

# Active at night, sleepy all day – Sleep disturbances in patients with hepatitis C virus infection

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**Background & Aims:** More than 50% of patients with chronic hepatitis C with only mild liver disease complain about chronic fatigue, daytime sleepiness and poor sleep quality. The aim of the present study was to characterize and objectify the sleep disturbances in hepatitis C virus-infected patients.

**Methods:** Twenty-five women who had been infected with hepatitis C virus contaminated anti-D immunoglobulin in 1978/79 and 22 age-matched female healthy controls underwent actigraphy over a period of 5 days to measure motor activity and thereby sleep-wake-rhythm and in addition completed questionnaires for depression, health-related quality of life, fatigue and sleep, and a sleep diary. Liver cirrhosis, a history of neurological or psychiatric disease, history of intravenous drug abuse, shift work, or current medication with effect upon the central nervous system were exclusion criteria.

**Results:** The patients achieved higher scores for depression, fatigue and sleep disturbances and lower quality of life scores than the healthy controls. Actigraphy showed higher nocturnal activity and worse sleep efficiency in the patients, while the 24-h activity level did not differ between groups. Fatigue and quality of life scores correlated with bad sleep quality and daytime sleepiness.

**Conclusions:** Our data indicate that chronic fatigue is associated with bad sleep quality and increased nocturnal activity in HCV-infected patients suggesting an alteration of sleep architecture behind fatigue in HCV-associated encephalopathy.

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## Introduction

About 170 million people worldwide suffer from chronic infection with the hepatitis C virus (HCV), the global prevalence is about 2.35%. In 80% acute hepatitis C infection leads to chronic hepatitis whereas between 1 and 20% – dependent on the presence or absence of risk factors such as older age, male sex, alcohol abuse, or diabetes for example – develop liver cirrhosis [1,2]. An extremely low rate of liver cirrhosis has been observed in the so-called German Anti-D cohort, a group of women who have been infected with HCV contaminated anti-D immunoglobulin in 1978/79 in Eastern Germany. Wiese *et al.* reported that only 2% of 1980 women who underwent a follow-up examination 25 years after the infection showed liver cirrhosis or a pre-cirrhotic stage while 62% “complained of constitutional symptoms such as reduced exertional capacity, weakness and fatigue, abdominal distention, arthralgia, myalgia, or headaches” [2].

Hepatologists are well aware of the extrahepatic manifestations of the HCV-infection and it has been acknowledged recently that the central nervous system is most frequently affected [3]. Symptoms like chronic fatigue, mood disturbances and cognitive dysfunction occur in HCV infected patients with none or only mild liver disease and are accompanied with a reduction of health-related quality of life. The most frequent symptom with more than half of the patients affected is fatigue. Of note, this holds true also in the absence of advanced liver disease or ongoing antiviral therapy with interferon alpha [4]. In detail, chronic HCV infected patients complain about daytime fatigue and poor sleep quality. Up to now these symptoms are not well described in the literature and the pathogenesis is still unclear [5].

The purpose of this study was to characterize the feature and degree of sleep disturbances in HCV-infected patients without relevant liver disease and to clarify the patients' symptoms. Furthermore we were interested to analyze the relationship between sleep disturbances, neuropsychiatric symptoms and health-related quality of life.

**Keywords:** Actigraphy; Fatigue; Hepatitis C; Sleep disturbances.

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**Abbreviations:** APRI, aspartate aminotransferase-to-platelet ratio index; PSQI, Pittsburgh sleep quality index; ESS, Epworth sleepiness scale; FIS, fatigue impact scale; BDI, Beck depression inventory; HADS, hospital anxiety and depression scale; SF-36, short-form questionnaire; QoL, quality of life; WASO, wake after sleep onset; cpm, counts per minute; MVPA, moderate to vigorous physical activity; CSF, cerebrospinal fluid; FSS, fatigue severity scale.



