03.12.2018

SUPPLEMENTARY MATERIALS

Table S1 Registered parameters for blood sampling.

Registered parameters at each blood sampling time*
• Coffee during the day
• Fever during the day
• Time for last physical activity
• No change in sitting or lying position 15 min. prior to blood sample
• No alcohol during the day
• No meals one hour prior to blood sample
• No smoking 30 min. prior to blood sample
Registered parameters before first blood sampling
• Other time zone the last 14 days
• Night work within the last 14 days

*Between sample time the participants were informed to avoid these circadian disturbing parameters in order to decrease pre-analytical variability and generate standardization [14].

Table S2 Basic demographics of the excluded and included patients.

Characteristic	**Included (*N* = 43)**	**Excluded (*N* = 30)**	***p* value**
Age, mean years (range)	73.2	72.1	0.60
Sex,			0.14
Male, n (%)	29 (69)	15 (50)	
Female, n (%)	13 (31)	15 (10)	
Time from ictus to inclusion, mean days (±SD)	5.5 (±3.5)	8.8 (±9.8)	0.22
Admission length, mean days (±SD)	40.4 (±21.1)	40.3 (±16.8)	0.69
Smoker, n (%)	29 (70.7)	24 (80)	0.38
Hypertension, n (%)*	29 (70.7)	21 (70)	0.76
Diabetes			
Type 1, n (%)	2 (4.8)	0 (0)	0.23
Type 2, n (%)	6 (14.3)	7 (23.3)	0.33
Hypercholesterolemia, n (%)	9 (21.4)	11 (36.7)	0.15
Atrial fibrillation, n (%)	8 (19)	4 (13.3)	0.52
Depression, n (%)**	1 (2.4)	4 (13.3)	0.07
Barthel, mean score (±SD)	56.9 (±30.0)	39.1 (±31.2)	**0.02**
NIHSS, mean score (±SD)	5.0 (±4.2)	7.8 (±6.4)	**0.04**
MEQ total score, mean (±SD)	58.8 (±13.2)	63.3 (±7.5)	0.28
*Definitely Evening Type, n (%)****	1 (3)	0 (0)	
*Moderately Evening type, n (%)****	4 (12.1)	0 (0)	
*Neither Type, n (%)****	7 (21.2)	6 (25)	
*Moderately Morning Type, n (%)****	13 (39.4)	13 (54.2)	
*Definitely Morning Type, n (%)****	8 (24.2)	5 (20.8)	

*Hypertension defined as under medical treatment for hypertension at inclusion. **History of depression.

***Percentage of 33 included patients and 24 excluded patients.

Table S3 Calculated variance of melatonin blood levels between time-points.

Time	Percent variance	Percentage interval		*p* value
		Lower	Upper	
INTERVENTION UNIT				
Melatonin inclusion*	-	-	-	NS
08 a.m.	7.45	−14.46	34.97	NS
Noon	18.92	−5.33	49.37	NS
04 p.m.	13.87	−9.34	43.03	NS
08 p.m.	26.49	0.70	58.88	**0.044**
Midnight	19.37	−4.96	49.94	NS
04 a.m.	30.69	4.04	64.16	NS
Second 08 a.m.	0	.	.	.
Melatonin discharge*	-	-	-	**0.002**
08 a.m.	37.08	2.65	83.05	**0.033**
Noon	84.13	37.89	145.87	**<.0001**
04 p.m.	67.70	25.59	123.92	**0.001**
08 p.m.	44.86	7.98	94.36	**0.014**
Midnight	62.80	21.35	118.42	**0.001**
04 a.m.	36.94	2.07	83.72	**0.036**
Second 08 a.m.	0	.	.	.
Variance, inclusion vs. discharge*	-	-	-	**0.007**
08 a.m.	34.49	0.53	79.90	**0.046**
Noon	75.01	30.66	134.40	**0.0002**
04 p.m.	61.56	20.71	116.25	**0.002**
08 p.m.	33.22	−1.31	79.84	NS
Midnight	54.74	14.94	108.32	**0.004**
04 a.m.	27.80	−5.20	72.29	NS
Second 08 a.m.	0	.	.	.
CONTROL UNIT				
Melatonin inclusion*	-	-	-	**0.0003**
08 a.m.	2.73	−18.54	29.56	NS
Noon	34.52	6.66	69.65	**0.013**
04 p.m.	29.54	2.35	63.96	**0.032**
08 p.m.	41.15	11.92	78.02	**0.004**
Midnight	65.98	31.31	109.80	**<.0001**
04 a.m.	31.49	3.63	66.83	**0.025**
Second 08 a.m.	0	.	.	.
Melatonin discharge*	-	-	-	NS
08 a.m.	22.28	−6.03	59.12	NS
Noon	32.83	2.08	72.85	**0.035**
04 p.m.	33.43	2.54	73.63	**0.032**
08 p.m.	21.34	−6.75	57.89	NS
Midnight	37.74	5.56	79.73	**0.019**
04 a.m.	21.37	−6.99	58.37	NS
Second 08 a.m.	0	.	.	.
Variance, inclusion vs. discharge*	-	-	-	NS
08 a.m.	20.74	−7.12	56.95	NS
Noon	17.81	−9.86	53.98	NS
04 p.m.	19.46	−8.83	56.54	NS
08 p.m.	5.58	−19.37	38.24	NS
Midnight	12.26	−15.20	48.60	NS
04 a.m.	4.13	−20.79	36.88	NS
Second 08 a.m.	0	.	.	.

