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CASE REPORTS

Misdiagnosis of narcolepsy caused by a false-positive orexin-A/hypocretin-1 enzyme immune assay

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The diagnosis of narcolepsy is based on clinical history, sleep studies, and, in some cases, cerebrospinal fluid orexin-A/hypocretin-1 measurement. The gold standard for orexin measurement is the radioimmunoassay but other commercial kits are also available, such as the enzyme immune assay (EIA). The specificity of orexin EIA in humans is unknown. We report four cases where orexin levels were measured by EIA and resulted in false positives and the misdiagnosis of narcolepsy. Therefore, orexin EIA measurement should be strongly discouraged in a clinical setting.

Keywords: narcolepsy, orexin, hypocretin.

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INTRODUCTION

Narcolepsy type 1 (NT1) is a life-long brain disorder caused by the destruction of neurons producing orexin-A (also called hypocretin-1). The main features of NT1 are excessive daytime sleepiness (EDS), cataplexy, hallucinations, and paralyses occurring at transitions between sleep and waking. The diagnosis of narcolepsy is based on the medical history, a full-night polysomnography (PSG), a Multiple Sleep Latency Test (MSLT), and a cerebrospinal fluid (CSF) orexin-A measurement via lumbar puncture (LP). Unfortunately, MSLT is not very sensitive or specific for NT1, especially in the absence of cataplexy. The sensitivity of MSLT for narcolepsy is only around 70–90%.[1] Insufficient sleep, delayed sleep circadian rhythm, irregular sleep times, and medications affecting rapid eye movement (REM) sleep may decrease the reliability of the MSLT. For instance, altogether 1–6% of adults may fulfill the MSLT criteria for narcolepsy, although the prevalence of narcolepsy is only 0.02%, indicating a lack of specificity as well.[2] Therefore, the CSF orexin measurement is the most reliable way to confirm a diagnosis of NT1.

A low (< 110 pg/mL) concentration of CSF orexin measured by a radioimmunoassay (RIA) using a Stanford reference sample is a highly specific finding for NT1. However, the RIA method has some drawbacks. First, it requires radioactivity precautions. Second, it is liable to undergo cross-reactions with matrix constituents, generating interference and high interbatch variability.[3] Other diagnostic methods to measure CSF orexin, such as mass spectrometry, fluorescence immune assay, and enzyme immune assay (EIA), have also been developed.[3–5] EIA is a cheaper and more environmentally friendly method (no radioactive waters) than RIA, but it is not validated as a diagnostic test for narcolepsy. Nevertheless, CSF orexin EIA kits are commercially available and have been used in clinical and research settings.[4]

In Finland, from April 2017 until April 2018, orexin samples from 1 administrative health care region were subcontracted after a bidding competition to a laboratory where orexin analyses were made by EIA analysis. Here, we report 4 cases where the primary EIA analysis of CSF orexin provided a false low orexin level compared with the RIA method, leading to a misdiagnosis of narcolepsy (**Table 1**). The diagnosis of narcolepsy was made according to *International Classification of Sleep Disorders*, third edition (ICSD-3)—that is, patients presented with EDS, possible cataplexy, and were examined by PSG followed by MSLT.

REPORT OF CASES

Case 1

The first patient is a 40-year-old professional driver. He had a history of asthma, very mild sleep apnea, and a previous catheter ablation for atrial fibrillation, which had left him with susceptibility to tachycardia. He had experienced a gradual onset of EDS for 2 years and needed daily naps to maintain sufficient alertness throughout the day. He had fallen asleep at work while driving a forklift. He slept quite well and did not report unambiguous cataplexy, although he complained of occasional subjective muscle weakness. In a new PSG, he had a low apnea-hypopnea index of 0.5 events/h and an unremarkable sleep macro- and microarchitecture. In an MSLT, the mean sleep latency (MSL) was short, at only 5.4 min, but there were no sleep-onset REM sleep periods

Table 1—Characteristics of cases.