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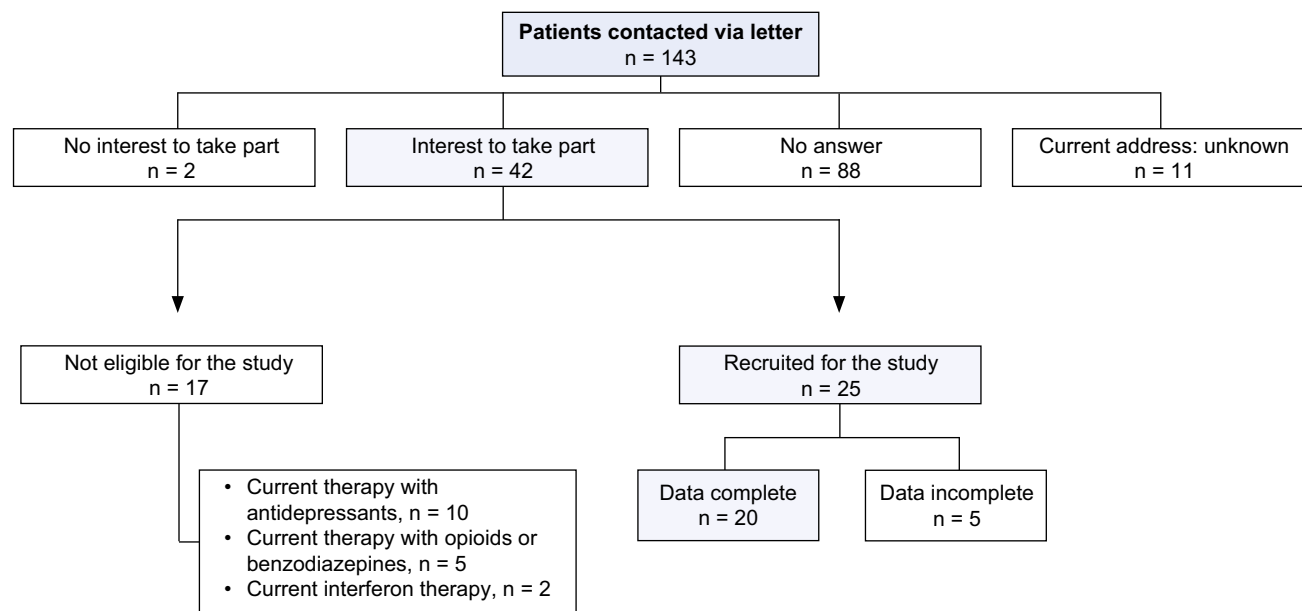


Fig. 1. Flow chart for patient recruitment.

## Patients and methods

### Patients and controls

One-hundred-forty-three members of HCV patient support groups for women who had been infected with HCV-contaminated anti-D immunoglobulin in 1978/79 were contacted in writing and asked if they were interested in taking part in the study. These patients were considered especially suitable to study the effect of HCV-infection upon sleep features and quality because neither co-infection, nor substance abuse or other concomitant disorders could probably bias the results. Eleven patients had moved and their current address was not available, two patients refused to take part in the study. Forty-two women answered our letter and showed interest (Fig. 1).

These 42 women were contacted via phone for a structured interview concerning the exclusion criteria such as liver cirrhosis, a history of neurological or psychiatric disease, history of intravenous drug abuse, shift work, or current medication with effect upon the central nervous system. Thereafter 17 patients had to be excluded from the study because of current therapy with antidepressants ( $n = 10$ ), opioids or benzodiazepine ( $n = 5$ ) or current interferon therapy ( $n = 2$ ) (Fig. 1).

Finally, twenty-five women were included into the study. The time span since infection was more than 30 years and the genotype of the virus 1b in all patients. Liver cirrhosis or severe fibrosis was excluded by calculation of the APRI (aspartate aminotransferase-to-platelet ratio index) score [6], by ultrasound of the liver and/or by liver biopsy.

22 age-adjusted female control subjects were recruited from the clinic staff as well as family and friends of working group members applying the same exclusion criteria as for the patients. Patients and controls gave their written informed consent.

The study has been performed according to the 1975 Declaration of Helsinki and has been approved by the local ethics committee.

### Questionnaires

The patients and controls completed several questionnaires including the Pittsburgh sleep quality index (PSQI) [7], the Epworth sleepiness scale (ESS) [8], the fatigue impact scale (FIS) [9], the Beck depression inventory (BDI) [10], the hospital anxiety and depression scale (HADS) [11] and the SF-36 [12]. The PSQI is a subjective measure of sleep quality over the previous 4 weeks. The questionnaire consists of 17 items based on components of quality, latency, duration, efficiency, disturbance, use of sleeping medication and daytime dysfunction. The global score varies between 0 and 21. A score  $\geq 5$  can be considered suggestive of significant sleep disturbance. The ESS intends to measure daytime sleepiness. Each item is rated on a 4-point scale. The global score ranges

between 0 and 24, with 10 as a cut-off score for pathological daytime sleepiness. The FIS is a self-report scale to measure the impact of fatigue upon patients' daily living activities. It contains 40 items with a scale from 0 to 4. The total score varies between 0 (no fatigue) and 160 (severe fatigue), we choose 50 as a suggested cut-off for pathological fatigue considering own data from healthy controls from former studies. The BDI is a self report scale to measure depression. The questionnaire contains 21 items and a 4-point response scale, ranging from 0 (minimal) to 3 (severe). The global score ranges from 0 to 63, a score  $\geq 18$  can be considered as pathologic. The Hospital Anxiety and Depression Scale (HADS) has been developed for use in in-hospital patients with internal disease to assess emotional alterations. It is a 14 items scale, each scored from 0 to 3. The global range varies between 0 and 21 for anxiety and depression, respectively, with a score  $\geq 11$  indicating anxiety or depression. The Short-Form questionnaire (SF-36) is a survey with 36 questions to measure health-related quality of life (QoL). It provides scores for 8 health domains which can be summarized to a physical and a mental score with a maximum of 400 points for each of the two domains. The cut-off depends on age and sex. The patients' results were evaluated accordingly.

