Intensive care unit-acquired weakness: early diagnosis, symptomatology and prognosis
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CHAP TER 7

AUTONOMIC DYSFUNCTION IN ICU-ACQUIRED WEAKNESS: A PROSPECTIVE OBSERVATIONAL PILOT STUDY

Luuk Wieske, Dean R. P. P. Chan Pin Yin, Camiel Verhamme, Marcus J. Schultz, Ivo N. van Schaik, Janneke Horn

ABSTRACT

Purpose
Intensive care unit-acquired weakness (ICU-AW) is a frequent complication of critical illness. It is unknown if patients with ICU-AW also have autonomic dysfunction, another frequent neurological complication of critical illness. We hypothesized that patients who develop ICU-AW also develop autonomic dysfunction. Furthermore, we hypothesized that patients with ICU-AW are more prone to develop autonomic dysfunction compared to patients without ICU-AW.

Methods
This was an observational cohort study of patients newly admitted to the ICU. Autonomic dysfunction was measured daily using heart rate variability (HRV) to a maximum of 15 days after admission. ICU-AW was diagnosed using the Medical Research Council score. Abnormal HRV was defined using age-matched reference values. The association between ICU-AW and HRV was analyzed using linear mixed effects models.

Results
We included 83 patients, 15 (18 %) of whom were diagnosed with ICU-AW. Of 279 HRV measurements, 204 could be analyzed. Abnormal HRV was found in all critically ill patients irrespective of the presence of ICUAW (ICU-AW 100 % (IQR: 71–100) vs. no ICU-AW 100 % (IQR: 40–100); p=0.40). Mechanical ventilation, sedation, norepinephrine, heart rate, and HRV artifacts were identified as confounders for HRV. ICU-AW was not associated with HRV.

Conclusion
Abnormal HRV is frequent in critically ill patients, both with and without ICU-AW. It is unlikely that patients with ICU-AW are more prone to develop abnormal HRV. However, we found that abnormal HRV may not be an accurate indicator of autonomic dysfunction because of confounders.
INTRODUCTION

Intensive care unit-acquired weakness (ICU-AW) is a frequent and important neurological complication of critical illness. In ICU-AW, critically ill patients develop severe weakness and sensory symptoms because of dysfunction of peripheral motor and sensory nerves and/or muscles. Despite the fact that autonomic dysfunction is a neurological complication frequently found in critically ill patients, it is unknown if patients with ICU-AW have autonomic dysfunction.

Autonomic function comprises the combined function of central autonomic nuclei, peripheral autonomic nerves, and effector organs. In critically ill patients, autonomic function has been investigated most often by measuring heart rate variability (HRV). Decreased HRV, a sign of autonomic dysfunction, has been found in patients with multiple organ dysfunction syndrome (MODS) and sepsis.

If autonomic dysfunction is present in patients with ICU-AW, this could have implications for outcome because autonomic dysfunction is associated with increasing severity of illness and mortality. Also, co-occurrence of both neurological complications in critically ill patients would suggest a common pathophysiology etiology, guiding future research.

We hypothesized that patients with ICU-AW also have autonomic dysfunction and are more prone to develop autonomic dysfunction, as assessed with HRV, compared to patients without ICU-AW. Therefore, we investigated autonomic function in critically ill patients with and without ICU-AW, using HRV.

METHODS

Design and population

This manuscript was drafted using the STROBE guidelines on reporting of observational studies. The population for this prospective observational cohort study was selected on the mixed medical/surgical ICU of the academic medical center between February and August 2012. All new admissions were eligible for inclusion except for patients who met the following criteria: neuromuscular disorder, stroke, traumatic brain injury, infection of the central nervous system, or cardiac arrest as reasons for admission; previously documented autonomic neuropathy; elective post-surgical care; pre-hospital poor functional status (Rankin ≥4), and (previous) spinal injury. Informed consent for this study was waived by the medical ethical committee of the academic medical center (W11_171 # 11.17.1015) because all study measurements were part of routine clinical care.

