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BRIEF REPORT

Hourly assessments of single-item immune fitness demonstrate its consistency during the day

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Abstract

Immune fitness is the body's capacity to respond to health challenges (such as infections) by activating an appropriate immune response. Adequate immune fitness is crucial to maintaining health and preventing and resolving disease. Control day data from an experimental study among 29 healthy volunteers were evaluated to determine the consistency of immune fitness ratings throughout the day. The single-item immune fitness scale was completed hourly, from 09:30 to 15:30. As a comparison, mood was assessed with the Profiles of Mood States-Short Form, including subscales for vigor, fatigue, tension, depression, and anger. The analyses revealed that single-item immune fitness scores were stable throughout the day. In contrast, as expected, assessments of fatigue and vigor varied throughout the day, showing more energy in the morning assessments and less energy in the afternoon assessments. Tension, depression, and anger scores also remained stable throughout the day. In conclusion, this study demonstrates that single-item immune fitness ratings remain consistent and stable throughout the day. Therefore, the time of day at which participants assess their immune fitness does not influence the outcomes in clinical trials. Future studies in larger, more diverse samples can be conducted to confirm these findings.

Keywords: Immune fitness; Immunity; Self-report; Mood; Anxiety; Depression; Tension; Anger; Vigor

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1. Introduction

Immune fitness is the body's capacity to respond to health challenges (such as infections) by activating an appropriate immune response.[1] Immune fitness represents the overall perception of how well one's immune system functions. A single-item assessment of immune fitness is assumed to encompass various aspects of experiencing immune-related complaints, including the type, severity, duration, frequency of occurrence, and their impact on daytime functioning.[1] Immune fitness is a vital determinant of health

that is associated with various health correlates (e.g., having an underlying disease or not) and lifestyle factors (e.g., attaining a healthy diet), quality of life,[1] as well as salivary concentrations of immune biomarkers.[2]

Adequate assessment of momentary immune fitness is essential in clinical practice and research. A quick and reliable way to assess immune fitness is through a single-item scale, ranging from 0 (very poor) to 10 (excellent).[1]

Previous research revealed that the single-item immune fitness scale has an excellent test-retest reliability,[3] and scores are stable throughout the year, notwithstanding variability in immune-related disease risk between the seasons.[4] This study evaluates the extent to which immune fitness ratings are stable throughout the day. The latter is essential for clinical trial design, as these findings can inform whether multiple assessments throughout the day are needed or whether a single assessment will provide the required information on immune fitness. To achieve this objective, the single-item immune fitness scale was completed hourly, from 09:30 to 15:30. It was expected that immune fitness ratings would remain stable during the day, unless in the case of acute illness. Mood was compared with the Profiles of Mood States-Short Form (POMS-SF).[5] The POMS-SF has five subscales: vigor, fatigue, tension, depression, and anger. For vigor and fatigue, changes throughout the day have been shown previously, with lower fatigue and greater energy in the morning compared to the afternoon.[6] In contrast, within-day fluctuations of tension, depression, and anger are usually small or absent,[6] although they may vary depending on the day of the week the assessment was conducted.[7] Therefore, the POMS-SF was included as a comparator.

2. Methodology

2.1. Patient recruitment

Subjects were recruited through local advertisements to participate in a study on alcohol hangover.[8] The University of Groningen Psychology Ethics Committee approved the study (protocol code: ppo-015–002, approval date: 3 September 2015), and written informed consent was obtained from all study participants. All participants were social drinkers. Both males and females, 18–30 years old, were included if they were healthy (no acute or chronic disease) and were not using medication or illicit drugs. Exclusion criteria included individuals experiencing minor (immune-related) illness, smokers, individuals who had poor sleep the night before the test day, and those who had consumed alcohol the day before the assessment. A medical examination by the study physician confirmed the health status and the medication history of the participants. A urine drug test (Alfa Scientific Designs Inc., United States) was conducted to verify the absence of illicit drug use (including amphetamines, barbiturates, cannabinoids, benzodiazepines, cocaine, and opiates). The absence of recent alcohol consumption was verified through a breath alcohol test with the Alcotest 7410 Breath Alcoholmeter (Dräger, the Netherlands). Participants were financially reimbursed (€120) for participating in the study.

