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TIMP-1 as a biomarker in obstructive sleep apnea: screening, monitoring, risk stratification, and a step towards precision medicine

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Abstract. – OBJECTIVE: The diagnosis of obstructive sleep apnea (OSA) is a complex time- and resource-intensive diagnostic procedure. Since tissue inhibitors of matrix metalloproteinases (TIMP's) are involved in various pathophysiological processes and are correlated with a high cardiovascular risk, TIMP's appear to be a suitable candidate for an OSA-biomarker.

PATIENTS AND METHODS: In a prospective controlled diagnostic study, TIMP-1 serum levels of 273 OSA-patients and controls were analyzed for correlation with OSA severity, BMI, age, sex, cardio-/ cerebrovascular comorbidities. Furthermore, longitudinal medium- and long-term effects of CPAP-treatment (n=15) on TIMP-1-levels were investigated.

RESULTS: TIMP-1 was clearly linked to OSA as well as to disease severity (mild, moderate, severe; each $p<0.001$) and was not influenced by age, gender, BMI, or cardio-/cerebrovascular comorbidities. ROC curve analysis revealed an AUC of 0.91 ± 0.017 SE ($p<0.001$), suggesting a TIMP-1 cut-off value of 75 ng/ml (sensitivity 0.78; specificity 0.91) being especially sensitive for patients with severe OSA (sensitivity 0.89; specificity 0.91). The likelihood ratio was 8.88, while the diagnostic odds ratio was 37.14. CPAP-treatment led to a significant decrease of TIMP-1 after 6-8 months ($p=0.008$).

CONCLUSIONS: TIMP-1 seems to fulfill the preconditions for a circulating OSA-biomarker: disease-specific with a mandatory presence in affected patients, reversible on treatment, reflects disease severity and provides a cutoff value between the healthy state and disease. In the clinical routine, TIMP 1 may help to stratify the individual OSA-associated cardiovascular risk and to monitor the treatment response to CPAP-therapy as a further step towards providing a personalized therapy.

Key Words:
Sleep apnea, OSA, Biomarker, Personalized medicine, TIMP, Cardiovascular risk stratification.

Introduction

Obstructive Sleep Apnea (OSA) is the most prevalent form of sleep-disordered breathing in the general population; it may limit quality of life and is verifiably associated with many common comorbid conditions such as hypertension, coronary artery disease, congestive heart failure, cerebrovascular events, and cardiac arrhythmias. In addition to this, patients with OSA have an increased risk of all-cause mortality[1]. Among 30-70-year-old adults, the prevalence of OSA (AHI $\geq$ 15) for men and women is currently estimated to be approximately 13% and 6%, respectively[2]. Based on the fact that some 82% of men and 93% of women still remain undiagnosed, the prevalence of OSA in the general population is unfortunately underestimated[3].

The gold standard for the diagnosis of OSA is a sleep laboratory-based overnight polysomnography, which is an expensive and labor-intensive diagnostic procedure that is not accessible for every patient at any given time or place[4]. Because of its obvious limitations in terms of accessibility and costs, studies[4] have been trying to find an easier-to-use alternative for the diagnosis of OSA, thereby restricting the use of polysomnography in the sleep laboratory setting to only those patients with a high pretest probability. The search for this alternative includes questionnaires, clinical morphometric models, and biomarkers. The assessment of potential biomarkers was based on quantifying several proteins and metabolites from blood, urine, saliva, and exhaled breath condensate[5]. In this regard, a biomarker is a measurable parameter that is able to represent a biological function. It needs to be a useful aid for the screening, diagnosis, monitoring of therapeutic response, prognosis, and risk prediction in specific clinical situations and in terms of personalized medicine[6].

