95%CI, -8.15% to 4.73%) and tibialis (0.52%; 95%CI, -5.81% to 3.40%) thickness did not change. Among the entire group, 47% (14/30) experienced diaphragm atrophy (defined apriori as 10% decrease in thickness). Eighty three percent of patients (25/30) experienced atrophy in one muscle group, and 47% (14/30) in two muscle groups. Only two factors, increasing age and traumatic brain injury (TBI) were associated with greater muscle loss, but this study did not collect any nutritional data.  However, although PICU acquired weakness is a multifactorial neuromuscular disease; it is different from the other neuromuscular diseases which are congenital and chronic. PICU skeletal muscle atrophy and the associated acquired weakness is transient and potentially reversible. Although we know that recovery is possible, due to an absence of research in this population we do not know how long after critical illness recovery of muscle mass begins, thus the importance of exploring factors that can impact upon the magnitude of deterioration while in PICU (such as nutrition) with a view to be able to develop interventions to reduce its development and speed up recovery.

Building on previous work 16Valla et al. 17 undertook a prospective observational study in two PICUs and refined and validated this technique of quadriceps muscle thickness measurement. In this study he established the inter and intra-rater reliability of this technique in 17 critically ill children, finding the mean relative difference in quadriceps femoris muscle thickness was 0.36% ± 2.5% (lower and upper limits of agreement: −4.5/+5.2%), and the intraclass correlation coefficient was 0.998. In the 17 children they were able to monitor over their PICU stay, quadriceps femoris thickness significantly decreased at day 5 by 9.8% (*p* = 0.006) and by 13.3% (< 0.001). In the children with a PICU length of stay > 5 days, he found a decrease of quadriceps thickness of 13.6% (+/-8%) in the first 7 days 17.

Once children are stabilized after admission to intensive care, nutritional support will be started in the form of tube feeding into the gut (enteral feeding) (80% of the time) or given via an intravenous line (parenteral) (<20% of the time) if enteral feeding is not possible.  Nutrition (energy) needs to be provided to reduce catabolism and provide energy for physiologic functions such as breathing. In the study intensive care unit, the target time to start enteral nutrition is within 6 hours of admission. Despite having estimated energy and protein goals for children, based on predictive equations 18,19,20,21, the amount of energy and protein children receive varies. This happens for several patient related reasons: feeding intolerance, fasting for procedures and fluid restriction. Adult evidence indicates that lower protein delivery in the acute phase of illness, promotes more muscle mass loss 8 yet some pediatric evidence 22,23 suggests that higher protein delivery in the early phase of critical illness may be harmful. By undertaking an observational study of real-life practice, of children’s actual energy and protein intake and relating this to the child’s severity of illness, inflammatory and nutritional markers and subsequent muscle mass loss, we believe will generate some evidence around this relationship. No previous study has done this in critically ill children; thus, our proposed study plans to build on the existing evidence and establish the relationship between this muscle mass loss over time and after discharge with nutritional intake and markers of critical illness.

**2.1 Study aim and objectives:**

**AIMS AND OBJECTIVES**:

Our overall study aim is to examine the relationships between muscle mass loss (measured via non-invasive ultrasound of the muscles) with nutritional intake and inflammatory markers during and after critical illness using standard, readily available bedside equipment. Figure 1 is a visual representation of the study.

**Primary:**

1. To assess the relationship and determine any correlation between PICU-muscle wasting (assessed on ultrasound) and protein intake during critical illness (from admission to PICU discharge)