2.2. Data collection

For the current analyses, only data from a control day (no intervention, no previous day alcohol consumption) were used. The study was conducted at Utrecht University. A standardized breakfast was served at 09.00 and a standardized lunch at 12:00. Participants were allowed to drink only moderate, standardized amounts of water, with no other beverages or food intake. Immune fitness was assessed hourly (09:30–15:30), with a single-item scale ranging from 0 (very poor) to 10 (excellent). The immune fitness score was indicated on a 10-cm Visual Analog Scale by placing an "X" and measured in cm, with higher scores implying better immune fitness. Mood was assessed with the POMS-SF.[5] The Dutch version of the POMS-SF has 32 items, which are scored on a 5-point Likert scale, ranging from 0 (not at all) to 4 (extremely). Sum scores for five subscales can be calculated, including vigor, fatigue, tension, depression, and anger. Higher scores reflect greater severity.

2.3. Statistical analysis

Statistical analyses were conducted with the Statistical Package for the Social Sciences software (Version 30). Means and standard deviations were computed. The data were not normally distributed; therefore, multiple timepoint assessments throughout the day were compared using Friedman's two-way analysis of ranks test. After Bonferroni's correction for multiple comparisons, differences were considered significant if $p<0.0083$. Spearman's correlations were computed between the assessments at different time points. After Bonferroni's correction for multiple comparisons, the correlations were considered significant if $p<0.0083$. To further evaluate the stability of the assessments over time, the intraclass correlation and the corresponding 95% confidence interval (CI) were computed using a single measurement absolute-agreement, a 2-way mixed-effects model. The limits of the 95% CI interval provide information about the stability of the assessments over time, and these values were interpreted as follows: <0.5 (poor stability), 0.5–0.75 (moderate stability), 0.75–0.9 (good stability), and >0.90 (excellent stability).[9]

3. Results

A total of 29 subjects participated in the study, including 15 males and 14 females. Their mean age was 21.1 ± 2.0 years old. Single-item immune fitness ratings, listed in Table 1, remained stable throughout the day (p=0.572), with no significant differences between the assessments (Figure 1A). Spearman's correlations between the immune fitness assessments were highly significant (all p<0.001), and ranged from r = 0.760 (between 09:30 and 14:30) to r = 0.942 (between 10:30 and 11:30). The intraclass correlation was 0.913 with a 95% CI ranging from 0.860 to 0.953, suggesting good to excellent stability between the assessments.[9]

As a control, mood assessments were assessed through the POMS-SF. The data are summarized in Table 1. An expected significant decrease in vigor was observed throughout the day (p < 0.001) (Figure 1B). Significant differences in vigor were observed between 09:30 and 15:30 (p<0.001), between 10:30 and 15:30 (p<0.001), and between 11:30 and 15:30 (p<0.001). Significant fluctuations throughout the day were also found for fatigue (p=0.021) (Figure 1C). After an initial decrease in fatigue in the morning, fatigue scores increased in the afternoon. The greatest differences in fatigue were observed between 09:30 and 11:30 (p=0.033) and between 11:30 and 15:30 (p=0.030); however, these were not statistically significant after applying the stringent Bonferroni correction to account for multiple comparisons. No significant fluctuations throughout the day were observed for depression (p=0.618), tension (p=0.344), and anger (p=0.090) (Figure 1D-F).

4. Discussion

The analyses revealed that single-item immune fitness scores were stable throughout the day. These findings support previously published high correlations between same-day test–retest assessments of immune fitness.[3] In contrast, as expected, assessments of vigor varied significantly throughout the day, showing more energy in the morning and less energy in the afternoon assessments. Variations in fatigue and other mood scales were modest and not statistically significant.