### Sleep diary

The patients were requested to fill in a sleep diary to record the "in bed time", time to sleep onset, number of awakenings, "out of bed time", daytime nap, coffee and alcohol consume for the days studied.

### Actigraphy

Actigraphy was used to measure motor activity of the subjects throughout a period of 5 days. The actigraph is a small device (38 mm  $\times$  37 mm  $\times$  18 mm, weight 26 g) which is worn on the wrist of the non-dominant hand. We used the accelerometer ActiGraph Monitor GT1M and GT3X (ActiGraph, Pensacola, USA). Both contain a sensor which detects motion with linear piezoelectric accelerometer in a vertical axis [13]. The signal is summarized over epochs of 60 s, and given as counts per minute (cpm). Patients and controls were advised to wear the device from Sunday to Saturday 24 h per day and to only take it off for washing the dishes or taking a shower. The data stored were downloaded to a computer using the ActiLife 5.0 software. Because of the varying sleep-wake-rhythm during the weekend only data covering the time span from Monday 12 pm until Friday 12 pm were considered for further analysis.

We used the floating window algorithm and 20 min of consecutive zero counts to identify the non-wear time. Afterwards the data were visually inspected to ensure that information recorded in the sleep diary corresponded with the accelerometer output. The periods of non-wear time as indicated by the participants were excluded from further analysis.

For the sleep scoring we completed the stored data by adding the "in bed time" and "out of bed time" from the sleep diary. The software provides the following parameters:

Sleep onset (the first minute that the algorithm scores "asleep"), total sleep time (the total number of minutes scored as "asleep"), sleep efficiency (number of sleep minutes divided by the total number of minutes the patient was in bed), wake after sleep onset (WASO; the total number of minutes the subject was awake after sleep onset occurred), average awakening (the average length in minutes of all awakening episodes), total counts (total actigraphy counts summed up for the entire sleep period).

The total numbers of epochs stored during the measurement ( $4 \times 24$  h) were averaged to measure different categories of activity intensity in a 24 h period. We selected 100 counts per minute (cpm) as a cut-point for sedentary behaviour, 100–759 cpm for light physical activity, 760–1951 cpm for lifestyle physical activity, 1952–5724 cpm for moderate physical activity and  $>5725$  cpm for vigorous physical activity [14,15]. In addition we combined the moderate physical activity and vigorous physical activity counts into one class (MVPA  $>1952$  cpm) for further analysis as described by Matthews *et al.* [14]. In addition to the "counts" the data provided bouts of activity. A minimum bout length was 10 min, the minimum count level to be considered as bout was 1953 cpm and the drop time was 2 min [15,16].

#### Statistical analysis

Statistical analysis was done using SPSS Version 20. If the data were normally distributed, the results of the questionnaires and actigraphy parameters are summarised as mean values and standard deviations. For not normally distributed data the results are given as median and 25th–75th percentile. A comparison between patients' and control data was performed using the unpaired Student's *t* test or the Mann-Whitney-U-test accordingly. Spearman correlation was used to look for a relationship between fatigue, QoL and sleep parameters and the actigraphy results because they were not normally distributed. The level of significance was set at a *p* value of  $<0.05$ . In case of multiple comparisons the Bonferroni-Holm correction was applied.

## Results

The data of 5 patients and 3 controls had to be excluded from further analysis due to device dysfunction ( $n = 3$ ), insufficient number of monitoring days ( $n = 4$ ) or missing sleep diary ( $n = 1$ ). Thus finally data of 20 patients (age  $56.8 \pm 4.2$ ) and 19 controls (age  $55.3 \pm 4.2$ ) were considered for analysis. Six patients had been treated with interferon more than 7 years ago. Eight patients were HCV PCR negative, seven spontaneously and one after therapy.

Liver cirrhosis was excluded based on clinical, ultrasound and laboratory data. Liver biopsy has in addition been performed in 3 patients and showed ISHAK 1 fibrosis in 2 and ISHAK 2 fibrosis in one patient. In the other 17 patients liver biopsy was not indicated considering the clinical course as well as ultrasound and laboratory findings. In these patients the APRI score was in median 0.14 (25th–75th percentile: 0.120–0.265).

Five patients are current smokers and 9 patients reported light to moderate alcohol consumption. In the control group 4 women are current smokers and 14 reported light-to-moderate alcohol consumption.

#### Questionnaires

The results of the questionnaires differed significantly between the HCV-infected patients and the controls, while there was no difference between PCR positive and PCR negative patients (Table 1), or patients with or without alcohol or nicotine consumption. The patients scored higher in the FIS than the controls ( $p < 0.001$ ) showing pathological FIS scores ( $>50$ ) in 14 (70%). They also showed significantly worse sleep quality. Nineteen of the 20

patients (95%) had a PSQI score indicating sleep disturbances. The patients had also significantly higher ESS scores; 50% showed scores representing pathological daytime sleepiness. Also the depression, anxiety and QoL scores differed significantly between patients and controls (all  $p < 0.05$ ). Thirteen patients (65%) revealed a pathological result in the anxiety subscore and 8 (40%) in the depression subscore of the HADS. Fifty percent of the patients showed pathological results in the BDI (Table 1). The patients had significantly worse quality of life scores compared to the controls (for SF-36 sum-score, physical and mental sub-score  $p < 0.001$ ).

