Increased Early Systemic Inflammation in ICU-Acquired Weakness; A Prospective Observational Cohort Study*

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Objectives: To investigate whether patients who develop ICU-acquired weakness have a different pattern of systemic inflammatory markers compared with critically ill patients who do not develop ICU-acquired weakness.

Design: Prospective observational cohort study.

Setting: Mixed medical-surgical ICU of a tertiary care hospital in the Netherlands.

Patients: Newly admitted critically ill patients, greater than or equal to 48 hours on mechanical ventilation with a nonneurologic ICU admission diagnosis, were included.

Interventions: A panel of systemic inflammatory markers and soluble vascular adhesion molecules were measured in plasma samples of day 0, 2, and 4 after ICU admission. ICU-acquired weakness was diagnosed by manual muscle strength testing as soon as patients were awake and attentive.

Measurements and Main Results: Ninety-nine of 204 included patients developed ICU-acquired weakness. Principal component regression analysis, adjusted for confounders, showed that principal component 1, mainly loaded with interleukin-6, interleukin-8, interleukin-10, and fractalkine, was significantly higher in patients who developed ICU-acquired weakness (odds ratio, 1.35 [95% CI, 1.18–1.55]). Partial least squares-discriminant analysis also showed that these markers were the most important discriminative markers. Mixed-effects models of these markers showed that ICU-acquired weakness was associated with an independent 1.5- to two-fold increase in these markers.

Conclusions: Systemic inflammation is increased in patients who develop ICU-acquired weakness compared with patients who do not develop ICU-acquired weakness in the first 4 days after ICU admission. This finding is consistent when adjusted for confounders, like disease severity. A group consisting of interleukin-6, interleukin-8, interleukin-10, and fractalkine was identified to be the most important. (*Crit Care Med* 2017; 45:972–979)

Key Words: critical illness neuromyopathy; cytokines; inflammation; inflammatory markers; intensive care unit-acquired weakness

ICU-acquired weakness (ICU-AW) is a serious complication of critical illness (1) and causes increased morbidity and mortality (2, 3).

The exact pathogenesis of ICU-AW is unidentified and probably multifactorial. As sepsis, the systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) are the main risk factors; an inflammatory pathogenesis is assumed (4).

A key element of these risk factors is activation of systemic inflammatory pathways, such as induction of cytokines (5). The development of ICU-AW might be associated with the extent to which these factors are activated.

Elevated systemic levels of cytokines and endothelial cell activation can induce increased permeability of vascular endothelium leading to MODS (6). This may also lead to impaired oxygenation of muscle and nerve tissue, causing muscle and nerve damage (7). Inflammatory mediators and endothelial cell activation markers have been found in muscle and nerve tissue of patients with ICU-AW, but histological evidence is limited (8). However, whether an association exists between increased systemic inflammation and development of ICU-AW remains unclear. A limited number of systemic inflammatory markers were investigated previously in patients with ICU-AW (9). These studies showed varying results, and in none of them, patterns of inflammatory markers were investigated.

To explore systemic inflammation as a possible pathophysiologic mechanism in ICU-AW, we investigated whether critically ill patients who develop ICU-AW have a different pattern of systemic inflammatory markers in the first 4 days after ICU admission, compared with critically ill patients who do not develop ICU-AW. We did not aim to predict the presence or absence of ICU-AW.

METHODS

Design and Ethical Approval

This prospective observational study was performed within the framework of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) study (ClinicalTrials.gov, NCT01905033). The Institutional Review Board gave approval for an opt-out consent method (protocol number 10-056C).

Study Setting

The study was performed in a mixed medical-surgical ICU of the Academic Medical Center in Amsterdam. Several standards of care are applied in this ICU, such as glucose control between 90 and 144 mg/dL, restricted use of neuromuscular blocking agents, and early mobilization. Sedation is stopped as soon as possible.

Inclusion and Exclusion Criteria

Patients newly admitted to the ICU between January 1, 2011, and December 31, 2012, greater than or equal to 18 years old, were eligible for inclusion. We included consecutive patients who were greater than or equal to 48 hours on mechanical ventilation. Patients admitted because of stroke, traumatic brain or spinal injury, a neuromuscular disorder, central nervous system infection, or cardiac arrest were excluded. Patients with preexisting spinal injury or poor prehospital functional status (modified Rankin score [10] > 3) were also excluded. For this article, only patients with both blood samples and the outcome (ICU-AW or no ICU-AW) available were analyzed.

Collection of Clinical Data and Blood Samples

Data on patient and disease characteristics were prospectively collected by trained observers (11). Presence of sepsis was scored when patients had SIRS (according to the Bone criteria [12]) and antibiotic administration for the suspicion of an infection. Immune insufficiency at admission was defined by use of immunosuppressive medication at admission, and/or chemo/radiotherapy in the year before ICU admission, and/or a documented humoral or cellular immune deficiency.

All patients with sepsis were managed according to protocols following the Surviving Sepsis Campaign guidelines (13).

