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DOCTORAL SCHOOL
BIOMEDICAL SCIENCES

THE LONG-TERM LEGACY OF CRITICAL ILLNESS

MECHANISMS AND CLINICAL IMPACT

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2021

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Abbreviations

Abbreviation	Explanation
Numeric/symbolic/units	
5STS	Five-repetition sit-to-stand test
6MWD	Six-minute-walk-distance
%pred	Percentage of predicted value
cmH_2O	Centimetres of water
$\Delta\Delta C_T$	Delta-delta threshold cycle method
E3	Enzymatic step 3 (of ubiquitine proteasome system, ligase)
IU	International Units
kcal	kilocalories
kg	kilograms
Kg/m^2	Kilograms per squared metre
µg	microgram
µg/l	micrograms per litre
µV	microvolt
mg/dl	Milligrams per decilitre
ml/min/kg	Millilitres per minute per kilogram body weight
mmHg	Millimetres of mercury
min	minute
PO_2/FIO_2	ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen
U/d	Units per day
A	
Achε,γ	Achetylcholine receptor, subunit epsilon and gamma
ADL	Activities of daily living
aHR	Adjusted hazard ratio
AMP(K)	5' adenosine monophosphate (activated protein kinase)
anti-Xa	Anti-coagulation Factor ten activity
APACHE II	Acute Physiology and Chronic Health Evaluation
ARDS	Acute Respiratory Distress Syndrome
AT	Anaerobic threshold
ATS	American Thoracic Society
B	
Bca	bias-corrected accelerated
BMI	Body mass index
C	
CI	Confidence interval
CIM	Critical illness myopathy
CIP	Critical illness neuropathy
CINMP	Critical illness neuromyopathy
CMAP	compound muscle action potential
COPD	chronic obstructive pulmonary disorder
COVID-19	Coronavirus-19 disease
CPET	Cardiopulmonary exercise test(ing)
CRP	C-reactive protein
CS	Corticosteroids
CT	Computed Tomography

Abbreviation	Explanation
D	
D	Day
DLCO	Diffusion capacity (of carbon monoxide)
DNA	Desoxyribonucleic Acid
E	
ECMO	Extracorporeal membrane oxygenation
EOLIA	ECMO to Rescue Lung Injury in Severe ARDS trial
EPaNIC	Early Parenteral Nutrition in Intensive Care trial
EPFU	Early Parenteral Nutrition in Intensive Care Follow-Up cohort
F	
FEV_1	Forced expiratory volume in 1 second
FOXO	Forkhead boxO
FVC	Forced Vital Capacity
H	
HCS	Hydrocortisone
H&E	Hematoxylin and Eosin
HD	Hemodynamic
HDAC	Histone Deacetylase
HGF	Hand grip force/strength
HHD	Hand held dynamometry
HOS	Hospitalisation
HR	Hazard ratio
HRmax	Maximal heart rate
HRpeak	Peak heart rate
HRR	Heart rate reserve
HR/WR ($\Delta HR/\Delta VO_2$)	ratio of heart rate change to oxygen consumption change during exercise
I	
IBW	Ideal Body Weight
ICDSC	Intensive Care Delirium Screening Checklist
ICU	Intensive care unit
ICUAW	Intensive care unit acquired weakness
IL	Interleukine
IMS	ICU mobility scale
IMV	Invasive mechanical ventilation
IQR	Interquartile range
L	
LML	Log-minus-log
LMWH	Low-molecular weight heparin
LOESS	Local(ly weighted) regression lines
LOS	Length of stay

Abbreviation	Explanation
M	
MCS	Mental component subscore of the 36-item short form health survey
MIP	Maximal inspiratory pressure
MRC(-ss)	Medical research council (sum score)
MURCE	Muscle Research Centre Erlangen
MuRF	Muscle Ring Finger
MV	Mechanical ventilation
MVV	Maximal voluntary ventilation
Myf	Myogenic factor
MyHC	Myosin heavy chain
MYOD	Myoblast Determination protein
N	
NA	Not available/applicable
NF-κB	Nuclear Factor kappa B
NIV	Non-invasive ventilation
NMBA	Neuromuscular blocking agent(s)
NMD	Neuromuscular disease
NRS	Nutritional risk score
O	
O_2-pulse	Oxygen pulse
P	
PAD	Pain Agitation Delirium bundle
Pax7	Parired box protein 7
PCS	Physical component subscore of the 36-item short form health survey
PEEP	Positive end-expiratory pressure
PET	End-tidal partial pressure
PF-SF-36	Physical function of the 36-item short form health survey
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PICS	Post-intensive care syndrome
PN	Parenteral nutrition
PR	Partial residuals
R	
RCT	Randomized controlled trial
Rehab	Rehabilitation
REML	restricted maximum likelihood
RER	Respiratory exchange ratio
RMW	Respiratory muscle weakness
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
RRT	Renal Replacement Therapy
RT-PCR	Real Time – Polymerase Chain Reaction

Abbreviation	Explanation
S	
SAPSII	Simplified Acute Physiology Score II
SARS-CoV-2	Severe Acute Respiratory Syndrome – Coronavirus-2 (previously 2019-novel coronavirus or human coronavirus-2019)
SBPbase	Baseline systolic blood pressure
SBPpeak	Peak systolic blood pressure
SICU	Surgical intensive care unit
SF-36	36-item short form health survey/quality of life questionnaire, Medical Outcomes Report Short Form 36
SOFA	Sequential Organ Failure Assessment score
SpO_2	Oxygen saturation
T	
Tiff	Tiffeneau index
TLC	Total lung capacity
TNF	Tumor necrosis factor
TTM	Targeted temperature management
TV	Tidal volume
V	
VE	Minute ventilation
VEpeak/MVV	Ratio of peak minute ventilation to maximal voluntary ventilation, ventilator reserve
VC	Vital capacity
VCO_2	Carbon dioxide production rate
VE/VCO_2	Rate of ventilation versus rate of carbon dioxide production, ventilator equivalent of carbon dioxide
vif	variance inflation factor
VO_2	Oxygen consumption rate
VO_2AT	Oxygen consumption rate at anaerobic threshold
VO_2base	Baseline oxygen consumption rate
VO_2peak	peak oxygen consumption rate
VO2max	Maximal oxygen consumption rate
VO_2/WR ($\Delta VO_2/\Delta WR$)	Rate of change of oxygen consumption for change of work rate, metabolic efficacy for mechanical work
VE/VO_2	Rate of ventilation versus rate of oxygen consumption, ventilator equivalent of oxygen
V-slope (method)	inflection of the VCO_2 versus VO_2 slope
VT	Ventricular tachycardia
Y	
Y	Year

Medicine is the art of possibility and the science of probability

– Sir William Osler –

Chapter 1: General introduction

PROLONGED INTENSIVE CARE: TOO MUCH OF A GOOD THING?

Critical illness includes any medical or surgical condition that acutely requires vital organ support to sustain life. Care for patients who suffer from life-threatening conditions occurs at designated wards, termed intensive care units (ICUs), that are equipped with advanced treatment modalities and staffed with specialized health care workers. Continued advances in intensive care management allow bridging of progressively more complicated states of organ failure. In parallel, however, there is an increase in patients unable to recover promptly from the initial insult, consequently developing a prolonged dependency on intensive care [1, 2]. During their stay on an ICU, patients are vulnerable to several complications specific to this unique environment that may impede liberation from organ supportive treatments. One particularly disabling complication is intensive care unit-acquired weakness (ICUAW).

INTENSIVE CARE UNIT-ACQUIRED WEAKNESS: WHAT'S IN A NAME

The first medical account of intensive care unit-acquired weakness (ICUAW) is credited to Sir William Osler, who depicted the progressive loss of skeletal muscle function and mass occurring over the course of a variety of life-threatening conditions two centuries ago [3]. Presently, the term still refers to the striking clinical syndrome of *de novo* skeletal muscle weakness and wasting in critically ill patients that has no cause other than critical illness [4].

The incidence of ICUAW depends on the patient profile and clinical setting, increasing with illness severity and duration [5-10]. ICUAW complicates the ICU-trajectory in 50-75% of patients with a bloodstream infection [10, 11], and characteristic features of sepsis – including bacteremia, excessive inflammation and multiple-organ dysfunction – have been linked to the development of weakness [5, 9, 10, 12-14]. In patients suffering from respiratory dysfunction requiring invasive ventilatory support, the incidence of ICUAW ranges from 25 to 60% at awakening [15-18], depending on severity of the respiratory and concomitant organ failure. The likelihood of weakness increases with duration of mechanical ventilation [5, 19, 20] and, presumably, of associated treatments including neuromuscular blocking agents [7, 14, 21, 22], and corticosteroids [5, 14, 21-24]. In patients who had been mechanically ventilated for at least one week, ICUAW was still present in 35% at the time of ICU-[5] and even hospital discharge [25]. The prevalence of ICUAW in relation to illness factors appears to be further modulated by comorbidities and demographics. Increasing age [14, 21] is an independent risk factor of ICUAW, and premorbid chronic disease and frailty may carry an increased risk of ICUAW through their association with a more complicated disease course [26, 27], and possibly pre-existing neuromuscular disease. These data are concerning given the increasing prevalence of such demographics among patients admitted to the ICU [27-29].

ICUAW typically presents as a flaccid paresis of (lower) limb muscles, variably accompanied by loss of tendon reflexes [30-33]. In the ICU, bedside strength assessment can be done in a standardised semi-quantitative manner using the Medical Research Council sum score (MRC-ss) [34]. Isometric strength is manually tested in six muscle groups of the upper and lower limb bilaterally and scored from 0 to 5, with higher scores indicating better strength. Clinically relevant weakness in critically ill patients situates at values lower than a validated cut-off score of 48 out of a maximal score of 60 [4, 35]. This clinical significance comprises an increased duration of ICU- and hospital stay [5, 36-38], and hospital mortality [5, 7, 15, 16, 38-40] in weak patients. Prolonged mechanical ventilation plays an important role in the observed increase in short-term morbidity associated with ICUAW [41], and historically has been attributed to respiratory muscle involvement delaying the weaning process [41-44]. Recently, however, prospective research assessing both respiratory and limb muscle weakness found several discrepancies in risk factors and short-term outcomes between both entities [36, 45-50]. Arguably, these data suggest that respiratory muscle weakness may be a separate condition overlapping with ICUAW rather than a different manifestation of a single problem[46-50].

Lack of consensus with respect to the classification of ICU-acquired neuromuscular complications partly stems from their complex pathophysiology, which to this day remains incompletely understood. Animal models of critical illness have shown that combinations of immobilisation with mechanical ventilation, sepsis, and therapies including corticosteroids and parenteral nutrition can mimic pathological findings in patients with ICU-acquired neuromuscular dysfunctions [51, 52]. These comprise primary motor nerve axonal degeneration [30, 53], termed critical illness neuropathy (CIP), as well as a spectrum of myopathic alterations without implicit neural involvement referred to as critical illness myopathy (CIM), including amongst others myonecrosis, loss of myofibrillary proteins, and type II myofibre atrophy [54-57]. Clinical studies in critically ill patients prospectively assessed for neuromuscular impairments further added to the body of evidence, by illustrating the importance of cellular homeostasis in maintaining neuromuscular health, and revealing – sometimes unexpected – modulating effects of the ICU-management on these processes. The detrimental effect of uncountered stress hyperglycemia on the peripheral nervous system – presumably by direct neurotoxic effects – was alleviated by tight glycemic control [58]. Parenteral feeding, and timing of its initiation in particular, was shown to critically influence the risk of ICUAW [9, 14, 59], challenging the concept that early protein provision would prevent muscle wasting. Indeed, withholding parenteral nutrition reduced the incidence of ICUAW through improved cellular housekeeping - and thereby preservation of myofibre integrity - via the process of autophagy [14]. Other pathways appeared even more difficult to fathom. Modulation of endocrine regulation of trophic muscle state is practically challenging [60] and potentially harmful [61]. Interactions between the immune system, inflammation, and neuromuscular excitability remain poorly understood, and hence challenging to intervene upon [62]. Avoidance of unloading and mechanical silencing has been attempted in many ways. Electrical muscle stimulation has thus far provided conflicting results [63-65]. Early mobilization of ICU-patients appears feasible, safe, and effective in protecting and improving strength and short- and intermediate term-outcomes [25, 66-68], although results seemed variable and any observed benefit transient [69]. Again, timing appears crucial [70, 71]. Rehabilitation success is possibly further affected by treatment modality [72], prioritization and staff experience [73], and patient profile [25, 62], although trial heterogeneity impedes definite conclusions [69, 71].

Collectively, these data illustrate that the origin of ICUAW involves a complex and potentially highly variable interplay of critical illness, the host homeostatic reserve and response to the insult – appropriate or not – and iatrogenic interventions affecting their course. The resulting disruptions of cellular metabolism and housekeeping, microcirculation and electrical signaling, trophic state, and excessive inflammation translate into a spectrum of neuromuscular impairments including critical illness neuropathy, critical illness myopathy, and their overlap. A unifying pathophysiological pathway charting to what extent each of these neuromuscular dysfunctions may precede or affect the development of the other is still missing [74], as longitudinal studies are scarce and clinical distinction at the patient level is challenging.

Strength assessment in patients with neuropathy, myopathy or their overlap can reveal weakness, but is non-discriminative with respect to its origin. The neurophysiological pattern of axonal neuropathy and primary myopathy upon nerve conduction studies and electromyography are also similar, consisting of reduced compound motor action potentials with near normal nerve conduction velocity, and variable presence of positive sharp waves and fibrillation potentials [11, 12, 75]. Additional neurodiagnostic studies, including the evaluation of motor unit potentials (duration, amplitude, and number of phases, as well as recruitment-interference patterns), and – more recently – the differential responsiveness of muscle to direct or nerve evoked electrical stimulation [76-82], may differentiate between the two, or suggest overlap. However, these examinations are time-consuming, and either dependent on a patient's volitional contribution or experimental in nature, limiting their routine implementation. Exploitation of electrophysiological studies is further impeded by the lack of clear implications with respect to patient management.

Currently, there is no cure for ICU-acquired muscle dysfunctions. Prevention involves minimising exposure to risk factors, through early goal-directed therapy for sepsis, minimising sedation, tight glycemic control, withholding parenteral nutrition during the first week of critical illness, and early mobilisation [14, 21, 25, 67, 83-85]. Notwithstanding considerable effort, ICUAW remains highly prevalent, presumably affecting up to a million people annually worldwide [4].

POST-INTENSIVE CARE SYNDROME: THE BURDEN OF SURVIVORSHIP

The ongoing reduction in case-fatality rate of critical illness [1, 86] is an important achievement realised through improved general and disease- or syndrome-specific management, enabled by evidence-based protocols for goal-directed therapy including those mentioned earlier. Unfortunately, available longitudinal data present a rather bleak prospect of long-term health for patients surviving the ICU.

Mortality rates in ICU-survivors are increased relative to the general population and to non-critically ill hospitalized patients up to years after the ICU-stay. Importantly, and contrasting the evolution for the acute phase, this excess long-term mortality of critical illness survivors has changed remarkably little over the past two decades [87-91], notwithstanding increased notion of its importance [92]. This may indicate that targeted determinants of short-term survival are inappropriate surrogates for long-term mortality and health deterioration, or alternatively, that some treatments may present a long-term trade-off, possibly depending on illness or patient characteristics [93-102].

The possibility of the ICU-trajectory mortgaging long-term perspectives is particularly relevant for those who do remain alive against the odds, as survival does not imply recovery. It is becoming increasingly obvious that ICU-survivors suffer from a multitude of functional deficits [26, 103-106]. The observed variable constellations of acquired chronic organ dysfunctions, neuropsychological and cognitive impairments, and neuromuscular and musculoskeletal disorders are collectively referred to as the post-intensive care syndrome [107-109]. Up to 60% of ICU-survivors are affected, not seldom by multiple morbidities [110] that herald important implications across illness and patient profiles. In cohorts of survivors of acute respiratory failure due to the Acute Respiratory Distress Syndrome (ARDS), clinically relevant physical impairments have been documented up to five years after the index ICU-stay, even in those young and previously healthy [106, 111]. In elderly sepsis survivors, the odds of cognitive impairment after ICU-discharge were tripled, and accumulation of subsequent deficits was accelerated [112]. Failure to return to their premorbid health status and society participation, and loss of functional independence, amount to important reductions in quality of life consistently documented among survivors of critical illness [113].

The implementation of long-term functional outcome and quality of life [113, 114] as a target outcome in critical care RCT's has been slow [115] and, consequently, represents an unmet need in patient's perceptions [116]. Furthermore, post-ICU follow-up services providing rehabilitation have failed to achieve persistent improvement in acquired impairments [117]. Accrual of knowledge that might better focus resources to improve results is impeded by between-trial heterogeneity [117-121]. Additionally, insufficient awareness of the possible importance of rehabilitation in patients [122] and primary care givers [123-125] may hamper home-based continuation and possibly corroboration of results. As unemployment rates of previously working ICU-survivors vary between 33-50%, and annual health care costs per head exceed hundreds of thousands dollars, ICU-survivorship imposes an important personal and societal burden [104, 126-130], which is why it has been referred to as the defining challenge for critical care of the 21st century [131, 132].

LEGACY OF CRITICAL ILLNESS: KNOWLEDGE GAPS

Important knowledge gaps remain when considering the relationship between prognosis in long-term ICU-survivors, critical illness, and neuromuscular function – both during and after an ICU-stay.

Prolonged critical illness and long-term outcome

It is debated whether the legacy of critical illness is due to the critical illness and related treatments and events, or rather to the pre-morbid constitution of affected patients resulting in a reduced recovery potential. Indeed, older age [106, 111, 133-135], comorbidities [111, 135], and pre-ICU disabilities [29, 136, 137] not only predispose to a more adverse disease course [26, 27], they are also independent predictors of impaired post-ICU outcomes [29, 93, 136, 138]. Conversely, survivors with poor functional outcome tend to be older and more comorbid than those with better post-ICU outcomes [127]. Also, type and severity of illness upon admission are important forebodes of post-ICU health trajectories relative to the general population [93, 111, 138] and, more importantly, among critically ill patients [139-141]. Disentangling the impact of the ICU trajectory per se from confounding effects on long-term prognosis is challenging [142, 143], and insufficiently addressed in available research given the questionable comparability of an ICU-population preadmission to the general population or hospitalized non-ICU patients. Nonetheless, this question is of increasing relevance given the increased burden of negative prognostic demographics among critically ill patients [28]. Any added effect to the already jeopardised long-term outcomes of these patients should be avoided if possible, certainly if iatrogenic.

ICU-acquired neuromuscular dysfunctions and long-term outcome

The matter of whether ICU-acquired neuromuscular impairments directly translate into increased mortality and morbidity post-ICU is particularly difficult to unravel. Neuromuscular complications of critical illness are associated with increased mortality in the acute hospitalization phase [5, 15, 16, 36, 38, 144, 145], and ICUAW predicts increased mortality post-ICU up to 1 year [36, 146-148]. ICUAW can have both resolving and worsening trajectories in the first year after hospital discharge [149-153]. Although persisting weakness after hospital discharge heralds even worse outcome, the presence of weakness at the time of ICU-discharge independently relates with physical function and physical limitations up to 1 year [154, 155]. Data beyond this time frame are scarce, but in ARDS, this relationship appears to dissipate by 5 years of follow-up [111, 153]. With respect to electrophysiological abnormalities, an abnormal Compound Muscle Action Potential (CMAP) 1 week after ICU admission independently predicts one-year mortality [146]. Electrophysiological abnormalities have consistently been associated with short-term morbidities including prolonged duration of mechanical ventilation, ICU and hospital stay and physical impairments prior to hospital discharge [31, 144-146]. Whereas some studies have described persistent physical disability in the months to years post-ICU in relation to electrophysiological abnormalities [31, 156-158], others found evidence of resolving trajectories[146]. Should a negative impact of abnormal electrophysiology persist up to 5-years, prognostication would also be possible for patients unable to perform an MRC-assessment due to insufficient consciousness- and attention levels, and further incentives may be needed to evaluate the pathophysiological processes translating into aberrant electrophysiological behavior, as they remain incompletely understood.

When considering the long-term outcome of ICU-acquired respiratory muscle weakness, even more controversy exists. Due to its conceptual positioning as an integral component of ICUAW, prospective research assessing the post-ICU burden of respiratory muscle weakness per se is limited. Two case series with follow-up limited to 2 years presented conflicting results for mortality and little information on morbidity [159, 160]. Reduced respiratory muscle strength has been reported in long-term ARDS-survivors [151], and research in patients with cardiorespiratory conditions has shown that respiratory

muscle weakness and its rehabilitation affect exercise tolerance and capacity [161-163]. Should an independent effect of respiratory muscle weakness on long-term outcome be present, then inspiratory muscle training – a feasible technique [164] in ICU-patients – needs reassessment as an intervention to improve long-term prospects.

Resolving the aforementioned questions, and establishing an association between particular ICU-related treatments and complications, and a long-term health burden may help select patients at higher risk of adverse long-term outcomes to post-ICU follow-up clinics and to clinical trials aiming to reduce the long-term burden. Hypothetically, targeted provision of rehabilitation and psychosocial programmes may succeed where such interventions in unselected patient cohorts failed [121].

Multiple organ failure and long-term aerobic exercise capacity

Exercise intolerance is invariably documented in long-term survivors of critical illness, but quantitative data on cardiorespiratory fitness and qualitative assessment of the physiological response to exercise and its limiting factors are scarce. One case series of ARDS-survivors indicated impaired aerobic exercise capacity at 3 months, explained by neuromuscular limitation in 33% of patients [165]. It is noteworthy that multiple organ failure (MOF), an important risk factor for ICU-acquired neuromuscular dysfunctions [5-9, 166], has consistently been associated with adverse functional outcome [106, 111, 112] and with impaired functional quality of life [90, 104, 113, 167], but that, to date, this relationship has not been evaluated in terms of aerobic exercise capacity in cohorts of general long-term ICU-survivors. Hypothetically, impaired strength and physical function in ICU-survivors may reflect a lasting disruption of skeletal muscle homeostasis due to MOF. Possibly, this may include decreased metabolic responsiveness to increasing energy demands, which is an important determinant of aerobic exercise capacity [168]. This highly dynamic process is incompletely captured by static clinical or biochemical evaluations currently available in longitudinal cohorts of ICU-survivors [106, 112, 151, 169]. Characterisation of a patient's level of cardiorespiratory fitness and origin of exercise limitation may additionally be of value for tailored rehabilitation prescription.

Survivorship of critical illness by COVID-19

There is a particularly pressing need to resolve the knowledge gaps on the impact of a complicated ICU-stay on long-term health to ensure appropriate focus of research efforts, as the ongoing pandemic of the novel SARS-Cov2-coronavirus has induced a surge of intensive care unit admissions for ARDS [170], a condition known to be intimately linked with both a long-term health detriment and with ICUAW. Although early reports suggested a more favourable demographic profile of critically ill COVID-19 patients (high proportion of males [5, 171-173], obesity [173-175] and possibly a relatively young age distribution[172, 173]) as compared with the current demography of ARDS patients not affected by COVID-19, early advocacy of prolonged and deep sedation [176] and usage of corticosteroids [177] may be associated with a higher risk of ICU-acquired neuromuscular complications. The particular demographic and management characteristics of this patient population invalidate extrapolation of the incidence of ICUAW from historical ARDS-cohorts. Charting the ICU-profile of this patient population, and clarifying if any of their particular ICU-exposures may predispose to an (iatrogenic) long-term health detriment, will be crucial to guide post-ICU practice given possibly millions of people affected [178]. As the hypothetical loss of Quality Adjusted Life Years is substantial, research aimed at minimising this burden is of prime concern.

Molecular correlates of weakness in long-term ICU-survivors

Ultimately, mitigating the burden of neuromuscular impairments after an ICU-stay may require increased mechanistic understanding. Indeed, the mediators of impaired physical function in the long-term are basically unexplored.

Research on the molecular basis of long-term physical impairments is limited to a single case series, which suggests that mechanisms of muscle damage in the acute phase – including disruptions of proteolysis, autophagy and mitochondrial biogenesis – normalise, whereas disabled regeneration might be involved in long-term neuromuscular impairments [179, 180]. Whether these findings in 10 long-term survivors of prolonged mechanical ventilation are sufficient to direct further research incentives in all critical illness survivors is questionable.

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Chapter 2: Research hypothesis and objectives

The main research hypothesis of this PhD-project is that the rapid decline of muscle function during critical illness, inducing ICU-acquired neuromuscular complications, can persist beyond the ICU phase and independently contributes to the legacy of prolonged critical illness.

This hypothesis will be addressed in three key objectives throughout the following six chapters.

OBJECTIVE 1: TO STUDY WHETHER LONG-TERM MORTALITY AND MORBIDITY ARE A LEGACY OF CRITICAL ILLNESS

The first aim (1) was to investigate whether long-term morbidity and mortality are a legacy of prolonged critical illness. This comprised a staged analysis approach, as detailed in chapter 3 to 6.

In chapter 3 (1.1), we investigated whether a prolonged ICU-stay, with its associated exposures and complications, is independently associated with 5-year all-cause mortality and 5-year morbidity. Five-year morbidity was assessed in this and subsequent chapters by measures of strength, physical function, and quality-of-life that previously were shown to be affected in long-term ICU-survivors as part of the post-intensive care syndrome. These included hand-grip-strength, six-minute-walk-distance, and the physical function subscore of the 36-item short form health survey.

In chapter 4 (1.2), we investigated whether ICU-acquired neuromuscular dysfunctions associate with 5-year strength, physical function, quality of life, and 5-year mortality, independently from confounders including duration of ICU-stay. This research question addressed this relationship for ICU-acquired weakness, as well as for ICU-acquired electrophysiological abnormalities.

In chapter 5 (1.3), we investigated whether ICU-acquired respiratory muscle weakness per se contributes to the long-term adverse outcome of ICU-survivors, independent of duration of ICU-stay and of ICUAW. In addition to 5-year all-cause mortality and 5-year morbidity endpoints listed previously, the relationship between ICU-acquired respiratory muscle weakness and respiratory muscle strength at 5-year follow-up was evaluated.

In chapter 6 (1.4), we studied aerobic exercise capacity in long-term ICU-survivors with cardiopulmonary exercise testing. Our objectives for this study were to assess, first, whether cardiorespiratory fitness is impaired in ICU-survivors throughout long-term follow-up, second, whether aerobic capacity relates to organ failure severity during the ICU-stay, and third, which exercise limiting factors are present in patients with an abnormal aerobic exercise capacity and to what extent muscular impairments may contribute.

OBJECTIVE 2: TO STUDY THE EPIDEMIOLOGY AND SHORT-TERM BURDEN OF ICUAW IN CRITICALLY ILL COVID-19 PATIENTS

The second aim (2) was to explore the incidence, risk factors, and ICU- and hospital outcomes of ICUAW in critically ill COVID-19 patients, detailed in chapter 7.

OBJECTIVE 3: TO STUDY THE MOLECULAR CORRELATES OF WEAKNESS IN LONG-TERM SURVIVORS OF CRITICAL ILLNESS

The third aim (3) was to explore plausible mechanisms implicated in weakness in long-term ICU-survivors, via analysis of molecular and histological alterations in skeletal muscle that may hallmark physically impaired long-term ICU-survivors.

In chapter 8, we studied muscle biopsies collected 5 years after ICU admission with the aim of unravelling mechanisms potentially explaining the reduced strength 5 years after critical illness,

focusing on morphological muscular abnormalities and differential gene expression patterns known to contribute to intensive care unit acquired weakness.

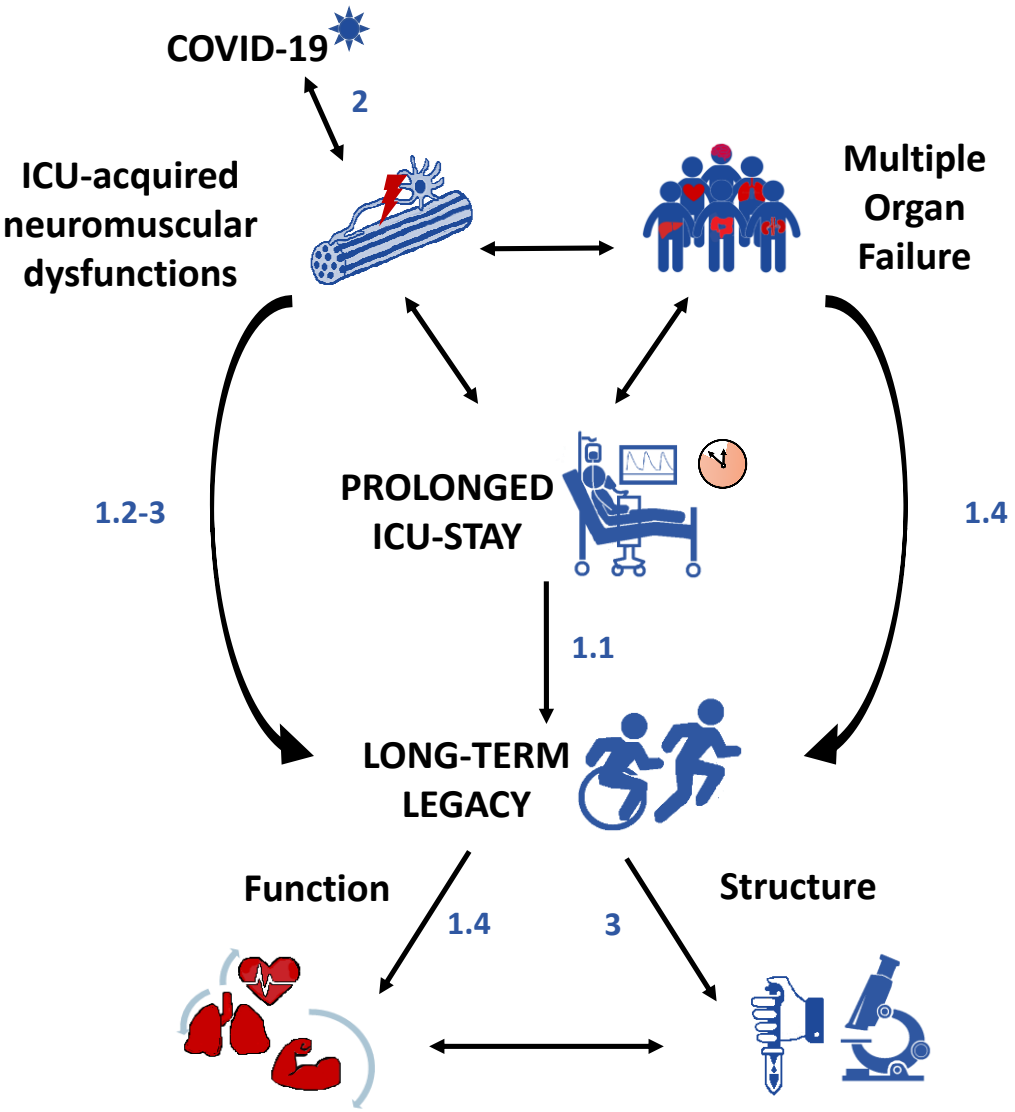

Fig. 1: Research questions and objectives (1 through 3) on the study of the long-term legacy of critical illness

Chapter 3: Five-year mortality and morbidity impact of a prolonged ICU stay

Adapted from:

Hermans G*, **Van Aerde N***, Meersseman P, Van Mechelen H, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Dubois J, Wouters PJ, Van den Berghe G. (2019) Five-year mortality and morbidity impact of prolonged versus brief ICU stay: a propensity score matched cohort study. Thorax 74: 1037-1045.

*Equally contributed

ABSTRACT

Purpose

Long-term outcomes of critical illness may be affected by duration of critical illness and intensive care. We aimed to investigate differences in mortality and morbidity after short (<eight days) and prolonged (≥eight days) ICU stay.

Methods

Former EPaNIC-trial patients (ClinicalTrials.gov:NCT00512122) were included in this pre-planned prospective cohort, five-year follow-up study. Mortality was assessed in all. For morbidity analyses, all long-stay and -for feasibility- a random sample (30%) of short-stay survivors were contacted. Primary outcomes were total and post-28-day five-year mortality. Secondary outcomes comprised handgrip strength (HGF, %pred), 6-minute-walking distance (6MWD, %pred) and SF-36 Physical Function score (PF-SF-36). One-to-one propensity-score matching of short- and long-stay patients was performed for nutritional strategy, demographics, co-morbidities, illness severity and admission diagnosis. Multivariable regression analyses were performed to explore ICU factors possibly explaining any post-ICU observed outcome differences.

Results

After matching, total and post-28-day five-year mortality were higher for long-stayers [48.2% (95% CI: 43.9%-52.6%) and 40.8% (95% CI: 36.4%-45.1%)] versus short-stayers [36.2% (95% CI: 32.4%-40.0%) and 29.7% (95% CI: 26.0%-33.5%), *P*<0.001]. ICU risk factors comprised hypoglycaemia, use of corticosteroids, neuromuscular blocking agents, benzodiazepines, mechanical ventilation, new dialysis, and the occurrence of new infection and liver dysfunction, whereas clonidine could be protective. Among 276 long-stay and 398 short-stay five-year survivors HGF, 6MWD and PF-SF-36 were significantly lower in long-stayers (matched subset HGF: 83% (95% CI: 60%-100%) versus 87% (95% CI: 73%-103%), *P*=0.020; 6MWD: 85% (95% CI: 69%-101%) versus 94% (95% CI: 76%-105%) *P*=0.005; PF-SF-36: 65 (95% CI: 35-90) versus 75 (95% CI: 55-90), *P*=0.002).

Conclusion

Longer duration of intensive care is associated with excess five-year mortality and morbidity, partially explained by potentially modifiable ICU factors.

INTRODUCTION

Critical illness can be defined as any acute, life-threatening condition requiring vital organ support in an intensive care unit (ICU) to avoid imminent death. While survival of the acute phase of critical illness has improved over the past decades [1], critical illness is associated with increased long-term mortality and morbidity [2-4], implicating major socio-economic impact [2, 4]. It is debated whether this so-called 'legacy of critical illness' is due to the critical illness itself and related ICU treatments and events, or rather to a frail premorbid constitution of these patients, predisposing them to ICU admission, and to the type and severity of illness necessitating ICU stay [5].
Several studies underlined the importance of the pre-morbid functional and health status, including age [2, 6-9], comorbidities [6, 9], frailty [10], pre-ICU disabilities, and functional trajectory [11, 12] as risk factors for increased long-term mortality and morbidity. In addition, type and severity of illness upon ICU admission [9, 13] predict outcomes. In contrast, it is challenging to quantify the actual contribution of critical illness and its treatments to long-term outcomes. This is because first, capturing the complex pre-morbid status and inherent susceptibility to ICU admission is virtually impossible and second, critically ill patients are fundamentally different from other hospitalized patients due to the severity of illness. Hence, the ideal control population is hard to define. Unravelling attributable mortality and morbidity of critical illness and its treatment is nonetheless crucial to guide the development of treatments to reduce this late burden of mortality and morbidity.
We hypothesized that a prolonged ICU stay, defined using a datadriven cut-off of an ICU stay of eight days or longer, and associated treatments contribute to the long-term burden. We compared five-year outcomes of patients with a prolonged ICU stay to those with a short ICU stay (less than eight days). By introducing another ICU population as a reference, we tried to adjust for the susceptibility for ICU admission and the overall severity of illness. To determine the excess five-year burden associated with prolonged ICU stay, we matched short- and long-stayers for randomization to early or late parenteral nutrition (PN), demographics, co-morbidities, type, and severity of illness upon ICU admission, hence rendering a group of patients comparable upon ICU admission. We further explored which interventions and treatments related to prolonged ICU stay could possibly explain any role of prolonged ICU stay on five-year outcomes.

METHODS

Ethics

The study protocol and informed consent forms were approved by the Leuven University Hospital Ethics Committee (ML4190). Patients gave separate informed consent for the five-year morbidity evaluations.

Study design and participants

This is a pre-planned prospective observational five-year follow-up study of patients included in the EPaNIC trial (Clinical trials.gov:NCT00512122) [14], an investigator-initiated randomized controlled trial conducted in 7 medical/surgical ICUs from the University Hospitals Leuven and Jessa Hospitals, examining early (≤ 48 hours) versus late (> 7 days) parenteral supplementation of deficient enteral nutrition. Study design, methodology, eligibility and primary outcomes have been reported [14]. On the ICU, standardized rehabilitation protocols were applied [15], however dose and duration of treatments sessions were not registered. Five-year mortality data were collected for all EPaNIC patients (N=4640). From June 2012 onwards, five-year survivors previously admitted to the Leuven Hospitals were invited to a follow-up clinic and recruited for the long-term morbidity follow-up study. All long-stay patients and, for feasibility purposes, a random subset of short-stay patients were eligible. The cut-off for defining prolonged ICU stay at eight days was based on the 75th percentile of duration of ICU stay. The subset of short-stay patients was a random, computer-generated sample

(3/10). To reduce selection bias, sampling was weighed within admission diagnostic categories to obtain a similar distribution as among long-stayers. Patients with pre-ICU neuromuscular disorders, unable to walk without assistance prior to ICU or other disabilities present before follow-up potentially confounding morbidity endpoints, refusing participation or not contactable were excluded (Supplementary Table 1). Home-visits were proposed to patients unable or declining a hospital visit. In parallel, as a healthy reference, individuals never admitted to the ICU were recruited from primary care givers' practices and outpatient clinics.

Outcomes

Primary endpoints were five-year all-cause mortality and post 28-day five-year mortality (further referred to as 'post-acute phase five-year mortality'), with day 0 defined as time of randomisation. This distinction was made as early (≤ 28 days) mortality, in contrast with post-acute phase mortality, is more impacted by illness severity [16]. The cut-off at > 28 days was defined by a fixed time-point, on the 75th percentile of duration of hospitalization. Mortality data were collected from the national registry and for foreigners, by hospital records or phone contacts.
Secondary endpoints comprised three distinct measures of clinical status including evaluation of muscle strength with handgrip strength (HGF, %pred), exercise capacity with 6-minute-walk distance (6MWD, %pred) and physical functioning with the Physical Function score of the SF-36 quality-of-life measure (PF-SF-36, range 0-100 with higher values indicating better scores) as well as additional physical and functional evaluations (details online supplement).

Statistics

Explanatory analyses of the association of prolonged ICU stay with five-year outcomes: Propensity score matching

To compare five-year outcomes between short- and long-stay patients, subsets of patients with short and prolonged ICU stay, matched for randomization to early or late PN, baseline risk factors (age, gender, BMI, nutritional risk score), co-morbidities (diabetes, malignancy, pre-admission dialysis), type of illness (cardiac surgery, emergency admission to surgical ICU, elective admission to surgical ICU, medical ICU and sepsis upon admission), presence or absence of sepsis and severity of illness (APACHE II) were selected. Matching was based on propensity scores obtained by logistic regression and using one-to-one nearest neighbour matching without replacement with prolonged ICU stay as the dependent variable. A calliper of 0.2 was used and satisfactory matching was obtained as indicated by an absolute standardized difference in means less than or equal to 0.1 for all variables. The distribution of propensity scores is provided in Supplementary Figure 3. Three matched sets of short- and long-stayers were created, respectively for comparison of the total and post-acute phase 5-year mortality as well as for morbidity analyses. Indeed, for each of these outcomes the available patient population was different (see Figure 1) and as such, we attempted optimal bias reduction in all analyses. For morbidity outcomes, patients were referenced to 50 controls, and hence a fourth subset was created of patients and controls, matched for demographics including age, sex, and BMI (see online supplement).
Outcomes of matched short-and long-stay patients were compared with Mann-Whitney U, chi-square, and Fisher exact test as appropriate. For completeness, also comparisons of outcomes between short- and long-stayers of the total patient samples for which mortality and morbidity data were available are provided. Differences were considered significant when two-sided *P*-values were 0.05 or less. For time-to-event analyses, comparisons for patients with short and prolonged ICU stay were performed with the log-rank test and visualized with Kaplan-Meier plots. Effect size was calculated with univariable Cox-proportional hazard regression analyses. Imputation of 6MWD was performed given meaningfull missingness, as explained in the online supplement. No further imputation for missing data was performed.

Exploratory analyses of factors possibly explaining the association of prolonged ICU stay with five-year outcomes

To explore which characteristics of the prolonged ICU stay may explain its possible adverse association with total, post-acute phase five-year mortality and with the three distinct measures of clinical status at five years, uni- and multivariable regression analyses were performed on all available data. These analyses included potentially modifiable ICU factors, such as treatments and exposures, which are amendable to intervention. Multivariable models were performed backward, probability to enter 0.05, removal 0.2. For time-to-event data, a Cox-regression model was applied. For each of the morbidity outcomes, linear regression models were applied, if necessary after appropriate transformation. Further details on these analyses are reported in the online supplement.
First, multivariable models were built, introducing prolonged ICU stay along with confounders comprising baseline risk factors (randomization, co-morbidities, type and severity of illness) that showed a *P*-value ≤ 0.2 with the outcome in univariable regression analyses. The 16 admission categories were grouped into four main categories for these analyses as done previously [15]. Next, another set of multivariable models were created in which prolonged ICU stay was replaced by ICU interventions and events that showed a *P*-value ≤0.2 in univariable analyses with the outcome studied. Continuous ICU variables were dichotomized on median values for the total population to provide sufficient overlap between short-and long-stay patients. Liver dysfunction was defined as bilirubin > 3 mg/dl [14]. Prior to entering these variables, collinearity was checked and judged problematic in case of variation inflation factor > 5 or tolerance < 0.2. Accuracy of these factors to discriminate prolonged ICU stay was evaluated with receiver operating characteristic (ROC) curve and c-statistic.
Bootstrapping (n=1000) was performed on the final multivariable models to obtain robust estimators of the confidence intervals for each of the regression coefficients.

Sensitivity analyses

To validate our definition of prolonged ICU stay, we evaluated the optimal cut-off for ICU stay to predict total five-year mortality, based on martingale residual plots with LOcal regrESSion (LOESS) lines [17]. At each step of the Cox-regression analyses, the proportional hazard assumption was checked for each variable retained in the model and sensitivity analyses were performed by adding the factors for which this assumption were violated as time-dependent covariates.

All analyses were performed with IBM SPSS-24 (IBM, Armonk, NY). Propensity score matching was performed with IBM SPSS-24 with a custom SPSS application [18] and R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics included median and interquartile ranges for continuous variables and numbers and percentages for categorical variables.

RESULTS

Patient subsets and characteristics

Five-year mortality data were available for 4619/4640 EPaNIC patients (99.6%), among whom 3410 were short- and 1209 were long-stayers (Figure 1). We created 964 matched pairs of short- and long-stayers, matching 79.7 % of long- and 28.2% of short-stayers. 4315/4619 patients survived the first 28 days of illness and were included in the post-acute phase five-year mortality analyses. Of these patients, 824 matched pairs were generated, matching 78.4% of long- and 33.8% of short-stayers. For morbidity analyses, 674 of 3014 eligible patients, including 398 short- and 276 long-stayers were recruited, as well as 50 controls. Reasons for exclusion are listed in Supplementary Table 1.

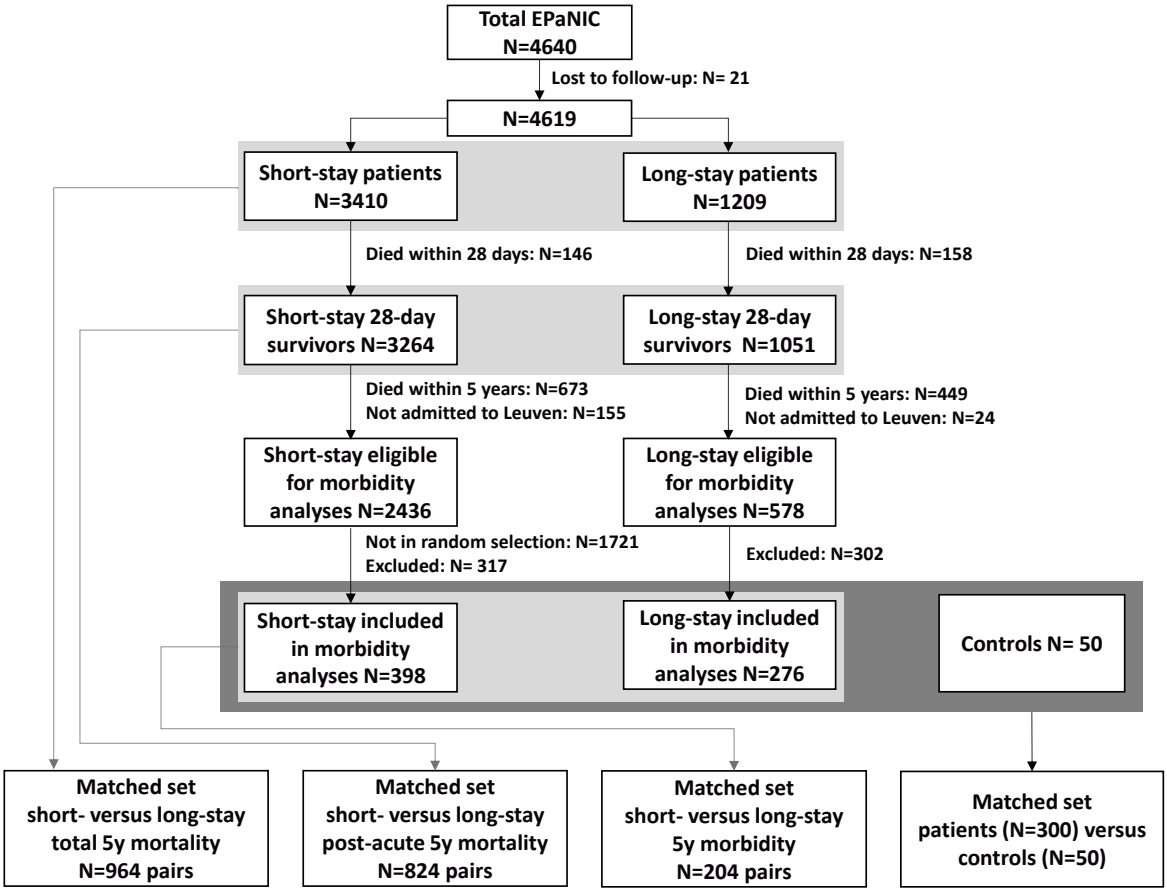

Fig.1: Flow chart of patients and controls and matched subsets for long-term mortality and morbidity analyses

Characteristics of in- and excluded patients are depicted in Table 1 and Supplementary Table 2. The subset of long-stayers included in the morbidity analyses was representative of the total population of long-stayers, whereas the subset of short-stayers included expectedly were sicker, had more co-morbidities and less frequently were cardiac admissions as compared to the short-stayers not included. We matched 300 patients to 50 controls and created 204 pairs of short- and long-stayers (representing 73.9% of evaluated long- and 51.2% of short-stayers). Patients with home visits were older, had more comorbidities and worse outcomes as compared to patients examined in the hospital (data not shown).

In both mortality end-points analyses and in the morbidity analyses, long-stay patients were younger, had a higher severity of illness, more frequently received early PN and had different admission diagnoses. Additional differences are highlighted in Supplementary Table 3 and 4. No residual imbalances remained in the matched sets (Table 1 and Supplementary Table 5).

Table 1: Baseline characteristics and ICU factors in short- versus long-stay patients included in the matched mortality and morbidity analyses

	All cause 5-year mortality analyses			All cause post-acute phase 5-year mortality analyses			Morbidity analyses		
	Matched population N= 1928			Matched population N= 1648			Matched population N= 408		
	Short-stay N= 964	Long-stay N= 964	*P*-value	Short-stay N= 824	Long-stay N= 824	*P*-value	Short-stay N=204	Long-stay N=204	*P*-value
Baseline factors									
Age, median (IQR)	64.9 (53.5-74.3)	64.4 (53.6-74.8)	0.892	64.5 (54.3-73.5)	63.4 (51.7-74)	0.240	60.4 (50.7-68.2)	58.4 (49.1-70.1)	0.820
Sex, male N (%)	608 (63.1)	605 (62.8)	0.888	531 (64.4)	518 (62.9)	0.506	145 (71.1)	140 (68.6)	0.590
BMI, median (IQR)	25 (22.5-28.1)	25.2 (22.6-28.9)	0.181	25.4 (22.8-28.6)	25.2 (22. .6-289)	0.895	25.6 (23.2-28.3)	25.7 (22.8-29)	0.927
NRS ≥ 5, N (%)	269 (27.9)	265 (27.5)	0.839	217 (26.3)	211 (25.6)	0.736	40 (19.6)	41 (20.1)	0.901
Diabetes mellitus, N (%)	164 (17)	157 (16.3)	0.669	132 (16)	127 (15.4)	0.735	31 (15.2)	33 (16.2)	0.785
Malignancy, N (%)	220 (22.8)	222 (23)	0.914	175 (21.2)	181 (22)	0.719	28 (13.7)	34 (16.7)	0.408
Pre-admission dialysis, N (%)	21 (2.2)	19 (2)	0.749	13 (1.6)	17 (2.1)	0.461	0	0	NA
Randomisation, late PN, N (%)	470 (48.8)	469 (48.7)	0.964	401 (48.7)	395 (47.9)	0.767	101 (49.5)	97 (47.5)	0.692
APACHE II, median (IQR)	31 (22-36)	30 (22-36)	0.930	30 (21-35)	29.5 (22-35)	0.773	28 (18-33)	27.5 (20-34)	0.588
Admission category, N (%)			0.811			0.802			0.968
Cardiac surgery	355 (36.8)	335 (34.8)		311 (37.7)	297 (36)		82 (40.2)	80 (39.2)	
Emergency SICU	429 (44.5)	442 (45.9)		363 (44.1)	383 (46.5)		102 (50)	103 (50.5)	
Elective SICU	53 (5.5)	53 (5.5)		51 (6.2)	48 (5.8)		9 (4.4)	8 (3.9)	
MICU	127 (13.2)	134 (13.9)		99 (12)	96 (11.7)		11 (5.4)	13 (6.4)	
Sepsis upon admission, N (%)	334 (34.6)	365 (37.9)	0.142	286 (34.7)	289 (35.1)	0.877	50 (24.5)	53 (26)	0.732

Continued Table 1: Baseline characteristics and ICU factors in short- versus long-stay patients included in the matched mortality and morbidity analyses

	Matched population N= 1928			Matched population N= 1648			Matched population N= 408		
	Short-stay N= 964	Long-stay N= 964	*P*-value	Short-stay N= 824	Long-stay N= 824	*P*-value	Short-stay N=204	Long-stay N=204	*P*-value
ICU-related exposure variables									
Mean morning glycaemia > 103 mg/dl	489 (51.3)	517 (53.6)	0.298	409 (50)	444 (53.9)	0.115	107 (52.5)	114 (55.9)	0.487
Mean insulin dose > 43.43 U/d	462 (47.9)	679 (70.4)	<0.001	401 (48.7)	574 (69.7)	<0.001	102 (50)	155 (76)	<0.001
Hypoglycaemia during intervention[a]	26 (2.7)	50 (5.2)	0.005	18 (2.2)	36 (4.4)	0.013	1 (0.5)	8 (3.9)	0.037
Corticosteroids	339 (35.2)	476 (49.4)	<0.001	260 (31.6)	380 (46.1)	<0.001	69 (33.8)	84 (41.2)	0.125
NMBA	107 (11.1)	532 (55.2)	<0.001	61 (7.4)	448 (54.4)	<0.001	16 (7.8)	113 (55.4)	<0.001
Benzodiazepines > 1 day	307 (31.8)	841 (87.2)	<0.001	267 (32.4)	712 (86.4)	<0.001	67 (32.8)	177 (86.8)	<0.001
Opioids > 3 days	319 (33.1)	885 (91.8)	<0.001	277 (33.6)	759 (92.1)	<0.001	71 (34.8)	193 (94.6)	<0.001
Propofol > 1 day	406 (42.1)	793 (82.3)	<0.001	360 (43.7)	681 (82.6)	<0.001	90 (44.1)	180 (88.2)	<0.001
Clonidine	26 (2.7)	185 (19.2)	<0.001	25 (3)	171 (20.8)	<0.001	4 (2)	53 (26)	<0.001
Ketamine	6 (0.6)	37 (3.8)	<0.001	5 (0.6)	31 (3.8)	<0.001	3 (1.5)	9 (4.4)	0.079
Mechanical ventilation > 2 days	337 (35)	891 (92.4)	<0.001	276 (33.5)	763 (92.6)	<0.001	68 (33.3)	191 (93.6)	<0.001
Vasopressors/ inotropes > 2 days	319 (33.1)	799 (82.9)	<0.001	261 (31.7)	676 (82)	<0.001	67 (32.8)	162 (79.4)	<0.001
Bilirubin>3 mg/dl	151 (15.7)	290 (30.1)	<0.001	111 (13.5)	225 (27.3)	<0.001	21 (10.3)	57 (27.9)	<0.001
New dialysis	39 (4)	208 (21.6)	<0.001	13 (1.6)	157 (19.1)	<0.001	1 (0.5)	31 (15.2)	<0.001
New infection	84 (8.7)	736 (76.3)	<0.001	69 (8.4)	630 (76.5)	<0.001	13 (6.4)	157 (77)	<0.001

[a]Intervention involved early (within 48h) versus late (not within the first week) parenteral substitution of deficient enteral nutrition

Abbreviations: *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *SICU:* Surgical Intensive Care Unit; *MICU:* Medical Intensive Care Unit; *NMBA:* neuromuscular blocking agents.

Primary outcomes: total and post-acute phase five-year mortality

In the matched analysis, total five-year mortality was higher in long-stayers as compared to short-stayers [48.2% (95% CI: 43.9%-52.6%) versus 36.2% (95% CI: 32.4%-40.0%), *P* < 0.001], rate differences are reported in Figure 2. As compared to short-stayers, long-stayers experienced a higher likelihood of death during the five-year follow-up (HR: 1.447 [95%CI: 1.286-1.697], *P* < 0.001) (Figure 2).
Post-acute phase five-year mortality in matched patients was higher in long-stayers as compared to short-stayers [40.8% (95% CI: 36.4%-45.1%) versus 29.7% (95% CI: 26.0%-33.5%), *P* < 0.001]. Long-stayers experienced a higher likelihood of late death than short-stayers (1.556 [1.320-1.834], *P* < 0.001) (Figure 2).

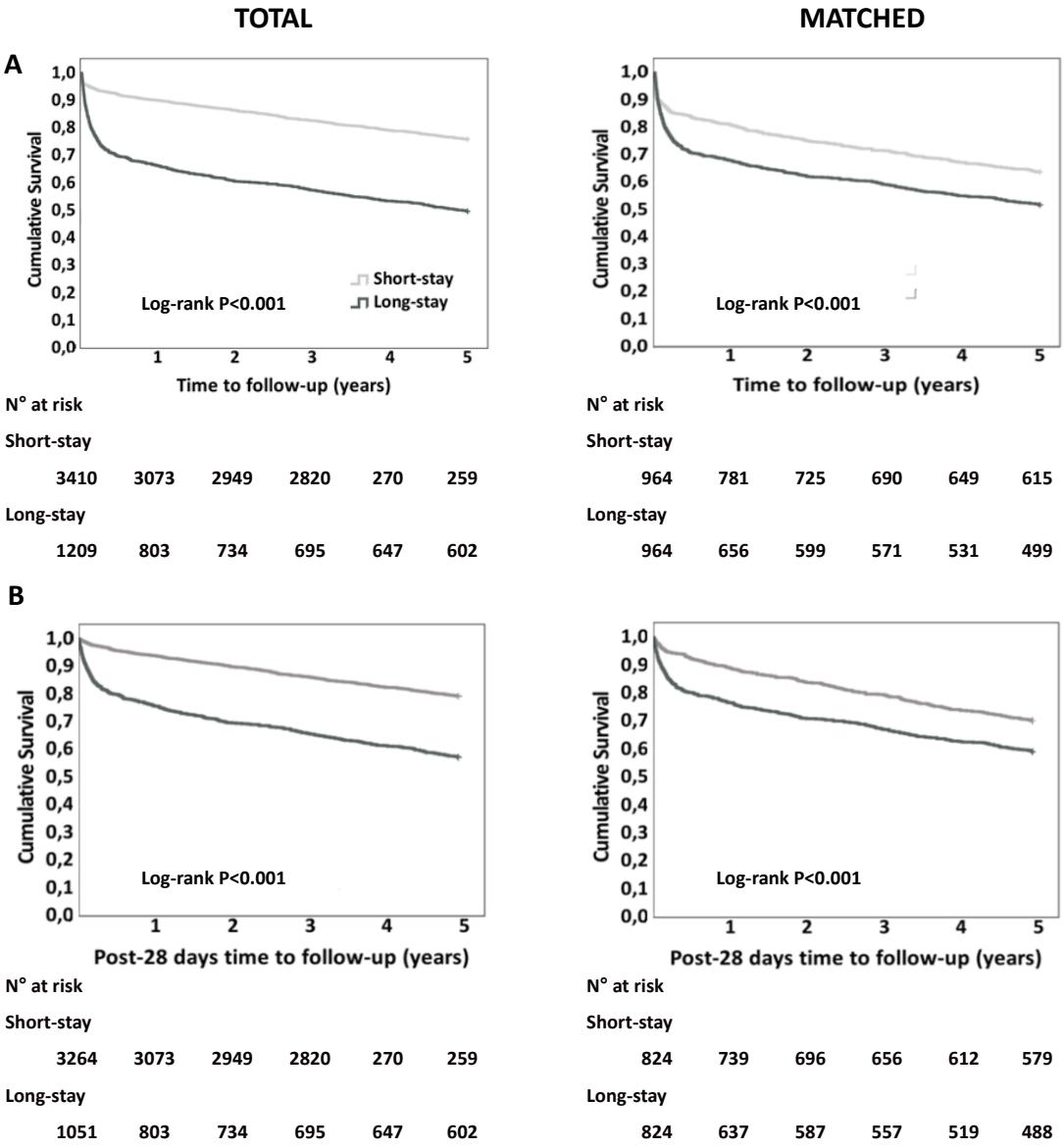

Fig.2: Kaplan Meier survival curves for total (panel a) and post-acute phase 5-year mortality (panel b) in the total and matched subset of patients with short (less than 8 days) and prolonged (8 days or more) ICU stay. Restricted mean survival times for matched patients in the total 5-year mortality analyses are 1134 days (95% CI: 1084-1185 days) for long-stayers, 1353 days (1308-1398 days) for short-stayers, difference -219 days (95% CI: -287 to -151 days), *P* < 0.001. Restricted mean survival times for matched patients in the post-acute phase 5-year mortality analyses are 1293 days (95% CI: 1243-1343 days) for long-stayers, 1503 days (95% CI: 1463-1543 days) for short-stayers, difference -209 days (95% CI: -274 to -146 days), *P* < 0.001.

Secondary outcomes: five-year morbidity

To evaluate how prolonged ICU stay associates with long-term morbidity, five-year survivors were evaluated at a mean 5.5 ± 0.2 years following ICU admission.
In the matched subset, long-stayers demonstrated more impairment in muscle strength, more activity limitation and worse self-reported physical functioning. This is demonstrated by lower handgrip strength: 83% (95% CI: 60%-100%) versus 87% (95% CI: 73%-103%), *P* = 0.019, 6MWD: 85% (95% CI: 69%-101%) versus 94% (95% CI: 76%-105%), *P*=0.005 and PF-SF-36: 65 (95% CI: 35-90) versus 75 (95% CI: 55-90), *P* = 0.002 (Fig.3, Supplementary Table 6). No difference in MRC sum score was present in the matched subset, which is not unexpected given the ceiling effect of this test [7]. Hand-held dynamometry indicated lower hip and ankle strength, and inspiratory muscle strength was reduced in matched long-stayer as compared to short-stayers. Long-stayers performed worse in daily life activities and rated their overall physical health inferior as compared to short-stayers (Supplementary Table 6).

Exploratory analyses

Five-year mortality

The ICU exposures and events explored to explain the detrimental impact of prolonged ICU stay on outcomes were highly discriminative for prolonged ICU stay (AUC = 0.968, Supplementary Figure 1).
Multivariable analyses adjusting for potential confounders showed that new dialysis (1.528 [95% CI: 1.289-1.812], *P* < 0.001), new infection (1.207 [95% CI: 1.050-1.387], *P* = 0.008), any use of corticosteroids (1.534 [95% CI: 1.348-1.745], *P* < 0.001), benzodiazepines > 1 day (1.195 [95% CI: 1.036-1.379], *P* = 0.015), hypoglycaemia (1.361 [95% CI: 1.068-1.733], *P* = 0.013), liver dysfunction (1.166 [95% CI: 1.012-1.343], *P* = 0.034), mechanical ventilation > 2 days (1.210 [95% CI: 1.034-1.417], *P* = 0.018), and any use of neuromuscular blocking agents (1.222 [95% CI: 1.054-1.417], *P* = 0.008) were associated with the excess total five-year mortality, whereas clonidine use was associated with improved outcome (0.792 [95% CI: 0.649-0.965], *P* = 0.021) (Table 2).
Excess post-acute phase 5-year mortality was possibly explained by use of new dialysis (1.474 [95% CI: 1.213-1.791], *P* < 0.001), occurrence of new infection (1.434 [95% CI: 1.228-1.676], *P* < 0.001), any use of corticosteroids (1.697 [95% CI: 1.466-1.964], *P* < 0.001), and use of benzodiazepines > 1 day (1.206 [95% CI: 1.038-1.401], *P* = 0.014) (Table 2).

Five year morbidity

Multivariable regression analyses identified benzodiazepines (HGF and PF-SF-36), vasopressors (PF-SF-36), and opioids (6MWD) as ICU exposure variables, possibly explaining increased morbidity five-years following ICU admission in long-stayers (Supplementary Table 7). Former ICU patients performed worse on all measured outcomes as compared to controls who never experienced critical illness. (Supplementary Table 6, Figure 3).

Sensitivity analyses

Sensitivity analyses accounting for the change over time of hazards ratios for those variables in the Cox proportional hazards models violating the proportional hazard assumption, implicating that the association of these variables with mortality changed within the five-year observation period, did not alter these conclusions (data not shown).
Post-hoc analyses of the optimal cut-off to define short-and long-stay patients suggests a break-point at six to eight days, supporting the *a priori* selected cut-off of eight days (Supplementary Figure 2).

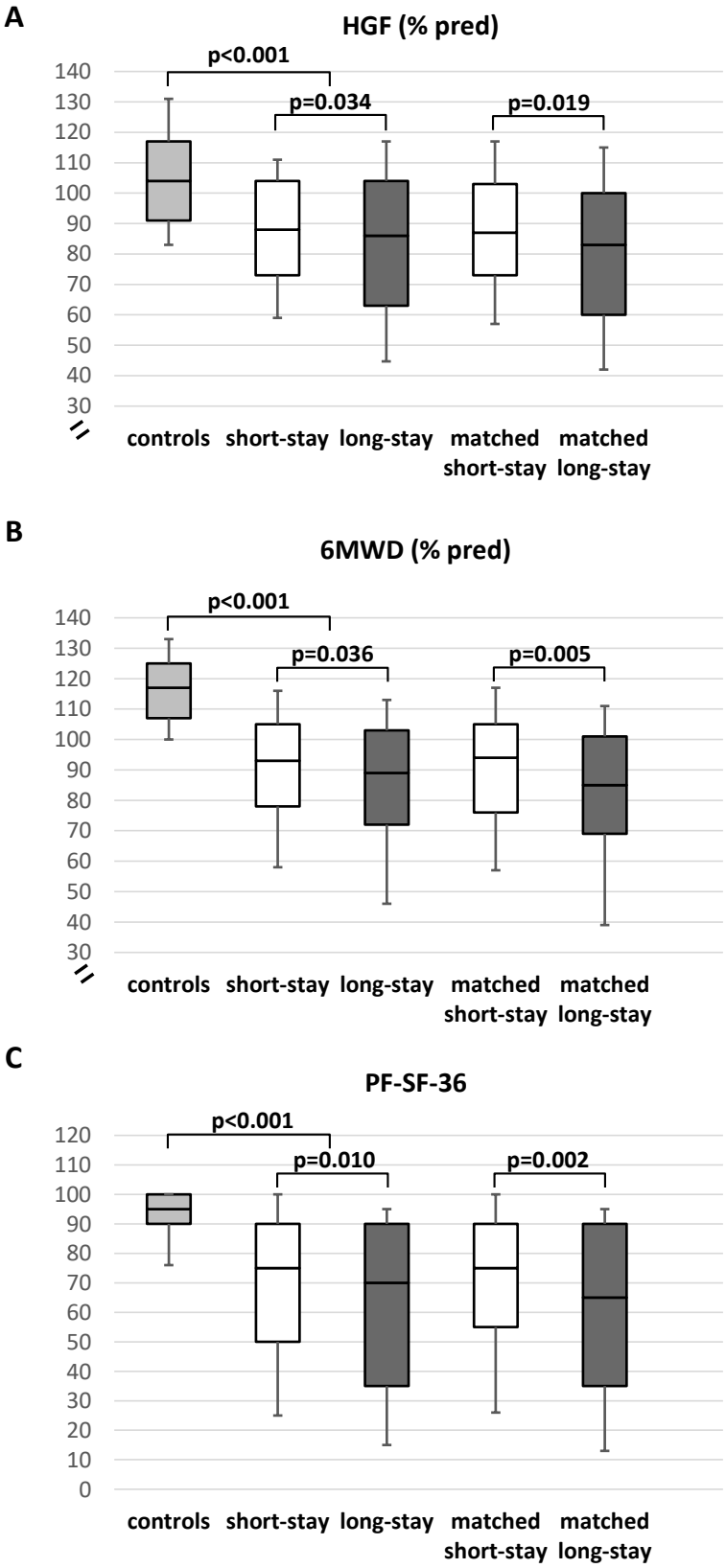

Fig.3: Boxplots of morbidity for short-and long-stay patients (white versus dark grey plots, respectively) referenced to controls (light grey plots). Panel A: Handgrip strength as percentage predicted. Panel B: 6-minute-walk distance as percentage predicted. Panel C: Physical Function of the Short Form 36 questionnaire. Whiskers represent percentiles 10 and 90. Comparisons were performed with Mann-Whitney U test.