	Case 1	Case 2	Case 3	Case 4
Age at onset, y	38	15	10	30
Age at presentation, years	40	24	17	34
Sex	Male	Female	Female	Female
EDS	Yes	Yes	Yes	Yes
CPL	Absent	Atypical	Atypical	Atypical
HHs	No	Yes	No	No
SP	No	Yes	No	No
DNS	No	Yes	No	Yes
MSLT MSL, min	5.4	11.7	10.6	Unavailable†
MSLT SOREMPs	0	0	0	Unavailable†
Orexin by EIA, pg/mL	<40	102	<40	114
Orexin by RIA, pg/mL	340	314	404	378
HLA DQB1*06:02	Not done	Negative	Not done	Not done
Possible diagnosis	Hypersomnia of unknown cause	Unclear	ADD, depression	Functional neurologic disorder, pain, medications‡

†Primary sleep studies not done (see text), follow-up studies with normal results. ‡Carbamazepine, pregabalin, amitriptyline, ADD = attention-deficit disorder, CPL = cataplexy, DNS = disturbed nocturnal sleep, EDS = excessive daytime sleepiness, EIA = enzyme immune assay, HH = hypnagogic hallucination, HLA = human leukocyte antigen, MSL = mean sleep latency, MSLT = Multiple Sleep Latency Test, RIA = radioimmunoassay, SOREMP = sleep-onset REM (rapid eye movement) sleep period, SP = sleep paralyses.

(SOREMPs). An LP was done simultaneously and showed undetectable (< 40 pg/mL) orexin levels with EIA, which led to a narcolepsy diagnosis. The traffic authorities were informed that he would not be able to continue in his work, and occupational rehabilitation was started. Modafinil caused palpitations, methylphenidate was not tried due to previous cardiac issues, and sodium oxybate caused multiple side effects. He was referred to a tertiary sleep center, where a new LP and orexin analysis was made by the RIA method and completely normal orexin levels (340 pg/mL) were found.

Case 2

A 24-year-old female with a history of hypothyroidism had been examined at a central hospital due to tremor, muscle twitches, and coordination problems that she had suffered for around 10 years. During the last couple of years, she had developed EDS and an increased need for sleep. She also expressed some auditory hypnagogic hallucinations and sleep paralyses. She said that, since childhood, when she laughed heartily, she felt that she lost the muscle tone in her arms and might drop things resembling cataplexy. In a clinical examination, she had exaggerated deep tendon reflexes and a positive plantar sign. In a thorough diagnostic workup, including a whole central nervous system magnetic resonance imaging scan, nerve conduction studies, electromyography, plasma oxysterols, and genetic ataxia screening, including also a DNMT1 gene test, the results were negative. In an MSLT, she had a normal MSL of 11.7 minutes without SOREMPs. However, her CSF orexin concentration was only 102 pg/mL. Due to the atypical clinical picture, she was referred to a university hospital for a second opinion. Fortunately, there was still some of her CSF left at the central hospital. A new orexin analysis from the same sample was performed with the RIA method, showing a completely normal level of 314 pg/mL.

Case 3

A 17-year-old woman had mild subclinical hypothyroidism. She had some depressive symptoms during high school. She had experienced EDS since she was 10 years old. She also recalled that she had started to experience a loss of muscle tone in her whole body while laughing at around the same time. Atypical for cataplexy, she had short spells of derealization, during which she would seem unresponsive. She experienced severe sleep inertia, also not typical for narcolepsy. She had mild to moderate depression. In an MSLT, the MSL was 10.6 minutes without SOREMPs. CSF orexin by EIA was less than 40 pg/mL. For some reason, the results took 4 months to arrive. During this period, she had been diagnosed with an attention-deficit disorder and started prolonged methylphenidate 36 mg daily, which did alleviate the sleepiness. Because of the aforementioned atypical features, a second LP was made and now the orexin levels were measured by RIA, yielding a result of 404 pg/mL.

Case 4

A 39-year-old woman had a history of chronic pains, hypothyroidism after radioiodine therapy for hyperthyroidism, celiac disease, and muscle cramps. Four years earlier, she had started to experience EDS and sleep attacks. During these episodes, her limbs felt weak, she had difficulties speaking, and occasionally experienced diplopia. These attacks lasted from minutes to longer periods, up to a maximum of 2 days. They were not associated with emotional

triggers. The fourth patient had difficulties sleeping in the sleep laboratory for the PSG and MSLT, and therefore an LP was done to analyze the orexin levels. The orexin concentration by EIA was low, at 114 pg/mL, indicating narcolepsy or borderline narcolepsy. She started modafinil but could not increase the dose above 200 mg in the morning since larger or later doses caused sleep problems and gastrointestinal symptoms. A new LP and orexin analysis with RIA was made because of the earlier false-positive results at this same central hospital. The orexin concentration was normal at 378 pg/mL.

