Plasma Levels of Tumor Necrosis Factor α and Soluble Tumor Necrosis Factor Receptors in Patients With Narcolepsy

Hubertus Himmerich, MD; Pierre A. Beitinger, MD; Stephany Fulda, MSc; Renate Wehrle, MSc; Jakob Linseisen, PhD; Günther Wolfram, MD; Stephanie Himmerich, MSc; Kurt Gedrich, PhD; Thomas C. Wetter, MD; Thomas Pollmächer, MD

Background: Narcolepsy is a disabling sleep disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis. Recent studies suggest that the immune system might play a pathogenic role pointing to a possible involvement of inflammatory cytokines.

Methods: We investigated a sample of 30 patients with narcolepsy in comparison with 120 sex- and age-matched and 101 sex-, body mass index (BMI)-, and age-matched randomly selected normal controls. In these groups, plasma concentrations of tumor necrosis factor α (TNF-α) and its soluble receptors p55 and p75 (soluble TNF receptor [sTNF-R] p55 and sTNF-R p75) were measured using commercial enzyme-linked immunosorbent assays.

Results: The narcoleptic patients showed a significantly higher BMI compared with controls of the same age. Soluble TNF-R p75 levels were consistently elevated in the narcoleptic patients compared with their sex- and age-matched (P=.001) as well as sex-, BMI-, and age-matched counterparts (P=.003). Female narcoleptic patients exhibited higher sTNF-R p55 levels compared with their sex- and age-matched controls (P=.01), but this difference disappeared when comparing patients with sex-, BMI-, and age-matched normal controls. Tumor necrosis factor α levels did not differ significantly between groups.

Conclusion: Narcoleptic patients show increased plasma levels of sTNF-R p75, suggesting a functional alteration of the TNF-α cytokine system, further corroborating a possible pathogenic role of the immune system in this sleep disorder.

Arch Intern Med. 2006;166:1739-1743
be elevated in disorders associated with excessive daytime sleepiness, such as sleep apnea and idiopathic hypersonnia, and total sleep loss has been shown to produce significant increases in plasma levels of the soluble TNF-R (sTNF-R) p55. Because sTNF-Rs are a component of normal human cerebrospinal fluid, it is possible that TNF-α and TNF-Rs are involved in the central regulation of sleep-wake behavior.

Okun et al reported significantly higher TNF-α levels in narcoleptic patients compared with controls. Another study, however, could not confirm these results. Okun et al also analyzed group and group interactions across the ladder of powers were sought to achieve normality of the data and thus allow for parametric modeling including the evaluation of possible interaction effects. None of the parameters (TNF-α, sTNF-R p55, and sTNF-R p75) had a normal distribution (K-S test, P = .23), whereas for sTNF-R p75 (K-S test, P = .33), all were significantly skewed. Log transformations were used to normalize the distribution for TNF-α and its soluble receptors. For all analyses the intra-assay and interassay coefficients were below 7% and 9%, respectively.

DATA ANALYSIS

We matched up to 4 subjects from the BVS II sample to 1 narcoleptic patient according to sex and age. Matching criteria were the same sex and ±1 year of age. In a second step, we matched up to 4 controls to 1 narcoleptic patient according to sex, BMI, and age. Respectively to the narcoleptic patient, matching criteria were the same sex, BMI ±5%, and age ±10 years. For 11 narcoleptic patients, fewer than 4 control subjects could be found according to these matching criteria (3 controls for 4 patients, 2 controls for 6 patients, and 1 control for 1 patient).

Cytokine levels were compared between narcoleptic patients and controls using a linear mixed model with a random intercept for each group consisting of 1 narcoleptic patient and his or her matched control. This allows for exploring the differences between the narcoleptic subjects and controls within each group of matched subjects while taking the variability between the control subjects into account and allowing for an unequal number of control subjects for each patient. In both the age-matched and the BMI- and age-matched samples, we assessed differences between groups (narcoleptic patients vs controls) and possible group × sex interaction effects. For the age-matched sample we also analyzed group and group × sex interaction effects after controlling for differences in BMI.

In the complete BVS II population sample, the distribution of cytokine levels was tested for normality using the Kolgomorov-Smirnov (K-S) test, and suitable transformations across the ladder of powers were sought to achieve normality of the data and thus allow for parametric modeling including the evaluation of possible interaction effects. None of the parameters (TNF-α, sTNF-R p55, and sTNF-R p75) had a normal distribution (K-S test, P < .05), and all were significantly skewed. Log transformations were used to normalize the distribution for TNF-α (K-S test, P = .22) and sTNF-R p55 (K-S test, P = .23), whereas for sTNF-R p75 a power transformation with α = 1 resulted in a normal distribution of sTNF-R p75 (K-S test, P = .33).
When comparing narcoleptic patients with sex- and age-matched controls, narcoleptic patients showed a significantly higher BMI compared with normal controls of the same age and sex (Table 1).