Table 2. Multivariable Cox-regression analyses for total 5-year mortality and post-acute phase 5-year mortality

	5-year mortality		Post-acute phase 5-year mortality	
	HR (95%BCaCI)	***P*-value**	**HR (95%BCaCI)**	***P*-value**
Baseline factors and prolonged ICU stay				
Age	1.038 (1.033-1.045)	0.001	1.042 (1.037-1.049)	0.001
BMI other than 25-40	1.262 (1.131-1.415)	0.001	1.319 (1.166-1.480)	0.001
NRS≥5	1.193 (1.050-1.346)	0.009	1.208 (1.024-1.407)	0.013
Diabetes mellitus	1.245 (1.057-1.438)	0.002	1.262 (1.065-1.488)	0.004
Malignancy	1.641 (1.429-1.877)	0.001	1.895 (1.636-2.230)	0.001
Pre-admission dialysis	2.454 (1.799-3.370)	0.001	2.861 (2.070-4.018)	0.001
APACHE II	1.031 (1.023-1.040)	0.001	1.017 (1.008-1.027)	0.001
Admission category (relative to cardiac surgery)				
Emergency SICU	1.439 (1.193-1.798)	0.001	1.590 (1.296-1.965)	0.001
Elective SICU	3.296 (2.655-4.068)	0.001	3.600 (2.886-4.533)	0.001
MICU	2.344 (1.863-2.992)	0.001	2.527 (1.953-3.270)	0.001
Sepsis upon admission	1.106 (0.943-1.294)	0.180	NA	NA
ICU stay, prolonged	1.596 (1.381-1.850)	0.001	1.851 (1.578-2.167)	0.001
Baseline factors and ICU factors				
Age	1.042 (1.036-1.048)	0.001	1.046 (1.040-1.052)	0.001
BMI other than 25-40	1.248 (1.109-1.427)	0.001	1.297 (1.150-1.488)	0.001
NRS≥5	1.165 (1.014-1.332)	0.023	1.182 (0.986-1.420)	0.048
Diabetes mellitus	1.278 (1.094-1.497)	0.001	1.320 (1.135-1.569)	0.001
Malignancy	1.767 (1.543-2.004)	0.001	2.030 (1.736-2.421)	0.001
Pre-admission dialysis	2.702 (1.914-3.777)	0.001	2.987 (2.182-4.093)	0.001
APACHE II	1.013 (1.003-1.023)	0.009	NA	NA
Admission category (relative to cardiac surgery)				
Emergency SICU	1.572 (1.298-1.856)	0.001	1.741 (1.445-2.066)	0.001
Elective SICU	3.314 (2.742-4.101)	0.001	3.675 (3.033-4.456)	0.001
MICU	2.527 (1.985-3.178)	0.001	2.683 (2.039-3.465)	0.001
Mean insulin dose >43.43 U/day	0.918 (0.813-1.053)	0.163	0.899 (0.786-1.047)	0.119
Hypoglycaemia during intervention[a]	1.361 (1.019-1.774)	0.027	1.235 (0.839-1.716)	0.225
Corticosteroids	1.534 (1.334-1.771)	0.001	1.698 (1.437-2.043)	0.001
NMBA	1.222 (1.051-1.446)	0.014	NA	NA
Benzodiazepines > 1 day	1.195 (1.026-1.382)	0.020	1.194 (1.036-1.387)	0.019
Propofol > 1 day	NA	NA	1.141 (0.974-1.310)	0.073
Clonidine	0.792 (0.637-0.985)	0.047	NA	NA

Continued Table 2. Multivariable Cox-regression analyses for total 5-year mortality and post-acute phase 5-year mortality

	5-year mortality		Post-acute phase 5-year mortality	
	HR (95%BCaCI)	***P*-value**	**HR (95%BCaCI)**	***P*-value**
Baseline factors and ICU factors				
Mechanical ventilation > 2 days	1.210 (1.029-1.416)	0.030	NA	NA
Vasopressors/ inotropes > 2 days	NA	NA	1.119 (0.962-1.293)	0.148
Bilirubin >3 mg/dl	1.166 (0.986-1.387)	0.066	NA	NA
New dialysis	1.528 (1.232-1.919)	0.002	1.472 (1.131-1.947)	0.004
New infection	1.207 (1.050-1.417)	0.017	1.429 (1.211-1.705)	0.001

[a]Intervention involved early (within 48h) versus late (not within the first week) parenteral substitution of deficient enteral nutrition

Abbreviations: *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *SICU:* Surgical Intensive Care Unit; *MICU:* Medical Intensive Care Unit; *NMBA:* neuromuscular blocking agents; *NA:* not applicable; *BCa:* bias-corrected accelerated confidence intervals obtained by bootstrap sample procedure (n=1000).

DISCUSSON

In this follow-up study of former EPaNIC patients, total and post-acute phase five-year mortality was higher among long-stayers as compared to short-stayers. Increased mortality could not entirely be explained by differences in demographics, co-morbidities, type, and severity of illness. Furthermore, long-stayers who did survive five years as compared to short-stayers had worse functional status, including decreased muscle strength, activity limitation, and reduced physical functioning, again not entirely explained by differences upon ICU admission. Former ICU patients clearly performed worse than matched controls. These data support that prolonged critical illness and associated exposure to the ICU environment itself may contribute to long-term mortality and morbidity, and hence, to the so-called 'legacy' of critical illness.

As compared with short-stayers who were comparable to long-stayers upon ICU admission following careful matching, long-stay patients had an absolute 44.7% and 55.6% increase in respectively total and post-acute phase five-year mortality. Furthermore, in those who did survive five years, prolonged ICU stay as compared to short ICU stay increased the burden of morbidity, not explained by the aforementioned baseline confounders. Importantly, we consistently identified across both mortality endpoints, several ICU-related exposures that could possibly explain the excess long-term mortality, independent from co-morbidities, severity, and type of illness upon ICU admission. These included new dialysis and infection, and any treatment with corticosteroids or >1 day of benzodiazepines. Several ICU exposures were identified possibly explaining the association of prolonged ICU stay with long-term morbidity, including use of benzodiazepine, vasopressors, and opioids. As several of these factors may be modifiable, the excess mortality and morbidity in prolonged ICU stay may be, to a certain extent, amendable. These analyses should be considered hypothesis-generating and need further confirmation.

Our findings, demonstrating high total and post-acute phase five-year mortality in patients with prolonged ICU stay, are consistent with previously reported high five-year mortality in critically ill patients as compared with hospitalized controls[4], further increasing with longer ICU stay [19, 20]. Our five-year morbidity data also align with other work indicating reduced physical function, and quality of life in specific sub-populations of mainly ARDS survivors as compared to healthy peers or reference values [2, 6]. In these studies, duration of ICU stay [7], or indicators hereof, such as prolonged mechanical ventilation [8], and duration of immobilization, were identified as risk factors. Our study further advances current literature by studying morbidity in a general and heterogeneous population of critically ill patients. Our data do not contradict nor refute the importance of pre-morbid factors on outcomes, but extend on this knowledge by the comparison of matched short-and long-stayers, indicating that about one out of three five-year deaths in prolonged ICU stay and a small but consistent reduction in physical outcomes including handgrip strength, 6MWD and PF-SF-36, are incremental to any baseline risk. The identification of exposures during ICU stay that may explain these adverse long-term outcomes, further offers the opportunity to avert poor outcomes rather than resigning on the poor prognostic value of prolonged ICU stay.

Our study has several strengths. First, it is to date and to the best of our knowledge, the largest study on long-term mortality and morbidity following critical illness in a general population. Second, we used a population previously included in a randomized trial, from which baseline and ICU characteristics were carefully and prospectively documented. Third, although we cannot definitely prove the causal relationship between prolonged ICU stay and the observed burden, by comparing this long ICU-stay population with matched, hence comparable, short ICU-stayers, this appears to be the best possible attempt disentangling this issue. We chose this propensity score matching method, and accordingly prospectively recruited short-and long-stayers, because it is more effectively reduces bias than multivariable regression analysis [21-23]. As the long-stayers who remained unmatched had higher severity of illness and even worse outcomes than those who were matched (data not shown),

propensity score matching represents a conservative approach to assess the long-term burden of prolonged critical illness.

Our study has some limitations. First, we chose to define prolonged ICU stay from day eight onwards, coinciding with the 75th percentile of ICU stay. Different cut-offs, such as more than ten[16, 20] or more than 14 days [24] were previously used. Post-hoc analyses of the relationship between duration of ICU stay and mortality supported our choice as we found a breaking point after six to eight days. Second, consistent with other reports [13], we pragmatically defined post-acute phase mortality from day 28 onwards, a fixed time point. Persistent critical illness, defined as the time-point beyond which outcome ceases to be determined by illness severity and diagnosis upon admission [16] likely is individually variable. Nevertheless, we found that 'post-acute phase' mortality was no longer dependent of illness severity upon admission or admission diagnosis, such as sepsis. Therefore, this time point appeared to be a sensible cut-off within our population. Third, though we attempted to adjust for pre-morbid factors, as for any matched cohort studies, we cannot exclude unmeasured confounding, which may lead to overestimation of attributable morbidity and mortality of long-stayers. Fourth, we did not include SF-36 data and Barthel-index upon ICU admission in the matching as these data may have been flawed by being collected post-hoc [25]. Nevertheless, our data indicate that prior to admission, if anything, long-stayers had better premorbid physical function than short-stayers. Fifth, during EPaNIC, dexmedetomidine was not available on the Belgian market but has currently largely replaced and expanded the use of clonidine. Sixth, some identified ICU exposures may be unavoidable in specific situations. Hence, attributable mortality and morbidity of prolonged ICU stay may not be entirely preventable. Seventh, for feasibility purposes, only 30% of short-stayers were contacted for the morbidity follow-up. The short-stayers included were a computer-generated sample, randomly selected to match with the diagnostic categories of the long-stayers and consequently included less cardiac patients, were sicker and had more co-morbidities than short-stayers who were not included, whereas the long-stayers included were representative for all long-stayers**.** Finally, external validity may be limited by the exclusion criteria of the randomized trial from which all patients were drawn. Nevertheless, the trial population does reflect a severely ill patient group (mean APACHE 23 ±10) and general population. Of note, other post-ICU cohort studies have also built on clinical trial populations [7, 26-29]. Morbidity analyses evidently were limited to survivors and additional exclusion criteria, including disabilities potentially confounding morbidity endpoints were applied in the follow-up study, which may have introduced selection bias.

In conclusion, five-year mortality and morbidity was higher among long-stayers than among short-stayers, a difference that was supplementary to any baseline pre-morbid vulnerability for ICU admission, and to type and severity of illness necessitating critical care. Identified ICU-related exposures may offer opportunities to reduce the long-term burden of prolonged ICU stay.

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SUPPLEMENTARY MATERIALS

Supplementary methods

Outcomes

Secondary endpoints comprised three distinct measures of clinical status reported to be impaired in survivors up to years following ICU admission [1-4], including evaluation of muscle strength with handgrip strength (HGF, %pred) [5], exercise capacity with 6-minute-walk distance (6MWD, %pred) [6], indicative for activity limitation and physical functioning with the Physical Function score of the SF-36 quality of life measure (PF-SF-36, range 0-100 with higher values indicating better scores) [7, 8].
To further characterize physical and functional limitations, additional measurements were performed. These comprised global assessment of muscle strength with the MRC sum score (range 0-60, higher values indicating higher strength), isometric muscle strength with hand held dynamometry for the same muscle groups as included in the MRC evaluation, maximal inspiratory pressure, the physical and mental component score of the SF-36, representing the respective health summaries of the questionnaire (PCS and MCS, range 0-100 with higher values indicating better scores) and the Barthel-index, a scale to measure performance in daily life activities (range 0-20, lower values indicating more disability) [9].

Handgrip strength

Handgrip strength was measured with a hydraulic handgrip dynamometer (Jamar Preston, Jackson, Michigan, USA) as previously described [10]. The dynamometer was regularly calibrated. Measurements were performed by the same four experienced physiotherapists (TVH, SV, TVA, HVM). Measurements were standardly performed on the right side. In case of focal or regional problems for certain muscle groups, evaluation was performed on the contralateral side. Care was taken to perform measurements with the elbow in 90 degrees flexion. Handgrip strength was determined as the highest of 3 attempts. Values were expressed as percent of predicted values for sex and age [5].

6-minute walk distance

The 6-minute walking distance (6MWD) was performed in a 30-meter corridor or on the side-walk during home visits, according to the ATS guidelines [11]. Results were expressed as percent predicted [6]. For patients unable to perform the test due to physical limitations, a zero value was imputed as performed in earlier work [12, 13].

Quality of life questionnaire

The quality of life was assessed with The Medical Outcomes Report – Short Form 36 (SF-36) questionnaire [7, 8]. The SF-36 includes 8 scores, consisting of multiple items, that assess 8 domains, including physical and social functioning, physical and emotional role, mental health, pain, vitality and general health. In addition, 2 summary scores, physical component summary (PCS) and mental component summary (MCS) are calculated. Scores range from 0 – 100 with lower scores indicating more disability and higher scores indicating less disability.

Medical Research Council sum score

MRC sum score was measured as described earlier [10]. Six muscle groups were evaluated (abduction of the shoulder, flexion of the elbow, extension of the wrist, flexion of the hip, extension of the knee and dorsal flexion of the foot) bilaterally and scored between 0 and 5 (0 = no visible/palpable contraction, 1 = visible/palpable contraction without movement of the limb, 2 = movement of the limb but not against gravity, 3 = movement against gravity (almost full passive range of motion) but not against resistance, 4 = movement against gravity and resistance, arbitrarily judged to be submaximal for sex and age, 5 = normal). Measurements were performed by one of four physiotherapists (TVH,

SV, TVA, HVM) who were extensively trained before the start of the study.

Hand held dynamometry

Isometric muscle force was measured using a hand held dynamometry (CompuFet®2; Biometrics, Almere, The Netherlands) that was connected to a laptop as previously described [14]. The same muscle groups involved in the MRC sum score were evaluated. Measurements were performed by the same four experienced physiotherapists (TVH, SV, TVA, HVM). Measurements were standardly performed on the right side. In case of focal or regional problems for certain muscle groups, evaluation was performed on the contralateral side. Values were expressed as percent of predicted values for sex and age [15].

Maximal Inspiratory Pressure

We measured maximal static inspiratory pressure (MIP) according to the ATS guidelines [16]. Measurements were performed by the same four experienced physiotherapists (TVH, SV, TVA, HVM), specifically trained for this by the pulmonary function technicians. We used a mouthpiece that incorporated a small leak to prevent glottis closure during the inspiratory manoeuvre. Pressures were measured with the Micro Medical respiratory pressure meter, CareFusion® using Puma PC software. The patient was asked to perform a maximal inspiratory manoeuvre starting from functional residual capacity. Maximal static inspiratory pressure was determined as the mouth pressure measure at the side port of a mouthpiece, maintained for 1 second. The best of 3 consecutive measurements was recorded.

Activities of daily living

The Barthel Index was used to assess independence during 10 daily life activities, including presence or absence of faecal and urinary incontinence, need of help with grooming, toilet use, feeding, transfers, walking, dressing, climbing stairs and bathing. Higher scores indicate higher level of independence, with a maximum score of 20 [9].

Statistics

Total and post- acute phase five-year mortality: Propensity score matching

To compare total and post-acute phase five-year mortality between short-and long-stay patients, we selected a subset of patients with short and prolonged ICU stay matched for randomization to early or late PN, baseline risk factors (age, gender, BMI, nutritional risk score), co-morbidities (diabetes, malignancy, pre-admission dialysis), type of illness (cardiac surgery, emergency admission to surgical ICU, elective admission to surgical ICU, medical ICU and sepsis upon admission), presence or absence of sepsis and severity of illness (APACHE II). Baseline characteristics accounted for are listed in Suppl. Table 3. Matching was based on propensity scores obtained by logistic regression and using one-to-one nearest neighbour matching without replacement with prolonged ICU stay as the dependent variable. A calliper of 0.2 was used and satisfactory matching was evaluated based on an absolute standardized difference in means less than or equal to 0.1 for all variables.
Comparisons between short and long-stayers were made in the matched population, and for completeness, also in the total population. Total and post-acute phase five-year mortality for short- and long-stayers were reported as proportions and compared by Chi-square statistics. For time-to-event analyses, comparisons for patients with short and prolonged ICU stay were performed with the log-rank test and visualized with Kaplan-Meier plots. Effect size was calculated with univariable Cox-proportional hazard regression analyses.

Total and post- acute phase five-year mortality: Exploratory analyses of factors explaining effects of prolonged ICU stay on total and post-acute phase five-year mortality

To further explore which characteristics of the prolonged ICU stay could explain its possible adverse effects on mortality, a stepwise multivariable Cox proportional hazards analysis was performed in the total EPaNIC population with a backward model, likelihood ratio, probability to enter 0.05, removal

0.2.
This involved a *first step*, introducing the baseline risk factors, including co-morbidities, type and severity of illness that showed at least a p value of 0.2 with five-year survival in univariable Cox proportional hazard analysis. In the *second* step, prolonged ICU stay was added to the model. In the *third* step, prolonged ICU stay was replaced by ICU interventions and events, which showed at least a p-value of 0.2 in univariable survival analyses. Prior to entering these variables, collinearity was checked and judged problematic in case of variation inflation factor > 5 or tolerance < 0.2. Predictive value of these factors for prolonged ICU stay was evaluated with receiver operating characteristic (ROC) curve and c-statistic. Continuous ICU variables were dichotomized on median values for the total population to provide sufficient overlap between short-and long-stay patients. Liver dysfunction was defined as bilirubin > 3 mg/dl [17].
Bootstrap sample procedure (n=1000) was performed on the final multivariable models to obtain robust estimators of the confidence intervals for each of the regression coefficients.

Total and post- acute phase five-year mortality: Sensitivity analyses

To validate our definition of prolonged ICU stay, we evaluated the optimal cut-off for ICU stay to predict total and post-acute phase five-year mortality, based on martingale residual plots with LOcal regrESSion (LOESS) lines [18].
At each step of the cox regression analyses, the proportional hazard assumption was checked for each variable retained in the model with use of log-minus-log (LML) plots for categorical variables, partial residuals (PR) plots for continuous variables and, if unclear, by entering the variable as a time-dependent covariate. At each step of the multivariable Cox regression analyses, sensitivity analyses were performed by adding the factors for which this assumption were violated as time-dependent covariates.

Five-year morbidity: Propensity score matching

To compare five-year morbidity between short-and long-stay patients, we selected a subset of patients with short and prolonged ICU stay, matched for randomization, baseline risk factors, including co-morbidities, type and severity of illness. Matching for demographics, co-morbidities and severity of illness upon admission was performed as described above. Post-hoc, ICU admission quality of life data (SF-36 questionnaire) and performance in daily life activities (Barthel index) were collected at the five-year follow-up visit as supplementary information on the pre-morbid status. These data were not used in the matching procedure as recollection of pre-morbid state may have been flawed by the post-ICU trajectory [19]. For completeness, also comparisons of outcomes between short-and long-stay patients in the total sample for which morbidity data were available were provided.

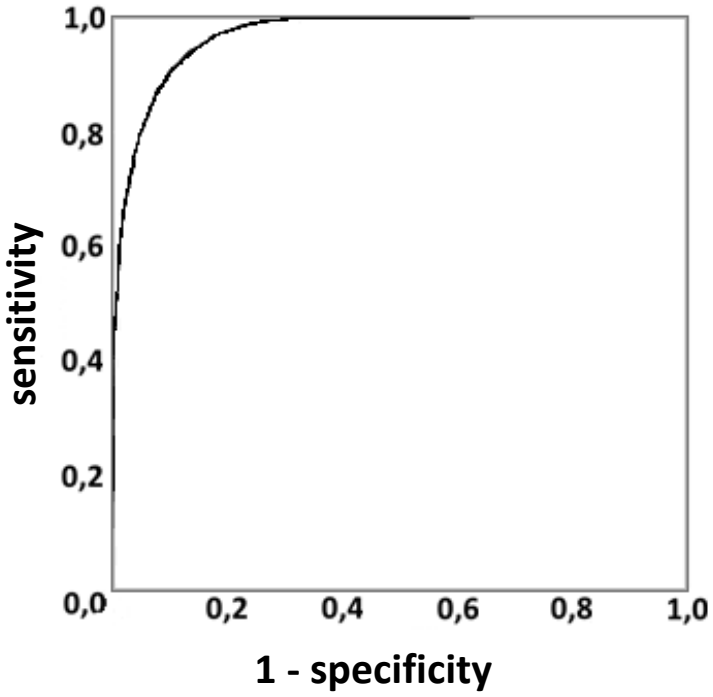
Morbidity outcomes of the former ICU patients were further referenced to controls in a subset matched for demographics including age, sex and BMI, and for completeness, also in the total population of patients and controls. To obtain satisfactory matching, repeated one-to-one nearest neighbour matching without replacement was performed based on propensity scores obtained by logistic regression with patients versus controls as dependent variable and age as covariate. This procedure was repeated as long as satisfactory matching for all 50 controls was obtained within the remaining patient population. This resulted in a 1:6 matching (50 controls and 300 patients). Final balance for each of the covariates was checked by chi-square and Mann-Whitney U test before examining any results.

Five-year morbidity: Exploratory analyses of factors explaining effects of prolonged ICU stay on five-year morbidity

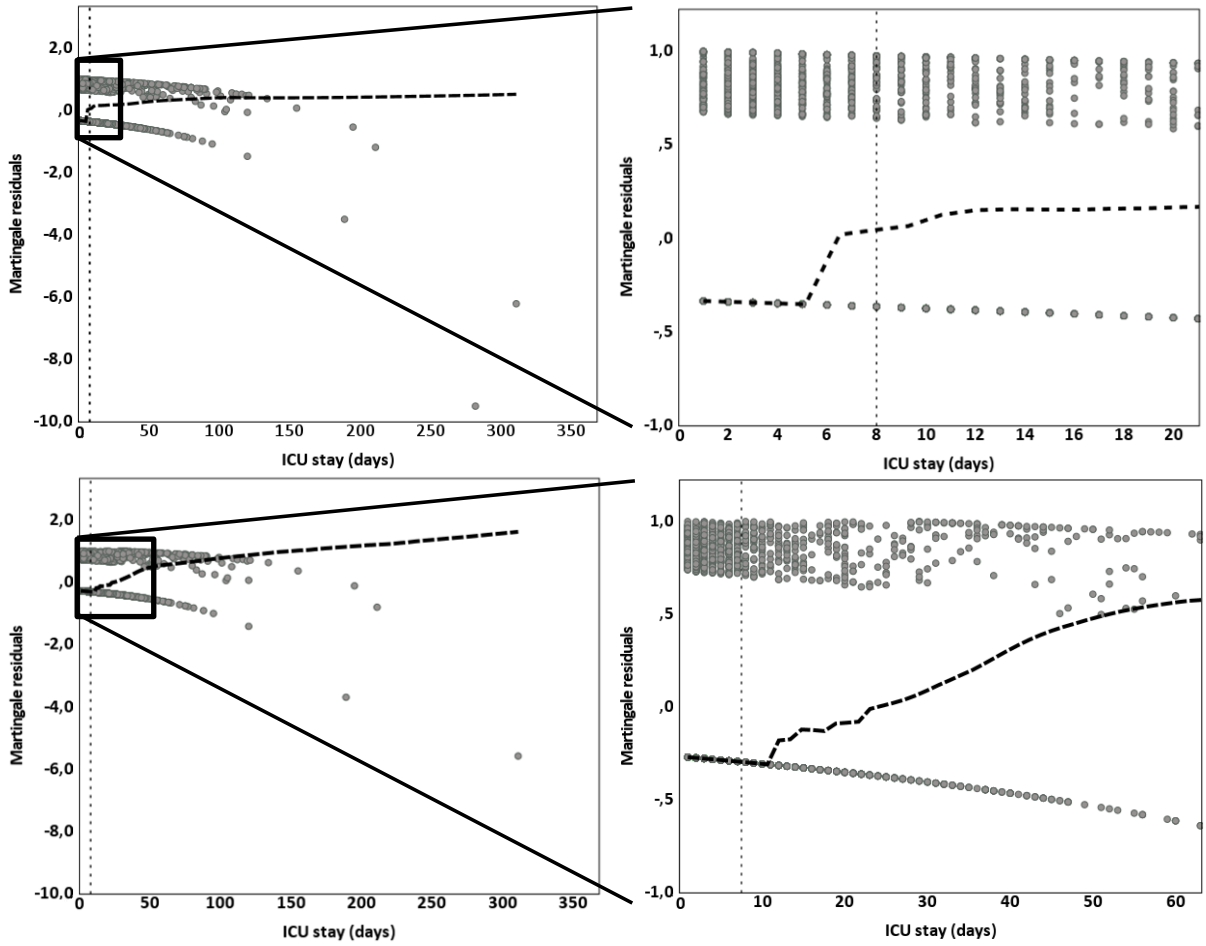
To further explore which characteristics of the prolonged ICU stay could explain any possible adverse effect on the secondary outcomes, a stepwise linear regression model was performed in the subset of five-year EPaNIC survivors who were evaluated at five years, with a backward model, probability for enter 0.05, removal 0.2.

This involved a *first step*, introducing baseline risk factors, including co-morbidities and severity of illness that showed at least a p value of 0.2 with the outcome of interest in univariable analysis. In the *second step*, prolonged ICU stay was added to the model. In the *third step*, prolonged ICU stay was replaced by treatments and events, which showed at least a p-value of 0.2 in univariable regression for the outcome studied. In order to obtain adequate model fit, the 6MWD data were transformed to power 2 and the PF-SF-36 were reversed (100 minus actual value) and subsequently transformed to power 0.54.

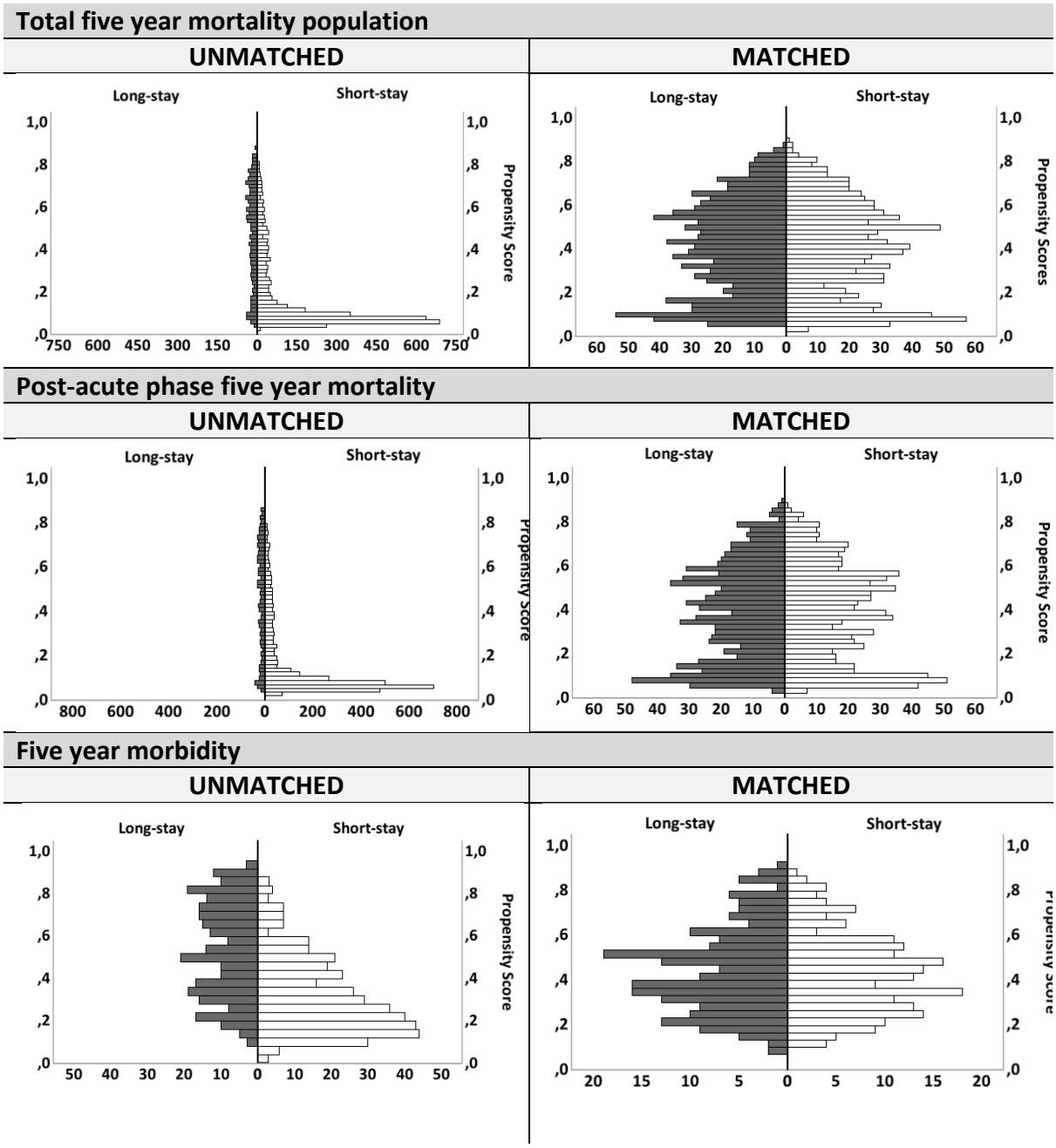
Supplementary figures



Suppl. Fig. 1: Receiver operating characteristic (ROC) curve, generated from the binary logistic regression analyses with prolonged ICU stay as the dependent variable and ICU-related exposure variables as the independent variables. Area under the curve=0.968.
These ICU-related exposure variables included: age; sex (male versus female); BMI; NRS (≥5 versus <5); Diabetes mellitus Malignancy; Pre-admission dialysis; Randomization (late versus early PN); APACHE II; Admission categories (cardiac surgery, emergency surgery, elective surgery, MICU); sepsis upon admission
Abbreviations: *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *MICU:* Medical Intensive Care Unit.



Suppl. Fig. 2: Plot of martingale residuals, for total 5-year mortality (panel A) and post-28 days 5-year mortality (panel B) versus duration of ICU stay. Martingale residuals were calculated form univariable Cox-proportional hazard analyses of total five-year mortality with ICU stay as the independent variable. LOESS lines (black dashed lines) indicate a breaking point around eight days. The right panel provides details of the main area of interest from the left panel. The dotted black lines represent the actually applied cut-off to define long-stayers.



Suppl. Fig. 3: Distribution of propensity scores of unmatched and matched subsets of EPaNIC patients, for five year mortality, post-acute five year mortality and five year morbidity analysis.

Supplementary tables

Supplementary Table 1: Reasons for exclusion from morbidity analyses within eligible patients[a]

	Short-stay eligible N= 2436	Long-stay eligible N=578
Short stay not in random selection	1721	NA
Died after five years, before planned testing	6	12
Lost to follow-up/ living abroad	42	51
Time window passed	14	8
Language barrier	0	2
Pre-existent (pre-ICU) neuromuscular disorder[a]	18	25
Unable to walk without assistance prior to ICU	2	3
Cardiac assist device[b]	1	0
Pulmonary resection (pneumonectomy)[b]	6	10
Psychiatric disease[b]	8	11
Dementia[b]	4	1
Vegetative state[b]	0	2
Hospitalized/Rehabilitation center/Nursing home[b]	17	20
Refusal:		
Good health	17	8
Poor health	15	20
Old age	8	2
Practical	23	24
Other follow-up programs	67	34
Emotional reasons	12	7
Not interested	35	32
No reason	22	30

[a]Pre-ICU neuromuscular disorders were excluded by manual chart review of the medical history performed by study nurses, and when in doubt, discussed with the Principal Investigator.
[b]present before follow-up evaluation
Abbreviations: *ICU:* intensive care unit; *NA*: not applicable.

Supplementary Table 2: Characteristics of eligible patients according to inclusion status

	Short-stay eligible patients N=2436			Long-stay eligible patients N=578		
	Included N=398	**Not included N=2038**	***P*-value**	**Included N=276**	**Not included N=302**	***P*-value**
Baseline factors						
Age	61.4 (50.8-70.3)	66.3 (55.7-74.2)	<0.001	58.1 (48.4-68.8)	57.1 (43.8-69.7)	0.514
Gender, male	252 (63.3)	1309 (64.2)	0.728	198 (71.7)	178 (58.9)	0.001
BMI	25.6 (23-28.4)	26 (23.5-29.3)	0.009	25.8 (22.9-29.4)	25.2 (22.4-28.4)	0.206
NRS >5	69 (17.3)	204 (10.0)	<0.001	60 (10.4)	80 (13.8)	0.183
Diabetes mellitus	57 (14.3)	330 (16.2)	0.350	42 (15.2)	36 (11.9)	0.247
Malignancy	65 (16.3)	234 (11.5)	0.007	41 (14.9)	40 (13.2)	0.578
Preadmission dialysis	3 (0.8)	19 (0.9)	1	0	2 (0.7)	0.500
Randomisation, late PN	222 (55.8)	1014 (49.8)	0.028	122 (44.2)	147 (48.7)	0.282
APACHE II	20 (14-32)	16 (13-21)	<0.001	30 (23-30)	31 (24-36)	0.558
Admission category			<0.001			0.109
Cardiac surgery	203 (51.0)	1732 (85)		98 (35.5)	80 (26.5)	
Elective SICU	36 (9.0)	52 (2.6)		8 (2.8)	7 (2.3)	
Emergent SICU	144 (36.2)	201 (9.9)		139 (50.4)	178 (58.9)	
MICU	15 (3.8)	53 (2.6)		31 (11.2)	37 (12.3)	
Sepsis on admission	62 (15.6)	165 (8.1)	<0.001	114 (41.3)	138 (45.7)	0.288
ICU stay, days	3 (1-5)	2 (1-3)	<0.001	15 (11-25)	15 (11-23)	0.674

Continuous variables are expressed as median (IQR), dichotomous variables are expressed as numbers (percentages).

Abbreviations: *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *SICU:* Surgical Intensive Care Unit; *MICU:* Medical Intensive Care Unit.

Supplementary Table 3: Baseline characteristics and ICU factors of the total population of short- versus long-stay patients included in the mortality and morbidity analyses

	All cause five-year mortality analyses			All cause post-acute phase five-year mortality analyses			Morbidity analyses		
	Total population N= 4619			Total population N= 4315			Total population N= 674		
	Short-stay N=3410	Long-stay N=1209	*P*-value	Short-stay N= 3264	Long-stay N= 1051	*P*-value	Short-stay N=398	Long-stay N=276	*P*-value
Baseline factors									
Age, median (IQR)	67.1 (56.6-75.1)	64.5 (53.9-74.5)	<0.001	66.8 (56.5-74.8)	63.7 (53.3-73.9)	<0.001	61.4 (50.8-70.3)	58.1 (48.4-68.8)	0.052
Sex, male (%)	2187 (64.1)	774 (64)	0.943	2093 (64.1)	677 (64.4)	0.864	252 (63.3)	198 (71.7)	0.022
BMI, median (IQR)	25.9 (23.2-29.1)	25.3 (22.6-28.9)	0.002	25.9 (23.3-29.1)	25.3 (22.7-29)	0.003	25.6 (23-28.4)	25.8 (22.9-29.4)	0.419
NRS ≥ 5 (%)	471 (13.8)	385 (31.8)	<0.001	422 (12.9)	315 (30)	<0.001	69 (17.3)	60 (21.7)	0.153
Diabetes mellitus (%)	602 (17.7)	204 (16.9)	0.539	570 (17.5)	166 (15.8)	0.211	57 (14.3)	42 (15.2)	0.747
Malignancy (%)	603 (17.7)	288 (23.8)	<0.001	564 (17.3)	241 (22.9)	<0.001	65 (16.3)	41 (14.9)	0.605
Pre-admission dialysis (%)	46 (1.3)	23 (1.9)	0.173	40 (1.2)	18 (1.7)	0.233	3 (0.8)	0	0.273
Randomization, late PN (%)	1748 (51.3)	570 (47.1)	0.014	1678 (51.4)	488 (46.4)	0.005	222 (55.8)	122 (44.2)	0.003
APACHE II, median (IQR)	17 (13-26)	32 (25-38)	<0.001	17 (13-24)	32 (24-37)	<0.001	19.5 (14-32)	30 (23-36.8)	<0.001
Admission category (%)			<0.001			<0.001			<0.001
Cardiac surgery	2463 (72.2)	347 (28.7)		2413 (73.9)	313 (29.8)		203 (51)	98 (35.5)	
Emergency SICU	552 (16.2)	607 (50.2)		493 (15.1)	543 (51.7)		144 (36.2)	139 (50.4)	
Elective SICU	222 (6.5)	54 (4.5)		217 (6.6)	49 (4.7)		36 (9)	8 (2.9)	
MICU	173 (5.1)	201 (16.6)		141 (4.3)	146 (13.9)		15 (3.8)	31 (11.2)	
Sepsis upon admission (%)	423 (12.4)	586 (48.5)	<0.001	360 (11.0)	492 (46.8)	<0.001	62 (15.6)	114 (41.3)	<0.001

Continued Supplementary Table 3: Baseline characteristics and ICU factors of the total population of short- versus long-stay patients included in the mortality and morbidity analyses

	All cause five-year mortality analyses			All cause post-acute phase five-year mortality analyses			Morbidity analyses		
	Total population N= 4619			Total population N= 4315			Total population N= 674		
	Short-stay N=3410	Long-stay N=1209	*P*-value	Short-stay N= 3264	Long-stay N= 1051	*P*-value	Short-stay N=398	Long-stay N=276	*P*-value
ICU-related exposure variables									
Mean morning glycaemia > 103 mg/dl	1658 (49)	629 (52)	0.075	1587 (49)	541 (51.5)	0.171	202 (50.8)	153 (55.4)	0.231
Mean insulin dose >43.43 U/d	1451 (42.6)	855 (70.7)	<0.001	1382 (42.3)	741 (70.5)	<0.001	170 (42.7)	216 (78.3)	<0.001
Hypoglycaemia during intervention[a]	60 (1.8)	66 (5.5)	<0.001	50 (1.5)	51 (4.9)	<0.001	4 (1)	9 (3.3)	0.036
Corticosteroids	531 (15.6)	621 (51.4)	<0.001	460 (14.1)	525 (50)	<0.001	99 (24.9)	124 (44.9)	<0.001
NMBA	214 (6.3)	673 (55.7)	<0.001	159 (4.9)	578 (55)	<0.001	28 (7)	157 (56.9)	<0.001
Benzodiazepines > 1 day	719 (21.1)	1062 (87.8)	<0.001	646 (19.8)	922 (87.7)	<0.001	103 (25.9)	244 (88.4)	<0.001
Opioids > 3 days	729 (21.4)	1102 (91.1)	<0.001	669 (20.5)	969 (92.2)	<0.001	113 (28.4)	261 (94.6)	<0.001
Propofol > 1 day	1005 (29.5)	973 (80.5)	<0.001	952 (29.2)	858 (81.6)	<0.001	139 (34.9)	243 (88)	<0.001
Clonidine	60 (1.8)	245 (20.3)	<0.001	57 (1.7)	227 (21.6)	<0.001	9 (2.3)	79 (28.6)	<0.001
Ketamine	9 (0.3)	50 (4.1)	<0.001	8 (0.2)	48 (4.6)	<0.001	3 (0.8)	9 (3.3)	0.019
Mechanical ventilation > 2 days	632 (18.5)	1130 (93.5)	<0.001	553 (16.9)	978 (93.1)	<0.001	100 (25.1)	261 (94.6)	<0.001
Vasopressors/ inotropes> 2 days	805 (23.6)	1002 (82.9)	<0.001	731 (22.4)	868 (82.6)	<0.001	102 (25.6)	224 (81.2)	<0.001
Bilirubin>3 mg/dl	244 (7.2)	389 (32.2)	<0.001	207 (6.4)	317 (30.2)	<0.001	36 (9.1)	84 (30.4)	<0.001
New dialysis	54 (1.6)	304 (25.1)	<0.001	20 (0.6)	242 (23)	<0.001	2 (0.5)	55 (19.9)	<0.001
New infection	229 (6.7)	897 (74.2)	<0.001	199 (6.1)	790 (75.2)	<0.001	24 (6)	206 (74.6)	<0.001

[a]Intervention involved early (within 48h) versus late (not within the first week) parenteral substitution of deficient enteral nutrition

Abbreviations: *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *SICU:* Surgical Intensive Care Unit; *MICU:* Medical Intensive Care Unit; *NMBA:* neuromuscular blocking agents.

Supplementary Table 4: Post-hoc pre-morbid status in short- versus long-stay patients for total and matched population included in the morbidity analyses

	Matched population N= 408			Total population N= 674		
	Short-stay N=204	**Long-stay N=204**	***P*-value**	**Short-stay N=398**	**Long-stay N=276**	***P*-value**
PF-SF-36, median (IQR)	70 (30-100)	85.0 (45.5-100)	0.100	75 (40-95)	85 (45-100)	0.016
PCS, median (IQR)	43.1 (31.9-56.0)	48.4 (34.7-57.9)	0.029	44.6 (33.8-55.6)	49.6 (35.4-57.9)	0.005
MCS, median (IQR)	52.4 (43.4-58.0)	51.6 (42.8-57.7)	0.659	52.7 (44.1-57.9)	51.7 (41.1-57.6)	0.155

Abbreviations: *PF-SF-36:* physical function of the 36-item short form health survey; *PCS:* physical component score; *MCS:* mental component score.

Supplementary Table 5: Demographics of patients and controls in morbidity analyses

	Matched population N= 350			Total population N= 724		
	Patients N=300	**Controls N=50**	***P*-value**	**Patients N= 674**	**Controls N= 50**	***P*-value**
Age	62 (58-68)	61 (57-66)	0.218	65 (55-75)	61 (57-66)	0.058
Sex, male (%)	205 (68.3)	35 (70)	0.814	450 (66.77)	35 (70)	0.639
BMI	27.3 (24.0-30.8)	26.4(23.7-29.3)	0.282	26.8 (23.8-30.0)	26.4 (23.7-29.3)	0.653

Abbreviations: *BMI:* body mass index.

Supplementary Table 6: Morbidity analyses in five-year survivors and controls

	Matched population N= 408			Total population N= 674		
	Short-stay patients N= 204	**Long-stay patients N= 204**	***P*-value**	**Short-stay patients N= 398**	**Long-stay patients N= 276**	***P*-value**
Strength						
MRC sum score	60 (57-60)	60 (57-60)	0.254	60 (58-60)	60 (58-60)	0.049
HGF (%pred)	87 (73-103)	83 (60-100)	0.020	88 (73-104)	86 (63-104)	0.035
HHD (%pred)						
Shoulder	88 (75-102)	85 (65-104)	0.250	91 (77-108)	88 (68-105)	0.007
Elbow	83 (71-97)	79 (64-97)	0.148	86 (73-100)	82 (66-97)	0.003
Wrist	97 (81-112)	94 (77-111)	0.126	99 (84-116)	96 (79-112)	0.004
Hip	146 (119-170)	134 (110-157)	0.001	149 (125-174)	135 (113-158)	<0.001
Knee	50 (42-62)	49 (38-59)	0.083	53 (44-64)	51 (39-59)	0.002
Ankle	72 (58-86)	67 (52-79)	0.004	75 (61-90)	68 (54-81)	<0.001
MIP (%pred)	92 (71-115)	87 (64-105)	0.021	91 (71-113)	88 (65-105)	0.077
Exercise capacity						
6MWD (%pred)	94 (76-105)	85 (69-101)	0.005	93 (78-105)	89 (72-103)	0.036
Quality of life						
PF-SF-36	75 (55-90)	65 (35-90)	0.002	75 (50-90)	70 (35-90)	0.010
PCS	47 (38-55)	43 (32-52)	0.003	47 (37-55)	45 (33-52)	0.013
MCS	56 (48-60)	54 (45-59)	0.053	55 (47-59)	54 (45-59)	0.206
ADL						
Barthel index	20 (19-20)	20 (18-20)	<0.001	20 (19-20)	20 (18-20)	0.001

Continued Supplementary Table 6: Morbidity analyses in five-year survivors and controls

	Matched population N= 350			Total population N= 724		
	Patients N= 300	**Controls N= 50**	***P*-value**	**Patients N= 674**	**Controls N= 50**	***P*-value**
Strength						
MRC sum score	60 (58-60)	60 (60-60)	<0.001	60 (58-60)	60 (60-60)	<0.001
HGF (%pred)	89 (73-104)	104 (91-117)	<0.001	87 (70-104)	104 (91-117)	<0.001
HHD (%pred)						
Shoulder	91 (76-106)	98 (86-119)	0.006	89 (74-106)	98 (86-119)	0.001
Elbow	84 (72-97)	101 (87-116)	<0.001	84 (71-99)	101 (87-116)	<0.001
Wrist	97 (84-115)	109 (93-122)	0.004	98 (82-115)	109 (93-122)	0.004
Hip	144 (123-171)	165 (134-183)	0.006	143 (120-168)	165 (134-183)	0.001
Knee	52 (43-60)	65 (53-74)	<0.001	52 (42-62)	65 (53-74)	<0.001
Ankle	72 (60-85)	91 (74-108)	<0.001	73 (59-86)	91 (74-108)	<0.001
MIP (%pred)	90 (71-11)	104 (85-124)	0.004	90 (69-111)	104 (85-124)	0.001
Exercise capacity						
6MWD (%pred)	95 (79-107)	117 (107-125)	<0.001	92 (75-104)	117 (107-125)	<0.001
Quality of life						
PF-SF-36	75 (50-90)	95 (90-100)	<0.001	75 (45-90)	95 (90-100)	<0.001
PCS	48 (37-55)	55 (51-58)	<0.001	46 (35-54)	55 (51-58)	<0.001
MCS	55 (49-59)	58 (53-60)	0.049	55 (46-59)	58 (53-60)	0.046
ADL						
Barthel index	20 (19-20)	20 (20-20)	0.020	20 (19-20)	20 (20-20)	0.006

Abbreviations: *MRC:* Medical Research Council; *HGF:* hand grip strength; *HHD:* hand held dynamometry; *MIP:* maximal inspiratory pressure; PF-SF-36: physical function of the 36-item short form health survey; *PCS:* physical component score; *MCS:* mental component score; *ADL:* activities of daily living.

Supplementary Table 7: Multivariable analyses of hand grip strength (% predicted), 6MWD (% predicted) and PF SF36 in five-year survivors

	Hand grip strength		6-MWD[b]		PF-SF-36[c]	
	Unstandardized coefficients (B) (95% BCa confidence intervals)[a]	***P*-value**	**Unstandardized coefficients (B) (95% BCa confidence intervals)[a]**	***P*-value**	**Unstandardized coefficients (B) (95% BCa confidence intervals) [a]**	***P*-value**
Models with baseline factors and prolonged ICU stay						
Age	NA	NA	NA	NA	0.068 (0.051-0.084)	0.001
Sex, male	14.098 (10.176-18.333)	0.001	984.794 (306.466-1650.549)	0.003	-1.195 (-1.693 to -0.715)	0.001
BMI other than 25-40	-4.554 (-8.333 to -0.852)	0.017	NA	NA	-0.412 (-0.899-0.027)	0.101
NRS≥5	-3.598 (-8.677 to 1.394)	0.189	NA	NA	NA	NA
Diabetes mellitus	-8.526 (-13.989 to -3.343)	0.006	-2024.754 (-2932.654 to -1179.825)	0.001	1.233 (0.504-1.901)	0.002
Malignancy	NA	NA	NA	NA	0.715 (0.075-1.377)	0.029
Admission category - emergency surgery	NA	NA	NA	NA	0.391 (-0.108-0.905)	0.147
ICU stay, prolonged	-6.272 (-9.915 to -2.592)	0.002	-913.743 (-1592.534 to -285.686)	0.003	0.923 (0.408-1.446)	0.001

Continued Supplementary Table 7: Multivariable analyses of hand grip strength (% predicted), 6MWD (% predicted) and PF SF36 in five-year survivors

	Hand grip strength		6-MWD[b]		PF-SF-36[c]	
	Unstandardized coefficients (B) (95% BCa confidence intervals)	***P*-value**	**Unstandardized coefficients (B) (95% BCa confidence intervals)**	***P*-value**	**Unstandardized coefficients (B) (95% BCa confidence intervals)**	***P*-value**
Models with baseline factors and ICU factors						
Age	NA	NA	NA	NA	0.066 (0.049-0.083)	0.001
Sex, male	13.830 (9.957-17.728)	0.001	946.598 (274.580-1667.701)	0.008	-1.128 (-1.655 to -0.610)	0.001
BMI other than 25-40	-4.694 (-8.430 to -1.166)	0.019	NA	NA	-0.426 (-0.938 -0.100)	0.107
NRS≥5	-3.636 (-8.578 to 1.152)	0.168	NA	NA	NA	NA
Diabetes mellitus	-9.177 (-14.510 to -3.605)	0.002	-2005.767 (-2885.473 to -1159.171)	0.001	1.359 (0.544-2.163)	0.001
Malignancy	NA	NA	NA	NA	0.732 (0.144 -1.321)	0.022
Admission category, emergency surgery	NA	NA	NA	NA	0.355 (-0.198-0.873)	0.170
Hypoglycemia	NA	NA	-1764.531 (-3363.715 - 43.651)	0.034	NA	NA
Mean morning glycaemia > 103 mg/dl	2.991 (-0.913-6.826)	0.113	NA	NA	-0.471 (-1.014-0.034)	0.066
NMBA	NA	NA	-692.840 (-1437.932 - 130.452)	0.085	NA	NA
Benzodiazepines > 1 day	-8.225 (-12.816 to -3.929)	0.001	NA	NA	0.572 (0.046-1.076)	0.056
Opiates > 3 days	NA	NA	-743.458 (-1362.968 to -74.954)	0.027	NA	NA
Clonidine	5.055 (-0.589-11.256)	0.104	NA	NA	NA	NA
Vasopressors/ inotropes > 2 days	NA	NA	NA	NA	0.557 (0.035 - 1.138)	0.056

[b]Values were transformed to power 2. [c]Values were reversed (100 minus PF-SF-36) and subsequently transformed to power 0.54.

Abbreviations: *NRS:* nutritional risk score; *BMI:* body mass index; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *BCa:* bias-corrected accelerated confidence intervals obtained by bootstrap sample procedure (n=1000).

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Chapter 4: Five-year mortality and morbidity impact of ICU-acquired neuromuscular complications

Adapted from:

Van Aerde N, Meersseman P, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Bruyninckx F, Wouters PJ, Gosselink R, Van den Berghe G*, Hermans G*. (2020) Five-year impact of ICU-acquired neuromuscular complications: a prospective, observational study. Intensive Care Med 46(6): 1184-1193.

*Equally contributed

ABSTRACT

Purpose

To assess the independent association between ICU-acquired neuromuscular complications and 5-year mortality and morbidity. To explore the optimal threshold of the Medical Research Council (MRC) sum score, assessing weakness, for prediction of 5-year outcomes.

Methods

Sub-analyses of a prospective, 5-year follow-up study including 883 EPaNIC patients (Early Parenteral Nutrition in Intensive Care) (Clinicaltrials.gov:NCT00512122), systematically screened in-ICU for neuromuscular complications with MRC sum score ('MRC-cohort', N=600), electrophysiology on day 8±1 to quantify compound muscle action potential ('CMAP-cohort', N=689), or both ('MRC&CMAP-cohort', N=415). Associations between ICU-acquired neuromuscular complications and 5-year mortality, hand-grip-strength (HGF, %predicted), six-minute-walk-distance (6MWD, %predicted) and physical function of the SF-36 quality-of-life questionnaire (PF-SF-36) at 5-years, were assessed with Cox-regression and linear regression, adjusted for confounders. The optimal threshold for MRC at ICU-discharge to predict 5-year outcomes was determined by martingale residual plots (survival) and scatterplots (morbidity).

Results

Both lower MRC sum score at ICU-discharge, indicating less strength [HR per-point-increase: 0.946 (95%CI: 0.928-0.968), p=0.001], and abnormal CMAP, indicating nerve/muscle dysfunction [HR: 1.568 (95%CI: 1.165-2.186), p=0.004], independently associated with increased 5-year mortality. In the MRC&CMAP-cohort, MRC [HR: 0.956 (95%CI: 0.934-0.980), p=0.001] but not CMAP [HR: 1.478 (95%CI: 0.875-2.838), p=0.088] independently associated with 5-year mortality. Among 205 survivors, low MRC independently associated with low HGF [0.866 (95%CI: 0.237-1.527), p=0.004], low 6MWD [105.1 (95%CI: 12.1-212.9), p=0.043] and low PF-SF-36 [-0.119 (95%CI: -0.186 to-0.057), p=0.002], whereas abnormal CMAP did not correlate with these morbidity endpoints. Exploratory analyses suggested that MRC ≤ 55 best predicted poor long-term morbidity and mortality. Both MRC ≤ 55 and abnormal CMAP independently associated with 5-year mortality.

Conclusions

ICU-acquired neuromuscular complications may impact 5-year morbidity and mortality. MRC sum score, even if slightly reduced, may affect long-term mortality, strength, functional capacity and physical function, whereas abnormal CMAP only related to long-term mortality.

INTRODUCTION

ICU-acquired weakness (ICUAW), as diagnosed by a Medical Research Council (MRC) sum score <48, and electrophysiological signs of neuromuscular dysfunction often occur during critical illness and are associated with in-ICU morbidity and up to 1-year mortality [1, 2]. In particular, weakness persisting until ICU discharge and reduced compound muscle action potential (CMAP) on screening electrophysiology after 1 week of intensive care strongly and independently associate with 1-year mortality [3, 4]. Beyond this time-frame, few data suggest that, for patients with acute respiratory distress syndrome (ARDS), the association between weakness at hospital discharge and mortality attenuates over time and loses significance by 5 years [5]. Regarding long-term morbidity, ICU survivors report reduced physical function and quality of life, imposing a burden on patients, their families and on society [2, 6-8]. ICU-acquired neuromuscular dysfunctions presumably contribute to this so-called 'legacy of critical illness' or 'post-intensive care syndrome' [9-11], though clear evidence of an independent relationship with long-term outcomes is lacking. Indeed, complaints of persisting weakness and disabilities were documented in 5-year ARDS survivors, but weakness could not be objectified [6]. Quantifying the degree to which in-ICU neuromuscular abnormalities contribute to long-term adverse outcomes could be important for risk stratification and for targeting strategies to prevent or reduce such long-term burden of critical illnesses.

We investigated a large cohort of former general ICU patients, who received systematic in-ICU clinical and electrophysiological neuromuscular screening, 5 years after ICU admission. We hypothesized that muscle strength at ICU discharge, as assessed by the MRC sum score and abnormal CMAP on electrophysiological screening after 1 week in ICU continue to independently associate with 5-year mortality (primary outcome) and morbidity (secondary outcome). We further aimed to explore the optimal threshold of the MRC sum score at ICU discharge, for predicting 5-year morbidity and mortality.

METHODS

Ethics

The study protocol and informed consent forms were approved by the Leuven University Hospital Ethics Committee (ML4190). Patients gave separate informed consent for the five-year morbidity evaluations.

Study design and participants

This was a sub-analysis of a prospective 5-year follow-up study, involving 883 EPaNIC (Clinical trials.gov:NCT00512122) patients who received systematic neuromuscular evaluation in the ICU. The EPaNIC trial was a large, randomised controlled trial (RCT) performed in 7 medical/surgical ICUs of the University Hospitals Leuven and Jessa Hospitals Hasselt, comparing early (≤ 48 hours) with late (>8days) parenteral supplementation of insufficient enteral nutrition in critically ill patients [12]. In the Leuven ICUs, as part of EPaNIC and to investigate effects of the intervention on neuromuscular outcomes, 730 patients received electrophysiological screening weekly from day 8±1 onwards [4]. In 698 of these patients, CMAP evaluation was technically feasible. Furthermore, 600 patients were clinically evaluated for weakness with the MRC sum score from day 8 onwards, 3-times weekly from awakening until ICU discharge or death [3, 13]. For both electrophysiology and strength assessment, we included long-stayers at risk for neuromuscular complications, as well as a randomly selected subgroup of short-stayers who were assessed on the ward at day 8±1. Further details on CMAP assessment and evaluation criteria are provided in the online supplement.

As part of the prospective post-EPaNIC follow-up study, 5-year mortality was assessed for all EPaNIC patients, whereas 5-year morbidity was assessed for survivors during hospital or home visits from June 2012 onwards [14]. Exclusion criteria included the inability to walk without assistance prior to ICU admission, pre-existing neuromuscular disease, other pre-ICU disabilities potentially confounding the morbidity endpoints, and refusal for participation [14]. Hence, for the following sub-analyses involving patients with in-ICU neuromuscular evaluation, we define three populations, comprising the 'MRC-cohort', the 'CMAP-cohort', and the overlapping cohort who received both assessments, further referred to as 'MRC&CMAP-cohort' (Figure 1).

Outcomes

To investigate long-term outcomes in relation to ICU-acquired neuromuscular dysfunctions, we defined all-cause 5-year mortality, obtained from the national registry, as the primary endpoint. We further assessed the association of ICU-acquired neuromuscular dysfunctions with 5-year morbidity, with three distinct measures of physical function as secondary endpoints. These included hand-grip strength (HGF, %predicted), six-minute-walk-distance (6MWD, %predicted), and the physical function of the SF-36 quality-of-life questionnaire (PF-SF-36, range 0-100, higher values indicating better scores) at 5-years follow-up. Other outcomes comprised evaluation of peripheral strength with the MRC-sum score and hand-held dynamometry of the muscle groups involved in the MRC sum score, as well as respiratory muscle strength, assessed by maximal inspiratory pressure [15]. Additionally, we assessed the Physical and Mental Component Score (PCS and MCS) of the SF-36 [16, 17] and Barthel-index [18, 19] (range 0-20, higher scores indicating higher degree of physical independence).

Additional exploratory analyses involved the assessment of linearity between the MRC sum score and the primary outcomes and, if appropriate, identification of the optimal threshold for the MRC sum score to predict 5-year outcomes.

Statistics

Study of the independent association between ICU-acquired neuromuscular dysfunctions and 5-year mortality

We explored in unadjusted analyses, whether last MRC at (or close to) ICU discharge (further referred to as 'MRC at ICU discharge') and abnormal CMAP on day 8±1 in ICU were associated with 5-year mortality in respectively the MRC- and CMAP-cohorts. Hazard ratios for both predictors were calculated with univariable Cox-regression analyses. MRC sum score was entered as a continuous variable [5]. For CMAP, data were visualised with Kaplan-Meier plots. If a univariable association was present between MRC or abnormal CMAP and 5-year mortality, adjusted hazard ratios were calculated for the cohort(s) of interest by adding literature-based, a priori defined confounders to the models [20]. Potential confounders included demographics, comorbidities and ICU treatments and events. Details on the search strategy, confounders identified, check of collinearity, and bootstrapping are provided in the online supplement.

If both predictors showed an independent association with five-year mortality, the added value of the combined information of MRC and CMAP was assessed within the MRC&CMAP-cohort.

Study of the independent association between ICU-acquired neuromuscular dysfunctions and 5-year morbidity

The association between the MRC sum score at ICU discharge and CMAP on ICU day 8±1 with the morbidity endpoints was explored in unadjusted analyses. For the MRC sum score, we performed

linear regression analyses. If necessary, the morbidity endpoints were transformed to obtain adequate model fit (see online supplement) [14]. For CMAP, outcomes for patients with normal and abnormal values were compared with Mann-Whitney-U or Fisher-exact test, as appropriate. If a univariable association was demonstrated for either of the three distinct measures of physical function, multivariable models were constructed for each of the cohorts of interest by introducing literature-based, a priori defined confounders, as covariates (see online supplement for search strategy, confounders identified, and modelling).

If appropriate, further analyses on the MRC&CMAP-cohort were performed to explore any additional information provided by the combination of data.

Exploratory analyses for defining the optimal threshold of MRC-sum score at ICU discharge for prediction of 5-year outcomes

Linearity of the relationship between MRC sum score and 5-year outcomes was assessed (see online supplement) and if appropriate, multivariable analyses were repeated with MRC as a binary factor.

Sensitivity analyses

The proportional hazard assumption was checked for each variable in each of the Cox-regression models with the Schoenfeld residuals test. If appropriate, sensitivity analyses were performed by adding all factors for which the assumption was violated as time-dependent co-variates.

Analyses were performed with SPSS version 25 (IBM corporation) and R version 3.6.1. Descriptive statistics included median and interquartile ranges for continuous variables and numbers and percentages for categorical variables. Continuous data were compared with Mann-Whitney-U test and categorical variables with Chi-square test or Fisher-exact test, as appropriate. Two-sided p-values $\leq$ 0.05 were considered statistically significant.

RESULTS

Patient cohorts and characteristics

MRC sum scores at ICU discharge were obtained for 600 patients. Four of these were lost to follow-up and lack 5-year mortality data. Five-year morbidity was assessed in 205 of these patients (Figure 1). Of the 730 patients with electrophysiological screening on ICU day 8±1, 698 patients had available CMAPs. Within this CMAP-cohort, 5 patients were lost to follow-up and lack 5-year survival data and 184 received 5-year morbidity assessment. The overlapping cohort, with both MRC and CMAP assessment, consisted of 415 patients, of whom 3 were lost to follow-up and 134 were assessed for 5-year morbidity. Baseline and ICU characteristics of these 3 cohorts are provided in Table 1 and Supplementary Table 1.

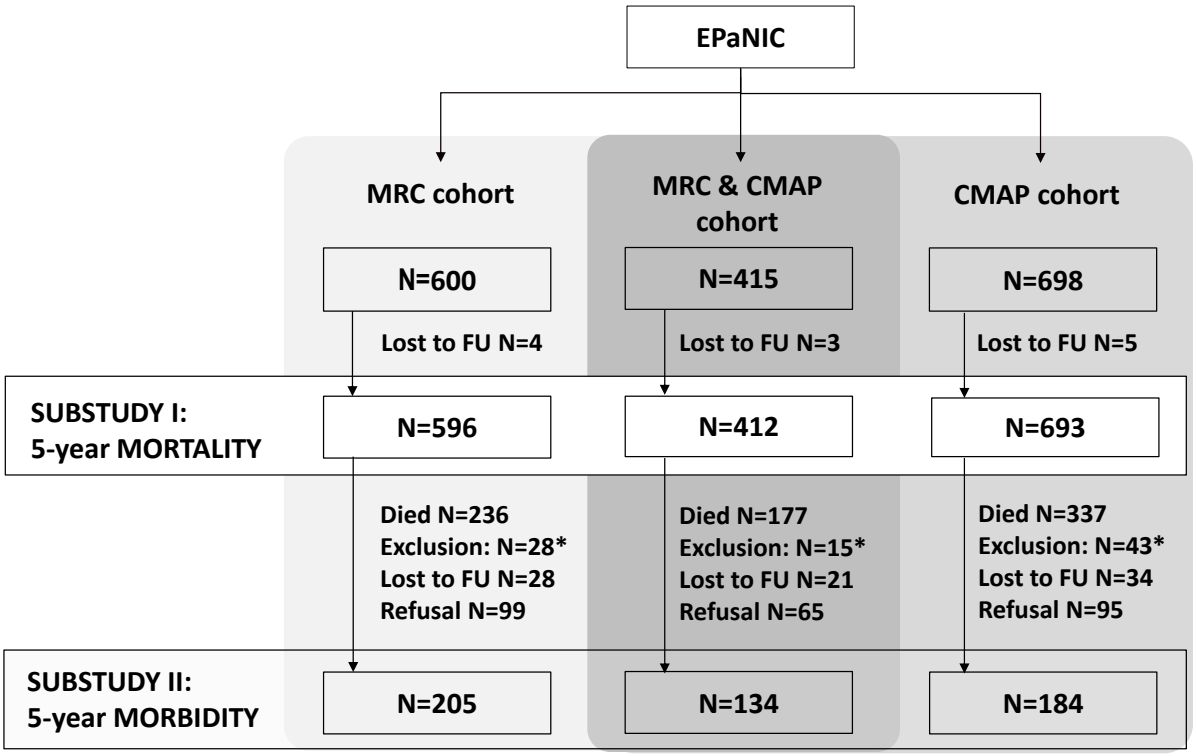

Fig. 1: Patient flow-chart for the 5-year mortality and morbidity analyses. The first cohort, labeled as 'MRC-cohort', is composed of EPaNIC patients in whom last in-ICU MRC-sum score was available, based on 3 times weekly screening. The second cohort consists of patients who underwent screening electrophysiological testing in ICU on D8±1 and in whom CMAP was evaluable. This cohort is further referred to as 'CMAP-cohort'. The third cohort is the overlapping population who received both MRC-sum scores and electrophysiological screening in ICU, labeled as the 'MRC&CMAP cohort'. *Exclusions: MRC-cohort: N=9: Practical reasons, N=6: Preexisting NM disorder, N=4: Severe neurocognitive disorder, N=8: Pulmonary resection, N=1: Unable to walk without assistance prior to ICU-stay; CMAP-cohort: N=20: Practical reasons, N=7: Preexisting NM disorder, N=7: Severe neurocognitive disorder, N=6: Pulmonary resection, N=3: Unable to walk without assistance prior to ICU-stay; CMAP & MRC cohort: N=4: Practical reasons, N=2: Preexisting NM disorder, N=2: Severe neurocognitive disorder, N=6: Pulmonary resection, N=1: Unable to walk without assistance prior to ICU-stay.

