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ORIGINAL ARTICLE

Very Low Frequency Heart Rate Variability Predicts the Development of Post-Stroke Infections

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Abstract

Stroke-induced immunodepression is a major risk factor for severe infectious complications in the immediate post-stroke period. We investigated the predictive value of heart rate variability (HRV) to identify patients at risk of post-stroke infection, systemic inflammatory response syndrome, or severe sepsis during the post-acute interval from days 3 to 5 after stroke onset. A prospective, observational monocentric cohort study was conducted in a university hospital stroke unit of patients with ischemic infarction in the territory of the middle cerebral artery without an ongoing infection at admission. Standard HRV indices were processed from Holter ECG. Recording started within the first day after the onset of stroke. Infection (primary endpoint: pneumonia, urinary tract, unknown localization) was assessed between days 3 and 5. The predictive value of HRV adjusted for clinical data was analyzed by logistic regression models and area under the receiver operating characteristic curve (AUC). From 287 eligible patients, data of 89 patients without event before completion of 24-h Holter ECG were appropriate for prediction of infection (34 events). HRV was significantly associated with incident infection even after adjusting for clinical covariates. Very low frequency (VLF) band power adjusted for both, the National Institutes of Health Stroke Scale (NIHSS) at admission and diabetes predicted infection with AUC = 0.80 (cross-validation AUC = 0.74). A model with clinical data (diabetes, NIHSS at admission, involvement of the insular cortex) performed similarly well (AUC = 0.78, cross-validation AUC = 0.71). Very low frequency HRV, an index of integrative autonomic-humoral control, predicts the development of infectious complications in the immediate post-stroke period. However, the additional predictive value of VLF band power over clinical risk factors such as stroke severity and insular involvement was marginal. The continuous HRV monitoring starting immediately after admission might probably increase the predictive performance of VLF band power. That needs to be clarified in further investigations.

Keywords Stroke · Infection · Pneumonia · Autonomic nervous system · Immune system · Heart rate variability · PRED-SEP

Introduction

Infection is the most common complication following acute ischemic stroke contributing to unfavorable functional outcome and mortality [1–3]. In intensive care, the incidence rates of pneumonia, urinary tract infection, and infections with unknown localization are 45%, 28%, and 29%, respectively [4]. In patients with acute ischemic stroke, preventive antibiotic therapy did not reduce the risk of poor functional outcome or death at the end of follow-up [5, 6]. Hence, timely and targeted antibiotic treatment should be based on assessment of individual risk of developing infectious complications.

Clinical risk factors for developing infectious complications after stroke include in particular stroke severity (indicated by the National Institutes of Health Stroke Scale (NIHSS)

Dirk Brämer, Heike Hoyer and Dirk Hoyer contributed equally to this work.

The study was registered under the acronym PRED-SEP (German Clinical Trials Register DRKS00003392).

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at admission), age, and dysphagia [7–9]. Two clinical scores that assess the risk of post-stroke pneumonia comprise the A^2DS^2 score (age, atrial fibrillation, dysphagia, sex, stroke severity) [8] and the PANTHERIS score (Glasgow Coma Scale, age, arterial blood pressure, white blood cells (WBCs)) [10].

Susceptibility to infections after stroke is increased by central nervous system injury-induced immunodepression (CIDS), characterized by downregulation of systemic cellular immune responses [9, 11, 12]. The cytokines released in the immune system are controlled by humoral and autonomic pathways [11, 13]. CIDS is partly mediated by alterations in the function of the autonomic nervous system (ANS). The insular cortex is a major modulator of ANS activity, and autonomic dysregulation is frequently observed in acute ischemic stroke [14]. It provides the potential linkage between CIDS, autonomic dysfunction, and increased susceptibility to infections.

Since alterations in central autonomic tone are reflected in cardiac autonomic outflow, heart rate variability (HRV) provides a noninvasive diagnostic tool to detect stroke-induced alterations in autonomic function and its impact on the development of infections. Associations between proinflammatory state and HRV were shown in the general population as well as patients with cardiovascular disease, depression, type 1 diabetes, and HIV infection [15–23]. HRV predicted related complications in neonatal sepsis [24] and subarachnoid hemorrhage [25]. Our pilot study indicated HRV as an early marker of stroke-induced infections [26].

The present study aims at investigating the predictive value of HRV adjusted for clinical data to identify patients at risk of post-stroke infection, systemic inflammatory response syndrome (SIRS) or severe sepsis during the post-acute interval between days 3 and 5 after stroke onset. The conclusive role of autonomic control will be evaluated with respect to the established clinical markers. Based on the mandatory electrocardiogram (ECG) and heart rate monitoring in the stroke unit, continuous HRV risk markers would allow early detection of developing infections, initiating additional diagnostic and timely therapy.

Materials and Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request. The PRED-SEP study was performed at the Hans Berger Department of Neurology and the Integrated Research and Treatment Center for Sepsis Control and Care (CSCC) at the Jena University Hospital and based on our pilot study [26]. The study protocol was published elsewhere [27].

Design, Study Population, and Setting

PRED-SEP was a prospective observational monocentric cohort study. Patients with acute ischemic infarction in the territory of the middle cerebral artery were eligible for enrollment. Further inclusion criteria were the following: age ≥ 18 years, NIHSS at admission ≥ 8, and written informed consent from patients or legal guardians and/or representatives. The exclusion criteria comprised a history of previous stroke, cardiogenic arrhythmias, or an ongoing infection at admission. In the subsets for the endpoints (infection, SIRS, severe sepsis), only data without a respective event within < 48 h after admission were considered.

The study was approved by institutional review committee of the Jena University Hospital and registered in the German Clinical Trials Register (DRKS00003392).

Data Acquisition and Processing

Patients were enrolled within 24 h after the onset of stroke symptoms (day 1). Clinical signs of infection, SIRS, or severe sepsis was documented daily from day 1 up to day 5 together with neurologic status and laboratory data. Baseline and follow-up data were captured electronically in the OpenClinica study database.

