No association between systemic complement activation and intensive care unit-acquired weakness

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Background: The main risk factors for intensive care unit-acquired weakness (ICU-AW) are sepsis, the systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction. These risk factors are associated with systemic complement activation. We hypothesized that critically ill patients who develop ICU-AW have increased systemic complement activation compared to critically ill patients who do not develop ICU-AW.

Methods: Complement activation products C3b/c, C4b/c and C5a were measured in plasma of ICU patients with mechanical ventilation for ≥48 hours. Samples were collected at admission to the ICU and for 6 consecutive days. ICU-AW was defined by a mean Medical Research Council (MRC) score <4. We compared the level of complement activation products between patients who did and who did not develop ICU-AW.

Results: Muscle strength measurements and complement assays were available in 27 ICU patients, of whom 13 patients developed ICU-AW. Increased levels of C4b/c were seen in all patients. Neither admission levels, nor maximum, minimum and mean levels of complement activation products were different between patients who did and did not develop ICU-AW.

Conclusions: Complement activation is seen in critically ill patients, but is not different between patients who did and who did not develop ICU-AW.

Keywords: Complement activation; critical illness myopathy; critical illness polyneuropathy; intensive care unit-acquired weakness (ICU-AW); systemic inflammation

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Introduction

The pathogenesis of intensive care unit-acquired weakness (ICU-AW) is probably multi-factorial (1). The main risk factors are sepsis, systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) (1). Activation of the complement system plays an important role in these risk factors and is associated with increased occurrence of shock and fatal outcomes in sepsis (2-4). Complement activation also plays a role in the pathogenesis of acute inflammatory polyneuropathies and

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myopathies (5-7). Thus, muscle and nerve damage in ICU-AW might also be complement-mediated.

Complement activation may lead to ICU-AW by anaphylatoxins (C3a, C4a, C5a), which can induce unbalanced systemic and local inflammatory responses, leading to MODS (8,9) and probably to 'failure' of muscles and nerves. C5a can also increase vascular permeability, leading to tissue edema and possibly nerve and muscle tissue damage (9). The final pathway of complement activation results in the membrane attack complex (MAC), causing direct cell damage (9). MAC depositions have been found in muscles of patients with ICU-AW (10-12).

In this pilot study, we tested the hypothesis that patients who develop ICU-AW have increased systemic complement activation compared to critically ill patients who do not develop ICU-AW.

Methods

This was a sub-study of a prospective observational cohort study (BASIC study, Biomarker Analysis in Septic Intensive Care patients), performed on the mixed medical-surgical intensive care unit (ICU) of the Academic Medical Center Amsterdam. The institutional review board approved the BASIC study protocol (No. NL34294.018.10). Informed consent from patients or their legal representatives was obtained before study participation.

Patients newly admitted to the ICU having sepsis or SIRS [Bone criteria (13)], mechanically ventilated for ≥48 hours, and in whom muscle strength assessment was performed, were eligible for inclusion. Exclusion criteria included antibiotic treatment for >48 hours, expected ICU stay <24 hours, no informed consent within 24 hours after ICU admission, pre-existing poor functional status [Modified Rankin score ≥4 (14)] and any central nervous system disorder, spinal cord injury or neuromuscular disorder as reason for ICU admission.

Blood samples were collected as soon as possible after ICU admission, and thereafter daily (about 3:00 PM) for 6 consecutive days. Blood was collected in vacutainer tubes, containing an inhibitor mix (with final concentrations of 10 mM benzamidine, 100 µg/mL soy bean trypsin inhibitor and 10 mM ethylene-diamine-tetra-acid) to prevent *in vitro* complement activation. Samples were centrifuged (1,500 g, 15 min, room temperature) within 1 hour after collection and plasma was stored in aliquots at –80 ℃ until assayed.

To determine complement activation of the common pathway and initial classical/lectin or alternative pathway, plasma levels of complement activation products C3b/c and C4b/c were measured using previously described enzyme-linked immunosorbent assays (ELISAs) (Sanquin, Amsterdam, The Netherlands) (15,16). These ELISAs do not distinguish C3b from C3bi and C3c, and C4b from C4bi and C4c and are therefore referred to as C3b/c and C4b/c. The normal reference values (from local healthy controls) are <57 nmol/L for C3b/c and <8 nmol/L for C4b/c.

Further downstream complement activation was assessed by measuring levels of C5a (no reference value available), using a commercial ELISA kit (MicroVue, Quidel, San Diego, CA, USA).