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Metabolites of oxidative stress caused by the intermittent nocturnal hypoxia and the production of inflammatory mediators might be a promising approach with regard to a potential biomarker. In this context, activated metalloproteinases (MMPs) are proteinases that participate in extracellular matrix degradation and have been reported to be elevated in patients with OSA[7-10]. MMPs are involved in the development and progression of atherosclerotic lesions and, together with oxidative stress, are strongly correlated with a high cardiovascular risk[8]. On the other hand, tissue inhibitors of MMPs (TIMPs) are specific inhibitors of MMPs that participate in controlling local activities in tissues[11]. An excess of MMPs may be responsible for the structural degradation of tissue, whereas an excess of TIMPs may promote excessive tissue repair processes and fibrosis[10]. The balance of the extracellular matrix depends largely on the close interaction between MMPs and TIMPs[12]. Hence TIMPs are also involved in various MMP-mediated pathophysiological processes, such as coronary syndrome, vascular disease, heart failure, and immunopathogenesis[13,14]. Until now, no ideal biomarker has been found for early diagnosis, establishing the severity of disease, prognosis, or prediction of response to OSA treatment[15]. In this prospective controlled diagnostic study, we focused on biomarker-based primary diagnostics and monitoring of therapeutic response in obstructive sleep apnea using TIMP-1, and on the future potential of generalized (cardiovascular) risk stratification in the context of personalized medicine.

Patients and Methods

This single center prospective controlled diagnostic study was conducted in accordance with the amended Declaration of Helsinki and was approved by the Ethics Committee of the Friedrich-Alexander-University Erlangen-Nürnberg (FAU) (Ref-Nr.103_18B). Between October 2014 and April 2018, a total of 273 subjects, 171 patients with OSA confirmed by polysomnography (PSG) and 102 healthy subjects, were prospectively investigated in the Sleep Department of the Department of Otorhinolaryngology, Head and Neck Surgery, Friedrich-Alexander-University Erlangen-Nürnberg (FAU). Inclusion criteria were men and women with untreated obstructive sleep apnea (n=171), healthy volunteers with an unremarkable sleep history without PSG (n=67) and healthy volunteers with a history of snoring and for whom obstructive sleep apnea had been excluded according to the ICSD-3 criteria by means of PSG (n=35). Exclusion criteria were patients with central or mixed apnea, patients under the age of 18, pregnant women, patients with significant cognitive impairment or a poorly controlled psychiatric disorder and patients with treated OSA. Prior to enrollment, all participants received an adequate explanation of the study plan and provided their written informed consent. This diagnostic study is reported according to the STARD requirements. The diagnosis of OSA was obtained by PSG in all patients. Each patient provided a venous blood sample for the determination of serum TIMP-1 between 8 and 12 a.m. in the morning after cardiorespiratory PSG.

Cardiorespiratory Polysomnography (PSG) and CPAP-Titration

PSG was carried out using the 33-channel cardiorespiratory SOMNOscreen diagnostic system (SOMNOmedics, Randersacker, Germany). The technical implementation of the PSG followed the recommendations of the American Academy of Sleep Medicine (AASM)[16]. The sleep stages and associated events were analyzed and scored visually according to the AASM criteria (Version 2.1, 2014)[17]. OSA was defined using the diagnostic criteria of the International Classification of Sleep Disorders (ICSD-3) for mild and moderate OSA and AHI>30/h for severe OSA[18]. In the night following PSG, CPAP titration was performed according to the clinical guidelines of the Positive Airway Pressure Titration Task Force of the AASM.

Analysis of TIMP-1

The analysis of TIMP-1 was performed with enzyme-linked immunosorbent assay (ELISA; Human TIMP-1 Development Kit, Peprotech, Hamburg).

Investigation of the Dian Rhythm of TIMP-1

Serum concentrations of TIMP-1 of 4 healthy subjects were analyzed throughout the day from 8 a.m. to 6 p.m. In total, 40 samples were analyzed, with 4 blood samples excluded due to their hemolytic appearance. The non-parametric Wilcoxon's test was chosen to compare blood samples, with the sample from each collection time being compared to that from 8 a.m. The Bonferroni correction was applied to avoid alpha inflation, with p_{crit} = 0.05 / # calculated tests = 0.005.

Differences Between Healthy Controls and OSA Subjects Regarding TIMP-1

The serum levels of the healthy controls and OSA group were compared *via* an independent *t*-test. The influence of OSA severity on TIMP-1 serum values were examined with a univariate ANOVA with the between factor "group" (Control *vs.* OSA mild *vs.* moderate *vs.* severe).

TIMP-1 Cut-off Value for OSA

To identify a cut-off value for the diagnosis of OSA, a Receiver Operating Characteristic (ROC) curve analysis was conducted. Sensitivity, specificity, positive and negative predictive value, likelihood ratio as well as the diagnostic odds ratio were calculated.

