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The Actigraphy Sleep Score: A New Biomarker for Diagnosis, Disease Staging, and Monitoring in Human African Trypanosomiasis

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Abstract. Human African trypanosomiasis (HAT) remains a serious public health problem with diagnostic and treatment challenges in many African countries. The absence of a gold-standard biomarker has been a major difficulty for accurate disease staging and treatment follow-up. We therefore attempted to develop a simple, affordable, and noninvasive biomarker for HAT diagnosis and staging. Simultaneous actigraphy and polysomnography as well as cerebrospinal fluid (CSF) white blood cell (WBC) count, trypanosome presence, and C-X-C motif ligand (CXCL)-10 cytokine levels were performed in 20 HAT patients and nine healthy individuals (controls) using standard procedures. The International HIV Dementia Scale (IHDS) was scored in some patients as a surrogate for clinical assessment. From actigraphic parameters, we developed a novel sleep score and used it to determine correlations with other HAT markers, and compared their performance in differentiating between patients and controls and between HAT stages. The novel actigraphy sleep score (ASS) had the following ranges: 0–25 (healthy controls), 67–103 (HAT stage I), 111–126 (HAT intermediate), and 133–250 (HAT stage II). Compared with controls, stage I patients displayed a 7-fold increase in the ASS ($P < 0.01$), intermediate stage patients a 10-fold increase ($P < 0.001$), and HAT stage II patients an almost 20-fold increase ($P < 0.001$). CXCL-10 showed high interindividual differences. White blood cell counts were only marked in HAT stage II patients with a high interindividual variability. The International HIV Dementia Scale score negatively correlated with the ASS. We report the development and better performance of a new biomarker, ASS, for HAT diagnosis, disease staging, and monitoring that needs to be confirmed in large cohort studies.

INTRODUCTION

Human African trypanosomiasis (HAT) is commonly known as sleeping sickness and affects thousands of people, with 70 million at risk in 37 sub-Saharan African countries.[1,2] Human African trypanosomiasis is caused by a protozoan parasite of the *Trypanosoma* genus transmitted to humans by the tsetse fly (*Glossina* genus).[3,4] Human African trypanosomiasis infection is still a very serious public health problem in some African countries as it continues to kill many who are affected, usually in remote villages that are not always accessible to national control programs for proper treatment because of various reasons.[5–8] This disease is considered a "neglected disease" in Africa with neglected associated stigma,[9,10] where it exists in two clinical forms: the West and Central African form caused by *Trypanosoma brucei (T.b.) gambiense*, representing the vast majority of reported cases (97%) and the East African form caused by *T.b. rhodesiense* (3%).[5,11] Furthermore, to make the situation even more complicated, HAT evolves classically in two main stages: hemolymphatic (stage 1) and meningoencephalitic (stage 2), each stage requiring different treatment regimens, some of them being very toxic.[9,12,13] Although in recent years a lot of effort has been made to develop newer, more effective, and less toxic HAT treatments such as fexinidazole, it is premature to think that all the outstanding issues with these new treatment regimens will be resolved, especially when their clinical performance is compared with the currently used nifurtimox–eflornithine combination therapy regimen.[9]

The problem of proper HAT staging and follow-up remains central to the management of patients, and we have recently proposed an alternative "reverse" approach to address the somewhat "circular" nature of the problem.[1] We conceptualized that using appropriate statistical methods, we could test the performance of combinations of established laboratory variables as staging biomarkers to correlate with the cerebrospinal fluid (CSF) white blood cell (WBC)/trypanosomes and clinical features of HAT. Our previous work suggested that actigraphy could objectively be used for clinical evaluation and monitoring in HAT.[14] Actigraphy uses simple, battery-run, wrist-worn appliances to record the circadian variation of rest–activity signals over several days, and these measurements have been largely validated against sleep–wake records as obtained from polysomnography (PSG); a fuller review of this can be found in the study by Njamnshi et al.[14] Building then on our past work and as a proof of concept in this study, we aimed at developing an actigraphy sleep score (ASS) in HAT patients and controls, and using this score to compare its performance in the same study sample with that of other biomarkers that have been used for disease staging such as CSF C-X-C motif ligand (CXCL) 10, CSF WBC count, CSF trypanosome, and PSG.[1,15,16]

MATERIALS AND METHODS

Study sites, patient recruitment, and clinical evaluation. We recruited 29 consenting subjects for this study, working in close collaboration with the national HAT control programs of

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Cameroon and the Democratic Republic of Congo (DRC), including 20 HAT patients (six early-stage patients, five intermediate patients, and nine late-stage patients) and nine control subjects. The HAT patients consisted of one from Cameroon (C20) and 19 from the DRC (including unpublished data from seven patients, Z1–Z7 from Njamnshi et al.[14]). All HAT patients were referred by the national control programs after a positive screening test for antibodies against *T.b. gambiense* in the blood using the card agglutination test for trypanosomiasis (CATT). The control subjects were CATT-negative subjects from the same villages as the patients in the DRC. HIV serology was negative in all the patients and control subjects. All clinical and actigraphic evaluations were performed by the same neurologist (A. K. N.), before treatment for the HAT patients, in the Neurology Department of the Central Hospital of Yaoundé, the "Centre Hospitalier Roi Baudouin I" in Kinshasa or in the HAT control field stations (DRC). The patients had a thorough general and neurological examination. The international HIV Dementia Scale (IHDS) score was performed on the controls and some of the patients as described in the following text. The patients kept a diary of daily activities throughout the investigation.