#### Wrist actigraphy

The patients showed a significantly higher nocturnal activity than the controls while the time they spent in bed was significantly longer. The time the patients were awake after sleep onset (WASO) and average awakening length at night were also significantly longer. Accordingly they had a worse sleep efficiency than the healthy women (Table 2, Fig. 2).

A subgroup analysis between the PCR positive and negative patients did not show significant group differences except of for the minutes of wake after sleep onset ( $p = 0.036$ ), where the PCR negative patients exceeded the PCR positive ones (Table 2).

For analysis on a daily basis we separated for the activity level. In a 24 h period the patients and controls engaged most of the time in sedentary. We could only show that the controls stay a longer time in light physical activity in comparison to the patients. The other activity parameters did not differ significantly between both groups (Table 3). In contrast to the activity level for the night (total counts in entire sleep period, Table 2) the activity level over a whole day (total counts in bouts/24 h) was almost the same in the two groups thereby indicating a higher daytime activity in the controls compared to the patients (Table 3).

Using Spearman rank correlation analysis we found a significant relationship in the patient group between the FIS, the PSQI and the ESS respectively ( $p < 0.001$ ). Patients with a higher fatigue score had worse sleep quality and more severe daytime sleepiness. There was no significant correlation between the results of the FIS, the PSQI, the ESS and the actigraphy parameters (Tables 4 and 5). Accordingly the SF 36 results (sum-score, physical and mental score) correlated negatively with the sleep questionnaires ( $p < 0.05$ ) but not with the actigraphy data (Table 4).

FIS and SF-36 scores correlated significantly with the sleep questionnaires but not with the actigraphy parameters also in the control group (data not shown).

## Discussion

In the last two decades there has been growing evidence that infection with the hepatitis C virus can lead to brain dysfunction in the absence of severe liver disease [3,17–20]. The patients complain about chronic fatigue, cognitive dysfunction, a reduced quality of life and depression. Furthermore they report sleep disturbances such as daytime sleepiness and poor sleep quality [21]. The pathology behind is unclear, as is the interrelationship between these different symptoms.

In the present study we examined 20 women, who had been infected with HCV more than 30 years ago but had not developed

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Table 1. Mood and sleep scores for patients and controls.

	HCV PCR+ (n = 12)	HCV PCR- (n = 8)	p value PCR+/ PCR-	Patients (P) (n = 20)	Controls (C) (n = 19)	p value P/C
Age (yr)	56.2 (4.40)	57.6 (4.10)	0.464	56.8 (4.20)	55.3 (4.20)	0.292
FIS	93.5 (56.75-110.00)	83.5 (5.5-110.75)	0.335	91.5 (46.25-110.00)	15 (8.0-33.0)	<b>&lt;0.001</b>
HADS-A	11.5 (9.25-15.75)	11.5 (9.0-12.75)	0.727	11.5 (9.0-14.75)	5 (2.0-6.0)	<b>&lt;0.001</b>
HADS-D	10 (7.0-12.0)	10.5 (2.25-12.5)	0.969	10.0 (6.25-12.00)	1 (0.0-4.0)	<b>&lt;0.001</b>
BDI	16 (10.25-24.50)	20 (8.5-26.0)	0.643	16.5 (8.5-26.0)	5 (2.0-11.0)	<b>&lt;0.001</b>
SF-36 mental	154.4 (75.20)	178.8 (139.60)	0.661	164.2 (103.00)	303.7 (70.60)	<b>&lt;0.001</b>
SF-36 physical	173 (66.60)	193.9 (129.40)	0.683	181.3 (94.10)	330.3 (48.90)	<b>&lt;0.001</b>
SF-36 sum score	285.5 (215.13-438.35)	307.3 (136.25-622.13)	0.817	285.5 (215.0-486.08)	666 (593.5-717.0)	<b>&lt;0.001</b>
ESS	10.8 (6.20)	9.3 (5.90)	0.596	10.2 (6.00)	6.6 (3.40)	<b>0.03</b>
PSQI	13 (10.25-16.75)	11 (7.0-14.0)	0.230	13.0 (8.25-16.00)	5 (3.0-7.0)	<b>&lt;0.001</b>

Normally distributed values as mean with standard deviation in parenthesis; not normally distributed values a median with 25th–75th percentiles in parenthesis.

HCV PCR positive (PCR+) and negative (PCR-) patients.

FIS, fatigue impact scale; HADS, hospital anxiety and depression scale; BDI, Beck depression inventory; SF-36, short-form questionnaire; ESS, Epworth sleepiness scale; PSQI, Pittsburgh sleep quality index.