Table 1. Baseline characteristics, indicators of neuromuscular dysfunction in ICU and ICU factors of patients included in the five year mortality and morbidity analyses

	MRC-cohort		CMAP-cohort		MRC&CMAP-cohort	
	5-year mortality N=596	5-year morbidity N = 205	5-year mortality N= 693	5-year morbidity N = 184	5-year mortality N=412	5-year morbidity N=134
Baseline factors						
Age, median (IQR)	63.4 (52.7-73.1)	60.0 (51.2-70.1)	64.1 (53.8-74.2)	59.7 (51.0-71.0)	63.6 (53.3-73.4)	59.7 (51.2-71.3)
Gender, male (%)	352 (59.1)	128 (62.4)	439 (63.3)	119 (64.7)	245 (59.5)	85 (63.4)
BMI, median (IQR)	25.0 (22.8-28.5)	25.4 (23.1-29.4)	25.3 (22.8-29.1)	25.6 (23.3-29.4)	25.0 (22.9-28.7)	24.8 (23.1-29.7)
NRS ≥5 (%)	174 (29.2)	39 (19.0)	206 (29.7)	37 (20.1)	123 (29.9)	26 (19.4)
Diabetes mellitus (%)	95 (15.9)	29 (14.1)	121 (17.5)	30 (16.3)	66 (16.0)	19 (14.2)
Malignancy (%)	162 (27.2)	38 (18.5)	173 (25.0)	28 (15.2)	108 (26.2)	24 (17.9)
Pre-admission dialysis (%)	6 (1.0)	0 (0)	11 (1.6)	0 (0)	6 (1.5)	0 (0)
Randomisation, late PN (%)	303 (50.8)	109 (53.2)	349 (50.4)	92 (50.0)	213 (51.7)	72 (53.7)
APACHE II, median (IQR)	31 (20-37)	27 (17-35)	32 (24-38)	30 (20-37)	33 (23-38)	29 (18-38)
Admission category (%)						
Cardiac surgery	202 (33.9)	99 (48.3)	196 (28.3)	72 (39.1)	129 (31.3)	58 (43.3)
Emergency SICU	256 (43.0)	75 (36.6)	329 (47.5)	82 (44.6)	192 (46.6)	54 (40.3)
Elective SICU	35 (5.9)	7 (3.4)	28 (4.0)	5 (2.7)	22 (5.3)	5 (3.7)
MICU	103 (17.3)	24 (11.7)	140 (20.2)	25 (13.6)	69 (16.7)	17 (12.7)
Sepsis upon admission (%)	261 (43.8)	71 (34.6)	335 (48.3)	73 (39.7)	197 (47.8)	55 (41.0)

Continued Table 1. Baseline characteristics, indicators of neuromuscular dysfunction in ICU and ICU factors of patients included in the five year mortality and morbidity analyses

	MRC-cohort		CMAP-cohort		MRC&CMAP-cohort	
	5-year mortality N=596	5-year morbidity N = 205	5-year mortality N= 693	5-year morbidity N = 184	5-year mortality N=412	5-year morbidity N=134
Indicators of neuromuscular function in the ICU						
MRC-sum score at ICU discharge	51 (47-57)	54 (48-58)	50 (46-56)	51 (47-56)	50 (46-56)	52 (47-56)
Abnormal CMAP D8±1, N (%)	300/412 (72.8)	87/134 (64.9)	523 (75.5)	121 (65.8)	300 (72.8)	87 (64.9)
ICU-related exposure variables						
Corticosteroids, days, median (IQR)	0 (0-7)	0 (0-3.5)	0 (0-9)	0 (0-6)	0 (0-10)	0 (0-6)
NMBA, days, median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
New infection (%)	306 (51.3)	85 (41.5)	445 (64.2)	107 (58.2)	252 (61.2)	72 (53.7)
Benzodiazepines, days, median (IQR)	4 (1-11)	3 (0-9)	6 (2-13)	5 (1-10)	6 (2-14)	5 (1-10)
Mechanical ventilation, days, median (IQR)	6 (2-14)	4 (1-11)	9 (5-17)	7 (3-14)	8 (4-17)	7 (2-13)
ICU stay, days, median (IQR)	12 (4-21)	9 (2-18)	14 (9-23)	12 (8-20)	14 (9-25)	12 (6-20)

Abbreviations: *BMI:* body mass index, *NRS:* nutritional risk score, *PN:* parenteral nutrition, *APACHE II:* Acute Physiology And Chronic Health Evaluation, *SICU:* Surgical Intensive Care Unit, *MICU:* Medical Intensive Care Unit, *NMBA:* neuromuscular blocking agents, *MRC:* Medical Research Council, *CMAP:* compound muscle action potential, *D:* day.

Primary outcome: five-year mortality

Within the MRC-cohort, 231/596 (38.8%) died during the 5-year follow-up (Supplementary Table 1). MRC at ICU discharge was significantly lower in non-survivors [48 (43-54)] than in survivors [54 (48-58), p<0.001]. Lower MRC was independently associated with higher 5-year mortality [HR per-point-increase: 0.946 (95%CI: 0.928-0.968), p=0.001](Table 2). In the CMAP-cohort, 328/693 (47.3%) deaths occurred within 5 years (Supplementary Table 1). Patients with abnormal CMAP on ICU day 8±1 had higher 5-year mortality as compared to patients with normal CMAP [281/523 (53.7%) versus 47/170 (27.6%), p<0.001](Figure 2) and abnormal CMAP was independently associated with increased 5-year mortality [HR: 1.568 (95%CI: 1.165-2.186), p=0.004](Table 2). When combining the clinical and electrophysiological information in the MRC&CMAP-cohort, low MRC remained independently associated with worse 5-year survival [HR: 0.956 (95%CI: 0.934-0.980), p=0.001], whereas for abnormal CMAP, this relationship was no longer significant [HR: 1.478 (95%CI: 0.875-2.838), p=0.088].

Secondary outcomes: five-year morbidity

Within the MRC-cohort, MRC at ICU discharge was significantly and independently associated with with hand-grip strength [adjusted effect size: 0.866 (95%CI: 0.237-1.527), p=0.004], 6MWD [adjusted effect size after transformation to normality: 105.1 (95%CI: 12.1-212.9), p=0.043] and PF SF-36 [adjusted effect size after transformation to normality: -0.119 (95%CI: -0.186 to -0.057), p=0.002] (Table 3). MRC at ICU discharge was also associated with most of the other morbidity outcomes (Supplementary Table 2). No univariable association was found between abnormal CMAP on ICU day 8±1 and any of the 5-year morbidity endpoints, except for hip strength and Barthel index (Supplementary Table 2).

Exploratory analyses

Assessment of the linearity of the relationship between MRC at ICU discharge and 5-year mortality and morbidity suggested an optimal threshold of MRC ≤ 55 for predicting 5-year mortality as well as 5-year hand-grip strength and 6MWD (Supplementary Figure 1). Within the MRC-cohort, MRC was ≤ 55 in 401/596 (67.3%) and >55 in 195/596 (32.7%) patients. 5-year mortality was higher in patients with MRC ≤ 55 as compared to patients with MRC > 55 [187/401 (46.6%) versus 44/195 (22.6%), p<0.001] and MRC ≤ 55 independently associated with increased 5-year mortality [HR: 1.584 (95%CI: 1.106-2.266), p=0.014] (Figure 2 and Supplementary Table 3). Combining this clinical and electrophysiological information indicated that both MRC ≤ 55 and abnormal CMAP provided additional predictive information with respect to 5-year mortality (Figure 2 and Supplementary Table 3).

Reassessment of morbidity revealed that patients with MRC≤55 at ICU discharge had worse outcomes for all 5-year morbidity endpoints (Supplemental Figure 2 and Supplementary Table 2). Indeed, patients with MRC≤55 had a 25% (or 7kg), 11% and 25-point reduction in respectively median hand-grip force, 6-MWD and PF-SF-36. These associations remained significant when adjusted for confounders (Table 3). Readmission rate was not different (Supplementary Table 4).

Table 2. Primary outcomes: Association between indicators of neuromuscular dysfunction in ICU and 5-year mortality

	HR (95% Bca CI) [b]	*P*-value
MRC-cohort		
MRC-sum score at ICU discharge, continuous variable		
Unadjusted		
MRC-sum score at ICU discharge (per point increase)	0.930 (0.915-0.945)	0.001
Adjusted [a]		
MRC-sum score at ICU discharge (per point increase)	0.946 (0.928-0.968)	0.001
CMAP-cohort		
Unadjusted		
Abnormal CMAP on day 8±1	2.406 (1.782-3.330)	0.001
Adjusted [a]		
Abnormal CMAP on day 8±1	1.568 (1.165-2.186)	0.004
MRC&CMAP-cohort		
MRC-sum score at ICU discharge, continuous variable		
Unadjusted		
MRC-sum score at ICU discharge (per point increase)	0.945 (0.926-0.967)	0.001
Abnormal CMAP on day 8±1	1.866 (1.214-3.320)	0.007
Adjusted [a]		
MRC-sum score at ICU discharge (per point increase)	0.956 (0.934-0.980)	0.001
Abnormal CMAP on day 8±1	1.478 (0.875-2.838)	0.088

[a]HR were calculated by multivariable Cox regression analyses correcting for a priori defined confounders including: age, diabetes mellitus, malignancy, preadmission dialysis, admission APACHE II-score, sepsis upon admission, ICU length-of-stay, days of in-ICU treatment with corticosteroids and neuromuscular blocking agents, and acquisition of new infection in ICU. Days of in-ICU treatment with benzodiazepines and duration of mechanical ventilation were eliminated from the model due to collinearity with ICU length-of stay;
[b]BCa: bias-corrected accelerated confidence intervals, obtained by bootstrap resample procedure (n=1000).
Abbreviations: *MRC:* Medical Research Council; *CMAP:* Compound Muscle Action Potential; *HR:* hazard ratio.

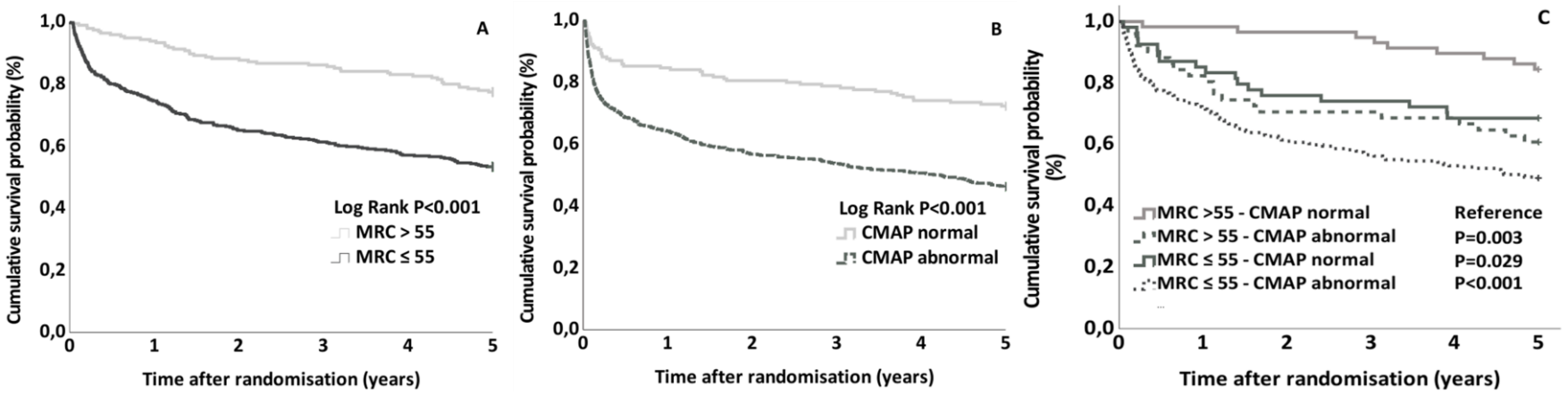

Fig. 2 Kaplan-Meier survival plots depicting the proportion of patients alive up to 5 years following ICU admission according to (A) MRC-sum score at (or close to) ICU discharge ≤55 or not (Log-rank test p<0.001) (B) CMAP D8±1 normal or abnormal (Log-rank test p<0.001) and (C) the combined information of MRC-sum score at ICU discharge ≤55 or not and CMAP D8±1 normal or abnormal (Log-rank test –as compared to normal CMAP and MRC-sum score at ICU discharge >55: for MRC>55 & abnormal CMAP: p=0.003; for MRC≤55 and normal CMAP: p=0.029; for MRC≤55 and abnormal CMAP: p<0.001). Abbreviations: *MRC:* Medical-Research-Council; *CMAP:* compound muscle action potential; *D:* day.

Table 3. Secondary outcomes: Association between indicators of neuromuscular dysfunction in ICU and primary outcomes of 5-year morbidity

	Hand-grip strength (%pred)		6MWD (%pred) [c]		PF-SF-36 [c]	
	B (95% Bca CI) [b]	*P*-value	B (95% Bca CI) [b]	*P*-value	B (95% Bca CI) [b]	*P*-value
COHORT 1: MRC-cohort						
MRC sum score at ICU discharge, continuous variable						
Unadjusted						
MRC sum score at ICU discharge (per point increase)	1.116 (0.526 - 1.714)	0.001	102.6 (-1.6 - 213.1)	0.051	-0.144 (-0.212 to -0.073)	0.001
Adjusted [a]						
MRC sum score at ICU discharge (per point increase)	0.866 (0.237 - 1.527)	0.004	105.1 (12.1 - 212.9)	0.043	-0.119 (-0.186 to -0.057)	0.002
MRC sum score at ICU discharge, dichotomous (exploratory analyses)						
Unadjusted						
MRC sum score at ICU discharge ≤55	-16.441 (-24.386 to -7.925)	0.001	-1618 (-2898.4 to -303.6)	0.013	1.700 (0.765 - 2.634)	0.002
Adjusted [a]						
MRC sum score at ICU discharge ≤55	-14.674 (-23.284 to -5.092)	0.002	-1648.2 (-2978.7 to -290.9)	0.023	1.464 (0.306 - 2.570)	0.007
COHORT 2: CMAP-cohort						
Unadjusted						
Abnormal CMAP on day 8±1	-5.921 (-14.009 - 2.483)	0.163	-171.6 (-1563.2 – 1132.0)	0.804	0.718 (-0.309 - 1.806)	0.168

[a] Regression coefficients were calculated with multivariable linear regression analyses, correcting for a priori defined confounders including: (1) for hand grip strength: demographics (age, gender, BMI), comorbidities (diabetes mellitus, malignancy, preadmission dialysis) and ICU features (maximal SOFA score, ICU length-of-stay), days of benzodiazepine treatment in ICU was omitted due to collinearity with ICU length-of-stay (2) for 6MWD: demographics (age, gender), comorbidities (diabetes mellitus, malignancy, preadmission dialysis) and ICU features (in-ICU hypoglycaemia and ICU length-of-stay). Days of in-ICU opioid treatment was omitted due to collinearity with ICU length-of-stay (3) for PF-SF-36: demographics (age, gender), comorbidities (diabetes mellitus, malignancy) and ICU features (duration of treatment with inotropics and/or vasopressors and ICU length-of-stay). Duration of in-ICU treatment with benzodiazepines was omitted due to collinearity with duration of ICU stay. Preadmission dialysis was dropped from analysis as no patients in the studied cohorts received dialysis prior to admission[b] *BCa* bias-corrected accelerated confidence intervals, obtained by bootstrap resampling procedure (n=1000) [c] In order to obtain adequate model fit, the 6MWD data were transformed to power 2 and PF-SF-36 scores were reversed (100 minus actual value) and subsequently transformed to power 0.54.

Abbreviations: *MRC:* Medical Research Council; *CMAP:* Compound Muscle Action Potential; *6MWD:* six minute walk distance; *PF-SF-36:* Physical Function domain of the SF-36 quality of life questionnaire.

Sensitivity analyses

The proportional hazard assumption was violated for MRC, indicating that the association of MRC at ICU discharge per point decrease with 5-year mortality attenuated over time (Supplementary Table 5). However, linear modelling of MRC may not have been optimal as indicated by the Martingale residual plot. Indeed, the association of MRC≤55 with mortality persisted throughout the 5-year observation period. The effect of abnormal CMAP on ICU day 8±1 on 5-year mortality also remained constant during the 5-year period.

DISCUSSION

The primary outcome of this 5-year follow-up study of patients systematically screened in ICU for neuromuscular dysfunctions showed that both more pronounced loss of strength, as measured with the MRC sum score at ICU discharge, as well as abnormal CMAP assessed after 1 week of intensive care were independently associated with higher 5-year mortality. Secondary outcomes showed that, among survivors, more pronounced loss of strength at ICU discharge but not abnormal CMAP assessed after 1 week of intensive care independently associated with poor 5-year morbidity. Furthermore, exploratory analyses indicated that even mildly reduced strength (MRC ≤ 55) may identify patients with worse long-term outcomes. These data support that neuromuscular complications of critical illness impact long-term survival as well as physical function.

Neuromuscular complications of critical illness, diagnosed clinically or with electrophysiology, relate with mortality in the acute hospitalization phase [3, 21-24], up to 90 days [25], and 1 year [3, 4]. Beyond this time frame, few data are available. We found a 4.4% decrease in the risk of death within 5 years per point increase in MRC-sum score. This is remarkably similar to the findings of Dinglas et al. in ARDS patients [5] and extends these findings to a general ICU population. While in the acute setting MRC<48 is a well-validated cut-off, differentiating populations with distinct clinical outcomes [3, 21, 26], Dinglas et al. showed that the effect of MRC < 48 at hospital discharge on 5-year mortality attenuated over time and was no longer significant at 5 years [5]. Our study provides additional exploratory data indicating that dichotomizing patients at an MRC of 55 at ICU discharge best describes the relationship between strength and 5-year mortality. This cut-off indeed defined a group of patients in whom the increased mortality risk persisted throughout the 5-year follow-up period. Hence, our data suggest that even a slightly submaximal MRC-sum score is prognostically detrimental. We also further extend on our previous data, indicating that CMAP on day 8±1 independently related with increased 1-year mortality, and expand these findings up to 5-years follow-up. The excess mortality in patients with abnormal CMAP is in the same order of magnitude as for patients with MRC ≤ 55 and therefore provides similar prognostic information if MRC is not available. Moreover, and similar to the findings at 1 year [4], both MRC and CMAP provided complementary information on 5-year mortality. These data suggest that neuromuscular complications of critical illness are a major contributor to the increased long-term mortality in critically ill patients and stress the relevance of both clinical as well as simple electrophysiological evaluation.

We further demonstrated an independent relationship between strength at ICU discharge and 5-year morbidity, including persisting weakness, reduced functional capacity and physical function. These findings are consistent with the widely accepted paradigm that ICUAW contributes to long-term disabilities. Recent studies showed that 6 months following ICU discharge, ICUAW independently related

with physical function [27] and physical limitations persisted up to 1 year in patients with ICUAW [28]. Previous work identified age, comorbidities and number of organ failures as risk factors for an episode of strength decline during 5 years following ARDS [8]. Nevertheless, recovery of the MRC-sum score above the generally accepted threshold defining ICUAW mostly occurs within 12 to 24 months [11, 29-31]. In the small percentage of patients with persisting weakness following ICU discharge, unadjusted analyses showed a correlation with activity limitation and reduced physical function up to 2 years [29]. The MRC cut-off of 48 may be insufficient to capture subtle changes in strength after the acute phase of critical illness and we show that strength at ICU discharge, even if mildly reduced, remains independently associated with morbidity up to five years post-ICU. Indeed, patients with MRC ≤ 55, compared to MRC > 55 at ICU discharge, exhibited reductions in hand-grip force, 6MWD and PF-SF-36, exceeding minimal important clinical differences (MICD respectively 5-6.5 kg [32], 3-5% [33], and 5 point reduction [16, 34], respectively.

Unexpectedly and in contrast with the mortality data, we could not confirm an independent relationship between CMAP on day 8 and 5-year morbidity outcomes. In-ICU electrophysiological abnormalities are related with short-term morbidity, including prolonged duration of mechanical ventilation, ICU and hospital stay and physical impairments prior to hospital discharge [4, 22, 23, 35]. Data on the relationship between electrophysiological abnormalities and long-term outcomes are confined to small case series, describing delayed rehabilitation, disability and persistent motor handicap months to years following ICU admission in patients with abnormal electrophysiology in the ICU [35-38]. In this large series, we could not confirm a 5-year functional impact of electrophysiological abnormalities 8±1 days following ICU admission. A first explanation could be our focus on abnormal CMAPs 8 days following ICU admission, as based on our previous findings [4]. It remains to be explored whether other electrophysiological characteristics or different timing relate with the 5-year morbidity. Though we cannot exclude lack of power, these findings confirm that electrophysiological information and strength assessment are not commutable [4, 23, 39]. These data underscore the need to unravel differential pathophysiological mechanisms involved in the clinical and electrophysiological phenotypes of neuromuscular involvement during critical illness and to explore the hypothesis that reduced CMAP may be an epiphenomenon, marking other non-neuromuscular derangements, causally associated with increased mortality.

This study has several strengths. To the best of our knowledge, this is the largest, prospective 5-year mortality and morbidity follow-up study in patients systematically assessed for neuromuscular complications of critical illness with clinical and electrophysiological screening in ICU. As patients were enrolled in an RCT, data were of high quality. This study has potential limitations. First, given the unpredictable nature of ICU admission, we do not have baseline strength measurements and, for feasibility purposes, no admission electrophysiology was performed. Second, also for practical reasons, electrophysiology did not include direct muscle stimulation to differentiate between critical illness polyneuropathy and myopathy. As myopathy may recover faster and more complete than neuropathy [38, 40-42], we cannot exclude a differential impact of both entities on 5-year outcomes. Third, as mortality data were collected from the national registry, we could not provide information on the cause of death. Fourth, obviously, morbidity analyses were limited to survivors and we excluded patients with disabilities potentially confounding morbidity endpoints, which may have introduced selection bias. Fifth, according to recent guidelines [20], we adjusted analyses for confounders identified through a systematic literature search. We cannot exclude unmeasured confounding. Sixth, as this is a single observational study, no definite causal conclusion can be drawn and the MRC threshold should be further validated.

Finally, generalizability may be limited due to study of an RCT population. Also, subgroup analyses (eg sepsis and SICU patients) would be interesting for further studies.

We conclude that the impact of neuromuscular complications of critical illness extends well beyond the acute phase and associates with 5-year outcomes, confirming the long-standing hypothesis of its role in the legacy of critical illness. We demonstrated that poor strength at ICU discharge independently associated with 5-year mortality and morbidity. Furthermore, even mildly reduced strength at ICU discharge (MRC ≤ 55) independently related with worse 5-year outcomes. Reduced CMAP documented after 1 week of intensive care independently associated with worse 5-year mortality but not morbidity. Our findings are important as they describe the population, which should be targeted in future studies, attempting to reduce the burden of critical illness. Meanwhile, our data underscore that lower strength at ICU discharge, even if only mildly reduced, and CMAP on day 8, may provide guidance for clinicians towards prognosis and may assist in targeting post-ICU services.

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SUPPLEMENTARY MATERIALS

Supplementary methods

Study design and participants

This was a sub-analysis of a prospective 5-year follow-up study, involving 883 EPaNIC (Clinical trials.gov:NCT00512122) patients who received neuromuscular evaluation while in ICU. The EPaNIC trial was a large, randomised controlled trial (RCT) performed in 7 medical/surgical ICUs of the University Hospitals Leuven and Jessa Hospitals Hasselt, comparing early (within 48 hours) with late (not before day 8) parenteral supplementation of insufficient enteral nutrition in critically ill patients [1]. As part of EPaNIC and to investigate effects of the intervention on neuromuscular outcomes, 730 patients received electrophysiological screening weekly from day 8±1 onwards [2]. In 698 of these patients, CMAP evaluation was technically feasible. Furthermore, 600 patients were clinically evaluated for weakness with the MRC-sum score from day 8 onwards, 3 times weekly from awakening until ICU discharge or death [3, 4]. For both electrophysiology and strength assessment, we included long-stayers at risk for neuromuscular complications, as well as a randomly selected subgroup of short-stayers who were assessed on the ward at day 8±1. CMAP was quantified for 1 motor nerve of upper and lower limbs (standardly, the tibial and median nerves, or alternatively the peroneal and ulnar nerves) and classified as "abnormal" when below the limit of normal in both nerves of both limbs. Based on reference values generated in the KU Leuven electrophysiology laboratory, CMAP was classified as below the limit of normal: for the median nerve when <6000 μV, for the ulnar nerve when <4500 μV, for the peroneal nerve when <1000 μV and for the tibial nerve when <2500 μV [2].

As part of the prospective post-EPaNIC follow-up study, 5-year mortality was assessed for all EPaNIC patients, whereas 5-year morbidity was assessed for survivors during hospital or home visits from June 2012 onwards [5]. Exclusion criteria included the inability to walk without assistance prior to ICU admission, pre-existing neuromuscular disease, other pre-ICU disabilities potentially confounding the morbidity endpoints, and refusal for participation [5]. Hence, for the here presented sub-analyses involving patients with in-ICU neuromuscular evaluation, we define three populations of interest, comprising the 'MRC-cohort', the 'CMAP-cohort', and the overlapping cohort who received both assessments, further referred to as 'MRC&CMAP-cohort' (Figure 1).

Outcomes

To investigate long-term outcomes in relation to ICU-acquired neuromuscular dysfunctions, we defined all-cause 5-year mortality as the primary endpoint. Mortality data were obtained from the national registry. We further assessed the association of ICU-acquired neuromuscular dysfunctions with 5-year morbidity, with three distinct measures of physical function as secondary endpoints. These included hand-grip strength (HGF, %predicted), six-minute-walk-distance (6-MWD, %predicted), and the physical function of the SF-36 quality-of-life questionnaire (PF SF-36, range 0-100, higher values indicating better scores) at 5-years follow-up. Other outcomes comprised extensive evaluation of peripheral muscle strength, with the MRC-sum score and hand-held dynamometry of the muscle groups involved in the MRC-sum score, as well as respiratory muscle strength as assessed by maximal inspiratory pressure [6]. Additionally, we assessed the Physical and Mental Component Score (PCS and MCS) of the SF-36 [7, 8] and Barthel-index [9, 10] (range 0-20, higher scores indicating higher degree of physical independence).

Additional exploratory analyses involved the assessment of the linearity of the relationship between the MRC-sum score and the primary outcomes and, if appropriate, the identification of the optimal threshold for the MRC-sum score to predict 5-year outcomes.

Statistics

Descriptive statistics included median and interquartile ranges for continuous variables and numbers and percentages for categorical variables. Continuous data were compared with Mann-Whitney-U test and categorical variables with Chi-square test or Fisher-exact test, as appropriate.

Study of the independent association between ICU-acquired neuromuscular dysfunction and 5-year mortality

We explored in unadjusted analyses, whether last MRC at (or close to) ICU discharge (further referred to as 'MRC at ICU discharge') and abnormal CMAP on day 8±1 in ICU were associated with 5-year mortality in respectively the MRC- and CMAP-cohorts. Hazard ratios for both predictors were calculated with univariable Cox-regression analyses. MRC-sum score was entered as a continuous variable [11]. For CMAP, data were visualised with Kaplan-Meier plots. If a univariable association was present between MRC or abnormal CMAP and 5-year mortality, adjusted hazard ratios were calculated for the cohort(s) of interest by adding literature-based, a priori defined confounders to the models[12]. The search strategy and results are presented below. Relevant confounders included age [3, 5, 13-21], comorbidities (diabetes mellitus, malignancy, preadmission dialysis) [5, 15, 18-20], admission APACHE II score [3, 5, 14-16, 18-20, 22-26], sepsis upon admission [3, 16, 20, 25, 26], duration of ICU-stay [3, 5, 14, 18, 19, 27], days of treatment with corticosteroids [3, 5, 13, 24, 26, 28], neuromuscular blocking agents [3, 5, 13, 26, 29], benzodiazepines [5], duration of mechanical ventilation [5, 15, 17, 24], and acquisition of a new infection while in the ICU [3, 5]. Prior to entering the variables as covariates to the models, collinearity was checked and judged problematic if variance inflation factor >5 or tolerance <0.2. Robust estimations of confidence intervals were created through bootstrap resampling (n= 1000).
If both predictors showed an independent association with five-year mortality, the added value of the combined information of MRC and CMAP was assessed within the MRC&CMAP-cohort.

Study of the independent association between ICU-acquired neuromuscular dysfunction and 5-year morbidity

The association between the MRC-sum score at ICU discharge and CMAP on ICU day 8±1 with the morbidity endpoints was explored in unadjusted analyses and was restricted to survivors. No imputation was performed for non-survivors. For the MRC-sum score, this was performed with linear regression analyses. If necessary, the morbidity endpoints were transformed to obtain adequate model fit[5]. This process involved checking linearity assumptions (linear relationship between variables, homoscedasticity and normal distribution of error terms). If these were not met, transformations were attempted. The Box-Cox algorithm for power transformations [30-32] was applied and when transformation did not result in satisfactory linear model diagnostics, Spearman's ranked correlation coefficient was used to express the relationship between the morbidity outcome of interest and MRC at ICU-discharge. For CMAP, outcomes for patients with normal and abnormal values were compared with Mann-Whitney-U or Fisher-exact test, as appropriate. If a univariable association was documented, multivariable models were constructed for each of the cohorts of interest. Again, these analyses included literature-based, a priori defined confounders, identified through a systematic literature search, as covariates. The search strategy and results are presented below. Confounders comprised of (1) for hand grip strength: demographics (age [5, 11, 33, 34], gender [24], BMI [5]), comorbidities (diabetes mellitus, malignancy, preadmission dialysis)[5, 33], and ICU-features (in-ICU treatment with benzodiazepines[5], maximal SOFA score in ICU [33], ICU length-of-stay [11, 34]; (2) for 6-MWD: demographics (age [33, 35], gender [24]), comorbidities (diabetes mellitus, malignancy, preadmission dialysis) [5, 33, 35], and ICU-features (in-ICU hypoglycaemia [5], in-ICU treatment with opioids [5], and ICU length-of-stay [5, 11]);(3) for PF SF-36: demographics (age [33, 35], gender [24]), comorbidities (diabetes mellitus, malignancy, preadmission dialysis) [5], and ICU features (in-ICU treatment with benzodiazepines [5], inotropics and/or vasopressors [5], and ICU length-of-stay [5, 11]).

Prior to entering variables as covariates to the models, collinearity was checked and judged problematic if variance inflation factor >5 or tolerance <0.2. Robust estimations of confidence intervals were created through bootstrap resampling (n= 1000).
If appropriate, further analyses on the MRC&CMAP-cohort were performed to explore any additional information provided by the combination of data.

Exploratory analyses for defining the optimal threshold of MRC upon ICU discharge for prediction of 5-year outcomes

We anticipated that linear modelling of the MRC-sum score may not be optimal and we hypothesised that the optimal MRC threshold for predicting long-term outcomes might not be identical to the well-established cut-off of 48 (and 36), associating with ICU, hospital and 1-year outcomes. Hence, assessment of the linearity of the relationship between last ICU MRC and 5-year outcomes was performed with martingale residual plots with Local regrESSion (LOESS) lines (for survival analysis) and scatterplots of the outcome versus MRC sum score with LOESS lines (for the morbidity outcomes). If appropriate, these plots were used to define an optimal threshold for MRC-sum score for the 5-year outcomes and mortality and morbidity multivariable analyses were repeated with this binary factor.

Sensitivity analyses

The proportional hazard assumption was checked for each variable in each of the Cox-regression models with use of the Schoenfeld residuals test. If appropriate, sensitivity analyses were performed by adding all factors for which the assumption was violated as time-dependent co-variates.
Analyses were performed with SPSS version 25 (IBM corporation) and R version 3.6.1.
Two-sided p-values ≤0.05 were considered statistically significant.

Search strategy to identify known confounders of the relationship between ICU-acquired neuromuscular dysfunctions and 5-year mortality and morbidity.

The Medline database was searched through Ovid and PubMed platforms for full-text, human studies, English language original research articles (prospective as well as retrospective observational design, randomized controlled trials) of patients admitted to an intensive care unit, reporting on the association between demographic factors, co-morbidities and ICU treatments and events with 5-year outcomes. Systematic reviews and meta-analyses retrieved with the search were screened for referenced original research articles.
We used Mesh terms and additional exploded searches centering around key concepts ‘critical illness’, ‘intensive care unit acquired neuromuscular dysfunctions’, ‘potential confounders’ and ‘mortality/morbidity outcomes’. All used Mesh terms and additional search terms (using relevant entry terms based on the tree built on each Mesh-term) are listed below. We included papers published within the past 10 years previous to October 2019. Due to limited available evidence on 5-year morbidity outcomes, we included studies with shorter follow-up (up to one year post-discharge) for the long-term morbidity association.
A confounder was defined as a factor independently associated with both ICU-acquired neuromuscular dysfunction, and long-term outcome in ICU-patients [36]. Confounders identified are provided in Supplementary Table 6.

5-year mortality

Pubmed search

((("Critical Illness"[Mesh] OR "Critical Care"[Mesh] OR "Intensive Care Units"[Mesh] OR "Acute Disease"[Mesh] OR "Shock"[Mesh] OR "Systemic Inflammatory Response Syndrome"[Mesh] OR "Multiple organ failure"[Mesh] OR critical-illness* OR critical-care* OR intensive-care* OR acute-disease* OR shock* OR Systemic-Inflammatory-Response-Syndrome* OR multiple-organ-failure*)) AND ("Polyneuropathy"[Mesh] OR "Paresis"[Mesh] OR "Muscle weakness"[Mesh] OR "Muscle strength"[Mesh] OR "Electromyography"[Mesh] OR polyneuropath* OR paresis* OR muscle-

weakness* OR muscle-strength* OR neuromuscular-inexcitabilit* OR electromyography* OR neuromuscular-inexcitability* or muscle-wasting*)) AND ("Length of Stay"[Mesh] OR "Severity of illness index"[Mesh] OR "Simplified acute physiology score"[Mesh] OR "APACHE"[Mesh] OR "Artificial respiration"[Mesh] OR "Neuromuscular blocking agents"[Mesh] OR "Kidney, artificial"[Mesh] OR "Vasoconstrictor agents"[Mesh]OR "Comorbidity"[Mesh] OR "Body mass index"[Mesh] OR length-of-stay*or severity-of-illness-index* OR simplified-acute-physiology-score* OR SOFA-score* OR APACHE* OR comorbidit* OR multimorbidit* OR gender* OR age* OR body-mass-index* or artificial-respiration* OR mechanical-ventilation* OR neuromuscular-blocking-agent* OR artificial-kidney*OR hemodialyser*OR renal-dialysis* OR blood-dialyser*OR vasoconstrictor*OR vasopress* OR vasoactive-agonist*)) AND ("Critical care outcomes"[Mesh] OR "Mortality"[Mesh] OR *mortality* OR critical-care-outcome*OR follow-up* OR survival*)))

Ovid search search

Critical Illness OR Critical Care OR Intensive Care Units OR Multiple organ failure AND Polyneuropathy OR Paresis OR Muscle weakness OR Muscle strength OR Electromyography OR neuromuscular-inexcitability or muscle-wasting AND Critical care outcomes OR Mortality OR critical-care-outcome OR follow-up OR survival

5-year morbidity

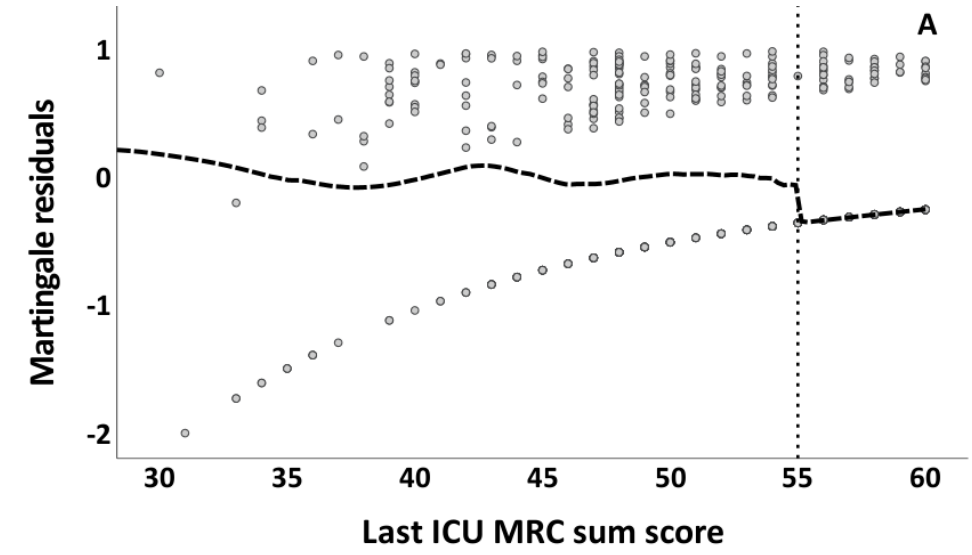
Pubmed search

(((("Critical Illness"[Mesh] OR "Critical Care"[Mesh] OR "Intensive Care Units"[Mesh] OR "Shock"[Mesh] OR "Systemic Inflammatory Response Syndrome"[Mesh] OR "Multiple organ failure"[Mesh] OR critical-illness* OR critical-care* OR intensive-care* OR shock* OR Systemic-Inflammatory-Response-Syndrome* OR multiple-organ-failure*) AND ("Polyneuropathy"[Mesh] OR "Paresis"[Mesh] OR "Muscle weakness"[Mesh] OR "Muscle strength"[Mesh] OR "Electromyography"[Mesh] OR polyneuropath* OR paresis* OR muscle-weakness* OR muscle-strength* OR neuromuscular-inexcitabilit* OR electromyography*[tiab] OR neuromuscular-inexcitability* or muscle-wasting*)) AND ("Morbidity"[Mesh] OR "Muscle strength"[Mesh] OR "Hand strength"[Mesh] OR "Pinch strength"[Mesh] OR "Walk test"[Mesh] OR "Activities of Daily Living"[Mesh] OR "Quality of life"[Mesh] OR morbidit* OR muscle-strength* OR hand-strength* OR pinch-strength* OR 6-min-walk-test*OR activities-of-daily-living* OR ADL* OR barthel-index* OR quality-of-life* or SF36* OR physical-component-score*))

Ovid search

Critical Illness OR Critical Care OR Intensive Care Units OR Multiple organ failure AND Polyneuropathy OR Paresis OR Muscle weakness OR Muscle strength OR Electromyography OR neuromuscular-inexcitability or muscle-wasting AND Critical care outcomes OR Morbidity OR Muscle strength OR Walk test OR Quality of life OR Activities of Daily Living OR Barthel-index OR SF36

Supplementary figures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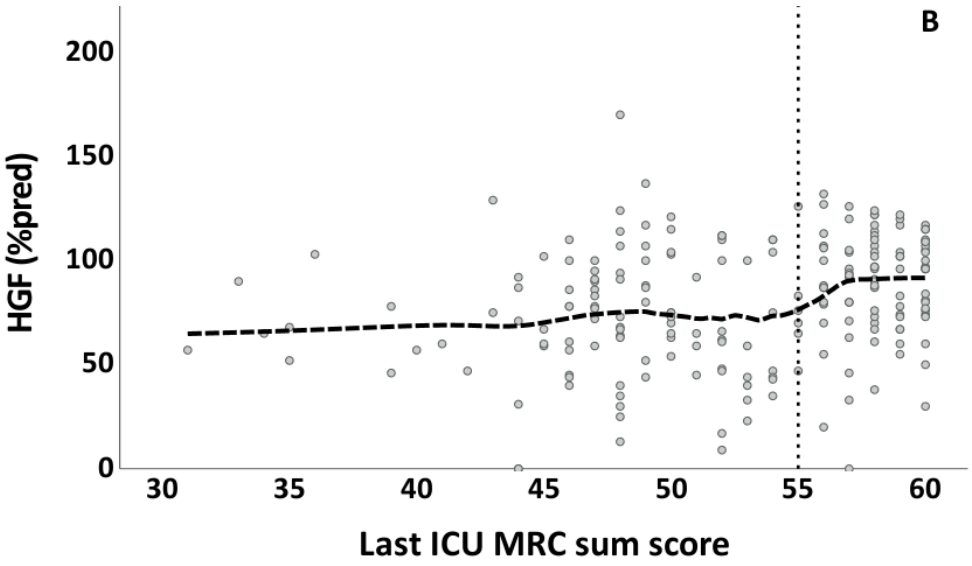
Last ICU MRC sum score


B
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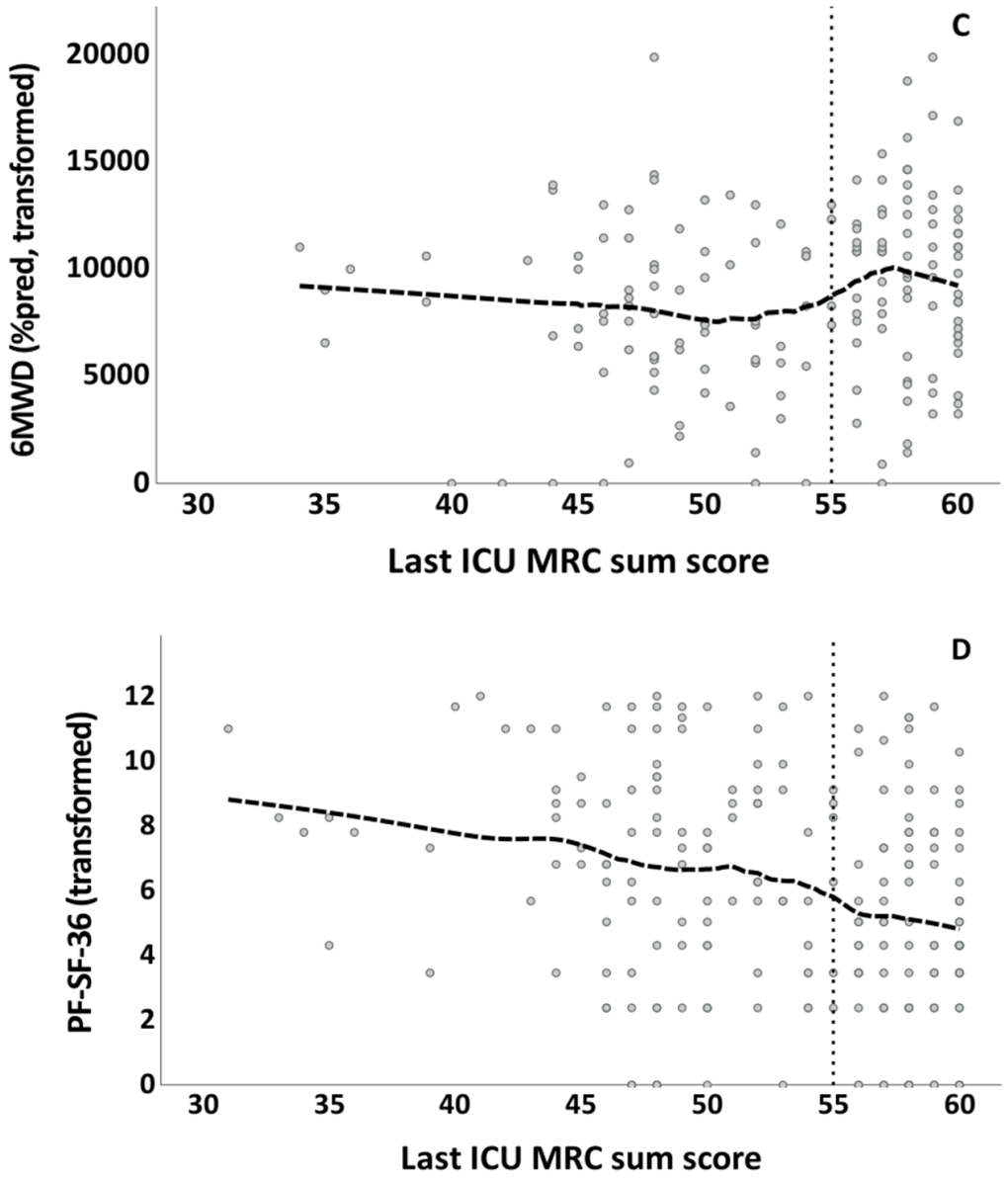
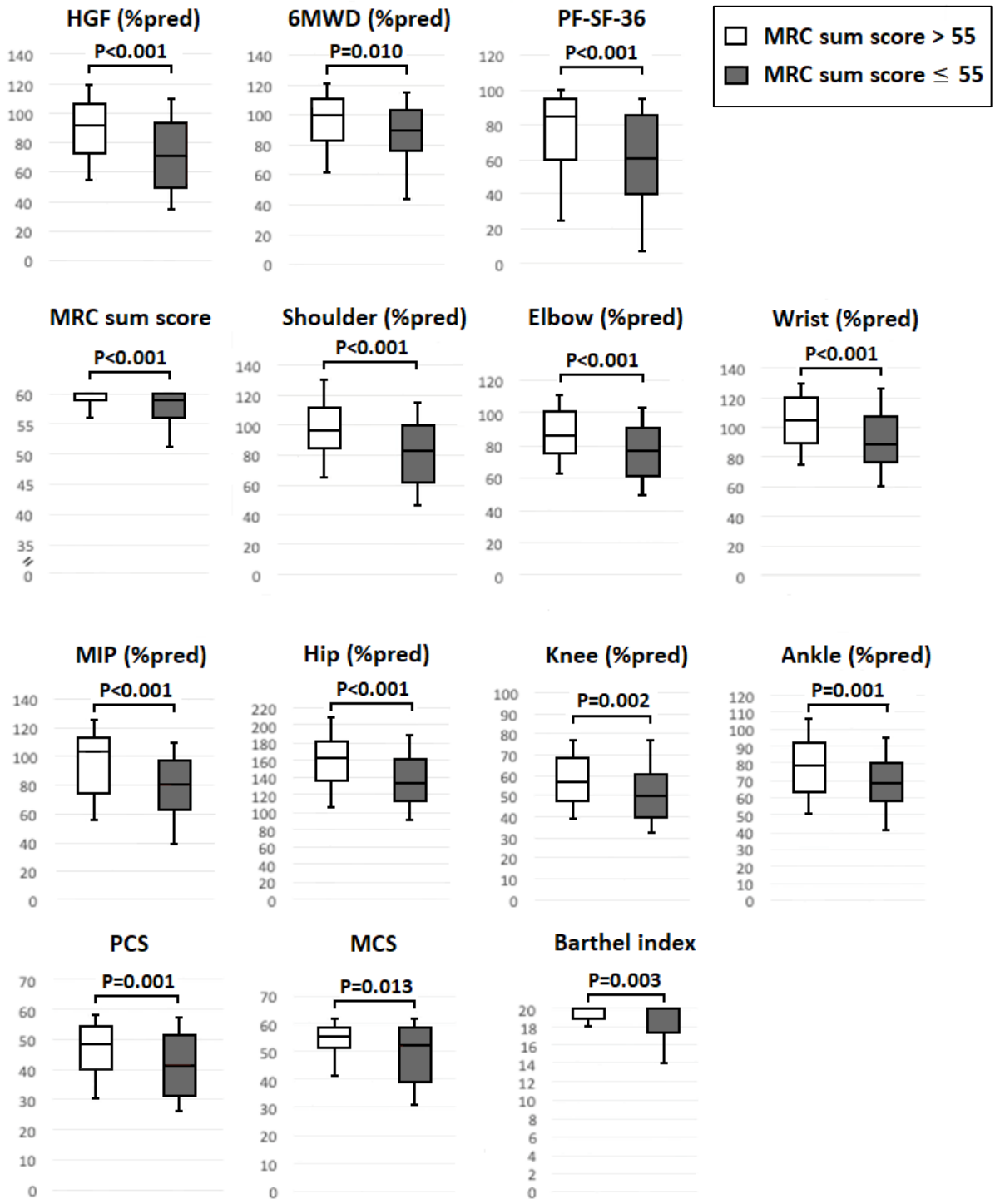
Last ICU MRC sum score



Suppl. Fig. 1: Exploration of the linearity assumption for MRC-sum score at ICU discharge and 5-year mortality and morbidity outcomes. *Panel A:* LOESS-smoother on the Martingale residuals plot for 5-year mortality by MRC-sum score at ICU discharge, indicating a possible cut-off at approximately 55; *Panel B:* LOESS-smoother on the scatter plot for hand-grip strength at 5-year follow-up (% predicted) by MRC-sum score at ICU discharge, indicating a possible cut-off at approximately 55; *Panel C:* LOESS-smoother on the scatter plot for transformed values of PF-SF36 at 5-year follow-up by MRC-sum score at ICU discharge, indicating a linear relationship; *Panel D:* LOESS-smoother on the scatter plot for transformed values of 6-MWD at 5-year follow-up by MRC-sum score at ICU discharge, indicating a possible cut-off at approximately 55. The following transformations were performed: the 6-MWD data were transformed to power 2 and the PF SF-36 were reversed (100 minus actual value) and subsequently transformed to power 0.54 (higher transformed values of PF SF-36 correspond to lower actual values of PF SF-36).



Suppl. Fig. 2: Morbidity outcomes according to MRC-sum score at ICU discharge with a cut-off at 55 (exploratory analyses). *Panel A:* handgrip-strength (HGF, % predicted), 6-minute walk distance (6-MWD, % predicted) and Physical function of the SF-36 (PF SF-36, range 0-100 with higher values indicating better scores); *Panel B:* measures of peripheral muscle strength: Medical-Research-Council (MRC) sum-score, hand-held dynamometry for shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension, ankle dorsiflexion (% predicted), and respiratory muscle strength (maximal inspiratory pressure, MIP), Physical (PCS) and Mental Component score (MCS) of the SF-36 questionnaire and Barthel index (range 0-20, higher values indicating better scores). P- values were obtained by Mann-Whitney U tests. No patient required home ventilation.

Supplementary tables

Supplementary Table 1. Baseline characteristics, ICU factors and outcomes of patients included in the five year mortality analyses, MRC cohort compared to CMAP cohort

	MRC-cohort (N=596)	CMAP cohort (N=693)	*P*-value[a]
Baseline factors			
Age, median (IQR)	63.4 (52.7-73.1)	64.1 (53.8-74.2)	0.256
Gender, male (%)	352 (59.1)	439 (63.3)	0.115
BMI, median (IQR)	25.0 (22.8-28.5)	25.3 (22.8-29.1)	0.696
NRS ≥5 (%)	174 (29.2)	206 (29.7)	0.835
Diabetes mellitus (%)	95 (15.9)	121 (17.5)	0.466
Malignancy (%)	162 (27.2)	173 (25.0)	0.365
Pre-admission dialysis (%)	6 (1.0)	11 (1.6)	0.362
Randomisation, late PN (%)	303 (50.8)	349 (50.4)	0.864
APACHE II, median (IQR)	31 (20-37)	32 (24-38)	0.001
Admission category (%)			0.039
Cardiac surgery	202 (33.9)	196 (28.3)	
Emergency SICU	256 (43.0)	329 (47.5)	
Elective SICU	35 (5.9)	28 (4.0)	
MICU	103 (17.3)	140 (20.2)	
Sepsis upon admission (%)	261 (43.8)	335 (48.3)	0.102
ICU-related exposure variables			
Corticosteroids, days, median (IQR)	0 (0-7)	0 (0-9)	0.108
NMBA, days, median (IQR)	0 (0-1)	0 (0-1)	<0.001
New infection (%)	306 (51.3)	445 (64.2)	<0.001
Benzodiazepines, days, median (IQR)	4 (1-11)	6 (2-13)	<0.001
Mechanical ventilation, days, median (IQR)	6 (2-14)	9 (5-17)	<0.001
ICU stay, days, median (IQR)	12 (4-21)	14 (9-23)	<0.001
Five-year outcomes			
Mortality (%)	231 (38.8)	328 (47.3)	0.002
HGF, % pred (IQR)	80 (60.5-103)	82 (60.5-99.3)	0.646
6MWD, %pred (IQR)	93 (77.5-107)	91 (73.5-104)	0.114
PF-SF36 (IQR)	72.5 (45-90)	70 (35-85)	0.142

[a]Comparisons were made stringently by use of independent samples Mann-Whitney-U test, as these cohorts do not completely overlap.

Abbreviations: *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *SICU:* Surgical Intensive Care Unit; *MICU:* Medical Intensive Care Unit; *NMBA:* neuromuscular blocking agents; *MRC:* Medical Research Council; *CMAP:* compound muscle action potential; *D:* day.

Supplementary Table 2. Association between indicators of neuromuscular dysfunctions in ICU and other 5-year morbidity outcomes [a]

	MRC-cohort					CMAP-cohort		
5-year outcomes	**MRC-sum score at ICU discharge, continuous variable**		**MRC-sum score at ICU discharge, dichotomized variable (exploratory analyses)**					
	B or correlation coefficient (95% Bca CI) [d]	***P*-value**	**Last MRC sum score in-ICU ≤55[b]**	**Last MRC sum score in-ICU >55 [b]**	***P*-value**	**CMAP D8±1 abnormal [b]**	**CMAP D8±1 normal [b]**	***P*-value**
MRC-sum score	0.290 (0.162-0.408)[c]	<0.001	59 (56-60)	60 (59-60)	<0.001	60 (57-60)	60 (56-60)	0.872
HHD (%pred)								
Shoulder	1.359 (0.731-1.949)	0.001	83.0 (63.0-101.0)	96.0 (84.2-111.7)	<0.001	84.0 (62.0-105.0)	87.0 (71.0-106.5)	0.257
Elbow	0.949 (0.509-1.415)	0.001	77.0 (61.5-91.5)	86.0 (75.5-100.5)	<0.001	77.0 (60.2-92.0)	78.0 (65.0-101.0)	0.185
Wrist	1.059 (0.609-1.545)	0.001	89.0 (77.0-109.0)	104.0 (90.5-118.5)	<0.001	91.0 (75.0-110.0)	95.0 (77.0-111.0)	0.535
Hip	2.183 (1.404-3.001)	0.001	134.0 (113.2-160.7)	162.5 (136.7-181.7)	<0.001	133.5 (109.5-153.7)	148.0 (129.0-174.0)	0.006
Knee	0.726 (0.416-1.044)	0.001	50.0 (39.7-60.5)	57.0 (48.0-68.0)	0.002	51.0 (40.0-62.0)	52.0 (42.0-62.0)	0.963
Ankle	0.856 (0.382-1.372)	0.001	68.0 (58.0-80.0)	78.5 (63.5-91.7)	0.001	69.0 (57.0-81.0)	72.0 (58.7-85.0)	0.297
MIP (%pred)	1.315 (0.624-1.953)	0.001	80.0 (63.0-97.0)	103.0 (74.0-113.0)	<0.001	84.0 (61.0-105.0)	91.0 (68.5-108.2)	0.258
PCS	0.428 (0.190-0.670)	0.001	41.9 (32.6-51.6)	48.9 (40.5-54.7)	0.001	42.0 (32.1-52.3)	46.9 (36.3-52.6)	0.144
MCS	0.115 (-0.033-0.265)[c]	0.108	52.6 (39.7-58.6)	55.4 (50.5-58.6)	0.013	53.0 (44.0-58.7)	54.1 (46.3-58.4)	0.678
Barthel index	0.251 (0.119-0.371)[c]	<0.001	20.0 (17.7-20.0)	20.0 (19.7-20.0)	0.003	20.0 (17.0-20.0)	20.0 (19.0-20.0)	0.020

[a]unadjusted analyses; [b]data are provided as median (IQR);

[c]Because of violation of linearity assumptions and inability to produce satisfactory transformation, Spearman's rank correlation coefficients are provided;

[d]*BCa* bias-corrected accelerated confidence intervals were obtained through bootstrap resampling (n=1000).

Abbreviations: *MRC:* Medical Research Council; *HHD:* hand held dynamometry; *MIP:* maximal inspiratory pressure; *PCS:* Physical Component Score; *MCS:* Mental Component Score.

Supplementary Table 3. Association between indicators of neuromuscular dysfunction in ICU and 5-year mortality, exploratory analyses

	HR (95% Bca CI) [b]	*P*-value
MRC-cohort		
MRC-sum score at ICU discharge, dichotomous		
Unadjusted		
MRC-sum score at ICU discharge ≤55	2.573 (1.917-3.401)	0.001
Adjusted [a]		
MRC-sum score at ICU discharge ≤55	1.584 (1.106-2.266)	0.014
MRC&CMAP-cohort		
MRC-sum score at ICU discharge, dichotomous		
Unadjusted		
(MRC-sum score at ICU discharge *>55 and normal CMAP as a reference)*		
MRC-sum score at ICU discharge >55 and abnormal CMAP	3.037 (1.439-8.449)	0.002
MRC-sum score at ICU discharge ≤55 and normal CMAP	2.340 (1.090-6.209)	0.031
MRC-sum score at ICU discharge ≤55 and abnormal CMAP	4.491 (2.492-12.528)	0.001
Adjusted [a]		
(MRC-sum score at ICU discharge *>55 and normal CMAP as a reference)*		
MRC-sum score at ICU discharge >55 and abnormal CMAP	2.477 (1.196-6.699)	0.014
MRC-sum score at ICU discharge ≤55 and normal CMAP	1.878 (0.861-4.669)	0.090
MRC-sum score at ICU discharge ≤55 and abnormal CMAP	2.707 (1.543-7.164)	0.003

[a]HR were calculated by multivariable Cox regression analyses correcting for a priori defined confounders including: age, diabetes mellitus, malignancy, preadmission dialysis, admission APACHE II-score, sepsis upon admission, ICU length-of-stay, days of in-ICU treatment with corticosteroids and neuromuscular blocking agents, and acquisition of new infection in ICU. Days of in-ICU treatment with benzodiazepines and duration of mechanical ventilation were eliminated from the model due to collinearity with ICU length-of stay;
[b]BCa: bias-corrected accelerated confidence intervals, obtained by bootstrap resample procedure (n=1000).
Abbreviations: *MRC:* Medical Research Council; *CMAP:* Compound Muscle Action Potential; *HR:* hazard ratio.

Supplementary Table 4. Readmissions according to MRC-sum score at ICU discharge

	Last MRC≤55 N=114	Last MRC>55 N=91	*P*-value
Total N of patients with readmission[a], N (%)	71 (62.3)	48 (52.7)	0.169
N of patients readmitted[a] to the ICU, N (%)	21 (18.4)	9 (9.9)	0.086
Total N of readmissions[a] per patient, median (IQR)	1 (0-2)	1 (0-2)	0.093
N of readmissions[a] to the ICU per patient, median (IQR)	0 (0-0)	0 (0-0)	0.092

[a]Readmissions were defined as stay in the index hospital for ≥24h between hospital discharge and the 5-year follow-up visit. Abbreviations: *MRC:* Medical Research Council; *N:* number; *IQR:* interquartile range.

Supplementary Table 5. Multivariable Cox-regression model for 5-year mortality including time-dependent covariates for variables with changing hazards over time

	HR (95% CI) [a]	*P*-value
MRC-cohort		
Model with MRC-sum score at ICU discharge as a continuous variable[b]		
MRC-sum score at ICU discharge, baseline hazard (per point increase)	0.924 (0.905-0.943)	<0.001
MRC-sum score at ICU discharge, time dependent covariate (per year)	1.026 (1.013-1.039)	<0.001
Model with MRC-sum score at ICU discharge as a dichotomous variable (exploratory analyses)[c]		
MRC-sum score at ICU discharge ≤ 55	1.578 (1.098-2.270)	0.014
CMAP-cohort		
Abnormal CMAP D8±1 [d]	1.589 (1.152-2.192)	0.005
MRC&CMAP-cohort		
Model with MRC-sum score at ICU discharge as a continuous variable[e]		
MRC-sum score at ICU discharge, baseline hazard (per point increase)	0.931 (0.908-0.955)	<0.001
MRC-sum score at ICU discharge, time dependent covariate (per year)	1.025 (1.010-1.041)	0.001
Abnormal CMAP D8±1	1.534 (0.976-2.412)	0.064
Model with MRC-sum score at ICU discharge as a dichotomous variable (exploratory analyses)[f]		
MRC-sum score at ICU discharge > 55 – Abnormal CMAP D8±1	2.467 (1.116-5.454)	0.026
MRC-sum score at ICU discharge ≤ 55 – Normal CMAP D8±1	1.858 (0.817-4.228)	0.140
MRC-sum score at ICU discharge ≤ 55 – Abnormal CMAP D8±1	2.701 (1.338-5.451)	0.006

[a] HR are calculated by multivariable Cox regression analyses including a prior defined confounders (age, diabetes mellitus, malignancy, preadmission dialysis, APACHE II-score, sepsis upon admission, days ICU-stay, days of treatment with corticosteroids and with neuromuscular blocking agents, and occurrence of new infection) as well as time-dependent covariates for factors with changing hazards over time. Assessment of violation of the proportional hazards was performed with Schoenfeld residuals test, time-dependent covariates included to correct for non-proportional hazards for the respective analyses were: [b] MRC-sum score at ICU discharge, sepsis upon admission and ICU length of stay, [c] sepsis upon admission, ICU length of stay, duration of treatment with corticosteroids and duration of treatment with NMBA, [d] malignancy, ICU length of stay, days of treatment with NMBA, occurrence of new infection, [e] sepsis upon admission, ICU length of stay and last ICU MRC sum score, [f] sepsis upon admission, ICU length of stay, days of treatment with corticosteroids and days of treatment with NMBA

Abbreviations: *MRC:* Medical Research Council; *CMAP:* Compound Muscle Action Potential; *D:* Day; *CI:* Confidence Interval.

Supplementary Table 6. Results of the search strategy identifying potential confounders for the 5-year mortality and morbidity analyses

Five-year mortality analyses	
Demographics	age [3, 5, 13-20, 37]
Co-morbidities	diabetes mellitus, malignancy, preadmission dialysis [5, 15, 18-20]
Admission factors	admission APACHE II score [3, 5, 14-16, 18-20, 22-26], sepsis upon admission [3, 16, 20, 25, 26]
ICU treatments and events	duration of ICU-stay [3, 5, 14, 18, 19, 27], days of treatment with corticosteroids [3, 5, 13, 24, 26, 28], neuromuscular blocking agents [3, 5, 26, 29, 38], benzodiazepines [5], duration of mechanical ventilation [5, 15, 17, 24], acquisition of a new infection while in the ICU [3, 5]
Five-year morbidity analyses	
Hand grip strength	
Demographics	age [5, 11, 33, 34], gender [24], BMI [5]
Co-morbidities	diabetes mellitus, malignancy, preadmission dialysis [5, 33]
ICU treatments and events	in-ICU treatment with benzodiazepines[5], maximal SOFA score in ICU [33], ICU length-of-stay [11, 34]
6-min walk distance	
Demographics	age [33, 35], gender [24]
Co-morbidities	diabetes mellitus, malignancy, preadmission dialysis [5, 33, 35]
ICU treatments and events	in-ICU hypoglycaemia [5], in-ICU treatment with opioids [5], and ICU length-of-stay [5, 11]
PF-SF-36	
Demographics	age [33, 35], gender [24]
Co-morbidities	diabetes mellitus, malignancy, preadmission dialysis [5]
ICU treatments and events	in-ICU treatment with benzodiazepines [5], inotropics and/or vasopressors [5], and ICU length-of-stay [5, 11]

Abbreviations: *PF-SF-36:* Physical Function domain of the SF-36 quality of life questionnaire.

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Chapter 5: Five-year outcome of respiratory muscle weakness at intensive care unit discharge

Adapted from:

Van Aerde N, Meersseman P, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Wauters J, Wouters PJ, Gosselink R, Van den Berghe G*, Hermans G*. (2021). Five-year outcome of respiratory muscle weakness at intensive care unit discharge: secondary analysis of a prospective cohort study. Thorax. epub ahead of print. http://dx.doi.org/10.1136/thoraxjnl-2020-216720.

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ABSTRACT:

Purpose

To assess the association between respiratory muscle weakness (RMW) at intensive care unit (ICU)-discharge and 5-year mortality and morbidity, independent from confounders including peripheral muscle strength.