Twenty-four-hour Holter ECG recordings (Lifecard 12; Spacelab Healthcare, Washington) were started at 9:00 a.m. on the first day after stroke onset. Normal-to-normal (NN) interbeat intervals were exported, and standard HRV indices [28] (own software, Matlab R2012, DFT Welch power spectral density) were calculated for daytime (1:00–4:00 p.m.) and nighttime (1:00 a.m.–4:00 a.m.). Only sections with error and arrhythmia rate < 5% were considered. The following HRV indices were considered for prediction: mean heart rate (mHR), total band power (TP), very low frequency band power (VLF), normalized low frequency band power (LFnorm), ratio of low- to high-frequency band power (LF/HF), standard deviation of NN intervals (SDNN), root mean square of square-sum of adjacent NN interval differences (RMSSD).

Endpoints

Primary endpoint was an incident infection including pneumonia, urinary tract infections, and infections with unknown localization occurring during the post-acute interval from days 3 to 5 after stroke onset. Infection was defined according to the criteria of PANTHERIS study [1]: (I) pneumonia as the presence of at least one of the A and one of the B criteria ((A) abnormal respiratory examination and pulmonary infiltrates in chest X-ray and (B) productive cough with purulent sputum, microbiological cultures from the lower respiratory tract or blood cultures, leukocytosis, and elevation of CRP), (II) urinary tract infection as the presence of two of the criteria (fever (> 38.0 °C), urine sample positive for nitrite, leukocyturia, and significant bacteriuria), and (III) infection also if body temperature was above 38.0 °C on at least two determinations with

additional leukocytosis and positive blood cultures, but no determined focus.

Secondary endpoints comprised the SIRS and severe sepsis occurring in the same post-acute interval. Both endpoints were defined according to the guidelines of the German Sepsis Society [29].

All data contributing to the complex endpoints had to be documented on a daily basis up to day 5 post stroke onset. To ensure valid day 3 to day 5 diagnoses, we defined an endpoint as unknown, if the required data was incomplete.

Statistical Analysis

The sample size was calculated in order to verify the main results of our pilot study [26]. LFnorm(daytime) and VLF(nighttime) were specified as most promising predictors. Assuming an infection risk of 35%, a sample size of 136 patients was needed for the primary analysis [27].

Baseline data and HRV indices have been displayed by descriptive statistical measures according to scale. The discriminatory performance was quantified by areas under the curves (AUC) of receiver operating characteristics (ROCs). HRV indices were chosen for subsequent modeling if their AUC was significantly > 0.5 (p value, Mann-Whitney U test < 0.05, respectively).

Primarily, we evaluated the predictive value of LFnorm(daytime) and VLF(nighttime) on incident infection using several logistic regression models. We prespecified age, NIHSS at admission, diabetes, beta-blocker medication, and characteristics of infarction (size, side, affected insular cortex) as potential covariates. Covariates with a p value < 0.2 were eligible for forward selection. We limited the total number of covariates in the model according to the rule of ten events per variable [30]. Alternatively, the validated A^2DS^2 score was used as single covariate. Three odds ratios (ORs) with 95% Wald confidence intervals (CIs) and Wald test p values were estimated per HRV index: unadjusted OR, OR adjusted for

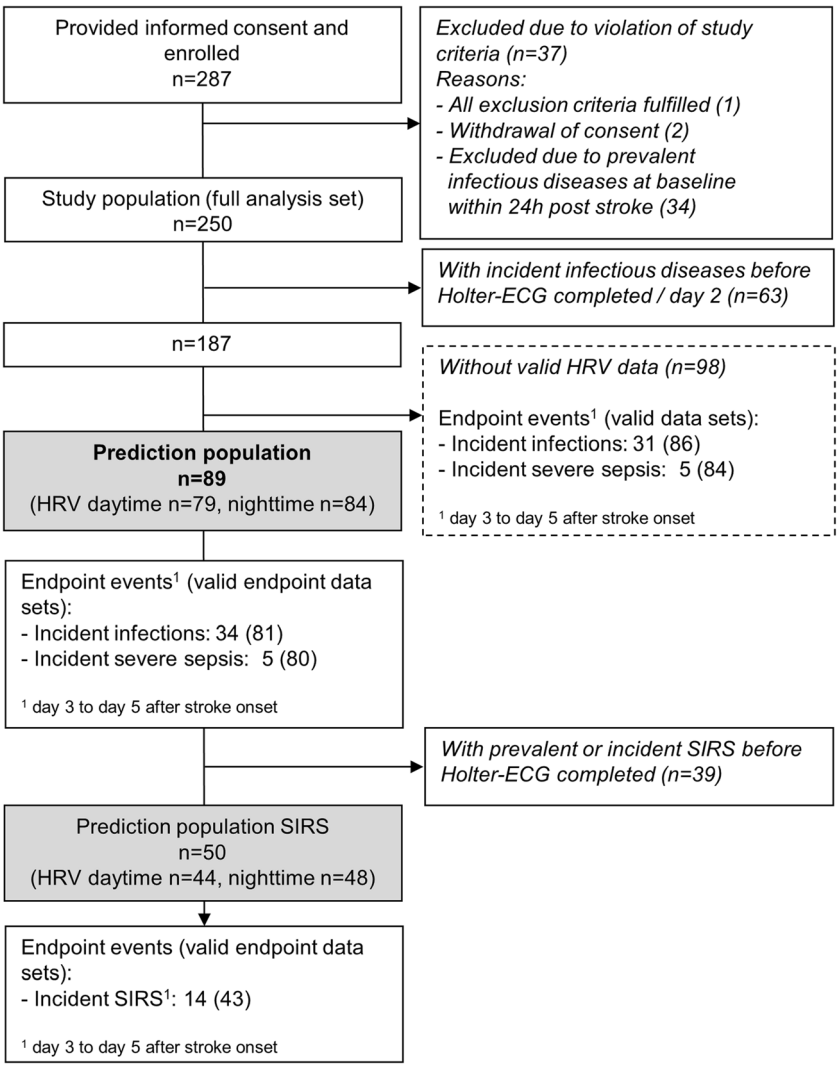

Fig. 1 Flowchart of the study population. ECG electrocardiography, HRV heart rate variability, SIRS systemic inflammatory response syndrome

selected covariates, and OR adjusted for A^2DS^2 score. We evaluated model performance by means of the Hosmer-Lemeshow test, the area under the model ROC curve (*c*-index), and AUC using cross-validation. Similarly, we investigated prediction models for severe sepsis and SIRS. SAS 9.4 was used.