Rest–activity (actigraphic) and polysomnographic recordings. Rest–activity and polysomnographic recordings were performed using actigraphs and polysomnographs as described previously.[14] Briefly for rest–activity, octagonal BASIC motion logger H actigraphs (Ambulatory Monitoring, Inc., Ardsley, NY) were worn continuously on the nondominant wrist by the subjects, and actigraphs were only removed when the subject was taking a bath to avoid eventual damage to the device by water. For patients who were recruited in the field or at home in their villages during screening campaigns by the control programs, they were clinically evaluated immediately and actigraphs placed for recording. Furthermore, PSG was performed using standard procedures, using a computerized battery-run portable field system (Vitaport 3®, Temec Technologies B.V., Heerlen, the Netherlands): a robust 16-channel electroencephalogram (EEG) and eight-channel module, allowing excellent recording even in rural field conditions in the primary healthcare facility. Electroencephalogram electrodes were placed on the scalp of the subjects to record the EEG, using the standard 10/20 system. Polysomnography data (EEG, electrooculogram, chin and leg electromyograms, SpO_2, chest and abdominal movements, and nasal sensor signals) collected via the Vitaport-3 system were stored into the system flash-RAM cards and later transferred onto a laptop PC for visual data integrity and quality verification. The data were then archived and later exported and uploaded to a PSG scoring workstation for data processing and analysis using the Neuron Spectrum PSG software (Neuronsoft®, SAS NEUROMED, Entraigues sur la Sorgue, France) or the Neurolite® system (Neurolite AG, Belp, Switzerland).

Actogram analysis and ASS. Actograms and actigraphy waves where analyzed to create an ASS allowing to discriminate the early, intermediate, and late stages of HAT using MATLAB and Action 4 software (Ambulatory Monitoring, Inc.). More specifically, the following 33 absolute and relative actigraphy parameters were assessed in patients: three rhythm parameters (mesor, amplitude, and acrophase), the daily average activity, the *F*-distribution index of the actogram (*F*-ratio), the total sleep time estimated from actograms, the total wakefulness time estimated from actograms, the time spent sleeping at night, the time spent awake during the day, the number of wake–sleep transitions, the ratio of transition number to age, the ratio of *F*-ratio to age, the ratio of mesor to age, the ratio of amplitude to age, the ratio of night sleep time to age, the ratio of wakefulness time during the day to age, the ratio of transition number to *F*-ratio, the ratio of transition number to mesor, the ratio of transition number to amplitude, the ratio of transition number to night sleep time, the ratio of transition number to wakefulness time during the day, the ratio of *F*-ratio to amplitude, the ratio of *F*-ratio to mesor, the ratio of *F*-ratio to night sleep time, the ratio of *F*-ratio to wakefulness time during the day, the ratio of mesor to night sleep time, the ratio of mesor to wakefulness time during the day, the ratio of amplitude to night sleep time, the ratio of amplitude to wakefulness time during the day, the daily sleep ratio, the sleep onset latency, the number of awakenings during night sleep, and the ratio of night sleep time to wakefulness time during the day.

For each actigraphy parameter that offered clear inter-clinical stage differences, a score of 0 was given when values were similar to the values of control subjects (when they belonged to the range of values shared by the group), a score of 1 when values were similar to those of the early-stage patients, a score of 10 when values were alike to intermediate-stage patients' values, and a score of 20 when values were within the range of those of the late-stage patients.

International HIV Dementia Scale score. Procedures for the IHDS score were performed according to the adaptation described by Njamnshi et al.[17] In summary, the IHDS score consists of three subsets: timed finger tapping which is a measure of motor speed, timed alternating hand sequence which assesses the psychomotor speed, and recall of four items at 2 minutes which assesses memory registration and recall. Each of these subtests is rated on a scale of 0–4. A total IHDS score of ≤ 10 was considered abnormal for this study. We choose to use this clinical score as it takes a short time to perform (about 5 minutes) and can be performed by a trained nonmedical staff, which would be an advantage in rural field evaluations by national HAT control programs that hardly have specialized medical personnel.

CXCL-10 assay and WBC count in the cerebrospinal fluid. CSF was withdrawn from HAT patients following standard laboratory procedures by the national control program team in the study sites in Cameroon and the DRC. Patients were classified as early, intermediate, and late stages based on the CSF WBC count. CSF white blood count was performed following standard laboratory procedures by the national control program team. Patients' CSF was immediately aliquoted (using study codes for identification) and stored at −80°C for CSF from the Neurology Department of the Yaoundé Central Hospital (Cameroon) or stored in liquid nitrogen in the Institut National de la Recherche Biomédicale, Kinshasa (DRC). All samples were later packaged in dry ice and shipped to the Karolinska Institute for analysis. Human CXCL-10 (IP-10) BioLegend (San Diego, CA) ELISA MAX™ was used and instructions from the manufacturer followed. In brief, capture antibody was diluted 1/200 (50 µL/well) and incubated on 4C in Corning™ 96-well half-area plates (Life Sciences, Tewksbury, MA). Undiluted CSF, 50 µL/well were incubated for 2 hours at room temperature. Plates were washed, incubated with 1/200 detection antibodies for 1 hour, washed, and 1/1,000 streptoavidin-horseradish peroxidase (HRP) added. The sensitivity of the assay was 8 pg/mL.

Data analysis. Actograms were analyzed using the Action 4 software (Ambulatory Monitoring, Inc.). As described previously,[14] sleep–wake parameters were approximated from activity recorded using the algorithm of Sadeh in MATLAB (MathWorks, Natick, MA), and raw activity values were analyzed to obtain the parameters of the circadian activity rhythm using the cosinor rhythmometry method, also using MATLAB. Data of the different disease stages were compared with those of healthy controls using Analysis of Variance followed by Least Significant Difference (LSD) post hoc test for intergroup comparisons. Differences with $P < 0.05$ were significant. Data were presented as mean ± SEM. Correlations between actigraphy parameters and physiological and CSF parameters were explored using SPSS and Microsoft Excel.

Ethics statement. The study was conducted according to the principles expressed in the Declaration of Helsinki. All patients recruited received written and verbal information explaining the purpose of the study, and informed consent was obtained from subjects or parents (in case of children). Ethical consent forms were designed in English and French in Cameroon and in French in the DRC, and were also translated into local languages when necessary during administration. The study protocol was approved by the Cameroon National Ethics Committee and the Ministry of Public Health of Cameroon, as well as by the National Ethics Committee of the DRC and the Ministry of Health National Sleeping Sickness Control Program. All patients were hospitalized and cared free of charge in the Neurology Department of the Yaoundé Central Hospital for the Cameroonian patients and in the "Centre Hospitalier Roi Baudouin I" for the Congolese patients. All hospitalization charges were paid by the research project funds.