Blood samples were collected from leftover plasma drawn for routine care. Plasma samples were stored at –80°C within 4 hours after collection from the patient.

Muscle Strength Assessment

Trained physiotherapists performed manual muscle strength testing (MMT), using the Medical Research Council (MRC) scale. As soon as patients were awake (defined as Richmond Sedation and Agitation Scale between –1 and 1) and attentive (able to adequately respond to verbal commands with eyelids), six muscle groups were tested bilaterally: shoulder abductors, elbow flexors, wrist flexors, hip flexors, knee extensors, and ankle dorsiflexors. Our outcome ICU-AW was defined as a mean MRC score less than 4, in accordance with the international consensus statement (2).

Inflammatory Marker Assays

All inflammatory marker measurements were done in EDTA anticoagulated plasma obtained within 24 hours after ICU admission (day 0) and on day 2 and 4 after ICU admission. We analyzed a panel of inflammatory markers, all of which are assumed to play a role in sepsis. The panel consisted of pro- and anti-inflammatory cytokines, a chemokine and soluble vascular adhesion molecules: interleukin-1 beta (IL-1β), IL-6, IL-8, IL-10, IL-13, tumor necrosis factor (TNF) α, interferon gamma (IFNγ), granulocyte macrophage colony-stimulating factor (GM-CSF), fractalkine, soluble intercellular adhesion molecule-1 (sICAM-1), soluble E-selectin (sE-selectin), and soluble P-selectin (sP-selectin). For further details on these measurements, see the **online data supplement** (Supplemental Digital Content 1, http://links.lww.com/CCM/C547).

Statistical Analysis

See the online data supplement (Supplemental Digital Content 1, http://links.lww.com/CCM/C547) for details on the statistical methods. We compared two outcomes: ICU-AW or no ICU-AW. Unless otherwise stated, levels of inflammatory markers of all time points together were used for analyses.

As the inflammatory response is a cascade of activated inflammatory markers, we considered it better to look at patterns of markers instead of solitary markers, since these are highly correlated. In this way we looked at the whole complex network and avoided multiple testing. Multiple methods to investigate patterns of inflammatory markers and their association with ICU-AW were used. First, to visualize patterns, a heat map was created with hierarchical clustering of columns (inflammatory markers).

Second, principal component (PC) regression analyses (14, 15) were performed with a priori selected possible confounders for ICU-AW. Based on the literature, we used age, gender, presence of sepsis, immune insufficiency at ICU admission, corticosteroid use, and Sequential Organ Failure Assessment (SOFA) score. By inclusion of the SOFA score, which is a score for degree of organ failure based on six organ systems, we prevented the use of too many variables in our model. The Acute Physiology and Chronic Health Evaluation (APACHE) IV score was not included in the model because of collinearity with the SOFA score. We also visualized the course of PC 1–3 over time and assessed the difference between patients with and without ICU-AW with linear mixed-effects models.

Next, partial least squares-discriminant analysis (PLS-DA) was used to get a maximum separation of components between patients with and patients without ICU-AW. We also used PLS-DA for different ICU-AW severities (severe ICU-AW: mean MRC < 3; moderate ICU-AW: mean MRC < 4 and > 3) and an analysis stratified by sepsis.

To quantify the effect of the inflammatory markers and to account for the repeated measurements structure in our data, we used mixed-effects models. To restrict multiple testing, only the variables with a variable importance in projection (VIP) score greater than 1 in the PLS-DA were selected for mixed-effects models. The effect of ICU-AW on selected inflammatory markers, adjusted for confounders, was assessed.

As a sensitivity analysis, we assessed the influence of missing values.

RESULTS

eFigure 1 (Supplemental Digital Content 1, http://links.lww.com/CCM/C547) shows the flowchart of screened and included patients. For this study, MRC measurements and plasma samples were available for 204 patients, of whom 99 patients developed ICU-AW. Patient characteristics are presented in **Table 1**.

After Bonferroni correction, IL-1β, IL-6, IL-8, IL-10, IFNγ, fractalkine, and sICAM-1 were significantly higher in patients with ICU-AW (**eTable 1**, Supplemental Digital Content 1, http://links.lww.com/CCM/C547). Graphs of inflammatory marker values over time are presented in **eFigure 2** (Supplemental Digital Content 1, http://links.lww.com/CCM/C547). Several measurements of IL-13, GM-CSF, TNFα, and IFNγ were below detectable limits (**eTable 2**, Supplemental Digital Content 1, http://links.lww.com/CCM/C547).

Roughly three main clusters of inflammatory markers were identified using heat map analyses (**Fig. 1**): cluster 1 (including IL-6, IL-8, IL-10, IFNγ, and fractalkine), cluster 2 (GM-CSF, IL-1β, TNFα, IL-13), and cluster 3 (sP-selectin, sE-selectin, sICAM-1). Especially, markers in cluster 1 were higher in patients with ICU-AW (Fig. 1***B***).