DISCUSSION

CSF orexin measurement by RIA is the gold standard for narcolepsy diagnosis, although the current ICSD-3 criteria do not classify the certainty of diagnosis. The Brighton Collaboration Criteria with 3 levels of diagnostic certainty for narcolepsy were developed after the abrupt increase in the incidence of narcolepsy in countries where the pandemic H1N1 vaccine Pandemrix (GlaxoSmithKline, Dresden, Germany) was used.[6] In these criteria, the highest level 1 certainty is achieved with EDS or cataplexy and low CSF orexin. A recent reappraisal by European sleep experts also suggests certainty classification including orexin measurement.[7] The CSF orexin RIA measurement has some methodological limitations, as described in the Introduction. This is further complicated by a lack of laboratory standards and quality assurance for orexin RIA. In addition, orexin levels seem to have diurnal and seasonal variation.[8,9] Despite these limitations, the specificity of a low or undetectable orexin level in the CSF for narcolepsy is 99% if measured by RIA. Therefore, it is highly indicative for narcolepsy and, in conjunction with sleepiness as a complaint, it leads easily to a narcolepsy diagnosis.

Lin et al[10] reported the correlation of EIA with the RIA method in rat brain, but there are no validation studies for orexin EIA measurement for human narcolepsy. Blood testing for orexin has also been studied but with modest and inconsistent results.[11,12] Our case series shows that, in humans, EIA and RIA results can vary greatly.

In our third case, EDS was probably explained by attention-deficit disorder and depression. The exact cause of the reported EDS and other symptoms remain open in the other 3 cases, but NT1 was excluded. In the same hospitals, credible but unconfirmed low orexin concentrations by EIA were also found in patients with typical clinical phenotype for NT1 (total of approximately 20 to 30 samples). All of the reported cases here had somewhat atypical features for narcolepsy. This emphasized the careful examination of the patients' history and clinical judgment. Their sleep study results were also not consistent with narcolepsy, but due to the moderate sensitivity of MSLT and PSG, they are not exclusive to a narcolepsy diagnosis.

Some research groups have introduced mass spectrometry methods for CSF orexin measurement.[5,13,14] Mass spectrometry seems to detect considerably lower levels of orexin in the CSF, which raises an intriguing question of what we actually measure with orexin RIA.[15] It seems that, actually, RIA detects, in addition to intact orexin-A, also its breakdown products. The clinical usefulness of the different methods (RIA and mass spectrometry) is still unclear. Therefore, mass spectrometry still needs further validation in larger studies and especially in other diseases than NT1 before replacing RIA as a gold standard for orexin measurement.

It is also worth noting that none of the 4 cases had unambiguous cataplexy in their clinical history. Absence of unambiguous cataplexy with low levels of orexin is possible in NT1, as cataplexy may develop several years after onset of sleepiness. However, if EIA has been used, one cannot make a diagnosis of NT1 unless cataplexy is unambiguous.[6]

Accurate laboratory diagnostics are crucial for the reliability of the diagnosis of chronic debilitating disorders. Misdiagnosis of narcolepsy can cause psychological distress and reduced quality of life and mask other treatable causes of sleepiness. Most narcolepsy medications are controlled substances with severe potential adverse effects. Narcolepsy diagnosis also has implications on driving, employment, and insurance policies. The use of EIA for the diagnosis of narcolepsy should be avoided.

ABBREVIATIONS

CSF, cerebrospinal fluid
EDS, excessive daytime sleepiness
EIA, enzyme immune assay
LP, lumbar puncture
NT1, narcolepsy type 1
MSL, mean sleep latency
MSLT, Multiple Sleep Latency Test
PSG, polysomnography
RIA, radioimmunoassay
SOREMP, sleep-onset REM sleep period

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