Methods

Secondary analysis of the prospective 5-year follow-up of the EPaNIC cohort (clinicaltrials.gov:NCT00512122), limited to 366 patients screened for respiratory and peripheral muscle strength in the ICU with maximal inspiratory pressure (MIP) after removal of the artificial airway, and the Medical-Research-Council (MRC) sum-score. Respiratory muscle weakness was defined as an absolute value of MIP<30 cmH_2O. Associations between RMW at (or closest to) ICU discharge and all-cause 5-year mortality, and key measures of 5-year physical function, comprising respiratory muscle strength (MIP), hand-grip strength (HGF), six-minute-walk-distance (6MWD), and physical function of the SF-36 quality-of-life questionnaire (PF-SF-36), were assessed with Cox-proportional hazards and linear regression models, adjusted for confounders including peripheral muscle strength.

Results

RMW was present in 136/366 (37.2%) patients at ICU discharge. RMW was not independently associated with 5-year mortality [hazard ratio with 95% confidence interval: 1.273 (0.751-1.943), p=0.352]. Among 156 five-year survivors, those with, as compared to those without RMW demonstrated worse physical function [MIP (absolute value, cmH_2O): 62 (42-77) versus 94 (78-109), p<0.001; HGF (%pred): 67 (44-87) versus 96 (68-110), p<0.001; 6MWD (%pred): 87 (74-102) versus 99 (80-111), p=0.009; PF-SF-36 (score): 55 (30-80) versus 80 (55-95), p<0.001]. Associations between RMW and morbidity endpoints remained significant after adjustment for confounders [effect size with 95% confidence interval: MIP: -23.858 (-32.097 to -15.027), p=0.001; HGF: -18.591 (-30.941 to -5.744), p=0.001; 6MWD (transformed): -1587.007 (-3073.763 to -179.253),p=0.034; PF-SF-36 (transformed): 1.176 (0.144-2.270), p= 0.036].

Conclusions

Respiratory muscle weakness at ICU discharge is independently associated with 5-year morbidity but not 5-year mortality.

INTRODUCTION

Respiratory muscle weakness (RMW), in particular diaphragm dysfunction, is a frequent complication of critical illness, occurring in over 60% of patients requiring mechanical ventilation [1-3]. Risk factors include higher age, infection, systemic inflammation, illness severity, mechanical ventilation- both over and under-assistance, and certain drugs such as sedatives [1-5]. RMW often co-occurs and shares common features with peripheral muscle weakness acquired in the intensive care unit (ICU), labeled as ICU-acquired weakness (ICUAW). Both are associated with increased ICU and hospital mortality[6]. However, recent research revealed that RMW is more frequent than ICUAW, risk factors do not completely overlap, and short-term impact on morbidity differs. Whereas RMW contributes to weaning failure, increased ICU mortality [3, 7, 8] and early readmission [1, 6], ICUAW predominantly affects the duration of mechanical ventilation, ICU-, and hospital stay [6, 9]. As such, RMW and ICUAW may be considered separate, though overlapping, entities [1, 10]. In contrast to ICUAW, of which we and others recently described the impact on long-term outcomes [11, 12], studies of RMW and its associated outcomes beyond the index hospitalisation, when patients have overcome the major short-term risks of RMW, are scarce. Current evidence is confined to two case series with follow-up periods of respectively 1 and 2 years that revealed conflicting results [10, 13]. Identifying a possible relationship between respiratory muscle weakness in the ICU and the legacy of critical illness is highly relevant as preventive strategies for the long-term, multidimensional limitations reported for survivors of critical illness, are lacking. Respiratory muscle strength training in ICU survivors may hold promise in improving the long-term outcomes of critically ill patients.

We hypothesized that RMW in critically ill patients, as assessed by the maximal inspiratory pressure (MIP) after removal of (or without) artificial airway at (or closest to) ICU discharge, associates with 5-year mortality and morbidity, independent of confounders, including peripheral muscle strength. To this aim, we investigated the 5-year outcomes of a subgroup of the post-EPaNIC prospective follow-up cohort [14], who received systematic in-ICU respiratory and peripheral muscle strength testing.

METHODS

Ethics

The study protocol and informed consents of EPaNIC and its long-term follow-up were approved by the Ethical Committee Research UZ/KU Leuven (ML4190).

Study design and patient population

This is a secondary analysis of the prospective 5-year follow-up study of EPaNIC patients. Study design and methods of the EPaNIC trial and the EPaNIC follow-up study (clinicaltrials.gov:NCT00512122) have been published previously[14, 15] and are summarized in the online supplement. We here focus on those patients who were systematically screened in the ICU for respiratory and peripheral muscle strength. This screening was performed for long-stay patients (ICU stay ≥8 days). Additionally, a random set of short-stayers (ICU stay <8 days) were tested on the ward on day 8±1. The last evaluations performed in the ICU closest to ICU discharge, or on day 8±1 on the ward are further referred to as values ‘at ICU discharge’.

Measurement of respiratory muscle strength and diagnosis of respiratory muscle weakness

In patients without an artificial airway, MIP was measured according to the ATS guidelines [16] as described earlier[9]. Contra-indications included flail chest, pneumothorax, hemodynamic instability, intracranial hypertension, respiratory distress, or high flow oxygen therapy. A mouthpiece, incorporating a small leak to prevent glottic closure during the forceful inspiration was used.

Measurements were performed with the Micro Medical respiratory pressure meter, CareFusion® with Puma PC software. The patients were instructed to perform a maximal inspiratory maneuver starting from FRC. Maximal static inspiratory pressure was determined as the pressure maintained for 1 second. The best of 3 consecutive measurements was recorded. RMW was defined as an absolute value of MIP <30 cmH_2O [13, 17].

Outcomes

The primary endpoint was all-cause 5-year mortality. Secondary endpoints included respiratory muscle strength (MIP, cmH_2O) at 5-years follow-up as well as 3 key measures of physical function frequently compromised in ICU survivors, amounting to the so-called legacy of critical illness. These comprise hand-grip strength (HGF, %predicted), 6-minute-walk-distance (6MWD, %predicted), and the physical function domain of the SF-36 quality-of-life questionnaire (PF SF-36, range 0-100, higher values indicating better scores). Additionally, the associations between RMW and other 5-year morbidity outcomes were explored, including peripheral muscle strength (MRC-sum score and hand-held dynamometry), Barthel index (indicating the degree of physical independence), and the Physical and Mental Component Score (PCS and MCS) of the SF-36.

Statistics

Descriptive statistics included median and interquartile ranges for continuous variables and numbers and percentages for categorical variables. Continuous data were compared with the Mann-Whitney-U test, categorical variables with the Chi-square or Fisher-exact test, as appropriate. As this was a secondary analysis, sample size was not modifiable, however, it was estimated that the sample size was sufficient for the primary endpoint (details online supplement). Bootstrap resampling (N=1000) was performed to obtain more robust estimates of effect sizes (denoted as bias-corrected and accelerated confidence intervals, BcaCI). Analyses were performed with SPSS version 26 (IBM corporation) and R version 3.6.1. Two-sided p-values ≤0.05 were considered statistically significant.

Primary endpoint: explanatory modelling of the association between respiratory muscle weakness at ICU discharge and all-cause 5-year mortality

Crude 5-year mortality of patients with and without RMW was compared as a time-to event analysis with log-rank test and visualized with Kaplan-Meier plots. The effect size was estimated with Cox proportional hazard analyses, adjusting for a priori defined confounders, identified through a systematic literature search [18]. Potential confounders comprised demographic variables, comorbidities, and ICU treatments and events, particularly including peripheral muscle strength. To identify the latter, the MRC sum-score at ICU discharge was dichotomised at 55, according to recent findings concerning its association with long-term outcomes [11]. Further details on the search strategy, confounders identified and modelling are provided in the online supplement.

Secondary endpoints: explanatory modelling of the association between respiratory muscle weakness at ICU discharge and 5-year morbidity

The association between 5-year morbidity outcomes and RMW at ICU discharge was explored with the Mann-Whitney-U test. The association between RMW at ICU discharge and respiratory muscle strength at 5 years, as well as the three key measures of physical function, comprising hand-grip strength, 6-minute-walk-distance and the physical function domain of the SF-36, was further assessed by multivariable linear regression analyses, adjusted for confounders identified through a systematic literature search (see online supplement for further details).

Exploratory analyses

As the cut-off for MIP to define RMW has not been validated for long-term outcomes, we assessed the functional form of the relationship between MIP at ICU discharge and the primary and secondary outcomes through a LOESS-smoother on the Martingale residuals plot [19] for survival analyses and on the scatter plots for linear regression analyses. If appropriate, analyses were repeated with alternative modelling of MIP.

Sensitivity analyses

Multiple sensitivity analyses were performed. This involved restricting 5-year mortality analyses to hospital survivors. Also, the proportional hazard assumption was checked for each variable in each of the Cox-regression models with the Schoenfeld residuals test. Where appropriate, sensitivity analyses were performed by adding factors for which the assumption was violated as time-dependent covariates. For both 5-year mortality and morbidity, sensitivity analyses also involved adjusting for MRC at ICU discharge as a continuous variable as well as dichotomized for the previously defined cut-off of 48.

RESULTS

Patient cohorts and characteristics

The patient cohort with both peripheral and respiratory muscle strength assessment at ICU discharge consisted of 368 patients (Figure 1). Five-year mortality data were available for 366 patients. Among 246 survivors, 156 (63.4%) were evaluated for 5-year morbidity. For the 5-year mortality cohort, the median age was 60 (50-72) years, duration of mechanical ventilation 4 (2-10) days, and ICU-stay 10 (3-17) days. For the 5-year morbidity cohort, the median age was 60 (51-70) years, duration of mechanical ventilation 3 (1-9) days, and ICU-stay 8 (2-15) days. Further details are provided in Table 1 and Supplementary Table 1. RMW was present in 136/366 (37.2%) patients at ICU discharge. Patients with RMW were older, more likely to be female, to have a high nutritional risk score, and to suffer from diabetes. Patients with RMW were sicker upon admission and were longer exposed to corticosteroids and benzodiazepines, more frequently acquired a new infection in the ICU, and had a longer duration of mechanical ventilation and ICU stay. Interestingly, the incidence of sepsis upon admission was not significantly different for patients with and those without RMW.

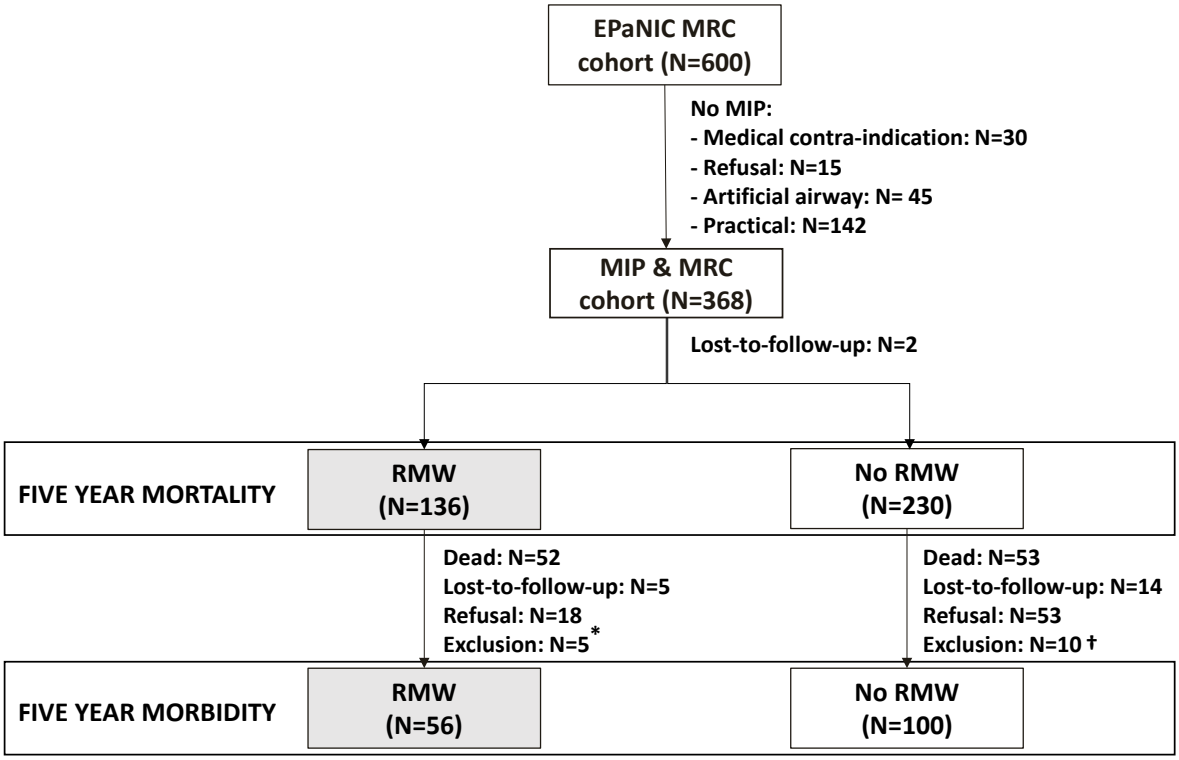

Fig. 1: Patient flow chart for the 5-year mortality and morbidity analyses. The patient cohort is composed of EPaNIC patients in whom MIP was measured in the ICU as part of a systematic screening protocol in long-stay, cooperative patients without (after removal of the) artificial airway and in a random sample of short-stay patients on the ward. The same cohort was also systematically screened for peripheral muscle strength with the MRC-sum score.

* Reasons for exclusion involved: rehab/nursing facility: N=1, pulmonary resection: N=1, dementia: N=1, neuromuscular disease: N=1, exceeded time window: N=1;

† Reasons for exclusion involved: rehab/nursing facility: N=3, pulmonary resection: N=2, psychiatric disease: N=1, neuromuscular disease: N=4.

Abbreviations: *EPaNIC:* Early versus late Parenteral Nutrition in Critically ill adults; *MIP:* Maximal Inspiratory Pressure; *MRC:* Medical Research Council; *RMW:* Respiratory Muscle Weakness.

Table 1: Baseline characteristics, indicators of muscle strength in the ICU, and ICU factors of patients included in the five year mortality and morbidity analyses

	5-year mortality				5-year morbidity			
	Total population (N=366)	**RMW at ICU discharge (N=136)**	**No RMW at ICU discharge (N=230)**	***P*-value**	**Total population (N=156)**	**RMW at ICU discharge (N=56)**	**No RMW at ICU discharge (N=100)**	***P*-value**
Baseline factors								
Age on admission	60 (50-72)	67 (55-76)	58 (47-69)	<0.001	60 (51-70)	61 (52-73)	60 (50-67)	0.203
Gender, male	222 (60.7)	70 (51.5)	152 (66.1)	0.006	97 (62.2)	27 (48.2)	70 (70)	0.007
NRS>5	95 (26.0)	49 (36.0)	46 (20.0)	0.001	30 (19.2)	11 (19.6)	19 (19.0)	0.922
BMI	25.1 (23.0-29.0)	25.0 (22.8-30.4)	25.2 (23.0-28.1)	0.474	25.4 (23.3-28.4)	25.6 (23.4-31.9)	25.2 (23.2-27.7)	0.190
Diabetes mellitus	58 (15.8)	30 (22.1)	28 (12.2)	0.012	24 (15.4)	12 (21.4)	12 (12.0)	0.117
Malignancy	87 (23.8)	35 (25.7)	52 (22.6)	0.497	30 (19.2)	11 (19.6)	19 (19.0)	0.922
Pre-admission dialysis	3 (0.8)	2 (1.5)	1 (0.4)	0.558	0 (0)	0(0)	0(0)	NA
Randomisation, late PN	191 (52.2)	75 (55.1)	116 (50.4)	0.383	83 (53.2)	35 (62.5)	48 (48.0)	0.082
APACHE II	30 (19-36)	33 (22-38)	27 (17-34)	<0.001	27 (17-35)	30 (18-36)	26 (16-34)	0.128
Admission category				0.305				0.801
Cardiac surgery	133 (36.3)	47 (34.6)	86 (37.4)		77 (49.4)	25 (44.6)	52 (52.0)	
Emergency SICU	149 (40.7)	61 (44.9)	88 (38.3)		52 (33.3)	20 (35.7)	32 (32.0)	
Elective SICU	20 (5.5)	4 (2.9)	16 (7.0)		6 (3.8)	2 (3.6)	4 (4.0)	
MICU	64 (17.5)	24 (17.6)	40 (17.4)		21 (13.5)	9 (16.1)	12 (12.0)	
Sepsis upon admission	141 (38.5)	59 (43.4)	82 (35.7)	0.142	47 (30.1)	19 (33.9)	28 (28.0)	0.439

Continued Table 1: Baseline characteristics, indicators of muscle strength in the ICU, and ICU factors of patients included in the five year mortality and morbidity analyses

	5-year mortality				5-year morbidity			
	Total population (N=366)	**RMW at ICU discharge (N=136)**	**No RMW at ICU discharge (N=230)**	***P*-value**	**Total population (N=156)**	**RMW at ICU discharge (N=56)**	**No RMW at ICU discharge (N=100)**	***P*-value**
Muscle strength at ICU discharge								
MIP (absolute value in cmH_2O)	35 (25-49)	21 (17-26)	45 (37-58)	<0.001	38 (23-51)	21 (17-25)	46 (38-59)	<0.001
Timing of MIP	9 (8-15)	12 (8-19)	8 (8-14)	0.001	8 (8-15)	12 (8-19)	8 (8-11)	<0.001
MRC-sum score	54 (48-58)	50 (47-54)	56 (50-58)	<0.001	55 (49-58)	50 (47-54)	58 (52-59)	<0.001
Timing of MRC-sum score	9 (8-16)	12 (8-19)	8 (8-14)	<0.001	8 (8-15)	12 (8-19)	8 (8-11)	<0.001
ICU-related variables								
Corticosteroids, days	0 (0-5)	0 (0-8)	0 (0-4)	0.071	0 (0-4)	0 (0-8)	0 (0-2)	0.028
NMBA, days	0 (0-1)	0 (0-1)	0 (0-1)	0.293	0 (0-1)	0 (0-1)	0 (0-0)	0.269
New infection	157 (42.9)	78 (57.4)	79 (34.3)	<0.001	61 (39.1)	36 (64.3)	25 (25.0)	<0.001
Benzodiazepines, days	3 (0-8)	4 (1-10)	2 (0-7)	0.002	1 (0-7)	5 (0-12)	1 (0-6)	0.008
Mechanically ventilated	350 (95.6)	131 (96.3)	219 (95.2)	0.617	154 (98.7)	55 (98.2)	99 (99.0)	0.676
Mechanical ventilation, days	4 (2-10)	6 (2-13)	3 (1-8)	<0.001	3 (1-9)	6 (2-14)	2 (1-7)	0.001
ICU stay, days	10 (3-17)	13 (6-20)	8 (2-15)	<0.001	8 (2-15)	13 (6-22)	4 (1-11)	<0.001

Continuous variables are reported with median and interquartile range, dichotomous variables are reported with number and percentage. *P*-values reflect differences between patient with and without RMW.

Abbreviations: *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *SICU:* Surgical Intensive Care Unit; *MICU:* Medical Intensive Care Unit; *NMBA:* neuromuscular blocking agents; *MRC:* Medical Research Council; *MIP:* maximal inspiratory pressure.

Primary outcome: five-year mortality

Total 5-year mortality in the studied cohort was 104/366 (28.4%), involving 51/136 (37.5%) of patients with RMW and 53/230 (23.0%) patients without RMW. As mortality data were obtained from the national registry, no information on cause of death was available. Time-to-death was significantly different for patients with versus those without RMW [Log-Rank p=0.002], the former experiencing a higher event rate (Supplementary Figure 1). However, when adjusted for confounders, RMW was not associated with 5-year mortality [HR: 1.273 (95% BcaCI: 0.751-1.943), p=0.352](Table 2).

Secondary outcomes: five-year morbidity

Five-year survivors with RMW at ICU discharge had worse 5-year morbidity outcomes than those without RMW [MIP (absolute value): 62 (42-77) cmH_2O versus 94 (78-109) cmH_2O, p<0.001; HGF (%pred): 67% (44%-87%) versus 96% (68%-110%), p<0.001; 6MWD (%pred): 87% (74%-102%) versus 99% (80%-111%), p=0.009; PF-SF36: 55 (30-80) versus 80 (55-95),p<0.001]. Further exploratory analyses showed that patients with RMW also scored worse for all other 5-year morbidity endpoints, including MRC sum-score, hand-held dynamometry, Barthel index and quality-of-life questionnaires (Supplementary Figure 2). Adjusted for confounders, RMW remained independently associated with all secondary endpoints [MIP (absolute value, cmH_2O): B:-23.858 (95%BcaCI:-32.097 to -15.027),p=0.001; HGF (%pred): B: -18.591(95% BcaCI: -30.941 to -5.744, p= 0.001; 6MWD(%pred, transformed) B: -1587.007(95%BcaCI:-3073.763 to -179.253) p=0.034; PF SF-36 (transformed with higher transformed values indicating lower scores): B: 1.176 (95% BcaCI: (0.144-2.270), p= 0.036] (Table 2). Additional information on rehabilitation trajectory, working and living conditions is provided in Supplementary Table 3.

Exploratory analyses

Martingale residual plots and scatter plots did not support a clear cut-off at 30 cmH_2O for MIP at ICU discharge, for prediction of the studied long-term endpoints (Supplementary Figure 3). Re-analyses with both MIP as a continuous variable and with MIP at a cut-off of 45 cmH_2O, possibly suggested by the plots as a better cut-off, did not alter the conclusions (Supplementary Table 2).

Sensitivity analyses

RMW at ICU discharge was not independently associated with 5-year mortality, neither when the analysis was restricted to hospital survivors, nor when MRC was modelled as a continuous variable or when dichotomised at 48. Also, the addition of time-dependent covariates to the survival model for those factors violating the proportional hazard assumption did not alter the conclusion (Supplementary Table 2). For the morbidity endpoints, alternative modelling strategies for the MRC sum-score did not affect the conclusions (Supplementary Table 2).

Table 2. Association between RMW at ICU discharge and 5-year outcomes

All-cause five-year mortality	**HR (95% BCa CI)**	***P*-value**
RMW at ICU discharge, unadjusted	1.837 (1.242-2.689)	0.002
RMW at ICU discharge, adjusted [a]	1.273 (0.751-1.943)	0.352
Five-year morbidity	**B (95% BCa CI)**	***P*-value**
MIP at 5-year follow-up (cmH_2O)		
RMW at ICU discharge, unadjusted	-31.285 (-39.911 to -22.972)	0.001
RMW at ICU discharge, adjusted [b]	-23.858 (-32.097 to -15.027)	0.001
Hand-grip strength at 5-year follow-up (%pred)		
RMW at ICU discharge, Unadjusted	-24.595 (-33.407 to -15.349)	0.001
RMW at ICU discharge, adjusted [c]	-18.591 (-30.941 to -5.744)	0.001
6MWD at 5-year follow-up (%pred) [f]		
RMW at ICU discharge, unadjusted	-2134.881 (-3575.090 to -739.109)	0.004
RMW at ICU discharge, adjusted [d]	-1587.007 (-3073.763 to -179.253)	0.034
PF-SF-36 at 5-year follow-up [f]		
RMW at ICU discharge, unadjusted	2.078 (1.107-3.005)	0.001
RMW at ICU discharge, adjusted [e]	1.176 (0.144-2.270)	0.036

[a] HR were calculated by multivariable Cox regression analyses correcting for a priori defined confounders including: age, BMI, diabetes mellitus, malignancy, preadmission dialysis, diagnostic category, APACHE II-score, sepsis upon admission, ICU length-of-stay, days of in-ICU treatment with corticosteroids and neuromuscular blocking agents, acquisition of new infection in ICU and MRC sum-score $\leq$ 55. Days of in-ICU treatment with benzodiazepines and duration of mechanical ventilation were eliminated from the mortality model due to collinearity with ICU length-of stay.

[b-e] B-estimates were calculated by multivariable linear regression analyses correcting for a priori defined confounders including, for MIP[b]: age, gender, BMI, diabetes mellitus, malignancy, preadmission dialysis, duration of treatment with benzodiazepines, duration of mechanical ventilation, maximal SOFA score, ICU length of stay, MRC sum-score $\leq$ 55; HGF[c]: age, gender, BMI, diabetes mellitus, malignancy, preadmission dialysis, duration of treatment with sedatives ie benzodiazepines, duration of mechanical ventilation, maximal SOFA score, ICU length of stay, MRC sum-score $\leq$ 55; 6MWD[d]: age, gender, diabetes mellitus, malignancy, preadmission dialysis, duration of treatment with opioids, duration of mechanical ventilation, hypoglycaemia, length of ICU stay, MRC sum-score $\leq$ 55; PF-SF-36[e]: age, gender, diabetes mellitus, malignancy, preadmission dialysis, duration of treatment with inotropics/vasopressors, duration of treatment with benzodiazepines, duration of mechanical ventilation, length of ICU stay, MRC sum-score $\leq$ 55. In these models [b,c,d,e], duration of in-ICU treatment with sedatives was eliminated due to collinearity with ICU length-of stay.

[f] 6MWD data were transformed to power 2 and the PF-SF-36 were reversed (100 minus actual value) and subsequently transformed to power 0.54 to obtain adequate model fit (with higher transformed values corresponding to lower values of PF-SF-36)

Abbreviations: *BCa:* bias-corrected accelerated confidence intervals obtained by bootstrap resample procedure (N=1000); *MIP:* Maximal Inspiratory Pressure; *6MWD:* 6-minute-walk-distance; *PF-SF-36:* Physical function component of the SF-36 quality of life questionnaire; *HR:* hazard ratio; *MRC:* Medical Research Council.

DISCUSSION

In this patient cohort of mixed adult critically ill patients, systematically screened for respiratory and peripheral muscle strength at ICU discharge and prospectively followed, we demonstrated that RMW at ICU discharge was independently associated with lower respiratory muscle strength at 5 years follow-up. Furthermore, RMW at ICU discharge independently associated with key morbidity outcomes that characterize the post-intensive care syndrome [11, 12, 14, 20-23], comprising handgrip strength, 6-minute walk distance, and the physical function component of the SF-36 quality-of-life questionnaire. Importantly, these analyses were adjusted for confounders, including peripheral muscle strength at ICU discharge. In contrast, RMW at ICU discharge did not independently associate with 5-year mortality.

The incidence of RMW in this mixed cohort of critically ill patients, as assessed by a volitional maximal inspiratory pressure maneuver in cooperative patients without artificial airway at ICU discharge, was 37.2%. Previous research reported diaphragm dysfunction in 64% of patients upon ICU admission, strongly associated with the presence of sepsis and with illness severity [3]. At the start of the weaning process, the incidence of RMW ranged between 54% and 63% [1, 17], with severe diaphragm dysfunction present in 31% [10]. In patients with peripheral weakness, up to 80% also suffer from diaphragm dysfunction [7]. The lower incidence of RMW in our study may have several explanations. First, factors contributing to RMW may improve over time. Indeed, in contrast to the findings upon ICU admission [3] or up to a week prior to successful extubation [24], we and others who assessed RMW at a later stage in the ICU [1, 13] found no association between sepsis and RMW. Second, by measuring RMW after removal of the artificial airway at ICU discharge, we evidently eliminated the sickest patients from this analysis, including those who were never weaned or were not cooperative after weaning. As such, we likely excluded patients with severe RMW and poor short-term outcomes. This allowed us to focus on the long-term impact of RMW, studying only patients who overcame the major short-term risks.

The detrimental short-term impact of RMW has been well documented [6]. This includes increased ICU and hospital mortality rates [1, 3, 6], possibly linked with longer duration of mechanical ventilation [25], later weaning [24] and increased risk of weaning failure [1, 2, 13]. The combination of respiratory and peripheral muscle weakness in particular associates with poor outcomes [17]. Data on the impact of RMW beyond the acute timeframe are scarce. We demonstrated increased 5-year mortality in patients with RMW (37.5%) at ICU discharge as compared to patients without RMW (23.0%). However, when adjusted for baseline characteristics, illness severity, co-morbidities, ICU exposures, and peripheral muscle strength at ICU discharge, the risk of death within 5 years was not increased in patients with RMW. These data were confirmed in all sensitivity analyses, including the scrutinized analysis limited to hospital survivors. Previous data obtained from 124 patients suggested that 1-year mortality was significantly increased in patients with RMW (31% versus 7%) at the time of successful extubation [13]. However, 21% of patients with RMW experienced extubation failure, and 47.6% of deaths occurred on the ICU. The difference in mortality after hospital discharge for those with, and those without RMW, was not statistically significant. Saccheri recently showed that 2-year mortality was not different between patients with and those without diaphragmatic dysfunction at the start of the weaning process (36% versus 29%) [10]. These data were confirmed when analyses were limited to hospital survivors. Survival at 2 years was worse for patients suffering from both ICUAW and RMW (36%), though the patient sample was too small to allow adjustment for confounders. Our study extended the follow-up time window and suggests that, if patients with RMW overcome the acute challenges, long-term survival is not independently affected by RMW.

We subsequently evaluated the association between RMW at ICU discharge and 5-year morbidity. We demonstrated that, among 5-year survivors, RMW was independently associated with reduced inspiratory

muscle strength, but also with peripheral muscle weakness as assessed with handgrip strength, poor physical function as assessed with six-minute-walk-distance, and low quality-of-life as assessed with PF-SF-36. These findings are novel, as data on the association between RMW at ICU discharge and morbidity beyond the index hospitalization are virtually absent. Saccheri recently found no association between RMW in critically ill patients and the quality-of-life assessed in 40 survivors at 2 years follow-up [10]. In light of the data we here present, the lack of effect in the aforementioned paper may be due to a lack of power. The finding of an independent relationship between RMW at ICU discharge and typical features of the long-term legacy of critical illness may have important consequences as, currently, strategies to prevent or cure this long-term burden of critical illness are lacking [26, 27]. Respiratory muscle training has demonstrated multiple positive effects when performed in various patient populations, including patients with COPD, chronic heart or kidney disease, as well as in healthy volunteers and athletes. These benefits surpass mere improvement of respiratory muscle strength and include improved submaximal and maximal exercise capacity, functional capacity, and quality-of-life [28-32]. Several mechanisms have been proposed to explain such beneficial effects [28, 33, 34], which are relevant at both strenuous and submaximal exercise effort, and reversible by inspiratory muscle training, in the aforementioned patient populations [35]. The role for inspiratory muscle training as an intervention to specifically reduce the long-term burden of critical illness therefore deserves consideration and further evaluation through randomised clinical trials.

Our study has several strengths. To the best of our knowledge, this is the largest dataset on the association between respiratory muscle weakness in critically ill patients and long-term outcomes. By focusing on RMW at ICU discharge, in contrast to previous studies, we dissociated the outcomes of interest from the acute risks of RMW. Furthermore, the dataset is unique, as we have concomitant information on peripheral muscle strength, well-known to contribute to the long-term sequelae of critical illness, which allowed us to assess the independent effect of RMW. Finally, this is the first study to assess the association between RMW at ICU-discharge and morbidity at five years post-ICU, a time-window of follow-up far exceeding currently available perspectives. This study also has several limitations. First, we measured respiratory muscle strength with the maximal inspiratory pressure, rather than with transdiaphragmatic (or transtracheal) twitch pressure generated in response to bilateral magnetic phrenic nerve stimulation (BAMPS), which is considered to be the golden standard in critically ill patients [3, 25, 36]. MIP is a simple screening measure of global inspiratory muscle strength, not limited to evaluation of the diaphragm. MIP correlates with twitch tracheal pressure [7, 37] and several researchers have demonstrated its clinical relevance in the ICU setting [6, 13, 17, 24, 37]. MIP requires consciousness and cooperation, while BAMPS is not dependent on patient effort. As we performed measurements at ICU discharge and after removal of the endotracheal tube aiming to study post-acute phase impact of RMW, conscious cooperation was a reasonable inclusion criterion, and MIP was considered a suitable measure for our research purpose. Additionally, while MIP can be easily performed at the bedside, BAMPS is a time-consuming and technically challenging procedure, requiring expensive equipment that is only available in a few ICUs worldwide [16, 36, 38]. Second, although we adjusted our analyses for confounders identified through a systematic literature search, we cannot exclude unmeasured confounding. Consequently, as this is an observational study, we cannot draw causal conclusions. Third, morbidity endpoints are subject to selection bias, as not all survivors were evaluated. Fourth, as no systematic evaluations at intermediate time points were made, no conclusions can be drawn with regard to differential recovery trajectories of respiratory muscle strength. Fifth, as maximal expiratory pressure (MEP) was not a pre-specified outcome in our study, we cannot draw any conclusions on a potential association of expiratory muscle weakness with long-term outcomes. However, recent data could not define an independent effect of MEP when MIP was taken into account with regard to short-term outcomes [39]. Finally, all patients were included in the EPaNIC trial, possibly limiting generalisability.

We conclude that RMW at discharge from the ICU did not independently associate with 5-year mortality. However, RMW at ICU discharge was associated with peripheral and respiratory muscle strength, physical function, and quality-of-life in 5-year survivors, independent of baseline characteristics, comorbidities, illness severity, ICU exposures, and peripheral muscle strength. As inspiratory muscle training has the potential to improve respiratory muscle strength as well as other outcomes, our findings suggest that inspiratory muscle training is an attractive and promising intervention that should be investigated in ICU survivors in randomised studies with the aim to improve long-term outcomes of critical illness.

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SUPPLEMENTARY MATERIALS

Supplementary methods

EPaNIC and post-EPaNIC follow-up study

The EPaNIC trial (Clinical trials.gov: NCT00512122, N=4640) was a multicentre, randomised controlled trial (7 medical and surgical intensive care units) conducted at the University Hospitals Leuven and Jessa Hospitals Hasselt, comparing early (within 48 hours) with late (not before day 8) parenteral supplementation of insufficient enteral nutrition to reach caloric goals in adult critically ill patients [1]. Main exclusion criteria included age < 18 years, moribund state or DNR-coding, enrollment in other trial, short-bowel syndrome, home ventilation, diabetic coma, referred with nutritional regime, pregnant or lactating women, no central catheter, on oral nutrition, readmission, BMI<17 kg/m², nutritional risk score < 3, or refusal to participate. The study showed that late initiation of parenteral nutrition was associated with faster recovery and fewer complications [1]. The post-EPaNIC follow-up study prospectively assessed 5-year mortality in all, and 5-year morbidity in a subgroup of long-term surviving EPaNIC-patients through hospital or home visits from June 2012 onwards [1-4]. Recruitment for the 5-year morbidity follow-up focussed on patients with prolonged ICU-stay (≥8 days), and for feasibility purposes, a random sample (398/2436) of eligible short-stay patients was included, matched to the diagnostic category distribution of long-stayers. Exclusion criteria for the 5-year morbidity analysis involved pre-existing neuromuscular disease, other pre-ICU disabilities potentially confounding the morbidity endpoints, and patients refusing participation [3, 4].

Statistics

Primary outcome: explanatory modelling of the association between respiratory muscle weakness at ICU discharge and all-cause 5-year mortality

Crude 5-year mortality of patients with and without RMW was compared as a time-to event analysis with log-rank test and visualized with Kaplan-Meier plots. The effect size was estimated with Cox proportional hazard analyses, adjusting for a priori defined confounders, identified through a systematic literature search [5]. The search strategy and results are presented below. Prior to entering the variables as covariates to the models, collinearity was checked and judged problematic in case of variance inflation factor >5 or tolerance <0.2. Potential confounders comprised demographic variables, comorbidities, and ICU treatments and events, particularly including peripheral muscle strength. Potential confounders identified included age, BMI, diabetes mellitus, malignancy, preadmission dialysis, diagnostic category, APACHE II-score, sepsis upon admission, ICU length-of-stay, days of in-ICU treatment with corticosteroids and neuromuscular blocking agents, acquisition of new infection in ICU and MRC sum-score<55. MRC sum-score at ICU discharge was dichotomised at 55, according the recent findings with regard to long-term outcomes [4]. Of the pre-specified ICU-factors, days of in-ICU treatment with benzodiazepines and duration of mechanical ventilation were eliminated from the final model due to collinearity with ICU length-of stay.

Secondary outcomes: explanatory modelling of the association between respiratory muscle weakness at ICU discharge and 5-year morbidity

The association between 5-year morbidity outcomes and RMW at ICU discharge was explored with Mann-Whitney U test. The association between RMW at ICU discharge and respiratory muscle strength at 5 years as well as the 3 key measures of physical function, comprising hand-grip strength, 6-minute-walk-distance and the physical function domain of the SF-36, was further assessed by multivariable linear regression analyses, adjusted for confounders identified through a systematic literature search.

In order to enable the adjusted analyses through linear regression and obtain adequate model fit for 6-MWD and PF SF-36, we performed Box-Cox based transformations [6, 7] as we did previously [4]. This involved transformation of the 6-MWD to power 2. For PF SF-36, data were reversed and subsequently transformed to power 0.54. Agreement with assumptions for linear regression (linearity, homoscedasticity, independence of errors and normal distribution of residuals) was assessed through normal probability plot, histogram of standardized residuals, scatter plot of the standardised residuals versus the standardised predicted values.

The search strategy and results for relevant confounders are presented below. Again, collinearity was checked and judged problematic if variance inflation factor >5 or tolerance <0.2. The following factors were identified as potential confounders, for MIP: age, gender, BMI, diabetes mellitus, malignancy, preadmission dialysis, duration of mechanical ventilation, maximal SOFA score, ICU length of stay, MRC sum-score<55; for HGF: age, gender, BMI, diabetes mellitus, malignancy, preadmission dialysis, duration of mechanical ventilation, maximal SOFA score, ICU length of stay, MRC sum-score<55; for 6-MWD: age, gender, diabetes mellitus, malignancy, preadmission dialysis, duration of mechanical ventilation, hypoglycaemia, length of ICU stay, MRC sum-score<55; for PF SF-36e: age, gender, diabetes mellitus, malignancy, preadmission dialysis, duration of treatment with inotropics/vasopressors, duration of mechanical ventilation, length of ICU stay, MRC sum-score<55. Duration of in-ICU treatment with sedatives (for MIP, HGF and PF-SF-36: duration of treatment with benzodiazepines; for 6MWD: duration of treatment with opioids) was eliminated from the final models due to collinearity with ICU length-of stay.

As this was a secondary analysis, sample size was not modifiable. However, we anticipated based on prior data, 40% patients would have RMW. For the primary outcome, given a hazard ratio of 4.4 at 1-year [8], and assuming this difference would reduce to a HR of 2 at 5 years, with an overall 5-year mortality in critically ill patients of 30% [9], with type I error at 0.05 and power of 80% this would require a total sample size of 227 patients, which was covered by the available sample. As the number of deaths was 104, and 11 confounders were identified to be included in the Cox-regression analysis, the likelihood of problematic error was small [10]."

Search strategy to identify known confounders of the relationship between respiratory muscle weakness in ICU and 5-year mortality and morbidity.

The Medline database was searched through Ovid and PubMed platforms for full-text, human subject, English language original research journal articles (prospective as well as retrospective observational studies, randomized controlled trials) of patients admitted to an intensive care unit, reporting on the association between demographic factors, co-morbidities and ICU treatments and events with 5-year outcomes in relation to measures of respiratory muscle strength in the ICU. Systematic reviews and meta-analyses retrieved with the search were screened for referenced original research articles.
We used Mesh terms and additional exploded searches centering around key concepts 'critical illness', 'intensive care unit acquired respiratory muscle weakness/ventilator-induced diaphragm dysfunction', 'potential confounders' and 'mortality/morbidity outcomes'. All used Mesh terms and additional search terms (relevant entry terms based on the tree built on each Mesh-term, as well as recently introduced professional language not indexed in the Mesh-database including 'ventilator-induced diaphragm dysfunction') are listed below. We included papers published within the past 20 years previous to January 2020. Due to limited available evidence on 5-year outcomes, we included studies with shorter follow-up after ICU-discharge and with measures of respiratory muscle weakness other than the MIP. A confounder was defined according to recent guidelines on causal inference studies, as a factor independently associated with both ICU-acquired respiratory muscle weakness, and with long-term outcome in ICU-patients[5]. For verification of the latter requirement, given sparsity of data in particular for 5-year morbidity, we extrapolated evidence from research on the adverse long-term outcome of prolonged mechanical ventilation and sepsis [11-14], and refer to

previous work centering more specifically on the association of prolonged ICU-stay and ICUAW with long-term outcome [3, 4, 11, 12, 15, 16]. Final construction of the search string as presented below and article retrieval to create Supplemental Table 3 was conducted between the 4th and 10th of January 2020.

Five-year mortality

- *Pubmed search*

(((“Critical Illness”[Mesh] OR “Critical Care”[Mesh] OR “Intensive Care Units"[Mesh] OR “Acute Disease”[Mesh] OR “Shock”[Mesh] OR “Systemic Inflammatory Response Syndrome”[Mesh] OR “Multiple organ failure”[Mesh] OR “Respiratory insufficiency”[Mesh] OR critical-illness* OR critical-care* OR intensive-care* OR acute-disease* OR *shock* OR Systemic-Inflammatory-Response-Syndrome* OR multiple-organ-failure* OR respiratory-insufficienc*)) AND (“Respiratory paralysis”[Mesh] OR respiratory-paralys* OR Respiratory-Muscle-Paralysis* OR Diaphragm-atrophy* OR Diaphragm-dysfunction* OR Diaphragm/pathology* OR Diaphragmatic-Paralysis* OR Diaphragm-paralysis* OR Respiratory-muscle-weakness* OR Respiratory-muscle-paresis* OR diaphragm-paresis* OR Ventilator-induced-diaphragmatic-dysfunction* OR Ventilator-induced-diaphragm* OR inspiratory-pressure* OR MIP* OR transtracheal-pressure*) AND (“Length of Stay”[Mesh] OR “Severity of illness index”[Mesh] OR “Simplified acute physiology score”[Mesh] OR “APACHE”[Mesh] OR “Artificial respiration”[Mesh] OR “Neuromuscular blocking agents”[Mesh] OR “Kidney, artificial”[Mesh] OR “Vasoconstrictor agents”[Mesh]OR “Comorbidity”[Mesh] OR “Body mass index”[Mesh] OR length-of-stay*or severity-of-illness-index* OR simplified-acute-physiology-score* OR SOFA-score* OR APACHE* OR comorbidit* OR multimorbidit* OR gender* OR age* OR body-mass-index* OR artificial-respiration* OR mechanical-ventilation* OR neuromuscular-blocking-agent* OR artificial-kidney* OR vasoconstrictor* OR vasopress* OR vasoactive-agonist* OR “Polyneuropathy”[Mesh] OR “Paresis”[Mesh] OR “Muscle weakness”[Mesh] OR “Muscle strength”[Mesh] OR polyneuropath* OR paresis* OR *muscle-weakness* OR muscle-strength* OR muscle-wasting*) AND (“Critical care outcomes”[Mesh] OR “Mortality”[Mesh] OR *mortality* OR critical-care-outcome*OR follow-up* OR survival* OR “Long Term Adverse Effects”[Mesh] OR Long-Term-Adverse-Effect*)))

- Ovid search

(Critical Illness OR Critical Care OR Intensive Care Units OR Multiple organ failure) AND (Respiratory paralysis OR Diaphragm-atrophy OR Diaphragm-dysfunction OR Diaphragm/pathology OR Respiratory-muscle-weakness OR Ventilator-induced-diaphragmatic-dysfunction) AND (Critical care outcomes OR Mortality OR critical-care-outcome OR follow-up OR survival)

Five-year morbidity

- Pubmed search

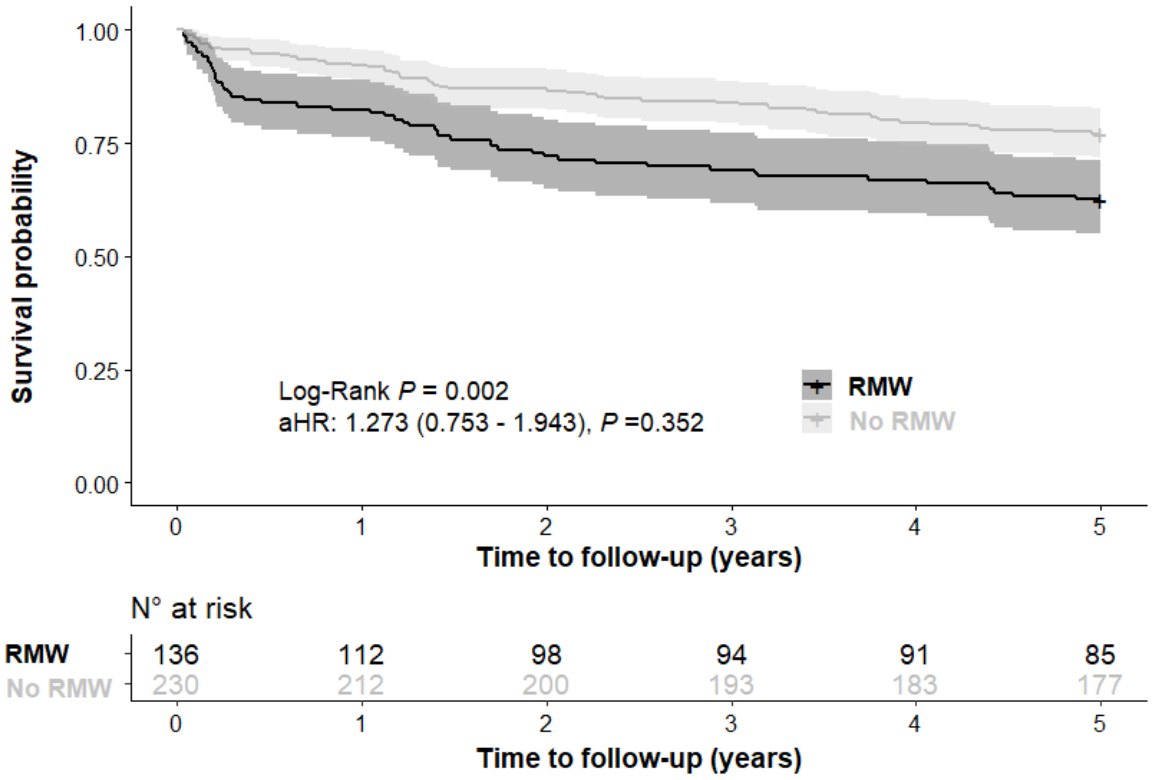
(((“Critical Illness”[Mesh] OR “Critical Care”[Mesh] OR “Intensive Care Units"[Mesh] OR “Acute Disease”[Mesh] OR “Shock”[Mesh] OR “Systemic Inflammatory Response Syndrome”[Mesh] OR “Multiple organ failure”[Mesh] OR critical-illness* OR critical-care* OR intensive-care* OR acute-disease* OR *shock* OR Systemic-Inflammatory-Response-Syndrome* OR multiple-organ-failure*)) AND (“Respiratory paralysis”[Mesh] OR respiratory-paralys* OR Respiratory-Muscle-Paralysis* OR Diaphragm-atrophy* OR Diaphragm-dysfunction* OR Diaphragm/pathology* OR Diaphragmatic-Paralysis* OR Diaphragm-paralysis* OR Respiratory-muscle-weakness* OR Respiratory-muscle-paresis* OR diaphragm-paresis* OR Ventilator-induced-diaphragmatic-dysfunction* OR Ventilator-induced-diaphragm* OR inspiratory-pressure* OR MIP* OR transtracheal-pressure*) AND (“Length of Stay”[Mesh] OR “Severity of illness index”[Mesh] OR “Simplified acute physiology score”[Mesh] OR

"APACHE"[Mesh] OR "Artificial respiration"[Mesh] OR "Neuromuscular blocking agents"[Mesh] OR "Kidney, artificial"[Mesh] OR "Vasoconstrictor agents"[Mesh]OR "Comorbidity"[Mesh] OR "Body mass index"[Mesh] OR length-of-stay*or severity-of-illness-index* OR simplified-acute-physiology-score* OR SOFA-score* OR APACHE* OR comorbidit* OR multimorbidit* OR gender* OR age* OR body-mass-index* OR artificial-respiration* OR mechanical-ventilation* OR neuromuscular-blocking-agent* OR artificial-kidney* OR vasoconstrictor* OR vasopress* OR vasoactive-agonist* OR "Polyneuropathy"[Mesh] OR "Paresis"[Mesh] OR "Muscle weakness"[Mesh] OR "Muscle strength"[Mesh] OR polyneuropath* OR paresis* OR *muscle-weakness* OR muscle-strength* OR muscle-wasting*) AND ("Morbidity"[Mesh] OR "Muscle strength"[Mesh] OR "Hand strength"[Mesh] OR "Pinch strength"[Mesh] OR "Walk test"[Mesh] OR "Activities of Daily Living"[Mesh] OR "Quality of life"[Mesh] OR morbidit* OR muscle-strength* OR hand-strength* OR pinch-strength* OR 6-min-walk-test*OR activities-of-daily-living* OR ADL* OR barthel-index* OR quality-of-life* or SF36* OR physical-component-score* OR "Respiratory paralysis"[Mesh] OR Respiratory-muscle-weakness* OR Respiratory-muscle-paresis* OR Diaphragm-dysfunction* OR inspiratory-pressure* OR MIP*)))

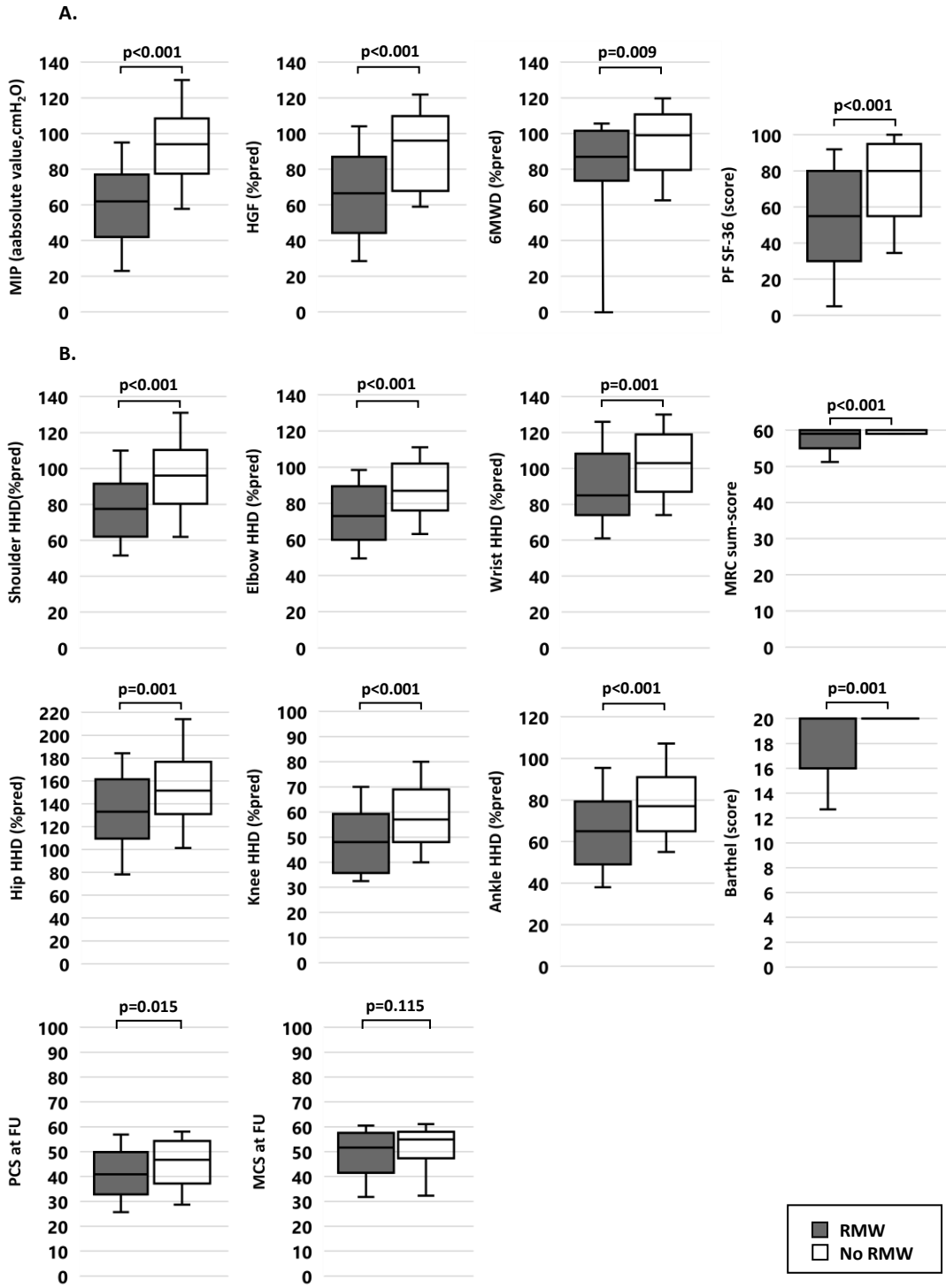
- Ovid search

(Critical Illness OR Critical Care OR Intensive Care Units OR Multiple organ failure) AND (Respiratory paralysis OR Diaphragm-atrophy OR Diaphragm-dysfunction OR Diaphragm/pathology OR Respiratory-muscle-weakness OR Ventilator-induced-diaphragmatic-dysfunction) AND (Critical care outcomes OR OR Morbidity OR Muscle strength OR Walk test OR Quality of life OR Activities of Daily Living OR Respiratory-muscle-weakness OR MIP)

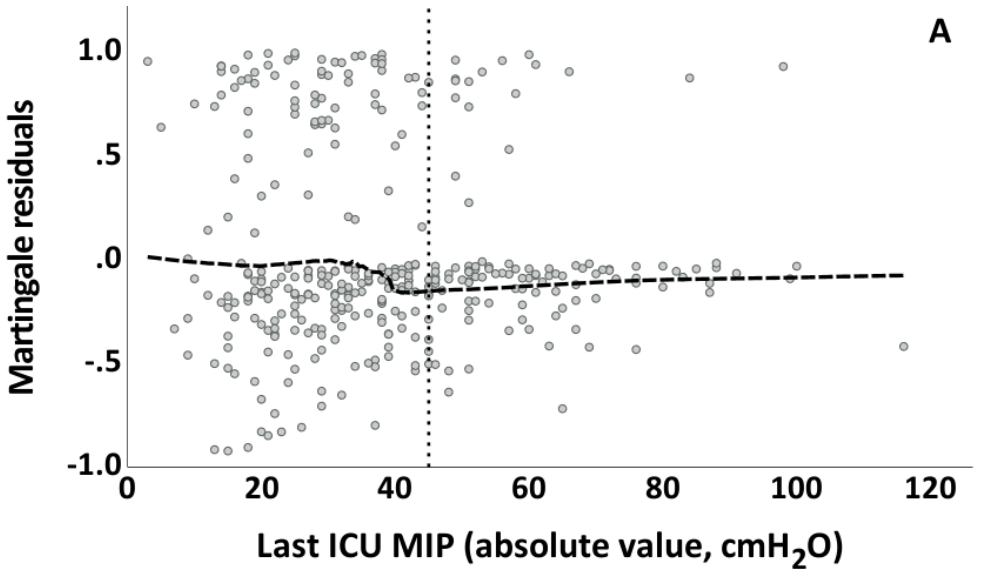
Supplementary figures

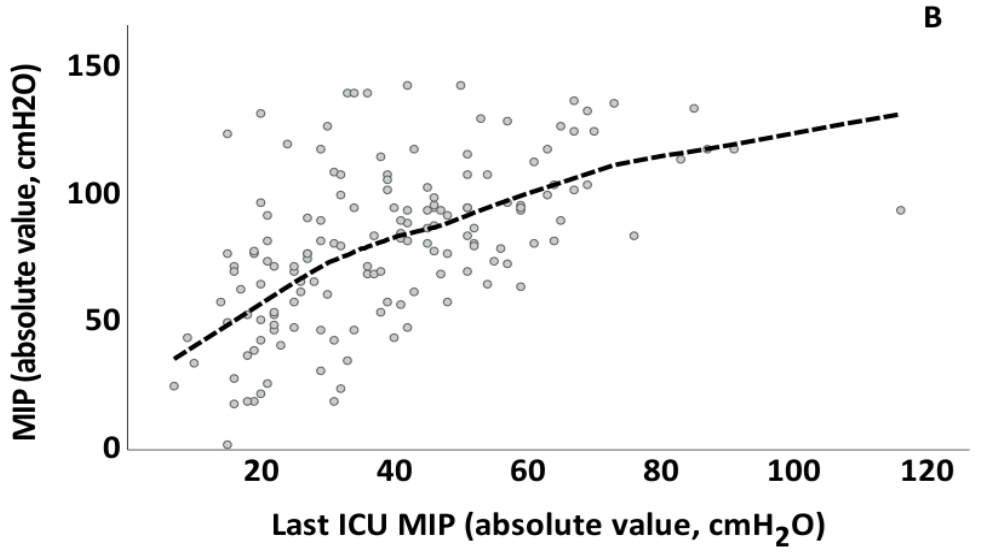


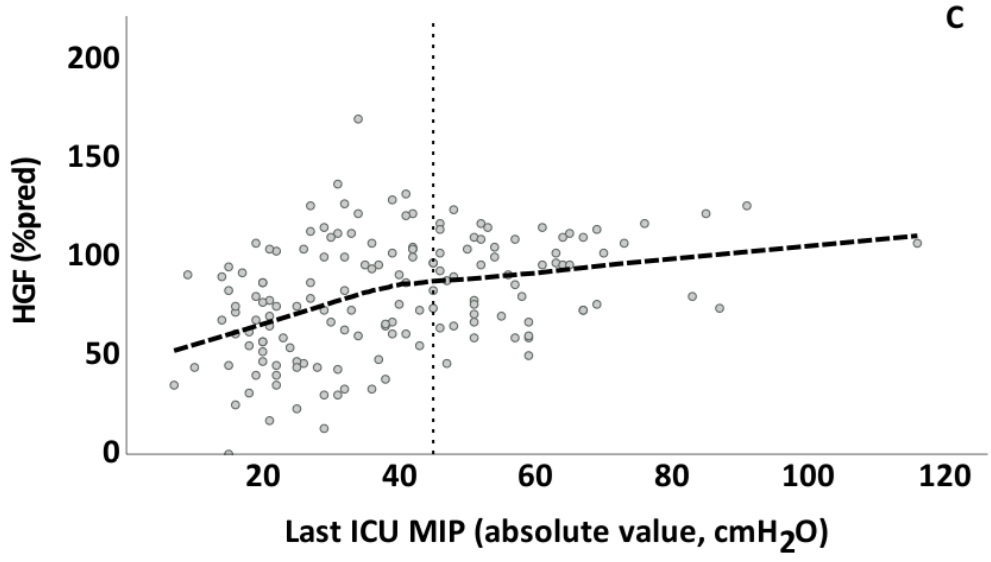
Suppl. Fig. 1: Kaplan-Meier survival plots with 95% confidence intervals depicting the proportion of patients alive up to 5 years following ICU admission according to the presence or absence of RMW at ICU discharge. RMW is defined as an absolute value of MIP <30cmH_2O. Effect size is expressed as Log-rank test and adjusted hazard ratio, correcting for potential confounders, was calculated with multivariable cox regression analysis. Abbreviations: *RMW*: Respiratory Muscle Weakness; *MIP*: Maximal Inspiratory Pressure.

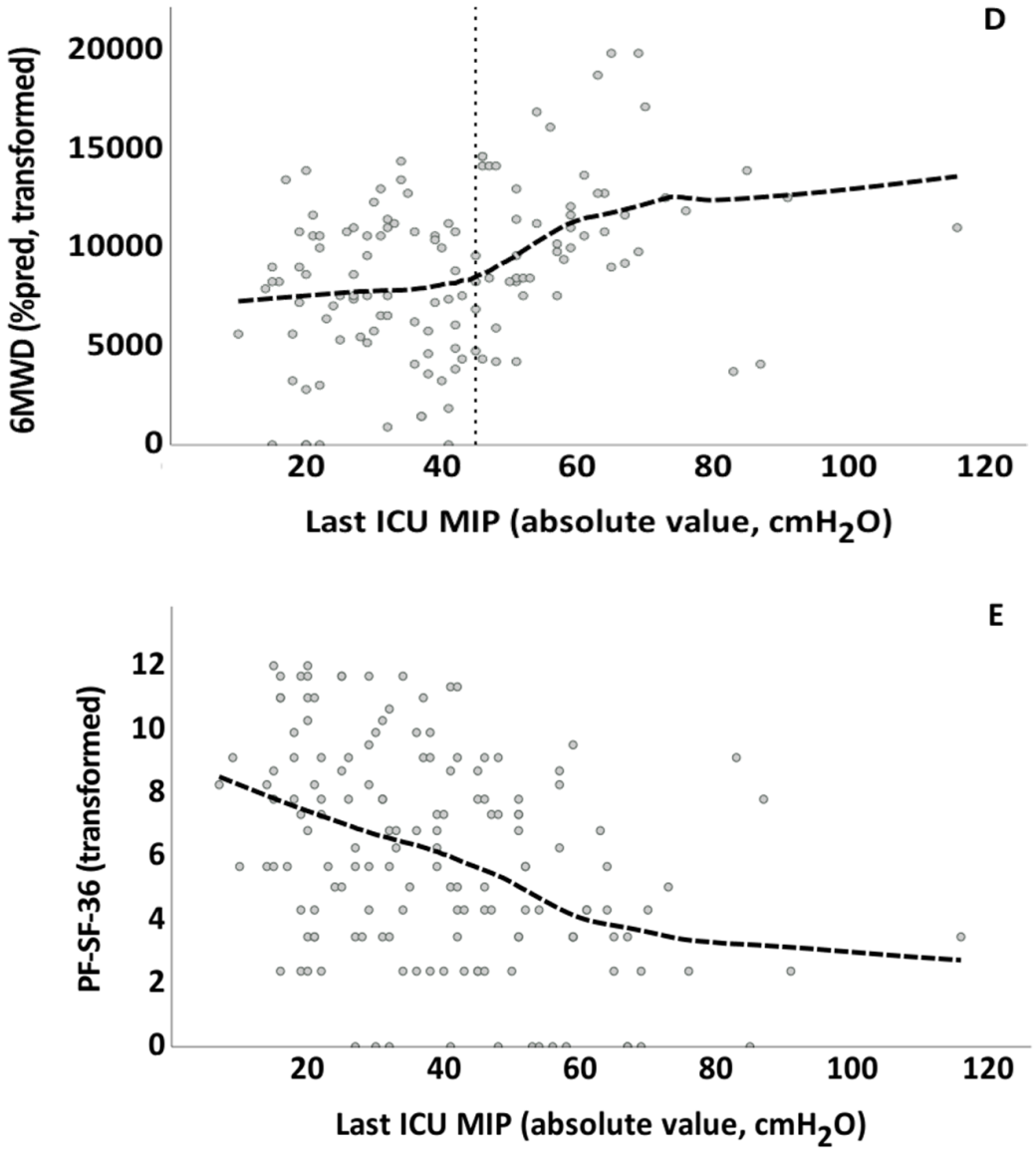


Suppl. Fig. 2: Morbidity outcomes at 5 years according to presence or absence of RMW at ICU discharge. RMW is defined as an absolute value of MIP <30cmH_2O. *Panel A:* maximal inspiratory pressure (MIP, cmH_2O), handgrip-strength (HGF, % predicted), 6-minute walk distance (6-MWD, % predicted) and Physical function of the SF-36 (PF SF-36, range 0-100 with higher values indicating better scores). *Panel B:* measures of peripheral muscle strength: hand-held dynamometry (HHD) for shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension, ankle dorsiflexion (% predicted), Medical-Research-Council (MRC) sum-score, Barthel index (range 0-20, higher values indicating better scores), Physical (PCS) and Mental Component score (MCS) of the SF-36 questionnaire and. P-values were obtained by Mann-Whitney U tests. Abbreviations: *RMW:* Respiratory Muscle Weakness, *MIP:* Maximal Inspiratory Pressure, *HGF:* Handgrip-strength, *6MWD:* 6-minute walk distance, *PF-SF-36:* Physical function of the *SF-36* questionnaire, *HHD:* Hand-Held Dynamometry, *MRC:* Medical-Research-Council, *PCS:* Physical Component score, *MCS:* Mental Component score.