All measurements were done batch-wise and in duplo. Samples were analyzed blinded to all patients' data. Measurements with a coefficient of variation (CV) value of >30% were excluded from the analysis. The ELISA was successfully performed (CV of <30%) in 99% of C3b/c measurements, 98% of C4b/c measurements and 100% of C5a measurements. The lower limit of detection [determined by the mean of blanks plus 3 times the standard deviation (SD) of the blanks] for the C3b/c, C4b/c and C5a assays were 0.001 nmol/L, 0.002 nmol/L and 0.004 ng/mL, respectively.

Manual muscle strength was assessed as soon as patients were awake and attentive. Using the Medical Research Council (MRC) scale, six muscle groups were tested, bilaterally. ICU-AW was defined by a mean MRC score <4 (17,18).

The following clinical characteristics were collected: age, gender, admission reason, presence of sepsis at admission, length of stay on the ICU, number of days with mechanical ventilation, days from admission to muscle strength assessment, ICU mortality, Acute Physiology and Chronic Health Evaluation IV (APACHE IV) score and Sequential Organ Failure Assessment (SOFA) scores at days of blood sampling.

This study is an exploratory pilot study. Therefore, no formal power calculation was performed.

Mean values are presented with SD, median values with interquartile range (IQR) and proportions with total numbers and percentages. Differences between proportions were assessed using Fisher's exact test, between normally distributed continuous variables using Welch's *t*-test and between non-normally distributed continuous variables using Mann-Whitney U test.

To assess our primary endpoint, the difference between systemic complement activation and the presence of ICU-

Table 1 Patient characteristics

Characteristic	ICU-AW (n=13)	No ICU-AW (n=14)	P value
Age, median years (IQR)	72.0 [63–76]	58.0 (43.5–64.8)	0.01
Males, n (%)	7 (53.8)	6 (42.9)	0.71
Sepsis at admission, n (%)	12 (92.3)	10 (71.4)	0.33
Admission reason, n (%)			0.76
Medical	9 (69.2)	10 (71.4)	
Planned surgical	1 (7.7)	2 (14.3)	
Emergency surgical	3 (23.1)	2 (14.3)	
APACHE IV score, mean (SD)	91.5 (28.9)	76.4 (34.2)	0.23
Maximal SOFA score on day of blood sample, median (IQR)	13.0 (9.0–14.0)	8.0 (5.3–12.3)	0.12
Number of blood samples per patient, median (IQR)	7.0 (6.0–7.0)	7.0 (6.0–7.0)	0.74
Mean MRC score, median (IQR)	2.8 (1.3–2.9)	4.5 (4.1–4.9)	–
Day of MRC score, median (IQR)	7.0 (6.0–11.0)	7.0 (6.0–9.0)	0.88
Days with MV, median (IQR)	10.0 (6.0–13.0)	5.0 (4.3–7.8)	0.07
LOS ICU, median days (IQR)	13.0 (8.0–17.0)	8.0 (7.0–11.0)	0.13
Died on the ICU, n (%)	5 (38.5)	0 (0.0)	0.02

ICU-AW, intensive care unit-acquired weakness; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; MRC, Medical Research Council; MV, mechanical ventilation; LOS, length of stay; ICU, intensive care unit.

AW, and to account for repeated measurements we used summary statistics, which included: admission complement levels, maximum, minimum and mean values per patient during the first 7 days in ICU. A P value <0.05 was considered statistically significant (P<0.004 after Bonferroni correction). Analyses were done using R (version: 3.0.2).

Results

Data and plasma samples of 27 patients were available; 13 patients who developed ICU-AW and 14 patients who did not develop ICU-AW. Patient characteristics are presented in *Table 1*. A total of 167 plasma samples were analyzed (median of seven samples/patients). The median time from ICU admission to the first sample was 15.8 hours (IQR, 12.8–22.5 hours) in the ICU-AW group versus 17.3 hours (IQR, 14.1–22.2 hours) in the no ICU-AW group (P=0.74).

Levels of C3b/c, C4b/c and C5a fluctuated considerably in individual patients during the first 7 days in ICU. Median levels and IQR of C3b/c, C4b/c and C5a at each time point are presented in *Figure 1*.

There was no difference in admission, maximum, minimum or mean levels of C3b/c, C4b/c or C5a between patients who developed and did not develop ICU-AW (*Table 2*).

Discussion

This pilot study shows no difference in systemic complement activation in the first 7 days after ICU admission between patients who did and who did not develop ICU-AW.