Influence of CPAP Therapy on TIMP-1

After obstructive sleep apnea was diagnosed *via* polysomnography, CPAP therapy was initiated after appropriate CPAP pressure titration. Compliance was defined as CPAP use for ≥ 4 h per night. TIMP-1 was re-analyzed after 3 and 6-8 months under ongoing CPAP treatment. To avoid a listwise exclusion of patient samples due to missing values in one of the two follow-up samples, a linear mixed model with the fixed effect "time" (baseline *vs.* 3 months *vs.* 6-8 months) was computed. Post-hoc testing for differences between the subsequent measurements were conducted within the linear mixed model. To test for differences between the TIMP-1 values of patients and the control group at the 6-8-month follow up, an independent *t*-test was calculated.

Cardio- and Cerebrovascular Comorbidities and TIMP-1

To analyze the influence of pre-existing cardio- and cerebrovascular comorbidities (arterial hypertension, coronary heart disease, diabetes mellitus and apoplex) as well as demographic variables (sex, age, and BMI) on TIMP-1, a multiple linear regression was performed. Data were collected on the basis of a detailed medical history and review of the patient files.

Statistical Analysis

Data were analyzed in SPSS 25 (IBM Corp., Armonk, NY, USA). The significance level was chosen at $p<0.05$. Continuous variables are presented as mean ± 1 SD (standard deviation). The mean ± 1 SE (standard error) is only reported when performing the linear mixed model. Baseline characteristics were analyzed with cross tables and Chi^2-tests in the case of nominal variables, or with univariate ANOVAs and independent *t*-tests in the case of continuous variables. Effect sizes were calculated to illustrate the importance of the effect: r = 0.1 displayed a small, r = 0.3 a medium and r = 0.5 a huge effect. In the case of explorative testing, Bonferroni correction was performed.

Results

A total of 273 patients with a mean age of 48.4 years ± 13.1, a BMI of 29.1 ± 5.7 and an ESS of 8.7±4.6 were recruited for this study. This group consisted of 171 patients with OSA diagnosed by PSG (26 mild, 66 moderate and 79 severe OSA) and 102 healthy control subjects, of which 67 reported an unremarkable sleep history without PSG. Another 35 patients reported snoring and - as a precaution - underwent PSG to exclude OSA. Further demographic investigations of both control groups revealed a significant difference between the two subgroups regarding sex and BMI. While controls with PSG showed higher BMIs than those without (27.17 ± 4.34 *vs.* 24.18 ± 3.69, $t_{(86)} = 3.429$, $p = 0.001$) and included more men (56.7% *vs.* 77.1%, $\chi^2_{(1)} = 4.15$, $p = 0.042$), there was no difference between the two subgroups regarding age (41.99 ± 12.36 *vs.* 44.70 ± 3.69, $t_{(99)} = 0.941$, $p = 0.349$). After OSA had been carefully excluded in both control groups, they were merged and further analyzed as one control population.

Details of baseline characteristics of the two main groups (OSA patients and healthy controls) as well as of the OSA subgroups (mild, moderate, severe) are shown in Tables I and II.

The influence of OSA severity on TIMP-1 serum values was examined with a univariate ANOVA with the between factor "group" (Control *vs.* OSA mild *vs.* moderate *vs.* severe).

Dian Rhythm and TIMP-1

There was slight increase of TIMP-1 (ng/ml) during the day (8 a.m.: 81.44 ± 7.32 *vs.* 6 p.m.: 104.67 ± 8.20). There were no significant differences between the blood sample taken at 8 a.m. and any of the samples taken later during the day ($z < 1.604$, $p > 0.068$, Table III).

In summary, no significant dian rhythm of TIMP-1 serum levels could be determined (Table III).

Table I. Baseline characteristics of OSA patients and healthy controls.

	Control	OSA	Control *vs.* OSA
Male/Female n	65/37	137/34	$\chi^2_{(1)} = 8.92$ $p = 0.003$
Age years (±SD)	**43.8** (±13.8)	**51.2** (±12.0)	$t_{(187.12)} = 4.53$ $p < 0.001$
BMI kg/m² (±SD)	**25.3** (±4.2)	**31.2** (±5.4)	$t_{(217.93)} = 9.69$ $p < 0.001$
ESS score (±SD)	**6.5** (±3.8)	**9.6** (±4.6)	$t_{(150.93)} = 5.27$ $p < 0.001$

SD: Standard Deviation, BMI: Body Mass Index, ESS: Epworth Sleepiness Scale.