Significance level  $p < 0.05$ ; significant values in bold.

relevant liver disease, and 19 age-adjusted female healthy controls using a battery of sleep questionnaires and wrist actigraphy.

Like HCV-infected patients in former studies [4,19,20] the patients revealed increased scores for depression and anxiety, and decreased QoL scores. Sleep questionnaires indicated a significant alteration of sleep quality, and in accordance with the characteristic complaints wrist actigraphy showed sleep disturbances reflected by a higher nocturnal activity and poorer sleep efficiency.

It must be emphasized that our patients had no significant liver disease, since sleep disturbances are known as a frequent complication of liver cirrhosis. Córdoba *et al.* [22] studied 20 patients with liver cirrhosis and 20 healthy controls with a sleep questionnaire and actigraphy for 5 consecutive days. The cirrhotic patients showed a fragmented sleep at night, a higher nocturnal activity and a reduced physical activity over a 24 h period compared to the controls. Patients with unsatisfactory sleep showed a shift of activity toward later hours of the day. In 2008 Mostacci *et al.* [23] studied 178 patients with a diagnosis of non-alcohol-related liver cirrhosis and 178 healthy controls. Patients with cirrhosis showed a significantly higher prevalence of daytime sleepiness and bad sleep quality with difficulties in falling asleep and frequent nocturnal awakenings. Those patients with minimal hepatic encephalopathy had more pronounced daytime sleepiness and night time sleep disturbances in contrast to the other cirrhotic patients. A comparison between the patient groups with pathological and normal blood ammonia level did not show any differences in the parameters of sleep quality and sleep habits. Montagnese and co-workers [24] assessed sleep-wake-disturbances in 87 patients with liver cirrhosis with and without minimal/overt hepatic encephalopathy and 19 healthy controls. They found higher PSQI and ESS scores in the patients compared to the control group. In contrast to the study of Mostacci and co-workers there was no significant correlation between the sleep questionnaires and the presence of hepatic encephalopathy. In concordance to the findings of Córdoba and co-workers evening type cirrhotic patients took longer to fall asleep and

the sleep quality was worse than in patients with earlier sleep timing.

In summary sleep-wake disturbances are common in cirrhotic patients. The patients suffer from daytime sleepiness and bad sleep quality and some studies showed a phase delay in the sleep patterns of cirrhotic patients.

Our patient group with only mild liver disease showed a different feature of sleep disturbances. Although they showed a higher nocturnal activity as well, they had a similar 24 h activity level compared to controls and no shift in the sleep-wake-rhythm. Thus the pathogenesis behind is probably different from that in cirrhotic patients with sleep disorder.

Our patient group consists of women who had been infected with hepatitis C virus contaminated anti-D immunoglobulin in the 1970s. Patients with risk factors for developing fatigue (independent of the HCV infection) like intravenous drug abuse and co-infection with the human immunodeficiency virus (HIV) were excluded. Fatigue and depression are known as side effects of interferon therapy in HCV patients [25]. Of note, interferon therapy had been finished at least 7 years ago in the 6 patients of our group who had ever been treated. None of the subjects in this study took any medication that could be held responsible for fatigue and sleep disturbances. However, nearly half of the patients (and 73% of the controls) reported slight to moderate alcohol consumption and 25% had a smoking habit. According to Yamini *et al.* [26] both factors are associated with the presence of depression, fatigue and sleep disturbances in patients with HCV-infection. They evaluated clinical data of 800 HCV-infected patients for the impact of past and current tobacco use or drinking habits, comorbidities such as diabetes mellitus or hyperlipidemia, history of interferon treatment, fibrosis stage and others upon the presence of depression, fatigue and difficulty sleeping in these patients. The analysis showed a significant relationship between past and present tobacco use and past alcohol use and the presence of depression, between past and present tobacco use and current alcohol use and fatigue, as well as between past and present tobacco use and difficulty sleeping in these patients. In



Table 2. Sleep actigraphy data in patients and controls.

	HCV PCR+ (n = 12)		HCV PCR- (n = 8)		p value PCR+/ PCR-	Patients (P) (n = 20)		Controls (C) (n = 19)		p value P/C
Total counts (entire sleep episode/night)	59,342.6	(41,650.81- 79,376.38)	78,174.5	(54,697.0- 125,885.0)	0.165	64,218.6	(44,899.88- 87,556.25)	46,409.0	(26,275.5- 58,025.5)	<b>0.012</b>
Average awakening length (min/night)	4.7	(1.40)	5.3	(1.00)	0.326	4.9	(1.20)	3.9	(1.40)	<b>0.015</b>
WASO (min/night)	73.1	(24.60)	104.2	(37.00)	<b>0.036</b>	85.5	(33.10)	58.3	(23.50)	<b>0.005</b>
Number of awakenings/night	18.6	(14.94-20.44)	23.4	(16.63-29.00)	0.153	19.1	(16.25-23.81)	19.3	(14.5-23.0)	0.978
Sleep efficiency (%/night)	82.7	(5.70)	78.2	(6.50)	0.121	80.9	(6.30)	84.5	(4.60)	<b>0.046</b>
Sleep latency (min)	8.4	(3.0-15.94)	7.3	(5.18-12.31)	1.000	7.6	(4.38-12.75)	5.5	(0.5-13.25)	0.255
Total sleep time (min/night)	404.8	(71.90)	406.5	(48.90)	0.955	405.5	(62.20)	376.0	(32.90)	0.075
Time in bed (min/night)	487.9	(70.10)	518.9	(49.10)	0.294	500.3	(63.00)	441.3	(48.00)	<b>0.002</b>