**Secondary:**

1. To assess the relationship and determine any correlation between PICU-muscle wasting and energy intake during critical illness (from admission to PICU discharge)
2. To assess the relationship and determine any correlation between PICU-muscle wasting and nitrogen balance during critical illness (from admission to PICU discharge)
3. To describe the relationship between PICU-muscle wasting and inflammatory markers (CRP) that relate to the severity of illness, over the PICU stay
4. To assess and further describe muscle wasting and function changes during PICU admission and from PICU discharge to 3 months after PICU discharge
5. To describe and further quantify risk factors for quantify PICU-muscle wasting
6. To examine and quantify PICU-muscle wasting and muscle function and its impact on short term (Length of ventilation, PICU Length of stay, Hospital length of stay) and longer-term outcomes (Functional physical state (FSS), muscle function recovery (Bayley’s motor score, MFM 20 or MFM 32) and Quality of Life (PedsQL) at 3 months after PICU discharge)
   1. **STUDY DESIGN**

**DESIGN**: A prospective observational single centre study to determine the association between muscle mass loss and function, nutrition, and protein intake during and after pediatric critical illness.

**Duration:** 24 months

**SETTING:** We will recruit children from a single large mixed general and cardiac PICU (and funded Extra Corporeal Membrane oxygenation (ECMO) centre) at Alder Children’s NHS Foundation Trust Liverpool, UK, admitting around 1200 children a year, 85% of whom are invasively ventilated.

**3.1 TARGET POPULATION**

**INCLUSION CRITERIA**: Invasively ventilated children (Term neonate (>37 weeks gestational age) -16 years) expected to stay >48 hours in PICU receiving some form of nutrition (enteral and/or parenteral) whose parents consent to the study. Children included will be normal neurologically or with mild impairment only, and if of walking age, will be independently ambulatory pre-admission, with no previous > 3-day PICU admission within the last 5 years and with no known neuromuscular disease.

**EXCLUSIONS**: Children will be excluded if they are extubated (not on a breathing machine) at PICU admission, they are expected to stay < 24 hours, are not expected to survive, are undergoing palliative care, have an existing or acute muscle disease or we are unable to perform muscle ultrasound or in cases where no follow up possible (out of region referrals).

**4.1 STUDY OUTCOME MEASURES**

**OUTCOME MEASURES (**Table 1 in Appendix 1 shows data collection points**)**

Muscle mass will quantitatively be assessed by thigh ultrasound in the technique described and validated by Valla 17: Quadriceps muscle thickness will be measured using a standardized technique, based on this previous study. PICU Muscle wasting will be defined as a decrease in quadriceps muscle thickness >10%. We will also use a continuous scale for muscle thickness values instead of a cut-off; in addition to a >10% cut off. We will also calculate variability and measurement error at baseline, so that we can determine if any observed changes are greater than the measurement error associated with this cohort. This measurement will be performed a key time points along PICU stay (and for some where possible, pre-operatively) on day 1, 3, 5, 7 and 10 of PICU stay.

1. Muscle Function assessment will be assessed using the Motor Function Measure Bayley scale (<2 years)25, MFM-20 (2-6 years)26 or MFM-32 (>6 years)27 will be used to assess muscle function and follow muscular disability. The scoring will be performed by a trained operator (the RA), at PICU discharge, hospital discharge and 3 months later.
2. The inflammatory biomarker assessed is the C-reactive protein (CRP). C-reactive protein (mg/L) will be measured from plasma samples collected on days 1, 3, 5, 7 and 10 of the study (this is a routine daily blood test in intensive care) and no extra blood will be taken.
3. Nutritional outcomes assessed are theNitrogen balance (g/day) calculated using both the British Dietetic Association’s Parenteral and Enteral Nutrition Group recommended equation and the Deacon equation for estimating nitrogen excretion, assuming nitrogen balance equals nitrogen intake minus nitrogen excretion and the WHO equation that estimates faecal and other losses in g/kg (these are very low compared to urinary losses) 18,19,20,21

Total nitrogen losses (g/d) = urinary nitrogen (g/d) + faecal losses (0.021 g/d \* body weight) + miscellaneous losses (0.001 g/d \* body weight).