Assessment of ICU-AW

The Medical Research Council (MRC) score was determined as part of routine care by the attending physical therapists. Six muscle groups were investigated bilaterally when the patient was awake [Richmond Sedation and Agitation Scale (RASS) between -1 (“drowsy”) and 1 (“restless”)] and attentive (able to follow verbal commands using arms or eyelids). Individual muscle group scores were summated to calculate the MRC-sum score (MRC-SS). ICU-AW was defined as an MRC-SS less than 48 (max 60) or an average MRC less than 4 (MRC-SS divided by the number of muscle groups tested), when it was not possible to test all six muscle groups bilaterally. For analysis, we used the average MRC score.
Assessment of autonomic dysfunction

Autonomic dysfunction, using HRV, was measured daily to a maximum of 15 days after admission. HRV measurements, processing, and analysis were performed according to the following protocol built upon guideline recommendations.7

First, a 10-min continuous ECG registration was made daily from a bedside monitor during sinus rhythm. From the ECG signal, intervals between sequential R peaks were extracted, stored digitally using custom-made software (based on National Instruments LabVIEW, Austin, Texas, USA; sampling frequency, 250 Hz; high pass filter, 100 Hz) and processed offline.

Second, the 10-min period was checked for artifacts caused by interference or ectopic heartbeats using automated artifact detection software (ARTiiFACT8). The first 5 min containing the lowest percentage of artifacts was selected for further processing and the number of artifacts and total number of RR intervals in those 5 min were noted. Detected artifacts were then replaced by estimations based on cubic spline interpolation (ARTiiFACT8). HRV recordings with a percentage of artifacts greater than 20 % were excluded from analysis.9

Third, variability of the corrected RR intervals was assessed using frequency domain analysis calculated with fast Fourier transformation based on Welch’s periodogram (Kubios HRV software; version 2.010). Frequency powers, i.e., the area under the curve, of two frequency ranges were used for analysis: low frequency (LF) power (in milliseconds squared; frequency range 0.04-0.15 Hz) and high frequency (HF) power (in milliseconds squared; frequency range 0.15-0.4 Hz).7

Finally, after frequency domain transformation, group distributions of LF and HF power were investigated and recordings with either absolute LF or HF power greater than the 97th percentile were screened manually for remaining artifacts. If RR intervals less than 325 or greater than 2000 ms (indicative of a heart rate above 185 or below 30 beats per minute) were found, these were removed and the measurement was re-analyzed. Artifact detection, correction, and analysis of HRV measurements were done blinded for study outcomes.

For every HRV measurement the total sequential organ failure assessment (SOFA) score of that day and the following possible confounders were scored: patient’s age, mechanical ventilation during HRV measurement (yes/no), sedation during HRV measurement dichotomized into sedated (RASS ≤ -3 (“moderate sedation’’)) and awake (RASS > -3), treatment with and dosage of norepinephrine (microgram/kilogram body weight) during HRV measurement, and treatment with a β-blocker (yes/no) on the same day prior to HRV measurement.

Clinical data collection
From the patient file, clinical characteristics were collected including age, gender, admission type, presence of sepsis during admission11, acute physiology and chronic health evaluation IV (APACHE IV) score, presence of MODS during admission (defined as organ failure (organ-specific SOFA score >2) in two or more organ systems occurring on the same day12), diabetes mellitus and alcohol abuse.

Analysis
Depending on the distribution of the data, means with standard deviation (±SD), medians with interquartile range (IQR), or range and proportions with percentages and total numbers
are presented. Differences between proportions were assessed using Fisher’s exact test. Differences between normally distributed variables were assessed using Welch’s t test; differences between non-normally distributed continuous variables were assessed using the Wilcoxon rank-sum test.

HRV was analyzed using two approaches. First we analyzed if and how often patients had abnormal HRV. Abnormal HRV was defined as both LF and HF power below the 2.5th percentile of the corresponding age group reported by Ziegler et al.\textsuperscript{13}. Percentages of abnormal HRV measurements per patient were calculated and grouped for patients with and without ICU-AW. Differences between these grouped percentages were compared using the Wilcoxon rank-sum test. Also for patients with or without sepsis and for patients with or without MODS, grouped percentages of abnormal measurements were compared using the Wilcoxon rank-sum test.