RESULTS

Actograms, activity rhythm, and hypnograms. Circadian activity rhythms and 24-hour actograms of patients classified according to increasing ASS are shown in Figure 1. Increasingly marked decreases in daily activity were observed in intermediate- and late-stage cases, as well as more fragmented actograms (Figure 1A–U). The analysis of circadian activity rhythms of patients revealed lower amplitudes in most intermediate-stage and late-stage patients, and a phase shift in some late-stage cases (Figure 1V–Y).

Figure 2 shows the hypnogram and actogram of a representative HAT stage, one case during night sleep and early morning. Coherence between hypnograms and actograms was observed in early-stage (Figure 2) and intermediate-stage patients, but not in late-stage patients (not shown here as this was not the focus of this article).

Actigraphy parameter changes and HAT stages. Table 1 presents the changes in the 19 actigraphy parameters that had good intergroup differences (clear inter-clinical stage differences) (Table 1), thus good potential for developing an ASS, among the 33 absolute and relative actigraphy parameters analyzed. The 19 actigraphy parameters that had good

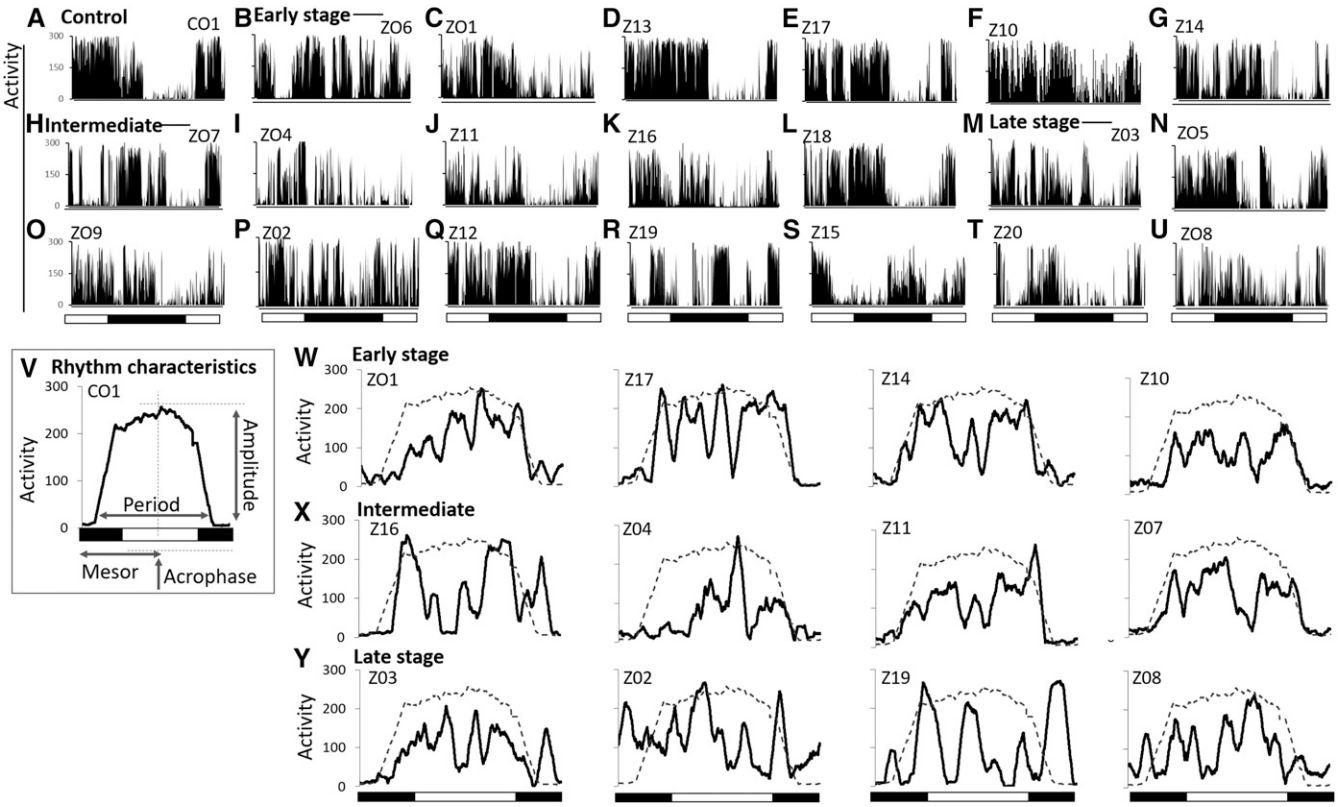

FIGURE 1. Actograms and activity rhythm. (**A**–**U**) Twenty-four–hour actograms of patients presented according to disease stage and increasing actigraphy sleep score. Note the more fragmented actograms and the marked decreases in daily activity in the intermediate- (**H**–**L**) and late-stage cases (**M**–**U**), and the phase shifts in some late-stage cases (**S** and **R**) compared with the representative control subject (**A**). (**V**–**Y**) Rhythm characteristics of a representative control subject. (**V**) Circadian rhythms of representative cases of human African trypanosomiasis patients in early (**W**), intermediate (**X**), and late (**Y**) stages. Note the lower amplitudes in most intermediate- (**X**) and late-stage (**Y**) patients.