PC regression analysis showed that the first three PCs accounted for 57.7% of the variance in the data. All PCs showed a significant difference between patients with and without ICU-AW (**Table 2**). PC1, loaded by IL-6, IL-8, IL-10, and fractalkine, had the largest effect size (odds ratio [OR], 1.35 [95% CI, 1.18–1.55]). Loading coefficients are presented in **eTable 3** (Supplemental Digital Content 1, http://links.lww.com/CCM/C547).

eFigure 3 (Supplemental Digital Content 1, http://links.lww.com/CCM/C547) shows the course of PC1 to PC3 over time. PC1 is significantly higher in patients with ICU-AW than in those without ($p < 0.0001$; p adjusted = 0.0001).

PLS-DA identified IL-6, IL-8, IL-10, and fractalkine to be the most important discriminative markers (VIP scores: IL-8, 1.56; fractalkine, 1.45; IL-10, 1.40; IL-6, 1.20). Scores and loadings plots of the first two components of PLS-DA are presented in **Figure 2**, ***A*** and ***B***.

Although there is some variation in loadings between the different time points, IL-6, IL-8, IL-10, and fractalkine are consistently identified as the most important markers (Fig. 2***C***). On the first day of ICU-admission, IL-1β and IFNγ have also a VIP score greater than 1. This was not found at the other time points.

PLS-DA analysis for groups in which ICU-AW was split in severe and moderate ICU-AW showed that the first component (including IL-6, IL-8, IL-10, fractalkine, ICAM, and IL-13) was increasing with increasing ICU-AW severity (**eFig. 4**, Supplemental Digital Content 1, http://links.lww.com/CCM/C547).

Separate PLS-DA for patients with sepsis and without sepsis showed similar results, with IL-6, IL-8, IL-10, and fractalkine as the most discriminant markers (**eFig. 5**, Supplemental Digital Content 1, http://links.lww.com/CCM/C547).

Mixed-effects models showed that ICU-AW was associated with an independent 1.5- to two-fold increase in the markers IL-6, IL-8, IL-10, and fractalkine (**Table 3**).

In the PC regression analysis of imputed datasets, only PC1 remained significantly higher between patients with and patients without ICU-AW (OR, 1.33 [95% CI, 1.17–1.49]). In all imputed datasets, PC1 was loaded by IL-6, IL-8, and IL-10, and in six of 10 imputed datasets also by fractalkine. PC2 and PC3 were not different in imputed datasets.

PLS-DA of imputed datasets showed corresponding results with the nonimputed dataset, with IL-6, IL-8, IL-10, and fractalkine having VIP scores greater than 1 in all imputed datasets.

In the mixed-effects models, imputed datasets did not change the estimates for the effect of ICU-AW (Table 3).

DISCUSSION

To our knowledge, this is the first study investigating patterns of systemic inflammatory markers in ICU-AW. The

TABLE 1. Patient Characteristics of Patients With and Without ICU-Acquired Weakness

Characteristic	ICU-AW ($n = 99$)	No ICU-AW ($n = 105$)	p
Male (%)	50 (50.5)	66 (62.9)	0.101
Age (median [IQR])	64.0 (55.5–72.0)	61.0 (50.0–70.0)	0.070
Admission type (%)			0.469
Medical	57 (57.6)	62 (59.0)	
Planned surgical	16 (16.2)	22 (21.0)	
Emergency surgical	26 (26.3)	21 (20.0)	
Systemic inflammatory response syndrome[a]	99(100)	102 (97.1)	0.266
Any sepsis[a]	87 (87.9)	75 (71.4)	0.006
Primary site of infection			0.010
Pulmonary	40 (46.0)	38 (50.7)	
Cardiovascular	6 (6.9)	9 (12.0)	
Abdominal	29 (33.3)	9 (12.0)	
Urinary tract	1 (1.1)	5 (6.7)	
Other	11 (12.6)	14 (18.7)	
Immunodeficiency prior to ICU admission	32 (32.3)	29 (27.6)	0.562
Corticosteroids on ICU[a]	80 (80.8)	60 (57.1)	< 0.001
Acute Physiology and Chronic Health Evaluation IV score (median [IQR])	90.0 (74.5–103.0)	69.0 (56.0–95.0)	< 0.001
Maximum sequential organ failure assessment on sample day (mean [SD])	11.7 (3.6)	9.2 (3.4)	< 0.001
Average MRC score (median [IQR])	2.5 (1.3–3.2)	4.7 (4.0–5.0)	Not applicable
Days from ICU admission to MRC (median [IQR])	9.0 (6.0–16.0)	7.0 (5.0–9.0)	< 0.001
Length of stay ICU (median [IQR])	16.0 (8.0–27.0)	7.0 (5.0–11.0)	< 0.001
Mechanical ventilation duration (median [IQR])	13.0 (6.0–22.0)	6.0 (4.0–8.0)	< 0.001
Death in ICU (%)	15 (15.2)	5 (4.8)	0.024

ICU-AW = ICU-acquired weakness, IQR = interquartile range, MRC = Medical Research Council.
[a]During first 4 d after ICU-admission.

results show that in the first 4 days after ICU admission, systemic inflammation is increased in critically ill patients who develop ICU-AW compared with critically ill patients who do not develop ICU-AW. Despite the fact that there is not a clear decision boundary between patients with and patients without ICU-AW in the biplot, a group of four markers, IL-6, IL-8, IL-10, and fractalkine, were identified to be the most discriminant at all time points, including at ICU admission. As ICU-AW is not yet present at ICU admission, this suggests that these increased markers may cause ICU-AW and are not a consequence of ICU-AW. Besides, inflammatory markers increased with increasing ICU-AW severity.