Second, we analyzed if ICU-AW was associated with HRV using total power and LF/HF ratio as HRV parameters. Total power (LF and HF power summed) is a reflection of heart rate modulations caused by sympathetic (LF power) and parasympathetic (HF power) influences.\textsuperscript{7} The LF/HF ratio is a measure for sympathetic/parasympathetic balance.\textsuperscript{7} We used univariate and multivariate linear mixed effects models to account for repeated measures and missing data. Total power and LF/HF ratio were logarithmically transformed (using the natural logarithm) to obtain normality. Taking into account the number of patients, we chose to correct for SOFA scores and the percentage of artifacts in the multivariate model. Also, we included a propensity score (probability for every observation calculated using a logistic mixed effects model using the SOFA score at the day of measurement, percentage of artifacts present in the measurement, mean heart rate during measurement, sedation at the moment of measurement, norepinephrine dosage at the moment of measurement, and mechanical ventilation at the moment of measurement as covariates) in the multivariate model. The effect of ICU-AW on total power and LF/HF ratio is reported as percentage change (with 95% confidence interval; CI) when ICU-AW is present. In the multivariate model interaction terms for confounders and ICU-AW were analyzed and left out because of non-significance. Model assumptions were verified. Additionally, we analyzed the influence of HRV measurements that could not be analyzed by repeating the analyses mentioned above on data where HRV measurements that could not be analyzed were imputed by multivariate imputation (see Table E2 in the data supplement for details).

We also analyzed associations between possible confounders and total power or LF/HF ratio. These associations were investigated corrected for same day total SOFA scores. The effects of possible confounders on total power and LF/HF ratio are reported as percentage change (with 95% CI) when either the confounder is present, in case of binary confounders, or with every unit increase of the confounder, for continuous confounders.

Data on HRV in patients with ICU-AW necessary for a power calculation are not available, so this could not be performed for this study. Statistical significance is defined as $p<0.05$. Analyses were done using R (version 2.15.1; R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

Figure 1 displays the study flow chart and patient characteristics are given in Table 1.
HRV MEASUREMENTS

Of the 279 HRV measurements recorded in 83 patients, 204 (73 %) could be used for analyses. HRV measurements could not be used for analyses because too many artifacts or no sinus rhythm was present (N = 67) and logistical reasons (N = 7). Characteristics of measurements that could or could not be analyzed are described in Table E1 in the data supplement.

HRV in patients with ICU-AW

Both patients with and without ICU-AW had abnormal HRV with a median of 100 % (IQR 71–100 and 40–100, respectively; p:0.40) of measurements, when compared to reference values. Also patients with or without sepsis and patients with or without MODS had abnormal HRV in 100 % of measurements (for sepsis, IQR 33-100 and 88-100, respectively, and p:0.16; for MODS, IQR 50-100 and 33-100, respectively, and p:0.50).
autonomic dysfunction in ICU–acquired weakness

Figure 2 displays the median and spread of LF/HF ratio and total power for patients with and without ICU-AW for the first 7 days of admission.

We identified several confounders that were associated with HRV. The percentage of artifacts was independently associated with both total power and the LF/HF ratio, whereas mechanical ventilation, sedation, norepinephrine dosage, and heart rate were independently associated with total power only (Table 2).

Using univariate analysis, we did not find an association between ICU-AW and total power or LF/HF ratio ($p$: 0.07 and 0.36 respectively; Table 3). Using multivariate analyses, when corrected for percentage of artifacts present in the HRV measurement and SOFA score at the day of HRV measurement, we found no association was between ICU-AW and total power or LF/HF ratio ($p$: 0.53 and 0.75 respectively; Table 3). Additionally, when corrected using a propensity score, no association between ICU-AW and total power or LF/HF ratio was found ($p$: 0.32 and 0.37, respectively; Table 3). Imputing missing HRV measurements did not change these results (Table E2 in the data supplement).