Table II. Baseline characteristics of OSA-subgroups: mild, moderate, severe.

	Mild OSA	Moderate OSA	Severe OSA	Mild *vs.* mod. *vs.* severe
Male/Female	18/8	53/13	66/13	$\chi^2_{(2)} = 2.52$ $p = 0.284$
Age years (±SD)	**51.3** (±11.6)	**50.9** (±11.2)	**51.4** (±12.8)	$F_{(2, 168)} = 0.03$ $p = 0.97$
BMI kg/m² (±SD)	**30.3** (±5.3)	**28.9** (±3.9)	**33.4** (±5.7)	$F_{(2, 166)} = 14.88$ $p < 0.001$
ESS (±SD)	**11.5** (±3.8)	**9.8** (±4.65)	**8.8** (±4.6)	$F_{(2, 162)} = 3.71$ $p = 0.027$
AHI /h (±SD)	**9.5** (±2.6)	**21.9** (±4.5)	**53.1** (±20.0)	$F_{(2, 168)} = 139.42$ $p < 0.001$

SD: Standard Deviation, BMI: Body Mass Index, ESS: Epworth Sleepiness Scale, AHI: Apnea Hypopnea Index.

Table III. Depiction of the descriptive statistics and mean difference of TIMP-1 (ng/ml) to that at 8 a.m. (Diff 8 a.m.) as well as a Wilcoxon test's Z- and asymptotic *p*-values corresponding to this difference.

Variable	Mean	SD	Diff 8 a.m.	Z	p
8 a.m.	81.44	7.32			
9 a.m.	70.67	3.57	-10.77	1.60	.109
10 a.m.	85.73	13.26	4.29	0.73	.465
11 a.m.	79.68	10.36	-1.76	0.73	.465
12 a.m.	87.17	33.66	5.73	0.37	.715
1 p.m.	101.54	34.71	20.10	1.07	.285
2 p.m.	93.50	18.54	12.06	1.10	.273
3 p.m.	98.49	32.39	17.05	0.73	.465
4 p.m.	100.56	14.66	19.12	1.83	.068
5 p.m.	115.01	5.06	33.57	1.60	.109
6 p.m.	104.67	8.20	23.23	1,60	.109

Relation between OSA and TIMP-1

We tested TIMP-1 levels between the two control groups to further exclude possible differences (Table IV). There was no significant difference between the two subgroups of the control group (controls w/ PSG *vs.* controls w/o PSG; $p = 0.467$).

Statistical analysis of TIMP-1 serum concentration between OSA and control patients showed a highly significant difference ($p < 0.001$, r = 0.61; Table IV) between both groups with a mean serum concentration of 114.98 ± 59.4 ng/ml in the OSA group *vs.* 51.2 ± 25.4 ng/ml in the control group (Figure 1).

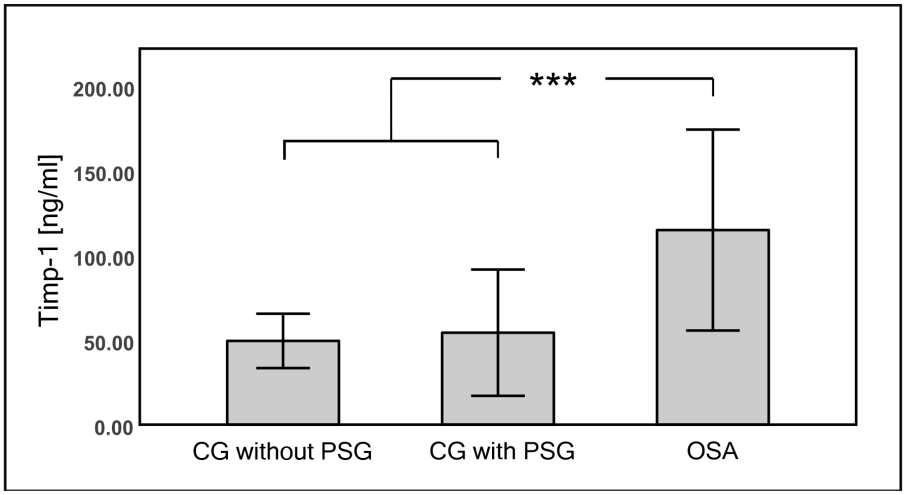

Figure 1. TIMP-1-levels (in ng/ml) of control groups (CG; n=102) and OSA-patients (n=171). ***p<0.001.

