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Sleep Duration and Cutaneous Melanoma Aggressiveness. A Prospective Observational Study in 443 Patients

Duración del sueño y agresividad del melanoma cutáneo. Un estudio observacional prospectivo con 443 pacientes

Dear Editor,

Both short and long sleep duration have been associated with an increased prevalence and incidence of cancer,[1,2] but its relationship with cancer aggressiveness remains unknown. We have previously described an association between cutaneous melanoma and sleep-disordered breathing based on a prospective multicentric cohort.[3] In the present paper, we describe the results of a post-hoc analysis aiming to evaluate the association between subjective sleep duration and objective parameters of melanoma aggressiveness.

This is an observational, cross-sectional, multicenter study that included 443 consecutive patients with a diagnosis of melanoma from 29 Spanish hospitals. Patients were excluded if they had in situ melanoma or had received previous treatment with continuous positive airway pressure. The study was approved by the ethics committees of all the hospitals, and all the patients gave their informed consent. Each patient completed a clinical questionnaire which included anthropometric measurements, relevant antecedents, medication, sleep apnea symptoms, presence of insomnia and somnolence (Epworth Sleepiness Scale).

Sleep duration was assessed by asking the patients the following question: How many hours of sleep (including naps) have you had on average in a 24-h period during the last year (year prior to the diagnosis of melanoma)? Participants estimated habitual sleep duration using full hour units. Snoring time was also quantified in the sleep study records.

All patients underwent a sleep study by means of a home respiratory polygraphy and a peripheral blood test. Patients were divided into three groups depending on their daily sleep duration: appropriate sleep duration (between 6 and 8 h), short sleepers (<6 h) and long sleep duration (>8 h). The independent relationship between melanoma aggressiveness factors and sleep duration was determined by introducing into a multivariate logistical regression analysis those variables which could, in the opinion of the researchers, also have clinical importance as confounders: age, sex, AHI, BMI and hypnotics intake. This relationship was evaluated by Hazard Ratio (CI95%), considering the group of patients with appropriate sleep duration as the control group. The degree of melanoma aggressiveness was measured by histological variables such as the Breslow thickness, the tumor mitotic rate ($\geq$5 vs. <5 mitoses per mm^2), the histological presence of ulceration and regression and positive sentinel lymph node (SLN) involvement. The cut-off points for the Breslow thickness were established at 1, 2 and 4 mm, according to international guidelines.[4]

443 patients were finally included in the study. Mean age was 55.9 ± 15.3 years, and 50.6% were male. The mean BMI was 27.3 ± 4.5 kg/m^2 and the median Epworth score was 6 (IQR: 3–8). The median of the Breslow thickness was 0.85 mm (IQR: 0.49–1.80). A Breslow thickness above 1, 2 and 4 mm was found in, respectively, 44, 22.3 and 8.8% of the patients. Ulceration was present in 16%, regression in 23.3%, and SLN was positive in 10.6% of patients. The median AHI was 8.6 (IQR: 2.8–20.2). The mean sleep duration was 7.4 ± 1.27 h, with 4.7% sleeping <6 h, 77.9% between 6 and 8 h and 17.4% > 8 h. 8.4% of the patients presented insomnia and 12% were taking hypnotic drugs. The sleep study time was 7.2 h (IQR: 6.6–8) and the snoring study time was 6.9 h (IQR: 6.5–7.7). The correlation between subjective sleep duration and snoring study time was $r = 0.86$, $p < 0.0001$.

There was a statistically significant correlation between sleep duration and the Breslow index (r: 0.26; $p = 0.001$). Those patients with more aggressive melanoma (Breslow $\geq$ 2 mm vs <2 mm, and