In the sex- and age-matched and the sex-, BMI-, and age-matched samples, narcoleptic patients did not differ from controls in TNF-α levels (Table 2). Patients had higher sTNF-R p55 levels compared with their sex- and age-matched controls, but the difference was apparent only in female participants (group × sex interaction, Table 2). However, compared with the sex-, BMI-, and age-matched sample, this difference was not statistically significant.

Soluble TNF-R p75 levels were consistently elevated in the narcoleptic patients compared with their sex- and age-matched as well as sex-, BMI-, and age-matched counterparts. Again, this difference was mostly apparent in female participants; however, a group × sex interaction was only found in the sex- and age-matched sample (Table 2).

### Comment

In the present study, we found a significantly higher BMI in narcoleptic patients compared with controls. Soluble TNF-R p75 levels were consistently elevated in the narcoleptic patients compared with their sex- and age-matched as well as sex-, BMI-, and age-matched counterparts, while the difference in sTNF-R p55 plasma levels between groups disappeared when matching according to the BMI. Levels of TNF-α did not differ significantly between the group of narcoleptic patients and the 2 control groups.

Using the present data, we could confirm that narcoleptic patients exhibit a significantly higher BMI compared with controls.12,21 Regarding TNF-α levels, previous studies revealed conflicting results,18,19 possibly because, in contrast to the present study, controls in these studies were not matched by sex, age, and BMI. Regarding sTNF-R p55 and sTNF-R p75 plasma levels in narcoleptic patients, no comparable data are available to our knowledge.

Possible causes of elevated sTNF-R plasma levels in narcoleptic patients could be due to differences in age, BMI, genetics, and/or disease-related activation of the TNF-α system. We could exclude age-, sex-, and BMI-related differences for differences in sTNF-R p75 plasma levels because sTNF-R p75 levels were consistently elevated in the narcoleptic patients, even when comparing sTNF-R p75 plasma levels with their sex-, BMI-, and age-matched counterparts.

One reason for sTNF-R p75 plasma level elevation in narcoleptic patients would be genetic differences such as TNF-R p75 gene polymorphisms, though previous results regarding the frequency of certain alleles are conflicting.23,22 Another reason for sTNF-R p75 plasma level elevation in patients with narcolepsy may be HLA-DR2 differences between narcoleptic patients and controls. However, HLA-DR2–positive narcoleptic subjects5,3 have been shown to have lower TNF-α plasma levels in vivo,26 and to our knowledge no literature is available regarding sTNF-R plasma levels and HLA-DR2 differences.

It could also be the case that sTNF-R p75 plasma levels are elevated secondary to other aspects in narcoleptic patients caused by differences regarding the leptin27 or hypocretin’s system or caused by the medication patients take. Comparable obese nonnarcoleptic subjects, however, are reported not to show an association between leptin and sTNF-R p75 plasma levels39 or even show a positive correlation between leptin and sTNF-R p75 plasma levels.29 However, because narcoleptic patients were reported to have decreased leptin levels,27 one would expect these patients to have even decreased sTNF-R p75 plasma levels. To our knowledge, no data exist regarding the influence of decreased hypocretin production on sTNF-R p75 plasma levels.

It is unlikely that the psychotropic medication taken by the patients is responsible for the elevation of sTNF-R p75 levels because stimulants such as modafinil, which was the preferred drug in the investigated narcoleptic sample, are not known to activate the TNF-α system. On the contrary, tricyclic and tetracyclic antidepressants,30 atypical neuroleptics,31 and mood stabilizers32 leading to daytime sleepiness are reported to activate the TNF-α system and to raise sTNF-R p55 and sTNF-R p75 plasma levels, whereas psychotropic drugs not leading to daytime sleepiness such as venlafaxine30 do not result in elevated sTNF-R p55 and sTNF-R p75 plasma levels. Therefore, the elevation of sTNF-R p55 and sTNF-R p75 plasma levels due to the administration of drugs leading to daytime sleepiness appears as an experimental model for the association between sTNF-R p55 and sTNF-R p75 plasma levels and sleepiness.

Questioning the possible functional significance of our findings leads to remarkable metabolic aspects of narcoleptic patients; levels of sTNF-Rs, known to be involved in the regulatory endocrine system of body adiposity independently of leptin and resistin axis in nonmorbidly obese patients,28 are elevated in obese subjects,28 and sTNF-R levels are reported to be associated with type 2 diabetes mellitus independently of body weight.33 Tumor necrosis factor signaling is known to lead to diabetes by decreasing insulin receptor signaling capacity and...
zyme37 and may therefore be associated with the amount of integrin metalloproteinase called TNF-bound form derived by the proteolytic actions of a dis-variants of the extracellular domains of their membrane-tosis.40

The elevation of sTNF-R p75 plasma levels in narcoleptic pa-tients is in line with the known impaired glucose toler-elevation of sTNF-R p75 plasma levels in narcoleptic pa-tients in comparison with normal controls. Narco-
eas is related to being overweight, and sleep apnea can affect inflammatory markers.14 To rule out BMI-related effects, we compared the patients with the sex-, BMI-, and age-matched controls.