HCV PCR positive (PCR+) and negative (PCR-) patients.

Normal distributed values with standard deviation in parenthesis; not normally distributed values with 25th–75th percentiles in parenthesis.

WASO, wake after sleep onset.

Significance level  $p < 0.05$ , significant values in bold.

contrast social drinking was associated with a lower risk of difficulty sleeping. Of note, their patient cohort differed significantly from that examined in our study: about half of their patients had a history of drug abuse, one third were heavy drinkers, more than 50% were male and about 50% had advanced fibrosis (28%) or cirrhosis (18%). Depression was present in about 30%, fatigue in 45% and difficulty sleeping in only 3.8%. Considering these differences and the fact that both, smoking and drinking habits of the patients in our cohort were only slight to moderate these factors are unlikely having influenced the results.

Eight of our 20 patients were HCV PCR negative, 7 of them had cleared the virus spontaneously. These HCV PCR negative patients were included into the study and treated as homologous to the HCV PCR positive patients because we have repeatedly shown in previous studies that both, HCV PCR positive and negative patients may develop HCV-infection-associated encephalopathy and present with identical symptoms and findings in brain imaging such as magnetic resonance spectroscopy, Fluor-Desoxy-Glucose positron emission tomography and single photon emission tomography [19,20,27]. Accordingly we did not find a significant difference between both patient groups in the present study as well.

In the past several studies have shown that the virus replicates in the brain [28–31]. Different quasiespecies of the virus have been detected post-mortem in the liver and the brain of HCV-infected patients. In 2002 Radkowski *et al.* detected negative strand HCV-RNA, a viral replication intermediary, in autopsy brain tissue of three out of six HCV-infected patients. As an indication for viral brain compartmentalization in two of these patients viral sequences amplified from the brain differed from those circulating in serum. Based on a sequence analysis of two different viral regions it was suggested that the brain-derived HCV variants were more closely related to the virus present in the lymphoid system than to virus circulating in serum [28]. Forton *et al.* also described a close relationship between HCV variants present in brain tissue and in lymph nodes [30]. HCV-RNA

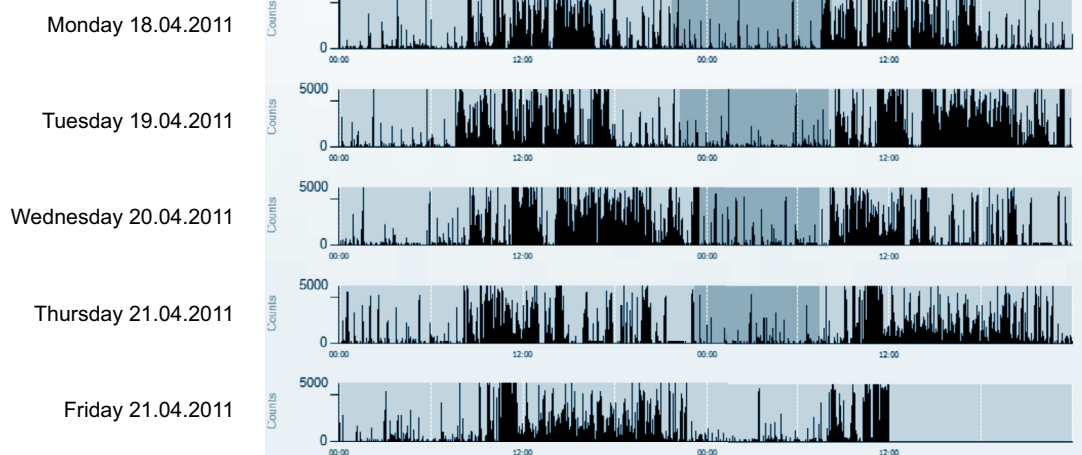
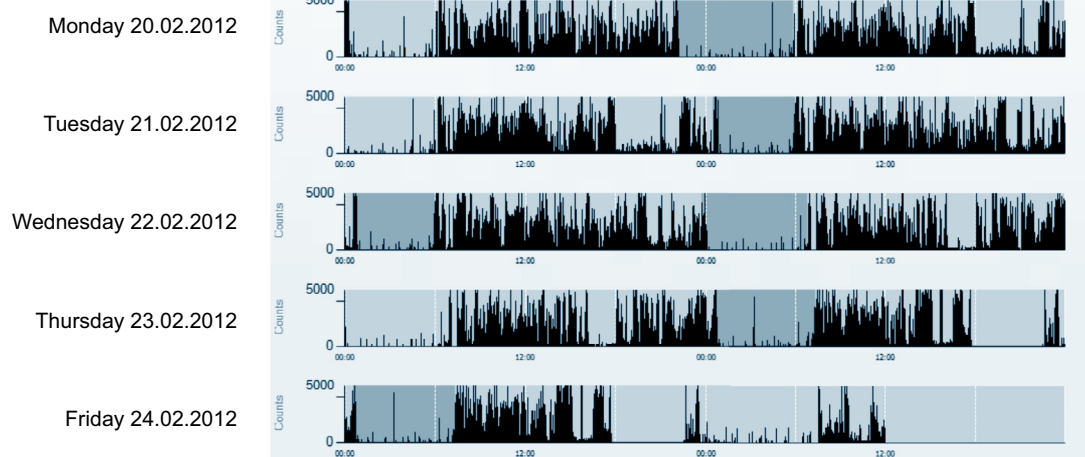
was also found in the cerebrospinal fluid (CSF) of eight out of 13 patients. Again the sequences of the brain (CSF) derived virus were closer to those found in peripheral blood mononuclear cells (PBMC) than to those circulating in serum of the patients [30,31]. HCV-RNA positive and negative strands were detected in CD68-positive cells in the brain, which indicates that the cells harboring HCV are macrophages/microglia. It is suggested that HCV-infected macrophages/monocytes enter the brain via the blood-brain-barrier and induce a neuroinflammatory response that might be causative for the development of neuropsychiatric symptoms [29].