A
Martingale residuals
1.0
.5
.0
-.5
-1.0
0
20
40
60
80
100
120
Last ICU MIP (absolute value, cmH2O)


B
MIP (absolute value, cmH2O)
150
100
50
0
20
40
60
80
100
120
Last ICU MIP (absolute value, cmH2O)


C
HGF (%pred)
200
150
100
50
0
20
40
60
80
100
120
Last ICU MIP (absolute value, cmH2O)



Suppl. Fig. 3: Exploration of the linearity assumption for MIP at ICU discharge in relation with 5-year mortality and morbidity. *Panel A:* LOESS-smoother on the Martingale residuals plot for 5-year mortality by MIP at ICU discharge. Martingale residuals were calculated from the adjusted Cox-regression model for 5-year mortality, including MIP at ICU discharge as a continuous variable and all confounders as covariates; *Panel B:* LOESS-smoother on the scatter plot for MIP (absolute value, cmH_2O) at 5-year follow-up by MIP at ICU discharge; *Panel C:* LOESS-smoother on the scatter plot for hand grip strength at 5-year follow-up (% predicted) by MIP at ICU discharge; *Panel D:* LOESS-smoother on the scatter plot for transformed 6-MWD at 5-year follow-up by MIP at ICU discharge; *Panel E:* LOESS-smoother on the scatter plot for of transformed PF-SF36 at 5-year follow-up by MIP at ICU discharge. The following transformations were performed: the 6MWD data were transformed to power 2 and the PF-SF-36 were reversed (100 minus actual value) and subsequently transformed to power 0.54 (higher transformed values of PF SF-36 correspond to lower actual values of PF SF-36). Abbreviations: *MIP:* Maximal Inspiratory Pressure, *HGF:* handgrip-strength, *6MWD:* 6-minute walk distance, *PF-SF-36:* Physical function of the SF-36 questionnaire.

Supplementary tables

Supplementary Table 1. Comparison of baseline and ICU-characteristics of eligible 5-year survivors included versus not-included in the five year morbidity analysis

	RMW at ICU discharge, included (N=56)	RMW at ICU discharge, not included (N=23)	*P*-value	No RMW at ICU discharge, included (N=100)	No RMW at ICU discharge, not included (N=67)	*P*-value
Baseline factors						
Age on admission	61 (52-73)	58 (40-76)	0.567	60 (50-67)	50 (37-63)	0.002
Gender, male	27 (48.2)	11 (47.8)	0.651	70 (70)	40 (59.7)	0.385
NRS>5	11 (19.6)	11 (47.8)	0.004	19 (19.0)	11 (16.4)	0.418
BMI	25.6 (23.4-31.9)	25.4 (21-29.4)	0.409	25.2 (23.2-27.7)	25 (23-29)	0.686
Diabetes mellitus	12 (21.4)	6 (26.1)	0.877	12 (12.0)	7 (10.4)	0.798
Malignancy	11 (19.6)	2 (8.7)	0.009	19 (19.0)	6 (9.0)	<0.001
Pre-admission dialysis	0 (0)	1 (4.3)	0.336	0 (0)	0 (0)	0.264
Randomisation, late PN	35 (62.5)	13 (56.5)	0.268	48 (48.0)	32 (47.8)	0.458
APACHE II	30 (18-36)	34 (25-37)	0.142	26 (16-34)	25 (14-33)	0.302
Admission category			0.449			<0.001
Cardiac surgery	25 (44.6)	6 (26.1)		52 (52.0)	23 (34.3)	
Emergency SICU	20 (35.7)	13 (56.5)		32 (32.0)	30 (44.8)	
Elective SICU	2 (3.6)	0 (0)		4 (4.0)	4 (6.0)	
MICU	9 (16.1)	4 (17.4)		12 (12.0)	10 (14.9)	
Sepsis upon admission	19 (33.9)	13 (56.5)	0.134	28 (28.0)	26 (38.8)	0.084
ICU factors						
Mechanically ventilated	55 (98.2)	22 (95.7)	0.607	99 (99.0)	63 (94.0)	0.040
Mechanical ventilation, days	6 (2-14)	8 (3-11)	0.914	2 (1-7)	3 (1-8)	0.491
ICU stay, days	13 (6-22)	12 (5-17)	0.593	4 (1-11)	9 (2-14)	0.083
Hospital stay, days	29 (19-59)	28 (15-40)	0.362	16 (10-30)	18 (10-36)	0.316

Continuous variables are reported with median and interquartile range, dichotomous variables are reported with number and percentage. *P*-values reflect differences between eligible patients included versus not included in the 5-year morbidity analyses. Eligible patients were those surviving at 5 years and not falling under the exclusion criteria.

Abbreviations: *RMW:* Respiratory Muscle Weakness; *BMI:* body mass index; *NRS:* nutritional risk score; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology And Chronic Health Evaluation; *SICU:* Surgical Intensive Care Unit; *MICU:* Medical Intensive Care Unit.

Supplementary Table 2. Exploratory analyses and sensitivity analyses

Five-year mortality analyses		
	HR (95%CI)[a]	***P*-value**
MIP (absolute value) at ICU discharge as a continuous variable, HR per unit increase	0.991 (0.976-1.004)	0.240
MIP (absolute value) at ICU discharge, dichotomised at 45 cmH_2O	1.381 (0.770-2.980)	0.220
RMW at ICU discharge, analysis limited to hospital survivors	1.217 (0.624-2.300)	0.518
RMW at ICU discharge (model including MRC sum score at ICU discharge as a continuous variable)	1.162 (0.658-1.970)	0.569
RMW at ICU discharge (model including MRC sum score at ICU discharge, dichotomised at 48)	1.346 (0.811-2.136)	0.240
RMW at ICU discharge (model including time-dependent covariates for factors violating the proportional hazard assumption[b])	1.248 (0.785-1.984)	0.348
Five-year morbidity analyses		
Maximal inspiratory pressure (cmH_2O)	**B (95%BcaCI)[a]**	***P*-value**
MIP (absolute value) at ICU discharge as a continuous variable	0.669 (0.389-0.975)	0.001
MIP (absolute value) at ICU discharge, dichotomised at 45 cmH_2O	-20.086 (-28.325 to -11.539)	0.001
RMW at ICU discharge (model including MRC sum-score at ICU discharge as a continuous variable)	-22.919 (-32.645 to -14.262)	0.001
RMW at ICU discharge (model including MRC sum-score at ICU discharge, dichotomised at 48)	-25.031 (-33.517 to -16.827)	0.001
Hand-grip strength (% pred)		
MIP (absolute value) at ICU discharge as a continuous variable	0.421 (0.200-0.676)	0.002
MIP (absolute value) at ICU discharge, dichotomised at 45 cmH_2O	-10.714 (-19.606 to -1.572)	0.020
RMW at ICU discharge (model including MRC sum-score at ICU discharge as a continuous variable)	-19.811 (-32.318 to -6.897)	0.001
RMW at ICU discharge (model including MRC sum-score at ICU discharge, dichotomised at 48)	-21.534 (-32.253 to -9.371)	0.001
6-minute walk distance (%pred, transformed) [c]		
MIP (absolute value) at ICU discharge as a continuous variable	78.778 (37.600-131.736)	0.001
MIP at ICU discharge, dichotomised at an absolute value of 45 cmH_20	-3464.1 (-5116.8 to -1959.9)	0.001
RMW at ICU discharge (model including MRC sum-score at ICU discharge as a continuous variable)	-1643.64 (-3395.4 to -106.2)	0.030
RMW at ICU discharge (model including MRC sum-score at ICU discharge, dichotomised at 48)	-1921.4 (-3282.3 to -651.2)	0.007

Continued Supplementary Table 2. Exploratory analyses and sensitivity analyses

Five-year morbidity analyses		
PF-SF-36 (score, transformed) [c]	**B (95%BcaCI)**[a]	***P*-value**
MIP (absolute value) at ICU discharge as a continuous variable	-0.049 (-0.075 to -0.020)	0.001
MIP (absolute value) at ICU discharge, dichotomised at 45 cmH_2O	1.755 (0.649-2.768)	0.002
RMW at ICU discharge (model including MRC sum-score at ICU discharge as a continuous variable)	1.183 (-0.072 - 2.337)	0.046
RMW at ICU discharge (model including MRC sum-score at ICU discharge, dichotomised at 48)	1.324 (0.218-2.379)	0.015

[a] All models are adjusted for confounders;
[b] The proportional hazards assumption was violated for BMI and for ICU length of stay, adjusted hazard ratio is based on adjustment of the model by adding a time dependent factor for BMI and for ICU length of stay;
[c] The following transformations were performed: the 6MWD data were transformed to power 2 and the PF-SF-36 were reversed (100 minus actual value) and subsequently transformed to power 0.54 (higher transformed values of PF-SF-36 correspond to lower actual values of PF-SF-36).
Abbreviations: *MIP*: maximal inspiratory pressure; *RMW*: respiratory muscle weakness, defined as an absolute value of MIP<30 cmH_2O.

Supplementary Table 3. Rehabilitation trajectory, working and living conditions at 5-year follow-up

	RMW at ICU-discharge (N=56)	No RMW at ICU discharge (N=100)	*P*-value
Rehabilitation between hospital discharge and 5-year follow up visit			
Any physiotherapy	37 (66.1)	67 (67.0)	0.906
Any in-patient rehabilitation	15 (26.8)	14 (14.0)	0.049
Living conditions			
Living at home	56 (100)	98 (98.0)	0.567
Working conditions			
Fulltime job	4 (7.1)	17 (17.0)	0.277
Part-time job	2 (3.6)	7 (7.0)	
House wife	2 (3.6)	3 (3.0)	
Invalidity	12 (21.4)	11 (11.0)	
Student	0	1 (1.0)	
Retirement	33 (58.9)	60 (60.0)	
Temporarily at home	1 (1.8)	0	
Unemployed	2 (3.6)	1 (1.0)	
Working condition as compared to pre-ICU [a]			
Same work as before	15/32 (46.8)	26/55 (47.3)	0.967
Readmissions			
N of patients readmitted to the ICU (%)[b]	11 (19.6)	13 (13.0)	0.270

[a]Calculated after exclusion of patients retired prior to ICU admission

[b]Readmissions to the ICUs of the University hospitals of Leuven following hospital discharge

Abbreviations: *RMW:* Respiratory Muscle Weakness; *ICU:* Intensive Care Unit.

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Chapter 6: Aerobic exercise capacity in long-term survivors of critical illness

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ABSTRACT

Purpose

To describe aerobic exercise capacity in 5-year survivors of intensive care (ICU) as compared to healthy controls and to assess the association between severity of organ failure in ICU and exercise capacity up to 5-years follow-up.

Methods

Secondary analysis of the EPaNIC follow-up cohort (NCT00512122) including 433 patients screened with cardiopulmonary exercise testing (CPET) between 1 to 5-years following ICU admission. Aerobic exercise capacity in 5-year ICU survivors (N=361) was compared to demographically matched controls (N=49). We described the incidence of abnormal exercise capacity, defined as peak oxygen consumption (VO_2peak) <85% of predicted maximal oxygen consumption (%predVO_2max) in maximal CPETs (defined as respiratory exchange ratio >1.05), and identified exercise limiting factors. To study the association between severity of organ failure, quantified as the maximal Sequential Organ Failure Assessment score during ICU-stay (SOFA-max), and aerobic exercise capacity as assessed with VO_2peak, a linear mixed model was built, adjusting for predefined confounders and including all follow-up CPET studies.

Results

Aerobic exercise capacity was lower in 5-year follow-up patients than in controls (VO_2peak: 24.0±9.7ml/min/kg versus 31.7±8.4ml/min/kg, p<0.001; %predVO_2max: 94%±31% versus 123%±25%, p<0.001; VO_2AT: 15.3±8.7ml/min/kg versus 18.5±6.0ml/min/kg, p<0.001; %predVO_2max: 55%±16% versus 71%±21%, p<0.001). Abnormal exercise capacity was present in 118/313 (37.7%) patients versus 1/48 (2.1%) controls, p<0.001. Muscular limitation frequently contributed to impaired exercise capacity at 5-years [69/118 (58.5%)]. SOFA-max independently associated with VO_2peak throughout follow-up.

Conclusions

Critical illness survivors often display abnormal aerobic exercise capacity, frequently involving muscular limitation. Severity of organ failure throughout the ICU stay independently associates with these impairments.

INTRODUCTION

The impact of severe illness requiring admission to an intensive care unit (ICU) extends long beyond the ICU stay. Survivors of critical illness frequently report exercise intolerance and demonstrate reduced muscle strength and six-minute-walk-distance as compared to healthy controls, features paralleled with reduced quality of life [1-11]. The post-ICU trajectories of patients may vary strongly [8, 9, 12, 13], but a particularly pertinent association between long-term physical impairments and illness severity has been suggested and illustrated in several cohorts of survivors of the acute respiratory distress syndrome (ARDS) and sepsis [3, 8, 9]. Static clinical evaluations of muscle strength and physical function however, incompletely capture physical fitness, which is a highly dynamic state [14]. Cardiopulmonary exercise testing (CPET) in contrast, represents a global assessment of the integrated physiological exercise response, and is the gold standard to assess exercise capacity. CPET better reflects exercise performance as compared to the evaluation of single components under resting circumstances [14, 15]. Importantly, it also allows to distinguish between cardiorespiratory and muscular impairment as factors contributing to exercise limitation [15]. Yet, aerobic exercise capacity is scarcely studied in ICU survivors. Three small case series reported significant exercise limitations within the first weeks [16, 17] and up to 3 months [18] post-ICU, and suggested an important contribution of deconditioning and muscle weakness. No data at later time points are available. Determining aerobic exercise capacity however is important as it not only mirrors functional performance in daily life [19, 20] and health-related quality of life [21], it independently predicts long-term health outcomes and mortality in the general population [22-24] as well as in a variety of clinical conditions [25-27]. Furthermore, information on aerobic exercise capacity in ICU survivors may be important as CPET-guided rehabilitation can improve cardiorespiratory fitness in heterogeneous patient populations known to suffer from muscular impairments [28-33], with small increments in exercise capacity entailing clinically relevant short- and long-term benefits [34, 35].

Here, we aimed to investigate cardiorespiratory fitness in long-term survivors of critical illness through CPET. We hypothesized that 5-year survivors of critical illness would have impaired aerobic exercise capacity as compared to healthy controls. We further hypothesized that in these patients, a muscular component frequently contributes to exercise limitation. Finally, we hypothesized that impaired aerobic exercise capacity in ICU survivors would independently associate with the severity of organ failure during critical illness.

METHODS

Ethics

The study protocol of EPaNIC and its long-term follow-up were approved by the Ethical Committee Research UZ/KU Leuven (ML4190). All patients gave informed consent.

Study design and participants

This is a secondary analysis of the prospective 5-year follow-up study of the EPaNIC trial (Clinicaltrials.gov:NCT00512122) in which we included patients assessed for cardiorespiratory fitness by cardiopulmonary exercise testing from 1-year, up to 5-years post-inclusion. The EPaNIC trial was a large randomised study (N=4640) comparing early (within 48h) versus late (not within the first week) parenteral supplementation of deficient enteral nutrition [36]. The post-EPaNIC follow-up study evaluated mortality in all, and morbidity in UZ Leuven survivors during follow-up hospital visits. If a hospital visit was refused or not feasible, a home visit was offered. All long-stayers (ICU stay ≥8 days), and a random set of short-stayers (ICU stay <8 days) were invited at 5-years, in the absence of pre-ICU diagnosis of a neuromuscular disease or the presence of other major pre-ICU disabilities as published previously [11, 13]. At intermediate time points, patients were invited according to residual time-slot

availability, with a priority for long-stayers. In patients attending the hospital, exercise capacity was assessed with CPET. Additional exclusion criteria for CPET are detailed in the online supplement and involved safety concerns, refusal or practical reasons.

To evaluate the exercise capacity in 5-year survivors, a group of 49 controls who never stayed in the ICU were selected to demographically match to this cohort and served as a healthy reference.

Cardiopulmonary exercise testing

An incremental, symptom-limited CPET was performed on an electrically braked cycle-ergometer (eBIKE, GE, provided by Acertys). The CPET was conducted by 1 of four trained physiotherapists (HVM, TVA, TVHB, SV) in the presence of a physician with advanced life support skills. After a 2-minute resting phase, followed by 3 minutes of cycling without resistance, an initial load of 20 Watt was imposed and subsequently increased with 20 Watt/minute until peak exercise was reached, by taking exercise to exhaustion. Safety criteria for immediate termination were predefined [15, 37] (see online supplement). The test was performed under continuous monitoring of heart rate, transcutaneous oxygen saturation, and 12-lead electrocardiography. Blood pressure was measured at rest and then every 2 minutes. Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and minute ventilation (VE) were measured on a breath-by-breath basis (JAEGER® Oxycon Pro® and Vyntus CPX® metabolic carts [38] provided by CareFusion). Variables were collected as 30 second averaged data. After termination of the test, patients were monitored until recovery to baseline values or for a maximum of 6 minutes.

Main CPET parameters

The main exercise parameters derived were obtained at two pivotal points during incremental exercise: (1) *peak exercise*, corresponding to the highest work rate achieved upon symptom limitation of exercise and maintained for 30 seconds; and (2) the *anaerobic threshold*, the point during exercise considered to mark the onset of anaerobic metabolism. This was determined noninvasively by concordance of the V-slope and ventilatory equivalent methods (at respiratory exchange ratio approaching 1) [15].

At *peak exercise*, we obtained (a) peak oxygen consumption rate (VO_2peak), a widely accepted approximation of VO_2max, which is the best index of cardiorespiratory fitness [15, 27]; (b) peak work rate; (c) peak heart rate (HRpeak) and heart rate reserve (HRR), defined as the difference between the predicted maximal heart rate and the heart rate achieved at peak exercise; (d) peak ventilation (VEpeak) and ventilatory reserve, defined as the ratio of VEpeak to Maximal Voluntary Ventilation (MVV). MVV was measured prior to performance of the exercise test; (e) peak oxygen pulse (O_2-pulse); the ratio of VO_2peak to peak heart rate, approximates the amount of O_2 extracted per heart beat; (f) respiratory exchange ratio (RER), calculated as VCO_2peak/VO_2peak.

(g) The *anaerobic threshold* is generally referenced to the VO_2 at which the metabolic changes occur (VO_2AT), and theoretically corresponds to the level of exercise that could be maintained aerobically for indefinite periods of time; (h) Additionally, the ratio of ventilation rate to CO_2-production rate at the anaerobic threshold (VE/VCO_2AT) or ventilatory efficiency, is considered the most informative point estimate of ventilation and gas exchange efficacy during exercise [39]. VE/VCO_2AT is considered abnormal if higher than 34 [15].

(i) In addition to estimates at *peak exercise* and at the *anaerobic threshold*, the metabolic efficacy for mechanical work, reflected by $\Delta VO_2/\Delta WR$, was calculated.

Predicted values were based on reference equations proposed by Jones *et al* [40].
Categorising CPET outcomes

The main goal of the process of classification of the CPET studies was to identify the patients in whom abnormal aerobic exercise capacity was primarily due to peripheral limitations, presumably due to muscular impairments or deconditioning, further referred to as muscular limitations. To avoid erroneous labelling of patients delivering insufficient effort, we first excluded patients without maximal CPET. Maximal effort was defined as RER > 1.05 [14]. Next, we identified patients who exhibited an abnormal exercise capacity, defined as peak oxygen consumption (VO_2peak) < 85% of predicted VO_2max [15, 41]. We than identified patients in whom ventilatory limitations were presumed, and in whom signs of cardiac disease were present. Patients without clear indication of such pathological exercise responses were considered likely to suffer predominantly from muscular limitation. Further details are provided in the online supplement.

Severity of organ failure

Severity of organ dysfunction was quantified as the maximal Sequential Organ Failure Assessment (SOFA-max) score during intensive care stay, ranging from 0 to 24, with higher values indicating more severe organ failure [42, 43].

Other outcomes

At follow-up, patients also underwent pulmonary function testing, evaluation of peripheral and respiratory muscle strength, functional exercise capacity as assessed with the 6-minute walking distance (6MWD), and quality-of-life assessment with the SF-36 questionnaire as reported previously [11, 13]. Further details are provided in the online supplement.

Statistics

The primary outcome was aerobic exercise capacity in 5-year survivors, as compared to healthy controls. Secondary outcomes included description of exercise limiting factors in 5-year survivors versus controls and the association between the severity of organ dysfunction and exercise capacity in ICU survivors.

Descriptive analysis

Descriptive statistics included mean and standard deviations or median and interquartile ranges for continuous variables as appropriate, and numbers and percentages for categorical variables. Comparisons were made with t-test or Mann-Whitney-U test for continuous variables, and Chi-squared or Fisher-exact tests for categorical variables, as appropriate.

Association between aerobic exercise capacity and severity of organ failure

To assess whether long-term aerobic exercise limitation was independently associated with severity of organ dysfunction throughout follow-up, data from all CPET studies between 1- and 5-year follow-up were used and we created an adjusted linear mixed model for VO_2peak. Fixed effects included the SOFA-max as key explanatory variable, time-to-follow-up in years, the interaction between SOFA-max and time, as well as a set of a priori selected confounders for adjustment [44]. Further details are provided in the online supplement.

Exploratory analyses

If an independent effect of the severity of organ failure on peak oxygen consumption was demonstrated, we further examined whether the effect of severity of organ failure could be explained by persisting weakness. For this purpose, we built additional linear mixed models in which both knee and hip strength at follow-up were added as covariates in separate models.

Descriptive analyses were conducted with SPSS version 27 (IBM), linear mixed modelling with the MIXED procedure in SAS version 9.4 (SAS institute).

RESULTS

Participants

Throughout the 5-year period, 1151 follow-up visits were performed in 774 unique patients. CPET was performed during 591 follow-up visits (in 40/76 visits at 1-year, 54/115 at 2-years, 72/152 at 3-years, 64/134 at 4-years and 361/674 at 5-years), involving 433 unique patients. Reasons for not performing CPET are provided in Figure 1. Characteristics of enrolled and non-enrolled 5-year follow-up patients are depicted in Table 1. Follow-up patients who performed a CPET were significantly younger, more frequently were male, had fewer comorbidities, received vasopressors less frequently and for shorter periods of time, less frequently acquired a new infection and had shorter ICU stays as compared to patients who did not perform a CPET. Mean time to follow-up was 5.5±0.2 years. In parallel, 49 controls were tested with similar demographics as the 5-year cohort (Supplementary Table 1). Baseline and ICU characteristics of the cohorts at intermediate time points are provided in Supplementary Table 2.

Cardiopulmonary exercise capacity in five-year survivors

VO_2peak was significantly lower in 5-year follow-up patients as compared to controls (24.0±9.7 ml/min/kg versus 31.7±8.4 ml/min/kg, $p < 0.001$; % predicted VO_2max: 94%±31% versus 123%±25%, $p < 0.001$)(Figure 2 & Table 2). The anaerobic threshold was identified in 331/361(92%) patients at 5-year follow-up and values were significantly lower in the 5-year follow-up cohort as compared to the healthy controls (VO_2AT: 15.3±8.7 ml/min/kg versus 18.5±6.0 ml/min/kg, $p < 0.001$; % predicted VO_2max: 55%±16% versus 71%±21%, $p < 0.001$). In the 5-year follow-up cohort, 313/361 (86.7%) patients performed a maximal test, of which 118 (37.7%) had impaired aerobic exercise capacity. This was significantly different from controls, in whom 48/49 (98.0%) performed a maximal test, of which 1/48 (2.1%) exhibited an abnormal aerobic exercise capacity ($p<0.001$). Other CPET results are shown in Table 2.

Factors limiting cardiopulmonary exercise capacity in five-year survivors

In 5-year follow-up patients exhibiting abnormal exercise capacity, the anaerobic threshold was abnormal in 78/118 (66.1), undetermined in N=4/118 (3.4%) and normal in 36/118 (30.5%) of patients. After elimination of patients in whom primary ventilatory [7/118 (5.9%)], gas exchange [5/118 (4.2%)], combined respiratory [3/118 (2.5%)], or cardiac limitation [12/118 (10.2%)] of exercise was suspected, a primary muscular limitation was presumed in 69/118 (58.5%) of these patients. (Supplementary Figure 1). In the single control subject, the anaerobic threshold was normal and there were no signs of ventilatory or gas exchange impairment, nor signs of cardiac ischemia, hence abnormal exercise capacity was likely due to muscular limitation.

Other physical outcomes

As reported previously in general ICU survivors, the 5-year follow-up patients performed worse on all other physical outcomes as compared to controls (Table 3)[11]. Patients who received a CPET had significantly higher peripheral and respiratory muscle strength, performed better on pulmonary function tests, 6-MWD, and functional quality-of -life as compared to those who did not perform CPET.

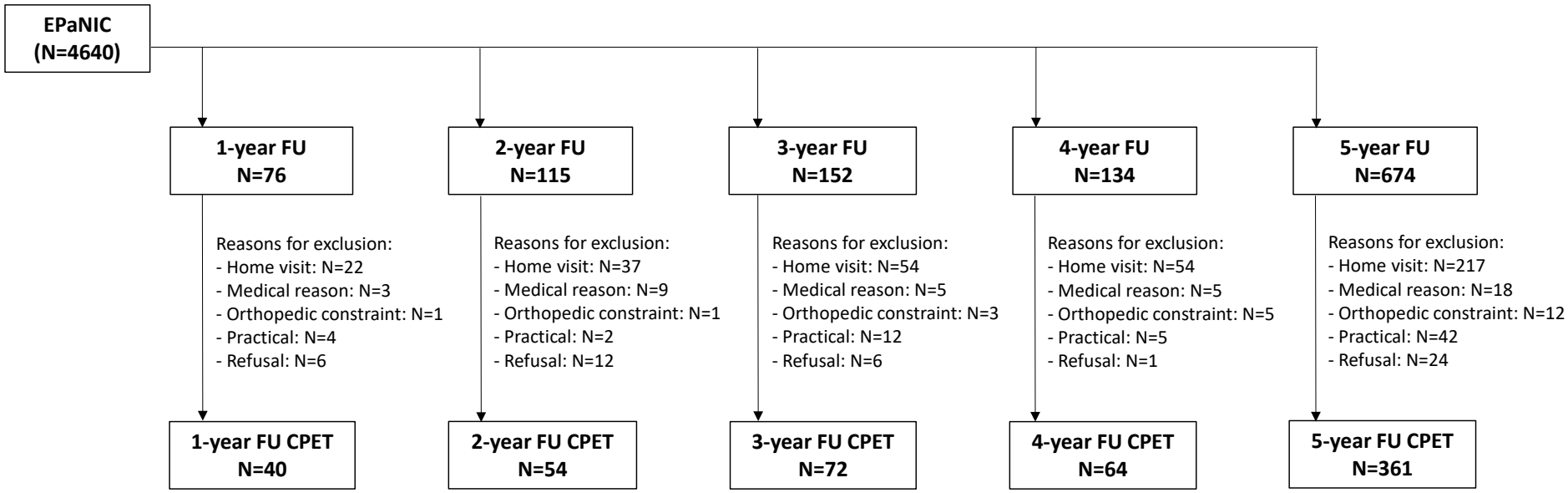

Fig. 1: Patient flow chart and reasons for not performing CPET during the 5-year follow-up period. Abbreviations: *EPaNIC*: Early Parenteral Nutrition in Intensive Care; *CPET*: Cardiopulmonary Exercise testing

Table 1: Admission and ICU-characteristics of patients evaluated at 5-year follow-up

	CPET (N=361)	No CPET (N=313)	*P*-value
ADMISSION			
Age*	59±14	68±15	<0.001
Gender, male	254 (70.4)	196 (62.6)	0.003
BMI*	27.5±4.7	26.8±5.0	0.056
Diabetes mellitus	40 (11.1)	58 (18.6)	0.006
Malignancy	47 (13.0)	59 (18.9)	0.036
Preadmission dialysis	3 (0.8)	0	0.253
Diagnostic category			0.131
Cardiac surgery	146 (40.4)	154 (49.4)	
Emergency SICU	164 (45.4)	119 (38.1)	
Elective SICU	26 (7.2)	18 (5.8)	
Medical ICU	25 (6.9)	21 (6.7)	
Randomisation, late PN	181 (50.1)	163 (52.2)	
APACHE II	26 (16-33)	24 (17-34)	0.543
Sepsis upon admission	88 (24.4)	88 (28.2)	0.260
ICU STAY			
MV	351 (97.2)	298 (95.5)	0.231
Duration of MV, days	3 (1-8)	3 (2-9)	0.190
Vasopressors/ inotropics	291 (80.6)	269 (86.2)	0.052
Duration of HD support, days	2 (1-5)	3 (2-9)	<0.001
New infection	110 (30.5)	120 (38.5)	0.029
New dialysis	26 (7.2)	31 (9.9)	0.204
Bilirubin>3 mg/dL	64 (17.8)	56 (17.9)	0.954
ICU LOS, days**	5 (2-12)	7 (3-14)	0.001
ICU-stay>8 days	136 (37.7)	140 (44.9)	0.058

Continuous variables are depicted as mean (standard deviation)* or median (interquartile range)**,categorical variables are depicted as number (percentages).

Abbreviations: *ICU:* intensive care unit; *CPET:* cardiopulmonary exercise test; *BMI:* Body Mass index; *SICU:* surgical intensive care unit; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology and Chronic Health Evaluation; *MV:* mechanical ventilation; *HD:* hemodynamic; *LOS:* Length of stay.

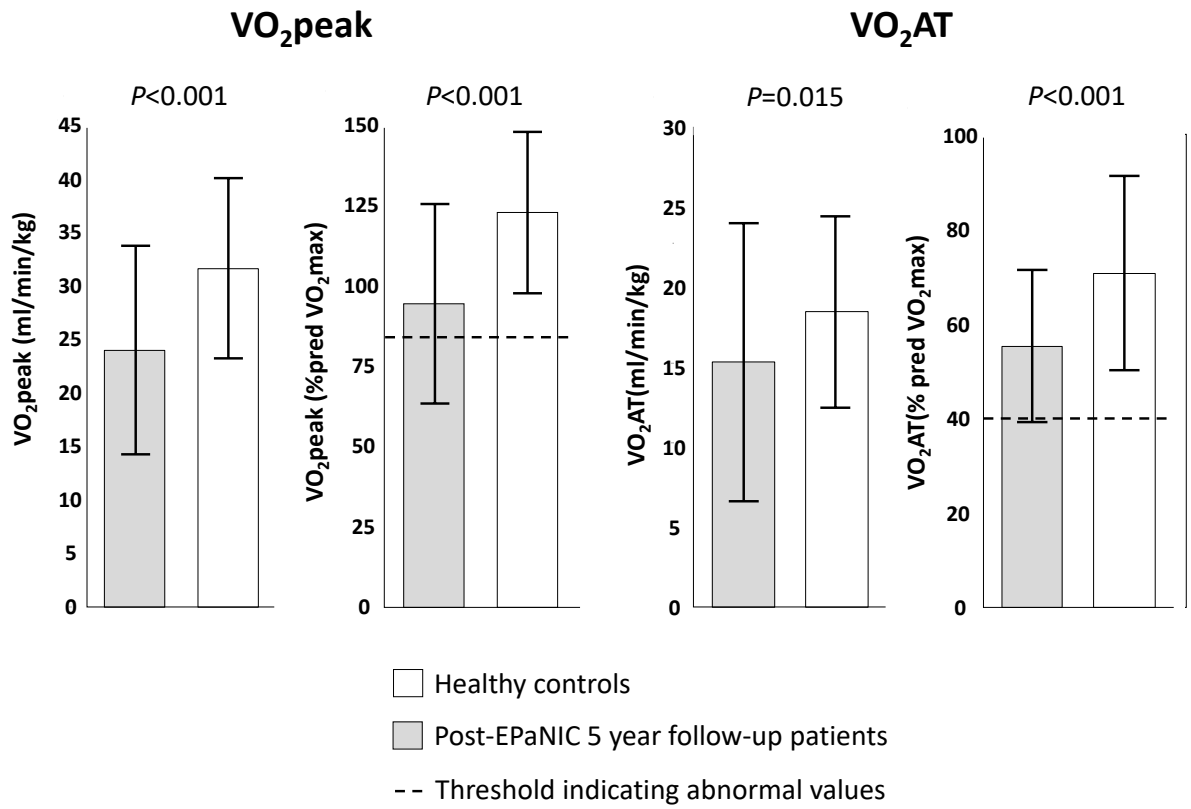

Fig. 2: Key outcomes of cardiopulmonary exercise testing in the post-EPaNIC 5-year follow-up cohort and controls, depicted as mean and standard deviation. *Panel A*: VO_2peak in ml/kg/min and as percentage predicted of VO_2max; *Panel B*: VO_2AT in ml/kg/min and as percentage predicted of VO_2max. Comparisons were performed with t-test. Abbreviations: *VO_2peak: peak oxygen consumption rate*; *VO_2AT*: oxygen consumption rate at the anaerobic threshold.

Table 2: Selected outcomes of cardiopulmonary exercise testing in the post-EPaNIC 5-year follow-up cohort and controls

	EPANIC 5-year follow-up	Controls	*P*-value
CPET – PEAK EXERCISE	**N=361**	**N=49**	
VO_2peak (ml/min/kg)	24.0±9.7	31.7±8.4	<0.001
VO_2peak (%pred)	94±31	123±25	<0.001
Work rate (%pred)	81±26	112±29	<0.001
Heart rate (beats per minute)	128±29	150±21	<0.001
Heart rate (%predHRmax)	80±15	95±12	<0.001
Heart rate reserve	31±24	7±20	<0.001
O_2-pulse (ml/beat)	14.8±4.8[a]	17.1±4.6	0.001
O_2-pulse (%pred)	110±31[a]	118±23	0.068
SpO_2 (%)	95±2[b]	96±2	0.574
VEmax (L/min)	67.3±27.2	85.2±23.4	<0.001
Ventilatory reserve (VE/MVV)	64±18[c]	66±15	0.370
RER	1.17±0.11	1.21±0.09	0.025
$\Delta VO_2/\Delta WR$ (ml/W/min)	13.4±5.1[d]	13.6±2.5[e]	0.955
CPET – ANAEROBIC THRESHOLD	**N=331**	**N=49**	
VO_2AT (ml/min/kg)	15.3±8.7	18.5±6.0	0.015
VO_2AT (%pred of VO_2max)	55±16	71±21	<0.001
VE/VCO_2AT	31.0±17.3	27.6±3.4	<0.001

Continuous variables are depicted as mean (± standard deviation), categorical variables are depicted as number (percentages). Measurement available in [a] N=357, [b] N= 338, [c] N=355, [d]N=345, [e]N=21.

Abbreviations: *CPET:* cardiopulmonary exercise testing; *HR:* heart rate; *VE:* ventilation; *VO_2:* oxygen consumption rate; *AT:* anaerobic threshold; *VCO_2:* carbon dioxide production rate; *RER:* respiratory exchange ratio; *O_2-pulse:* oxygen pulse; *VE/VCO_2AT:* ratio of ventilation to CO_2-production at the anaerobic threshold; *$\Delta VO_2/\Delta WR$:* metabolic efficiency, calculated as (VO_2peak-VO_2baseline)/peak work rate.

Table 3: Demographics, physical outcomes in the post-EPaNIC 5-year follow-up cohort and controls

	EPaNIC 5-year follow-up					Controls		
	No CPET	**N****	**CPET**	**N****	*P*-value		**N****	*P*-value*
Test location, hospital	94 (30.0)	**313**	361 (100)	**361**	<0.001	49 (100)	**49**	NA
PULMONARY FUNCTION								
FVC (%pred)	85±24	306	103±19	361	<0.001	114±14	49	<0.001
FEV1 (%pred)	78±24	306	94±20	361	<0.001	108±12	49	<0.001
Tiff	74±10	306	74±9	361	0.932	77±6	49	0.022
TLC (%pred)	92±15	82	98±15	331	0.001	NA	0	NA
DLCO (%pred)	72±19	97	81±17	349	<0.001	NA	0	NA
MUSCLE STRENGTH								
MRC-sum score	60 (57-60)	307	60 (58-60)	361	0.001	60 (60-60)	49	<0.001
HGF (%pred)	77±29	312	93±21	359	<0.001	104±19	49	<0.001
HHD (%pred)								
Shoulder	86±28	304	94±22	355	<0.001	104 ±27	49	0.002
Elbow	79±23	305	89±20	360	<0.001	103±21	49	<0.001
Wrist	95±27	298	102±23	360	<0.001	109±22	49	0.038
Hip	139±40	297	148±33	352	0.002	163±36	49	0.004
Knee	53±18	293	53±15	340	0.794	65±15	48	<0.001
Ankle	72±21	299	73±21	358	0.480	91±22	49	<0.001
Knee extension, (Biodex %pred)	79±28	79	86±26	333	0.037	99±30	46	0.002
MIP (%pred)	83±32	299	95±30	357	<0.001	105±27	49	0.029
PHYSICAL FUNCTION								
6MWD (%pred)	81±22	206	95±19	361	<0.001	115±18	49	<0.001
QUALITY OF LIFE								
PCS	42 (32-52)	290	49 (39-55)	344	<0.001	56 (52-58)	49	<0.001
PF-SF36	60 (30-85)	306	80 (60-95)	357	<0.001	95 (90-100)	49	<0.001
MCS	55 (45-59)	290	55 (49-59)	344	0.689	58 (53-60)	49	0.025

Continuous variables are depicted as mean± standard deviation or median (interquartile range) as appropriate, categorical variables are depicted as number (percentages).

* P-value for comparison of patients EPaNIC patients at 5-year follow-up who performed CPET and controls.

** Number of patients for whom data are available.

Abbreviations: *FVC:* Forced Vital Capacity; *FEV1:* Forced Expiratory Volume in 1 second; *TLC*: Total lung capacity; *VC:* Vital Capacity; *DLCO:* diffusion capacity; *MRC:* Medical Research Council sum score; *HGF:* Hand-grip-strength; *HHD:* Handheld dynamometry; *MIP:* maximal inspiratory pressure; *6MWD:* six-minute-walk-distance; *PF-SF36:* Physical function sub-score of the 36-item Short Form Health score; *MCS: Mental Component Score; PCS:* Physical component score.

Association between severity of organ failure and aerobic exercise capacity

Baseline characteristics (age, BMI, diabetes mellitus, preadmission dialysis, malignancy) and ICU-characteristics (feeding strategy during ICU-stay, diagnostic category and sepsis upon ICU-admission, and length of ICU-stay) [11, 44] were identified as potential confounders through a literature search and were included as fixed effects in the adjusted linear mixed models for both of the key CPET outcomes. Severity of organ dysfunction significantly associated with peak oxygen consumption rate with a reduction in VO_2peak of 0.345 ml/min/kg (95% CI: -0.669 to -0.021, $p = 0.037$) per point increase in SOFA-max throughout follow-up (Supplementary Table 3). This effect did not depend on the timing of evaluation during the 1- to 5-year study period. There was no significant difference between VO_2 peak at intermediate time points and VO_2peak at 5-year follow-up.

Exploratory analyses

Both knee extensors strength [increase in VO_2peak of 0.229 ml/min/kg per 10 N increase in strength (95% CI: 0.132-0.327, $p < 0.001$] and hip flexors strength at follow-up [increase in VO_2peak of 0.182 ml/min/kg per 10 N increase in strength (95% CI: 0.071-0.294), $p = 0.001$] independently associated with VO_2peak at follow-up. In these models adjusting for strength at follow-up, SOFA-max however remained independently associated with VO_2peak. Hence, strength at follow-up did not statistically explain the effect of SOFA-max on the VO_2peak (Supplementary Table 3).

DISCUSSION

In this cohort of critical illness survivors, we evaluated aerobic exercise capacity with cardiopulmonary exercise testing throughout a follow-up period of 5 years. Peak oxygen consumption rate and oxygen consumption rate at the anaerobic threshold were significantly lower in 5-year ICU-survivors as compared to a demographically matched control group and abnormal exercise capacity was present in 118/313 (37.7%) of patients. Muscular limitation contributed to abnormal exercise capacity in 58.5% of these patients. Adjusted for confounders, severity of organ failure throughout the ICU stay was independently associated with the peak oxygen consumption. The association between SOFA max and VO_2peak was not explained by strength at follow-up.

Though physical impairments after ICU- and hospital discharge and their association with illness severity are well-documented in various subpopulations of critical illness [3, 5, 7, 12], data on aerobic exercise capacity in ICU survivors are largely uncharted. In a cohort of 50 patients mechanically ventilated for at least 5 days, severe exercise limitation with VO_2peak at 56%±16% and an anaerobic threshold of 41%±13% of predicted VO_2max were described, 24±14 days after hospital discharge [16]. In 10 patients mechanically ventilated for acute lung injury, VO_2peak was significantly lower 6 weeks following hospital discharge as compared to controls (median 17.8 ml/kg/min versus 31.8 ml/kg/min)[17]. Ong et al. demonstrated reduced VO_2peak in 18/44 (41%) of SARS-survivors 3 months after hospital discharge, of which 10 had required intensive care[18]. Our study focused on the longer-term exercise limitations and showed overall improved aerobic exercise capacity as compared to results obtained very early after hospital discharge [16, 17]. However, exercise capacity was still reduced as compared to controls, and striking within-patients variability was observed, with 37.7% demonstrating reduced exercise capacity 5 years following ICU stay. Noteworthy, our data show no difference in exercise capacity at intermediate time points as compared to the 5-year follow-up point. Differences in age, ethnicity, critical illness history and CPET-protocol preclude direct comparison with the previous cohorts, but these findings suggest that some degree of recovery of exercise capacity may have occurred within the first year following ICU stay.

We further demonstrated an independent association between severity of organ dysfunction in ICU and VO_2peak in survivors of critical illness up to five years post-ICU. We chose the SOFA-max score to

reflect severity of illness and organ dysfunction throughout the entire ICU stay, rather than admission severity scores, which would not capture new organ failure due to complications occurring during ICU stay. Because of the observed wide variability in exercise capacity in ICU survivors, SOFA-max may assist in stratifying patient at risk for poor performance post-ICU.

Previous data showed that very early after hospital discharge, exercise limitations were multifactorial however, based on low RER, high breath and heart rate reserve at peak exercise, an important contribution of general deconditioning and weakness was proposed [16, 17]. These findings were consistent with Ong et al [18], who showed that reduced exercise capacity at 3 months was due to neuromuscular limitation in 33% of these patients and deficiencies could not be explained by impairment in pulmonary function. We demonstrated that at 5 years, muscular limitation was still a major cause of abnormal exercise capacity, present in 58.5% of patients. This is consistent with previous reports, indicating the presence of neuromuscular sequellae of critical illness, lasting up to 5 years following ICU stay [5, 11] in particular in patients who suffered from intensive care unit associated weakness (ICUAW) [13]. Furthermore, we here demonstrate that hip and knee muscle strength at follow-up also independently associate with exercise capacity. However, the impact of the severity of organ dysfunction – which itself is a strong risk factor for the development of ICUAW [45-50] – on exercise capacity, was not statistically explained by strength at follow-up, suggesting that also other mechanisms related to organ failure are involved. The number of patients with pulmonary factors contributing to exercise limitation was limited with ventilatory limitation in 5.9%, significant desaturation in 4.2%, and combined respiratory limitation in 2.5% of patients with an abnormal exercise capacity. These findings are in agreement with previous data [16, 18] suggesting that pulmonary function is not the main limiting factor of exercise capacity in ICU-survivors, notwithstanding some persistent abnormalities in static pulmonary function evaluations.

The observation that a significant amount of ICU survivors has reduced aerobic exercise capacity, not recovering between the 1 to 5-year follow-up period, may have important implications for the management ICU survivors. Efforts to improve outcomes of critical illness survivors after hospital discharge to date have been largely disappointing [51-54]. Multiple factors may contribute to the lack of success, including heterogeneity of the population, differential potential for rehabilitation, and likely need for an individualized approach [55]. As aerobic exercise testing was demonstrated to be an efficient tool to guide rehabilitation in variable populations including chronic heart failure [29], COPD [30], chronic kidney disease patients [31], cancer [56, 57], and even neuropsychiatric conditions [58, 59], including stroke [32], CPET may become a valuable instrument to stratify patients, and individualize rehabilitation programs in survivors of critical illness, in particular those who experienced more severe organ dysfunction [60]. Presently, data from a single pilot study suggested temporary benefit of tailored rehabilitation schemes on physical function and quality-of-life in survivors of critical illness that was lost upon cessation of the program [61]. Evaluation in larger cohorts seems warranted to identify the window of opportunity for initiation, and feasibility of home-based continuation of these rehabilitation programs.

This study has several strengths. To the best of our knowledge, this is the largest study assessing aerobic exercise capacity in ICU survivors, and the first to repeatedly evaluate cardiorespiratory fitness up to 5 years following ICU stay. As the data were collected in a cohort that was included in a randomized trial, the ICU stay was well documented, allowing to assess and confirm the independent association of exercise limitation with the severity of organ failure in ICU. There are also some limitations. First, patients who performed CPET represented a subgroup of ICU survivors with less severe illness, shorter stays and better overall outcomes as compared to the follow-up patients who did not perform CPET. This was inevitable as patients unable to attend the follow-up clinic, and patients with unstable medical condition could not be included. Despite this bias, we report exercise limitation in a substantial number of patients, suggesting that the actual proportion in the overall population of ICU survivors is likely even higher. Second, by only considering maximal CPETs for identifying exercise limitations, we may have missed some patients unable to perform this degree of

exercise due to muscular limitation (7). We lack invasive measurements and used a protocolized rather than integrative approach to categorize exercise limiting factors because of feasibility purposes in this large-scale research setting. This allowed to identify major cardiac and pulmonary limitations. We may however have missed subtle limitations. Third, for the mixed model, choice of confounders was based on general considerations for the interpretation of CPET and previous work with respect to physical outcomes post-ICU [44], as evidence on CPET in ICU-patients is limited. Unmeasured confounding cannot be excluded. Fourth, as muscle strength was not systematically assessed during ICU-stay, we could not directly assess whether the impact of SOFA-max on exercise capacity was mediated by ICUAW. Finally, as our population was derived from an RCT, generalizability may be limited.

In summary, survivors of critical illness have impaired aerobic exercise capacity. Severity of organ failure independently associates with exercise performance throughout follow-up. Reduced muscle strength at follow-up also independently associates with reduced exercise capacity but did not explain the effect of severity of organ failure on this outcome. Although causal pathways explaining lasting aerobic exercise inefficiency should be further explored, our data suggest a potential role of CPET to select proper candidates and guide individualized rehabilitation programs in survivors of critical illness.

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SUPPLEMENTARY MATERIALS

Supplementary methods

Cardiopulmonary exercise testing

Exclusion criteria

Exclusion criteria for CPET in patients who were eligible and willing to attend the follow-up clinic comprised safety concerns. Absolute contra-indications for CPET were unstable cardiac conditions (acute myocardial infarction < 5 days, unstable angina, uncontrolled arrhythmias causing symptoms or hemodynamic compromise, syncope, active endocarditis, acute peri(myo)carditis, symptomatic severe aortic stenosis, uncontrolled heart failure), vascular conditions (suspected dissecting aneurysm, thrombosis of lower extremities), or pulmonary conditions (acute pulmonary embolus or pulmonary infarction, uncontrolled asthma, respiratory failure, room air desaturation at rest ≤85%), acute non-cardiopulmonary disorders potentially affecting exercise performance (infection, renal failure, thyrotoxicosis), and mental impairment leading to inability to cooperate. Relative contra-indications that were evaluated by the principal investigator individually encompassed other severe cardiac conditions (left main coronary stenosis or its equivalent, moderate stenotic valvular heart disease, severe untreated arterial hypertension at rest, >200 mmHg systolic and >120 mmHg diastolic, tachy- or bradyarrhythmia, high-degree atrioventricular block, hypertrophic cardiomyopathy, significant pulmonary artery hypertension), advanced or complicated pregnancy, electrolyte abnormalities, orthopaedic impairment compromising exercise performance [1, 2]. Additional exclusion criteria involved practical issues, such as cycle-ergometer and/or supervising personnel not being available, and patient refusing CPET.

Protocol

An incremental symptom-limited CPET was performed on an electrically braked cycle-ergometer (eBike, GE®, provided by Acertys). The CPET was conducted by 1 of four trained physiotherapists (HVM, TVA, TVHB, SV) in the presence of a physician with advanced life support skills. Prior to the test, spirometry was performed and maximal voluntary ventilation was measured as the maximum minute volume of ventilation that the subject could maintain for 12 to 15 s. After a 2-minute resting phase, followed by 3 minutes of cycling without resistance, an initial load of 20 Watt was imposed and subsequently increased with 20 Watt/minute until exhaustion. Safety criteria for immediate termination were predefined according to general guidelines [1, 2]. These included complaints of chest pain or recording of ischemic electrocardiography alterations, arrhythmia (frequent multifocal or repeated couplets or triplets of ventricular extrasystole, (supra)ventricular tachycardia, atrial fibrillation with rapid ventricular response, second or third grade atrioventricular block), systolic blood pressure drop of 20 mmHg compared to the highest value or hypertension (systolic blood pressure >250 mmHg, diastolic blood pressure >120 mmHg), desaturation (percutaneous oxygen saturation <80% combined with symptoms and signs of hypoxemia), pallor, loss of coordination, confusion, (pre)syncope, signs of respiratory failure. After termination of the test, patients were monitored until recovery to baseline values or for a maximum of 6 minutes.

The test was performed under continuous monitoring of heart rate, transcutaneous oxygen saturation, and 12-lead electrocardiography. Blood pressure was measured at rest and then every 2 minutes. Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and minute ventilation (VE) were measured on a breath-by-breath basis (JAEGER® Oxycon Pro® and Vyntus CPX® metabolic carts [3] provided by CareFusion). Volumes were assed with a Triple V turbine volume transducer, VO_2 was

assessed by a high-speed analyser according to the differential paramagnetic principle, and VCO_2 was measured by a high-speed analyser using infrared absorption. Automated volume- and gas calibrations were done prior to each test according to manufacturer user manual's instructions. Variables were collected as 30 second-averaged data.

CPET outcomes and definition of abnormal values

The main exercise parameters derived were obtained at two pivotal points during incremental exercise: (1) peak exercise, corresponding to the highest work rate achieved upon symptom limitation of exercise and maintained for 30 seconds; and (2) the anaerobic threshold, the point during exercise theoretically marking the onset of significant contribution of anaerobic metabolism.

At peak exercise, we obtained:

a) *peak oxygen consumption rate* (VO_2peak), a widely accepted approximation of VO_2max, which is the best index of cardiorespiratory fitness [1, 4]. VO_2peak depends on age, sex, body build and may be affected by training level, and genetic factors. VO_2peak is expressed in absolute values (ml/min/kg body weight), and relative to its expected value adjusted for age, gender, and weight based on reference equations [5]. VO_2max below 85% of its predicted value is considered a clinically useful threshold indicating relevant limitation of maximal oxidative capacity [1]. A reduced VO_2max is not specific for an underlying condition, and may indicate cardiovascular limitation, pulmonary limitation peripheral limitation including muscular limitation and or effort [6].
b) *peak work rate:* highest work rate achieved at exercise exhaustion and at VO_2peak
c) *peak heart rate*: heart rate at peak exercise, and *heart rate reserve*, defined as the difference between the predicted maximal heart rate and the heart rate achieved at peak exercise. A HRR <15 bpm or HRpeak ≥ 90% of its predicted maximal value is considered indicative of cardiac limitation of exercise. In normal, healthy individuals, there is little to no HRR as exercise is limited by the cardiovascular system.
d) *peak ventilation* (VEpeak) and *ventilatory reserve*, defined as the ratio of VEpeak to Maximal Voluntary Ventilation (MVV). MVV was determined prior to performance of the exercise test as the maximum minute volume of ventilation that the subject could maintain for 12 to 15 s. In healthy individuals, approximately 70% of MVV is generally used during maximal exercise, with 85% being a reasonable upper limit in the general population [1]; If VEpeak exceeds 85% of MVV during exercise, this indicates that ventilatory limits are reached. This may point to underlying respiratory (muscle) pathology, although it can also be observed in (master) athletes [7].
e) *peak oxygen pulse* (O_2-pulse), the ratio of VO_2peak to peak heart rate, approximates the amount of O_2 extracted per heart beat
f) *respiratory exchange ratio* (RER), calculated as VCO_2peak/VO_2peak, is often used to indicate maximal effort and – somewhat arguably – achievement of VO_2max during incremental exercise testing. Given valid criticism with respect to use of stringent criteria, a RER>1.05 was perceived as indicating maximal effort given a heterogeneous sample of patients [8].

At anaerobic threshold:

g) *Oxygen consumption rate at anaerobic threshold (VO_2AT):* The oxygen consumption rate at the time point during incremental exercise where the rate of change in arterial lactate concentration rapidly increases – presumably marking increased anaerobic metabolism

resulting in lactate production in excess of maximal clearance capacity – is referred to as the lactate threshold, or more commonly the anaerobic threshold (AT) [8-10]. AT was determined noninvasively by concordance of the V-slope (inflection of the VCO_2 versus VO_2 slope) and ventilatory equivalent methods (nadir of fraction of oxygen ($PETO_2$) or of the VE/VO_2 versus work rate)[1, 9, 11]. VO_2AT depends on age and training, and is generally expressed as percentage of the predicted value of VO_2max: VO_2AT below 50% of the predicted value for VO2max is considered abnormal [6]. In certain cases, it is not possible to reliably derive the AT [12, 13], which may hold prognostic information.

h) *ventilatory efficiency (VE/VCO_2AT):* The ratio of ventilation rate to CO_2-production rate at the anaerobic threshold is considered the most informative point estimate of ventilation and gas exchange efficacy during exercise [14]. The generally accepted upper limit of normal is situated at a ratio of 34 [1]. Abnormal values may indicate ventilation–perfusion mismatch, either due to increased dead space ventilation or gas exchange abnormalities. Causes of abnormal VE/VCO_2AT include acute hyperventilation, lung diseases, pulmonary vascular disease and left-ventricular heart failure with increased filling pressures [8, 15].

In addition to point estimates at peak exercise and at the anaerobic threshold:

i) the metabolic efficacy for mechanical work was calculated as $\Delta VO_2/\Delta WR$.

Predicted values were based on reference equations proposed by Jones et al [5].

Classification process to explore exercise limiting factors

The main goal of the process of classification of the CPET studies was to identify the patients in whom abnormal aerobic exercise capacity was primarily due to peripheral limitations, presumably due to muscular impairments or deconditioning, further referred to as muscular limitations. For this purpose, a pragmatic flow-chart was built by NVA, GH and two senior CPET experts (KG and RG) with the aim to be practical and applicable in a research setting investigating various underlying health conditions. This protocol aimed to prioritize excluding cardiovascular disease contributing to exercise limitations, as well as ventilatory and gas exchange limitations as the more likely primary reason of exercise limitation. Patients without clear indication of such limitations are considered to suffer predominantly from muscular limitations. The process involved the following steps:

1. ***Was exercise maximal?***
   To avoid erroneous labelling of patients delivering insufficient effort, we first excluded patients without maximal CPET. Maximal effort was defined as *RER >1.05* [8].
2. ***Was exercise capacity abnormal?***
   Within the maximal tests, we next identified patients who exhibited an abnormal exercise capacity. This was define as *peak oxygen consumption (VO_2peak) <85% of predicted VO_2max* [1, 6].
3. ***Did the anaerobic threshold occur early?***
   Further evaluation of patients with abnormal exercise capacity depended on whether or not the anaerobic threshold was reached prematurely, defined as *VO_2 at the anaerobic threshold <50% of predicted VO_2max* [6].
   a. ***VO_2AT <50% pred VO_2 max or VO_2AT undetermined:***
      To discriminate premature acidosis reflecting insufficient oxygen delivery due to cardiorespiratory impairment rather than due to a muscular limitation, we first evaluated evidence of respiratory limitation, consisting of ventilatory limitation (defined as *VEpeak/MVV≥85%)* [1, 6] or abnormal gas exchange (defined as *desaturation ≥5% from*

baseline to peak exercise) [6]. In patients without evidence of respiratory limitation, we searched for evidence of pathological cardiac limitation of exercise.
When maximal heart rate was reached (*Peak HR ≥90% of* predicted maximal heart rate according to reference equations by Jones et al. [1, 5], we aimed to verify if the underlying cardiac response to exercise was abnormal, hence making a primary cardiac pathology as a cause of exercise limitation likely. A pathological systolic blood pressure response at peak exercise (*SBPpeak < SBPbase or SBPpeak > 210 mmHg*) [2] and abnormal ventilatory efficiency at the anaerobic threshold (*$VE/VCO_2AT > 34$*) [1] were considered to indicate an abnormal cardiac response, and thus suggesting cardiac disease limiting exercise.
In patients not reaching maximal heart, evidence of cardiac limitation due to chronotropic incompetence was defined as *$\Delta HR/\Delta VO_2 < 25$* [16].

b. VO_2AT > 50% predicted VO_2max:
Only in the strict absence of signs indicating respiratory or gas exchange impairments (*VEpeak/MVV < 85% and ΔSpO_2 < 5%*), and no signs of cardiac ischemia (*no electrocardiographic findings suspect for ischemia, defined as repolarization alterations or broad QRS-tachycardia suspect for VT during exercise or the recuperation phase, in presence or absence of angina pectoris*), we propose a primary muscular exercise limitation[6, 8]

Other outcomes

At follow-up, patients also underwent pulmonary function testing, evaluation of muscle strength [Medical Research Council (MRC)-sum score, hand-grip strength, hand-held dynamometry of the same muscle groups involved in the MRC-sum score, maximal inspiratory pressure], physical function as assessed with the 6-minute walk distance (6MWD), and quality-of-life assessment with the 36-item short form health survey (SF-36) [physical (PCS) and mental component score (MCS) as well as the physical function domain (PF-SF 36), range 0-100, higher values indicating better scores], as reported previously [17, 18]. Pulmonary function was reported as absolute values and percentage of predicted values.

Statistics

Linear mixed models

To assess whether long-term aerobic exercise performance was independently associated with severity of organ dysfunction throughout follow-up, we created an adjusted linear mixed model with VO_2 peak at follow-up as the dependent variable.

Fixed effects of main interest included the SOFA-max, time-to-follow-up in years, and the interaction between time-to-follow-up and SOFA-max. SOFA-max was entered as a continuous variable, time-to-follow-up was entered as a categorical variable, with the first-year follow-up time point as the starting ('zero') point. The five-year follow-up time point was set as the reference category. A set of a priori selected confounders was added for adjustment [19]. These included: age and BMI at ICU-admission as continuous variables and gender, diabetes mellitus at ICU admission, malignancy, preadmission dialysis, randomization strategy, diagnostic category and sepsis upon admission, and length of ICU-stay (dichotomized at an ICU-stay of 8 days as before) as categorical variables. To facilitate interpretation, yielding estimates for the representative patient, continuous values were centered at their mean in the sample [for SOFA-max: 9 (range: 1-18); age: 54 (range: 19-83); BMI: 26.4, (range: 17-48)] and for categorical variables, the reference category was set at the main category represented in

the sample (male, no diabetes, no malignancy, no preadmission dialysis, emergency surgery, late PN, no sepsis, short-stay).

We accounted for repeated patient measurements over the 5 time points by specification of an unstructured covariance matrix. The estimation method was restricted maximum likelihood (REML).

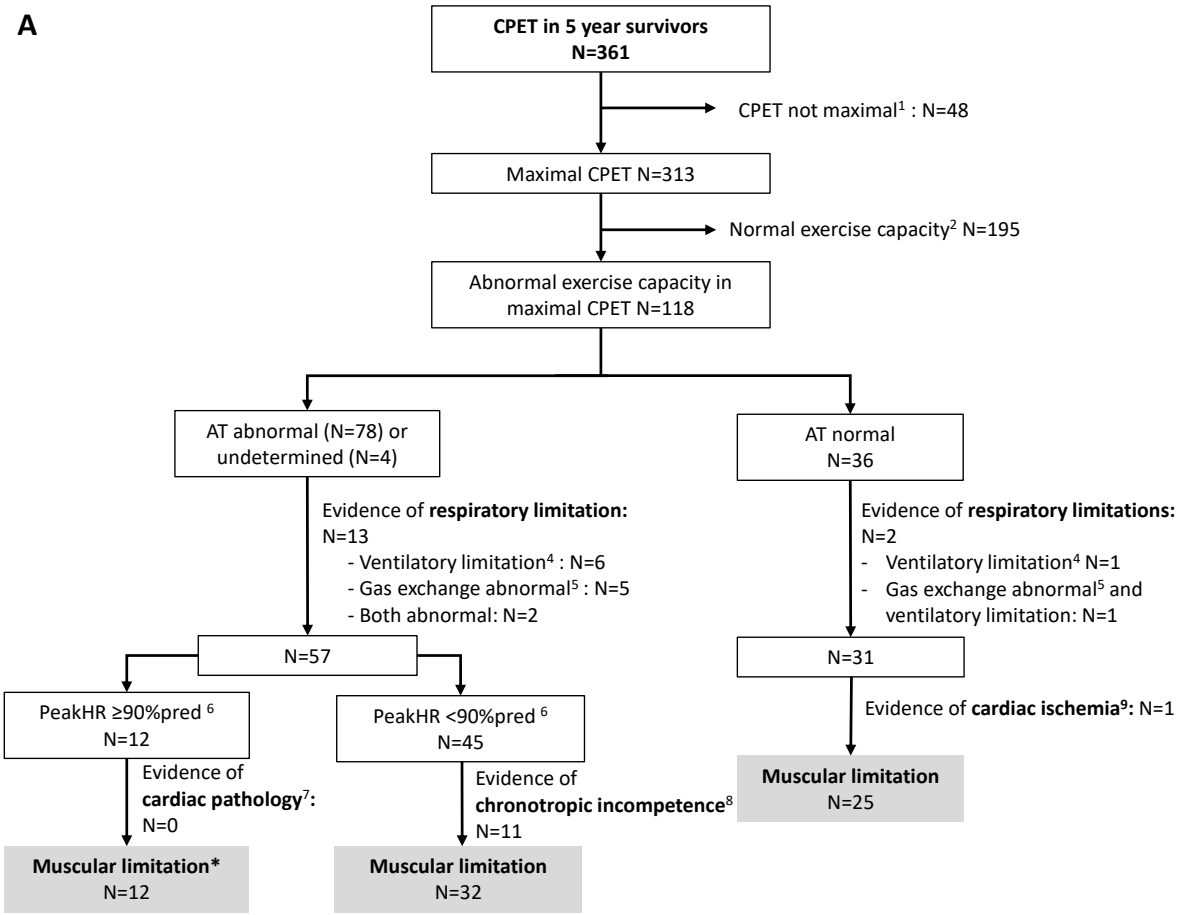
Exploratory analyses

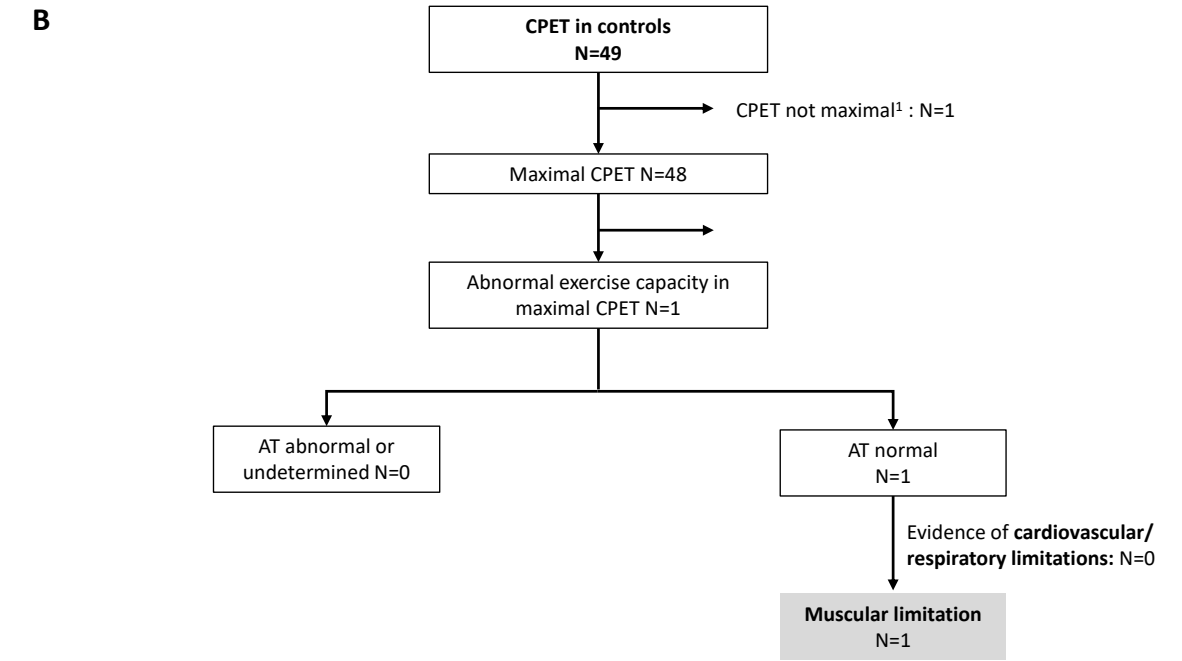
When an independent effect of the severity of organ failure on peak oxygen was demonstrated, we further examined whether the effect of severity of organ failure could be explained by persisting weakness. For this purpose, we built additional linear mixed models in which both knee and hip strength at 5-year follow-up were to be added as co-variates in separate models.

Model diagnostics

Prior to model construction, the distribution of the dependent variables was evaluated for normality. Collinearity of the independent variables was evaluated by fitting simple linear regression and was judged problematic if VIF >5. After modelling, model fit agreement with model assumptions was estimated through fit statistics and residuals plots [20].