Weight (kg), height (m) or length (cm) and body mass index (kg/m2), centiles and WAZ scores will be recorded, in addition to daily energy (kcal) and protein (grams) intakes. Recent weights and heights/length will be obtained, directly or from medical notes. Enteral feed tolerance will be monitored by assessing gastric residual volumes (ml) and incidence of vomiting as per unit protocol. Patients will be fed within 6 hours of ICU admission according to the usual PICU feeding protocol; energy requirements will be calculated using the Schofield predictive equation. Protein requirements will be estimated at 1.5 g/kg/day as per current recommendations. 21

1. Assessment at 3 months after PICU dischargewill include assessment for both muscle mass (using ultrasonography) functional status, quality of life and feeding disturbances will be assessed using a number of validated tools: the Functional Status Score (FSS)24, PedsQL, Bayley’s scale motor component (<2 years)25, MFM-20 26 (children aged 2-6 years and MFM-3227 (>6 years of age) scores. A typical week food diary (via Nutritics) and activity diary will also be completed by the parents/and or child within a week of this appointment and submitted at this appointment for analysis by the dietitian and exercise specialist (Comfort). This follow up will be done in conjunction with the nearest outpatient clinic appointment at Alder Hey Children’s (if possible). Any additional travel and parking costs for this visit will be covered. If parents cannot attend for muscle assessment the FSS can be assessed by telephone interview24.
2. Patient characteristics during critical illness will be recorded including:  At PICU admission (age, weight, gender, nutritional status, severity of illness and organ dysfunction scores (PELOD 2, PIM3), admission diagnosis, previous history, current medication, current feeding). Patient outcomes (death, acquired infections, length of stay, mechanical ventilation duration). We will also collect data relevant to interpret our outcomes (PICU nutrition and protein intake, PICU drugs, weight change over PICU stay, fluid balance, plasma albumin levels and haematocrit (as taken for clinical purposes)

**Table 2: Data collection items**

|  |  |  |
| --- | --- | --- |
| **Outcome** | **Completed by** | **Analysed by** |
| Muscle mass by US | RA or AW, or another trained team member | FV, PC, LT |
| Muscle Function | RA | LT, CV, FV, PC |
| CRP, other routinely collected bloods: albumin, Haematocrit | Taken routinely input into CRF by RA | LT |
| Energy and protein calculation | Entered into CRF by RA or LL | LL, FV. LT |
| Weight | Bedside nurse with RA | LL |
| Height | Bedside nurse or RA | LL |
| WAZ | LL | LL |
| Enteral feed volumes | Bedside nurse as routine, input into CRF by RA | LL, LT |
| PELOD, PIM3, Patient outcomes | AW extract from Unit PICANet and input into CRF by RA | LT, AW, FV |
| Weight change over PICU stay | Bedside nurse with RA | LT, FV, LL |
| PedsQL | RA | LT and CV |
| FSS | RA | LT, PC, FV |
| MFM 20 or MFM 32 or Bayley’s motor score | RA | LT, CV, FV, PC |
| Diet diary | Parent/or child | LL |
| Activity diary | Parent/or child | PC |

# 5.0 ENROLMENT PROCEDURE

**5.1 RECRUITMENT**: Our recruitment target would be 4 patients per month over 15 months.

In terms of study feasibility, analysis of last 12 months Alder Hey PICU data set (PICANet) revealed 150 children stayed more than 7 days, so with some exclusions and a cautious consent rate of 60%, (which is based upon the consent rate for the five most recent 2019 research studies in this PICU: DEPICT 86.9%, BESS 100%, P4 85.7%, NEUROPACK 83.3%) we believe it is feasible to undertake this study with a data collection/recruitment period of 15 months. If we include children who stay between 3-7 days (more children stay for up to 7 days), then our numbers would increase up to 386.