Furthermore, when taking the OSA severity into account, a highly significant main effect "group" was found ($F_{(3,271)}$ = 43.98, p < 0.001), revealing that each OSA group had significantly higher TIMP-1 levels than the control group (Figure 2).

Also, TIMP-1 levels of the moderate OSA groups were lower than those of the severe group (99.36 ± 47.42 *vs.* 132.40 ± 65.61, p < 0.001, r = 0.29). There was also a trend for lower TIMP-1 levels in the mild group compared to the severe OSA group (102.37 ± 54.46 *vs.* 132.40 ± 65.61, $t_{(138,77)}$ = 2.31, p = 0.038, r = 0.22). Based on the Bonferroni correction, we assess a p of 0.038 as a trend, as it is above the determined p_{crit} of 0.0083 (Table IV).

Receiver Operating Characteristic (ROC) Analysis and Likelihood Ratio (LR)

ROC curve analysis for OSA *vs.* healthy controls revealed a very high area under the curve (AUC) of 0.91 ± 0.017 SE (p <0.001, 95% CI: 0.88-0.95), with an optimal diagnostic TIMP-1 cut-off value of 75 ng/ml, which maximally increased sensitivity and specificity (sensitivity 78.2 %; specificity 91.2 %; positive predictive value 94

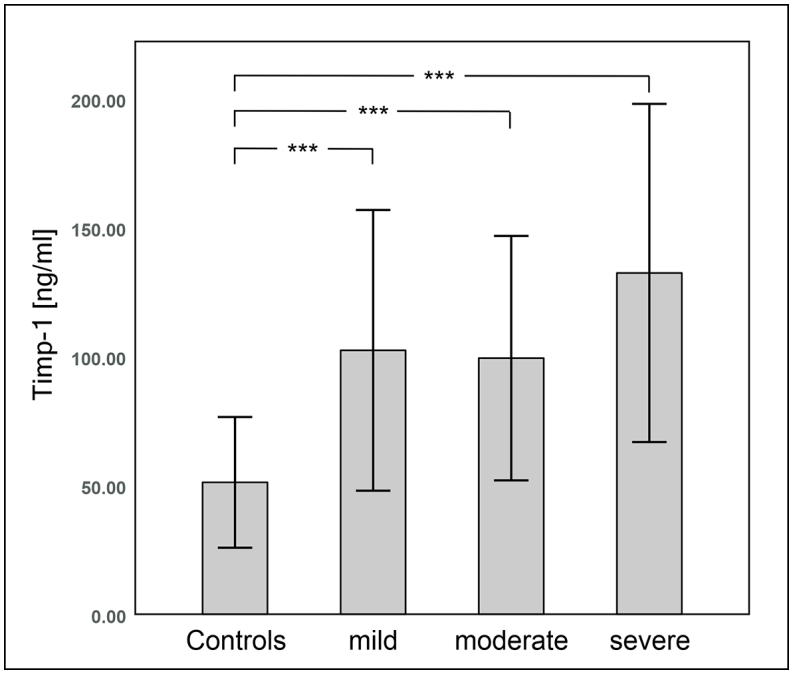

Figure 2. TIMP-1-levels (in ng/ml) of healthy controls and patients with mild and moderate OSA (according to ICSD-3) and severe OSA (AHI>30/h)[19]. ***p<0.001.

Table IV. Statistical analysis of TIMP-1 serum concentration of OSA patients and healthy controls. The serum levels were compared *via* an independent *t*-test. (PSG: Polysomnography). Statistical analysis of TIMP-1 serum concentration between OSA and control patients showed a highly significant difference [$p < 0.001$, r = 0.61 (Table IV)] between both groups with a mean serum concentration of 114.98 ± 59.4 ng/ml in the OSA group *vs.* 51.2 ± 25.4 ng/ml in the control group (Figure 1).