Table 1. Single-item immune fitness ratings and mood assessments throughout the day

Time	Immune fitness	Vigor	Fatigue	Depression	Tension	Anger
09:30	8.1 (1.4)	12.1 (2.5)*	1.4 (1.5)	0.5 (1.3)	0.8 (1.2)	0.4 (0.7)
10:30	8.2 (1.2)	11.7 (3.0)*	0.9 (0.9)	0.2 (0.8)	0.3 (0.7)	0.1 (0.4)
11:30	8.2 (1.2)	11.4 (2.9)*	0.6 (0.9)	0.2 (0.9)	0.5 (0.7)	0.1 (0.4)
12:30	8.1 (1.3)	10.1 (4.1)	0.9 (1.3)	0.5 (1.2)	0.5 (0.8)	0.4 (0.9)
13:30	8.3 (1.2)	9.9 (4.8)	1.2 (1.4)	0.3 (1.2)	0.7 (1.1)	0.2 (0.8)
14:30	8.3 (1.2)	10.3 (4.1)	1.0 (1.7)	0.4 (1.4)	0.7 (1.2)	0.2 (0.5)
15:30	8.2 (1.3)	9.3 (3.9)	1.5 (2.0)	0.3 (1.0)	0.4 (0.9)	0.3 (0.6)

Note: Data are presented as mean and standard deviation for each time point. Significant differences from the 15:30 assessment are indicated by * (Bonferroni-corrected threshold for six comparisons: P<0.0083).

A key strength of the study was the thorough screening and careful selection of participants. All were healthy male and female volunteers, free from any medical or psychological conditions that could have influenced the study outcomes. In addition, participants were screened for alcohol and drug use to ensure the reliability of the assessments. The participants were continuously monitored during the day, and no specific activities were scheduled. Most participants were relaxing between the assessments (for example, by reading a book). Furthermore, the POMS-SF is a highly validated and reliable scale to assess mood,[5] and the single-item immune fitness scale has also been previously validated and used in many studies.[1]

Notably, in the current study, an early version of the single-item immune fitness scale was used. Participants marked their immune fitness on a Visual Analog Scale ranging from 0 (very poor) to 10 (excellent), and immune fitness was measured in cm. In the latest version of the single-item immune fitness scale, immune fitness is assessed on an 11-point scale.[1] However, results would not be expected to differ if the study were repeated using the 11-point scale instead of the Visual Analog Scale. While it has been argued that the mode of administration of questions may affect scoring,[10] a previous systematic review comparing numerical rating scales with Visual Analog Scales for pain intensity measurement revealed no significant differences in outcomes between the two test formats.[11] Other studies indicated that the mode of administration (i.e., paper-based versus digital) also did not influence scoring on Visual Analog Scales.[12] These findings suggest that the stability in immune fitness scores observed with the Visual Analog Scale will be the same when using an 11-point numerical rating scale.

A limitation of the study was its relatively small sample size. While 29 was a sufficient number for the experimental study by Merlo *et al.*,[8] it is essential to further investigate and replicate the current findings in a larger sample. Although differences are expected, a larger sample would allow for evaluating potential sex and age differences in the consistency of immune fitness scores throughout the day. In addition, it is important to investigate whether the immune fitness ratings also remain consistent throughout the day in individuals with compromised, reduced immune