Results

From the 287 enrolled patients, only those fulfilling (i) the study criteria, (ii) having appropriate daytime or nighttime HRV data, and (iii) having a confirmed endpoint diagnosis were considered for analysis. Reasons for exclusion were as follows: violation of study criteria ($n = 37$) and incident infection before completion of Holter ECG ($n = 63$). Unfortunately, Holter recordings from further 98 patients were not available for study-specific analysis due to daily care logistic problems. After quality control, 15 inadequate ECG measurements had to be excluded (ten daytime and five nighttime measurements). Thus, HRV data of 89 patients were eligible for prediction of incident infection and severe sepsis and of 50 patients for prediction of incident SIRS. Accounting further for condition (iii), the effective sample sizes varied by the analysis of daytime or nighttime HRV data and the endpoint considered. Therefore, we defined the general prediction population for the primary endpoint by conditions (i) and (ii) and reported the specific results together with the effective sample sizes. The patient flow is summarized in Fig. 1.

Concerning the baseline characteristics, patients with and without infection differed in the following: NIHSS at admission, size of infarction, involvement of the insular cortex, medical history of diabetes, and A^2DS^2 score (comprising age, atrial fibrillation, dysphagia, NIHSS at admission). All

Table 1 Baseline characteristics of patients belonging to the study population ($n = 250$, full analysis set in Fig. 1)

	Study population (full analysis set)			Total ($n = 250$)
	Incident infection day 2 ($n = 63$)	HRV missing population ($n = 98$)	Prediction population: infection ($n = 89$)	
Male (gender)[a], *n* (%)	33 (52)	49 (50)	50 (56)	132 (53)
Age (years)[a], median (IQR)	74 (61–84)	70 (61–78)	65 (55–77)	69 (60–78)
Atrial fibrillation[a], *n* (%)	7 (11)	11 (11)	2 (2)	20 (8)
Dysphagia at admission[a], *n* (%)	55 (87)	46 (47)	38 (43)	139 (56)
NIHSS admission[a], median (IQR)	18 (11–24)	12 (9–16)	11 (8–15)	12 (9–18)
A^2DS^2 score, median (IQR)	7 (6–8)	5 (4–7)	5 (4–6)	6 (4–7)
Characteristics of stroke, *n* (%)				
Right side affected	31 (49)	49 (50)	42 (47)	122 (49)
Insular cortex affected	44 (70)	44 (45)	40 (45)	128 (51)
Right insular cortex affected	25 (40)	27 (28)	20 (22)	72 (29)
> 1/3 of media territory affected	37 (59)	30 (31)	23 (26)	90 (36)
Vegetative and laboratory data, median (IQR)				
Breathing rate (1/min)	20 (16–22)	19 (17–21)	18 (17–20)	19 (17–21)
Heart rate (1/min)	75 (66–86)	75 (70–88)	76 (70–80)	75 (70–85)
Systolic blood pressure (mmHg)	140 (130–150)	142 (130–150)	150 (130–155)	140 (130–150)
Diastolic blood pressure (mmHg)	60 (50–70)	62 (55–70)	70 (60–75)	60 (55–70)
White blood cells (Gpt/l)	10.0 (8.0–11.6)	9.2 (7.1–11.1)	8.5 (7.1–11.0)	9.3 (7.2–11.2)
C-reactive protein (mg/l)	18.4 (7.2–37.0)	9.1 (3.2–22.1)	6.1 (3.2–16.0)	10.0 (3.5–24.0)
Medical history and medication, *n* (%)				
Coronary heart disease	16 (25)	17 (17)	9 (10)	42 (17)
Diabetes	15 (24)	24 (24)	19 (21)	58 (23)
Arterial hypertension	43 (68)	64 (65)	61 (68)	168 (68)
Beta-blocker	26 (41)	50 (51)	31 (35)	107 (43)
Antihypertensive drugs	39 (62)	65 (66)	52 (58)	156 (62)

IQR interquartile range (25th–75th percentiles)

[a] Components of the clinical A^2DS^2 score to predict post-stroke pneumonia (8)

of these items belong to the set of predefined covariates for prediction modeling. Baseline markers of inflammation, not a focus in our study, were slightly elevated in patients who developed an infection (C-reactive protein: AUC = 0.62, 95% CI 0.49–0.75; WBC: AUC = 0.65, 95% CI 0.53–0.77) (study population in Table 1, prediction population in Table 2).

Prediction Models for the Primary Endpoint: Infection

An infection occurring between days 3 and 5 after stroke onset was observed in 34 (42%) out of 81 patients with confirmed primary endpoints. Twenty-two patients had pneumonia, 11 urinary tract infection, and one patient both. Quartiles of HRV indices according to infection and AUC of ROC curves are shown in Table 3. The AUC (95% CI) was 0.60 (0.47–0.74, $p = 0.14$) for LFnorm(daytime) and 0.58 (0.44–0.72, $p = 0.24$) for VLF(nighttime). Two other indices qualified for prediction were as follows: VLF(daytime) with AUC = 0.70 (0.57–0.82, $p < 0.01$) and mHR(daytime) with AUC = 0.64 (0.51–0.78, $p = 0.04$).

Three predictors were allowed at the most in a model (Table 4, the infection part). Generally, models adjusting a HRV index for forward selected clinical baseline data scored highest in the AUC. The increase of VLF(daytime) was significantly associated with a lower risk of infection in all three models (OR < 1). The highest AUC of 0.80 (0.70–0.91) was generated by the model including VLF(daytime), diabetes, and NIHSS at admission (Table 4). Surprisingly, in this model, diabetes was associated with a lower risk of infection incident from days 3 to 5 (OR = 0.22, 95% CI 0.03–1.36), a result discussed below as selection bias. In all tables *p*-values of significant discriminations in italics.