**6.1 PROJECT TIMETABLE**: The project will run for 24 months. During the set-up phase (months -3 -2) we will apply for approvals, recruit and train staff and ensure protocols are standardized and in place. Recruitment will begin at month 3 and end by month 18. Primary outcome data collection will be completed by month 18, with follow up outcomes completed by month 21. We have allowed 3 months for final analysis and dissemination of findings (see Gantt chart Figure 2)

**PROJECT MANAGEMENT:**The CI (Tume) will manage the project, recruit the research assistant, arrange the training and supervise the team ensuring the project is delivered within time and on budget.  She has experience in managing research teams on funded studies. She will also manage the ethical and R&D approvals. She will manage the project, but a study management group (SMG) involving the study team investigators will be set up and will meet monthly via teleconference. In conjunction with the study statistician (CH) a bespoke database will be set up for data entry.

**7.1 PPI INVOLVEMENT**: The development of this application has been informed by discussions with our main patient representative Mr Archie Veale. Archie was a patient on this PICU when he was 15 years of age and spent almost three months on intensive care, losing around 30 kg of body weight and muscle mass, which was a significant problem for him in his subsequent rehabilitation. Working with Archie and his family has revealed this issue to be immensely important and to find way to try to ameliorate this muscle mass loss. As such Archie and his father are co-applicants on this study and will provide his vitally important patient experience throughout this study, they will attend the study management meetings (all virtually) and help us to prepare the parent information sheets, information and in writing lay understandable study results for dissemination. The CI will ensure any training they require is addressed and she will meet with them virtually once a month to clarify any queries (before the SMG meeting).

**8 adverse events**

Due to the nature of the patient population, there is a high incidence of possible adverse events during their routine care and treatment. However, as this is an entirely observational and non-invasive study none of these are likely to be related to the study and therefore, we will only collect those adverse events identified as serious will be recorded for the study.

## 8.1 Definitions

**Serious Adverse Event** **(SAE)**

Adverse events are defined as serious if they:

* Result in death
* Are life-threatening

The term “life-threatening” refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

**9 DATA COLLECTION, ASSESSMENT AND FOLLOW-UP**

**9.1** Follow-up will be until three months after PICU discharge.

**9.2 Data collection** will be on paper CRF entered directly into the study database.

The PICU owns one Sonosite device (with another on loan) which will be used along with two linear probes (9-13Hz) for the ultrasound measurements.

The device is under are a service contract with annual safety check as required by the manufacturer to ensure accurate device function.