<table>
<thead>
<tr>
<th></th>
<th>ICU-AW (N:15)</th>
<th>no ICU-AW (N:68)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>male, n (%)</td>
<td>9 (60)</td>
<td>41 (60)</td>
<td>1.00</td>
</tr>
<tr>
<td>age, mean year ±SD</td>
<td>60 ± 13</td>
<td>59 ± 16</td>
<td>0.81</td>
</tr>
<tr>
<td>DM in medical history, n (%)</td>
<td>2 (13)</td>
<td>11 (16)</td>
<td>1.00</td>
</tr>
<tr>
<td>alcohol abuse in medical history, n (%)</td>
<td>1 (7)</td>
<td>2 (3)</td>
<td>0.45</td>
</tr>
<tr>
<td>admission type</td>
<td>medical, n (%)</td>
<td>12 (80)</td>
<td>47 (69)</td>
</tr>
<tr>
<td></td>
<td>surgical, n (%)</td>
<td>3 (20)</td>
<td>21 (31)</td>
</tr>
<tr>
<td>LOS ICU, median days (IQR)</td>
<td>11 (4-17)</td>
<td>3 (2-5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>APACHE IV score, mean ±SD</td>
<td>83 ± 27</td>
<td>66 ± 22</td>
<td>0.06</td>
</tr>
<tr>
<td>sepsis during admission, n (%)</td>
<td>14 (93)</td>
<td>50 (74)</td>
<td>0.17</td>
</tr>
<tr>
<td>MODS during admission, n (%)</td>
<td>14 (93)</td>
<td>31 (46)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>average MRC, median (IQR)</td>
<td>3.3 (2.6-3.6)</td>
<td>5 (4.3-5)</td>
<td></td>
</tr>
<tr>
<td>day of MRC examination, median (IQR)</td>
<td>10 (5-15)</td>
<td>4 (2-6)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

ICU-AW: Intensive Care Unit – acquired weakness; DM: diabetes mellitus; LOS ICU: length of stay in the intensive care unit; APACHE IV: Acute Physiology and Chronic Health Evaluation IV score; MODS: Multiple Organ Dysfunction Syndrome; MRC: Medical Research Council

Table 1. Patient characteristics. Table describing demographics, relevant medical history, admission characteristics and Medical Research Council scores for patients with and without Intensive Care Unit-acquired weakness.
Figure 2. Heart rate variability measurements
Distributions of LF/HF ratio (A) and total power (B) for patients with and without ICU-AW for the first 7 days after admission.

ICU-AW intensive care unit-acquired weakness, LF/HF ratio low frequency/high frequency ratio

Table 3. Association between Intensive Care Unit – acquired weakness and heart rate variability. Table displaying univariate and multivariate associations between Intensive Care Unit-acquired weakness and heart rate variability. The predicted effects are presented for the presence of Intensive Care Unit-acquired weakness.

<table>
<thead>
<tr>
<th>204 measurements for 69 patients</th>
<th>predicted effect (95% CI) on total power</th>
<th>p-value</th>
<th>predicted effect (95% CI) on LF/HF ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>univariate</td>
<td>-62.1 (-86.7 to 8.1)</td>
<td>0.07</td>
<td>-20.9 (-52.2 to 31.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>multivariate 1 corrected for percentage artifacts</td>
<td>-56.4 (-83.1 to 12.7)</td>
<td>0.09</td>
<td>-22.7 (-52.5 to 26.2)</td>
<td>0.30</td>
</tr>
<tr>
<td>multivariate 2 corrected for percentage artifacts and SOFA score</td>
<td>-26.4 (-71.9 to 92.5)</td>
<td>0.53</td>
<td>-8.0 (-44.8 to 53.4)</td>
<td>0.75</td>
</tr>
<tr>
<td>multivariate 3 corrected using propensity score*</td>
<td>-41.0 (-79.4 to 69.4)</td>
<td>0.32</td>
<td>-21.6 (-54.0 to 33.6)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*propensity score calculation based on: SOFA score at the day of measurement, percentage artifacts present in measurement, mean heart rate during measurement, sedation at moment of measurement, norepinephrine dosage at moment of measurement (μg/kg bodyweight), and mechanical ventilation at moment of measurement. CI: confidence interval; LF/HF ratio: low frequency/high frequency ratio; SOFA: Sequential Organ Failure Assessment score
Table 2. Associations between confounders and heart rate variability. Table displaying associations between confounders and heart rate variability, independent of severity of illness as scored by the Sequential Organ Failure Assessment score on the same day. Presented are the predicted effects for every unit increase of a confounder, when continuous, or for the presence of a confounder, when binary.