Variance between melatonin blood collection time-points. The values were log2 transformed before calculation due to non-parametric distribution. The calculated estimate was back-transformed from log2 to empirical fractiles to achieve parametric distribution, which were converted to percent variance ((x−1)*100). The percent variance at each time-point describes the percentage difference from the time-point reference “second 08 a.m.”. *Type 3 tests of fixed effects. NS = Not significant.

Table S4 Calculated variance between time-points for cortisol blood levels.

Time	Percent variance	Percentage interval		*p* value
		Lower	Upper	
INTERVENTION UNIT				
Cortisol inclusion*	-	-	-	**<0.0001**
08 a.m.	14.47	−8.50	43.21	NS
Noon	−4.67	−23.80	19.26	NS
04 p.m.	−37.00	−49.64	−21.19	**<0.0001**
08 p.m.	−47.64	−58.15	−34.50	**<0.0001**
Midnight	−62.24	−69.88	−52.66	**<0.0001**
04 a.m.	−35.31	−48.29	−19.07	**0.0002**
Second 08 a.m.	0	.	.	.
Cortisol discharge*	-	-	-	**<0.0001**
08 a.m.	4.74	−17.56	33.07	NS
Noon	−17.81	−35.31	4.41	NS
04 p.m.	−41.33	−53.81	−25.46	**<0.0001**
08 p.m.	−59.37	−68.09	−48.28	**<0.0001**
Midnight	−69.41	−75.97	−61.06	**<0.0001**
04 a.m.	−43.53	−55.64	−28.10	**<0.0001**
Second 08 a.m.	0	.	.	.
Variance, inclusion vs. discharge*	-	-	-	**<0.0001**
08 a.m.	0.21	−20.56	26.41	NS
Noon	−16.44	−33.68	5.28	NS
04 p.m.	−31.54	−46.27	−12.76	**0.002**
08 p.m.	−49.48	−60.87	−34.77	**<0.0001**
Midnight	−55.84	−66.81	−41.25	**<0.0001**
04 a.m.	−34.58	−48.70	−16.58	**0.001**
Second 08 a.m.	0	.	.	.
CONTROL UNIT				
Cortisol inclusion*	-	-	-	**<0.0001**
08 a.m.	17.13	−6.88	47.32	NS
Noon	−12.57	−30.49	9.97	NS
04 p.m.	−28.80	−43.40	−10.45	**0.004**
08 p.m.	−42.92	−54.62	−28.20	**<0.0001**
Midnight	−53.65	−63.31	−41.46	**<0.0001**
04 a.m.	−32.39	−46.35	−14.79	**0.001**
Second 08 a.m.	0	.	.	.
Cortisol discharge*	-	-	-	**<0.0001**
08 a.m.	−1.27	−22.34	25.52	NS
Noon	−39.07	−52.07	−22.54	**<0.0001**
04 p.m.	−53.65	−63.54	−41.08	**<0.0001**
08 p.m.	−70.93	−77.13	−63.04	**<0.0001**
Midnight	−74.82	−80.24	−67.91	**<0.0001**
04 a.m.	−53.53	−63.54	−40.77	**<0.0001**
Second 08 a.m.	0	.	.	**.**
Variance, inclusion vs. discharge*	-	-	-	**<0.0001**
08 a.m.	−5.65	−27.36	22.55	NS
Noon	−38.14	−52.36	−19.68	**0.0004**
04 p.m.	−50.90	−62.47	−35.78	**<0.0001**
08 p.m.	−67.76	−75.71	−57.22	**<0.0001**
Midnight	−69.42	−77.47	−58.48	**<0.0001**
04 a.m.	−50.25	−62.15	−34.60	**<0.0001**
Second 08 a.m.	0	.	.	.

Variance between cortisol blood collection time-points. Values were log2 transformed before calculation due to non-parametric distribution. The calculated estimate was back-transformed from log2 to empirical fractiles to achieve parametric distribution, which were converted to percent variance ((x−1)*100). The percent variance at each time-point describes the percentage difference from the time-point reference “second 08 a.m.”. *Type 3 tests of fixed effects. NS = Not significant.