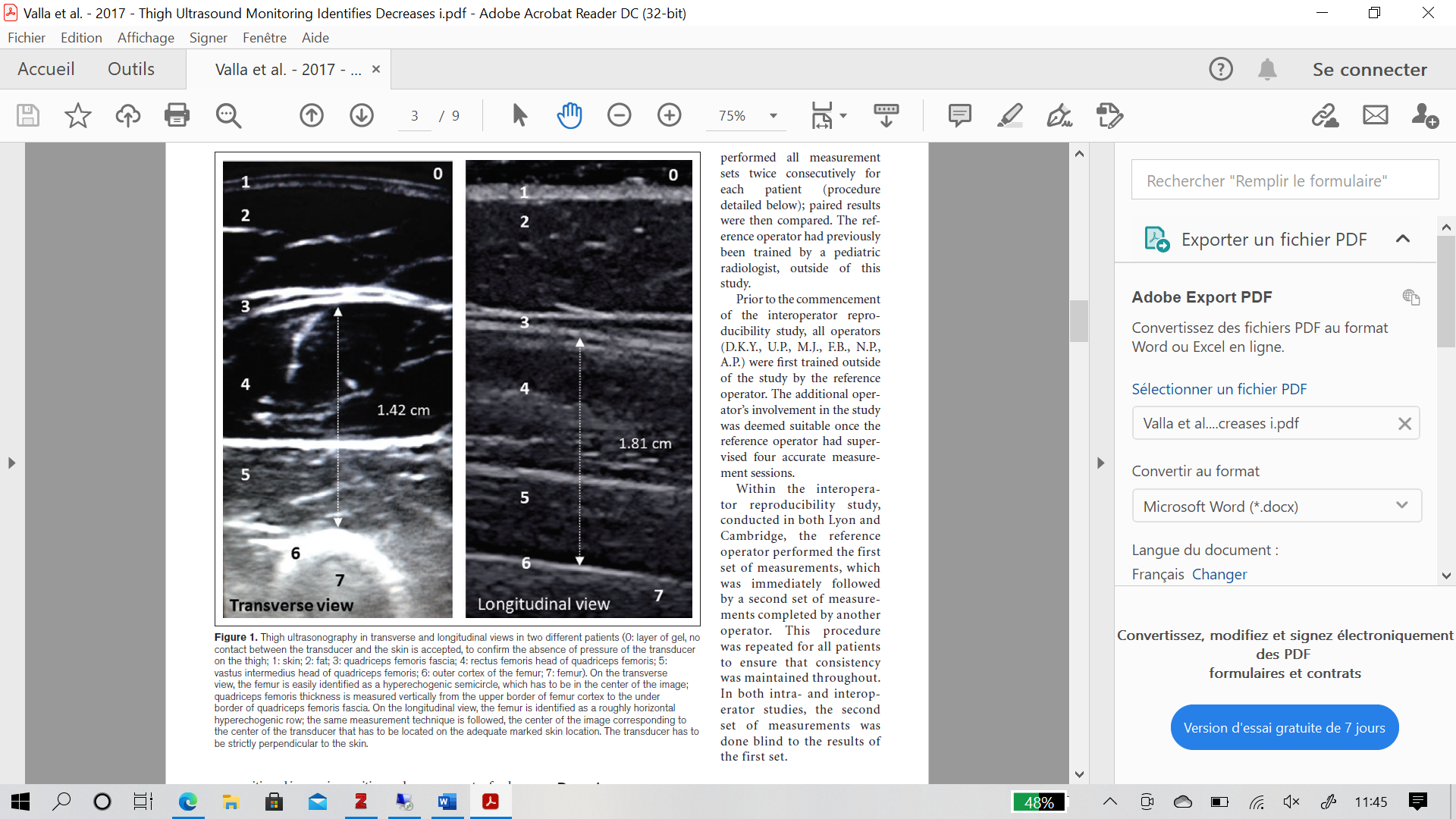
**The measurement technique:**

Ultrasonographic examination will be performed using B-mode ultrasonography. We will use a linear transducer which frequency is adapted according to the accumulative thickness of thigh muscle and fat, ranging from 9 to 13 Hz. The measurements will be performed strictly perpendicularly to the skin plane 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).

Quadriceps femoris (QF) muscle is composed of four heads, two of which (rectus femoris (RF) and vastus intermedius (VI) muscles) are located anteriorly. QF thickness will be defined as the sum of the anterior thickness of these two heads. Measurements will be obtained while recruited children are either sedated or fully cooperative to obtain muscle relaxation, therefore avoiding the confounding effects of muscle contraction on measured QF thickness.

To obtain the most accurate QF thickness and maintain consistency for all children, each patient will be positioned in a supine position and measurements of only one leg will be taken. On each occasion, the leg will be fully extended and positioned in a neutral rotation while external compression of the muscle will be avoided to limit artefactual deformation of the muscle shape (this includes removal of positioning aids and pillows). To overcome the progressive increase in thickness of the anterior QF muscle, we will use a measuring tape to identify the widest portion of the thigh and record 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 will then mark this point to ensure that all subsequent measurements will be taken at exactly the same location during hospital stay.

We will perform a set of four measurements of QF muscle anterior thickness. First, a transverse measurement will be performed (transducer placed perpendicularly to thigh axis) and then a longitudinal one (transducer placed parallel to thigh axis); both measurements will be repeated once. QF thickness will be measured vertically on the image, from the outer cortex of the femur to the internal border of the QF fascia, as shown in the Figure below. The average of the four measurements will then be calculated to account for operator-related error and will serve as the main criteria.