<table>
<thead>
<tr>
<th>independent association of confounders (corrected for same day SOFA score)</th>
<th>distribution (204 measurements/69 patients)</th>
<th>predicted effect (95% CI) on total power</th>
<th>p-value</th>
<th>predicted effect (95% CI) on LF/HF ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOFA score at the day of measurement</td>
<td>6 (4-9) median (IQR)</td>
<td>-20.3% (-28.2 to -11.4)</td>
<td>&lt;0.01</td>
<td>-5.3% (-11.0 to 0.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>age</td>
<td>59 ± 15 mean ±SD</td>
<td>1.9% (-1.0 to 4.9)</td>
<td>0.20</td>
<td>-1.3% (-2.8 to 0.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>percentage of artifacts in measurement</td>
<td>0 % (0-2.0) median (IQR)</td>
<td>18.7% (11.3 to 26.6)</td>
<td>&lt;0.01</td>
<td>-4.5% (-8.4 to -0.4)</td>
<td>0.03</td>
</tr>
<tr>
<td>mean heart rate during measurement (beats/minute)</td>
<td>96 ± 18.6 mean ±SD</td>
<td>-5.2% (-6.7 to -3.7)</td>
<td>&lt;0.01</td>
<td>0.3% (-0.8 to 1.3)</td>
<td>0.62</td>
</tr>
<tr>
<td>sedation (RASS ≤-3) at moment of measurement</td>
<td>47.0 % (N:95)</td>
<td>-54.0% (-75.0 to -15.3)</td>
<td>0.01</td>
<td>-71% (-36.5 to 35.9)</td>
<td>0.70</td>
</tr>
<tr>
<td>norepinephrine dosage at moment of measurement (μg/kg bodyweight)</td>
<td>0 (0-80.6) median (range)</td>
<td>-5.4% (-8.7 to -1.9)</td>
<td>&lt;0.01</td>
<td>0.1% (-2.2 to 2.4)</td>
<td>0.94</td>
</tr>
<tr>
<td>β-blocker at the day of measurement</td>
<td>8.3 % (N:17)</td>
<td>46.4% (-57.2 to 401.2)</td>
<td>0.54</td>
<td>659% (-18.3 to 237.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>mechanical ventilation at moment of measurement</td>
<td>58.3 % (N:119)</td>
<td>-71.2% (-84.4 to -47.0)</td>
<td>&lt;0.01</td>
<td>0.2% (-32.0 to 47.6)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

CI: confidence interval; LF/HF ratio: low frequency/ high frequency ratio; SOFA: Sequential Organ Failure Assessment score; RASS: Richmond Agitation and Sedation Scale.
DISCUSSION

Results from our study suggest that patients with ICU-AW frequently have abnormal HRV, however not more frequent than patients without ICU-AW. In fact, all critically ill patients in our study had abnormal HRV. This adds to previous studies that reported on abnormal HRV in specific groups of critically ill patients, like patients with sepsis or MODS. The high incidence of abnormal HRV could mean that autonomic dysfunction is present in all critically ill patients or, alternatively, that abnormal HRV in critically ill patients is influenced by confounders and as such is not a reliable indicator of autonomic dysfunction in critically ill patients.

Autonomic dysfunction in ICU-AW

We did not find that patients with ICU-AW are more prone to develop autonomic dysfunction with compared to patients without ICU-AW, when assessed with HRV, although this study has some methodological limitations, as discussed below. Some studies have provided clues that in patients with ICU-AW peripheral autonomic nerves may be affected and that autonomic dysfunction and ICUAW may be related. Autonomic dysfunction might also be explained by dysfunction in other parts of the autonomic nervous system or in the effector organs. Indeed, both have been found in critically ill patients, but these studies did not investigate patients with ICU-AW per se. We speculate that autonomic dysfunction and ICU-AW may have different pathophysiological etiologies. It might be that peripheral autonomic nerves are affected in ICU-AW but dysfunction in other parts of the autonomic nervous system is a more important cause of autonomic dysfunction. Alternatively, it might be that dysfunction of peripheral autonomic nerves is an important cause of autonomic dysfunction but peripheral autonomic nerves are affected more often and not concurrently with peripheral motor or sensory nerves, as seen in ICU-AW. However, because we found that abnormal HRV in critically ill patients can also be explained by confounders, we cannot reliably conclude that ICU-AW and autonomic dysfunction are unrelated disorders.