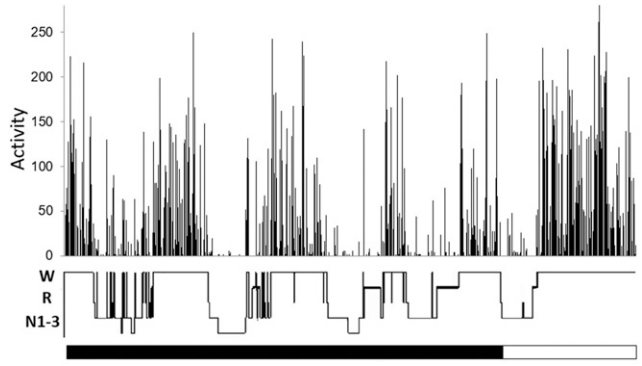

FIGURE 2. Hypnogram and actogram coherence. Coherence between hypnograms and actograms in an early-stage patient (adapted from ref. 14).

intergroup differences included the activity rhythm mesor, the *F*-ratio, the time spent sleeping at night, the time spent awake during the day, the ratio of transition number to age, the ratio of *F*-ratio to age, the ratio of mesor to age, the ratio of amplitude to age, the ratio of transition number to *F*-ratio, the ratio of transition number to mesor, the ratio of transition number to amplitude, the ratio of *F*-ratio to amplitude, the ratio of *F*-ratio to mesor, the ratio of *F*-ratio to night sleep time, the ratio of *F*-ratio to wakefulness time during the day, the ratio of mesor to night sleep time, the ratio of mesor to wakefulness time during the day, the ratio of amplitude to night sleep time, and the ratio of amplitude to wakefulness time during the day (Table 1). The ranges of values of these parameters observed in patients in the different clinical stages are presented in Table 2.

As also shown in Table 1 and 12 parameters offered statistically significant intergroup differences, namely, the activity rhythm mesor ($P < 0.05$), the *F*-ratio ($P < 0.01$), the time spent sleeping at night ($P < 0.05$), the time spent awake during the day ($P < 0.01$), the ratio of transition number to *F*-ratio ($P < 0.05$), the ratio of *F*-ratio to amplitude ($P < 0.01$), the ratio of *F*-ratio to mesor ($P < 0.01$), the ratio of *F*-ratio to night sleep time ($P < 0.05$), the ratio of *F*-ratio to wakefulness time during the day ($P < 0.05$), the ratio of mesor to night sleep time, the ratio of mesor to wakefulness time during the day ($P < 0.05$), and the ratio of amplitude to night sleep time ($P < 0.05$) (Table 1).

Actigraphy sleep score. Figure 3A presents the 15 actigraphy parameters finally used (offering better interstage cutoffs) for developing the ASS among the 19 potential parameters found, whereas Figure 3B shows the intergroup differences offered by the ASS. The 15 actigraphy parameters used included the F-ratio, the activity rhythm mesor, the time spent sleeping at night, the time spent awake during the day, the ratio of transition number to age, the ratio of F-ratio to age, the ratio of mesor to age, the ratio of amplitude to age, the ratio of transition number to *F*-ratio, the ratio of transition number to mesor, the ratio of transition number to amplitude, the ratio of mesor to night sleep time, the ratio of mesor to wakefulness time during the day, the ratio of amplitude to night sleep time, and the ratio of amplitude to wakefulness time during the day (Figure 3A). These parameters allowed for the development of an ASS with clear inter-HAT stage differences, that is, a score of 0–25 for healthy individuals, 67–103 for early-stage patients, 111–126 for intermediate-stage patients, and 133–250 for late-stage patients (Figure 3A). Compared with healthy individuals, early-stage patients displayed a 7-fold increase in the ASS ($P < 0.01$), intermediate-stage patients a 10-fold increase ($P < 0.001$), and late-stage patients almost a 20-fold increase ($P < 0.001$) (Figure 3B).

Intergroup differences in WBC count, CXCL-10 levels, and IHDS score. Figure 3B–E presents the intergroup differences offered by WBC count in the CSF (Figure 3C), CXCL-10 levels in the CSF (Figure 3D), and the IHDS score (Figure 3E), whereas Table 3 presents the clinical data, ASS, and IHDS score of patients investigated. White blood cell counts were only marked in late-stage patients and had a high interindividual variability (range: 2–5 in early stage, 6–11 in intermediate, and 22–935 in late stage) with no significant interstage difference (Figure 3C).

However, compared with healthy individuals, early-stage patients displayed a 2-fold increase in WBC count, intermediate-stage patients a 3-fold increase, and late-stage

TABLE 1
Changes in actigraphy parameters usable for human African trypanosomiasis staging