**Thigh fat thickness**: Ong et al.s 2021 study method will be followed. We will perform a set of four measurements of thigh anterior fat thickness. First, a transverse measurement will be performed (transducer placed perpendicularly to thigh axis) and then a longitudinal one (transducer placed parallel to thigh axis); both measurements will be repeated once. Thigh anterior fat thickness will be measured vertically on the image, from the internal border of the skin to the outer border of the QF fascia. The average of the four measurements will then be calculated to account for operator-related error.

**RF cross sectional area**: Ong et al. 2021 study method will be followed.

We will perform a set of 3 measurements of Rectus femoris cross sectional area (RF-CSA). A transverse measurement will be performed (transducer placed perpendicularly to thigh axis); measurements will be repeated twice. RF-CSA will be measured contouring the internal border of the RF fascia. The average of the three measurements will then be calculated to account for operator-related error.

Table 1 in Appendix 1 shows data collection at each time point.

Study data will be collected using (e) CRFs and transferred into the dedicated and secure trust study laptop for storage in the secure trust drive. Individual participant data will be identified by a study participant number only. Consent forms containing the patient and parent’s names will be kept securely within the PICU research office in a locked cabinet.

Archiving will follow the completion of the study and publication of results in line with NHS guidelines for a minimum of 5 years. At this point, the requirements to continue to archive these data will be reviewed in line with the applicable data protection guidelines. Electronic files will be stored on a restricted access (named individuals) server held in a secure location. In line with the trust security policy, authorised access to the PICU research office is via an electronic tag entry system and the room is kept locked when unoccupied. Authorised staff will process data via a secure network which requires individual login name and password (changed regularly). No data are stored on individual workstations/desktops. The data is backed up weekly to the secure drive.

All paper and electronic data will be stored securely in strict compliance with current data protection regulations.

No additional blood or tissue samples are required for this trial.

**10.3 Withdrawal from study**

Parent(s) may withdraw their child from the study at any time; they are not obliged to give a reason. Parents will be asked whether data already collected may be retained and used for the purposes of the trial and their decision will be recorded on the withdrawal form.

Parents will be made aware that this decision has no impact on any aspects of

their child’s continuing care. The attending clinician may withdraw the child from treatment if they consider this to be in the best interest of the infant’s health and well-being.

# 10 STATISTICs and data analysis

**10.1 SAMPLE SIZE**:

As a prospective observational study, we have based our sample size calculation on investigating the association between PICU-muscle wasting (via ultrasound) and protein intake during critical illness. Using the regression approach with muscle wasting as dependent variable, the sample size of n = 50 allow us to have protein intake as an independent variable, adjusting for 2-3 confounders 28 As each patient provides multiple measurements this should further increase the power of the analyses.

**10.2 STATISTICAL ANALYSIS PLAN**:

The reporting guideline of STROBE statement 29 will be followed for analysis and reporting of this study. All statistical tests and confidence intervals will be two-sided. Ninety-five percent confidence intervals will be presented with the significance of p value at 5% significance level. Continuous data will be tested for normality and presented as mean (SD), mean (95% confidence intervals) or median (IQR). Categorical data will be presented as frequencies. In-depth, graphical, exploratory analysis will allow us to visualise the relationship between muscle-wasting, nutrition/protein intake and other outcomes over time. It is through these graphs we would answer secondary research question 4.

For the primary research question a repeated measure mixed model will be used, with muscle wasting as the outcome (as a continuous scale and then as a binary outcome with 10% decrease as cut off) and protein levels as the independent variable. This type of model will allow us the flexibility of including potential confounders (neuro-blocking agents, energy-protein intake deficits and paediatric logistic organ dysfunction 2 score) as covariates and accounting for the longitudinal nature using fixed/random effects as appropriate. It may be determined is unlikely that CRP (protein) levels will be associated with concurrent muscle waste, but rather with future muscle waste (i.e. my protein levels today will determine my muscle wastage tomorrow). If this is the case, an alternate approach will be used to account for this lag, such as regressing future muscle wastage on past protein levels. Exploration of these models would also allow us to answer secondary research questions 5 and 6. One of these methods would be applied to the secondary research questions 1 and 2 also.