Confounders and HRV

In our study, we identified several confounders of HRV. We are the first to report the influence of artifacts and editing on HRV in critically ill patients, an effect known to occur in other patients. Additionally, we found that mechanical ventilation, sedation, norepinephrine dosage, and heart rate were independently associated with various parameters of HRV. In previous studies, mechanical ventilation and age were shown to influence HRV whereas sedation or administration of catecholamines were not. Furthermore, Schmidt et al. concluded that the importance of confounders is limited. Given our results, this conclusion may not be valid. We found that confounding effects were present independent of severity of illness, which is regarded to be the most important factor determining HRV.

Limitations of the study

Several limitations of this study exist. First, by not performing a power calculation, there is a risk that this study is underpowered and the lack of effect of ICU-AW on HRV could be a type II error. Also because of our sample size in relation to unexpected high number of confounders, we could not study the effect of ICU-AW on HRV adjusted for all identified confounders. Approximating this, by using a propensity score, did not lead to other results.
Second, the criteria used for selecting HRV measurements for analysis, although strict, may not have eliminated all errors from HRV measurements. Furthermore, our method for analyzing variability, using frequency domain analysis, may be inferior to other methods like non-linear analyses. However, autonomic nervous system correlates of non-linear variability analyses have not been established. Also, we did not measure HRV continuously.

Third, information bias may have been introduced because of missing MRC and HRV data. For HRV measurements, we did not find differences in measurements that could or could not be analyzed. Also, imputing HRV measurements that could not be analyzed because of too many artifacts did not change our results. However, we were not able to investigate the influence of HRV measurements missing because no sinus rhythm was present, because no reliable imputation model could be constructed for those measurements.

Finally, we did not differentiate between critical illness myopathy, critical illness neuropathy, or critical illness neuromyopathy as the underlying disorder for ICU-AW. Although critical illness neuromyopathy is thought to be most prevalent, we cannot exclude that some patients had critical illness myopathy and, in theory, autonomic dysfunction would be less likely in these patients.

Recommendations for future research
To further investigate the association between ICU-AW and autonomic dysfunction other autonomic function tests than HRV should be used. Examples of useful tests in the ICU might be the skin wrinkle test or the cold face test. It is not known if confounders also influence these tests. For HRV, a better understanding of the effect and importance of confounders may improve interpretation of results. Additionally, electrophysiological studies of peripheral nerves and muscles, and muscle biopsy can be used to investigate the relation between autonomic dysfunction and the underlying disorder (critical illness neuropathy, myopathy, or critical illness neuromyopathy) causing ICU-AW.

CONCLUSION
Abnormal HRV is frequent in all critically ill patients, both with- and without ICU-AW. It is unlikely that patients with ICU-AW are more prone to develop abnormal HRV, because no association between ICU-AW and HRV was found. However, abnormal HRV in critically ill patients may not be an accurate indicator of autonomic dysfunction because of confounders. Given these limitations, our study cannot provide a reliable conclusion on the presence or absence of autonomic dysfunction in ICU-AW. Other autonomic function tests should be used in future studies.

ACKNOWLEDGMENTS
The authors thank G. Sussenbach for his work on the development of the HRV recording equipment and software. This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl), project MARS (grant 04I-201). Dr. L. Wieske is supported by a personal grant from the Netherlands Organization for Health Research and Development (ZonMw–AGIKO grant [project number 40-00703-98-11636]).
CONFLICTS OF INTEREST
The authors declare that they have no conflict of interest.

DATA SUPPLEMENT

Table E1. Description of missing heart rate variability measurements. Table displaying distributions of confounders when heart rate variability could or could not be analyzed. Missing heart rate variability measurements because of logistical reasons (N: 7) are not included. Because the presence of sinus rhythm was evaluated throughout the day, confounders measured on specific time points are not reported for measurements that could not be analyzed because no sinus rhythm was present.