					P-value, ctrl vs.			*P*-value, early vs.		
	Control	Early	Inter	Late	Early	Inter	Late	Inter	Late	*P*-value, inter vs. late
F-ratio	664 ± 125	179 ± 53	192 ± 69	154 ± 58	0.010*	0.012*	0.007*	0.88	0.759	0.685
Mesor	150 ± 4.9	104 ± 8	89 ± 15	157 ± 19	0.002*	0.013*	0.716	0.40	0.031*	0.017*
Night sleep time (%)	63.8 ± 2	57.8 ± 2	65.3 ± 4	46.9 ± 5	0.124	0.774	0.026*	0.14	0.113	0.025*
Wakefulness time (%)	98 ± 1.1	85 ± 3.9	83 ± 6.5	74 ± 5	0.019*	0.080	0.002*	0.78	0.116	0.319
Ratio transitions/age	0.5 ± 0.1	0.8 ± 0.2	1.4 ± 0.6	1.5 ± 0	0.179	0.205	0.170	0.39	0.351	0.913
F-ratio/age	45.4 ± 29	15.4 ± 11	8.9 ± 4.2	7.3 ± 2	0.371	0.266	0.247	0.62	0.531	0.756
Mesor/age	8.2 ± 4.5	6.3 ± 4	3.6 ± 1.1	10.6 ± 3	0.748	0.358	0.681	0.52	0.411	0.074
Amplitude/age	6.7 ± 4.0	11.6 ± 9	5.5 ± 2	6.9 ± 1.3	0.644	0.797	0.967	0.54	0.636	0.578
Transitions/*F*-ratio	0.04 ± 0.02	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.133	0.190	0.032*	0.52	0.524	0.792
Transitions/mesor	0.1 ± 0.02	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.02	0.070	0.067	0.446	0.25	0.136	0.086
Transitions/amplitude	0.2 ± 0.04	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.375	0.177	0.594	0.59	0.682	0.348
F-ratio/amplitude	5.8 ± 0.8	1.4 ± 0.3	1.3 ± 0.3	1.0 ± 0.2	0.001*	0.001*	0.001*	0.74	0.259	0.404
F-ratio/mesor	4.5 ± 0.8	1.6 ± 0.4	2.2 ± 0.9	0.9 ± 0.3	0.019*	0.117	0.007*	0.57	0.160	0.226
F-ratio/night sleep time	10.3 ± 2	3.2 ± 1.1	3.1 ± 1.2	2.8 ± 0.8	0.013*	0.013*	0.010*	0.94	0.784	0.856
F-ratio/wake time	6.8 ± 1.3	2.2 ± 0.7	2.2 ± 0.7	2.0 ± 0.6	0.015*	0.015*	0.012*	0.97	0.845	0.876
Mesor/night sleep time	2.4 ± 0.2	1.8 ± 0.2	1.4 ± 0.3	4.0 ± 0.9	0.055	0.027*	0.102	0.28	0.038*	0.019*
Mesor/wake time	1.5 ± 0.1	1.3 ± 0.1	1.1 ± 0.2	2.3 ± 0.4	0.114	0.096	0.115	0.53	0.046*	0.028*
Amplitude/night sleep time	1.7 ± 0.2	2.3 ± 0.8	2.1 ± 0.6	2.9 ± 0.4	0.446	0.562	0.031*	0.81	0.551	0.337
Amplitude/wake time	1.1 ± 0.1	1.5 ± 0.4	1.5 ± 0.3	1.9 ± 0.4	0.349	0.337	0.115	0.94	0.532	0.466

* $P < 0.05$.

TABLE 2
Ranges of actigraphy parameters used to calculate the actigraphy sleep score

Parameter	As control	As early stage	As inter stage	As late stage
F-ratio	> 450	100–450	30–90	< 90
Mesor	150–160	122–149	100–121	< 100 or > 160
% Sleep time at night	> 55	48–55	45–48	< 45
% Wake time during day	> 93	85–93	50–84	< 50
Ratio of transitions to age	< 0.45	0.45–0.59	0.6–0.8	> 0.8
F-ratio to age	> 20	4–20	1–3.9	< 1
Mesor to age	2–4.85	> 20	< 2 0.4	5.86–20
Amplitude to age	1.85–4.6	> 26	< 1.85	4.7–16
Transitions to *F*-ratio	> 0.9	0.75–0.9	0.1–0.74	< 0.1
Transitions to mesor	> 0.75	0.22–0.75	0.18–0.21	< 0.18
Transitions to amplitude	> 0.55	0.3–0.55	0.1–0.29	< 0.09
Mesor to night sleep time	2.35–2.7	< 1.89	1.89–2.34	> 2.7
Mesor to day wake time	1.5–1.6	1.61–2	< 1.5	> 2
Amplitude to night sleep	1.5–2.5	> 5	< 1.5	2.5–5
Amplitude to day wake time	1.3–1.4	< 1.1	1.1–1.2	> 1.4
Score if in the range	0	1	10	20

patients almost an 8-fold increase ($P < 0.01$) (Figure 3D). Significant differences ($P < 0.05$) were also observed between the late and the earlier stages, but no significant difference was observed between the early- and intermediate-stage patients and healthy individuals (Figure 3D). CXCL-10 also displayed high interindividual differences: 6.1–699.8 pg/mL in early-stage, 19.7–690.4 pg/mL in intermediate-stage, and 30.5–1,570.1 pg/mL in late-stage patients (Table 3).

As shown in Figure 3E, the IHDS score was decreased in early-stage patients (–12.6%, not significant), intermediate-stage patients (–14.2%, not significant), and late-stage patients (–33.7%, $P < 0.05$) ($P < 0.01$) compared with healthy individuals (Figure 3E). No marked interstage difference in the IHDS score was observed (Figure 3E). The International HIV dementia scale score displayed the following ranges: 10–12 in healthy individuals, 8–10 in early-stage, 7–10 in intermediate-stage, and 6–8.5 in late-stage patients (Table 3). A significant correlation ($r = 0.69$, $P < 0.01$) was observed between the IHDS score and the ASS (Figure 3F).

Correlations of actigraphy parameters with physiological and CSF parameters. Figure 4 shows the correlation between the age and the ratio of night sleep time to the time spent awake during the day (Figure 4A), the eight actigraphy parameters that were correlated with WBC count in the CSF (Figure 4B–I), and the six actigraphy parameters that were correlated with CXCL-10 level in the CSF (Figure 4J–O). The age of the patients and control subjects correlated positively with the ratio of night sleep time to the time spent awake during the day ($r = 0.54$, $P < 0.05$) (Figure 4A).

The WBC count in the CSF was correlated negatively with two actigraphy parameters, namely, the relative night sleep time ($r = -0.68$, $P < 0.001$) (Figure 4B) and the ratio of night sleep time to the time spent awake during the day ($r = -0.51$, $P < 0.05$) (Figure 4I). The WBC count in the CSF was correlated positively with six actigraphy parameters, that is, the ratio of the number of transitions to age ($r = 0.73$, $P < 0.001$) (Figure 4C), the ratio of the mesor to age ($r = 0.58$, $P < 0.01$) (Figure 4D), the ratio of wake time to age ($r = 0.49$, $P < 0.05$) (Figure 4E), the ratio of transitions to night sleep time ($r = 0.64$, $P < 0.01$) (Figure 4F), the ratio of the mesor to the amplitude ($r = 0.64$, $P < 0.01$) (Figure 4G), and the ratio of the mesor to the night sleep time ($r = 0.58$, $P < 0.01$) (Figure 4H).