To assess whether there is a significant different between muscle levels at discharge and at 3 months follow up (secondary research question 3) we would use multiple linear regression with muscle mass as the outcome and the binary time point (discharge/follow up) as the independent variable. A similar approach would be used for secondary research question 5.

# 11 regulatory issues

## 11.1 Ethics approval

The study will only start after gaining approval from the University of Salford, the Health Research Authority (HRA), and a National Research Ethics Service (NRES) registered ethics committee. Additionally, and NHS Trust Research and Development (R&D). The CI will submit and, where necessary, obtain approval from the REC for any protocol amendments and changes to the parent information leaflet.

The study will be conducted in accordance with the recommendations for research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. This study will adhere to the principles outlined in the NHS UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, relevant Data Protection regulations, the principles of GCP and other regulatory requirements as appropriate.

Patients will be screened daily by the research assistant, members of the study team and the PICU research nurses for eligibility. Written informed consent will be obtained from the parent/primary carer before recruitment. For some elective children expected to stay for a prolonged time this may be done before PICU admission (eg on the cardiac surgical ward).

**Renumeration**

Neither trial participants nor their parents will be given any financial or material incentive or compensation to take part in this study, however expenses for additional visits (parking) will be covered.

## 11.2 Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the trial as registered under relevant Data Protection regulations.

## 11.3 Indemnity

Alder Hey Children’s NHS Foundation Trust holds negligent harm and non-negligent harm insurance policies which apply to this trial.

## 11.4 Sponsor

Alder Hey Children’s NHS Foundation Trust will act as the Sponsor for this trial. Delegated responsibilities will be assigned to the NHS Trusts taking part in this trial.

# 12 STUDY Management

The trial will be supervised on a day-to-day basis by the Study management team (SMT) this will comprise all members of the study team.

The study will also be overseen by a Study Advisory Group (SAG) who will advise on study development, progress, findings and recommendations. This will comprise our PPI expert representative Mr. Archie Veale (also qualified in Orthotics which provides prescriptions for insoles, braces, splints, callipers, footwear, spinal jackets and helmets which help people recover from or avoid injury), his father Lewis Veale along with Dr Carole Vuillerot (a pediatric rehabilitation specialist doctor from Lyon France who developed the MFM20 and 32 scales), Dr Barney Scholefield (a PICU Intensive care Physician from Birmingham and thee chief investigator for the NIHR HTA PERMIT study), Ms Laura Rimmer (a PICU physiotherapist with a special interest in early rehabilitation and Dr Luise Marino (a clinical-academic PICU dietician from Southampton UK), Dr Jan Hau Lee (Ped Intensivist Singapore), Dr Chengsi Ong (PICU dietician Singapore), Dr Zudin Puchuchaery (Adult Intensivist, London, with research program in muscle loss with critical illness)**.** This group will meet 6-monthly via teleconference.

**13.0 DISSEMINATION** **and IMPACT**

We intend to ensure that our findings impact on children and their families in the future. The results of this study will be presented at both locally within the trust, at the UK annual PICU congress (PCCS), the national PICU Study Group (PISG-SG) and European meetings (ESPNIC), then at least 2 papers will be submitted for publication in a high impact journal. From this exploratory study examining nutrition to muscle mass loss, we anticipate that a combined nutrition and rehabilitation intervention may be able to be developed and tested in a future study. This would be in collaboration with our colleagues (Scholefield B) undertaking the NIHR HTA-funded PERMIT Feasibility study which aims to determine if an early mobilization intervention is feasible in critically ill children in UK PICUs.  We will set up a study Twitter account which will also publicize our research to key parent groups.