We found a significant correlation between the degree of fatigue as represented by the FIS score and the daytime sleepiness and sleep quality (ESS and PSQI). The findings are in line with the results from Carlson *et al.* [32] who studied 59 hepatitis C patients with different stages of liver disease. They found a significant relationship between the PSQI and FSS (Fatigue Severity Scale) regardless of the stage of liver disease.

We could not find any significant correlations between the results of the fatigue and sleep questionnaires and the actigraphy parameters. In other words the subjective perception of bad sleep quality must not be accompanied with an equivalent amount of nocturnal motor activity. In sleep research actigraphy is a validated tool for activity based sleep-wake monitoring, which was established by The American Sleep Disorders Association [33–36]. Of note, some patients having difficulties in falling asleep may have the habit lying in bed for a longer period without movement, others move around in bed very often to try to fall asleep. Thus a possible limitation of the validity of actigraphy is detecting wakefulness during a sleep period [33].

A limitation of our study is the short actigraphy analysis period of 5 out of 7 recorded days. The International Classification of Sleep Disorders (established by the American Academy of Sleep Medicine) requires a minimum of 7 days of actigraphy performed in addition to a sleep diary for being able to demonstrate

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**A****B**

**Fig. 2. Actigraphy protocols of patient and control.** Examples of the actigraphy protocol of chronic Hepatitis C infected woman (A, 60 years) and healthy woman (B, 51 years). The amplitudes marked in black show the extent of motor activity in counts. The time span in bed (as indicated in the sleep diary) is marked in darker gray. The graphic is provided by the ActiLife software.

consistency in the pathology of circadian rhythm disorders. Acebo *et al.* [37] showed that 5 nights of measuring give reliable estimates for evaluating sleep start time, wake minutes and sleep efficiency. Therefore we choose the recording of the five working days of the week for further analysis taking into account that the weekends are usually characterized by alterations of the sleep habits.

The increased HADS and BDI scores and the significant correlation between HADS and BDI scores and ESS and PSQI scores suggest depression as a possible reason for altered sleep quality in our patients. Former studies in patients with depression had shown a relationship between the extent of depression and actigraphy parameters [38]. This could not be observed in our study. While these findings appear inconsistent on the first view they are comprehensible considering the findings of Golden *et al.* [39] who were able to show that HADS and BDI scores are falsely positive in patients with HCV infection and do not represent the extent of depression.

On the basis of experimental studies it is known that sleep disturbances are associated with alterations of the immune system. Krueger proposed that sleep is a process which is influenced by cytokines, a part of the peripheral and central immune system [40]. Gershon *et al.* discussed cytokine effects upon the brain as possible mechanism for the development of fatigue in HCV-infected patients [41]. In 2011 de Almeida studied chronic hepatitis C patients to find an association between the degree of sleep quality (provided by the PSQI and FSS) and peripheral blood immunological and virological biomarkers. They detected a different immunological profile in patients with good sleep quality compared to those with bad sleep quality [42]. Wilkinson *et al.* detected an upregulation of proinflammatory cytokines IL-1, TNF-alpha, IL-12, and IL-18 in post-mortem brain tissue of hepatitis C patients [43]. The alteration of cytokine levels both in the periphery as well as in the brain itself could well be involved in the development of sleep disturbances in HCV-infected patients.