All patients showed increased complement activation, as shown by C4b/c levels of nearly twice the reference value. The in- and exclusion criteria were rather strict and it is likely that we have selected a severely ill subpopulation with a high inflammatory state at admission. The severity of illness in the ICU-AW and no ICUAW group were comparable; both groups had high APACHE IV and SOFA scores.

Complement levels were lower than previously described in a study with patients with severe sepsis and septic shock (19), possibly due to a different case mix: not all patients in our cohort had sepsis, and patients might have

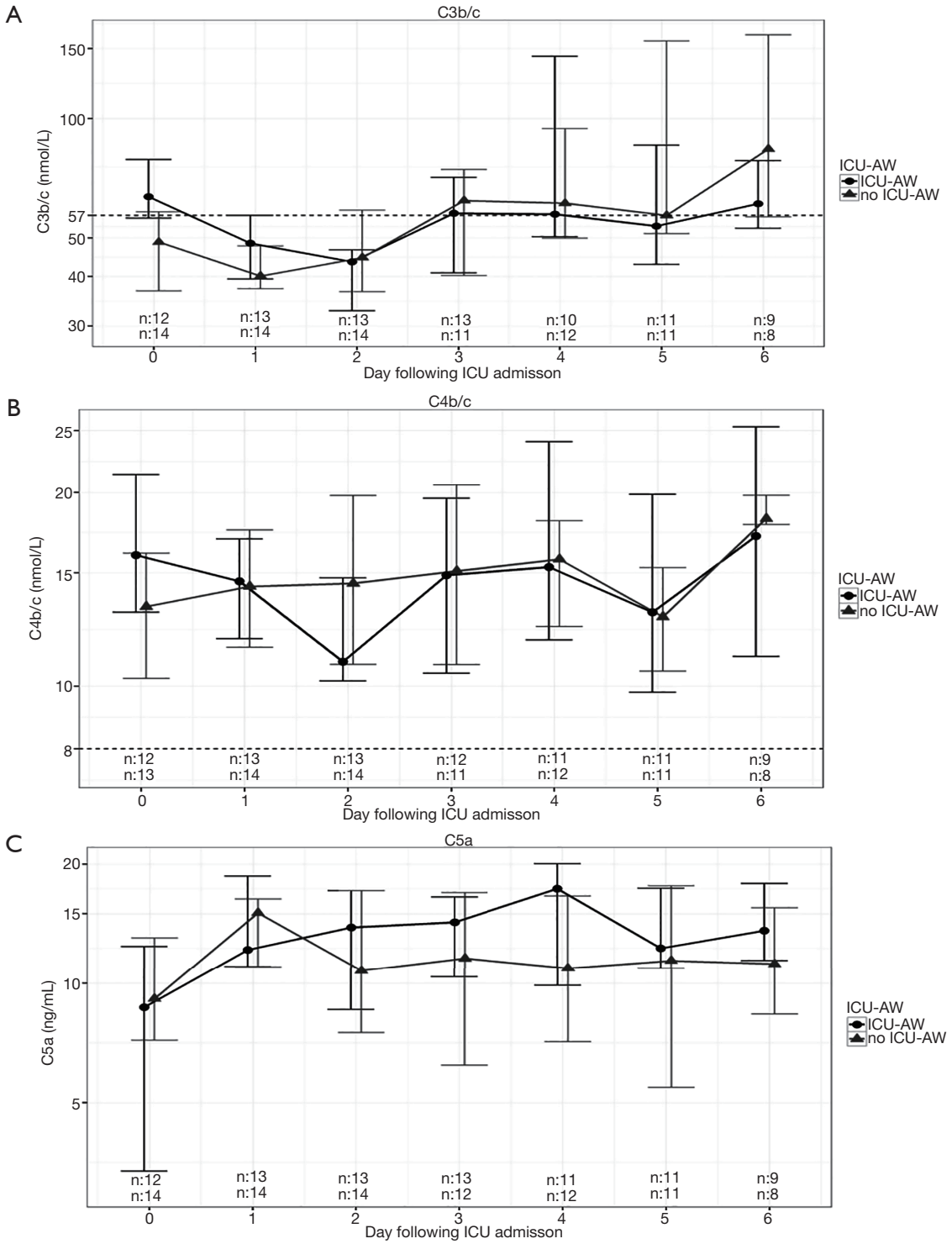

Figure 1 Levels of C3b/c, C4b/c and C5a in patients who developed and did not develop ICU-AW. Levels of C3b/c (A), C4b/c (B) and C5a (C) at admission (day 0) and 6 consecutive ICU days in patients who developed and who did not develop ICU-AW. Data are presented as median with interquartile range for each time point and numbers below the lines represent the number of samples of patients with ICU-AW (upper) and without ICU-AW (lower). Dotted lines (in A and B) represent the reference values. ICU, intensive care unit; ICU-AW, intensive care unit-acquired weakness.