The presence of ICU-AW was associated with a 1.5- to two-fold increase in these markers. This increase seems to be independent of potential confounders for development of ICU-AW, indicating that levels of IL-6, IL-8, IL-10, and fractalkine are higher in patients with ICU-AW, irrespective of disease severity.

Comparisons With Previous Studies

Previous studies investigated a limited number of systemic inflammatory markers and none of them investigated patterns.

Two studies showed an association between plasma cytokines and electrophysiological measurements in ICU patients (16, 17). Unfortunately, a diagnosis of ICU-AW based on MMT, which is the preferred method according to expert-based guidelines (18, 19), was not made in these studies. One study investigated IL-6 and IL-10 in 22 ICU patients with abnormal membrane excitability as a marker of myopathy and 18 patients with normal excitability (16). IL-6 was found to be an independent risk factor, with a small hazard ratio of 1.006 (95% CI, 1.003–1.009). For IL-10, no difference was found. In another study, in 20 ICU patients, IL-2 receptor levels were negatively correlated with compound muscle action potential amplitudes of median and tibial nerves (17). This was not found for IL-2, IL-6, IL-10, and complement factors C3 and C4.

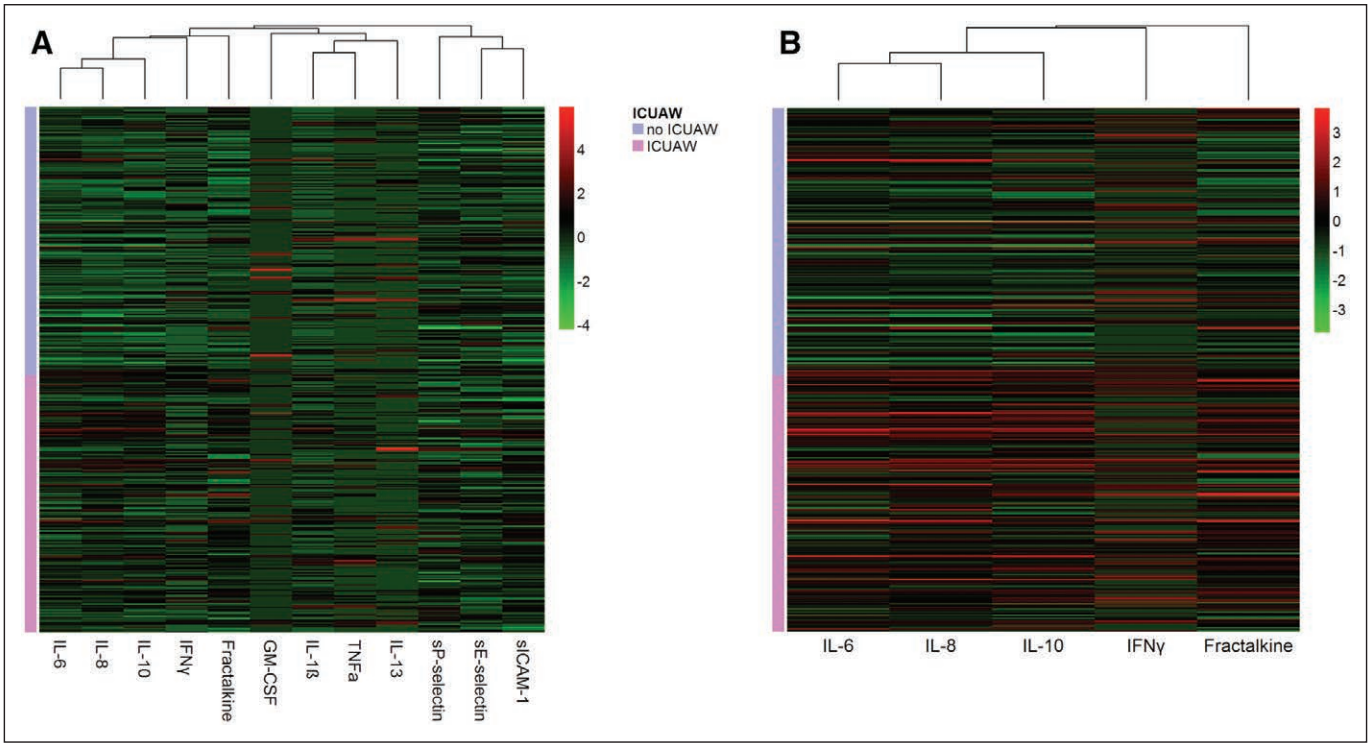

Figure 1. Heat maps of inflammatory markers. Standardized values of inflammatory markers with hierarchical clustering of columns (**A**, all markers; **B**, selected markers). Each row represents a single measurement of a patient. Rows are sorted on presence of ICU-acquired weakness (ICU-AW): rows with *purple* in front are patients without ICU-AW ($n = 275$ measurements), rows with *pink* are patients with ICU-AW ($n = 263$ measurements). Roughly, three main clusters can be identified in **A**. The markers in cluster 1 appear higher in patients with ICU-AW (**B**). GM-CSF = granulocyte macrophage colony-stimulating factor, IL = interleukin, IFNγ = interferon gamma, sICAM = soluble intercellular adhesion molecule, TNF = tumor necrosis factor.

TABLE 2. Principal Component Regression Analysis Showing the Association Between Principal Components of Inflammatory Markers and ICU-Acquired Weakness

PC	Crude OR (95% CI)	Adjusted OR (95% CI)	Main Loadings of PC
PC1	1.46 (1.31–1.63)	1.35 (1.18–1.55)	IL-6, IL-8, IL-10, fractalkine