Table 3. 24 h physical activity levels for patients and controls.

	Patients (n = 20) Mean (SD)	Controls (n = 19) Mean (SD)	p value
Daily sedentary time	654.7 (134.5)	642.3 (100.5)	0.747
Daily light time	227.3 (36.5)	260.0 (54.5)	<b>0.038</b>
Daily lifestyle time	255.6 (57.7)	245.7 (64.9)	0.616
Daily moderate time	219.8 (69.0)	211.6 (102.2)	0.773
Daily vigorous time	2.7 (9.8)	7.3 (19.7)	0.363
MVPA	222.5 (72.9)	214.9 (104.4)	0.793
Total counts in bouts/24 h	516,043.2 (193,788.9)	517,989.0 (411,773.6)	0.985
Total bouts	39.8 (13.6)	36.2 (24.7)	0.576

Normally distributed values as mean with standard deviation in parenthesis.

Daily time in minutes.

MVPA, moderate to vigorous physical activity.

Significance level  $p < 0.05$ , significant values in bold.

Table 4. Spearman correlation between fatigue impact scale, SF-36 and the actigraphy parameters in patients.

	FIS (n = 20)		SF-36 sum score (n = 20)		SF-36 physical (n = 20)		SF-36 mental (n = 20)	
	r	p value	r	p value	r	p value	r	p value
ESS (Epworth Sleepiness Scale)	0.704	<b>0.001</b>	0.596	<b>0.006</b>	0.585	<b>0.007</b>	0.561	<b>0.010</b>
PSQI (Pittsburgh Sleep Quality Index)	0.794	<b>0.000</b>	0.806	<b>0.000</b>	0.851	<b>0.000</b>	0.693	<b>0.001</b>
Total counts (entire sleep episode/night)	0.001	0.997	-0.096	0.686	-0.227	0.335	-0.081	0.734
Average awakening length (min)	-0.016	0.947	0.045	0.850	-0.101	0.672	0.080	0.738
WASO (min/night)	-0.030	0.900	0.030	0.900	-0.114	0.631	0.048	0.840
Number of awakenings/night	0.001	0.996	0.005	0.985	-0.046	0.846	-0.007	0.977
Sleep efficiency (%)	0.042	0.860	-0.071	0.767	0.038	0.875	-0.090	0.705
Sleep latency (min)	0.099	0.679	-0.032	0.895	-0.062	0.796	-0.017	0.945
Total sleep time (min/night)	-0.021	0.930	-0.185	0.435	-0.215	0.362	-0.152	0.523
Time in bed (min/night)	-0.044	0.855	-0.208	0.380	-0.275	0.240	-0.183	0.439
Total counts in bouts/24 h	-0.288	0.218	0.289	0.217	0.127	0.594	0.329	0.157

FIS, fatigue impact scale; SF-36, short-form questionnaire; WASO, wake after sleep onset.

Spearman correlation coefficient r.

Significance level after Bonferroni-Holm correction ( $p < 0.004$ ), significant values in bold.

Table 5. Spearman correlation between Epworth sleepiness scale, Pittsburgh sleep quality Index and the actigraphy parameters in patients.

	Epworth Sleepiness Scale (ESS) (n = 20)		Pittsburgh Sleep Quality Index (PSQI) (n = 20)	
	r	p value	r	p value
Total counts (entire sleep episode/night)	-0.207	0.381	0.051	0.832
Average awakening length (min)	-0.012	0.960	0.013	0.957
WASO (min/night)	-0.208	0.379	-0.002	0.995
Number of awakenings/night	-0.111	0.641	0.047	0.845
Sleep efficiency (%)	0.170	0.475	0.038	0.875
Sleep latency (min)	0.499	0.025	-0.003	0.990
Total sleep time (min/night)	0.101	0.672	0.162	0.492
Time in bed (min/night)	-0.047	0.842	0.135	0.570
Total counts in bouts/24 h	-0.112	0.637	-0.235	0.318

Significance level after Bonferroni-Holm correction ( $p < 0.004$ ).

WASO, wake after sleep onset; r, Spearman correlation coefficient.

## Research Article

Finally both pathways might play a role in the development of extrahepatic manifestations of chronic hepatitis C virus infection including sleep disturbances: The virus may induce the peripheral immune system which activates proinflammatory cytokines, while the cytokines may alter brain activity by modifying the neurotransmitter systems. Moreover the hepatitis C virus may induce cerebral cytokine production after crossing the blood brain-barrier and thereby alter neurotransmission.

In conclusion, our data showed altered sleep quality in a group of patients with HCV infection associated neuropsychiatric symptoms but only mild liver disease. The extent and the pathogenesis of sleep disorders in patients who had been infected by HCV has to be clarified in further studies considering the cytokine hypothesis as well as recent findings upon altered neurotransmission in HCV-associated encephalopathy [18,20,22,44].

### Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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