The CXCL-10 level in the CSF was positively correlated with six actigraphy parameters (Figure 4J–O), that is, the number of transitions ($r = 0.53$, $P < 0.05$) (Figure 4J), the activity rhythm mesor ($r = 0.73$, $P < 0.01$) (Figure 4K), the ratio of the transitions to the night sleep time ($r = 0.47$, $P < 0.05$) (Figure 4L), the ratio of the transitions to the time spent awake during daytime ($r = 0.48$, $P < 0.05$) (Figure 4M), the ratio of the mesor to the night sleep time ($r = 0.57$, $P < 0.01$) (Figure 4N), and the ratio of the mesor to the time spent awake during daytime ($r = 0.46$, $P < 0.05$) (Figure 4O).

DISCUSSION

In the present study, we developed and tested the performance of a novel actigraphy-based scoring system for HAT staging that we have termed the ASS, in an attempt to address the "circular problem" of finding a gold standard for HAT staging and monitoring,[1] especially given that PSG, which is the gold standard for sleep studies, is neither feasible nor specific as a routine procedure for HAT field studies in resource-limited contexts.[14] Of the 33 absolute and relative actigraphy parameters analyzed in the current study, 19 presented with clear inter-clinical–stage differences, of which 15 offered good inter-HAT–stage cutoffs, and were therefore used for developing the ASS.

The analysis of actograms revealed increasingly marked actogram fragmentation, decrease in daily activity, as well as lower amplitude and phase shift in circadian activity rhythm of intermediate- and late-stage patients, when compared with the controls. Given the coherence between actograms (rest–activity) and hypnograms (sleep–wake stage) that had already been observed in early-stage and intermediate-stage patients and disappearing in late-stage patients,[14] our results taken together further suggest that the ASS may compensate for the loss of coherence in late-stage HAT patients. This strengthens the argument for further investigation of the ASS on a larger scale with HAT patients at all stages of the disease. Interestingly, statistically significant intergroup differences were observed in 12 actigraphy parameters, supporting our earlier observation that actigraphy is a potential good tool for objective clinical evaluation and monitoring in HAT,[14] a disease for which biomarkers are direly needed to replace currently used inefficient, invasive, or cumbersome approaches for staging and posttreatment follow-up.[6,16,18–20]

The ASS evidently distinguished HAT patients (score range 67–250) from healthy individuals (score range 0–25) and