Supplementary figures





Suppl Fig 1. Flow chart indicating exercise limiting factors in 5-year ICU survivors (panel A) and controls (panel B). [1] Maximal CPET is defined by RER >1.05; [2] Abnormal exercise capacity is defined as VO_2peak <85%pred VO_2max; [3] AT is defined as normal if ≥ 50% predicted VO_2max; [4] Ventilatory limitation is defined as VEpeak ≥ 85% MVV; [5] Gas exchange abnormality is defined as saturation drop from baseline to peak exercise of ≥ 5%; [6] Peak heart rate is based on reference values by Jones et al [5]; [7] Evidence of cardiac disease contributing to exercise limitation is defined as a pathological blood pressure response (SBPpeak < SBPbase or SBPpeak > 210 mmHg) and VE/VCO_2AT >34; [8] Evidence of cardiac limitation due to chronotropic incompetence was defined as $\Delta HR/\Delta VO_2$ <25 [16]; [9]Cardiac ischemia was evaluated as the presence of electrocardiographic findings suspect for ischemia (repolarisation alterations or broad QRS-tachycardia suspect for VT, in presence or absence of angina pectoris). *In patients with low VO_2peak, low VO_2AT, and no respiratory limitation, those reaching cardiac limits (HR>90%pred) without evidence of cardiac pathology[7] were considered likely to have a peripheral limitation contributing to abnormal exercise capacity. Abbreviations: *CPET*: cardiopulmonary exercise testing; *VO_2peak*: peak oxygen consumption; *predVO_2max*: predicted maximal oxygen consumption; *VO_2AT*: oxygen consumption at anaerobic threshold; *RER*: respiratory exchange ratio; *VEpeak*: peak ventilation; *MVV*: minute volume ventilation; *SBPpeak*: peak systolic blood pressure; *SBPbase*: baseline systolic blood pressure; *VE/VCO_2AT:* ventilatory efficiency; *$\Delta HR/\Delta VO_2$*: ratio of heart rate change to oxygen consumption change during exercise; *VT:* ventricular tachycardia

Supplementary tables

Supplementary Table 1. Demographics of the 5-year follow-up cohort and healthy controls

	EPaNIC 5-year FU N=361	Controls N=49	*P*-value
Age	59±14	62±7	0.184
Gender, male	254 (70.4%)	34 (69.4)	0.889
BMI	27.5±4.7	26.8±4.0	0.349

Continuous variables are depicted as mean ± standard deviation, categorical variables are depicted as number (percentages). Abbreviations: *EPaNIC:* Early Parenteral Nutrition in Intensive Care; *BMI:* body mass index.

Supplementary Table 2: Admission and ICU-characteristics of patients at intermediate follow-up moments

	EPaNIC 1-year FU			EPaNIC 2-year FU			EPaNIC 3-year FU			EPaNIC 4-year FU		
	CPET (N=40)	No CPET (N=36)	*P*-value	CPET (N=54)	No CPET (N=61)	*P*-value	CPET (N=72)	No CPET (N=80)	*P*-value	CPET (N=64)	No CPET (N=70)	*P*-value
ADMISSION												
Age	56±14	62±15	0.066	58±13	65±16	0.013	57±14	65±16	0.001	59±12	69±14	<0.001
Gender, male	27 (67.5)	20 (55.6)	0.284	40 (74.1)	42 (68.9)	0.537	48 (66.7)	45 (56.3)	0.188	48 (75.0)	33 (47.1)	0.001
BMI	27.3±5.1	28.1±6.2	0.549	27.8±5.1	26.8±5.0	0.265	27.6±4.8	26.9±5.3	0.377	27.9±4.9	26.6±5.1	0.143
Diabetes mellitus	3 (7.5)	9 (25.0)	0.037	4 (7.4)	14 (23.0)	0.022	7 (9.7)	13 (16.3)	0.235	8 (12.5)	17 (24.3)	0.080
Malignancy	4 (10.0)	7 (19.4)	0.243	9 (16.7)	14 (23.0)	0.400	10 (13.9)	14 (17.5)	0.542	9 (14.1)	12 (17.1)	0.624
Preadmission dialysis	1 (2.5)	0	1	0	0	NA	0	0	NA	0 (0.0)	1 (1.4)	1
Diagnostic category			0.368			0.208			0.986			0.648
Cardiac surgery	13 (32.5)	8 (22.2)		20 (37.0)	19 (31.1)		25 (34.7)	34 (42.5)		22 (34.4)	30 (42.9)	
Emergency SICU	18 (45.0)	20 (55.6)		28 (51.9)	26 (42.6)		29 (40.3)	2 (2.5)		31 (48.4)	28 (40.0)	
Elective SICU	2 (5.0)	0		0 (0.0)	1 (1.6)		2 (2.8)	16 (20.0)		2 (3.1)	1 (1.4)	
Medical ICU	7 (17.5)	8 (22.2)		6 (11.1)	15 (24.6)		16 (22.2)	16 (20.0)		9 (14.1)	11 (15.7)	
Randomisation, late PN	18 (45.0)	16 (44.4)	0.961	25 (46.3)	28 (45.9)	0.966	38 (52.8)	43 (53.8)	0.905	30 (46.9)	38 (54.3)	0.391
APACHE II	29 (17-36)	34 (27-38)	0.062	29 (23-37)	30 (21-37)	0.920	30 (18-36)	31 (20-38)	0.420	30 (21-35)	31 (21-37)	0.525
Sepsis upon admission	14 (35.0)	19 (52.8)	0.118	24 (44.4)	24 (39.3)	0.580	35 (48.6)	32 (40.0)	0.286	29 (45.3)	27 (38.6)	0.429

Continued Supplementary Table 2: Admission and ICU-characteristics of patients at intermediate follow-up moments

	EPaNIC 1-year FU			EPaNIC 2-year FU			EPaNIC 3-year FU			EPaNIC 4-year FU		
	CPET (N=40)	No CPET (N=36)	*P*-value	CPET (N=54)	No CPET (N=61)	*P*-value	CPET (N=72)	No CPET (N=80)	*P*-value	CPET (N=64)	No CPET (N=70)	*P*-value
ICU STAY												
MV	39 (97.5)	35 (97.2)	1	54 (100)	58 (95.1)	0.246	70 (97.2)	75 (93.8)	0.447	64 (100)	69 (98.6)	1
Duration of MV, days	6 (3-12)	7 (4-15)	0.302	6 (3-11)	6 (4-10)	0.542	7 (4-12)	6 (2-12)	0.432	8 (4-16)	6 (2-13)	0.177
Vasopressors/ inotropics	31 (77.5)	34 (94.4)	0.036	45 (83.3)	53 (86.9)	0.592	64 (88.9)	69 (86.3)	0.623	57 (89.1)	65 (92.9)	0.442
Duration of HD support, days	5 (1-9)	6 (3-9)	0.257	5 (1-7)	4 (2-7)	0.993	5 (1-6)	5 (2-7)	0.792	5 (2-8)	4 (2-7)	0.588
New infection, yes	22 (55.0)	20 (55.6)	0.961	30 (55.6)	32 (52.5)	0.740	40 (55.6)	36 (45.0)	0.194	39 (60.9)	31 (44.3)	0.054
New dialysis, yes	11 (27.5)	9 (25.0)	0.805	11 (20.4)	14 (23.0)	0.738	12 (16.7)	15 (18.8)	0.737	12 (18.8)	8 (11.4)	0.235
Bilirubin>3 mg/dL	11 (27.5)	7 (19.4)	0.410	15 (27.8)	13 (21.3)	0.420	24 (33.3)	15 (18.8)	0.040	16 (25.0)	10 (14.3)	0.117
ICU-stay>8 days, yes	34 (85)	32 (88.9)	0.740	40 (74.1)	49 (80.3)	0.424	48 (66.7)	53 (66.3)	0.957	45 (70.3)	47 (67.1)	0.693
ICU LOS, days	12 (9-18)	13 (8-23)	0.398	11 (7-20)	12 (8-20)	0.670	11 (7-19)	10 (7-19)	0.846	12 (7-22)	10 (7-20)	0.545

Continuous variables are depicted as mean ± standard deviation or median (interquartile range) as appropriate, categorical variables are depicted as number (percentages). Abbreviations: *ICU:* intensive care unit; *EPaNIC:* Early Parenteral Nutrition in Intensive Care; *CPET:* cardiopulmonary exercise test; *BMI:* Body Mass index; *SICU:* surgical intensive care unit; *PN:* parenteral nutrition; *APACHE II:* Acute Physiology and Chronic Health Evaluation; *MV*: mechanical ventilation; *HD:* hemodynamic; *LOS:* Length of stay.

Supplementary Table 3: Relationship between multiple organ failure and long-term exercise capacity in survivors of critical illness

	Effect size (95% CI) [1]	*P*-value
Model 1. VO_2peak (ml/min/kg)		
SOFA-max (per point increase)	-0.345 (-0.669 to -0.0211) [2]	0.037 [2]
Time-to-follow-up		
1-year follow-up (5-year follow-up as a reference)	1.058 (-0.829 – 2.945) [3]	0.271 [3]
2-year follow-up (5-year follow-up as a reference)	-0.830 (-2.246 – 0.587) [3]	0.250 [3]
3-year follow-up (5-year follow-up as a reference)	-0.974 (-2.076 – 0.128) [3]	0.083 [3]
4-year follow-up (5-year follow-up as a reference)	-0.026 (-1.795-1.743) [3]	0.977 [3]
Interaction factor SOFA-max and time-to-FU	NA	0.350 [2]
SENSITIVITY ANALYSES		
Model 1a. VO_2peak (ml/min/kg) including FU knee strength		
SOFA-max (per point increase)	-0.313 (-0.624 to -0.003) [2]	0.048 [2]
Knee extensor strength at FU (per 10 Newton increase)	0.229 (0.132-0.327) [2]	<0.001 [2]
Model 1b. VO_2peak (ml/min/kg) including FU hip strength		
SOFA-max (per point increase)	-0.330 (-0.646 to -0.014) [2]	0.041 [2]
Hip flexors strength at FU (per 10 Newton increase)	0.182 (0.071-0.294) [2]	0.001 [2]

[1] Linear mixed models adjusted for: age and BMI upon admission (centered at their respective sample mean), gender, diabetes mellitus upon admission, randomisation group, diagnostic category, sepsis upon admission, prolonged ICU stay (with reference category set at the main category represented in the current sample). [2] *p*-value derived from Type III test, and effects size refers to the average effect throughout follow-up. [3] p-value derived from solution for fixed effects, and effect size refers to the effect of time-point of assessment as compared to the 5-year follow-up time point.

Abbreviations: *VO_2peak:* peak oxygen consumption rate, *VE/VCO_2AT:* ratio of ventilation to CO_2-production at the anaerobic threshold.

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Chapter 7: Intensive care unit acquired muscle weakness in COVID-19 patients

Adapted from:

Van Aerde N, Wilmer A*, Gosselink R*, Van den Berghe G*, Hermans G* for the COVID-19 consortium. (2020) Intensive care unit acquired muscle weakness in COVID-19 patients. Intensive Care Med, 46 (11), 2083–2085.

*Equally contributed

INTRODUCTION

Infection with the SARS-CoV-2 virus may lead to hypoxemic respiratory failure and acute respiratory distress syndrome (ARDS). ARDS is frequently complicated by intensive care unit acquired weakness (ICUAW) [1], which is associated with poor outcomes [2]. Critically ill COVID-19 patients may differ from typical ARDS-patients in baseline factors [3] and ICU exposures associated with ICUAW [4]. Of particular concern may be the need for deep sedation to avoid patient-ventilator dyssynchrony and ventilator-induced/self-inflicted lung-injury because of high respiratory drive [5]. We aimed to assess the incidence of ICUAW in critically ill COVID-19 patients, to identify factors associated with its occurrence, and to describe its short-term outcomes.

METHODS

This single-center, retrospective, observational study involved adult critically ill COVID-19 patients admitted to the University Hospitals Leuven, from March 13th until June 8th 2020. After April 1st, physiotherapists were re-engaged in patient care and performed daily strength-assessment when appropriate. Records of eligible patients were searched for baseline characteristics, ICU-exposures and outcomes. The primary outcome was the incidence of ICUAW, assessed with the MRC-sum-score [2], at awakening, at ICU- and hospital discharge in patients requiring invasive mechanical ventilation (IMV). Additionally, we evaluated factors and short-term outcomes associated with weakness at ICU discharge. To assess bias, we compared characteristics and outcomes for patients with and without MRC-sum-score, and studied patients without IMV.

RESULTS

Of 486 hospitalized COVID-19 patients, 114 required intensive care of whom 74 (64.9%) needed IMV (Supplementary Figure 1). Admission and ICU-characteristics are provided in the Online Supplement. Total hospital mortality was 60/486 (12.3%), ICU mortality was 11/114 (9.6%). All deaths occurred in IMV patients [11/74 (14.9%)]. In 50/74 (67.6%) assessed IMV patients, the incidences of ICUAW at awakening, ICU- and hospital discharge were 72.0%, 52.0% and 27.0% (Figure1).

Those without MRC-sum-score were older as compared to those with MRC-sum-score [67 (60-76) versus 60 (53-67), p=0.044] and comprised 9 patients who died before awakening, possibly introducing selection bias. Admission characteristics were similar between patients with and without ICUAW, but weak patients had prolonged ventilation (days) [24 (15-29) versus 12 (8-17), p<0.001], higher mean morning glycemia (mg/dl) [126 (119-134) versus 118 (110-129), p=0.041], and more frequently received dialysis [11/26 (42.3%) versus 4/24 (16.7%), p=0.048]. Exposure to corticosteroids, sedatives and analgesics, except for dexmedetomidine, and NMBAs was higher (see Online Supplement). Weak patients had longer ICU stays (days) [30 (19-42) versus 19 (12-25), p=0.008], lower mobility scores at ICU discharge [2 (2-2) versus 6 (4-6), p<0.001], but ICU readmission [0/26 (0%) versus 2/24 (8.3%), p=0.225] and mortality [2/26 (7.7%) versus 0/24 (0%), p=0.491] were not different. Handgrip-strength (%pred) [43% (28%-59%) versus 64% (36%-80%), p=0.045], and Barthel index at hospital discharge [8 (2.5-11.5) versus 10.5 (8-18), p=0.040], remained lower in weak patients (Figure 1). 15/26 (57.7%) weak versus 6/24 (25.0%) not-weak patients were referred for in-patient rehabilitation. In 6/40 (15.0%) assessed non-IMV patients, 1 patient was weak at ICU discharge and none at hospital discharge (see Online Supplement).

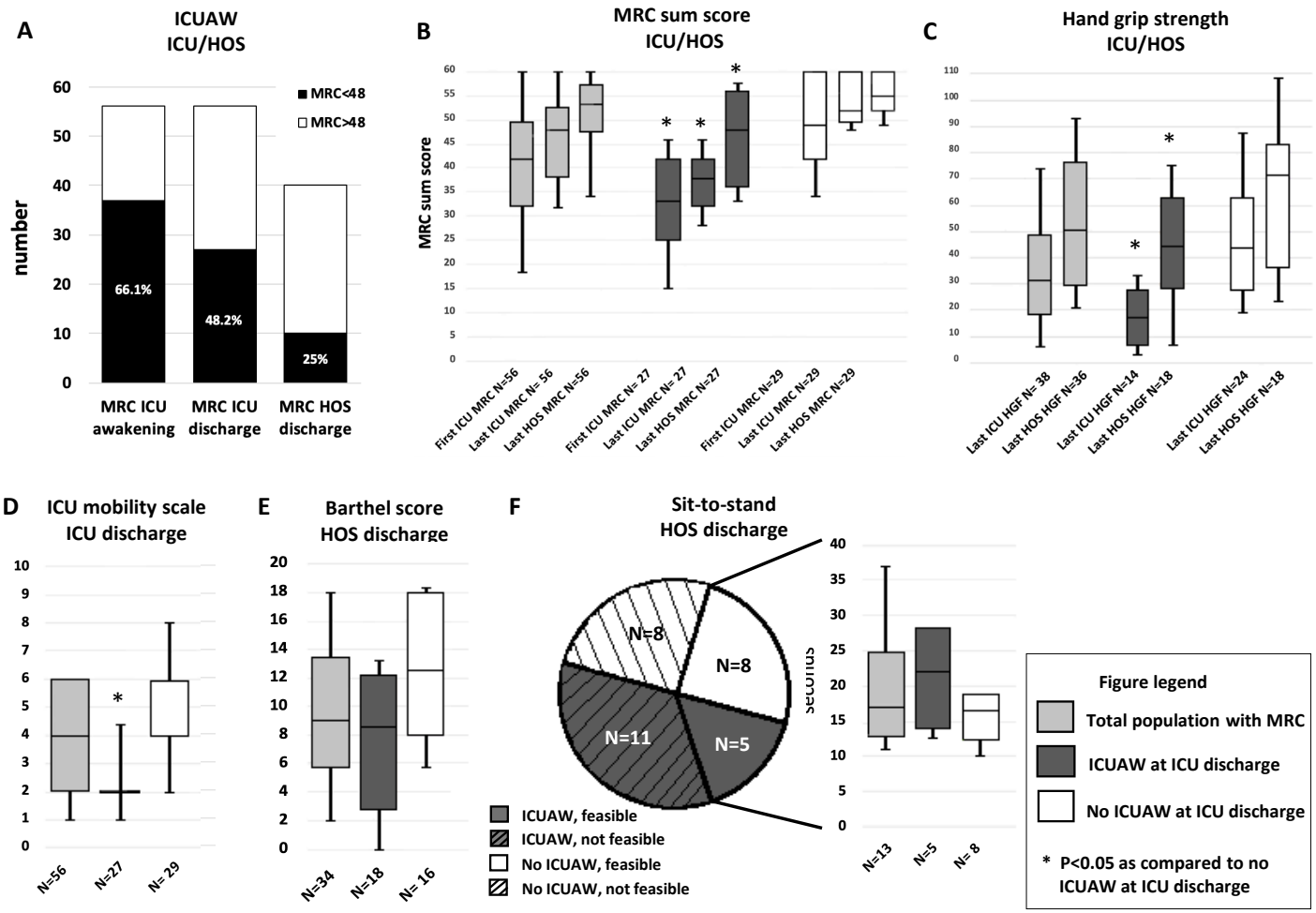

Fig. 1: Strength and functional outcomes in COVID-19 patients, requiring invasive mechanical ventilation, with and without weakness at ICU discharge. Panel A: Incidence of ICUAW at awakening, ICU discharge and hospital discharge. Panel B: MRC-sum-score at awakening, ICU discharge and hospital discharge. Panel C: ICU mobility score at ICU discharge. Panel D: Barthel score at hospital discharge. Abbreviations: *MRC*: Medical Research Council; *ICUAW*: intensive care unit acquired weakness; *ICU*: intensive care unit; *HOS*: hospitalization.

CONCLUSION

In this cohort of critically ill COVID-19 patients, survival was high, but those needing prolonged sedation frequently presented with ICUAW. Although strength improved throughout hospitalization, impact on functional status remained substantial. These data indicate that there may be a price to pay for allowing rigorous lung-protective ventilation and underscore the need for follow-up of post-ICU COVID-19 patients, to offer tailored rehabilitation, hopefully reducing long-term impact.

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SUPPLEMENTARY MATERIALS

Supplementary methods

Study design and patient population

This is a single-center, retrospective, observational study of critically ill adult patients, admitted to the University Hospitals Leuven, diagnosed with COVID-19 based on 1) a positive pharyngeal or lower respiratory tract sample polymerase chain reaction *or* 2) the presence of upper or lower respiratory infection symptoms (fever, cough, dyspnea, desaturation) and typical findings on chest CT scan [1], in the absence of other plausible diagnoses, since the start of the pandemic on March 13th 2020, until June 8th 2020. We here focus on patients who required invasive mechanical ventilation (IMV) as this population is expected to have the highest risk for developing neuromuscular complications. The ICU capacity prior to the pandemic consisted of 106 beds. During the pandemic, 56 of these beds were dedicated for COVID-19 patients, temporarily supplemented with 20 beds in a pop-up unit. The Belgian government instructed to regionalize COVID-19 admissions. As a tertiary center, we additionally admitted patients for whom added value of a tertiary setting was expected. Local ethical guidelines were developed on exclusion criteria for COVID-19 and other ICU admissions during the pandemic [2]. In the very early phase of the pandemic, due to hospital cohorting policy and while expanding ward capacity, the ICUs temporarily admitted COVID-19 patients without ICU profile. These patients, as well as ICU patients with pre-existing neuromuscular disease were excluded.

General management

Although a few older-generation ventilators were used, and switches between drugs of the same classes were made, there were no critical shortages in medication or materials. The ICU-nursing team was expanded with medium-care and post-anesthesia care nurses to guarantee the pre-COVID nurse-to-patient ratio (1:2). Medical staffing during week and night shifts was increased to equal staffing during weekdays, with the presence of a senior intensivist 24/7. Medical treatment involved hydroxychloroquine unless contra-indicated, according to Belgian guidelines. Patients were considered for compassionate use or inclusion in adaptive randomized trials involving treatment with remdesivir, itraconazole, azithromycin [3] or convalescent plasma [4]. Rescue treatment with corticosteroids, tocilizumab and anakinra was left to the physician's discretion. From March 31st onwards and while awaiting data from RCTs [5], all patients received anti-Xa guided high-intermediate doses of low-molecular weight heparin (LMWH), as coagulopathy, micro- and macrothrombosis occur frequently and are associated with worse outcomes [6, 7]. Respiratory management involved high-flow oxygen as a first approach as this may reduce the need for intubation [8-10]. As COVID-19 patients can deteriorate very quickly, hospital policy involved that administration of high-flow oxygen as a step-up approach, was not performed on the wards, and these patients were transferred to the ICU. This required expansion of ICU beds as indicated above, but was feasible as our health system was not completely overwhelmed. Non-invasive ventilation was not recommended as a step-up approach as NIV in hypoxemic respiratory failure has high failure rates, associated with increased mortality [9, 11]. Lung-protective ventilation, including prone ventilation, was performed according to ARDS guidelines [12]. From April 2nd onwards, particular attention was given to avoid excessive positive end-expiratory pressure (PEEP) when compliance was relatively preserved [13]. Extracorporeal membrane oxygenation (ECMO) was instituted according to EOLIA criteria [14]. Nutritional management involved early enteral nutrition but no caloric parenteral supplementation in the first week. Normoglycemia (80-110 mg/dl) was targeted with intravenous insulin. After April 1st, physiotherapists were re-engaged in patient care. Physiotherapists daily (5/7 days) assessed patients for awakening and cooperation [15]. If appropriate, assessment of strength was performed with MRC-sum-score and handgrip strength [16] as well as ICU mobility score [17] (range 0-10, higher scores indicating better mobility), as part of routine care [18], to the best of the team's ability (1 physiotherapist: 14 patients). On the wards, physiotherapists and rehabilitation physicians were re-involved in patient mobilization

from April 13nd onwards. Evaluation of post-ICU patients standardly involved weekly assessment of strength (MRC-sum-score, handgrip strength), activities of daily living (Barthel index, range 0-20, higher score indicating more independence) [19], and functionality (five repetition sit-to-stand test)[20], to the best of the team's ability.

Data collection

Electronic medical records of patients admitted to the COVID-19 ICUs during the pandemic were searched to identify eligible patients. We collected information on demographics, comorbidities, laboratory data, severity of illness and respiratory support within 24 hours of ICU admission. Also, treatments, metabolic control, nutrition and outcomes of the ICU stay were recorded. Physiotherapy files were searched for MRC sum scores, handgrip strength and mobility scores recorded during ICU stay. The electronic records from patients discharged to the ward were searched for evaluations of strength, physical function and general outcomes.

Outcomes

The primary outcome was defined as the incidence of ICUAW at key time points, including at awakening, at ICU discharge and at hospital discharge in patients requiring invasive mechanical ventilation. Secondary outcomes included the identification of factors associated with the presence of ICUAW at ICU discharge and related outcomes. To assess potential bias in the analyses, we compared characteristics and outcomes for patients with and without MRC-sum-score and studied data from patients who did not require IMV.

Assessments of strength and mobility in ICU

MRC sum score

The MRC sum score was measured as described earlier [16]. Six muscle groups were evaluated (abduction of the shoulder, flexion of the elbow, extension of the wrist, flexion of the hip, extension of the knee and dorsal flexion of the foot) bilaterally and scored between 0 and 5 (0 = no visible/palpable contraction, 1 = visible/palpable contraction without movement of the limb, 2 = movement of the limb but not against gravity, 3 = movement against gravity (almost full passive range of motion) but not against resistance, 4 = movement against gravity and resistance, arbitrarily judged to be submaximal for sex and age, 5 = normal). Measurements were performed by experienced ICU physiotherapists. ICUAW was defined as MRC sum score < 48 [21, 22].

Handgrip strength

Handgrip strength was measured with a hydraulic handgrip dynamometer (Jamar Preston, Jackson, Michigan, USA) as previously described. Measurements were performed by experienced physiotherapists and were standardly performed on the right side. In case of focal or regional problems for certain muscle groups, evaluation was performed on the contralateral side. Handgrip was assessed provided that the MRC for both forearm flexion and wrist extension were scored at ≥3. Care was taken to perform measurements with the elbow in 90 degrees flexion. Handgrip strength was determined as the highest of 3 attempts. Values were expressed as percent of predicted values for sex and age [23].

ICU mobility score

Patient maximal level of mobility was assessed at ICU discharge by experienced physiotherapists according to the ICU mobility scale (IMS) [17]. This includes the following levels of mobility:

	Classification	Definition
0	Nothing (lying in bed)	Passively rolled or passively exercised by staff, but not actively moving
1	Sitting in bed, exercises in bed	Any activity in bed, including rolling, bridging, active exercises, cycle ergometry and active assisted exercises; not moving out of bed or over the edge of the bed
2	Passively moved to chair (no standing)	Hoist, passive lift or slide transfer to the chair, with no standing or sitting on the edge of the bed
3	Sitting over edge of bed	May be assisted by staff, but involves actively sitting over the side of the bed with some trunk control
4	Standing	Weight bearing through the feet in the standing position, with or without assistance. This may include use of a standing lifter device or tilt table
5	Transferring bed to chair	Able to step or shuffle through standing to the chair. This involves actively transferring weight from one leg to another to move to the chair. If the patient has been stood with the assistance of a medical device, they must step to the chair (not included if the patient is wheeled in a standing lifter device)
6	Marching on spot (at bedside)	Able to walk on the spot by lifting alternate feet (must be able to step at least 4 times, twice on each foot), with or without assistance
7	Walking with assistance of 2 or more people	Walking away from the bed/chair by at least 5 m (5 yards) assisted by 2 or more people
8	Walking with assistance of 1 person	Walking away from the bed/chair by at least 5 m (5 yards) assisted by 1 person
9	Walking independently with a gait aid	Walking away from the bed/chair by at least 5 m (5 yards) with a gait aid, but no assistance from another person. In a wheelchair bound person, this activity level includes wheeling the chair independently 5 m (5 years) away from the bed/chair
10	Walking independently without a gait aid	Walking away from the bed/chair by at least 5 m (5 yards) without a gait aid or assistance from another person

Functional evaluations on the ward

Barthel index

The Barthel Index was used to assess independence during 10 daily life activities, including presence or absence of faecal and urinary incontinence, need of help with grooming, toilet use, feeding, transfers, walking, dressing, climbing stairs and bathing and scored by the rehabilitation physician. Higher scores indicate higher level of independence, with a maximum score of 20 [24]. Interpretation: 20: fully independent in basic ADL and mobility; 15-19: reasonably to well independent; 10-14: needs help but also does a lot by himself; 5-9: seriously in need of help; 0-4: completely in need of help.

Five-repetition sit-to-stand test

The five-repetition sit-to-stand test (5STS) is a test of lower limb function that measures the fastest time taken to stand five times from a chair with arms folded. The 5STS has been validated in healthy community-dwelling adults [20, 25] A straight-backed armless chair with a hard seat was stabilised by placing it against a wall. Floor to seat height was 47 cm. For participants unable to to stand up without using the upper limbs or who required assistance, the test was terminated. If successful, participants were then asked to stand up all the way and sit down landing firmly, as fast as possible, five times without using the arms. The time taken was recorded as the participant's score.

Statistics

Descriptive statistics include median and interquartile ranges for continuous variables and numbers and percentages for categorical variables. Continuous data were compared with Mann-Whitney-U test, categorical variables with Chi-square test or Fisher-exact test, as appropriate.

Analyses were performed with SPSS version 26 (IBM corporation). Two-sided p-values ≤0.05 were considered statistically significant.

Supplementary results

Patient cohort and characteristics

From March 13th until June 8th, 486 COVID-19 patients were hospitalized, including 114 critically ill patients admitted to the ICUs. Another 14 patients without ICU profile temporarily stayed in the ICUs because of hospital cohorting policy, and were excluded (Supplementary Figure 1). During ICU stay, 74/114 (66.7%) patients received IMV.

Median age, body mass index (BMI) and Charlson comorbidity index of IMV patients were 62 (54-71) years, 28.3 (25.7-31.4) kg/m^2 and 2 (2-4), respectively (Supplementary Table 1). Most patients [53/74 (71.6%)] were male. External referrals accounted for 26/74 (35.1%) patients, including 12/13 (92.3%) of the ECMO patients. IMV was started during the first day in ICU in 61/74 (82.4%) of these patients. Total respiratory system compliance was relatively preserved [34 (26-41) ml/cmH_2O] and ventilator settings included low tidal volume (TV) [6.1 (5.7-6.6) ml/kg Ideal Body Weight (IBW)] and moderate PEEP levels [12 (9-13) cmH_2O]. Further details are provided in Supplementary Table 1. A minority of patients were included in expanded access programs or RCTs involving antiviral treatments. Corticosteroids were frequently used as a rescue strategy, whereas tocilizumab and anakinra were scarcely used (Supplementary Table 2). 35/74 (47.3%) were ventilated in prone position. Treatment included ECMO in 13/74 (17.6%), vasopressors in 72/74 (97.3%) and renal replacement therapy in 21/74 (28.4%).

Total hospital mortality was 60/486 (12.3%). ICU mortality was 11/114 (9.6%). All deaths occurred in patients requiring IMV 11/74 (14.9%). When excluding ICU patients, 48/372 (12.9%) hospitalised patients died.

Incidence and risk factors associated with ICUAW at ICU discharge

Strength was measured in ICU in 50/74 (67.6%) IMV patients. Reasons for absent MRC data included prior neuromuscular disease (N=3), no physiotherapist available (N=3), never awake/cooperative prior to ICU discharge or death (N=14). This group comprised 5 patients who did not awaken prior to ICU discharge, including 3 patients still in ICU at the closure of the database, and 9 patients who died in the ICU before awakening. Final reason for absent MRC data included no priority because of short-stay/good functional status (N=4) (Figure 1). These data indicate that the subgroup without MRC was a heterogenous population, many of whom with poor prognosis. Median MRC-sum-score at

awakening was 42 (31-48), with 36/50 (72.0%) of patients being weak. At ICU and hospital discharge, median MRC-sum-score was 46 (38-52) and 53 (47-56) respectively, with 26/50 (52.0%) and 10/37 (27.0%) remaining weak at these time points. Patients with as compared to those without ICUAW at ICU discharge had similar admission characteristics. Patients with ICUAW at ICU discharge had higher mean morning glycemia [126 (119-134) mg/dl versus 118 (110-129) mg/dl, p=0.041], received longer duration of mechanical ventilation [24 (15-29) days versus 12 (8-17) days, p<0.001], and more frequently received renal replacement therapy [11/26 (42.3%) versus 4/24 (16.7%), p=0.048], as compared to patients without ICUAW. ICUAW was associated with longer duration of treatment with corticosteroids and NMBAs. Exposure to all sedatives and analgesics was higher, except for dexmedetomidine. However, doses administered on treatment days for these drugs were not different, except for higher dose of opioids (Supplementary Table 2). Supplementary Table 4 summarises the presence of risk factors and incidence of weakness in our COVID-19 ICU population and previously published ARDS cohorts.

Outcomes associated with ICUAW at ICU discharge

Patients with ICUAW had prolonged ICU stays as compared to patients without ICUAW [30 (19-42) days versus 19 (12-25) days, p=0.008] and exhibited reduced handgrip strength at ICU discharge as compared to those without ICUAW [17%pred (7.0%-28%) versus 40%pred (26%-57%), p<0.001](Table 3). ICU mobility score indicated severe impairments, more pronounced in patients with, as compared to those without ICUAW [2(2-2) versus 6(4-6), p<0.001]. ICU readmission rate [0/26 (0%) versus 2/24 (8.3%), p=0.225] and mortality [2/26 (7.7%) versus 0/24 (0%), p=0.491] were not different between patients with and without ICUAW. At hospital discharge, handgrip strength remained severely reduced in weak as compared to not-weak patients [43% (28%-59%) versus 64% (36%-80%), p=0.045]. Sit-to-stand test was not feasible in 11/15 (73.3%) patients with and 6/13 (46.2%) patients without ICUAW. In the other 11 patients, the time required was not significantly different [21 (13-29) sec versus 17 (12-19) sec, p=0.778]. Barthel index was worse in patients with ICUAW [8 (2.5-11.5) versus 10.5 (8-18), p=0.040]. As only 31/50 patients were assessed, missing data may have entailed selection bias. Also, weak patients had different discharge destinations than patients without weakness, with respectively 15/26 (57.7%) versus 6/24 (25.0%) being referred to an in-patient rehabilitation centre.

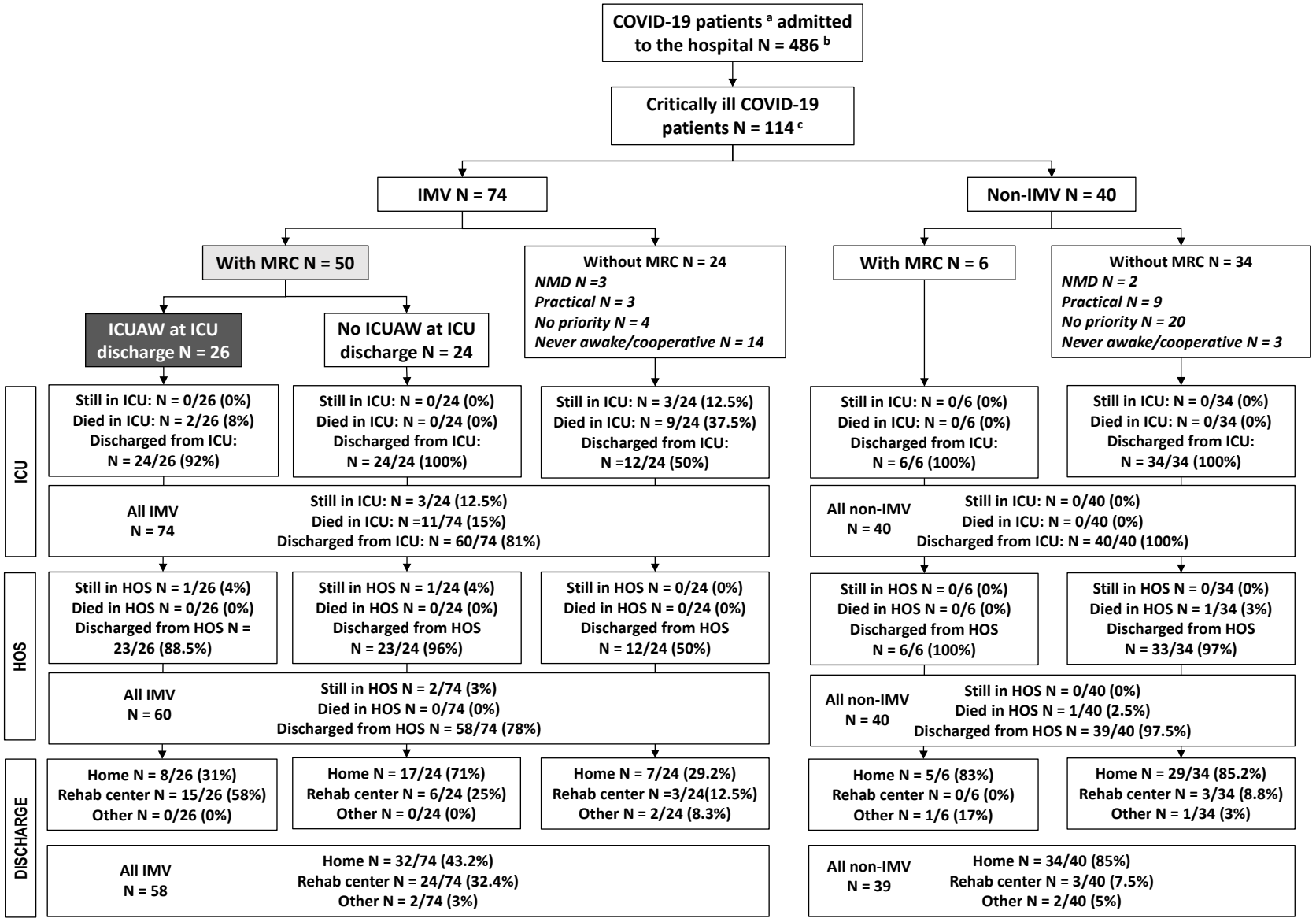
Comparison of characteristics and outcomes according to availability of MRC sum score

IMV patients not assessed for weakness [24/74 (32.4%)] were older [67 (60-76) verus 60 (53-67), p=0.044] and had a higher percentage of lymphocytes upon admission [10.6% (8.5%-16.4) versus 8.3% (5.9%-12.0%), p=0.051] as compared to IMV patients with MRC-sum score. (Supplementary Table 1). These patients had lower mean morning glycemia and mean daily caloric intake, received shorter duration of vasopressor therapy, and received sedatives and analgesics for shorter periods of time (Supplementary Table 2). However, this group also comprised 9 patients who died in the ICU before awakening (82% of ICU deaths).

Supplementary figures

Suppl. Fig. 1: Patient flow chart. A total of 486 COVID-19 patients were admitted to the hospital. Within this cohort, 114 critically ill patients were admitted to the ICUs, of which 74 required invasive mechanical ventilation. Muscle strength was assessed in 50 patients who required IMV and 6 patients without IMV. Abbreviations: *MRC*: Medical Research Council; *ICUAW*: intensive care unit acquired weakness; *ICU*: intensive care unit; *HOS*: hospitalisation.

[a]Diagnosis is based on (1) presence of positive pharyngeal or lower respiratory tract sample PCR (N=109) or (2) the presence of upper or lower respiratory infection symptoms (fever, cough, dyspnea, desaturation) and typical findings on chest CT scan and in the absence of other plausible diagnoses (N=5); b This comprises 14 patients, temporarily admitted to the ICU due to the hospital cohorting policy and while expanding ward capacity, in the very early phase of the epidemic (March 14th-16th and 24th-25th). None of these patients received high flow nasal canula, mechanical ventilation, vasopressor treatment, renal replacement therapy. Median duration of ICU stay for these patients was 1.8 (IQR 0.8-2.2) days; c This excludes patients temporarily admitted to the COVID-ICUs while awaiting diagnostics refuting COVID-19, and 2 patients with suspected but not confirmed COVID-19 (negative pharyngeal PCR, inconclusive CT scan, no lower respiratory tract sample obtained as patients were not intubated).


COVID-19 patients [a] admitted to the hospital N = 486 [b]
Critically ill COVID-19 patients N = 114 [c]
IMV N = 74
Non-IMV N = 40
With MRC N = 50
Without MRC N = 24
NMD N =3
Practical N = 3
No priority N = 4
Never awake/cooperative N = 14
With MRC N = 6
Without MRC N = 34
NMD N = 2
Practical N = 9
No priority N = 20
Never awake/cooperative N = 3
ICUAW at ICU discharge N = 26
No ICUAW at ICU discharge N = 24
ICU
Still in ICU: N = 0/26 (0%)
Died in ICU: N = 2/26 (8%)
Discharged from ICU: N = 24/26 (92%)
Still in ICU: N = 0/24 (0%)
Died in ICU: N = 0/24 (0%)
Discharged from ICU: N = 24/24 (100%)
Still in ICU: N = 3/24 (12.5%)
Died in ICU: N = 9/24 (37.5%)
Discharged from ICU: N =12/24 (50%)
Still in ICU: N = 0/6 (0%)
Died in ICU: N = 0/6 (0%)
Discharged from ICU: N = 6/6 (100%)
Still in ICU: N = 0/34 (0%)
Died in ICU: N = 0/34 (0%)
Discharged from ICU: N = 34/34 (100%)
All IMV N = 74
Still in ICU: N = 3/24 (12.5%)
Died in ICU: N =11/74 (15%)
Discharged from ICU: N = 60/74 (81%)
All non-IMV N = 40
Still in ICU: N = 0/40 (0%)
Died in ICU: N = 0/40 (0%)
Discharged from ICU: N = 40/40 (100%)
HOS
Still in HOS N = 1/26 (4%)
Died in HOS N = 0/26 (0%)
Discharged from HOS N = 23/26 (88.5%)
Still in HOS N = 1/24 (4%)
Died in HOS N = 0/24 (0%)
Discharged from HOS N = 23/24 (96%)
Still in HOS N = 0/24 (0%)
Died in HOS N = 0/24 (0%)
Discharged from HOS N = 12/24 (50%)
Still in HOS N = 0/6 (0%)
Died in HOS N = 0/6 (0%)
Discharged from HOS N = 6/6 (100%)
Still in HOS N = 0/34 (0%)
Died in HOS N = 1/34 (3%)
Discharged from HOS N = 33/34 (97%)
All IMV N = 60
Still in HOS N = 2/74 (3%)
Died in HOS N = 0/74 (0%)
Discharged from HOS N = 58/74 (78%)
All non-IMV N = 40
Still in HOS N = 0/40 (0%)
Died in HOS N = 1/40 (2.5%)
Discharged from HOS N = 39/40 (97.5%)
DISCHARGE
Home N = 8/26 (31%)
Rehab center N = 15/26 (58%)
Other N = 0/26 (0%)
Home N = 17/24 (71%)
Rehab center N = 6/24 (25%)
Other N = 0/24 (0%)
Home N = 7/24 (29.2%)
Rehab center N =3/24(12.5%)
Other N = 2/24 (8.3%)
Home N = 5/6 (83%)
Rehab center N = 0/6 (0%)
Other N = 1/6 (17%)
Home N = 29/34 (85.2%)
Rehab center N = 3/34 (8.8%)
Other N = 1/34 (3%)
All IMV N = 58
Home N = 32/74 (43.2%)
Rehab center N = 24/74 (32.4%)
Other N = 2/74 (3%)
All non-IMV N = 39
Home N = 34/40 (85%)
Rehab center N = 3/40 (7.5%)
Other N = 2/40 (5%)

Supplementary tables

Supplementary Table 1. Baseline and ICU admission characteristics

		IMV N=74						No IMV N=40		
	All patients N=74	IMV with MRC N=50	IMV without MRC N=24	*P*-value	ICUAW at ICU discharge N=26	No ICUAW at ICU discharge N=24	*P*-value	All patients N=40	No IMV with MRC N=6	*P*-value[d]
Baseline characteristics										
Age (years)	62 (54-71)	60 (53-67)	67 (60-76)	0.044	65 (54-71)	59 (49-65)	0.147	63 (51-73)	61 (45-74)	0.875
Gender, male	53 (71.6)	35 (70.0)	18 (75.0)	0.655	18 (69.2)	17 (70.8)	0.902	30 (75.0)	4 (66.7)	0.699
BMI	28.3 (25.7-31.4)	28.9 (26.3-31.4)	27.1 (23.5-31.8)	0.082	28.9 (26.1-31.0)	28.9 (27.2-32.0)	0.861	27.7 (24.2-29.7)	25.6 (24.1-36.1)	0.213
Charlson Comorbidity Index	2 (2-4)	2 (1-4)	3 (2-5)	0.059	2 (2-5)	2 (1-3)	0.289	3 (1-5)	4 (0-9)	0.933
Patient origin				0.732			0.251			0.018
Emergency department	25 (33.8)	16 (32.0)	9 (37.5)		11 (42.3)	5 (20.8)		14 (35.0)	2 (33.3)	
Ward	23 (31.1)	17 (34.0)	6 (25.0)		8 (30.8)	9 (37.5)		21 (52.5)	3 (50.0)	
Other hospital	26 (35.1)	17 (34.0)	9 (37.5)		7 (26.9)	10 (41.7)		5 (12.5)	1 (16.7)	

Continued Supplementary Table 1. Baseline and ICU admission characteristics

		IMV N=74						No IMV N=40		
	All patients N=74	**IMV with MRC N=50**	**IMV without MRC N=24**	***P*-value**	**ICUAW at ICU discharge N=26**	**No ICUAW at ICU discharge N=24**	***P*-value**	**All patients N=40**	**No IMV with MRC N=6**	***P*-value[d]**
ICU admission characteristics [a]										
Laboratory values upon ICU admission										
CRP (mg/l)	169 (78-278)	175 (108-285)	109 (58-248)	0.071	169 (113-218)	226 (86-312)	0.180	107 (42-209)	68 (29-220)	0.026
D-dimers (µg/l)	1603 (961-5027)	1485 (917-3867)	1967 (1022-6891)	0.131	1455 (935-4670)	1485 (706-3617)	0.605	989 (640-2368)	989 (648-1752)	0.012
White blood cell count (10**9/l)	9.2 (7.0-12.8)	9.2 (7.4-12.3)	7.4 (6.4-13.9)	0.432	9.2 (8.2-12.3)	9.1 (7.2-12.5)	0.522	6.8 (5.0-9.8)	5.3 (4.5-8.2)	0.001
Lymphocyte (%)	9.3 (6.3-15.5)	8.3 (5.9-12.0)	10.6 (8.5-16.4)	0.051	8.4 (5.5-12.0)	8.3 (6.3-12.0)	0.602	12.1 (10.1-17.1)	16.9 (9.5-19.2)	0.002
Lymphocyte count (10**9/l)	0.88 (0.60-1.14)	0.88 (0.59-1.11)	0.86 (0.73-1.44)	0.373	0.92 (0.59-1.10)	0.79 (0.59-1.11)	0.949	0.93 (0.64-1.27)	0.94 (0.63-1.01)	0.548
Severity of illness and respiratory support upon admission										
SOFA	8 (6-8.5)	8 (6-8)	7 (5-9)	0.812	8 (7-8.5)	7.5 (4-8)	0.403	3 (2-4)	4 (3-5)	<0.001
IMV	61 (82.4)	40 (80.0)	21 (87.5)	0.528	22 (84.6)	18 (75.0)	0.490	0 (0)	0 (0)	NA
PO_2/FIO_2 (worst value)	87 (60-134)	86 (58-128)	92 (68-179)	0.288	86 (56-122)	89 (60-134)	0.741	95 (83-128)	100 (81-236)	0.122
PEEP (cmH_20)	12 (9-13)	12 (10-14)	10 (8-12)	0.028	12 (10-14)	12 (10-14)	1	NA	NA	NA
Tidal volume (ml/kg/IBW)	6.1 (5.7-6.6)	6.3 (5.8-6.6)	6.0 (4.9-6.2)	0.051	6.3 (5.9-6.6)	6.3 (5.7-7.1)	0.900	NA	NA	NA
Inspiratory pressure (cmH_20) [b]	23 (21-27)	24 (21-28)	22 (20-27)	0.327	24 (21-26)	24 (21-30)	0.662	NA	NA	NA
Driving pressure (cmH_20)	12 (10-15)	12 (10-15)	12 (10-16)	0.776	12 (10-14)	12 (10-16)	0.567	NA	NA	NA
Total Respiratory System Compliance (ml/cmH_2O) [c]	34 (26-41)	35 (26-41)	34 (21-46)	0.704	36 (31-41)	34 (26-39)	0.242	NA	NA	NA

Continuous variables are reported as median (IQR), categorical values as number (%).

[a] Values within 24 hours of admission; [b] Inspiratory pressure is defined as the plateau pressure for patients in volume controlled ventilation or the inspiratory pressure for patients on pressure controlled ventilation; [c] Crs was calculated as tidal volume/driving pressure in patients receiving volume controlled mechanical ventilation or pressure controlled ventilation, provided actual breathing frequency was equal to mandatory breath rate, [d] P-values reflect the comparison between the total population of IMV and no IMV patients.

Abbreviations: *IMV*: invasive mechanical ventilation; *BMI*: body mass index; *CRP*: C-reactive protein; *SOFA*: sequential organ failure assessment; *PEEP*: positive end-expiratory pressure; *MRC:* Medical Research Council; *ICUAW*: Intensive Care Unit acquired weakness; *NA*: not applicable.

Supplementary Table 2. ICU treatments, complications and outcomes

		IMV N=74						No IMV N=40		
	All patients N=74	IMV with MRC N=50	IMV without MRC N=24	*P*-value	ICUAW at ICU discharge N=26	No ICUAW at ICU discharge N=24	*P*-value	All patients N=40	No IMV with MRC N=6	*P*-value[d]
Antiviral and anti-inflammatory treatments										
Expanded acces, adaptive				0.072			0.846			0.707
trial/rescue treatment, yes	19 (25.7)	16 (32)	3 (12.5)		8 (30.8)	8 (33.3)		9 (22.5)	2 (33.3)	
Itraconazole [a]	5 (6.8)	5 (10)	0 (0)		1 (3.8)	4 (16.7)		4 (10.0)	1 (16.7)	
Azithromycine [a]	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)		1 (2.5)	0 (0)	
Convalescent plasma [a]	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)		2 (5.0)	1 (16.7)	
Remdesivir	3 (4.1)	3 (6)	0 (0)		3 (11.5)	0 (0)		0 (0)	0 (0)	
Anti IL1	5 (6.8)	2 (4)	3 (12.5)		2 (7.7)	0 (0)		0 (0)	0 (0)	
Anti IL6	4 (5.4)	4 (8)	0 (0)		1 (3.8)	3 (12.5)		2 (5.0)	0 (0)	
Anti IL1 and itraconazole	1 (1.4)	1 (2)	0 (0)		0 (0)	1 (4.2)		0 (0)	0 (0)	
Anti IL6 and remdesivir	1 (1.4)	1 (2)	0 (0)		1 (3.8)	0 (0)		0 (0)	0 (0)	
CS, yes	53 (71.6)	39 (78)	14 (58.3)	0.079	23 (88.5)	16 (66.7)	0.063	7 (17.5)	0 (0)	<0.001
Duration (days)	7 (0-13)	9 (1-15)	1 (0-10)	0.077	11 (7-19)	3 (0-11)	0.014	0 (0-0)	NA	<0.001
Mean daily dose (methyl-prednisolone equivalent, mg)	17 (0-31)	19 (2-29)	8 (0-42)	0.250	24 (13-34)	12 (0-26)	0.921	0 (0-0)	NA	<0.001
Mean dose on treatment days (methylprednisolone equivalent, mg)	54 (39-77)	55 (39-77)	52 (39-68)	0.635	58 (38-76)	49 (39-81)	0.037	32 (4-80)	NA	0.286
Metabolic control and nutrition										
Mean morning glycemia (mg/dl)	121 (112-130)	123 (112-133)	117 (109-123)	0.038	126 (119-134)	118 (110-129)	0.041	104 (94-118)	108 (100-113)	<0.001
Mean daily insulin IU	47 (14.8-70.5)	54.5 (32.8-86.3)	16.5 (6-51.3)	0.001	55.5 (42.3-90.0)	53.0 (15.5-83.3)	0.290	0 (0-1.5)	3 (0-8.5)	<0.001
Mean daily caloric intake (kcal)	1164 (716-1473)	1284 (1010-1473)	761 (363-1505)	0.013	1310 (1090-1478)	1191 (789-1425)	0.168	357 (242-635)	479 (240-646)	<0.001
Mean daily protein intake (grams)	37 (21-51)	43 (27-51)	23 (8-55)	0.064	45 (33-52)	35 (23-50)	0.466	10 (0-19)	18 (8-26)	<0.001
Treatments and complications										
IMV, yes	74 (100)	50 (100)	24 (100)	.	26 (100)	24 (100)	.	0 (0)	0 (0)	NA
Duration (days)	14 (8-25)	16 (11-26)	9 (5-12)	<0.001	24 (15-29)	12 (8-17)	0.001	NA	NA	NA
High flow nasal oxygen, yes	59 (79.7)	44 (88.0)	15 (62.5)	0.015	23 (88.5)	21 (87.5)	1	26 (65.0)	5 (83.3)	0.085
Duration (days)	4 (1-6)	5 (3-7)	1 (0-2)	<0.001	4 (2-7)	6 (4-7)	0.324	5 (0-7)	7 (5-11)	0.998
Duration (days) if treated	4 (2-7)	5 (4-7)	2 (1-3)	<0.001	4 (3-8)	6 (4-7)	0.205	7 (5-8)	8 (6-11)	0.037

Continued Supplementary Table 2. ICU treatments, complications and outcomes, continued

		IMV N=74						No IMV N=40		
	All patients N=74	IMV with MRC N=50	IMV without MRC N=24	*P*-value	ICUAW at ICU discharge N=26	No ICUAW at ICU discharge N=24	*P*-value	All patients N=40	No IMV with MRC N=6	*P*-value[d]
Treatments and complications										
Prone ventilation, yes	35 (47.3)	27 (54.0)	8 (33.3)	0.096	17 (65.4)	10 (41.7)	0.093	NA	NA	NA
Duration (days) if treated	5 (2-6)	5 (2-7)	5 (2-6)	0.658	5 (4-9)	2 (2-6)	0.059	NA	NA	NA
ECMO, yes	13 (17.6)	7 (14.0)	6 (25.0)	0.329	4 (15.4)	3 (12.5)	1	NA	NA	NA
Duration (days) if treated	16 (11-35)	16 (11-21)	29 (9-55)	0.731	17 (12-22)	14 (11-.)	1	NA	NA	NA
Vasopressors, yes	72 (97.3)	48 (96.0)	24 (100)	1	26 (100)	22 (91.7)	0.225	2 (5.0)	0 (0)	<0.001
Duration (days)	8 (4-13)	10 (5-15)	6 (3-9)	0.027	12 (6-17)	9 (4-13)	0.073	0 (0-0)	NA	<0.001
Renal replacement therapy, yes	21 (28.4)	15 (30)	6 (25)	0.655	11 (42.3)	4 (16.7)	0.048	1 (2.5)	0 (0)	0.001
New bacterial infection, yes [b]	66 (89.2)	47 (94)	19 (79.2)	0.103	25 (96.2)	22 (91.7)	0.602	10 (25.0)	2 (33.3)	<0.001
New fungal infection, yes	13 (17.6)	8 (16)	5 (20.8)	0.746	6 (23.1)	2 (8.3)	0.250	0 (0)	0 (0)	0.004
Opiates, yes	74 (100)	50 (100)	24 (100)	.	26 (100)	24 (100)	.	1 (2.5)	0 (0)	<0.001
Duration (days)	14 (8-21)	15 (12-22)	7 (4-11)	<0.001	20 (14-26)	13 (9-18)	0.004	0 (0-0)	NA	<0.001
Mean daily fentanyl equivalent (µg)	2188 (1072-3986)	2311 (1097-3986)	1967 (1025-6177)	0.799	2914 (1666-5122)	1649 (893-2483)	0.036	0 (0-0)	NA	<0.001
Mean fentanyl equivalent (µg/kg/h) on treatment days	1.4 (0.8-2.3)	1.4 (0.9-2.3)	1.2 (0.8-2.8)	0.917	1.8 (1.3-2.9)	0.9 (0.6-1.6)	0.007	0.49	NA	0.133
Benzodiazepines, yes	59 (79.7)	45 (90.0)	14 (58.3)	0.004	26 (100)	19 (79.2)	0.020	1 (2.5)	0 (0)	<0.001
Duration (days)	6 (1-11)	7 (2-13)	2 (0-6)	0.011	10 (6-14)	6 (1-14)	0.048	0 (0-0)	NA	<0.001
Mean daily dose midazolam (mg)	18 (0-54)	21 (1-49)	15 (0-117)	0.635	22 (6-44)	17 (0-65)	0.382	0 (0-0)	NA	<0.001
Mean midazolam (mg/h) on treatment days	3.2 (1.2-4.8)	2.9 (1.0-4.1)	4.4 (2.6-8.3)	0.024	3.0 (0.9-3.7)	2.8 (1.0-4.5)	0.836	0.083	NA	0.067
Dexmedetomidine, yes	60 (81.1)	44 (88)	16 (66.7)	0.054	23 (88.5)	21 (87.5)	1	3 (7.5)	1 (16.7)	<0.001
Duration (days)	7 (3-15)	10 (4-16)	4 (0-6)	0.001	10 (5-19)	10 (3-16)	0.606	0 (0-0)	0 (0-1)	<0.001
Mean daily dose (µg)	435 (77-864)	696 (129-971)	152 (0-475)	0.004	553 (118-894)	788 (131-1044)	0.515	0 (0-0)	0 (0-66.4)	<0.001
Mean dose (µg/kg/h) on treatment days	0.6 (0.3-0.8)	0.6 (0.5-0.8)	0.3 (0.2-0.7)	0.047	0.7 (0.5-0.8)	0.6 (0.4-0.7)	0.235	0.3 (0.3-.)	0.3(0.3-0.3)	0.341
Clonidine, yes	27 (36.5)	20 (40)	7 (29.2)	0.365	14 (53.8)	6 (25.0)	0.038	NA	NA	NA
Duration (days)	0 (0-4)	0 (0-4)	0 (0-2)	0.318	2 (0-5)	0 (0-1)	0.095	NA	NA	NA
Mean daily dose (µg)	0 (0-62)	0 (0-78)	0 (0-41)	0.342	14 (0-115)	0 (0-7)	0.100	NA	NA	NA
Mean dose (µg) on treatment days	525 (300-684)	540 (300-729)	525 (360-629)	0.685	493 (272-744)	630 (410-719)	0.547	NA	NA	NA

Continued Supplementary Table 2. ICU treatments, complications and outcomes

		IMV N=74						No IMV N=40		
	All patients N=74	IMV with MRC N=50	IMV without MRC N=24	*P*-value	ICUAW at ICU discharge N=26	No ICUAW at ICU discharge N=24	*P*-value	All patients N=40	No IMV with MRC N=6	*P*-value[d]
Ketamine, yes	51 (68.9)	38 (76.0)	13 (54.2)	0.057	22 (84.6)	16 (66.7)	0.138	NA	NA	NA
Duration (days)	4 (0-11)	6 (1-12)	1 (0-7)	0.060	10 (3-13)	2 (0-9)	0.033	NA	NA	NA
Mean daily dose (mg)	78 (0-295)	99 (3-329)	12 (0-177)	0.098	169 (38-347)	31 (0-308)	0.132	NA	NA	NA
Mean dose (mg/kg/h) on treatment days	0.23 (0.13-0.32)	0.23 (0.13-0.33)	0.21 (0.10-0.29)	0.387	0.24 (0.16-0.34)	0.23 (0.12-0.30)	0.529	NA	NA	NA
Propofol, yes	73 (98.6)	50 (100)	23 (95.8)	0.324	26 (100)	24 (100)	.	NA	NA	NA
Duration (days)	11 (6-17)	13 (9-17)	8 (4-12)	0.007	15 (11-23)	11 (6-14)	0.007	NA	NA	NA
Mean daily dose (mg)	1840 (1014-3019)	1830 (1149-2667)	2225 (862-3696)	0.453	1862 (1401-3134)	1502 (1028-2520)	0.156	NA	NA	NA
Mean dose (mg/kg/h) on treatment days	1.4 (1.1-1.8)	1.4 (1.1-1.8)	1.3 (0.9-2.3)	0.859	1.3 (1.2-2.2)	1.4 (1-1.7)	0.130	NA	NA	NA
NMBA, yes	62 (83.8)	45 (90.0)	17 (70.8)	0.048	26 (100)	19 (79.2)	0.020	NA	NA	NA
any, duration (days)	5 (2-8)	6 (3-8)	3 (0-9)	0.146	8 (5-10)	4 (1-7)	0.001	NA	NA	NA
drip, duration (days)	2 (0-7)	4 (0-7)	0 (0-7)	0.171	7 (2-10)	0 (0-4)	0.001	NA	NA	NA
Tracheostomy, yes	3 (4.1)	3 (6.0)	0 (0)	0.546	3 (11.5)	0 (0)	0.236	NA	NA	NA
Delirium, yes [C]	50 (69.4)	41 (82)	9 (40.9)	<0.001	22 (84.6)	19 (79.2)	0.721	6 (17.6)	1 (16.7)	<0.001
Duration (days)	2 (0-5)	3 (1-6)	0 (0-2)	0.001	3 (2-6)	3 (1-6)	0.631	0 (0-0)	0 (0-1)	<0.001
ICU outcomes										
ICU stay (days)	19 (11-31)	22 (17-35)	11 (7-15)	<0.001	30 (19-42)	19 (12-25)	0.008	6 (3-8)	7 (5-11)	<0.001
ICU readmission	4 (5.4)	2 (4.0)	2 (8.3)	0.591	0 (0)	2 (8.3)	0.225	6 (15.0)	1 (16.7)	0.097
ICU mortality, yes	11 (14.9)	2 (4.0)	9 (37.5)	<0.001	2 (7.7)	0 (0)	0.491	0 (0)	0 (0)	0.008
HOS outcomes										
HOS stay (days)	30 (21-42)	36 (26-46)	21 (10-33)	<0.001	41 (30-50)	28 (21-39)	0.011	14 (10-19)	14 (12-17)	<0.001
Total HOS mortality	11 (14.9)	2 (4.0)	9 (37.5)	<0.001	2 (7.7)	0 (0)	0.491	1 (2.5)	0 (0)	0.054
Discharge destination				<0.001			0.019			<0.001
Home	32 (43.2)	25 (50.0)	7 (29.2)		8 (30.7)	17 (70.8)		34 (85.0)	5 (83.3)	
In-patient rehab center	24 (32.4)	21 (42.0)	3 (12.5)		15 (57.7)	6 (25.0)		3 (7.5)	0 (0)	
Still in ICU/HOS	3/2 (6.8)	0/2 (4.0)	3/0 (12.5)		0/1 (3.8)	0/1 (4.1)		0 (0)	0 (0)	
Other	2 (2.7)	0 (0)	2 (8.3)		0 (0)	0 (0)		2 (5.0)	1 (16.7)	

[a] treatments started on the ward; [b] defined as newly started/ expansion of antibiotic spectrum after initial antibiotic treatment; [c] delirium was assessed with the Intensive Care Delirium Screening Checklist (ICDSC); [d] P-values reflect the comparison between the total population of IMV and no IMV patients. Continuous variables are reported as median (IQR), categorical values as number (%).

Abbreviations: *IMV*: invasive mechanical ventilation; *MRC*: Medical Research Council; *ICUAW*: Intensive Care Unit acquired weakness; *ICU*: intensive care unit; *HOS*: hospitalization; *IL*: interleukine; *CS*: corticosteroids; *NMBA*: neuromuscular blocking agent; *ECMO*: extracorporeal membrane oxygenation.

Characteristics and outcomes of patients who did not require invasive mechanical ventilation

40/114 (35.1%) ICU patients did not require IMV. Non-IMV patients as compared to IMV patients expectedly had lower severity of illness upon presentation, as reflected by lower CRP (mg/l) [107 (42-209) versus 169 (78-278), p=0.026] and D-dimeres (ug/l) [989 (640-2368) versus 1603 (961-5027), p=0.012], higher percentage of lymphocytes [12.1 (10.1-17.1) versus 9.3 (6.3-15.5), p=0.002] and lower SOFA score [3 (2-4) versus 8 (6-8.5), p<0.001] (Supplementary Table 1). Evidently, exposure to corticosteroids, NMBA, sedatives and analgesics was significantly different between IMV and non-IMV patients (Supplementary Table 2). Median duration of ICU stay (days) [6 (3-8) versus 19 (11-31), p<0.001] and hospital stay (days) [14 (10-19) versus 30 (21-42), p<0.001] was lower for non-IMV versus IMV patients.

MRC sum score as assessed in 6/40 (15%) of non-IMV patients. The incidence of ICUAW at first evaluation and at ICU discharge was 1/6 (16.7%). None of the patients evaluated at hospital discharge [0/3(3%)] were weak. Other outcomes are depicted in Supplementary Table 3.