For comparison, the post-stroke pneumonia score A^2DS^2 (AUC = 0.69 (0.57–0.82)) had a similar predictive value like unadjusted VLF(daytime). The model that included diabetes,

Table 2 Baseline characteristics of patients belonging to the prediction population according to the primary endpoint

Baseline characteristics	Incident infection (day 3 to day 5)			
	Unknown ($n = 8$)	No ($n = 47$)	Yes ($n = 34$)	Total ($n = 89$)
Male (gender), *n* (%)	4 (50.0)	25 (53)	21 (62)	50 (56)
Age (years)[a], median (IQR)	60 (52–65)	68 (55–77)	65 (58–78)	65 (55–77)
Atrial fibrillation[a], *n* (%)	0 (0)	2 (4.3)	0 (0)	2 (2.2)
Dysphagia admission[a], *n* (%)	1 (12)	17 (36)	20 (59)	38 (43)
NIHSS admission[a], median (IQR)	9.5 (8.5–10)	9 (8–12)	13 (10–19)	11 (8–15)
A^2DS^2 score, median (IQR)	4 (3–4)	4 (4–6)	6 (5–8)	5 (4–6)
Characteristics of stroke, *n* (%)				
Right side affected	4 (50)	21 (45)	17 (50)	42 (47)
Insular cortex affected	3 (38)	16 (34)	21 (62)	40 (45)
Right insular cortex affected	2 (25)	8 (17)	10 (29)	20 (22)
> 1/3 of media territory affected	2 (25)	7 (15)	14 (41)	23 (26)
Vegetative and laboratory data, median (IQR)				
Breathing rate (1/min)	19 (18–19.5)	18 (16–21)	18 (18–20)	18 (17–20)
Heart rate (1/min)	76 (71.5–80)	76 (70–83)	74.5 (65–80)	76 (70–80)
Systolic blood pressure (mmHg)	162 (138–175)	150 (140–155)	140 (125–150)	150 (130–155)
Diastolic blood pressure (mmHg)	78 (70–85)	65 (60–70)	60 (55–75)	70 (60–75)
White blood cells (Gpt/l)	8.0 (6.8–9.3)	8.2 (6.4–10.8)	9.8 (8.1–12.2)	8.5 (7.1–11.0)
C-reactive protein (mg/l)	8.5 (3.3–13.7)	5.0 (2.4–12.5)	9.6 (3.8–24.2)	6.1 (3.2–16.0)
Medical history and medication, *n* (%)				
Coronary heart disease	1 (12)	5 (11)	3 (9)	9 (10)
Diabetes	3 (38)	12 (26)	4 (12)	19 (21)
Arterial hypertension	6 (75)	32 (68)	23 (68)	61 (68)
Beta-blocker	3 (38)	16 (34)	12 (35)	31 (35)
Antihypertensive drugs	4 (50)	27 (57)	21 (62)	52 (58)

IQR interquartile range (25th–75th percentiles)

[a] Components of the clinical A^2DS^2 score to predict post-stroke pneumonia

NIHSS at admission, and insular cortex revealed an AUC of 0.78 (0.68–0.88) (cross-validation AUC = 0.71 (0.59–0.83)) (Fig. 2).

Prediction Models for the Secondary Endpoints: Severe Sepsis and SIRS

The day 3 to day 5 incidence for severe sepsis was 6% (5/80). Descriptive statistics of HRV indices are displayed in Table 5. Due to the low number of endpoint events, neither of HRV indices qualified for prediction modeling. For completeness, unadjusted ORs for LFnorm(daytime) and VLF(nighttime) are displayed in Table 4 (the severe sepsis part). An incident SIRS occurred in 14 (33%) out of 43 patients eligible for prediction. VLF(nighttime) with AUC = 0.75 (0.59–0.90, $p = 0.01$) and TP(nighttime) with AUC = 0.77 (0.59–0.96, $p = 0.01$) were the most promising HRV candidates for prediction (Table 6). The results of prediction modeling are shown in Table 7. The model with TP(nighttime) adjusted for size of infarction (> 1/3 of the media territory affected) performed best with AUC = 0.90 (0.80–0.99). In addition, VLF(nighttime) was significantly related to SIRS and performed similarly well. The prediction performance for the two forward selected clinical covariates (diabetes and size of infarction) was lower AUC = 0.76 (0.64–0.89).

Discussion

The autonomic and humoral pathways controlling the immune system after stroke are known [11, 13]. But, this is the first study employing HRV within 48 h after stroke onset to predict stroke-induced infection, SIRS, and severe sepsis during the post-acute phase. We aimed to identify patients at risk before clinical symptoms and paraclinical markers of infection become visible to allow for early and appropriate therapy. Focusing on stroke patients with NIHSS ≥ 8 at admission, we addressed a high-risk population which was confirmed by the infection rate of 42% in our prediction population. This is a reasonable rate taking into account that in the PANTHERIS study that did not select patients by NIHSS, the rate of post-stroke pneumonias was 31.3% [10]. Our results show that VLF precedes and predicts the development of infectious complications after stroke with a roughly similar precision to that of clinical risk

Table 3 Descriptive statistics (Q1st–25th and Q3rd–75th percentiles) for HRV indices and estimation of the area under the curve (AUC) to discriminate between patients with and without infection (prediction population)