Table 2 Admission, maximum, minimum and mean levels of C3b/c, C4b/c and C5a

Complement activation product level	ICU-AW (n=13)	No ICU-AW (n=14)	P value
C3b/c admission levels	63.5 (56.1–78.8)	48.8 (36.8–58.2)	0.11
C3b/c max levels	107.0 (82.4–201.4)	90.0 (72.6–196.2)	0.55
C3b/c min levels	35.9 (32.3–39.8)	33.8 (26.5–38.6)	0.55
C3b/c mean levels	62.8 (56.9–94.4)	62.0 (50.4–95.4)	0.72
C4b/c admission levels	16.0 (13.0–21.4)	13.3 (10.3–16.1)	0.17
C4b/c max levels	23.1 (17.1–34.0)	24.0 (19.3–38.4)	0.37
C4b/c min levels	9.9 (7.0–10.9)	9.6 (6.6–11.2)	0.87
C4b/c mean levels	15.3 (12.0–19.9)	16.4 (13.3–23.6)	0.62
C5a admission levels	8.7 (3.4–12.4)	9.1 (7.2–13.0)	0.53
C5a max levels	18.6 (11.6–35.7)	16.6 (11.5–19.1)	0.58
C5a min levels	8.5 (3.3–11.0)	7.4 (4.4–9.9)	0.83
C5a mean levels	13.1 (9.7–18.8)	11.9 (7.7–16.0)	0.65

Admission levels, maximum levels per patient, minimum levels per patient and mean levels per patient of complement activation products in the first 7 days in ICU in patients who developed and who did not develop ICU-AW. Levels are presented as median and interquartile range (C3b/c and C4b/c in nmol/L and C5a in ng/mL). ICU, intensive care unit; ICU-AW, intensive care unit-acquired weakness.

died earlier in other cohorts, before muscle strength could have been measured.

Although we did not find a difference in complement activation, activated complement can still play a role in the pathophysiology of ICU-AW in the presence of another yet unknown factor, for example expression of membrane complement regulatory proteins (the sensitivity to complement-mediated injury) (20).

The difference in systemic complement levels between patients with and without development of ICU-AW has never been studied before. Previously, no correlation has been found between plasma C3 and C4 levels and compound muscle action potential (CMAP) amplitudes of three nerves in ICU patients, but muscle strength was not measured in this study (21).

The use of daily measurements is a strength of this study because it enabled us to investigate the time course of complement activation and to use summary statistics, such as maximum levels.

This study has some limitations. The sample size of this pilot study was small, limiting the robustness of our results. Furthermore, it was impossible to determine the exact moment at which the inflammatory process was triggered in individual patients, since the onset of this process may take place before ICU admission (22). As complement activation occurs very early in the inflammatory response, peaks of complement activation within the first hours after ICU admission may be missed. Hemodilution may also have decreased complement levels (15), but this is a difficult factor to correct for. Furthermore, it can be debated whether plasma levels of complement activation products, indicating systemic activation, adequately reflect the levels in muscle or nerve tissue, since complement can also be activated locally (12).

Muscle strength assessment by MRC is the recommended test for diagnosing ICU-AW (18,23). A diagnosis of ICU-AW by MRC is often delayed due to impaired consciousness. Therefore, the moment at which ICU-AW developed is unknown. ICU-AW may develop very early, because electrophysiological signs of ICU-AW have been found already within 3 days after ICU admission (24). We did not perform electrophysiological investigations. Therefore it is unknown if patients had electrophysiological alterations at the time the blood samples were taken. Finally, muscle strength might have returned to normal at the time patients woke up, because early detected electrophysiological alterations can be rapidly reversible (25).

Conclusions

This pilot study shows that systemic complement levels are

not different between patients with or without ICU-AW.

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Footnote

Conflicts of Interest: Prof. IN van Schaik received departmental honoraria for serving on scientific advisory boards and a steering committee for CSL-Behring. The other authors have no conflicts of interest to declare.

Ethical Statement: The institutional review board approved the BASIC study protocol (No. NL34294.018.10). Informed consent from patients or their legal representatives was obtained before study participation.

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