Supplementary Table 3. Strength, physiotherapy treatments and functional outcomes

	IMV N=74				No IMV N=40
	With MRC N=50	**ICUAW at ICU discharge N=26**	**No ICUAW at ICU discharge N=24**	***P*-value**	**With MRC N=6**
Evaluations in ICU					
MRC sum score					
MRC sum score at awakening	42 (31-48)	33 (25-42)	48 (40-54)	<0.001	55 (46-60)
MRC sum score <48 at awakening	36 (72.0)	26 (100)	10 (41.7)	<0.001	1 (16.7)
Days to MRC sum score at awakening	17 (12-25)	22 (14-33)	15 (11-19)	0.002	5.5 (4-8)
Time remaining under mechanical ventilation from the day of MRC sum score at awakening (days)	3 (0-8)	3 (1-14)	1 (0-4)	0.179	NA
Time remaining in the ICU from the day of MRC sum score at awakening (days)	3 (1-8)	3 (0-9)	4 (1-9)	0.667	3 (0-3)
MRC sum score at ICU discharge	46 (38-52)	39 (32-42)	52 (49-57)	<0.001	56 (46-60)
MRC sum score < 48 at ICU discharge	26 (52.0)	26 (100)	0 (0)	<0.001	1 (16.7)
Days to MRC sum score at ICU discharge	21.5 (14-33.5)	29 (16-37)	18.5 (11-23.5)	0.015	5.5 (4-9)
Time remaining in the hospital from the day the of MRC at ICU discharge (days)	12 (8-14)	13 (9-15)	10.5 (6-13)	0.139	8 (6-10)
Handgrip strength					
HGF at ICU discharge available	35 (70.0)	14 (53.8)	21 (87.5)	0.009	3 (50)
Reasons for no HGF				0.004	
MRC elbow < 3	6 (12)	6 (23)	0 (0)		0
No priority	1 (2)	0 (0)	1 (4)		0
Practical	8 (16)	6 (23)	2 (8)		3 (50)
HGF at ICU discharge (% pred)	30 (17-44)	17 (7-28)	40 (26-57)	<0.001	83 (58-.)
ICU mobility score					
ICU mobility score at ICU discharge	2.5 (2-6)	2 (2-2)	6 (4-6)	<0.001	6 (5-6)

Continued Supplementary Table 3. Strength, physiotherapy treatments and functional outcomes

	IMV N=74				No IMV N=40
	With MRC N=50	ICUAW at ICU discharge N=26	No ICUAW at ICU discharge N=24	*P*-value	With MRC N=6
Evaluations on the ward					
MRC sum score					
MRC sum score at HOS discharge available	37 (74.0)	21 (80.8)	16 (66.7)	0.256	3 (50)
MRC sum score at HOS discharge	53 (47-56)	48 (36-56)	55 (52-58)	0.007	60 (49-.)
MRC sum score < 48 at HOS discharge	10 (27.0)	10 (47.6)	0 (0)	0.002	0
Handgrip strength					
HGF at HOS discharge available	33 (66.0)	17 (65.4)	16 (66.7)	0.924	3 (50)
HGF at HOS discharge (%pred)	48 (29-73)	43 (28-59)	64 (36-80)	0.045	91 (83-.)
Barthel score					
Barthel score at HOS discharge available	31 (62.0)	17 (65.4)	14 (58.3)	0.608	3 (50)
Barthel score at HOS discharge	9 (5-13)	8 (2.5-11.5)	10.5 (8-18)	0.040	18 (13-.)
Sit-to-stand test					
Performed at HOS discharge	11 (22)	4 (36.4)	7 (63.6)	0.157	2 (33.3)
Not feasible	17 (34)	11 (64.7)	6 (35.3)		2 (33.3)
Time if feasible (sec)	17 (13-28)	21 (13-29)	17 (12-19)	0.788	18 (13-.)
Physiotherapy sessions ICU and ward	7 (3-16)	8 (5-20)	6 (2-12)	0.149	5 (3-9)

Abbreviations: *IMV*: invasive mechanical ventilation; *MRC*: Medical Research Council; *HGF*: handgrip force; ICU: intensive care unit; *HOS*: hospitalisation.

Supplementary Table 4: Comparison of characteristics between the IMV COVID-19 cohort and historical ARDS cohorts reporting on ICUAW

	Papazian et al. NEJM 2010 N=339	Fan et al. Crit Care Med 2014 N=222	Dinglas et al. Crit Care Med 2017 N=156	Moss et al. NEJM 2019 N= 1006	Van Aerde et al. N=74
Patient characteristics					
Age	I: 58±16 C: 58±15	49 (40-58)	47 (40-57)	I: 57±15 C:55±16	62 (54-71)
Gender, Male	NR	123 (55)	84 (54)	560 (56)	53 (72)
Comorbidity index score	NR	1 (1-3)	1 (1-3)	NR	2 (2-4)
Severity of illness					
SOFA	NR	9 (7-11)[2]	5 (4-7)[3]	I: 9±4 C: 9±4	8 (6-8.5)[4]
APACHEII	NR	23 (19-28)	23 (17-29)	NR	NR
SAPS II	I: 50±16 C: 47±14	NR	NR	NR	NR
PO_2/FIO_2 (worst value)	I: 106±36 C:115±41	NR	NR	I: 99±28 C:100±28	87 (60-134)
IMV, days	NR	9 (5-17)	10 (6-17)	NR	14 (8-25)
Proning, yes	97 (28.6)	NR	NR	159 (15.8)	35 (47)
Vasopressors	306 (90.2)	NR	NR	I:230/501(45.9)[5] C:185/505(36.6)[5]	72 (97)

Continued Supplementary Table 4: Comparison of characteristics between the IMV COVID-19 cohort and historical ARDS cohorts reporting on ICUAW

	Papazian et al. NEJM 2010 N=339	Fan et al. Crit Care Med 2014 N=222	Dinglas et al. Crit Care Med 2017 N=156	Moss et al. NEJM 2019 N= 1006	Van Aerde et al. N=74
Patient characteristics					
RRT, yes	118 (34.8)	51 (23)	NR	NR	21 (28)
NMBA, yes	267 (78.7)	49 (22)	32 (21)	574 (57.1)	62 (84)
Benzodiazepines,					
Yes	NR	NR	NR	NR	59 (78)
Cumulative dose (mg midazolam)	I:997 (459-2236)[1] C:1200 (657-2217)[1]	274 (72-922)	NR		105 (0.3-590)
Duration, days	NR	NR	NR		6 (1-11)
Opioids,					
Yes	NR	NR	NR	NR	74 (100)
Cumulative dose (µg fentanyl)	I:38 (18-97) [1] C:50 (17-100) [1]	16910 (5120-38710)	NR		25438 (10369-76029)
Duration, days	NR	NR	NR		14 (8-21)
Corticosteroids,					
Yes	143 (42.2)	NR	87 (56)	NR	53 (72)
Cumulative dose (mg HCS)	NR	400 (0-1909)	1464 (772-4316)[8]		695 (0-2310)
Duration, days	NR	NR	7 (4-13)[8]		7 (0-13)
Glucose control	NR	Mean blood glucose > 150 mg/dl: 38 (17%)	NR	NR	Mean morning glycemia (mg/dl): 121 (112-130)
Incidence of ICUAW					
ICUAW at awakening	NR	NR	NR	91/253 (36.0) [6]	36/50 (72)
MRC at awakening	NR	NR	NR	I:47±14 [6] C:50±12 [6]	42 (31-48)
ICUAW at ICU discharge	68/201 (33.8)	NR	NR	36/98 (36.7) [7]	26/50 (52)
MRC at ICU discharge	I: 55 (43-60) C: 55(44-60)	NR	NR	I:46±14 [7] C: 50±11 [7]	46 (38-52)
ICUAW at HOS discharge	NR	80/222 (36)	60/156 (38)	NR	10/37 (27)
MRC at HOS discharge	NR	50 (42-56)	50 (42-55)	NR	53 (47-56)

[1] Cumulative dose over first 7 days; SOFA score reported as: [2] maximum daily SOFA score, [3] mean daily SOFA-score, [4] maximum SOFA-score within first 24 hrs; [6] MRC was measured at predetermined time points during the trials, numbers correspond to MRC at day 7; [7] MRC was measured at predetermined time points during the trials, numbers correspond to MRC at day 28; [8] dose and duration provided for those who received CS. Abbreviations: *SOFA:* Sequential Organ Failure Assessment; *APACHEII*: Acute Physiology and Chronic Health Evaluation II; *SAPSII:* Simplified Acute Physiology Score II; *ICUAW:* intensive care unit acquired weakness; *PO_2/FIO_2:* ratio of partial arterial oxygen pressure to fraction of inspired oxygen; *RRT:* renal replacement therapy; *HCS*: hydrocortisone; *NR:* not reported.

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Chapter 8: Molecular mechanisms of long-term weakness in ICU-survivors

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ABSTRACT

Purpose

Many patients who have been critically ill are confronted with clinically relevant reductions in physical strength, even up to years after admission to the intensive care unit (ICU). We investigated several mechanisms potentially explaining reduced strength 5 years after critical illness, focusing on morphological muscular abnormalities and differential gene expression patterns known to contribute to intensive care unit acquired weakness.

Methods

This is a pre-planned subanalysis of the EPaNIC follow-up cohort, including 120 patients who underwent a vastus lateralis muscle biopsy at 5-year follow-up and 30 demographically similar controls. Muscle strength was evaluated with hand-held dynamometry of hip and knee, and quadriceps isometric peak torque. Morphological abnormalities and myofibre type and size distribution were examined by light microscopy. Targeted gene expression analysis was performed for myofibrillary proteins and markers of atrophy, denervation, and myogenesis/regeneration. Longitudinal analyses were performed for patients who also had an in-ICU biopsy (N=11).

Results

Strength measures of patients 5 years after ICU admission were 10-20% lower than those of controls ($p \leq 0.01$). Post-ICU patients more frequently showed abnormal myofibre shape (24.2% vs 0%, $p=0.001$), endomysial fibrosis (37.5% vs 6.7%, $p=0.0008$), and inflammation (27.5% vs 6.7%, $p=0.015$). Myofibre type distribution was similar in patients and controls, but patients had smaller type II myofibres. For none of the investigated genes, expression was significantly different in patients at 5 years relative to controls, with longitudinal within-patient evaluation suggesting recovery of acute in-ICU changes in these markers. Histological abnormalities did not significantly associate with reduced strength. Also, investigated molecular markers were not significantly different for patients with or without reduced strength, except for higher myogenin gene expression with reduced strength.

Conclusions

Several morphological abnormalities were observed in muscle 5 years after critical illness, though did not significantly associate with strength. Critical illness-induced alterations in markers of myofibrillary protein synthesis or breakdown, neural signaling/denervation, or muscle regenerative pathways may have resolved in the long-term. To explain reduced strength at 5 years, further research remains necessary, also including additional plausible pathways.

INTRODUCTION

Critical illness survivors display clinically relevant reductions in physical function up to 5 years after an intensive care unit (ICU) stay relative to demographically matched healthy control subjects [1]. A complicated ICU stay is an important risk factor for this adverse legacy of critical illness on long-term physical performance [2, 3]. Indeed, long-term strength and physical function appeared to be worse if critical illness is prolonged (Chapter 3) [4], and even slight reductions in isometric strength at ICU discharge were shown to be independently associated with decreased strength and physical performance at 5-year follow-up (Chapter 4-6) [3, 5].

Whereas the long-term clinical trajectories of critical illness survivors and their relation to ICU-acquired complications are increasingly being charted, the molecular mechanisms responsible for reduced strength in long-term ICU survivors have hardly been investigated. Indeed, such mechanisms have so far only been studied in a single, small cohort of critically ill patients, 6 months after ICU discharge. Histological analyses and a co-expression network analysis of transcriptomic data from muscle biopsies suggested that many pathways implicated in the development of intensive care unit-acquired weakness (ICUAW) [6, 7], including disrupted structural protein homeostasis, innervation, autophagy, mitochondrial biogenesis and inflammation, had been restored at 6 months follow-up, whereas aberrant expression of pathways involved in regeneration, extracellular matrix production and calcium signalling appeared to be associated with the strength deficit documented at this time point [8, 9]. Interestingly, atrophy persisted in 73% of patients but did not correlate with strength at 6 months [8]. The results of this study were hypothesis-generating, but insight and generalisability remained limited in view of the very small sample size of patients and controls, selection of patients who were mechanically ventilated for at least one week, and the relatively short follow-up of the patients up to 6 months after ICU admission.

In this study, we investigated specific molecular mechanisms potentially contributing to reduced strength after critical illness in a large, general population of ICU survivors who were followed-up 5 years after ICU admission. First, we investigated whether previously identified morphological abnormalities and differential gene expression patterns in skeletal muscle of critically ill patients, indicative of muscle damage and associated with the development of ICUAW [6, 7, 10], had recovered at 5-year follow-up. In patients with longitudinal biopsy samples, we further studied the temporal evolution of expression profiles of skeletal muscle between the ICU stay and the 5-year follow-up time point. Second, we aimed to investigate, should non-recovery of any alterations be observed, whether this associated with reduced strength at long-term post-ICU follow-up. In this regard, we also explored whether morphological or gene expression differences were present in patients depending on their post-ICU outcome, hypothesising that differences may exist between those with and those without strength deficit at long-term follow-up.

METHODS

Participants

This was a pre-planned sub-analysis of the prospective 5-year follow-up study of the EPaNIC cohort (ClinicalTrials.gov: NCT00512122) [11], where former ICU patients who gave written informed consent were evaluated for muscle strength, physical exercise capacity and physical functioning. Patients unable to walk without assistance prior to ICU admission and patients with pre-existing neuromuscular disease or other pre-ICU disabilities potentially confounding the morbidity endpoints had been excluded from participation in this follow-up study. In parallel to the 5-year follow-up cohort, a group of demographically similar controls had been recruited to serve as a healthy reference.
For the present study, we included all patients who provided a skeletal muscle biopsy during their

physical evaluation 5 years after ICU admission and all controls who provided a muscle biopsy. The Leuven University Hospital Ethics Committee (ML4190) approved the study. Details on the EPaNIC RCT and its long-term follow-up cohort have been published previously [3-5, 11, 12].

Clinical outcomes

Patients and controls had been assessed for isometric strength of the muscle from which biopsies were harvested, i.e. the quadriceps femoris [10, 11, 13, 14]. These strength measurements included hand-held dynamometry (microFET®2 using CompuFet®2 software; Biometrics, Almere, The Netherlands) of hip and knee, expressed as percentage of their predicted values [13]. Additionally, quadriceps isometric peak torque was assessed with a dynamometer (Biodex® Medical Systems, NY, USA) during a maximal isometric knee extension effort with the hip in a 90° flexion angle and the knee in a 60° flexion angle, expressed as percentage of its predicted value [14].

Muscle biopsy sampling

Skeletal muscle biopsy samples were obtained from the vastus lateralis of the quadriceps femoris muscle with the percutaneous needle biopsy technique [15, 16] after strength assessments. Under aseptic conditions, patients and controls underwent local anesthesia (skin and subcutaneous tissues) with Lidocaine 2% (Linisol, Braun). After a skin incision of 5 mm, a suction-modified Bergström needle – inserted perpendicular to the longitudinal direction of the muscle – was used to obtain muscle biopsy samples. The wound was closed with Steristrips® under a compressive bandage. For gene-expression analysis, a sample was snap-frozen in liquid nitrogen and stored at -80°C prior to further analyses. For histology, a separate sample was first fixed for 24 hours in 4% paraformaldehyde in phosphate-buffered saline and subsequently paraffin-embedded.

Morphological analyses of muscle biopsies

Hematoxylin-Eosin (H&E), and immuno-histochemical staining for type I and type II myofibres was performed on 5 µm paraffin sections after dewaxing. For slow myosin (type I), following preparation by antigen retrieval (Antigen Retrieval Solution, Dako), quenching of endogenous peroxidase (3% H_2O_2 solution) and blocking of non-specific binding sites (normal rabbit serum, Dako), sections were incubated at room temperature with the primary antibody (Sigma M8421, 1:200, 50 min) and subsequently with a horseradish peroxidase-conjugated secondary antibody (rabbit-anti-mouse, Dako P0447, 1:50, 1 hour) [10]. The reaction was developed (20 min) with the Vector SG substrate kit for peroxidase (Vector Laboratories SK-4700), resulting in a grey colour. For fast myosin (type II), preparation included quenching of endogenous alkaline phosphatase with 1 mM Levamisole, blocking with normal rabbit serum and incubation with alkaline phosphatase-conjugated fast myosin antibodies (Sigma A4335, 1:500, 1 hour) [10]. This reaction was developed (20 min) with the Vector Red Alkaline Phosphatase substrate kit (Vector Laboratories SK-5100), resulting in a red colour. For each participant, separate sections were stained for slow myosin, fast myosin, and both.

Cross-sectional area of stained myofibres was determined with a custom algorithm written for ImageJ (Fiji®) by an experienced researcher on image analysis from the Muscle Research Centre Erlangen (MURCE). Briefly, the algorithm was applied consecutively to pictures made with a Leica® microscope at 5x magnitude and comparable light settings of corresponding sections stained for either one of the myosin types. After converting the analysis scale from pixels to µm, and setting reasonable limits for fibre cross-sectional area and circularity based on previous experience [10, 17], the algorithm first required manual identification of a threshold that could separate stained from unstained myofibres through transformation of the image to a black-white scale. The resulting mask was applied on the stained section to identify individual myofibres and the cross-sectional area of each stained myofibre was computed in units of μm^2 based on the number of pixels within delineated regions of interest.

Manual quality checks of automatically identified regions of interest was performed for each picture to eliminate artefacts and to ensure proper identification of individual myofibres.

Qualitative analysis of H&E-stained sections was based on an in-house protocol, and involved scoring sections for fibre size and homogeneity, and presence of signs indicative of fibrosis, necrosis, regeneration and inflammation. The researcher performing the analyses (NVA) was unaware of whether sections were from a patient or control.

Gene expression analysis: targeted quantitative real-time polymerase chain reaction

RNA was extracted with an in-house protocol that made use of tissue lysis with QIAzol Lysis Reagent (Qiagen, Venlo, The Netherlands) and isopropanol precipitation, and was reverse-transcribed to cDNA. Targeted gene expression analyses were performed for myofibrillary proteins and markers of atrophy, neural signaling/innervation and myogenesis. Real-time PCR (StepOne Plus, Applied Biosystems, Carlsbad, CA, USA) was performed with TaqMan chemistry-based commercial kits (Applied Biosystems) or with use of primers and probes manufactured by Eurogentec (Seraing, Belgium). Relative gene expression was determined with the ΔΔCt method using CASC3 as housekeeping gene. Gene expression data are expressed relative to the median level of the healthy controls as reference. An overview of genes, assays and functional relevance of the targeted gene expression analyses is provided in Supplementary Table 1.

Statistics

First, we compared morphological aspects and gene expression of patients and healthy control subjects. In patients with available longitudinal biopsy samples, the temporal evolution of gene expression profiles of skeletal muscle between the ICU stay and the 5-year follow-up time point was explored. Second, we *a priori* planned to associate any abnormality documented in muscle biopsies 5 years after ICU admission with measures of strength at this 5-year follow-up time point, with use of Pearson or Spearman correlation analysis. Furthermore, in an exploratory analysis, we compared morphological aspects and gene expression for patients with and those without reduced strength at follow-up, which was defined as quadriceps peak torque less than 80% of its predicted value.

Statistical analyses were performed with SPSS version 27 (IBM corp, Chicago, IL, USA) and JMP (SAS Institute Inc., Cary, USA). Continuous data are expressed as medians and interquartile ranges, categorical variables as numbers and proportions. Differences were evaluated with Mann-Whitney U-test for continuous data, Chi^2- or Fisher exact-test for categorical variables as appropriate, and Wilcoxon signed-rank test for repeated measurements testing in the longitudinal sample. Differences were considered significant when two-sided *p*-values were 0.05 or less. No corrections were made for multiple comparisons.

RESULTS

Participants

Of the patients included in the EPaNIC follow-up cohort and evaluated at 5-year follow-up during a hospital visit, 120 underwent a percutaneous muscle biopsy (Figure 1). For 11 of the 120 patients also in-ICU biopsies were available, collected on day 8±1 in ICU. Muscle biopsies were also obtained from 30 healthy controls. Upon presentation at the follow-up clinic, age, BMI and sex distribution were comparable between patients and controls (Table 1). In-ICU characteristics of the patients are shown in Table 2.

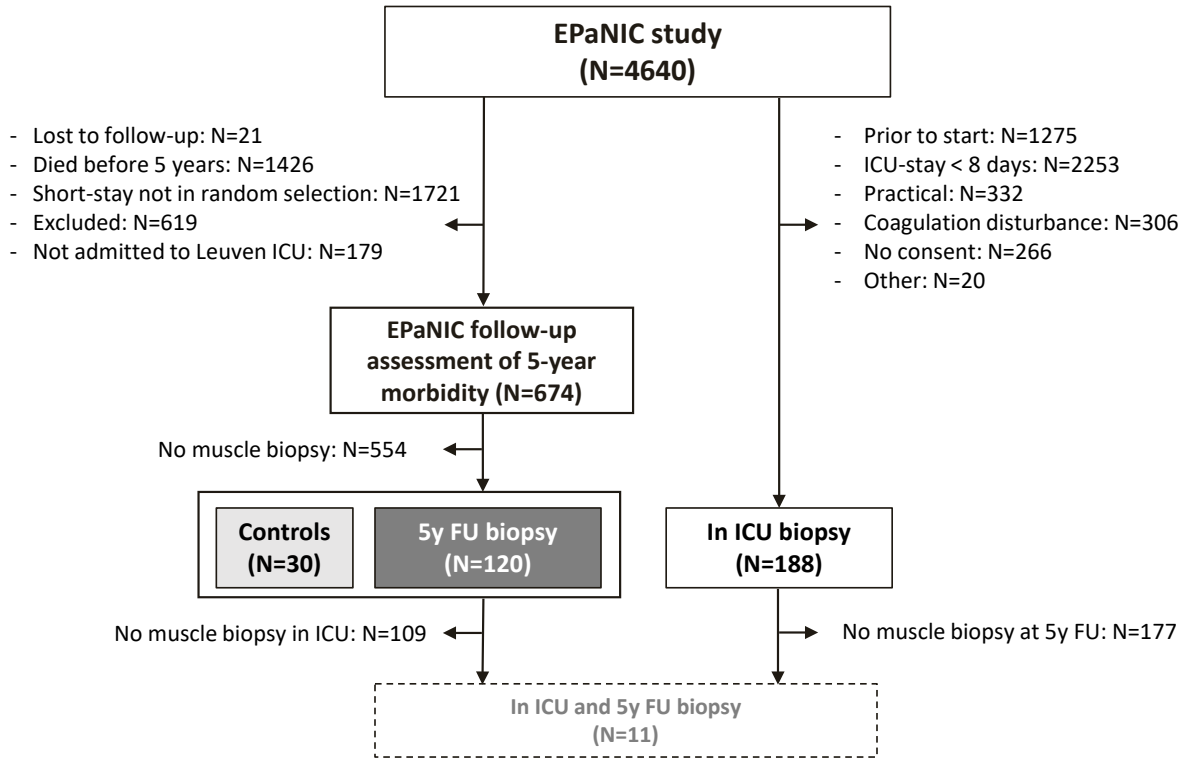

Fig. 1: Consort diagram of patients and controls included in the histological and differential gene expression analyses. Abbreviations: *EPaNIC:* Early versus Late Parenteral Nutrition in Intensive Care; *FU:* follow-up; *ICU:* intensive care unit; *y:* year.

Table 1: Characteristics upon presentation at the follow-up clinic of 5-year ICU-survivors and healthy controls with available muscle biopsy

	Controls (N=30)	EPaNIC FU 5 year (N=120)	*P*-value
Baseline factors			
Age at follow-up	61 (57-66)	58 (50-66)	0.195
Gender	23 (76.7)	96 (80.0)	0.719
BMI at follow-up	26.4 (23.4-28.6)	27.2 (23.7-30.4)	0.349
Strength assessments			
HHD			
Hip (%pred)	164 (133-181)	141 (122-159)	0.012
Knee (%pred)	66 (58-74)	53 (45-63)	<0.001
Biodex (%pred)	93 (88-108)	84 (68-99)	0.001

Continuous data are presented as median (interquartile range), categorical data are presented as number (percentage).
Abbreviations: *ICU:* intensive care unit; *EPaNIC FU:* Early versus late Parenteral Nutrition on the Intensive Care follow-up cohort; *BMI:* body mass index; *HHD:* hand-held dynamometry; *%pred:* percentage of predicted value according to reference values.

Table 2: Baseline comorbidities and ICU-characteristics of patients with a muscle biopsy at 5-year follow-up

Characteristic	EPaNIC FU 5y (N=120)
Baseline comorbidities	
Diabetes mellitus	10 (8.3)
Malignancy	15 (12.5)
Preadmission dialysis	0 (0)
ICU characteristics	
Randomisation to late PN	59 (49.2)
APACHE II score	26 (15-32)
Diagnostic category	
Cardiac surgery	44 (36.7)
Emergency SICU	58 (48.3)
Elective SICU	12 (10.0)
MICU	6 (5.0)
Sepsis upon admission	28 (23.3)
Mechanical ventilation, days	2 (1-9)
Vasopressors/inotropics, days	2 (0-4)
ICU stay, days	4 (2-14)

Continuous data are presented as median (interquartile range), categorical data are presented as number (percentage).
Abbreviations: *APACHE:* Acute Physiology And Chronic Health Evaluation; *EPaNIC FU:* Early versus late Parenteral Nutrition on the Intensive Care 5-year follow-up cohort; *MICU:* medical intensive care unit; *PN:* parenteral nutrition; *SICU:* surgical intensive care unit.

Strength at five-year follow-up

Patients had significantly worse strength at 5-year follow-up as compared to healthy controls (Table 1). Hand-held dynamometry revealed a median 14% decrease in hip strength, a median 20% decrease in knee strength, and a median 10% decrease in quadriceps peak torque as percentage of predicted ($p \leq 0.01$).

Muscle morphology

An overview of the comparison of morphological characteristics of muscle biopsies from patients 5 years after ICU admission and controls is presented in Table 3, and representative illustrations of the evaluated aspects are shown in Figure 2A-C. A remarkably variable myofibre size was observed in approximately a quarter of the biopsies from both patients and controls. However, whereas all healthy controls had myofibres with a polygonal shape, 24.2% of the patient biopsies showed the presence of myofibres with a rounded or angular shape (p=0.001). In addition, a significantly higher proportion of the patients showed a remarkably increased endomysial distance or endomysial connective tissue in their muscle biopsies (37.5%) as compared with healthy controls (6.7%, p=0.001), as well as more signs of inflammation (27.5% versus 6.7%, p=0.015). Presence of adipocytes, necrosis, centralized nuclei and vacuolization were rare findings, similarly in both patients and controls.
Classification of myofibres to type I and type II myofibres revealed that the proportion of type I myofibres was 44.8 (33.3-56.1) % in patients and 51.4 (41.9-64.8) % in controls (p=0.150). Quantification of myofibre size distribution suggested a shift towards smaller myofibres in patients as compared to controls, explained by smaller type II rather than type I myofibres (Figure 2).

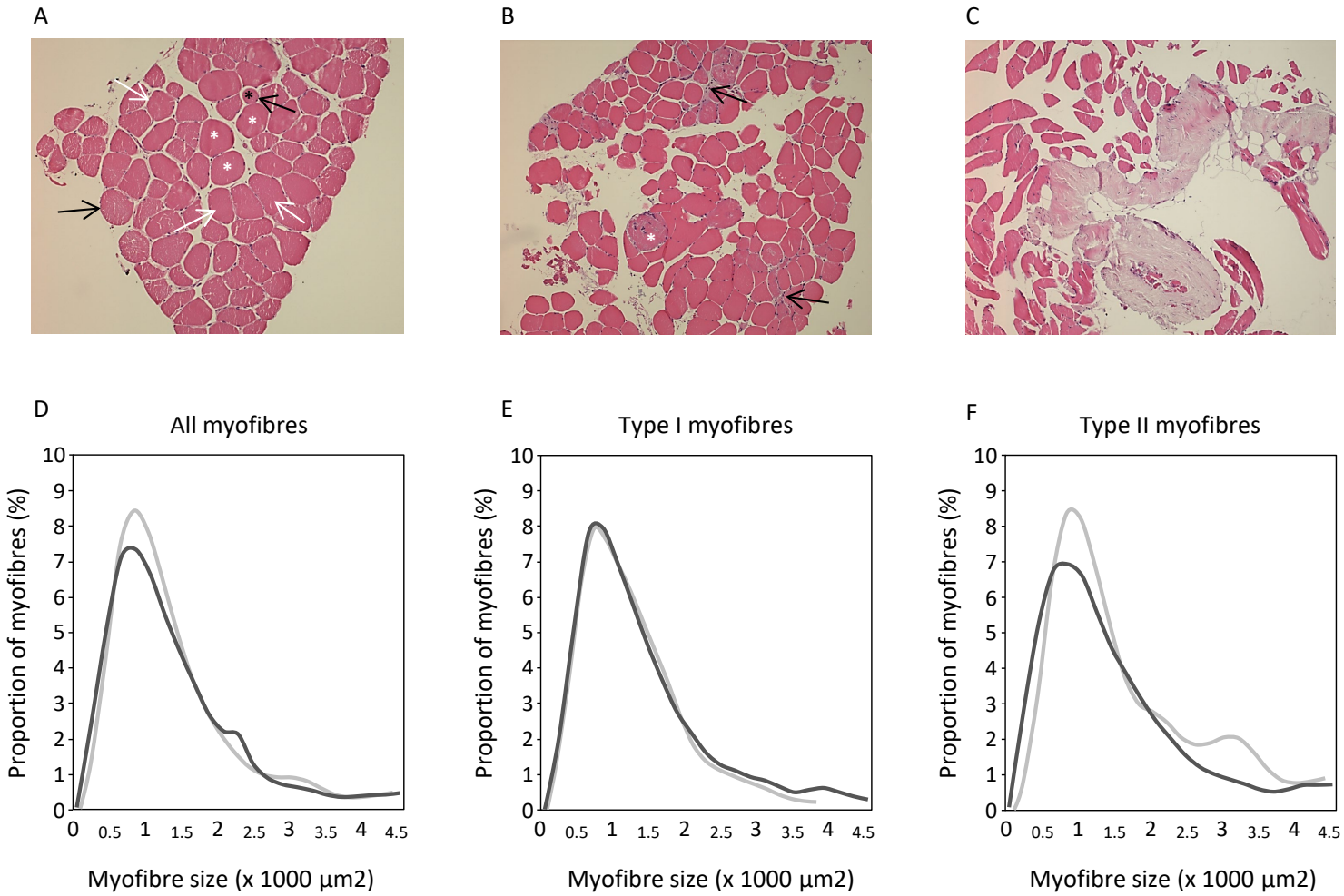

Fig. 2: Illustration of morphological abnormalities and myofibre size distribution in patients 5 years after ICU admission as compared with matched controls. (A) Illustration of heterogeneous fibre morphology, with polygonal (white arrows) and rounded (black arrows) myofibres, as well as large (white asterisk) and small (black asterisk) myofibres (B) Illustration of moderate endomysial fibrosis and inflammation (black arrows), with occasional infiltration of myofibres (white asterisk) by inflammatory cells (C) Illustration of severe fibrosis, (D) Size distribution of all myofibres, (E) Size distribution of type I myofibres, (F) Size distribution of type II myofibres. Dark grey line represents distribution of patients, light grey line represents distribution of controls.

Table 3: Morphological analyses of muscle biopsies of 5-year ICU-survivors and healthy controls

	Controls (N=30)	5y EPaNIC FU (N=120)	*P*-value
Myofibre homogeneity			
Highly variable myofibre size	7 (23.3)	31 (25.8)	0.778
Abnormal myofibre shape	0 (0)	29 (24.2)	0.001*
Adipocytes			
Adipocytes present	2 (6.7)	13 (10.8)	0.736*
Adipocytes replacing myofibres	1 (3.3)	8 (6.7)	0.688*
Increased endomysial distance/connective tissue	2 (6.7)	45 (37.5)	0.001*
Inflammation	2 (6.7)	33 (27.5)	0.0150*
Myofibre damage			
Necrosis	2 (6.7)	13 (10.8)	0.736*
Centralised nuclei	2 (6.7)	13 (10.8)	0.736*
Vacuolisation	1 (3.3)	3 (2.5)	>0.999*

Morphological analyses were performed on H&E-stained muscle biopsy sections. * Indicates Fisher exact test. Continuous data are presented as median (interquartile range), categorical data are presented as number (percentage).
Abbreviations: *ICU:* intensive care unit; *EPaNIC FU:* Early versus late Parenteral Nutrition on the Intensive Care follow-up cohort; *H&E:* Hematoxylin and Eosin

Gene expression analyses

We evaluated gene expression of several myofibrillary proteins (Myosin heavy chain (MyHC)-I, MyHC-IIa and α-actin) [18], markers of atrophy (MuRF-1 and Atrogin-1 [19, 20], and upstream pro-catabolic regulators including FOXO1 and FOXO3 [21-23]), markers of neural signaling/denervation (HDAC4, AChε and AChγ) [24-30], and markers of myogenesis/muscle regeneration (Pax7, MYF5, MYOD, myogenin, MYF6 and myostatin) [31-34] (Supplementary Table 1). None of the evaluated genes were differentially expressed between patients at 5-year follow-up and controls (Figure 3).
To assess temporal aspects of gene expression, we also compared the selected markers in available in-ICU and 5-year follow-up muscle samples from patients with longitudinal assessment, relative to controls. Differential gene expression was mostly consistent with previous findings, namely reduced expression of myofibrillary proteins, increased atrogene expression and signs of denervation during the phase of critical illness, resolving at long-term follow-up, although some interindividual variation was observed (Figure 4). Responses in markers of myogenesis were more variable, with most patients showing an increase in Pax7 and MyoD1 and a decrease in Myf5, myogenin and Myf6 from in ICU towards 5-year follow-up, but all towards levels comparable to controls.

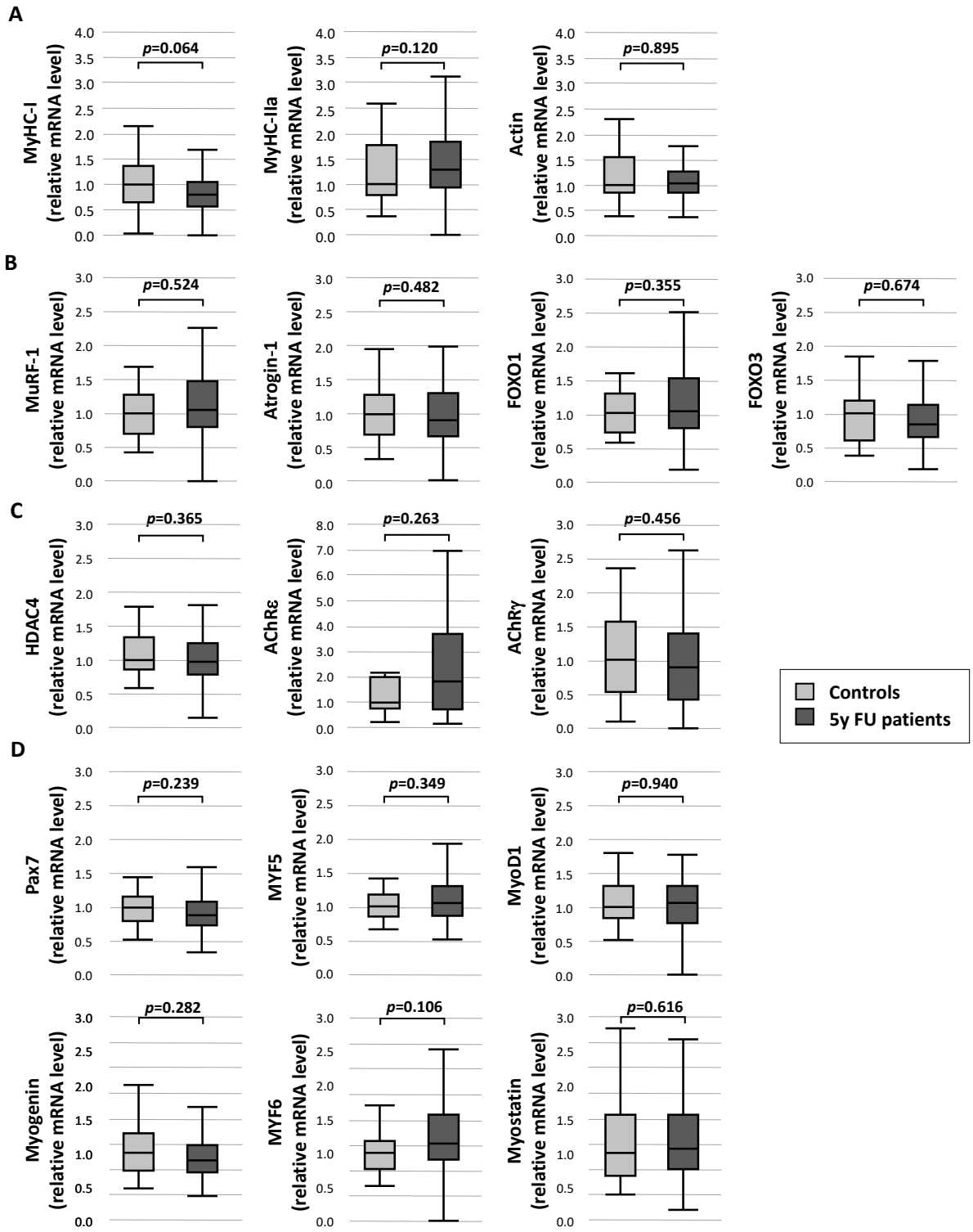

Fig. 3: Relative mRNA levels in 5-year survivors of critical illness as compared to demographically similar controls. (A) Myofibrillary proteins (MyHC-I, MyHC-IIa, α-actin), (B) Markers of atrophy (MuRF-1, atrogin-1, FOXO1, FOXO3), (C) Markers of neural signaling/denervation (HDAC4, AChRε and AChRγ), (D) Markers of the myogenic differentiation program (Pax7, MYF5, MyOD1, myogenin, MYF6, myostatin). *P*-values represent comparisons between patients and controls. Light grey boxplots represent control data, dark grey boxplots represent patient data. Boxes represent median and interquartile ranges (IQR), whiskers are drawn to the furthest point within 1.5 x IQR from the box. Abbreviations: *MHC:* myosin heavy chain; *MuRF:* muscle ring finger; *FOXO:* Forkhead Box O; *HDAC:* Histone Deacetylase; *AChR:* Acetylcholine receptor; *MYF:* myogenic factor; *MYOD:* myoblast determination protein; *Pax:* Paired box protein.

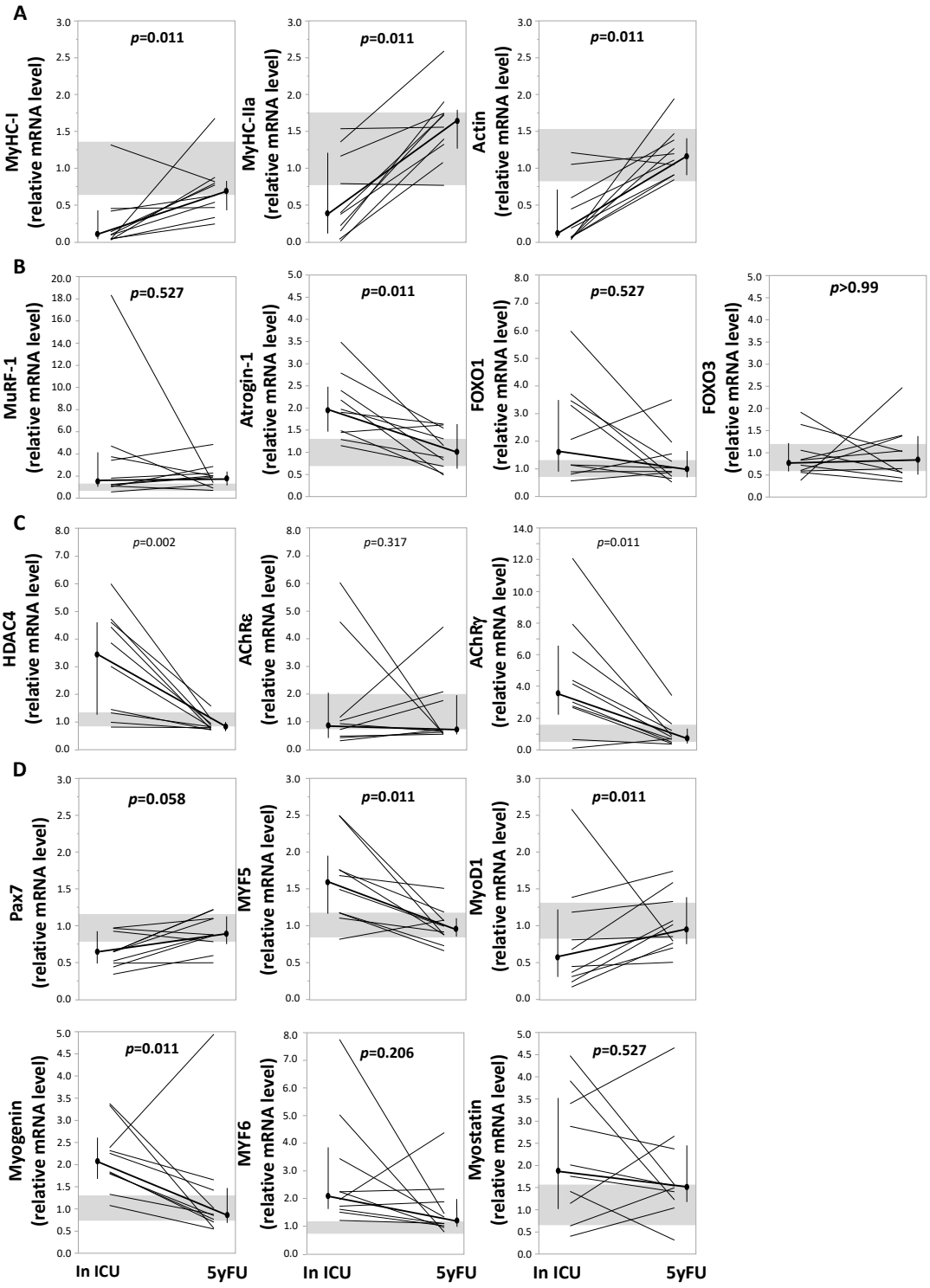

Fig. 4: Temporal patterns of gene expression profiles in critically ill patients from ICU-stay up to long-term follow-up. (A) Myofibrillary proteins (MyHC-I, MyHC-IIa, α-actin), (B) Markers of atrophy (MuRF-1, atrogin-1, FOXO1, FOXO3), (C) Markers of neural signaling/denervation (HDAC4, AChRε and AChRγ), (D) Markers of the myogenic differentiation program (Pax7, MYF5, MyOD1, myogenin, MYF6, myostatin). Spaghetti plots depict individual profiles for patients with available longitudinal data. Dots and whiskers depict the medians and interquartile ranges for all patients. The thick lines connect the medians, representing the overall evolution of expressions over time. The grey bar represents the interquartile range for the healthy controls. *P*-values represent comparisons of expression levels between patients in ICU and at 5-year follow-up. Abbreviations: *MHC:* myosin heavy chain; *MuRF:* muscle ring finger; *FOXO:* Forkhead Box O; *HDAC:* Histone Deacetylase; *AChR:* Acetylcholine receptor; *MYF:* myogenic factor; *MYOD:* myoblast determination protein.

Association of biopsy findings with muscle strength five years after ICU admission

Some measures of muscle strength were numerically, though not significantly lower in patients with certain histological abnormalities in muscle as compared with patients who did not show such abnormalities. More specifically, none of the strength measures were significantly different for patients with rounded or angular myofibres as compared with those with polygonal myofibres, for patients with or without a remarkably increased endomysial distance or endomysial connective tissue, or for patients with or without signs of inflammation in muscle (Supplementary Table 2). As none of the investigated genes were different for patients and controls, no further correlations with strength measures were performed for the total patient groups. Since differences between patients with reduced strength and those with normal strength at follow-up may be masked by analysing the total patient group, we also compared all studied morphological and molecular markers among these two subgroups. However, none of the markers were significantly different between 5-year follow-up patients with and those without reduced strength at follow-up, except for higher myogenin gene expression in patients with reduced strength (Supplementary Table 3 and 4).

DISCUSSION

In a large, heterogeneous cohort of general critical illness survivors, we performed a targeted analysis of abnormalities in muscle morphology and a number of pathways potentially involved in reduced strength 5 years after critical illness as compared to healthy controls. Morphological analysis revealed abnormalities in myofibre shape, more frequent signs of endomysial fibrosis and inflammation, as well as a shift towards smaller type II myofibres in ICU survivors at 5-year follow-up relative to controls. Investigated molecular markers of myofibrillary protein synthesis and breakdown, of neural signaling/denervation, and of myogenesis/muscle regeneration were comparable for patients at 5-year follow-up and controls, with longitudinal within-patient evaluation suggesting recovery of acute in-ICU changes in these markers. Except for a higher myogenin gene expression, none of the investigated markers were associated with reduced strength at follow-up, This suggests that other mechanisms may be involved.

Physical impairments are increasingly recognized as an important patient-centered outcome in critical illness survivors [35, 36]. Although clinical trajectories have been characterised [1-4, 37-40], insight into the molecular mechanisms of reduced strength in long-term ICU-survivors, and their possible heterogeneity, is lacking, partly due to limited tissue availability from long-term ICU-survivors across the illness spectrum. In this regard, the present study in a large follow-up population of general critical illness survivors with clinically discernable differences in muscle strength 5 years after ICU admission when compared to healthy controls, yielded a number of important findings.

Several morphological differences were present between muscle tissue sections of post-ICU patients and controls. Abnormal myofibre shape, with round or angular myofibres, was observed in a substantial proportion of post-ICU patients but not in controls. Widening of the endomysial space and increased presence of endomysial connective tissue, pointing to endomysial fibrosis, were more frequently observed in post-ICU patients, as were signs of inflammation. We further observed a shift towards smaller myofibres, explained by atrophy of type II rather than of type I myofibres. Chronic denervation could be involved in the observed histological abnormalities, as it can induce endomysial fibrosis [41] and angular fibre atrophy [13]. Additionally, neurodiagnostic studies and 1 small histological study suggested persistence of denervation up to two years after hospital discharge in functionally impaired ICU-survivors with documented critical illness neuro(myo)pathy or a history of weakness after an ICU stay of at least 28 days [42-45]. However, we did not observe fibre type grouping, a pathognomonic feature of denervation [17]. Also, preferential atrophy of type II muscle fibres as observed in our patients at follow-up is not typical for denervation [17], but has been

described in a variety of clinical settings including aging, cancer cachexia, diabetes and steroid myopathy that are also known to affect muscle strength [17, 46-49]. In the ICU, muscle atrophy appears to affect both type I and type II fibres [10, 50], involving myosin loss related to mechanical silencing and drug exposures [51, 52], although preferential type II myofibre involvement has been observed in patients with nonexcitable muscle membranes [53, 54]. Its etiology and clinical relevance in post-ICU patients based on current observations is unclear. Although our observations with respect to endomysial fibrosis agree with those at 6 months in the pilot study of Dos Santos *et al*, they observed smaller cross-sectional areas of both type I and type II myofibres in 7 of 10 patients studied at 6 months post-ICU [8]. Also, the inflammatory infiltrate of leukocytes that was present at 7 days after ICU discharge appeared to have resolved by 6 months after ICU discharge in the patients investigated in that study [8], unlike our observations at 5-year follow-up. These discrepancies may be related to the use of immunostaining versus H&E staining, or the latter study being limited to only 10 patients.

We further investigated gene expression of myofibrillary proteins as marker of muscle protein synthesis capacity, of E3 ligases of the ubiquitin-proteasome pathway as markers of muscle protein breakdown, of markers of neuronal signaling/denervation, and of markers of myogenesis/muscle regeneration. However, we did not observe any significant differences in these markers 5 years post-ICU as compared with controls. Hence, denervation-associated expression alterations (i.e. induction of MuRF-1, AChRγ, HDAC4, Myf5 and 6, myoD and myogenin [19, 27, 28]) were no longer apparent at 5-year follow-up. As similar trends have also been observed in experimental settings of chronically denervated muscle [55], this finding thus not necessarily contradicts the presence of some morphological signs of denervation. Lack of differences between post-ICU patients and controls suggests that changes occurring in the studied pathways during ICU stay had recovered in the long-term, further supported by longitudinal within-subject measurements in a small subgroup of patients. These data largely confirm the findings by Dos Santos et al. and Walsh et al. that suggested that changes in many pathways implicated in the development of ICUAW had been restored 6 months later [8, 9]. However, whereas these studies suggested that alterations in pathways involved in muscle regeneration may be involved in compromised muscle strength 6 months post-ICU, we did not observe any abnormalities in the expression of a whole range of genes involved in the myogenic program 5 years after critical illness.

Our study only revealed some abnormalities in muscle morphological aspects, but not in the studied molecular markers. Importantly, muscle strength was not significantly different for patients who presented with these morphological abnormalities as compared with those for whom these morphological aspects were normal. Furthermore, no significant differences were observed in any of the studied histological or molecular markers when comparing patients with versus those without reduced strength at long-term follow-up, with the exception of a higher myogenin gene expression with reduced strength. Although myogenin has been associated with muscle motor denervation and promotion of muscle reinnervation [28][ref], no differences according to degree of strength were found in other markers of such phenotype [56]. These results may imply that other pathways may mediate the long-term physical limitations that post-ICU patients are confronted with. Several plausible candidate mediators of long-term impairments in strength are interesting targets for future investigation. Metabolic responsiveness is of particular interest. Changes in physical activity or whole-body metabolic conditions dynamically regulate skeletal muscle oxidative metabolism through peroxisome proliferator–activated receptor gamma coactivator 1α (PGC-1α), with input from β-adrenergic receptors, AMP (5' adenosine monophosphate)-activated kinase, insulin, calmodulin-dependent kinase and calcineurin [57-63]. Failure to convey these signals results in impaired mitochondrial responsiveness to changing energy demands, which may affect physical performance and exercise endurance considerably. Interestingly, reduced mitochondrial biogenesis is a hallmark of aging-related sarcopenia [64, 65], a condition known to also involve selective atrophy of type II

myofibres [46, 49, 66]. Other interesting targets for further research comprise a range of calcium-dependent processes that are crucial for normal skeletal function, including excitation-contraction coupling, activity-dependent changes of oxidative metabolism, and proteostasis [34, 67-70], which are differentially regulated in type I and II myofibres as well [26, 59, 63, 71]. Calcium-related pathways have been implicated in the pathogenesis of ICUAW [72, 73] and, moreover, calcium signaling was one of the few pathways found to associate with strength at 6 months post-ICU [9]. As a final example, the immune system is increasingly considered to interact with skeletal muscle in health and disease, which could be relevant given the observation of inflammatory changes in our sample of post-ICU biopsies. Pro-inflammatory cytokines, mainly tumour necrosis factor (TNF), interleukin (IL)-1β, and IL-6, can induce procatabolic and anti-anabolic shifts in skeletal muscle [74, 75], contributing to the sarcopenia seen in chronic kidney disease, type 2 diabetes and aging [76-78].

Our study has important strengths. Our data represent the largest set of post-ICU muscle biopsies (N=120) and longest follow-up time window documented up to present. The use of the Bergström technique for collection of muscle biopsies contributed to this success as it enabled muscle biopsy sampling in an ambulatory research setting. This procedure is well-tolerated, safe and cost-effective with good sample yield in terms of quality and quantity [15, 79]. Our study also has some limitations. A first limitation relates to the selection of the samples. The sample size was determined by muscle biopsy availability and not by an *a priori* sample size calculation. Hence, we cannot exclude that statistical power could have been insufficient to demonstrate an association of biopsy findings with weakness. Further, with respect to the design of the study, incomplete longitudinal follow-up may have introduced selection bias, towards patients with better preserved muscle function allowing them to come to the hospital, and precluded time series analysis to elucidate factors potentially associated with recovery trajectories (i.e. speed and/or failure of recovery). Also, muscle biopsies before ICU admission were not available to assess baseline status, but patients with noticeable physical limitations prior to ICU admission had been excluded to avoid confounding. Second, the molecular studies were limited to gene expression analyses, precluding detection of potential changes at post-transcriptional level [34, 80]. Third, total muscle RNA had been extracted, which precluded the investigation of differential gene expression alterations according to myofibre type and differentiation stage (i.e. mature type I and II myofibres, muscle stem cells), which seems interesting given the morphological findings. Finally, choosing for a targeted analysis approach, as indicated, left many hypothetical pathways unexplored, which should be subject of further research.

In conclusion, this study in a large heterogeneous patient group suggested that reduced strength observed 5 years after critical illness and ICU admission may not primarily be explained by the documented morphological abnormalities in muscle visible with general light microscopic investigation, and may not support involvement of long-term changes in myofibrillary protein synthesis or breakdown, in neural signaling/denervation, or in muscle regenerative pathways. The study of other plausible candidate pathways involved in long-term weakness, possibly focused on myofibre-type/differentiation stage specific homeostasis, is warranted to yield further crucial insight.

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SUPPLEMENTARY MATERIALS

Supplementary tables

Supplementary Table 1: Genes, Taqman assays and functional relevance for targeted gene expression analysis on muscle biopsy samples of EPaNIC follow-up patients and controls

Gene	Assay*	Function
MyHC-I	F: 5'-AGCTGATGACCAACTTGCGC-3' R: 5'-CCCTGGAGACTTTGTCTCATTAGG-3' P: 5'-CACCCATCCCCACTTTGTACGCTTGT-3')	Cross-bridge structural component, slow-twitch myofibres
MyHC-IIa	F: 5'-GAAAGTCTGAAAGGGAACGCA-3' R: 5'-CGCCACAAAGACAGATGTTTTG-3' P: 5'-TGAGGCCCAGAATAGGCCCTTTGATG-3'	Cross-bridge structural component, fast-twitch myofibres
α-actin	F: 5'-AGGTCATCACCATCGGCAAT-3' R: 5'-AAGGAAGGCTGGAAGAGCGT-3' P: 5'-AGCGCTTCCGTTGCCCGGA-3'	Cross-bridge component, all myofibres
FOXO1	Hs01054576_m1	Pro-catabolic transcription factor
FOXO3	Hs00818121_m1	Autophagy-regulating transcription factor, also implicated in the induction of MYOD
MuRF-1	Hs00261590_m1	Atrophy inducing gene product
Atrogin-1	Hs01041408_m1	Atrophy inducing gene product
HDAC4	Hs01041648_m1	Regulator of gene expression reflecting neuronal input
AChRε	Hs00181084_m1	Nicotinergic acetylcholine receptor subunit expressed in adult muscle
AChRγ	Hs00183228_m1	Nicotinergic acetylcholine receptor subunit expressed in fetal muscle, induced by denervation
Pax7	Hs00242962_m1	Regulator of adult muscle satellite cell recruitment in response to muscle damage
MYOD1	Hs00159528_m1	Regulator of myogenic differentiation of satellite-cell derived myoblasts in adult muscle tissue
MYF5	Hs00929416_g1	commitment of skeletal myogenic cells to final differentiation into mature skeletal muscle cells
MRF4	Hs00231165_m1	late muscle differentiation gene to permit the formation of multinucleated myofibres
Myostatin	Hs00976237_m1	Inhibitor of myogenesis
Myogenin	Hs01072232_m1	Inducer of myogenesis

* Primers and probes manufactured by Eurogentec (Seraing, Belgium) or commercial assays manufactured by Applied Biosystems (Carlsbad, CA, USA).

Abbreviations: *AChRε:* acetylcholine receptor subunit ε (adult form); *AChRγ:* acetylcholine receptor subunit γ (fetal form); *F:* forward primer; *FOXO:* forkhead box O; *HDAC4:* histone deacetylase 4; *MRF4:* muscle-specific regulatory factor 4 (also called MYF6, myogenic factor 6); *MuRF-1:* muscle ring finger 1 (also known as TRIM63); *MyCH:* myosin heavy chain; *MYF5:* myogenic factor 5; *P:* probe; *R:* reverse primer. Atrogin-1 is also known as FBXO32.

Supplementary Table 2: Comparison of strength at 5-year evaluation by presence of histological abnormalities in muscle biopsy samples of EPaNIC follow-up patients

	Abnormal histology	N	Normal histology	N	*P*-value
Myofibre shape					
HHD hip (%pred)	132 (117-154)	29	143 (123-163)	89	0.142
HHD knee (%pred)	50 (41-60)	27	54 (47-65)	88	0.245
Biodex knee (%pred)	84 (63-93)	29	85 (69-100)	83	0.285
Endomysial distance/connective tissue					
HHD hip (%pred)	142 (127-163)	44	137 (119-157)	74	0.510
HHD knee (%pred)	54 (49-65)	43	53 (42-63)	72	0.551
Biodex knee (%pred)	79 (64-98)	40	85 (72-100)	72	0.565
Endomysial Inflammation					
HHD hip (%pred)	141 (131-166)	32	140 (119-156)	86	0.348
HHD knee (%pred)	54 (49-58)	30	53 (42-64)	85	0.894
Biodex knee (%pred)	83 (65-99)	28	84 (68-98)	84	0.869

Continuous data are presented as median (interquartile range), categorical data are presented as number (percentage).

Abbreviations: *EPaNIC:* Early versus late Parenteral Nutrition on the Intensive Care; *%pred*: percentage of predicted value according to reference values; *HHD*: hand-held dynamometry.

Supplementary Table 3: Comparison of presence of histological abnormalities in muscle biopsy samples of EPaNIC follow-up patients by quadriceps strength at 5-year evaluation

	Reduced quadriceps strength at FU (N=48)	Normal quadriceps strength at FU (N=64)	*P*-value
Abnormal myofibre shape (round/angular vs polygonal)	13 (27.1)	16 (25.0)	0.803
Increased endomysial distance/connective tissue	20 (41.7)	20 (31.3)	0.255
Endomysial Inflammation	13 (27.1)	15 (23.4)	0.659

Data are presented as number (percentage). Normal quadriceps strength at 5-year follow-up was defined as a value of at least 80% of its predicted value when assessed with a Biodex dynamometer.

Abbreviations: *EPaNIC:* Early Parenteral Nutrition in Intensive Care; *FU:* follow-up.

Supplementary Table 4: Comparison of relative gene expression in muscle biopsy samples of EPaNIC follow-up patients by quadriceps strength at 5-year evaluation

	Reduced quadriceps strength at FU (N=48)	Normal quadriceps strength at FU (N=64)	*P*-value
MyHC-I	0.84 (0.59-1.04)	0.80 (0.57-1.08)	0.583
MyHC-IIa	1.26 (0.96-1.79)	1.48 (0.94-1.96)	0.463
α-actin	1.06 (0.86-1.37)	1.04 (0.86-1.20)	0.635
MuRF-1	1.06 (0.84-1.65)	0.99 (0.77-1.43)	0.299
Atrogin-1	0.98 (0.68-1.32)	0.92 (0.72-1.33)	0.969
FOXO1	1.07 (0.81-1.70)	1.01 (0.80-1.54)	0.519
FOXO3	0.85 (0.67-1.03)	0.84 (0.65-1.15)	0.798
HDAC4	0.92 (0.77-1.32)	1.02 (0.80-1.24)	0.574
AChRε	1.67 (0.75-3.79)	2.10 (0.72-3.66)	0.620
AChRγ	1.00 (0.56-1.52)	0.73 (0.38-1.33)	0.152
Pax7	0.86 (0.68-1.13)	0.89 (0.80-1.08)	0.529
MYF5	1.00 (0.85-1.36)	0.97 (0.86-1.24)	0.457
MYOD1	1.09 (0.72-1.31)	1.04 (0.78-1.24)	0.847
Myogenin	0.99 (0.76-1.41)	0.84 (0.67-0.99)	0.028
MRF4	1.23 (0.83-1.74)	1.14 (0.90-1.48)	0.627
Myostatin	1.06 (0.79-1.74)	1.02 (0.67-1.45)	0.248

Data are presented as median (interquartile range). Normal quadriceps strength at 5-year follow-up was defined as a value of at least 80% of its predicted value when assessed with a Biodex dynamometer.

Abbreviations: *EPaNIC:* Early Parenteral Nutrition in Intensive Care; *FU:* follow-up; *AChRε:* acetylcholine receptor subunit ε (adult form); *AChRγ:* acetylcholine receptor subunit γ (fetal form); *F:* forward primer; *FOXO:* forkhead box O; *HDAC4:* histone deacetylase 4; *MRF4:* muscle-specific regulatory factor 4 (also called MYF6, myogenic factor 6); *MuRF-1:* muscle ring finger 1 (also known as TRIM63); *MyCH:* myosin heavy chain; *MYF5:* myogenic factor 5; *P:* probe; *R:* reverse primer. Atrogin-1 is also known as FBXO32.

Chapter 9: General discussion and future perspectives

CARING FOR THE CRITICALLY ILL: A BRAVE NEW WORLD

The field of intensive care medicine and its research has become exemplary of the so-called paradox of progress that our society presently faces. Rapidly evolving technologies have propelled forward both translational science and standards of daily care, but present practical and ethical challenges to research and practice frameworks currently in place. Increased pathophysiological understanding of critical illness and its associated complications raised notion of latent subgroups [1-7] and heterogeneity of treatment effect [8, 9], challenging protocolised care assuming "one size fits all". Furthermore, with improved survival of critical illness, the definition of patient-centered or 'meaningful' outcomes and at what cost they may be achieved is evolving [10]. Incorporating a longer-term perspective into treatment decisions will require intensivists to prospect on whether the cost or the benefit of certain interventions will be most consequential for the individual patient involved [11]. In this regard, the resolution of knowledge gaps on possible preventable factors contributing to adverse long-term outcomes of those surviving critical illness – and, arguably, intensive care, as well as identification of patients at risk– has become pressing, in particular in the face of an unprecedented cohort of ICU-survivors emerging from the ongoing COVID-19-pandemic.

During this PhD-project, supported by an experienced research group comprising field experts and relying on data obtained from two large patient cohorts, we investigated the relationship between the ICU-trajectory of patients requiring vital organ support to avoid imminent death and their long-term health outcomes.

More specifically, we aimed to investigate, first, if a prolonged ICU-stay predisposes to increased mortality and to the adverse functional long-term outcomes that are major pillars of the post-intensive care syndrome. We further explored whether *de novo* neuromuscular dysfunctions, frequently complicating prolonged ICU stay, associate with these poor long-term outcomes. Additionally, we extended the static clinical evaluations in ICU survivors with cardiopulmonary exercise testing to capture and assess the complex state of physical fitness in ICU survivors and its relation to in-ICU organ failure. Second, we set out to explore the incidence and risk factors for neuromuscular complications of severe COVID-19, a new disease that overwhelmed ICUs worldwide, as this group of patients may be at risk to suffer from such lasting health implications based on these neuromuscular complications. Third, we aimed to study the molecular correlates of long-term post-ICU disabilities, with the goal of increasing understanding and facilitating further research directed at the prevention or remediation of these abnormalities.

THE LEGACY OF PROLONGED CRITICAL ILLNESS

In chapter 3, we established an independent association between a prolonged ICU-stay, arbitrarily defined as an ICU-stay of at least 8 days, and adverse long-term outcomes, including 5-year mortality and morbidity, with clinically meaningful reductions in handgrip strength [12], 6-minute walk distance [13] and physical function of the SF-36 quality-of-life questionnaire [14, 15], relative to patients with a short ICU-stay (<8 days). Increased mortality rates and poor mental health, cognitive and physical function, associated with poor quality-of-life have been extensively documented in survivors of critical illness, a syndrome that has been labeled the post-intensive care syndrome [16-18]. As previous studies compared data from ICU survivors to other hospitalized patients or healthy controls [19-25], it remains unclear whether these poor outcomes merely reflect the pre-morbid function and health status rendering patients susceptible to ICU admission, rather than actually resulting from the ICU stay.

We here attempted to disentangle attributable mortality and morbidity of prolonged ICU stay. Therefore we introduced another ICU population as a reference to indirectly capture several

background factors that may predispose patients for poor outcomes, some of which may never be observable [26], minimising unmeasured confounding to the best of our abilities. In addition, we used a very stringent matching method to adjust for possible confounding. As such, we obtained groups of short-and long-stayers that were comparable upon ICU admission. Hence, our findings support the hypothesis that excess 5-year mortality and morbidity in long-term ICU survivors may partially result from the culmination of exposures and complications hallmarking a prolonged ICU-stay.

Establishing a contribution of prolonged ICU-stay to long-term adverse outcome in ICU-survivors has inherent value as it fuels the incentive for critical (re)appraisal of intensive care management. We explored the plausible role of several treatments associated with a prolonged ICU-stay in the observed association with adverse outcomes. These exposures – unlike the effect of duration of ICU-stay – could potentially be studied in randomised interventions, to assess whether modifying their use affects long-term outcome. Although our findings are strictly hypothesis-generating, several identified "suspects" are notable, including the use of sedatives, neuromuscular blocking agents, and corticosteroids.

Over the past decade, appropriate sedation has become a cornerstone of good clinical practice in critical care [27-29]. Although analgesia and sedation in critically ill patients are often required to perform procedures, increase tolerance for mechanical ventilation, and prevent harm from patient-ventilator asynchrony or excessive oxygen consumption, deep and prolonged sedation are associated with adverse short-term outcomes, including prolonged mechanical ventilation, and an increased incidence of ICUAW and of delirium [30-33]. Our data suggest that sedation practices may also be relevant to long-term outcomes. The use of benzodiazepines in particular may be detrimental, whereas dexmedetomidine, an alpha-2-agonist, may be protective. The latter has shown, in appropriately selected patients, to allow more controlled sedation, obviating the need of prolonged administration of opioids and benzodiazepines [34, 35]. This may have the potential to reduce the duration of mechanical ventilation and ICU stay [36], as well as to reduce the incidence of delirium [37-39].