HRV	Primary endpoint: infection (days 3–5)									
	Infection (days 3–5)			Without infection (days 3–5)			Discrimination			
	Q1	Median	Q3	Q1	Median	Q3	AUC	95% Wald CI		*p* value
Daytime, $n = 73$ (31 events)										
VLF	141.9	230.6	375.5	267.6	375.4	479.3	0.70	0.57	0.82	*< 0.01*
LFnorm (primary)	42.6	57.5	68.2	53.4	63.2	75.9	0.60	0.47	0.74	0.14
mHR	61.2	69.1	81.1	69.9	76.1	84.9	0.64	0.51	0.78	*0.04*
SDNN	18.6	25.6	49.2	23.3	32.6	42.8	0.55	0.40	0.69	0.50
RMSSD	10.5	15.3	22.2	10.9	13.8	22.1	0.53	0.39	0.67	0.68
TP	268.0	301.2	439.0	257.9	414.6	521.7	0.59	0.46	0.72	0.19
LFHF	0.75	1.38	2.94	1.22	2.11	3.94	0.61	0.47	0.74	0.11
Nighttime, $n = 76$ (31 events)										
VLF (primary)	165.2	318.7	416.5	259.7	348.3	441.5	0.58	0.44	0.72	0.24
LFnorm	39.2	49.8	68.6	46.1	60.1	74.8	0.57	0.44	0.71	0.29
mHR	61.6	71.3	78.9	64.0	68.1	77.6	0.51	0.37	0.65	0.89
SDNN	19.0	26.4	53.0	27.9	38.3	50.5	0.59	0.45	0.74	0.17
RMSSD	11.1	16.5	31.0	11.7	16.9	27.8	0.52	0.39	0.66	0.73
TP	162.5	308.9	471.2	300.3	396.5	469.0	0.61	0.47	0.75	0.11
LFHF	0.68	1.15	2.62	0.92	1.79	4.08	0.59	0.46	0.73	0.16

VLF very low-frequency band power, *LFnorm* normalized low-frequency band power, *mHR* mean heart rate, *SDNN* standard deviation of normal-to-normal interbeat intervals, *RMSSD* root mean square of square sum of adjacent normal-to-normal interbeat interval differences, *TP* total band power, *LFHF* ratio of low- to high-frequency band power

factors on admission such as stroke severity and insular cortex involvement. However, the additional predictive value of VLF over the clinical risk factors is marginal.

In our study, a reduced VLF performed best for predicting infections after stroke. This result is consistent with that reported relating to the development of infections and inflammation in different populations without stroke [22, 31–34]. The common predictive value of VLF may arise from its highly integrative nature that includes long-term regulatory circuits like thermoregulation, the renin-angiotensin system, and peripheral sympathetic vasomotor control, as well as faster autonomic control loops like vagal and sympathetic influences. Reduced VLF was found as a widespread index of globally reduced cardiac autonomic tone in different clinical conditions such as diabetes mellitus, after abdominal aortic surgery, cardiovascular disturbances, or the multiple organ dysfunction syndrome [28, 35–38].

In order to investigate the independent predictive value of VLF, we examined the influence of stroke characteristics such as age, comorbidities, paraclinical markers of infection, and the established clinical risk score A^2DS^2. We identified stroke severity, insular involvement, and diabetes as the most relevant (co-)predictors of infection. Prediction performance using this clinical data (AUC = 0.78) was higher compared to the A^2DS^2 pneumonia risk score (AUC = 0.69) and was similar to the HRV VLF model adjusted for NIHSS and diabetes (AUC = 0.80). Our results confirm previous studies demonstrating an increased susceptibility for infectious complications in patients with severe stroke [39–41]. Notably, involvement of the insular cortex as a major modulator of autonomic activity [39, 41, 42] seems to be an additional risk factor for infectious complications independent of stroke severity. Our results fit well within the experiment-based framework of

Table 4 Logistic regression models to predict infection (primary endpoint) and severe sepsis (secondary endpoint) occurring first within day 3 to day 5 after the onset of stroke based on the prediction population (the number of valid datasets is reduced due to missing data)

Endpoint/HRV variables/model	Logistic regression model			ROC model		ROC cross-validation		H-L test
	OR[a]	95% CI	*p* value	AUC	95% CI	AUC	95% CI	*p* value
Infection								
LFnorm daytime (primary), *n* = 73 (31 events)								
Unadjusted	0.79	0.59–1.05	0.11	0.60	0.47–0.74	0.55	0.41–0.68	0.53
Adjusted[b]	0.73	0.53–1.02	*0.06*	0.77	0.66–0.88	0.72	0.59–0.84	0.83
Adjusted[c]	0.84	0.62–1.14	0.27	0.71	0.59–0.83	0.66	0.53–0.79	0.51
VLF nighttime (primary), *n* = 76 (31 events)								
Unadjusted	0.98	0.94–1.01	0.13	0.58	0.44–0.72	0.51	0.37–0.65	0.55
Adjusted[d]	0.98	0.94–1.02	0.28	0.77	0.66–0.88	0.73	0.60–0.85	0.01
Adjusted[c]	0.99	0.95–1.03	0.54	0.71	0.59–0.83	0.65	0.52–0.78	0.40
VLF daytime, *n* = 73 (31 events)								
Unadjusted	0.96	0.93–0.99	*0.01*	0.70	0.57–0.82	0.66	0.54–0.79	0.46
Adjusted[b]	0.96	0.93–0.99	*0.02*	0.80	0.70–0.91	0.74	0.62–0.86	0.67
Adjusted[c]	0.96	0.93–1.00	*0.03*	0.76	0.64–0.88	0.72	0.59–0.84	0.55
Mean HR nighttime, *n* = 76 (31 vents)								
Unadjusted	0.69	0.46–1.05	0.08	0.64	0.51–0.78	0.59	0.45–0.72	0.79
Adjusted[b]	0.80	0.51–1.25	0.32	0.77	0.66–0.88	0.72	0.60–0.84	0.74
Adjusted[c]	0.75	0.48–1.15	0.19	0.71	0.59–0.83	0.66	0.52–0.79	0.19
Severe sepsis								
LFnorm daytime (primary), *n* = 72 (5 events)								
Unadjusted	0.83	0.05–1.39	0.48	0.55	0.17–0.93	–		0.48
VLF nighttime (primary), *n* = 75 (4 events)								
Unadjusted	0.95	0.89–1.02	0.19	0.63	0.19–1.00	–		0.26

[a] Per 10-unit change

[b] Adjusted for baseline NIHSS and diabetes

[c] Adjusted for A^2DS^2 (clinical score to predict post-stroke pneumonia)

[d] Adjusted for baseline NIHSS and affected insular cortex (no/yes)

stroke-induced disturbances of insular modulation of autonomic function and the increased susceptibility for infections. However, it must be noted that changes in HRV may not compulsorily reflect disturbances in insular autonomic modulation. Among others, alteration in cardiac signal transduction may play a key role. Bacterial toxins can affect cardiac pacemaker cells in the sinus node and alter cardiac signal transduction. The latter results in a depressed vagal and sympathetic signal transduction leading to reduced HRV [37, 43]. In conclusion, the predictive value of VLF seems to depend on the integrative behavior of several autonomic, humoral, and immune mediators on the systemic and cardiac levels, which are mechanisms that cannot clearly be distinguished by observing particular HRV indices. Therefore, VLF may constitute a substantiated, overarching, and robust risk marker.