PC2	1.18 (1.03–1.36)	1.17 (1.02–1.35)	(Negatively loaded) IL-1, IL-13, tumor necrosis factor-α
PC3	0.81 (0.70–0.94)	0.85 (0.72–0.99)	Soluble intercellular adhesion molecule-1, soluble P-selectin, soluble E-selectin

IL = interleukin, OR = odds ratio, PC = principal component.
Multivariable logistic regression with three principal components, unadjusted and adjusted for confounders.

Some studies did not find any differences in plasma cytokines. No associations were found between MMT composite scores of 36 ICU patients and sequentially measured cytokines IL-8, IL-15, and TNFα on three consecutive days from day 6 after ICU admission (20). In another study, mean and maximum levels of IL-6 and TNF did not differ between nine ICU patients with critical illness polyneuropathy (CIP) and 10 ICU patients without CIP (21). However, blood samples were taken after a longer duration of critical illness in patients with CIP (ranging from 12 to 55 d) compared to patients without CIP (0–12 d), limiting useful comparisons.

Identified Pattern of Increased Levels of Inflammatory Markers and ICU-AW

Our results suggest that IL-6, IL-8, IL-10, and fractalkine may be involved in the pathogenesis of ICU-AW. The both pro- and anti-inflammatory acting cytokine IL-6, pro-inflammatory acting cytokine IL-8, and anti-inflammatory cytokine IL-10 are important factors in the onset of the systemic inflammatory and anti-inflammatory responses. They play an important role in the disbalanced inflammatory response as is seen in sepsis and MODS (22). Fractalkine (CX3CL1) is a recently discovered inflammatory mediator, which can be expressed in several tissues, including skeletal muscle and neurons (23). It can act both as an adhesion molecule and as a soluble chemokine and is correlated with disease severity in sepsis patients in the ICU (24). IL-6, IL-8, IL-10, and fractalkine are described as prognostic biomarkers in sepsis: IL-6, IL-10, and fractalkine can distinguish between survivors and nonsurvivors at day 28, and IL-8 has been used for the prediction of MODS (25, 26). As these markers can predict severity of sepsis, an association with ICU-AW, a severe complication of sepsis, would not be surprising.

In vitro and in vivo experiments have shown that cytokines are implicated in muscle damage (27, 28) and that the systemic