A more restrictive sedation policy naturally extends to the use of neuromuscular blocking agents. In the ICU, NMBAs are used for various indications [40, 41], including facilitation of tracheal intubation [42, 43], optimisation of mechanical ventilation and oxygenation in acute respiratory disorders, prevention of shivering during targeted temperature management after cardiac arrest, management of increased intracranial pressure [44, 45], and management of increased intra-abdominal pressure. Evidence base for most indications is largely historical, often limited to intermediate end-points with conflicting data regarding effects on ICU-mortality and morbidity [40, 42, 43]. Additionally, continuous infusions of NMBAs possibly increase the incidence of ICUAW. Recommendations of the Society of Critical Care Medicine hence refer to NMBAs with reticence, in particular with respect to its prolonged use [41]. Although continuous NMBA infusions (48 hours) as adjunct in the management of ARDS have received support because of effects on oxygenation and lung injury, their routine use is increasingly contested by lack of a consistent morbidity or mortality benefit [46, 47]. Effects on long-term outcome are unclear but relevant given the high burden of long-term physical disability in this patient population [25, 48]. Evaluation of long-term outcome effects of NMBAs has received most attention in the context of targeted temperature management (TTM) after cardiac arrest. Whereas routine continuous infusion appears to hold no benefit [49, 50], observational data suggest possible neurological benefit after a TTM-strategy applying NMBA boluses as needed to prevent shivering when compared to a sedation escalation strategy, possibly through a sedation-sparing effect of NMBAs [51]. However, severity of post-anoxemic brain injury may have confounded these results, and it is notable that the American Heart Association guidelines on post-cardiac arrest care make no explicit recommendation with respect to NMBA use during TTM except from noting on the risk of continuous paralysis masking seizures [52]. Our finding of a possible long-term mortality detriment underscores the importance of careful consideration of NMBA use across its range of widely differing indications, and need of a more solid evidence base to guide these treatment choices.

The association of corticosteroids with poor long-term outcomes observed in our cohort of general ICU-patients adds another dimension to the debate concerning their in-ICU use. Systemic corticosteroid administration is life-saving in patients with known adrenal insufficiency experiencing severe illness, as well as in fulminant initial presentations or exacerbations of inflammation-mediated conditions including vasculitis [53, 54], moderate to severe exacerbations of chronic obstructive pulmonary disorder (COPD) and of asthma [55, 56]. Outside these indications, corticosteroids have been extensively investigated in critical illness for exploitation of their pleiotropic effects on inflammation, fluid balance, and vasomotor tone. Whereas high-dose schemes have been abandoned due to harm [57], equipoise remains on the appropriateness of lower-dose steroid regimes. In the context of septic shock, the latest European guidelines on sepsis management – dating from 2016 – proposed the use of low-dose (200 mg/day) hydrocortisone continuous infusion in case fluid resuscitation and vasopressor therapy do not restore hemodynamic stability, based on available evidence at the time [55, 58-60]. Two more recently published randomised controlled trials confirmed that hydrocortisone administration results in faster hemodynamic stabilisation [59, 61, 62] and furthermore indicated earlier liberation from mechanical ventilation [61, 62]. However, effects on survival were inconsistent, possibly dependent on adjunctive therapies and patient characteristics [61-63]. Additionally, long-term outcomes of these patients have not been reported.
In the acute respiratory distress syndrome (ARDS), timing, duration and modality of corticosteroid treatment has been given ample consideration for its possible disease modifying effects [64]. In absence of septic shock, current guidelines from the Society of Critical Care Medicine suggest consideration of low dose methylprednisolone in early moderate to severe ARDS within 14 days of onset [55] given reduced duration of mechanical ventilation [65-67]. These beneficial effects were supported by a recent RCT showing reduced duration of mechanical ventilation and 60-day mortality with low dose corticosteroids in early ARDS [65]. However, there may be a price to pay for these short-term benefits, and longer-term term outcomes need to be integrated in future trial designs.

THE LEGACY OF ICU-ACQUIRED NEUROMUSCULAR DYSFUNCTIONS

In chapters 4 and 5, we considered the hypothesis that ICU-acquired neuromuscular dysfunctions could further mortgage long-term outcome when taking into account the confounding effect of other prognostic factors, including ICU length-of-stay. Scrutinised subgroup analyses provided compelling results.

In chapter 4, we first analysed MRC-data at or closest to ICU-discharge in relation to long-term outcome. With respect to 5-year mortality, a more pronounced loss of strength at ICU-discharge – expressed by a point-wise decrease in MRC-sum score – was independently associated with worse outcomes, an effect that appeared to dissipate over time [68], consistent with earlier findings [69]. However, in an exploratory analysis, prognostic stratification using a threshold for MRC at a score of 55 better captured the data at hand, in particular when evaluating 5-year mortality. In fact, the 5-year mortality hazard of a patient with an MRC of 55 or lower was approximately 60% higher than the hazard of a patient with an MRC higher than 55, adjusted for confounders. In 205 long-term ICU-survivors, MRC at ICU-discharge was significantly associated with all morbidity measures at follow-up, and we established an independent association between MRC at ICU discharge and hand-grip-strength, six-minute-walk-distance, and the physical function sub-score of the 36-item short form health survey. For long-term strength and physical function, again, stratification of the outcome at 5-years was possible by an in-ICU MRC cut-off of 55. This MRC-threshold is considerably higher than the cut-off of 48, which has been validated for predicting short-term outcomes of critically ill patients [70, 71]. Consequently, this suggests that even slight reductions in muscle strength may be associated with an increased risk of compromised long-term outcomes.

Apart from muscle strength prior to ICU-discharge, systematically performed screening electrophysiological evaluation also provided long-term prognostic information. In the studied patient

cohort, an abnormal response to motor nerve stimulation 1 week after ICU admission, defined as a compound muscle action potential (CMAP) below the limit of normal in both an upper and lower limb motor nerve, was independently associated with 5-year mortality [68]. Given that a substantial number of critically ill patients are unable to perform an MRC-assessment due to insufficient consciousness and cooperation, in this setting, CMAP can provide insight into long-term mortality prospects. When taking into account the newly defined threshold of MRC at 55, CMAP as well as MRC were independently associated with an increased 5-year mortality hazard in patients who received both evaluations. This indicates that electrophysiological data also provide additional information to strength assessment with respect to 5-year mortality.

Interestingly, and in contrast to data on strength at ICU-discharge, abnormal CMAP was not independently associated with long-term morbidity. Although selection bias could play a role, an abnormal electrophysiological assessment may capture different aspects or pathophysiological pathways of neuromuscular involvements as compared to the strength assessment. Also, we chose to study results of CMAP on day 8 in relation to long-term outcomes, based on previous findings identifying this parameter as a crucial factor for 1-year outcomes [72]. Hence, we cannot exclude that other characteristics of the electrophysiological profile or different timing would yield important information on long-term functional status. Of particular interest would be to study the association of neuropathic and myopathic profiles with long-term outcome, as for the short-term a more favourable outcome has been described for myopathy as compared to neuropathy [73-76]. However, prospective follow-up of large patient cohorts in which repeated and extensive electrophysiological profiling is performed is expected to be very time-consuming. If associated with outcomes, translation into daily practice would rely on the extent to which electrophysiological subtypes can be linked to actionable targets affecting the patients' medical course.

In chapter 5, we addressed the question whether respiratory muscle weakness (RMW), which frequently co-occurs with peripheral muscle weakness, has an independent long-term prognostic impact, independent from limb weakness. To this end, we studied the subgroup of EPaNIC-follow-up patients weaned from, or not receiving respiratory support, and assessed for both peripheral and respiratory muscle strength in the ICU. Assessment of respiratory muscle strength was limited to the evaluation of inspiratory muscle strength. The resulting "better subset" selection is illustrated by the lower MRC scores in the total MRC population as compared to the subgroup evaluated in this sub-study, and the longer duration of mechanical ventilation and ICU stay in the former. Nonetheless, the prevalence of RMW at, or closest to ICU-discharge was still considerably high (37.2%), and in those affected, respiratory muscle strength at follow-up was significantly lower than in patients without RMW in the ICU. Furthermore, RMW at ICU discharge was associated with lower peripheral muscle strength, physical function and quality of life up to five years post-ICU, independent from ICU-acquired peripheral muscle weakness and other confounders [77].

Reduced inspiratory muscle strength relative to a healthy reference has been described after a protracted weaning process [78] and up to 2 years post-ICU in ARDS-patients [17, 48]. However, as in the absence of ICUAW resolving trajectories of respiratory muscle strength were described [48], and no consistent associations with longer-term outcome were found [79, 80], screening for inspiratory muscle strength at ICU-discharge appeared to be no priority. Our data indicate that RMW prior to ICU discharge per se, however, does carry additional prognostic information for long-term outcomes, further supporting recent concepts describing respiratory weakness in critically ill patients as a distinct entity, though overlapping with peripheral weakness. Mechanisms explaining the link between RMW and functional impairments in the long-term are still unclear. Inspiratory muscle weakness may affect measures of limb muscle performance, exercise capacity, and ultimately quality of life, through the respiratory muscle metaboreflex, a sympathetic vasoconstrictor response induced by fatiguing inspiratory muscle contractions that redistributes blood flow from peripheral to respiratory muscles [81, 82]. This mechanism was shown to be relevant – at both strenuous and submaximal exercise effort – and reversible by inspiratory muscle training in other clinical conditions [81, 83-87]. Although the

importance of this physiological response in survivors of critical illness has not been studied, it is an attractive hypothetical framework to consider. The plausible value of inspiratory muscle training in ameliorating long-term outcome may thus be in need of reappraisal, as it certainly is feasible and safe in ICU-patients but, given lack of data on long-term benefit [88], currently is mostly reserved to difficult weaning settings [89]. Importantly, as respiratory muscle strength training in other populations, such as heart failure and COPD, is effective in improving muscle strength as well as functional clinical outcomes it seems an attractive intervention to improve outcomes also in ICU survivors [85, 87, 90-92].

Collectively, these results support the concept that the various components of the spectrum of ICU-acquired neuromuscular dysfunctions occurring as complications of critical illness have distinct prognostic value with respect to patient's long-term outcomes. Manual assessment of limb muscle strength, motor nerve conduction studies and maximal inspiratory pressure measurement indeed provide complementary information. Whether they can help to strategise rehabilitation programs to ameliorate long-term functional outcome deserves further investigation.

THE LEGACY OF MULTIPLE ORGAN FAILURE

In chapter 3-5, we demonstrated reduced strength and physical function 5 years following ICU stay and identified several factors that independently associate with these outcomes. However, these assessments of physical function, and other static evaluations such as pulmonary function tests, insufficiently capture physical fitness, a largely uncharted field in ICU survivors. Therefore, in chapter 6, we additionally studied cardiorespiratory fitness in ICU survivors with use of cardiopulmonary exercise testing, being the gold standard for assessment of exercise capacity, and aimed to identify exercise limiting factors, as well as to study the relation between exercise capacity and in-ICU severity of organ failure.

In 361 5-year survivors who were medically stable and physically able to perform strenuous exercise, we found that aerobic fitness was impaired, relative to healthy controls. We observed a striking heterogeneity in exercise response, with abnormal aerobic exercise capacity being present in 37.7% of patients. As the studied population fit and able to perform cardiopulmonary exercise testing represents the better subgroup within the ICU survivors, this number is probably an underestimation of the actual extent of this problem. In approximately 60% of these patients, a primary muscular limitation of exercise was presumed. We further demonstrated that throughout a follow-up period of 1 to 5 years following ICU admission, severity of organ failure during critical illness independently associated with maximal oxygen consumption in a cohort of 433 patients. Interestingly, we found no difference in exercise capacity between the intermediate time points and the 5-year follow up point. Given the intimate relationship between severity of organ failure throughout the ICU-course and the development of ICUAW, and the association between ICUAW and impaired long-term physical function which we documented in chapter 4, we further explored whether persisting weakness would explain the effect of the severity of organ failure on aerobic exercise capacity. We found that lower limb strength independently associated with aerobic exercise capacity, but did not statistically explain the effect of severity of organ failure. This suggests that the impact of severity of organ failure on exercise capacity may involve other mechanisms that require further study. Possible candidate mechanisms may involve a lasting disruption of whole body homeostasis, with persisting metabolic [93] and microcirculatory [94] alterations.

Our data may offer valuable prospects with regard to the rehabilitation process in ICU survivors, which up to date has rather been disappointing [95-98]. First, our data suggest that, similar to other patient populations [99-106], cardiopulmonary exercise testing may provide a valuable tool to prescribe individualized rehabilitation programs and to monitor progress. Muscular limitation including deconditioning was an important contributor to exercise limitation in our population. In heart failure and COPD, it is recognised that deconditioning facilitates the negative spiral of muscular compromise

imposed by the primary disease and associated treatments, and that rehabilitation can break the perpetuation of this trend [107, 108]. Second, our data also provided insights into the possible window of opportunity for rehabilitation programs in ICU survivors. Based on 3 small studies performed in the first weeks up to 3 months following ICU stay reporting worse exercise capacity than in our cohort [109-111], with values that were not different between intermediate time points and 5-year evaluation, some degree of recovery may occur within the first year, which possibly could be optimised by tailored rehabilitation. We cannot exclude that further benefit may be obtained also after 1 year, as our cohort was not subjected to systematic rehabilitation programs.

In chapters 4-6, we focused in particular on the physical aspects of the post-intensive care syndrome. However, survivors of critical illness often suffer from limitations in other domains including mental health and cognitive function. Typical problems include anxiety, post-traumatic stress disorder and depression, disabilities involving executive function, memory, attention, visuo-spatial and mental processing speed all interacting in complex ways [112-116]. Extensive evaluation of all aspects of the post-intensive care syndrome unfortunately was not feasible in this research setting. However, when approaching individual patients, a holistic view considering all interrelated domains is crucial.

Another consideration involves the selection of confounders for adjusting the analyses. According to recent guidelines on causal inference studies [117], we performed systematic literature searches to identify possible confounders as to optimally adjust our explanatory analyses. However, as for any observational study, we cannot exclude residual bias through unmeasured confounding. Importantly, occurrence of delirium, which is a confounder for outcomes, was not systematically assessed during the EPaNIC study.

Delirium, a fluctuating state of altered consciousness, attention and cognition, which occurs in up to 40% of critically ill patients, but affects up to 80% of those sedated and mechanically ventilated [118, 119]. Although several non-modifiable factors – including age, baseline cognitive impairment, comorbidities and severity of illness – stratify a patient's risk of delirium, exposure to sedatives, in particular benzodiazepines and opioids, is a notorious precipitating factor [120, 121]. Delirium is associated with increased ICU-morbidity, including prolonged duration of mechanical ventilation, and ICU- and hospital stay, and with increased ICU-mortality [119, 122-124]. Furthermore, delirium is an independent predictor of long-term cognitive impairment and mortality [114, 125-129]. This possibly explains the association between sedative use, notably benzodiazepines, and long-term adverse outcome suggested by our analysis and other research as well [31].

CRITICAL ILLNESS DUE TO COVID-19: THE WORST IS YET TO COME?

As second objective of this PhD-project, in chapter 7 we documented the incidence, risk factors, and ICU- and hospital outcomes of ICUAW in a retrospective cohort study of 50 mechanically ventilated COVID-19 patients assessed for peripheral muscle strength. The context of the pandemic precluded the documentation of electrophysiological profiles and respiratory muscle strength in our cohort.

In this cohort, 72% of patients presented with ICUAW at awakening. This was much higher as compared to general ICU cohorts reporting incidences of ICUAW upon awakening, ranging between 25% and 55% [70, 130-132]. The incidence of ICUAW had dropped to 52% at ICU discharge, which is still very high as compared to historic ARDS cohorts, reporting rates around 35% [48, 69, 133]. Our patients were exposed to exceptionally prolonged sedation in combination with neuromuscular blocking agents to facilitate lung-protective mechanical ventilation [134] as well as to prolonged use of corticosteroids. These clinical features, in particular prolonged mechanical ventilation, as well as the challenging sedation and high use of NMBAs in COVID-19 patients, are quite similar to those reported in other COVID-19 ICU cohorts [135-139]. Markedly, doses of sedatives applied on treatment days were not different between patients with and without ICUAW, and well within ranges typically required to counter ventilator dyssynchrony [134]. We expectedly found that these factors, being markers of the

duration of immobilization, strongly associated with the presence of ICUAW and potentially explain the high incidence reported. These high incidences occurred despite glycemic control that appeared to be better as compared to the historical ARDS cohorts. Other differences between our ICU COVID-19 patients and classical ARDS patients involved a relatively high age, high proportion of males and high comorbidity index. However, these factors appeared not to be crucial in determining the risk for ICUAW in our ICU COVID-19 cohort, as none of these factors was found associated with the presence or absence of ICUAW in this population.

Strikingly, at hospital discharge, strength had further recovered with persisting weakness being present in 27%. This stands in contrast to data from historic ARDS-cohorts, where the incidence of ICUAW at hospital discharge ranged between 36%-39% [48, 69, 133]. These encouraging findings suggest a substantial recovery potential in COVID-19 patients, possibly facilitated by a rapid mobilisation strategy from the time of awakening onwards in our cohort. Notwithstanding this favourable evolution of strength, the functional impact of ICUAW was impressive, as illustrated by the ICU mobility scale, Barthel index and high need for in-patient rehabilitation. Our data on the high incidence of ICUAW have been confirmed in later cohorts of critically ill COVID-19 patients [140-142]. These observations are particularly concerning as in the previous chapters we demonstrated that even slightly reduced strength at ICU discharge independently associates with poor strength, physical function and quality-of-life 5 years following ICU admission [143] and may affect mortality up to 5 years following ICU stay. Given the high disease-burden of COVD-19, with over 125 million people infected worldwide of whom an estimated 5% [144] progressing to ARDS-like disease, this should be considered a global health care priority [145]. This should urge clinicians and the scientific community to carefully follow these patients and offer tailored rehabilitation programs in order to reduce the anticipated long-term impact of the ICU stay [146]. Follow-up of this specific patient cohort is crucially important as, to date, the long-term optimal trade-off between severe wasting and weakness and strong suppression of respiratory drive to allow rigorous lung-protective mechanical ventilation and its facilitating treatments in COVID-19 patients remains unclear.

A note of optimism should be taken from the observation that patients in whom invasive mechanical ventilation could be avoided by use of high flow nasal oxygen, despite the presence of severe hypoxemia, appeared to have much better outcomes. As only few such patients were evaluated, these data need further confirmation.

MOLECULAR MECHANISMS OF IMPAIRED PHYSICAL FUNCTION IN SURVIVORS OF CRITICAL ILLNESS

Notwithstanding the importance of epidemiological knowledge on the contribution of ICU-associated exposures and complications to the post-intensive care syndrome, ultimately, development of strategies to ameliorate the impact of physical impairments in long-term ICU-survivors is of interest. As this problem may only be solved through increased mechanistic understanding, in chapter 8, we studied histological aspects and gene expression alterations implicated in ICUAW for a candidate mediator role in the long-term legacy of critical illness.

Up to present, evidence was limited to a longitudinal sample of 10 survivors of prolonged mechanical ventilation, aiming to describe mediators of differential functional outcome of ICUAW. Molecular and morphological analyses suggested that structural protein homeostasis, autophagy, mitochondrial biogenesis and inflammation – associated with early weakness – were restored by 6 months post-ICU, whereas pathways involved in regeneration, extracellular matrix production and calcium signalling associated with the strength deficit documented at 6 months post-ICU [147, 148]. Atrophy per se, however, did not correlate with strength at 6 months [147]. Supposedly, differential (persistence of) impairment of factors involved in muscle force generation versus muscle mass regulation could explain these inconsistencies.

Insight and generalisability of these findings remained limited in view of the very small sample size and consequently low statistical power, selection of patients who were mechanically ventilated for at least one week, and the relatively short follow-up of the patients up to 6 months after ICU admission. We therefore studied a much larger cohort of survivors who also experienced a more diverse range of causes for critical illness at a longer-term follow-up 5 years after ICU admission. In ICU measures of strength of our sample were unfortunately largely unknown. The documented ranges of strength at follow-up were significantly lower as compared to healthy demographically matched controls. As compared to the cohort reported by Dos Santos and Walsh et al. [148], mean quadriceps peak torque was higher in our total patient cohort, but similar in the subgroup of patients with a quadriceps peak torque less than 80% of its predicted value.

Targeted gene expression analysis for myofibrillary proteins, markers of atrophy, denervation, and myogenesis/regeneration showed no differential expression patterns between patients and healthy controls overall. Given the possibility of a dose-response effect, we compared those with reduced strength and those with normal strength at follow-up, but apart from myogenin expression, none of the gene products were significantly different.

Our group previously documented a lower proportion of type I myofibres in muscle biopsies obtained on day 8 in ICU as compared with demographically matched controls [149]. Immunohistochemical staining suggested that the proportion of type I myofibres may remain relatively lower 5 years later [44.8 (33.3-56.1) %] as compared to that in controls [51.4 (41.9-64.8) %], although the difference was not statistically significant, possibly due to insufficient statistical power. It may nonetheless be clinically relevant, as previous studies in highly sedentary healthy subjects revealed similar type I myofibre proportions (41%) in vastus lateralis biopsies, correlating with a VO_2max range (20-25 ml/min/kg) close to the one observed in our cohort. In contrast, the percentage of type I myofibres in vastus lateralis of very active healthy subjects ranges between 60–65%, even in the age range of our population. As type I myofibres are energetically more efficient to perform work, a lower proportion of type I myofibres may affect the ability to perform (endured) muscular work [150, 151]. We additionally found a reduction in type II myofibres. We did not verify capillary density, a factor closely linked with myofibre trophic state and muscular plasticity: capillary rarefaction may accompany myofibre size reduction, which in turn may impair oxygen and nutrient delivery thereby further compromising muscle function [153-155]. Given possible implications with respect to ease of rehabilitation, this may be an interesting target to consider for further research.

The myofibre proportion alterations and smaller size of type II myofibres observed in our population may be related to muscle disuse and deconditioning, factors that certainly can contribute to long-term dysfunction in ICU-survivors [152]. Other findings are less likely to be explained by deconditioning, including irregular myofibre shape – possibly a sign of denervation –, endomysial fibrosis [156], and a persisting inflammatory infiltrate, not reported previously. It is noteworthy that several inflammatory molecules, including TNF-α[157, 158], have been implicated in the pathogenesis of critical illness neuromyopathy [159, 160], and play a role in models of axonal neuropathy as well as in steroid myopathy and cancer, which affect type II myofibre size [152]. Additionally, as exercise rehabilitation has been shown to reduce inflammatory signalling, these aspects deserve further investigation [161]. The (post)transcriptional pathways involved in inflammation and excitation-contraction coupling were not explored as they did not comprise prespecified targets. However, given signs of ongoing inflammation in light microscopy, dysregulation of inflammatory signalling may be involved. It is unclear why mechanisms involving inflammation may persist after critical illness has subsided. One possible explanation – not unsimilar to that in several myopathic conditions – is a self-perpetuating state of oxidative stress [162-165]. A major natural source of redox stress are mitochondria, and mitochondrial dysfunction is associated with both severe organ failure [93], and with the development of ICUAW [166, 167]. Notably, severity of organ failure has been linked to impaired aerobic capacity at long-term follow-up as well (chapter 6). As gene ontology enrichment was found for mitochondrial

related genes in early modules of ICUAW, and calcium signalling in modules of persisting weakness [148], persisting mitochondrial redox leakage evoking inflammatory cell attraction, as well as dysregulation of contractile apparatus calcium sensitivity and sarcoplasmatic reticulum calcium release, is a possible pathophysiological pathway which could translate into observations of inflammation, impaired myogenesis/regeneration, and weakness [165, 168, 169]. Plausible mediators in health [157, 170] and disease related to oxidative metabolism [171], redox signalling, and inflammation [172-174], notably peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1)-molecules [175-178] and their upstream regulators [179], as well as TNF-α and NF-κB [158, 180], are worth additional investigation.

FUTURE PERSPECTIVES AND CONCLUDING REMARKS

From a clinical point of view, the aforementioned findings hold value in raising awareness on the double-edged sword that certain ICU-related treatments present, and on clinical traits predicting unfavourable outcome in both the short- and long-term. As “time is muscle”, critical care clinicians should consciously engage in evaluating what treatments impede or facilitate reduction of the “ventilator-to-door time”.

Also, the knowledge obtained in this work could enable ICU-clinicians to better inform patients, relatives and primary care physicians on the presumed functional trajectory post-ICU [181]. This may facilitate discussions that allow the incorporation of patients’ values and preferences into decisions about goals of care during the ICU-stay [182, 183]. While awaiting more effective interventions both in the ICU and in the post-ICU setting, creating realistic expectations may create opportunities for psychological adaptation, allowing a meaningful redefinition of quality of life for both patients and their families [23, 184, 185].

From a research perspective, this work highlights the need for critical evaluation of the window of opportunity for multimodal interventions tackling all aspects of ICU-acquired morbidities through randomised controlled trials. Apart from the early in-ICU management [149, 186, 187], consideration should be given to continued engagement of affected or at-risk patients in multidimensional rehabilitation programs. The aforementioned novel insights may help in selecting patients at higher risk of adverse long-term outcomes to post-ICU follow-up clinics and to clinical trials specifically aiming to reduce the long-term burden. In particular, patients with prolonged ICU stay, those developing neuromuscular complications and those who suffered severe organ dysfunction may represent an interesting target population. Furthermore, we identified cardiopulmonary exercise testing as a possible tool to individualize rehabilitation programs.

From a more global perspective, results from the EPaNIC follow-up sub-studies each contribute to increased understanding of and raising awareness on the physical aspects of the post-intensive care syndrome. Together with our documentation on the short-term morbidities in critically ill COVID-19 patients, these data should alert clinicians and researchers on the realistic threat of a long-term health detriment in survivors of severe COVID-19. Fortunately, our data have indeed contributed to the call for action by the World Health Organisation, to alleviate the plausible individual, economical and societal aftermath of the COVID-19 pandemic [145]. Indeed, advocacy of early instalment of follow-up services will enable the study of functional trajectories in an impressive number of patients worldwide. Given the numerous collaborative efforts initiated during the pandemic with unprecedented focus on homogeneity in reporting of outcome targets [188, 189], this undoubtedly will help unravel mediators and moderators of the PICS.

Clearly, further mechanistic understanding of the temporal patterns and molecular mechanisms explaining (and possibly distinguishing) neuromuscular disabilities in the ICU and in long-term ICU-survivors, which currently unfortunately remain largely unresolved, will be important. Several

hypothetical mechanisms, including metabolic responsiveness and calcium signalling, especially if evaluated at single-cell resolution, hold promise in clarifying these remaining questions.

In conclusion, survivors of prolonged critical illness, those who experienced neuromuscular complications including limb and inspiratory muscle weakness and electrophysiological abnormalities, or severe organ failure, appear to be at particular risk for physical and functional sequellae. Suboptimal muscle health is an increasingly recognised independent determinant of long-term health in various clinical settings, and this work strengthens that similar reasoning may apply to long-term ICU-survivors. Latent mediators resulting in the clinical phenotype of reduced physical function remain incompletely understood. Hypothetically, lasting disruptions of muscle and whole-body resilience to homeostatic challenges in ICU-survivors could account for the observation that even slight reductions of MRC-sum score at ICU-discharge are associated with lasting mortality and morbidity detriments, and that in-ICU organ failure severity predicts long-term aerobic exercise capacity. Future basic science ideally should target (cell-specific) molecular signatures discriminating persisting and resolving trajectories of weakness post-ICU in larger cohorts. Clinical research should evaluate the timing and value of individualized rehabilitation programmes for ICU-survivors. The search for strategies to prevent ICU-related complications amounting to the post-intensive care syndrome remains vital. Collectively, these efforts will hopefully result in improvement of the long-term prospects of ICU-survivors. The expected individual and societal burden of the post-COVID-19 period certainly serves as incentive to bring research aimed at resolving these remaining questions to the forefront.

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Summary

Patients who experience life-threatening illnesses – including major trauma, surgery, medical illness, and severe burns – are cared for at intensive care units (ICUs). Advances in critical care have decreased acute mortality, but in parallel, the proportion of patients requiring prolonged intensive care to overcome vital organ dysfunction has increased. Patients surviving critical illness unfortunately continue to face increased mortality relative to healthy peers, and suffer from a range of new or worsened neurocognitive, psychiatric and physical impairments that extend well beyond the hospitalisation phase up to years following ICU-admission. This so-called 'legacy' of critical illness interferes with activities of daily life, and is associated with reduced quality of life and return to participation in the society .

It is unclear to what extent this excess mortality and morbidity burden results from a patient's premorbid health, or is attributable to the duration of the ICU stay, and to the exposures and complications occurring in the ICU. Indeed, during their ICU-stay, patients frequently develop complications related to the severity of their illness, their premorbid health, and to the supportive treatments required to avert death. Neuromuscular complications involving both respiratory and limb muscle function are frequent. They can be diagnosed in cooperative ICU patients by bed-side assessment of limb strength or respiratory muscle strength, or through electrophysiological testing if patients are unconscious. In the short-term, weakness of the limbs – labelled "intensive care unit-acquired weakness (ICUAW)" – as well as electrophysiological abnormalities are associated with delayed liberation from mechanical ventilation, increased ICU- and hospital- length-of-stay, and increased ICU-mortality. Respiratory muscle weakness in particular associates with delayed weaning from mechanical ventilation and with ICU mortality. The impact of these complications on long-term outcomes however remains unclear.

In this PhD-project, we aimed to assess whether the long-term limitations in ICU survivors are actually related to the duration of ICU stay, and to ICU exposures and complications. This was addressed through several studies in 2 patient cohorts.

In a large cohort of former ICU patients, we investigated the relationship between the ICU-trajectory of critically ill patients and their long-term health outcomes. These outcomes included 5-year mortality and 5-year morbidity, assessed with handgrip strength, distance walked in 6 minutes and physical function related quality-of-life assessment. In a first study, we demonstrated that both 5-year mortality and morbidity were higher among patients with an ICU stay of at least 8 days ('long-stayers') as compared to those with an ICU stay less than 8 days ('short-stayers'). This difference was supplementary to any baseline pre-morbid vulnerability for ICU admission, and to the type and severity of illness necessitating critical care. We further identified a number of ICU-related risk factors, of which some may be amendable to intervention. Hence, part of the so-called 'legacy' of critical illness may be preventable.

In a second study, we showed that also neuromuscular complications of critical illness further mortgage long-term outcome, independently from ICU-length of stay. Lower muscle strength at discharge from the ICU independently related with worse 5-year mortality and morbidity. Strikingly, this was the case even if strength was only mildly reduced. On the other hand, we demonstrated that abnormal motor nerve conduction studies after 1 week of intensive care also independently associated with worse 5-year mortality but not morbidity. These data underline that evaluation of muscle strength and electrophysiological alterations carry complementary information, and support a role of neuromuscular complications in the long-term outcomes of critically ill patients. This information may assist in prognostication and selecting of patients who may benefit from post-ICU follow-up services.

In a third study, we additionally demonstrated that respiratory muscle weakness at discharge from the ICU, assessed by maximal pressure generated during a forced inspiratory manoeuver associates with 5-year morbidity but not mortality, independent from duration of ICU stay and limb muscle strength. These findings support the theory that peripheral and respiratory muscle weakness are separate, though overlapping entities and suggest that inspiratory muscle training may have potential to improve outcomes in ICU survivors.

In a fourth study, we addressed the subset of ICU survivors from the same cohort in whom strenuous exercise could be safely performed. These patients were additionally evaluated with cardiopulmonary exercise testing – the gold standard to assess cardiorespiratory fitness – at the 5-year follow-up time point, as well as at intermediate (annual) time points starting from year 1 post-ICU. We demonstrated that ICU survivors performed worse than healthy controls. Exercise capacity appeared frequently limited by muscular factors, and the severity of organ failure in the ICU independently associated with exercise limitations. This suggests that, similar to other diseases, cardiopulmonary exercise testing may be useful to individualise rehabilitation programs in ICU survivors. As no change in aerobic exercise capacity in the studied post-1 year follow-up period was observed, the first year post-ICU may represent the window of opportunity for intervention.

We performed a fifth study in a cohort of critically ill COVID-19 patients. As this new disease caused by SARS-CoV-2 virus overwhelmed ICUs worldwide, with particular patient profiles and disease characteristics, we studied the incidence, risk factors and short-term outcomes of ICU-acquired weakness in this population. We demonstrated high survival but also a very high incidence of weakness upon awakening in these patients. Potential factors related to this high incidence of weakness include high requirements for sedation and paralysing agents to suppress the respiratory drive, frequent administration of corticosteroids, and mechanical ventilation for particularly long duration. Although strength improved throughout the hospitalisation, functional impact at discharge remained substantial. Given our previous findings, these patients may be at high risk for long-term sequellae.

In a final study, we performed a targeted analysis of abnormalities in muscle morphology and a number of pathways potentially involved in reduced strength 5 years after critical illness as compared to healthy controls. Several morphological abnormalities were observed in muscle of ICU-survivors 5 years after critical illness relative to controls, including abnormal myofibre shape, endomysial fibrosis and inflammatory infiltration, and a shift towards smaller type II myofibres. However, these changes did not significantly associate with strength. Investigated molecular markers of myofibrillary protein synthesis and breakdown, of neural signaling/denervation, and of myogenesis/muscle regeneration were comparable for patients at 5-year follow-up and controls, with longitudinal within-patient evaluation suggesting recovery of acute in-ICU changes in these markers. Except for a higher myogenin gene expression, none of the investigated markers were significantly different for patients with or without reduced strength at follow-up. This suggests that other mechanisms may be involved, although selection bias and insufficient power cannot be excluded. To explain reduced strength at 5 years, further research thus remains necessary.

In conclusion, our findings suggest that a prolonged ICU stay, ICU-acquired peripheral and respiratory neuromuscular complications of critical illness and the severity of organ failure associate with 5-year mortality and morbidity. Consequently, COVID-19 ICU-survivors, in whom we demonstrated a high incidence of weakness, may be at high risk for such long-term impairments. Unfortunately, molecular and histological analyses of muscle tissue left many questions with respect to the mechanisms explaining persisting weakness unanswered. Nonetheless, our data provide insights into potentially modifiable ICU factors with regard to long-term outcomes, and delineate the population which should

be targeted in future studies attempting to reduce the long-term burden of critical illness. Organized follow-up of post-ICU COVID-19 patients to offer tailored rehabilitation seems warranted.

Samenvatting

Patiënten met levensbedreigende aandoeningen – waaronder ernstig trauma, chirurgie, medische ziekte, infectie en ernstige brandwonden – worden verzorgd op intensieve zorgen eenheden (ICUs). Door de vooruitgang in kritieke zorg is de acute sterfte van orgaanfalen afgenomen. Tegelijkertijd is er echter een stijging in het aantal patiënten die nood hebben aan langdurige intensieve zorg om van vitale orgaandysfunctie te herstellen. Patiënten die verlengde kritieke ziekte overleven, blijven helaas geconfronteerd met een verhoogde mortaliteit in vergelijking met gezonde leeftijdsgenoten, en hebben te kampen met nieuwe of verergerde neurocognitieve, psychiatrische en fysieke beperkingen die persisteren tot jaren na het initiële intensieve zorgenverblijf. Dit zogenaamde post-intensive zorgensyndroom verstoort activiteiten van het dagelijks leven, vermindert levenskwaliteit en verhindert terugkeer naar normale maatschappelijk functioneren.

Het is onduidelijk in hoeverre deze toegenomen mortaliteit- en morbiditeitsbelasting het gevolg is van de premorbiede gezondheid van de patiënt, dan wel of deze toe te schrijven is aan de duur en de blootstellingen en complicaties van het intensieve zorgenverblijf. Tijdens hun opname op een intensieve zorgeneenheid ontwikkelen patiënten vaak complicaties die verband houden met de ernst van hun ziekte, met hun premorbiede gezondheid en met de orgaanondersteunende behandeling die ze krijgen. Neuromusculaire complicaties van skeletspieren – zowel ademhalings- als lidmaatspieren – zijn frequent. De diagnose kan gesteld worden door krachttesten van ledematen en ademhalingsspieren in coöperatieve patiënten, of met behulp van zenuwgeleidingsstudies en electromyografie in onbewuste patiënten. Op korte termijn zijn ICU-verworven zwakte en tekenen van abnormale neuromusculaire excitabiliteit geassocieerd met verlengde duur van mechanische ventilatie, verlengd verblijf op intensieve zorgen en in het ziekenhuis, en toegenomen mortaliteit op de intensieve zorgenafdeling. Respiratoire spierzwakte in het bijzonder is geassocieerd met verlengde duur van mechanische ventilatie en mortaliteit op de intensieve zorgenafdeling. De impact van deze complicaties op lange termijn is evenwel onduidelijk.

In dit PhD-project beoogden we na te gaan of de toegenomen morbiditeit en mortaliteit van patiënten na kritieke ziekte toe te schrijven zijn aan de duur van hun intensieve zorgenverblijf, aan blootstellingen tijdens hun opname, en aan opgelopen complicaties. We deden hiervoor beroep op verschillende substudies met betrekking tot 2 patiëntcohorten.

In een grote longitudinale cohorte van patiënten na een verblijf op intensieve zorgen onderzochten we de relatie tussen het intensieve zorgen traject van deze kritiek zieke patiënten en gezondheidsgerelateerde eindpunten op lange termijn. Deze eindpunten omvatten 5-jaar mortaliteit en 5-jaar morbiditeit, uitgedrukt als handknijpkracht, zes-minuten-wandel-afstand, en de fysieke functie deelscore van de 36-item gezondheidsvragenlijst (SF-36).

In een eerste studie toonden we aan dat zowel 5-jaar mortaliteit als 5-jaar morbiditeit hoger was in patiënten na een verlengd verblijf op intensieve zorgen (ten minste 8 dagen) in vergelijking met patiënten met een kort intensieve zorgenverblijf (minder dan 8 dagen). Dit verschil werd niet verklaard door en was onafhankelijk van pre-morbiede risicofactoren, en van type en ernst van kritieke ziekte bij admissie. We konden bijkomend een aantal risicofactoren gerelateerd aan intensieve zorg identificeren die mogelijk remedieerbaar zijn. Zodoende zou de ‘erfenis” van kritieke ziekte mogelijkerwijze gedeeltelijk voorkomen kunnen worden.

In een tweede studie toonden we vervolgens aan dat ook neuromusculaire complicaties van kritieke ziekte de lange-termijn prognose van patiënten bijkomend hypothekeren, onafhankelijk van de duur van het intensieve zorgenverblijf. Krachtsverlies vastgesteld op het moment van ontslag van een intensieve zorgeneenheid is onafhankelijk gerelateerd aan toegenomen 5-jaarsmortaliteit en –

morbiditeit. Deze relatie bleek relevant bij zelfs minimale krachtsreductie. Daarnaast toonden we aan dat een abnormale elektrofysiologische respons op zenuwgeleidingsonderzoeken na 1 week op een intensieve zorgenafdeling ook onafhankelijk geassocieerd is met toegenomen 5-jaars mortaliteit maar niet met morbiditeit. Deze gegevens onderstrepen het feit dat evaluatie van krachtsverlies en van elektrofysiologische veranderingen tijdens kritieke ziekte complementaire informatie aanleveren, en dat hun aanwezigheid ook met betrekking tot lange-termijn vooruitzichten prognostisch ongunstig is. Deze informatie kan bijdragen in prognosticatie en bij selectie van patiënten die baat zouden kunnen hebben bij multidisciplinaire opvolging na hun intensieve zorgenverblijf.

In een derde studie onderzochten we de relatie tussen respiratoire spierzwakte bij ontslag van een intensieve zorgeneenheid, gemeten door maximale negatieve druk gegenereerd tijdens een geforceerd inspiratie maneuver, en lange termijn prognose. We stelden vast dat respiratoire spierzwakte verworven tijdens kritieke ziekte geassocieerd is met toegenomen 5-jaar morbiditeit, onafhankelijk van duur van het intensieve zorgenverblijf en van perifere spierkracht. Er was geen onafhankelijk verband met 5-jaar mortaliteit. Deze bevindingen illustreren dat respiratoire en perifere spierzwakte verschillende entiteiten zijn met een zekere mate van overlap, en dat inspiratoire spierkrachttraining mogelijkerwijze de lange termijn prognose van kritiek zieke patiënten zou kunnen beïnvloeden.

In een vierde studie werd in de subgroep van deze cohorte van overlevenden van kritieke ziekte die in staat waren om op een veilige manier een piekinspanning te leveren, een cyclo-ergospirometrie – de gouden standaard voor inschatting van fysieke fitheid – uitgevoerd. Dit onderzoek werd verricht op het 5-year follow-up moment, evenals op de intermediaire (jaarlijkse) tijdspunten vanaf 1 jaar na het intensieve zorgenverblijf. In deze substudie toonden we aan dat patiënten na een intensieve zorgen verblijf een lagere aerobe inspanningscapaciteit hebben dan gezonde controles. De inspanningscapaciteit van patiënten bleek vaak gelimiteerd door musculaire factoren, en ernst van orgaanfalen tijdens kritieke ziekte was onafhankelijk geassocieerd met inspanningslimitatie. Deze data suggereren dat – naar analogie met andere ziektebeelden – overlevenden van kritieke ziekte baat zouden kunnen hebben bij een geïndividualiseerd revalidatieprogramma gebaseerd op resultaten van een cyclo-ergospirometrie. Aangezien we geen verandering in inspanningscapaciteit zagen tijdens de bestudeerde periode vanaf 1 jaar na intensieve zorgen zou het optimale therapeutische venster voor interventie zich in het eerste jaar na kritieke ziekte kunnen situeren.

We verrichtten een vijfde studie op een cohort van kritiek zieke patiënten door COVID-19. Deze nieuwe ziekte veroorzaakt door het SARS-CoV-2 virus heeft wereldwijd intensieve zorgeneenheden overspoeld met patiënten met acuut respiratoir falen. Gezien de unieke combinatie van patiëntprofielen, ziekte en behandelingskarakteristieken, besloten we de incidentie, risicofactoren en korte-termijn eindpunten van ICUAW in deze populatie te bestuderen. We noteerden hoge overleving maar ook een zeer hoge incidentie van spierzwakte bij het ontwaken op intensieve zorgen. Potentiële factoren gerelateerd aan deze hoge incidentie van zwakte zijn de noodzaak aan langdurige sedatie en neuromusculaire paralyse om de ademhalingsdrive van patiënten te onderdrukken, het frequent gebruik van corticosteroïeden, en verlengde duur van mechanische ventilatie. Hoewel kracht verbeterde doorheen de hospitalisatie was de functionele weerslag op het moment van ziekenhuisontslag indrukwekkend. Gezien onze voorgaande bevindingen hebben deze patiënten dus een belangrijk risico op lange termijn sekwellen.

In een laatste studie voerden we gericht onderzoek nar abnormale spiermorfologie en afwijkingen op genexpressieniveau die mogelijk betrokken zouden kunnen zijn in gereduceerde kracht 5 jaar na kritieke ziekte in vergelijking met gezonde controles. Verscheidene morfologische abnormaliteiten warden geobserveerd in spier van patiënten 5 jaar na kritieke ziekte in vergelijking met gezonde

controles, waaronder abnormale spiervezel morfologie, fibrose en ontsteking van het endomysium, en een shift naar kleinere type II spiervezels. Deze veranderingen waren echter niet geassocieerd met kracht. De onderzochte moleculaire markers van myofibrillaire eiwitsynthese en –afbraak, neurale signalisatie/denervatie, en myogenese/spierregeneratie waren vergelijkbaar voor patiënten bij 5 jaar follow-up evaluatie en controles. Longitudinale evaluatie binnen patiënten lijkt herstel van acute veranderingen tijdens kritieke ziekte te suggereren. Met uitzondering van een hogere myogenine genexpressie waren geen van de onderzochte merkers geassocieerd met verminderde kracht bij follow-up. Mogelijk zijn andere mechanismen betrokken, hoewel definitieve conclusies niet mogelijk lijken wegens selectie bias en mogelijk onvoldoende power. Om gereduceerde kracht op 5 jaar volledig te verklaren blijft verder onderzoek nodig.

Samenvattend suggereren onze bevindingen dat een verlengd verblijf op intensieve zorgen, perifere en respiratoire neuromusculaire complicaties verworven tijdens kritieke ziekte, en de ernst van orgaanfalen tijdens het intensieve zorgenverblijf, geassocieerd zijn met 5-jaar mortaliteit en morbiditeit. Bijgevolg lopen overlevenden van kritieke ziekte door COVID-19, bij wie we een hoge incidentie van zwakte aantoonden, potentieel risico op multipele beperkingen op lange termijn. Moleculaire en histologische analysen van spierweefsel van overlevenden van kritieke ziekte lieten helaas nog veel vragen onbeantwoord. Echter onze data bieden inzicht in potentieel modificeerbare factoren geassocieerd met het intensieve zorgenverblijf en met lange-termijn prognose. Bovendien helpen onze inzichten de populatie aflijnen die preferentiële doelgroep lijkt voor toekomstig onderzoek met als doel de lange-termijn erfenis van kritieke ziekte te reduceren. Georganiseerde opvolging van overlevenden van kritieke ziekte door COVID-19 met de mogelijkheid om aangepaste revalidatietrajecten aan te bieden, lijkt opportuun.

Scientific acknowledgments, personal contribution and conflict of interest

SCIENTIFIC ACKNOWLEDGEMENTS

I am indebted:

To all patients and controls for their participation in the studies;

To all intensive care unit staff of the University hospitals Leuven (chapter 3-8) and of the Jessa Hospital Hasselt (chapter 3) for their contribution to the studies and daily patient care;

To the funders of the scientific projects upon which this PhD-project was founded:

- the Fund for Scientific Research, Flanders Belgium: this work was funded by an aspirant PhD fellowship to myself: 1131618N, and additionally by a Fundamental Clinical Research fellowship to Prof. Dr. Greet Hermans: 1805116N (chapter 3-8), to Prof. Dr. Michaël Casaer: 1700111N (chapter 3-8), to Prof. Dr. Geert Meyfroidt: 1843118N (chapter 7),
- the clinical research fund (KOF) of the University Hospitals Leuven, Belgium: postdoctoral research fellowship to Prof. Dr. Jan Gunst (chapter 3-8),
- the Methusalem program of the Flemish Government [METH/14/06 via KU Leuven to Prof. Dr. Greet Van den Berghe (chapter 3-8),
- the European Research Council ERC Advanced grants: AdvG-2012-321670 from the Ideas Program of the EU FP7 and AdvG-2017-785809 from the Horizon 2020 Program of the EU to Prof. Dr. Greet Van den Berghe (chapter 3-8)

To Prof. Dr. Greet Van den Berghe, Prof. Dr. Greet Hermans, for concept and design of the studies and protocol writing, for data analysis, for critical appraisal of manuscript drafts, for obtained funding (chapter 3-8);

To Prof. Ilse Vanhorebeek, for concept of the studies and protocol writing, for data analysis, for critical appraisal of manuscript drafts, for obtained funding (chapter 8);

To Prof. Dr. Rik Gosselink, for concept and design of the studies, for his expert advice on the drafting of a classification protocol for ergospirometry analysis (chapter 7), and critical revision of manuscript drafts (chapter 3-7);

To Prof. Dr. Michael P. Casaer, Dr. Philippe Meersseman, Prof. Dr. Yves Debaveye, Prof. Dr. Alexander Wilmer, Prof. Dr. Jan Gunst, for data collection (chapter 3-8), for contribution to study design, for critical appraisal of manuscript drafts (chapter 3-5, 7), for obtained funding (chapter 3-8), for supervision of the clinical studies(chapter 3-8);

To Dr. Kaatje Goetschalckx, for her expert advice on the drafting of a classification protocol for ergospirometry analysis, and critical revision of the manuscript (chapter 7);

To Prof. Dr. Frans Bruyninckx (chapter 4) and Dr. Jasperina Dubois (chapter 3), for data collection and critical revision of manuscript drafts, to Prof. Dr. Joost Wauters for critical revision of manuscript drafts (chapter 5 and 7);

To all members of the COVID-19 consortium* for data collection and for critical appraisal of the manuscript draft (chapter 7);

To Helena Van Mechelen, Tine Vanhullebusch, Sanne Verweyen, and Tim Van Assche for acquisition of data, technical and administrative support (chapter 3-6, 8);

To Alexandra Hendrickx, Sylvia Van Hulle, Katleen Witpas, Heidi Utens, Liese Mebis, and Pieter J. Wouters (chapter 3-8), and to Bram Vercammen (chapter 7) for their technical and administrative support;

To Inge Derese and Francesca Mulazzani for performing RNA-isolation, cDNA preparation, RT-PCR analysis and logistic support (chapter 8), to Lies Pauwels for section preparation and staining (H&E and immunohistochemical) of muscle biopsies (chapter 8), to Chloë Goossens for her advice on quantitative analysis of IHC stained muscle biopsy sections (chapter 8), to Sarah Derde for her advice on and providing of a protocol for the qualitative analysis of H&E stained muscle biopsy sections (chapter 8), to Sarah Van Der Perre for her administrative support(chapter 8);

To Lucas Kreiss for writing the image analysis algorithm for Fiji (ImageJ) used for the analysis of the immunehistochemical stained muscle biopsies – type I/II myofibre typing (chapter 8);

To Steffen Fieuws (chapter 3-6), Martial Luyts (chapter 3) and Prof. Fabian Guizza (chapter 3), for their statistical advice;

To the entire staff of the Cardiology department of the University Hospitals Leuven, in particular to Dr. Kaatje Goetschalckx, Dr. Walter Droogné, Prof. Dr. Lucas Van Aelst, Dr. Tim Balthazar, Dr. Christophe Vandenbriele, Prof. Dr. Stefan Janssens, to enable me to perform both my clinical and research work.

PERSONAL CONTRIBUTION

Chapter 1-2, 9

Writing of the chapters, making of the figure

Chapter 3: Five-year mortality and morbidity impact of a prolonged ICU stay

Data collection: recruitment and clinical testing of the last members of the healthy control group for the EPaNIC-follow-up cohort (including the performance of muscle biopsies).
Data analysis: performing the statistical analyses
Writing of the manuscript
Figures and tables: figure 1 and 2 were adapted from the figures made by Prof. Dr Greet Hermans

Chapter 4: Five-year mortality and morbidity impact of ICU-acquired neuromuscular dysfunctions

Data collection: recruitment and clinical testing of the last members of the healthy control group for the EPaNIC-follow-up cohort (including the performance of muscle biopsies).
Data analysis: performing the statistical analyses
Writing of the manuscript
Making of figures and tables

Chapter 5: Five-year mortality and morbidity impact of respiratory muscle weakness

Data analysis: performing the statistical analyses
Writing of the manuscript

Making of figures and tables

Chapter 6: Aerobic exercise capacity of long-term ICU-survivors

Statistical planning: formulating of the research hypothesis, writing the statistical plan, writing the classification protocol for the qualitative analysis of ergospirometry data,
Data analysis: performing the statistical analyses, classification of the ergospirometry protocols according to the finalised classification protocol
Writing of the manuscript
Making of figures and tables

Chapter 7: ICUAW in critically ill COVID-19 patients

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Data analysis: statistical analyses
Writing of the manuscript
Figures and tables: figure 1 and 2 were adapted from the figures made by Prof. Dr. Greet Hermans

Chapter 8: Molecular aspects of weakness in long-term ICU survivors

Data collection: recruitment and clinical testing of the last members of the healthy control group for the EPaNIC-follow-up cohort (including the performance of muscle biopsies and strength assessments).
Wet lab work: RT-PCR: performance of RT-PCR analyses (MuRF, Atrogin, Achε and γ, FOXO1 and 3, HDAC4, Myf6, Myostatin, MyoD1)
Data analysis:

- IHC myofibre types: Taking pictures, preprocessing pictures and analysing pictures for type 1/2 myofibre staining.
- H&E analysis: qualitative analysis of H&E stainings based on an in-house protocol (courtesy of Sarah Derde). H&E staining of tissue sections was performed by an experienced laboratory technician (Lies Pauwels).
- Statistical analyses

Writing of the manuscript
Figures and tables: figure 1 and 2 were adapted from the figures made by Prof. Ilse Vanhorebeek

CONFLICT OF INTEREST

I have no conflict of interest to declare.

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Funding	Flemish fund of scientific research (FWO) Aspirant fellowship for the period 2017- 2019, which was renewed for the period 2019-2021

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Clinical course: BLS renewal (UZ Leuven)	01/2019 02/2021
Clinical course: Basics of radioprotectionǂ (KU Leuven)	04/11/2019
Doctoral course: VME in het labo (KU Leuven)	08/2017
Doctoral course: Biomedical writing skills for doctoral students (KU Leuven)	10/2017
Doctoral course: Biostatistics for doctoral students, basic courseǂ (KU Leuven)	11/2017
Doctoral course: Creating effective posters (KU Leuven)	12/2017
Doctoral course: Central Lecture Research Integrity (KU Leuven)	05/2018
Doctoral course: Writing for Medical Journals (KU Leuven) (Stuart Spencer, Editor of The Lancet))	05/2018
Doctoral course: Cochrane Belgium course for Flemish doctoral schools: How to write a protocol for systematic reviews and how to conduct SRs in the clinical context	06/2018
Doctoral course: Good clinical practiceǂ (KU Leuven)	09/2018
Doctoral course: Introduction to Next Generation Sequencing techniques (KU Leuven)	03/2019
Doctoral course: Good clinical practiceǂ (UZ Leuven)	09/2018
Doctoral course: Research integrity part II (KU Leuven)	12/2019
Doctoral course: Online Statistics courses: Data Science: R basics ǂ(Harvard by edx)	07/2019
Doctoral course: Creating effective presentation slides	03/2020

International training

Clinical course: Advanced Life Support - provider course† (European Resuscitation Council certified course for emergency care providers)	06/2018
Clinical course: ERS Virtual School on Clinical exercise testing - Core principles†	11/2020
Doctoral training: "Introduction to Bayesian Statistics using BUGS", biostatistics, MRC unit of the University of Cambridge, Cambridge, United Kingdom†	10/2019

PUBLICATIONS

Publications within the contents of the PhD-manuscript

First author paper, original research	• Van Aerde N, Meersseman P, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Bruyninckx F, Wouters PJ, Gosselink R, Van den Berghe G*, Hermans G*. Five-year impact of ICU-acquired neuromuscular complications: a prospective, observational study. Intensive Care Medicine, 2020, 46(6):1184-1193. • Van Aerde N, Meersseman P, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Wouters PJ, Gosselink R, Van den Berghe G*, Hermans G*. Five-year outcome of respiratory muscle weakness at intensive care unit discharge: secondary analysis of a prospective cohort study. Thorax, 2021 epub ahead of print, doi: http://dx.doi.org/10.1136/thoraxjnl-2020-216720.
First author paper, to the editor	• Van Aerde N, Wilmer A*, Gosselink R*, Van den Berghe G*, Hermans G* for the COVID-19 consortium. Intensive care unit acquired muscle weakness in COVID-19 patients. Intensive Care Medicine, 2020, 46(11), 2083–2085. • Van Aerde N, Van den Berghe G, Hermans G. Weakness in the ICU: the right weight on the right scale. Intensive Care Med, 2021, 47(1):137-138. • Van Aerde N, Van den Berghe G, Hermans G. Zwakte na kritieke ziekte door COVID-19: een last te zwaar om dragen? Ortho-Rheumato, 2020, 18(6):2.
Shared first author paper	• Hermans G*, Van Aerde N*, Meersseman P, Van Mechelen H, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Dubois J, Wouters P, Van den Berghe G. Five-year mortality and morbidity impact of prolonged versus brief ICU stay: a propensity score matched cohort study. Thorax, 2019, 74(11): 1037-1045.
Abstracts	• Van Aerde N, Hermans G, Meersseman P, Van Mechelen H, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Dubois J, Wouters P, Van den Berghe G. P469: Five-year mortality and morbidity impact of a prolonged versus a brief ICU stay: a propensity score matched cohort study, 39th International Symposium on Intensive Care and Emergency Medicine. Crit Care, 2019, 23, 72. • Van Aerde N, Meersseman P, Vlasselaers D, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Dubois J, Wouters PJ, Gosselink R, Van den Berghe G, Hermans G. P01-P061: Five-year outcomes in ECMO patients", Abstracts for the 8th EuroELSO Congress on ECMO-ECLS, 10 – 13 April 2019, Barcelona, Spain. Perfusion, 2019, 48:82-251. • Van Aerde N, Meersseman P, Debaveye Y, Wilmer A, Gunst J, Casaer MP, Bruyninckx F, Wouters PJ, Gosselink R, Van den Berghe G, Hermans G. P386: Five-year impact of ICU-acquired neuromuscular complications: a prospective, observational study", 40th International Symposium on Intensive Care & Emergency Medicine. Crit Care, 2020, 24, 87.

Publications not within the contents of the PhD-manuscript

Review	Van Aerde N, Dendale P. Cannabisgebruik: cardiovasculaire effecten. Tijdschrift voor Geneeskunde, 2014, 70(24): 1445-1450.
Co-authored paper	Kherad O et al. The challenge of implementing Less is More medicine: A European perspective. Eur J Intern Med, 2020, 76:1-7.
Abstracts	"Five-year outcomes in ECMO patients", 8th Euro-ELSO (European conference of the Extracorporeal Life Support Organisation), Barcelona, Spain, 2019
Book chapters	Van Aerde N, Van Dyck L, Vanhorebeek I, Van den Berghe G. Endocrinopathy of the Critically Ill. In: Post-Intensive Care Syndrome. Springer, Cham, 2020. p. 125-143.

PRESENTATIONS

Poster presentations	• "Five-year mortality and morbidity impact of a prolonged versus a brief ICU stay: a propensity score matched cohort study", 39rd ISICEM (international society of intensive care and emergency medicin), Brussel, België, 2019 • "Five-year outcomes in ECMO patients", 8th Euro-ELSO (European conference of the Extracorporeal Life Support Organisation), Barcelona, Spain, 2019 • "Five-year impact of ICU-acquired neuromuscular complications: a prospective, observational study", 40th ISICEM (international society of intensive care and emergency medicine), Brussels, Belgium, 2020: digital poster 09/2020
Course presentations	• Case presentation as Belgian resident op ESIM (European Schools of Internal Medicine) Winter schools in Internal Medicine, Levi, Finland, 01/2018. • Research presentation on PhD project, summarizing the results of the "Five-year mortality and morbidity impact of a prolonged versus a brief ICU stay: a propensity score matched cohort study", as Belgian resident at the 19th annual FDIME-EFIM Clinical Research Seminar, Paris, France, 06/2019. • Case presentation at ERS Virtual school on Clinical exercise testing: Core principles, online course, 11/2020
Invited presentations	• Presentation on the ESIM Winter schools hosted by the European Society of Internal Medicine at the annual conference of the Belgian Society of Internal Medicine, Dolce La Hulpe, Belgium, 2019

EXPERIENCE AND COMMITMENTS

Cultural work	• Guide at the Venetian art Biennale for the project 'Nato a Venezia', a collaboration on genetic diversity involving the "Universiteit Hasselt" (Faculty of Genetics) and artist Koen Vanmechelen (2011)
International courses	• Belgian representative at ESIM (European Schools of Internal Medicine) Winter schools in Internal Medicine, Levi, Finland, 01/2018. • Belgian representative at 19th annual FDIME-EFIM Clinical Research Seminar, Paris, France, 06/2019. • "Introduction to Bayesian Statistics using BUGS", course on biostatistics, University of Cambridge, Cambridge, 10/2019.
Data science	• Second best PhD-team participating at the annual KU Leuven Datathon edition 2020, organised by the Leuven Statistical Research Centre, using Leuven air pollution data our team had build a prediction model for air quality in Leuven of the following day, aimed at providing patients with respiratory disease guidance on the risk of respiratory symptoms (http://rrsymptoms.com)
Membership	• Invited member of the working group of ESIM: "Choosing wisely" and "EBIM in the loop"

Final thoughts: words of gratitude

I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference

From 'The Road Not Taken' by Robert Frost

There are countless interpretations of this poem, but supposedly Robert Frost wrote it referring to his close friend Edward Thomas, who always second-guessed his decisions, wondering whether he had pulled the shortest straw and if the grass was greener on the other side of his decision after all.

There is certainly a thing or two I've learned about travelling roads these 4 years.
The roads of which the end is out of sight, will take you to unexpected places. I never expected to study survival analysis or code models in three different statistics programms among statistics master students, to take muscle biopsies from critically ill patients and analyse them at the molecular and histological level, and to study exercise physiology through ergospirometry datasets from hundreds of clinical cases. These have been the most intense and cathartic years of my professional training thus far.
These roads tend to be obscure, and it is a bliss if you can ask for directions on the tricky parts. I had the privilege to have been guided by 3 women in science unafraid to walk their own path.
Prof. Dr. Greet Van den Berghe, who allowed me into her research group and showed me the importance of asking the right question.
My copromotor, Prof. Dr. Ilse Vanhorebeek, whose meticulous attention to detail never ceased to amase me.
My promotor, Prof. Dr. Greet Hermans, who showed me a new dimension of proficiency, perseverance, and passion for research.
I hope their light may guide generations of researchers to come.

While walking a road that can seem so otherworldly as a journey to Oz, the journey itself can give a sense of belonging when you travel in good company.
My PhD-colleges from Scriptorium 1 – Giorgia, Yuan, Grégoire, Chloë – and Prof. Fabian Guizza: thank you for giving a computer a heart, and reminding me of how small our wide world is, if the people living in it can find ways to connect. Bavo, you'll have a blast brainstorming with these coding wizards.
My collegues from the study office and from the lab: Sandra Hendrickx, Sylvia Vanhulle, Liese Mebis, Heidi Utens, Hanna Van Cleemput, Pieter Wouters, Lies Pauwels, Inge Derese, Sarah Vander Perre, Sarah Derde: you are worth a lion's courage, saying you provided technical and mental support will never cover the lifesaving actions you have been involved in. Prof. Lies Langouche, thank you for your constructive remarks during research presentations, they did encourage me to walk a little further.
My PhD-fellows from Scriptorium 2, your honest passion for research has been my pleasure to share, your friendship a support beyond compare. The Ladies, An, Lisa, Ines, Astrid, I admire how you manage to combine your compelling careers with renovating a house and running a household. The boys, Arno, Wouter, Ruben: mice surgeons and thankful echocardiography subjects, how amazing it is to hear the enthusiasm for your projects, on subcellular pathways that in most cases I only have a vague notion of. I look forward to seeing what you guys will do hereafter. Lauren, new and diligent, I hope you will enjoy the company and mentorship of the amasing collegues of your new office!

Cardiology has always been part of the planned final destination, and now that my enthousiasm for intensive care medicine has further rooted, I know that the road towards clinical proficiency is still a long one. I cannot express how thankful I am for all the help, explanation, guidance and patience I was given by so many professionals under oftentimes pressing circumstances in which I did my on call services.

The nurses and supporting staff from the department of heart failure and echocardiography, in particular Ilse, Joris, Christine, as well as the nurses from the medical and cardiac ICU, the physiotherapists, in particular Bieke, Bregje, Else and Inge.

The entire staff of the surgical ICU, in particular Prof. Dr. Michaël Casaer, Prof. Dr. Yves Debaveye, Prof. Dr. Jan Gunst, Prof. Dr. Geert Meyfroidt for their research input.

The entire staff of the medical ICU: Prof. Dr. Alexander Wilmer, Prof. Dr. Joost Wauters, Dr. Philippe Meersseman, and of course Prof. Dr. Greet Hermans, for their clinical and research input.

My fellow collegue cardiology trainees and PhD-students, the entire staff of the cardiology and CCU department of Prof. Dr. Stefan Janssens, and in particular Dr. Kaatje Goetschalckx, Dr. Walter Droogné, Prof. Dr. Lucas Van Aelst, Dr. Tim Balthazar, Dr. Christophe Vandenbriele, for their clinical and research input.

As the road - and those I encounter on the way - is bound to change again now, there have been some constants that I hope will remain.

My friends, you have been a source of support and joy that replenished my mind every time: I owe you guys quite a number of coffees, gin-tonics and time to properly thank you and catch up: Laura Coninckx, Karlijn Louwies, Marijke Timmers, Hélène Vanneste, Leticia Barrios.

The final credit goes to those without whom I literally would not have been able to finish this manuscript. My family.
“Oma en opa van Zwartberg”, “oma en opa van Begijnendijk”,
My godmother and godfather, and my aunts and uncles: “tante Sonia en nonkel Alain, nonkel Geert en tante Hilde, tante Henia en nonkel André, tante Christel en nonkel Alex”,
My cousins: “David en Maarten, Thomas en Alexander, Cédric, Vincent en Lorenz, Valerie en Julie”,
My brother Matthias – Matthi –,
My sister Eline, my brother-in-law, Filip, my favourite new cousin Simon,
Papa en Mama.
You have been the catalyst of any and every accomplishment I can take pride in.
Thank you, I love you all.

At the end of this road, I have one heartfelt hope, that somehow, in the smallest of ways, my work may have helped map a previously uncharted land, and that it will help lead the way this research will travel by hereafter. Or as Seneca said it so eloquently.

“The time will come when diligent research over very long periods will bring to light things which now lie hidden. A single lifetime, even though entirely devoted to the sky, would not be enough for the investigation of so vast a subject. And so this knowledge will be unfolded only through long successive ages. Let us be satisfied with what we have found out, and let our descendants also contribute something to the truth... ”