We also observed that diabetes mellitus, which is an established risk factor for post-stroke infections [8], was associated with less occurrence of infections in our observational period from days 3 to 5 after admission. Most of our patients with diabetes already developed infections until day 2 after admission (27 out of 70 diabetic patients and 70 out of 212 nondiabetic patients, unadjusted OR = 1.3). In contrast, in the prediction population with valid endpoint data ($n = 81$), day 3–5 infections occurred in 4 out of 16 diabetic and in 30 out of the 65 nondiabetic patients (unadjusted OR = 0.39, adjusted OR = 0.22). Thus, the exclusion of patients with early infections may explain the apparently *protective* effect of diabetes in our prediction model. This exclusion could be avoided by the usage of continuous VLF monitoring starting immediately after admission (see also "Practical Implications" section below).

VLF provided only a slight additional predictive value compared to the use of clinical characteristics and established risk scores. However, in contrast to the initial clinical markers at admission comprising stroke severity and localization, HRV analysis potentially allows continuous risk monitoring, whereby VLF can sufficiently be assessed from moving 30 min time windows [44]. In neonates, HRV monitoring could predict sepsis and sepsis-like illness [24]. In these trials, typical changes in the individual HRV time course were predictive. With respect to prediction of stroke-induced infections, corresponding dynamic intra-individual analysis is pending.

With regard to the circadian rhythm, we investigated daytime and nighttime separately. The development of *local*

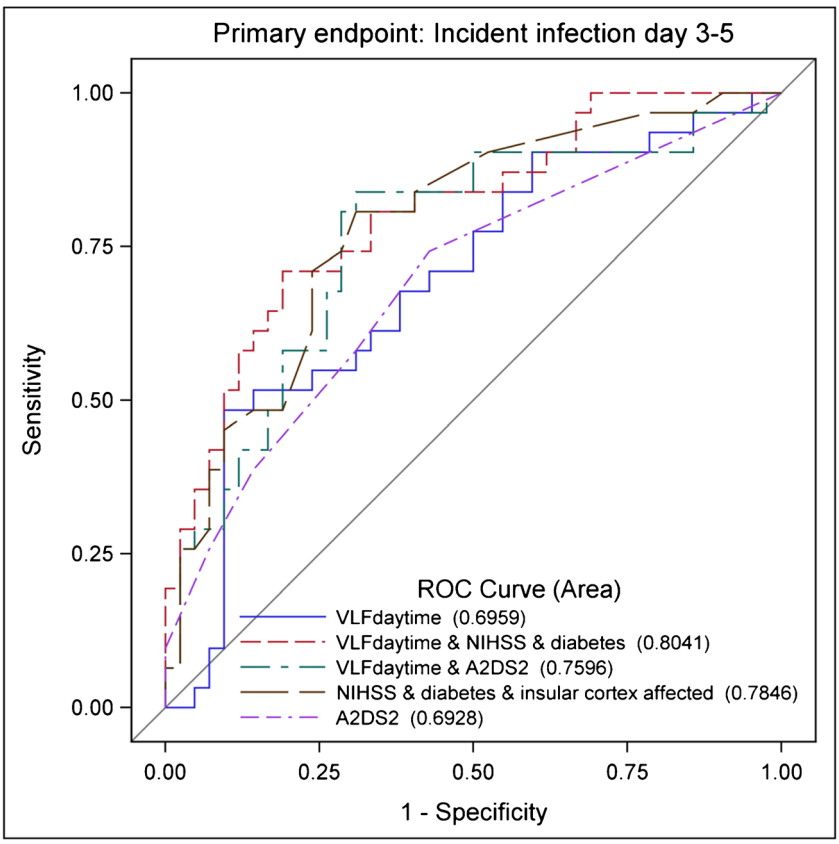

Fig. 2 Receiver operating characteristics (ROCs) resulting from the logistic regression models to predict incident infection within day 3 to day 5 after the onset of stroke. VLF very low-frequency band power, NIHSS National Institutes of Health Stroke Scale, A^2DS^2 clinical score to predict post-stroke pneumonia

infections such as pneumonia and urinary tract infections was associated with decreased VLF during daytime only. In contrast, the development of *systemic* infectious complications including SIRS and severe sepsis was associated with VLF decrease during nighttime only. This discrepancy may arise from pronounced disturbances in circadian rhythms in SIRS and sepsis. In agreement with this assumption, experimental human studies examining the effects of SIRS induced by LPS endotoxemia on day-night variation in HRV showed a more pronounced depression in HRV during nighttime compared to daytime [45]. Circadian variations in HRV in septic patients are less well studied, and their potential relevance for prognosis remains uncertain. Both autonomic immune response and cellular signal transduction in the sinus node are circadian regulated [46, 47]. Continuous HRV monitoring would be advantageous to clarify these open issues.

Strengths and Limitations

We performed a planned prospective prognostic study which adhered strictly to the previously published study protocol [27]. Clinical data was captured using electronic case report forms and managed in a quality-assured database. We used standardized algorithms for the calculation of HRV indices. A statistical analysis plan was developed for the modeling procedures prior to biometrical analysis.