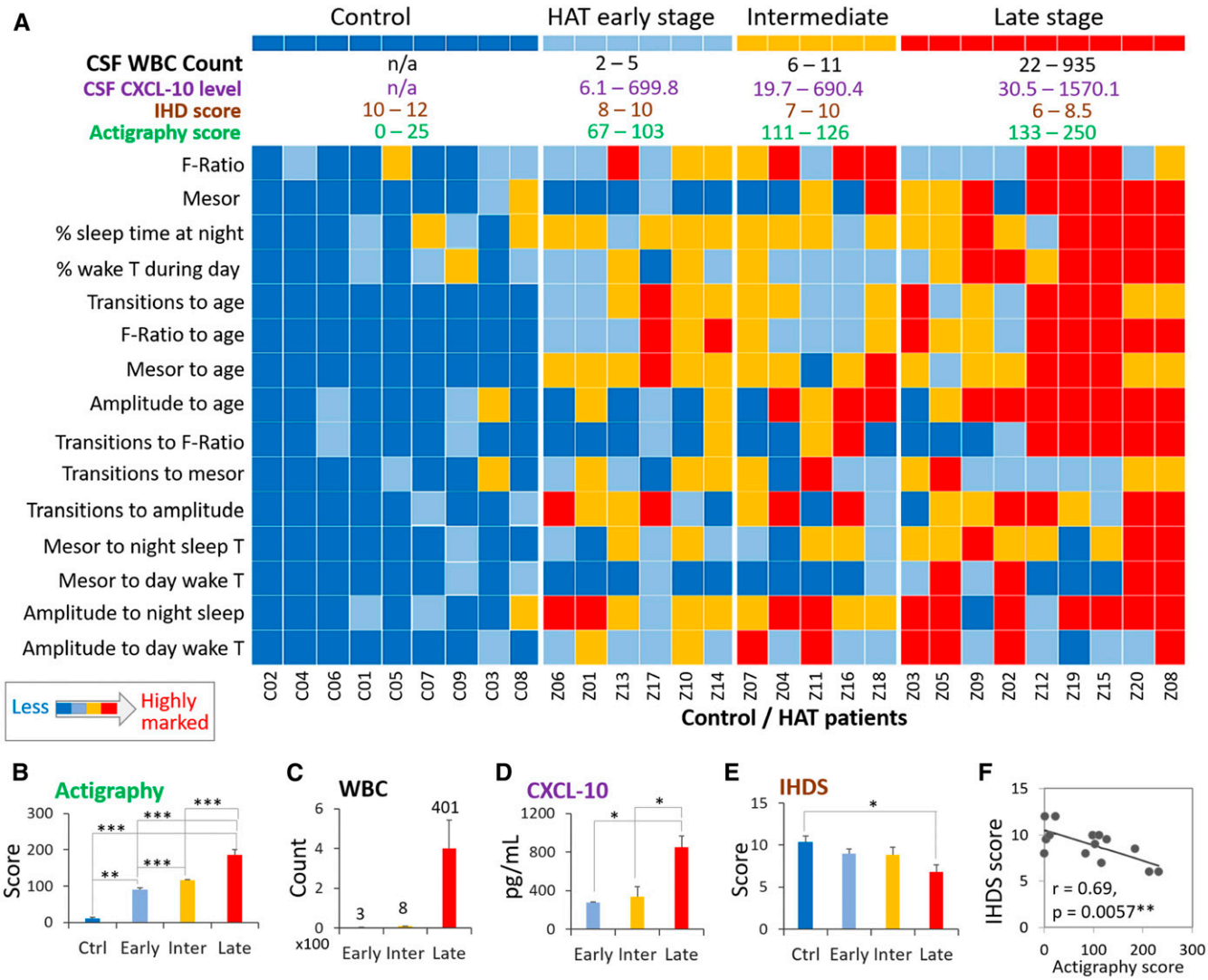

FIGURE 3. Functional scores and CSF parameters. (**A**) Ranges of CSF white blood cell (WBC) counts, CXCL-10 levels, the International HIV Dementia Scale (IHDS) score, and the actigraphy sleep score (ASS) according to disease stages, and severity of changes in actigraphy parameters used to calculate the ASS. Note that unlike CSF WBC counts and CXCL-10 levels, the ASS predicted the clinical stages accurately. (**B**–**E**) Inter-disease stage group differences in the average ASS (**B**), WBC counts (**C**), CXCL-10 level (**D**), and IHDS score (**E**). Note that intergroup differences are more marked with the ASS (**B**) than the other parameters presented (**C–E**). Data are mean ± SEM. ANOVA + LSD test: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. (**F**) Negative linear correlation between the IHDS score and the ASS. This figure appears in color at www.ajtmh.org..

offered clear inter-HAT–stage differences, with a range of 67–103 for early-stage patients, 111–126 for intermediate-stage patients, and 133–250 for late-stage patients. Early-stage patients displayed a 7-fold increase in the ASS compared with healthy individuals, intermediate-stage patients a 10-fold, and late-stage patients almost a 20-fold increase. On the other hand, concerning WBC count in the CSF, although early-stage patients displayed a 2-fold increase, intermediate-stage patients a 3-fold increase, and late-stage patients an almost 8-fold increase compared with healthy individuals (classically considered ≤ 5/mL), this parameter did not show any marked difference between the early- and intermediate-stage patients and healthy individuals, partly because of a high interindividual variability and to the occurrence of a large number of WBCs only in late-stage disease. This observation is not surprising considering the controversy on the usefulness of rather arbitrary WBC count in the CSF criterion for HAT staging.[1,5] Furthermore, our current study confirmed the observation that the demonstration of trypanosomes in the CSF, which is highly specific, however remains very insensitive,[1] as only in two of our 20 patients was the parasite detected. It is worth noting that experimental studies strongly suggest that trypanosomes traverse the blood–brain barrier even in the early stage and may be moving in and out in the late stage.[18,19,21–23] Our new biomarker, the ASS, appears to be a more objective and performing criterion for HAT diagnosis and staging into early, intermediate, and late stages than the arbitrary or insensitive criteria currently in use. In our earlier report, we already demonstrated the usefulness of actigraphy in disease monitoring through posttreatment follow-up.[14]

Considering the promising reports on the importance of CXCL-10 in the onset of disease, that is, brain invasion by trypanosomes,[16,19,22] we also looked at CXCL-10 levels in the CSF of our patients. Rather unexpectedly, CXCL-10 assay also failed to provide clear differences in value ranges between the

TABLE 3
Clinical data, ASS, and the International HIV Dementia Scale (IHDS) score

ID	Human African trypanosomiasis Stage	Actigraphy sleep score	Gender	Age (years)	CSF white blood cell	CXCL-10 (pg/mL)	Trypanosome in CSF	IHDS score Finger tapping	Hand alternate sequence	Three-word memory recall	Total
C02	–	0	M	27	–	–	No	4	4	4	12
C04	–	1	F	50	–	–	No	4	4	4	12
C06	–	2	F	5	–	–	No	–	–	–	–
C01	–	3	F	30	–	–	No	4	4	4	12
C05	–	11	M	62	–	–	No	3	3	4	10
C07	–	13	F	43	–	–	No	4	2	4	10
C09	–	13	F	30	–	–	No	–	–	–	–
C03	–	23	F	47	–	–	No	4	4	4	12
C08	–	25	M	39	–	–	No	–	–	–	–
Z06	Early	67	F	5	3	162.79	No	–	–	–	–
Z01	Early	84	F	30	5	6.08	No	–	–	–	–
Z13	Early	84	F	3	2	182.23	No	–	–	–	–
Z17	Early	98	M	12	2	462.88	No	2	3	3	8
Z10	Early	101	M	16	3	699.76	No	3	4	2	9
Z14	Early	103	F	28	3	154.08	No	3	4	3	10
Z07	Inter	111	F	50	6	19.66	No	–	–	–	–
Z04	Inter	113	F	51	6	294.48	No	–	–	–	–
Z11	Inter	114	M	48	9	469.89	No	4	4	2	10
Z16	Inter	116	M	42	11	690.35	No	2	3	2	7
Z18	Inter	126	M	24	8	213.76	No	3	3	3.5	9.5
Z03	Late	133	F	45	935	1,066.90	No	–	–	–	–
Z05	Late	144	M	62	27	30.49	No	–	–	–	–
Z09	Late	153	F	36	83	1,045.63	No	–	–	–	–
Z02	Late	155	M	25	1,150	239.77	Yes	–	–	–	–
Z12	Late	184	F	12	22	594.98	No	–	–	–	–
Z19	Late	211	F	30	258	1,399.63	No	3	3	2.5	8.5
Z15	Late	213	M	9	42	1,130.56	No	2	2	2	6
Y20	Late	232	F	43	396	573.88	Yes	–	–	–	–
Z08	Late	250	M	14	695	1,570.09	No	3	3	0	6

C = control; F = female; Inter = intermediate stage; M = male; Y/Z = HAT patient.

disease stages, partly because of high interindividual variability (6.1–699.8 pg/mL in early-stage patients, 19.7–690.4 pg/mL in intermediate-stage patients, and 30.5–1,570.1 pg/mL in late-stage patients). This further suggests that the ASS may be a better biomarker for HAT staging than CXCL-10.