Because narcoleptic patients showed a higher BMI com-pared with the sex- and age-matched controls, sleep apnea is related to being overweight, and sleep apnea can affect inflammatory markers.14 To rule out BMI-related effects, we compared the patients with the sex-, BMI-, and age-matched controls.

In conclusion, we investigated a sample of narcolep-tic patients in comparison with normal controls. Narco-leptic patients showed increased plasma levels of sTNF-R p75, suggesting a functional alteration of the TNF-α cyto-kine system and further corroborating a possible patho-logenic role of the immune system in this sleep disorder. These results highlight the important relationship be-tween sleep and sleep disorders and immune function.

Accepted for Publication: June 6, 2006.

Correspondence: Hubertus Himmerich, MD, Center for Neurosciences, Klinikum der Philipps-Universität, Klinik für Neurologie, Rudolf-Bultmann-Straße 8, 35039 Marburg, Germany (himmerich@mpipsykl.mpg.de).

Author Contributions: All authors had full access to all of the data in the study and take responsibility for the integ-rity of the data and the accuracy of the data analysis.

Financial Disclosure: None reported.

Funding/Support: The study was supported in part by grant MCRTN-CT-2004-512362 from the European Union. The BVS II was supported by funds of the Kurt-Eberhard-Bode-Stiftung and the Bavarian Ministry of En-vIRONMENT, Health, and Consumer Protection.

Acknowledgment: We thank Gabriele Kohl and Irene Gunst for excellent technical assistance regarding the cyto-kine measurement in the narcoleptic patients and BVS II study samples and Dorothea Skottke for help in pre-paring the manuscript. We especially thank the physi-cians from the health offices in Bavaria for providing study rooms and drawing the blood samples (BVS II). We ac-knowledge the cooperation of the narcoleptic patients from the German Narcoleptic Society and acknowledge the cooperating physicians for providing information.

Table 2. Plasma Concentrations of TNF-α and Its Soluble Receptors in Narcoleptic Patients and Normal Controls

<table>
<thead>
<tr>
<th>Parameter/Sample</th>
<th>All</th>
<th>Male</th>
<th>Female</th>
<th>Group</th>
<th>Group × Sex</th>
<th>Controlled for BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with narcolepsy</td>
<td>30 (8.39) [8.58]</td>
<td>9 (9.04) [7.31]</td>
<td>21 (9.75) [9.24]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S/A-MC</td>
<td>120 (10.84) [6.24]</td>
<td>36 (11.57) [4.69]</td>
<td>84 (10.58) [6.57]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S/A/B-MC‡</td>
<td>101 (10.58) [5.30]</td>
<td>34 (10.49) [4.65]</td>
<td>67 (10.58) [4.88]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTNF-R p55, ng/mL</td>
<td>30 (1.96) [0.59]</td>
<td>9 (1.93) [0.41]</td>
<td>21 (2.03) [0.59]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with narcolepsy</td>
<td>120 (1.77) [0.64]</td>
<td>36 (2.10) [0.46]</td>
<td>84 (1.66) [0.50]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-MC</td>
<td>101 (1.84) [0.49]</td>
<td>34 (1.86) [0.65]</td>
<td>67 (1.84) [0.42]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B-MC‡</td>
<td>30 (5.47) [1.41]</td>
<td>9 (4.81) [1.21]</td>
<td>21 (5.54) [1.21]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTNF-R p75, ng/mL</td>
<td>120 (4.38) [1.59]</td>
<td>36 (5.52) [1.71]</td>
<td>84 (4.15) [1.19]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with narcolepsy</td>
<td>101 (4.49) [1.60]</td>
<td>34 (4.90) [1.52]</td>
<td>67 (4.37) [1.58]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: A/B-MC, age- and BMI-matched controls; A-MC, age-matched controls; BMI, body mass index; S/A/B-MC, sex-, BMI-, and age-matched controls; S/A-MC, sex- and age-matched controls; sTNF-R, soluble tumor necrosis factor receptor; TNF-α, tumor necrosis factor α; ellipses, not applicable.

*pData are given as number (median) [interquartile range].

‡For 11 narcoleptic patients, there were fewer than 4 control subjects. For that reason age and BMI values are weighted summary statistics.

©2006 American Medical Association. All rights reserved.

(Reprinted) Arch Intern Med/Vol 166, Sep 18, 2006 www.archinternmed.com

1742

Downloaded From: https://archinte.jamanetwork.com/ by a Non-Human Traffic (NHT) User on 06/02/2019
about the narcoleptic patients. We thank the Kreiskrankenhaus Wolfratshausen for letting us use the laboratory equipment to directly process the blood of the patients with narcolepsy.

REFERENCES