However, some limitations need to be discussed. First, patients with known atrial fibrillation were not eligible for valid HRV measurement. Therefore, a substantial proportion of stroke patients (27% in [8]) were not addressed by our study, which limits the external validity of our results. Second, we defined the post-acute prediction interval from days 3 to 5 to make sure that HRV measurements actually precede the endpoints. Most incident post-stroke infections in our study occurred at day 2 (63(49%) of 128) and, hence, were excluded in our primary analysis. In a post hoc sensitivity analysis, our most promising HRV index VLF(daytime) performed similar as for the primary analysis when day 2 infections were included. The median (25th, 75th percentile) of VLF(daytime) in 21 patients with valid HRV data and a day 2 infection was 267.1 (174.3, 353.5) compared to 230.6 (141.9, 375.5) in 31 patients with day 3 to day 5 infections. Expanding the prediction period from days 2 to 5, we got the same

Table 5 Descriptive statistics (Q1st–25th and Q3rd–75th percentiles) for HRV indices and estimation of the area under the curve (AUC) to discriminate between patients with and without severe sepsis (prediction population)

HRV	Secondary endpoint: severe sepsis (days 3–5)									
	Severe sepsis (days 3–5)			Without severe sepsis (days 3–5)			Discrimination			
	Q1	Median	Q3	Q1	Median	Q3	AUC	95% Wald CI		*p* value
Daytime, $n = 72$ (5 events)										
VLF	134.7	180.5	375.5	225.8	323.0	468.0	0.65	0.34	0.96	0.28
LFnorm (primary)	29.8	62.2	69.1	47.4	62.6	72.8	0.55	0.17	0.93	0.72
mHR	74.4	81.9	87.0	66.6	73.5	82.6	0.62	0.32	0.93	0.38
SDNN	18.9	18.9	49.2	21.9	32.9	43.3	0.43	0.04	0.83	0.63
RMSSD	10.5	12.0	17.8	10.7	15.2	22.2	0.45	0.14	0.76	0.71
TP	162.0	294.6	325.0	260.2	347.2	512.7	0.66	0.39	0.93	0.24
LFHF	0.4	2.8	3.7	1.0	2.0	3.5	0.50	0.11	0.90	0.98
Nighttime, $n = 75$ (4 events)										
VLF (primary)	22.3	208.1	435.4	235.7	337.5	439.5	0.63	0.19	1.00	0.40
LFnorm	21.9	35.9	55.4	45.3	57.4	74.8	0.75	0.46	1.00	0.10
mHR	64.8	71.4	77.7	61.7	69.0	78.7	0.45	0.20	0.71	0.77
SDNN	9.5	16.8	56.2	24.6	38.0	53.0	0.70	0.25	1.00	0.19
RMSSD	11.0	16.4	27.5	11.1	16.7	28.5	0.55	0.22	0.88	0.77
TP	83.6	241.4	503.3	273.2	369.4	469.3	0.62	0.16	1.00	0.44
LFHF	0.3	0.6	1.8	0.9	1.6	3.9	0.77	0.46	1.00	0.07

VLF very low-frequency band power, *LFnorm* normalized low-frequency band power, *mHR* mean heart rate, *SDNN* standard deviation of normal-to-normal interbeat intervals, *RMSSD* root mean square of square sum of adjacent normal-to-normal interbeat interval differences, *TP* total band power, *LFHF* ratio of low- to high-frequency band power

OR of 0.96 (95% CI 0.93, 0.99) adjusted for baseline NIHSS and diabetes and a slightly lower AUC of 0.77 (95% CI 0.68, 0.87) for this model. Thus, by using real-time monitoring delivering continuous HRV data, the prediction interval could be extended, and more patients would probably benefit from early risk assessment. Third, appropriate Holter ECG recordings were missed in 98 patients due to daily care logistic problems or inadequate ECG recordings during the acute phase of stroke. Thus, we did not achieve the planned sample size by about 35%. Compared to the patients who had to be excluded due to logistic or technical problems, the prediction population was slightly younger, suffered less frequently from larger infarction, and used beta-blocker or antihypertensive drugs less frequently (Table 1). Therefore, our results apply to the characteristics of the investigated patient population only. Fourth, the smaller sample size implicates less precise parameter estimates and a lower number of endpoint events. This is especially true for SIRS and severe sepsis which, in turn, limit the number of covariates in the models and, probably, the prediction performance. Nevertheless, the sample size was sufficient to ascertain significant associations. Fifth, we used internal cross-validation but no external validation to evaluate the model performance. Sixth, our study was monocentric in order to facilitate the conduct. However, since diagnosis and treatment of patients at the stroke unit is standardized according to German guidelines, a center effect seems to be implausible.

Practical Implications

With respect to routine, we want to point out that the HRV data loss was mainly caused by technical and logistical problems of the Holter ECG management due to the limited/delayed availability of the Holter device at the time point of patient inclusion and/or correct handling of the device. These limitations can be easily overcome by the usage of ECG monitoring systems which are routinely used on the ICU and stroke units. After a minor technical extension, which is commercially available, HRV can continuously be calculated online and would immediately be available at the end of a running calculation time window of 30 min without the need of any additional Holter device. Since ECG monitoring starts immediately after admission in the ICU, such an approach would allow earlier prediction

Table 6 Descriptive statistics (Q1st–25th and Q3rd–75th percentiles) for HRV indices and estimation of the area under the curve (AUC) to discriminate between patients with and without SIRS (prediction population SIRS)

HRV	Secondary endpoint: SIRS (days 3–5)									
	SIRS (days 3–5)			Without SIRS (days 3–5)			Discrimination			
	Q1	Median	Q3	Q1	Median	Q3	AUC	95% Wald CI		*p* value
Daytime, $n = 38$ (14 events)										
VLF	149.7	271.4	403.4	270.2	416.3	514.7	0.72	0.55	0.89	*0.02*
LFnorm (primary)	46.0	64.5	69.1	59.8	67.4	78.5	0.62	0.43	0.81	0.23
mHR	65.6	78.7	85.3	69.9	76.1	82.3	0.49	0.26	0.71	0.90
SDNN	18.9	36.3	45.2	29.3	33.8	44.7	0.55	0.33	0.76	0.64
RMSSD	10.2	15.0	18.5	11.6	14.1	21.8	0.46	0.25	0.66	0.66
TP	268.0	332.7	424.2	241.9	415.6	532.4	0.59	0.40	0.78	0.36
LFHF	0.8	2.1	3.1	1.9	2.7	4.5	0.64	0.46	0.82	0.15
Nighttime, $n = 41$ (13 events)										
VLF (primary)	183.5	237.9	288.9	237.9	369.3	450.9	0.75	0.59	0.90	*0.01*
LFnorm	47.4	60.5	77.2	47.7	61.4	77.0	0.51	0.32	0.71	0.92
mHR	62.3	73.7	78.7	64.4	67.9	74.8	0.58	0.38	0.79	0.42
SDNN	18.8	39.7	53.0	28.2	40.2	58.7	0.60	0.39	0.80	0.33
RMSSD	8.5	12.3	21.8	12.3	16.8	27.7	0.63	0.43	0.83	0.19
TP	155.0	261.5	289.2	332.6	397.7	469.1	0.77	0.59	0.96	*0.01*
LFHF	1.0	1.6	3.7	1.1	1.9	4.3	0.54	0.34	0.73	0.73