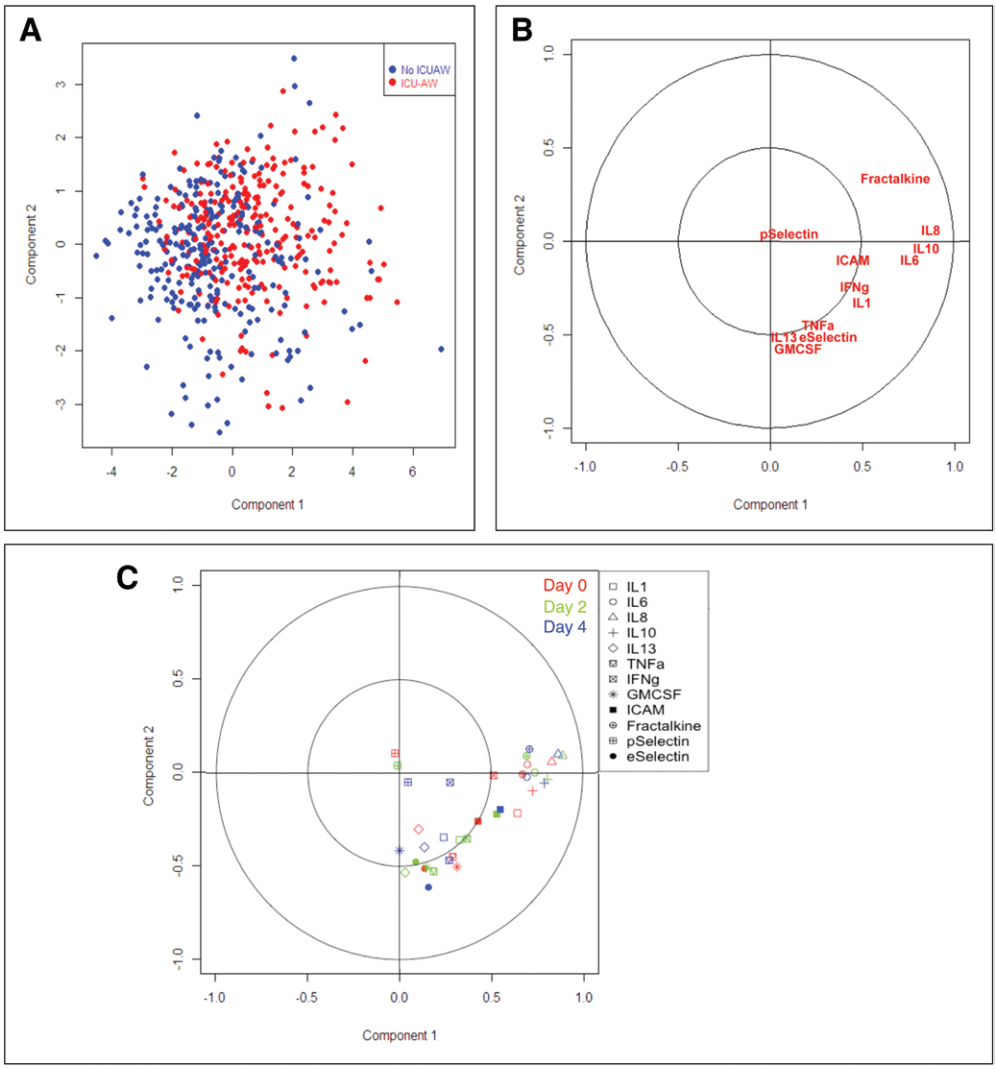

Figure 2. Scores plot and loadings plot of partial least squares-discriminant analysis (PLS-DA). Scores plot (**A**) and loadings plot (**B**) of first 2 components of PLS-DA of all plasma samples. Loadings plot of all time points (**C**). Patients with ICU-acquired weakness (ICU-AW) have a higher score on component 1 (**A**). Interleukin (IL)-8, IL-10, IL-6, and fractalkine are the highest loaded on component 1 (**B** and **C**). GMCSF = granulocyte macrophage colony-stimulating factor, IFNg = interferon gamma, ICAM = intercellular adhesion molecule, TNF = tumor necrosis factor.

inflammatory response results in local production of cytokines and acute phase proteins in muscle (29–31). IL-6 and fractalkine act as chemoattractants, recruiting cytokine producing leukocytes to the muscle, leading to proteolysis, myocyte degeneration, and muscle atrophy (27). IL-10 is an anti-inflammatory protein and can inhibit IL-6. It may not be the absolute values of pro-inflammatory cytokines that produce muscle damage, but the imbalance between pro- and anti-inflammatory cytokines (27). Capillary leakage and hypoxia causing muscle and nerve damage are an indirect consequence of activated inflammatory markers (9).

Interestingly, IL-6 and IL-8, along with IL-15, can be expressed by and also released from skeletal muscle; they are so-called myokines (32). Release of these myokines is mainly described after exercise, where it is believed to play a protective role in the local signaling and regulation of inflammatory markers (33). It might be possible that, in critical illness, release of myokines, especially IL-6, may contribute to systemic inflammation, perpetuating and disturbing the systemic inflammatory response, possibly adding to multiple organ failure and muscle and nerve damage.

The other markers and patterns we investigated in this study did not seem to be different in patients with and without ICU-AW. This was possibly limited by the fact that many measurements of IL-1β, IL-13, TNFα, IFNγ, and GM-CSF were below the detection limit. In PLS-DA, we found that IL-1β and IFNγ on day 0 were also important

TABLE 3. Mixed-Effects Models Showing the Predicted Effect of ICU-Acquired Weakness on Selected Inflammatory Markers

Marker	Predicted Effect of ICU-AW (95% CI) (Fold Increase)	Pooled Effect of ICU-AW (95% CI) Imputed Data Sets (Fold Increase)
IL-6	2.15 (1.39–3.32)	2.34 (1.38–3.96)
IL-8	2.27 (1.60–3.21)	2.21 (1.44–3.41)
IL-10	1.86 (1.34–2.57)	1.85 (1.21–2.83)
Fractalkine	1.65 (1.25–2.17)	1.57 (1.11–2.23)

ICU-AW = ICU-acquired weakness, IL = interleukin.