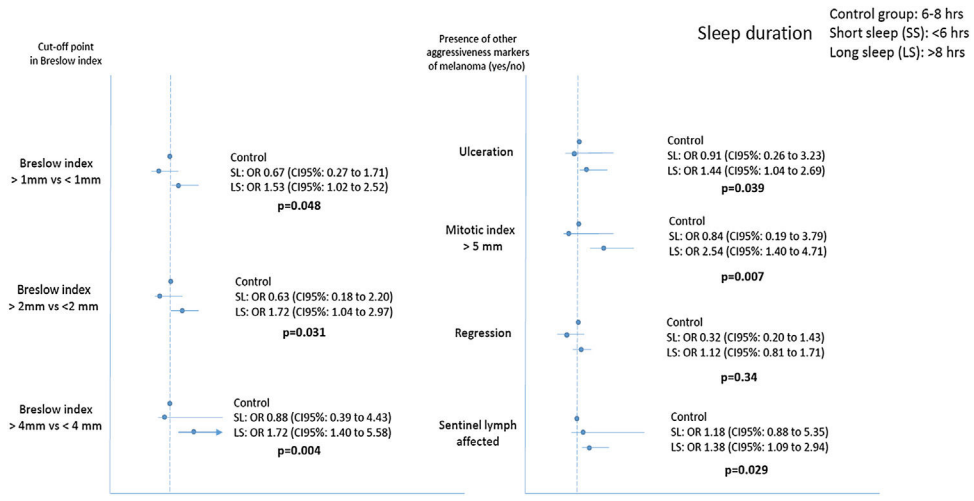

Fig. 1. Relationship between sleep duration and commonly used clinical markers of tumor aggressiveness in cutaneous melanoma. *p* values refer to sleep duration > 8 h versus control group (sleep duration between 6 and 8 h). Results were adjusted for age, sex, apnea-hypopnea index, body mass index, presence of insomnia and intake of hypnotics. SL: Short sleep; LS: Long Sleep.

Breslow ≥ 4 mm versus <4 mm) presented longer sleep duration (8.1 vs. 7.1 h; $p = 0.0001$ and 8.3 vs. 6.9 h; $p = 0.0001$, respectively). Long sleepers presented, in comparison with those patients who slept less than 8 h, a significant increase in various systemic markers of inflammation like high-sensitivity C-reactive protein (hs CRP) (2.35 [2.3) vs 1.33 [2.5] mg/L; $p = 0.03$), fibrinogen concentration (392 [97.1] vs.335 [92.7] mg/dL; $p = 0.01$) and erythrocyte sedimentation rate (ESR) (17.4 [13.9] vs. 11.2 [9.1] mm1 h; $p = 0.01$), as well as a higher AHI (19.8 [17.9] vs. 15.1 [16.1]; $p = 0.02$).

Fig. 1 shows that long sleepers were 1.53, 1.72 and 2.84 times more likely to present a more aggressive melanoma (Breslow index above 1, 2 and 4 mm) compared to the control group, after adjustment for age, gender, BMI, AHI, intake of hypnotic drugs and presence of insomnia. Similarly, long sleepers had an increased risk of SLN positive, presence of tumor ulceration and a high tumor mitotic rate.

As far as we know, this is the first study to analyze the relationship between longer sleep duration and the aggressiveness of a cancer. Of note, this association was not caused by the presence of confounders such as obesity, sleep apnea, age, gender, hypnotics intake or insomnia. Various mechanisms have been postulated to explain this relationship, including various deregulations of the immune system (especially reductions in natural killer cell activity),[5] increased sleep fragmentation[3] and increased systemic inflammation.[6,7] In fact, our study shows that long sleepers presented greater systemic inflammation (increased levels of US-CRP, ESR and fibrinogen).

The main limitation of our study was the fact that the evaluation of sleep duration was subjective, as is the case in most of the similar studies published to date on this topic. However, there was a very strong correlation between the subjective sleep duration and the snoring study time (while the patients are supposed to be sleeping). Moreover, some authors[8] have observed in large series that those patients with self-reported sleep > 7 h per night overestimated their real sleep duration by an average of 0.3 h, while this overestimate was even higher (1.3 h) in patients who slept < 5 h. These circumstances would minimize any error derived from our analysis since, even assuming this situation, the vast majority of the patients in our study would remain in the same analysis group. Moreover, the percentage of patients with abnormal sleep time was 22%, which could affect the conclusions. Finally, we did not include the presence of anxiety or depression as a covariate, but we did include the intake of hypnotics and other psychotropic medication, which is closely associated with anxiety and depression.

In conclusion, we found for the first time a positive relationship between a long sleep duration and some markers of melanoma aggressiveness. Future studies are needed to investigate the main pathophysiological mechanisms that could explain this association and the prognostic relevance of this finding.

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Conflict of interest

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Comparing Probe-Based Confocal Laser Endomicroscopy With Histology. Are We Looking at the Same Picture?

Comparando endomicroscopía confocal con histología. ¿Estamos mirando la misma imagen?