Given that there are no stage-specific clinical features of HAT, we decided to pilot-test in some of our HAT patients the performance of a simple clinical score, the IHDS that has been widely used as a screening tool in HIV-associated neurocognitive disorders.[17] Interestingly, although the IHDS score did not demonstrate clear differences between HAT stages, it displayed a progressive decrease with increasing disease severity (ranges: 8–10 in early-stage, 7–10 in intermediate-stage, and 6–8.5 in late-stage patients, with 10–12 for healthy individuals), with poor scores in late-stage patients. This observation may be due to the small numbers in the subanalysis. Furthermore, a significant negative correlation was observed between the IHDS score and the ASS, suggesting that a combination of the two scoring methods may be more performing and useful to the current clinical practice for accurate staging of HAT. The IHDS score is a very simple clinical score, lasting about 5 minutes on the average and can be performed by nonphysician staff in field studies. Collection of actigraphy data can equally be carried out in the field continuously for several days by nonphysician personnel, and the results can be easily transmitted even in poor Internet connectivity situations, given the small data size, to the expert for detailed analysis.[12] The importance of multiple-day actigraphy in patients lies in the possibility of better harnessing the changes in rest–activity circadian rhythm for more precise follow-up evaluation.[14,24] However at this stage, we think that it is premature to base the initial HAT diagnosis on the ASS alone. CSF examination as well as this new actigraphy test should be performed if it is possible to do both to get maximal information. If for various reasons it is not possible to do a lumbar puncture, then this actigraphy test should be the only one performed to diagnose the disease after CATT screening. Nevertheless, the ASS can already be used first line to diagnose a relapse of HAT.

Large cohort studies now need to be carried out to improve these tools, considering their potential use for better staging and posttreatment follow-up in HAT, thus eventually eliminating the need for invasive and painful methods such as lumbar puncture for CSF collection, which may alarm some patients and prevent them from returning for posttreatment follow-up.[12,25,26] After validation in large studies, the ASS could become a useful tool for the monitoring of new treatment regimens for HAT.

CONCLUSION

We report here the development and performance testing of a new biomarker, the ASS, for diagnosis, disease staging, and monitoring in HAT. The ASS appears to perform better than the currently used biomarkers and may be even better in combination with simple clinical scores such as the IHDS. There is therefore a need for large cohort studies to confirm these results that would influence national policies on HAT

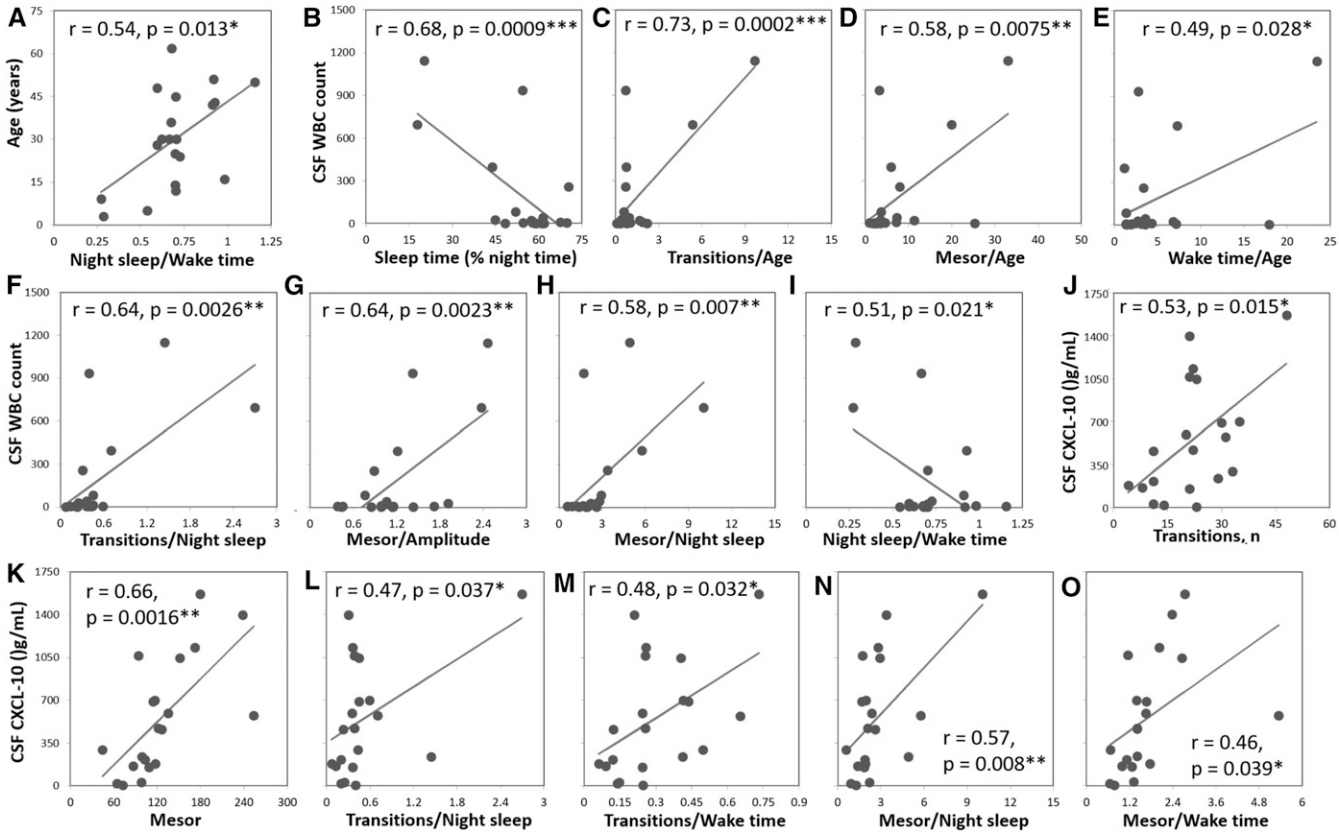

FIGURE 4. Correlations revealed by factor analysis. Correlations of 24-hour actigraphy parameters and physiological parameters with age (**A**), CSF white blood cell (WBC) counts (**B**–**I**), and CXCL-10 levels (**J**–**O**).

management and contribute to the global process of developing and testing new therapeutic approaches to HAT and contribute toward HAT elimination.

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