Linear mixed-effects models showing the association between ICU-acquired weakness (ICU-AW) and inflammatory markers. Predicted effects are fold increase in pg/mL in patients with ICU-AW compared to patients without ICU-AW.

factors, but this was not seen in PLS-DA of day 2 and 4. In contrast, in multivariable logistic regression with PCs, ICU-AW was associated with lower values of IL-1, IL-13, and TNFα (PC2), but this PC was not significant in pooled analysis of imputed datasets.

Sepsis is associated with increased expression of vascular adhesion molecules on endothelium and increased shedding of these molecules, leading to accumulation of soluble forms in the blood (34). Increased shedding seems to diminish inflammation and high levels of soluble adhesion molecules are associated with better outcomes in sepsis (35). In accordance with this, we found that PC3 representing soluble vascular adhesion molecules gave a lower risk of ICU-AW, although this was not significant in pooled data from imputed datasets and should therefore be interpreted with caution.

Strengths and Limitations

The large sample size and serial collection of blood samples are the main strengths of this study. Taking the complexity of the systemic inflammatory response into consideration, we are the first to investigate patterns of inflammatory markers using statistical procedures like PC analysis. By including also patients without sepsis and performing stratified analysis, we showed that the association found was not restricted to patients with sepsis. ICU-AW was systematically diagnosed, using MMT. Even though MMT has its limitations, it is the most reliable test and the experts' recommended method to diagnose ICU-AW (18, 19). We diagnosed ICU-AW at the earliest time point possible. This leads to a variable time window to the diagnosis of ICU-AW, but it is our experience that by choosing a set time for MMT assessment, there is an increased risk for missing patients without ICU-AW because they will in general be discharged earlier from the ICU.

This study also has limitations. First of all, we did not perform a power calculation, because data from previous studies did not allow a reliable sample size calculation. Second, corticosteroid use on the ICU, APACHE IV score, and maximal SOFA score were higher in the group with ICU-AW. Although we included these factors (except APACHE IV, because of collinearity) as confounders in our statistical analysis, we cannot completely rule out some residual confounding.

Furthermore, we described an association between ICU-AW and increased inflammatory markers, but no causal relations can be deduced from this observational study.

Finally, serial measurements of inflammatory markers early after ICU admission restrict our study to statements about inflammation in the first 4 days after ICU admission. The time of onset of ICU-AW in our patients is unknown, because muscle strength can only be evaluated when patients are awake and attentive, in our study after a median of 9 days. However, ICU-AW is assumed to develop early, since electrophysiological studies have described abnormalities within 3 days after ICU admission (36). The use of electrophysiological measurements in our study could have been of additional value, but this was not possible in our study set-up.

Recommendations for Future Research

Our recommendation for future research is to explore IL-6, IL-8, IL-10, and fractalkine and their possible pathophysiologic role in ICU-AW in animal or laboratory studies. It should be further investigated if these inflammatory markers play a causal role in the development of ICU-AW and whether this is via a direct or indirect pathway. It would also be interesting to investigate whether these markers differ between patients with a polyneuropathy and patients with a myopathy, although most patients with ICU-AW have a combined polyneuropathy and myopathy (2).

The focus should not be on the individual markers but on their combined functions as these markers interact with each other in complex inflammatory networks. Further unraveling of the involved pathways may open a way to modulate the inflammatory response and possibly prevent ICU-AW.

CONCLUSIONS

Systemic inflammation is increased in the first 4 days after ICU admission in critically ill patients who develop ICU-AW compared with critically ill patients who do not develop ICU-AW. ICU-AW is independently associated with increased levels of IL-6, IL-8, IL-10, and fractalkine. These four markers may be important in the pathophysiology of ICU-AW.

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ICU-Acquired Weakness, Chronic Critical Illness, and the Persistent Inflammation-Immunosuppression and Catabolism Syndrome

To the Editor:

We congratulate Witteveen et al (1) of the Molecular Diagnosis and Risk Stratification of Sepsis consortium for their prospective investigation of systemic inflammatory marker patterns among patients with ICU-acquired weakness (AW) in a recent issue of *Critical Care Medicine*. This well-designed observational study provides a detailed description of a morbid condition that is becoming more and more common. Almost 50% of critically ill patients with at least 48 hours of mechanical ventilation demonstrated evidence of ICU-AW.

Although the enrollment strategy successfully identified a relatively heterogeneous population of critically ill patients, there was a common theme that was unrelated to inclusion criteria: 79% of the total study population and 88% of all patients with ICU-AW had sepsis, defined as presence of systemic inflammatory response syndrome plus antibiotic administration. Patients with ICU-AW also had a median ICU length of stay of 16 days, versus only 7 days among patients without ICU-AW, and had higher Sequential Organ Failure Assessment (SOFA) scores. These findings suggest that the ICU-AW cohort was composed of patients who would have succumbed to multiple organ failure in previous eras; in modern ICUs, these patients survive and develop chronic critical illness (CCI) (2) and the persistent inflammation-immunosuppression and catabolism syndrome (PICS) (3). ICU-AW may be a manifestation of these conditions.