VLF very low-frequency band power, *LFnorm* normalized low-frequency band power, *mHR* mean heart rate, *SDNN* standard deviation of normal-to-normal interbeat intervals, *RMSSD* root mean square of square sum of adjacent normal-to-normal interbeat interval differences, *TP* total band power, *LFHF* ratio of low- to high-frequency band power

Table 7 Logistic regression models to predict SIRS (secondary endpoint) occurring first within day 3 to day 5 after the onset of stroke based on the prediction population SIRS (the number of valid datasets is reduced due to missing data)

Endpoint/HRV variables/model	Logistic regression model			ROC model		ROC cross-validation		H-L test
	OR[a]	95% CI	*p* value	AUC	95% CI	AUC	95% CI	*p* value
SIRS								
LFnorm daytime (primary), $n = 38$ (14 events)								
Unadjusted	0.70	0.43–1.14	0.15	0.62	0.43–0.81	0.54	0.34–0.74	0.14
Adjusted[b]	0.72	0.42–1.22	0.22	0.78	0.62–0.93	0.67	0.47–0.86	0.38
Adjusted[c]	0.67	0.39–1.13	0.13	0.70	0.53–0.88	0.59	0.40–0.78	0.35
VLF nighttime (primary), $n = 41$ (13 events)								
Unadjusted	0.93	0.88–0.99	*0.01*	0.75	0.59–0.90	0.69	0.51–0.87	0.62
Adjusted[b]	0.90	0.83–0.98	*0.02*	0.88	0.78–0.99	0.83	0.70–0.95	0.82
Adjusted[c]	0.93	0.88–0.99	*0.03*	0.76	0.59–0.90	0.66	0.46–0.81	0.81
VLF daytime, $n = 38$ (14 events)								
Unadjusted	0.94	0.90–0.99	*0.02*	0.72	0.55–0.89	0.67	0.49–0.85	0.26
Adjusted[b]	0.94	0.89–1.00	*0.05*	0.82	0.55–0.89	0.76	0.60–0.91	0.95
Adjusted[c]	0.94	0.89–0.99	*0.02*	0.78	0.62–0.94	0.69	0.50–0.88	0.56
TP nighttime, $n = 41$ (13 events)								
Unadjusted	0.94	0.89–0.99	*0.03*	0.77	0.59–0.96	0.73	0.52–0.93	< 0.01
Adjusted[b]	0.92	0.86–0.98	*0.01*	0.90	0.80–0.99	0.83	0.70–0.97	0.41
Adjusted[c]	0.94	0.89–0.99	*0.03*	0.77	0.59–0.95	0.77	0.59–0.96	0.36

[a] Per 10-unit change

[b] Adjusted for infarct size > 1/3 media territory (no/yes)

[c] Adjusted for A^2DS^2 (clinical score to predict post-stroke pneumonia)

of incident infections starting directly after admission to ICU. The ECG quality of both, Holter and routine ICU monitoring, is sufficient for continuous HRV monitoring. Particular limitations for routine applications are identified [48]. According to the lowest frequency of VLF and previous systematic analyses [26, 44], we recommend the calculation of VLF values from 30 min recording windows and their averaging over 3 h. This procedure decreases the variability and allows the exclusion of disturbed windows.

In the present data, the autonomic tone, namely VLF of one daytime period, had a similar predictive value like the clinical predisposing data. The resulting question is now whether a continuous analysis of VLF starting on admission would be superior in the early identification of developing infections compared to the established paraclinical markers.

Summary/Conclusions

VLF predicts infectious complications in the immediate post-stroke period. The additional predictive value of VLF from predefined time periods compared to clinical risk factors such as stroke severity and insular involvement was marginal. However, VLF as a complementary, continuously available index of the autonomic-humoral control may have implications for early diagnosis and, consequently, timely and appropriate treatment of infections.

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Compliance with Ethical Standards The study protocol was approved by the local ethic committee of the Jena University Hospital and registered at the German Clinical Trial Register DRKS00003392. Each patient gave written informed consent.

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Appendix

Two logistic regression models for the prediction of incident infection occurring first within day 3 to day 5 after the onset of stroke

HRV-based model with covariates
1 = diabetes (no = 0, yes = 1)
$x2$ = NIHSS at admission
$x3$ = VLF (daytime)/10
Logistic regression model
Logit P (infection from day 3 to 5) = $-0.822 - 1.537 \times x1 + 0.165 \times x2 - 0.042 \times x3$
Model performance
AUC (95% CI) model = 0.80 (0.70–0.91), AUC cross-validation = 0.74 (0.62–0.86)
Hosmer-Lemeshow $p = 0.67$, R^2 (max-rescaled) = 0.35

Clinical data based model with covariates
$x1$ = diabetes (no = 0, yes = 1)
$x2$ = NIHSS at admission
$x3$ = involvement of the insular cortex (no = 0, yes = 1)
Logistic regression model
Logit P (infection from day 3 to 5) = $-2.466 - 1.096 \times x1 + 0.138 \times x2 + 1.190 \times x3$
Model performance
AUC (95% CI) model = 0.78 (0.68–0.89), AUC cross-validation = 0.71 (0.59–0.83)
Hosmer-Lemeshow $p = 0.91$, R^2 (max-rescaled) = 0.28

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