Dear Editor,

Probe-based confocal laser endomicroscopy (pCLE) provides real-time vision of respiratory tissues at the cellular level through a flexible bronchoscope. This technique might guide sampling of pulmonary nodules,[1–4] lymph nodes[5] or pleural biopsies.[6] First studies of the respiratory tract were performed with a 488 nm wavelength probe that allows elastin visualization without adding fluorophores in the tissue. Because cells are not visualized, lung cancer pattern descriptions were limited to changes on the stromal component.[1,7–9] Later studies used fluorophores like methylene blue or fluorescein that could be excited at a 488 or a 660 nm wavelength, respectively, to visualize cell nuclei.[2,4,10] In these studies, different imaging patterns were described. In particular, healthy tissue was described as having homogeneous architecture with bright, partially overlapping nuclei. Inflammation was considered when heterogeneous tissue architecture without overlapping nuclei but expanded cytoplasms were observed, and neoplasia was reported as a chaotic distribution of dark cells with heterogeneous nuclei.[2,8,9,11,12] Although pictures of these patterns were frequently presented together with pictures of histology samples, none of the studies correlated the measurements performed in pCLE images to those of histology samples. In this pilot study, we aimed to explore the feasibility of correlating pCLE and optical microscopy features of normal and pathological airway samples.

Under general anesthesia and orotracheal intubation with a rigid bronchoscope (Efer-Dumon type, La Ciotat, France) pCLE was performed after applying a drop of 1% MB in nine regions of normal mucosa and in six tumors, as determined with white-light bronchoscopy. The AlveoFlex® probe and a laser scanning unit equipped with 660 nm laser wavelength (Cellvizio®, Mauna Kea Technologies, Paris, France) were used. After pCLE image registration, biopsies were taken and histology was studied with optical microscopy following haematoxylin-eosin (H&E) staining. A total of 15 patients were studied. The biopsies revealed normal epithelium in 6 cases, inflammatory infiltrate in 3 cases and thoracic tumors in 6 cases, which included B cell lymphoma, adenocarcinoma, squamous cell carcinoma, small cell lung cancer, and non-small cell lung cancer. The study was approved by the local ethics review board (Clinical Research Ethics Committee of Bellvitge University Hospital – Act 08/13) and written informed consent was obtained from all participants.

One representative image from the pCLE registration was selected and compared to one image of the H&E-stained sample. Nuclei were segmented in all images using ImageJ software (National Institutes of Health, Bethesda, MD, USA).[13] In the pCLE images, we accounted for the number and mean size of nuclei, the relative area occupied by nuclei and the intensity of fluorescence. In the H&E-stained histological sample images, we accounted for the number and mean size of nuclei and the relative area occupied by nuclei. We chose these features because these structures can be identified both in the optical and the pCLE images.

We made two different comparisons. First, we compared the patterns of pCLE alone. A logistic regression model was fitted to predict the probability of pathological tissue (inflammation or malignancy) based on every feature. We observed that changes in these features were associated with variations in the probability of disease. In particular, we observed that the odds of disease increased by a factor of 1.03 (95% CI: 0.99–1.07) for every one unit increase in the mean size of nuclei, by a factor of 1 (95% CI: 1–1) for every one unit increase in the intensity of fluorescence, and by a factor of 1.088 (95% CI: 1.01–1.224) for every one unit increase in the relative area occupied by nuclei. Next, we registered the measurements performed in the optical microscopy and pCLE images (see Table 1) and compared their distributions. After a Wilcoxon rank sum exact test we observed that the distributions of the mean size and relative area occupied by nuclei were different in the images of optical compared to pCLE microscopy. Although this small analysis is not adequately powered to achieve statistical significance, our results of the pCLE alone patterns are in line with the previously mentioned studies showing that it is possible to discern between normal and pathological tissue patterns, while the measurements performed in the optical microscopy and pCLE images cannot be correlated to the tissue structures observed in the biopsy specimens.

Our pilot study supports the use of pCLE to identify patterns of normal and pathological airway tissue but discourages comparisons between pCLE and histology images. These findings might reflect that the final histology diagnosis is based on a set of information not obtainable from a sequence of nuclei as it is observed in pCLE images. To empower pCLE as a useful tool for diagnosis of lung cancer, future studies should focus on identifying further discriminative features[14] or specific tumor markers. Otherwise, this technique will be limited to the identification of pathological areas for biopsy guidance.