Although the study by Witteveen et al (1) was not designed to assess whether the subjects had persistent inflammation juxtaposed with simultaneous suppression of adaptive immunity or whether patients with ICU-AW were also experiencing protein catabolism, these scenarios seem likely based on previous findings (3). Although plasma elevations in interleukin (IL)-6, IL-8, IL-10, and fractalkine generally occurred prior to measurement of muscle strength testing in both groups, elevated concentration of these cytokines may be best understood as representative of persistent inflammation rather than its cause. To understand what drives persistent inflammation and elevation of inflammatory cytokines on a mechanistic level, we must also consider noninfectious conditions like extended ventilatory support, immobility, exposure of extracellular matrix by injured tissues, and chronic low-grade organ injury (SOFA) driving the release of endogenous danger signals (4).

Persistent inflammation in critically ill patients is generally associated with protein wasting, abnormal hematopoiesis, and immune suppression, often leading to secondary infections and/or viral reactivation. Although not reported here, one could reasonably expect that patients with extended ICU stays and prolonged mechanical ventilation would likely have increased frequency of secondary infections (5). These infectious events would only serve to exacerbate inflammation, immune suppression, and protein wasting.

Witteveen et al (1) are to be congratulated for making this important contribution to our understanding of ICU-AW. Importantly, ICU-AW needs to be considered in the context of a holistic approach to an understanding of CCI. PICS was originally proposed as a hypothesis that could be experimentally tested in preclinical and clinical settings (3). As evidence accumulates for the existence of a common underlying pathophysiology for many of the conditions that adversely affect critically ill patients, we hope that the scientific community will continue to work together in elucidating this pathway and to promote the development of novel management strategies and therapies targeting this pathway.

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The authors reply:

We thank Loftus et al (1) for their kind comments and interest in our article (2), recently published in *Critical Care Medicine*. In this study (2), we measured systemic levels of inflammatory markers on days 0, 2, and 4 after ICU admission and found that plasma levels of interleukin (IL)-6, IL-8, IL-10, and fractalkine were increased in patients with ICU-acquired weakness (ICU-AW). Loftus et al (1) propose that these elevated plasma levels can be considered as representative of persistent inflammation. The persistent inflammation-immunosuppression and catabolism syndrome (PICS) is seen in patients with a prolonged ICU stay (> 14 d) and consists of both ongoing inflammation and immunosuppression with increased susceptibility to secondary infections (3). It is likely that patients with ICU-AW will have PICS, because the ICU admission is usually longer in these patients (4). As we only measured plasma levels of inflammatory markers in the first 4 days after ICU admission, we cannot determine if these same markers are also involved in persistent inflammation. The assumption of Loftus et al (1) is however supported by the fact that it has been previously shown by the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) consortium that IL-6, IL-8, IL-10, matrix metallopeptidase-8, and fractalkine were also elevated in the first 4 days after ICU admission in patients who develop an ICU-acquired infection (5). This hyperinflammatory state was sustained up to the day of the infection (median 10 d after ICU admission). This indeed suggests an association between patients with ICU-AW and patients with a secondary infection/PICS. It would be interesting to investigate this further.

It is thought that ICU-AW develops early after ICU admission. Electrophysiological signs of ICU-AW have been found already within 3 days (6). Unfortunately, the use of the Medical Research Council (MRC) score to diagnose ICU-AW often leads to a delay in diagnosis, because it cannot be used in sedated patients. In our study, ICU-AW was diagnosed at a median of 9 days. It seems likely that ICU-AW already develops days before it can officially be detected with the MRC score. A pathophysiological relationship between ICU-AW and early hyperinflammation, as opposed to persistent inflammation, is therefore likely. However, persistent inflammation and the catabolic responses associated with it may possibly contribute to the persistence of weakness. Furthermore, a long period of immobilization, despite early and intensive physiotherapy, may result in further muscle breakdown. Whether the release of myokines (cytokines produced by skeletal muscle tissue) due to muscle damage might play a role in persistent inflammation also needs further investigation.

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Ultrasound in Confirming Central Catheter Position: (Almost) Ready for Prime Time

To the Editor:

We read with great interest the article published in a recent issue *of Critical Care Medicine* by Galante et al (1) which describes a single-operator ultrasound-guided central venous catheter insertion technique. We recently reported a very similar technique using visualization of guidewire tip and the catheter in the right atrium to confirm catheter position and established chest ultrasound findings to exclude pneumothorax (2). The technique was associated with shorter times to catheter use with significant reductions in chest radiography (CXR) usage when tested in a randomized controlled experimental design. We believe that the pullback technique is feasible and effective. Nonetheless, we have noticed some differences between our techniques, and we would ask Galante et al (1) to clarify certain points for the reader:

1) How many machines/probes were used during the procedure? If one probe is taped to the abdomen, was there another machine at bedside to aid with catheter insertion? This will have cost and resource implications. If a single machine was used, how did the single operator toggle between the probes without breaking sterility? In our