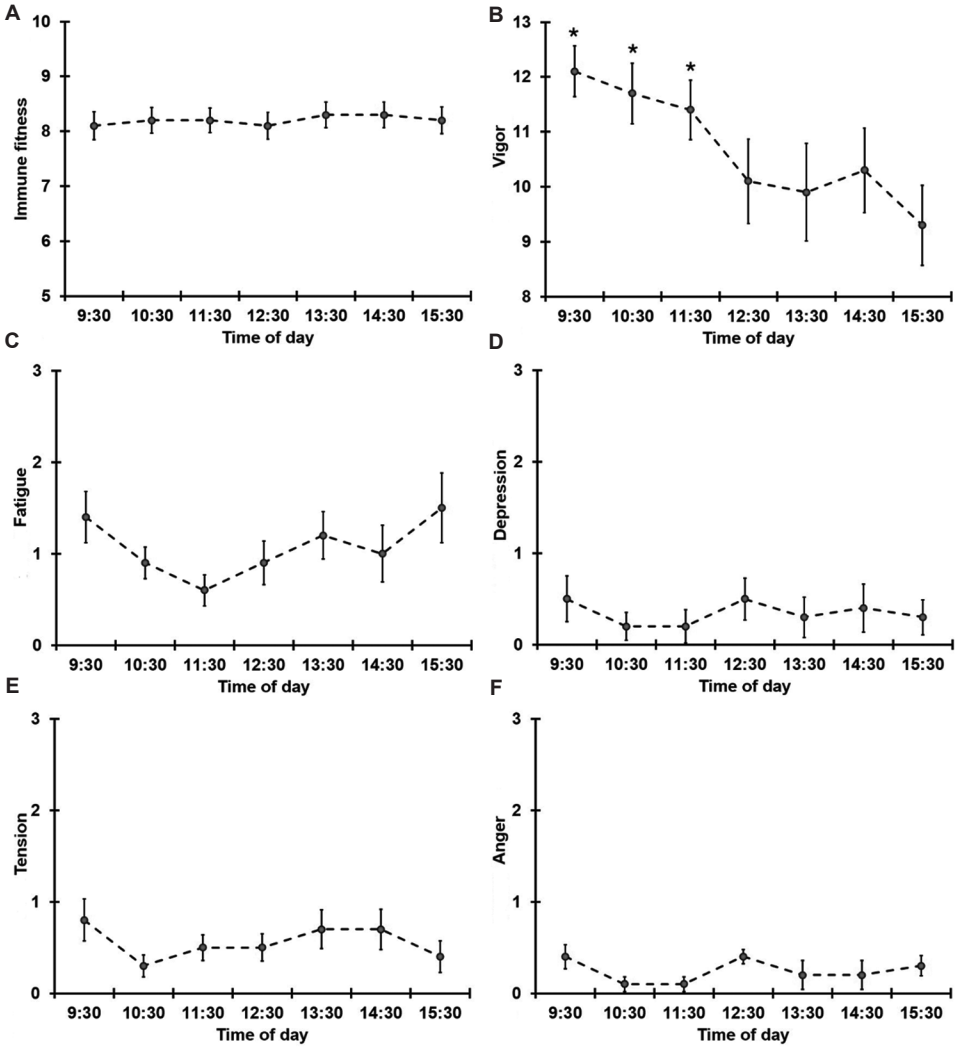

Figure 1. Single-item immune fitness ratings and mood assessments throughout the day. Mean and standard errors are shown for each time point for (A) immune fitness, (B) vigor, (C) fatigue, (D) depression, (E) tension, and (F) anger. Significant differences from the 15:30 assessment (Bonferroni-corrected threshold for six comparisons: $p<0.0083$) are indicated by*.
Abbreviation: POMS-SF: Profile of Mood States-Short Form

fitness, such as those with underlying diseases. Another limitation is that the assessments were only conducted between 09:30 and 15:30. To fully confirm the reliability of immune fitness ratings throughout the day, future studies should consider extending the hourly assessments until participants go to sleep, allowing for a more complete evaluation of potential diurnal variation.

The implications of the current findings are critical to future clinical trials that assess immune fitness. The findings suggest that the timing of evaluation during the test day does not affect immune fitness outcomes. This allows researchers more freedom to implement the immune fitness assessment into their study design. The results further suggest that immune fitness can be reliably assessed without needing multiple assessments, in contrast to other constructs that show variability during the day, such as daytime sleepiness and vigor.

5. Conclusion

This study revealed that single-item immune fitness ratings are consistent and stable throughout the day. Therefore, immune fitness can be reliably assessed at any time during a test day in clinical trials, without requiring multiple assessments. Future studies in larger, more diverse samples should confirm these findings.

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Conflict of interest

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Author contributions

Conceptualization: All authors
Formal analysis: Joris Verster
Investigation: Aurora J.A.E. van de Loo
Methodology: Joris Verster
Writing–original draft: Emina Išerić, Joris Verster
Writing–review & editing: All authors

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the University of Groningen Psychology Ethics Committee (protocol code: ppo-015–002). Written informed consent was obtained from all subjects involved in the study.

Consent for publication

Permission for publication was obtained from all subjects involved in the study.

Availability of data

The data are available from the corresponding author upon reasonable request.

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