**IMPACT AND FUTURE RESEARCH** This study in intended to establish what the relationship is between nutrition and energy and protein delivery and muscle-wasting in critically ill children, with the intended aim of being able to develop a targeted nutritional intervention to test in a future trial. This study ties in with current HTA-funded feasibility work around early rehabilitation and mobilization in critically ill children (the PERMIT study REF 17/21/06), but they have not collected data on nutrition. We believe that any future trial looking to improve rehabilitation outcomes for critically ill children will almost certainly have to involve a combined nutrition and early mobilization intervention, thus we believe this work will provide the knowledge required for us to develop and refine the nutritional aspect of a future intervention. We will work in collaboration with the PERMIT study investigators to ensure a future trial would incorporate both nutrition and early rehabilitation intervention if both studies indicate these are feasible.

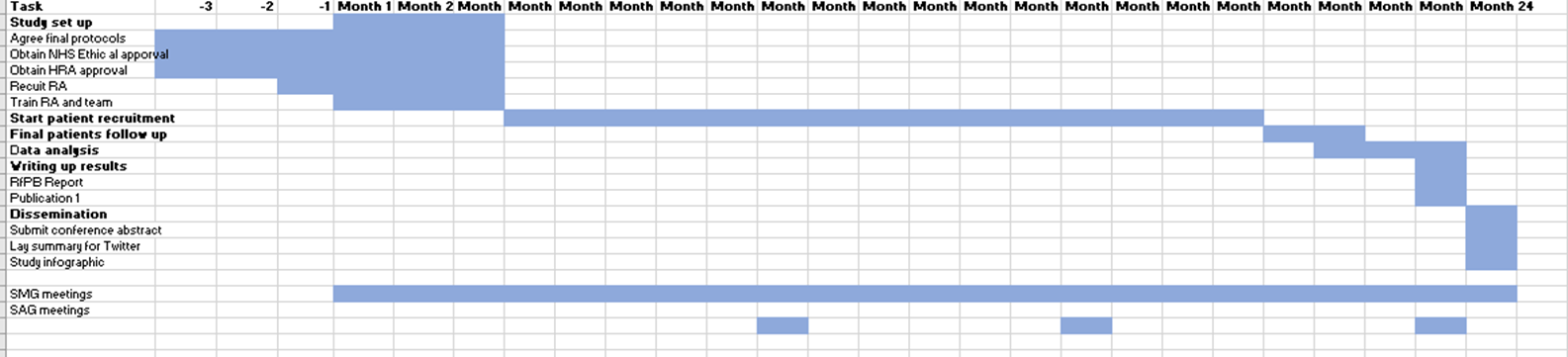
**Record of changes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Version Stage** | **Versions No** | **Version Date** | **Protocol updated & finalised by** | **Detail the reason(s) for the protocol update** |
| V1.1 | V1.1 | 7 May 2021 | SMG | TO BE ADDED |

**Table 1: Proposed data collection points**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter**  **(and data type)** | **Baseline at PICU admission Day 1** | **3 days** | **5 days** | **7 days** | **10 days** | **PICU discharge** | **Hospital discharge** | **3 months follow up** |
| Weight (kg) | **x** |  | **x** | **x** |  | **x** | **x** | **x** |
| Length/height (cm) | **x** |  |  |  |  |  |  |  |
| Centile/WAZ score  BMI HAZ WHA MUACz | **x** |  |  |  |  | **x** | **x** |  |
| Muscle mass 1  % change from baseline  Muscle function | **X Pre-op if possible**  **x** | **X**  **x** | **X**  **x** | **X**  **x** | **X**  **x** | **X**  **x** | **X**  **x** | **X**  **x** |
| CRP (mg/l) as long as a line insitu to draw blood from | **x** | **x** | **x** | **x** | **x** |  |  |  |
| Bayley’s Scale (gross motor skills scale), MFM-32 or MFM-20 scores  A composite score (Bayley’s) or 0-3 scores | **X**  **pre-op if possible** |  |  |  |  | **x** | **x** | **x** |
| Paediatric Functional status score (FSS) Score ranges from 6 – 30 | **Pre-op if possible** |  |  |  |  |  | **x** | **x** |
| PedsQL  Score 0 - 100 |  |  |  |  |  |  |  | **x** |

**Figure 2: Study gantt chart**

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