<table>
<thead>
<tr>
<th>confounders</th>
<th>HRV analyzed (N:204)</th>
<th>HRV not analyzed (N:67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>measurements in patients with ICU-AW</td>
<td>77.3 % (N:136)</td>
<td>14.8 % (N: 26)</td>
</tr>
<tr>
<td>measurements in patients without ICU-AW</td>
<td>71.6 % (N: 68)</td>
<td>21.1 % (N: 20)</td>
</tr>
<tr>
<td>SOFA score at the day of measurement, median (IQR)</td>
<td>6 (4-9)</td>
<td>8 (5-11)</td>
</tr>
<tr>
<td>percentage of artifacts in measurement, median (IQR)</td>
<td>0 % (0-2.0)</td>
<td>n.a.</td>
</tr>
<tr>
<td>mean heart rate during measurement (beats/minute), mean ±SD</td>
<td>96 ± 18.4</td>
<td>n.a.</td>
</tr>
<tr>
<td>sedation (RASS ≤-3) at moment of measurement</td>
<td>47 % (N: 95)</td>
<td>n.a.</td>
</tr>
<tr>
<td>norepinephrine dosage at moment of measurement (μg/kg bodyweight), median (range)</td>
<td>0 (0-80.6)</td>
<td>n.a.</td>
</tr>
<tr>
<td>β-blocker at the day of measurement</td>
<td>8.3 % (N:17)</td>
<td>n.a.</td>
</tr>
<tr>
<td>mechanical ventilation at moment of measurement</td>
<td>58.3 % (N:119)</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

* p-value for the difference in model fit between a model with constant and a model using HRV analyzed (categories: yes/no sinus rhythm/too many artifacts) as independent variables and each confounder as dependent variable (linear or logistic mixed effects models); †because of low prevalence of cases convergence of logistic mixed effect model was not reached

HRV: heart rate variability; ICU-AW: Intensive Care Unit-acquired weakness; SOFA: Sequential Organ Failure Assessment score; RASS: Richmond Agitation and Sedation Scale; n.a.: not applicable
Table E2. Influence of missing data on the association between Intensive Care Unit – acquired weakness and heart rate variability analyzed using multiple imputations. Table displaying univariate and multivariate associations between Intensive Care Unit-acquired weakness and heart rate variability with missing data imputed for measurements that could not be analyzed because of too many artifacts (N: 21). Missing data is imputed using multivariate imputation by chained equations corrected for a multilevel structure. The imputation model was build using the following variables: age, presence of Intensive Care Unit-acquired weakness, SOFA score, percentage artifacts, mean heart, sedation, norepinephrine dosage, β-blocker and mechanical ventilation. Pooled predicted effects for 10 iterations of 10 imputations are presented for the presence of Intensive Care Unit-acquired weakness.

<table>
<thead>
<tr>
<th>225 measurements for 74 patients (original data: 204 measurements for 69 patients)</th>
<th>predicted effect (95% CI) on total power</th>
<th>p-value</th>
<th>predicted effect (95% CI) on LF/HF ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>univariate</td>
<td>-63.4 (-88.0 to 11.4)</td>
<td>0.08</td>
<td>-16.8 (-49.8 to 38.1)</td>
<td>0.47</td>
</tr>
<tr>
<td>multivariate 1 corrected for percentage artifacts</td>
<td>-62.1 (-87.3 to 13.3)</td>
<td>0.08</td>
<td>-17.9 (-50.2 to 35.5)</td>
<td>0.44</td>
</tr>
<tr>
<td>multivariate 2 corrected for percentage artifacts and SOFA score</td>
<td>-35.3 (-78.3 to 92.8)</td>
<td>0.43</td>
<td>-5.6 (-45.4 to 63.3)</td>
<td>0.84</td>
</tr>
<tr>
<td>multivariate 3 corrected using propensity score*</td>
<td>-50.5 (-83.7 to 50.2)</td>
<td>0.21</td>
<td>-9.4 (-46.9 to 54.4)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*propensity score calculation based on: percentage artifacts present in measurement, mean heart rate during measurement, sedation at moment of measurement, norepinephrine dosage at moment of measurement (μg/kg bodyweight), and mechanical ventilation at moment of measurement.

CI: confidence interval; LF/HF ratio: low frequency/ high frequency ratio; SOFA: Sequential Organ Failure Assessment score
REFERENCES