Timp-1 [ng/ml]	n	Mean	SD	Statistics
Controls w/ PSG *vs.*	35	54.34	37.21	
Controls w/o PSG	67	49.49	16.07	$t_{(40.71)} = 0.734$, $p = 0.467$
Controls *vs.*	**102**	51.15	25.36	
mild OSA	26	102.38	54.46	$t_{(27.82)} = 4.67$, $p < 0.001$
moderate OSA	66	99.36	47.42	$t_{(89.31)} = 7.59$, $p < 0.001$
severe OSA	79	132.40	65.61	$t_{(95.65)} = 10.36$, $p < 0.001$
OSA	**171**	114.98	59.4	$t_{(248.9)} = 12.28$, $p < 0.001$

%; and negative predictive value 72 %) (Figure 3). The likelihood ratio was 8.88, while the diagnostic odds ratio was 37.14. This cut-off value was especially sensitive for patients with severe OSA (sensitivity 0.89; specificity 0.91).

CPAP Therapy and TIMP-1

In total, 15 patients with moderate (n = 7) and severe (n = 8) OSA were longitudinally monitored (mean age of 47.13 years ± 12.29 SD; BMI 31.89 ± 3.5 SD; ESS 6.81, ± 5.21 SD, mean AHI of 36.45 ± 22.25 SD). Linear mixed model analyses revealed a significant fixed effect "Time" for TIMP-1 ($F_{(2,11,67)} = 11.46$, $p = 0.002$, n = 15), with a trend for a significant decrease of TIMP-1 from baseline to 3 months of CPAP usage (112.34 ± 9.68 SE *vs.* 93.93 ± 7.59 SE, $p = 0.038$), and a significant decrease from 3 months to 6-8 months of CPAP usage after baseline (93.93 ± 7.59 SE *vs.* 76.39 ± 4.00 SE, $p = 0.008$, Figure 4). Please note, that $p = 0.038$ was evaluated as a trend for significance based on the Bonferroni correction ($p_{crit} = 0.025$).

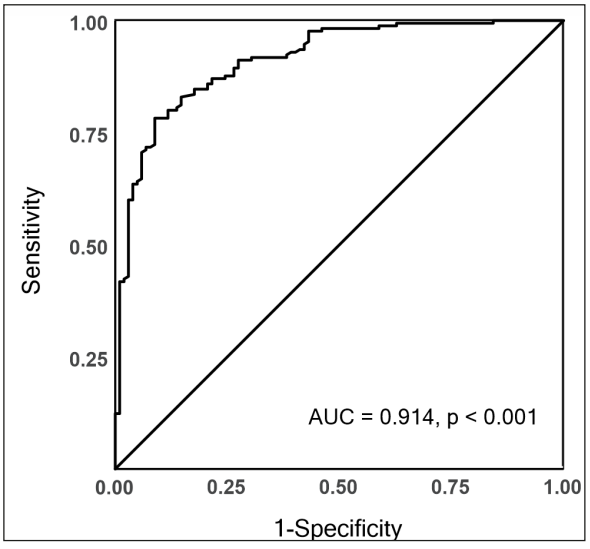

Figure 3. ROC curve: Recommending the optimal diagnostic TIMP-1 cut-off value to allow the separation of healthy controls from patients with OSA.

BMI, Age, Sex, and TIMP-1

Tested *via* multiple linear regression, neither age (beta = -0.008, $p = 0.889$), nor BMI (beta = -0.082, $p = 0.647$) nor sex (beta = 0.030, $p = 0.980$) predicted TIMP-1 levels significantly.

Cardio- and Cerebrovascular Comorbidities and TIMP-1

There was no significant relationship between TIMP-1 and arterial hypertension (beta = -0.090, $p = 0.779$), coronary heart disease (beta = -0.077, $p = 0.972$), diabetes mellitus (beta = -0.097, $p = 0.978$), or apoplex (beta = - 0.044, $p = 0.992$).

Discussion

The generally under-estimated prevalence of OSA and the individually varying cardiovascular risk necessitates an easy-to-use, easily available, cost-effective, and time-, personnel- and resource-saving screening method as a means to providing personalized medicine. Compared to healthy controls, men with severe and untreated OSA have an approximately three times higher risk for a fatal or non-fatal cardiovascular event[19]. Furthermore, the most challenging task in the management of OSA remains the identification of patients with a high long-term risk of developing OSA-associated complications. Identifying high-risk OSA patients is important in terms of providing prognostic information about the potential fu-

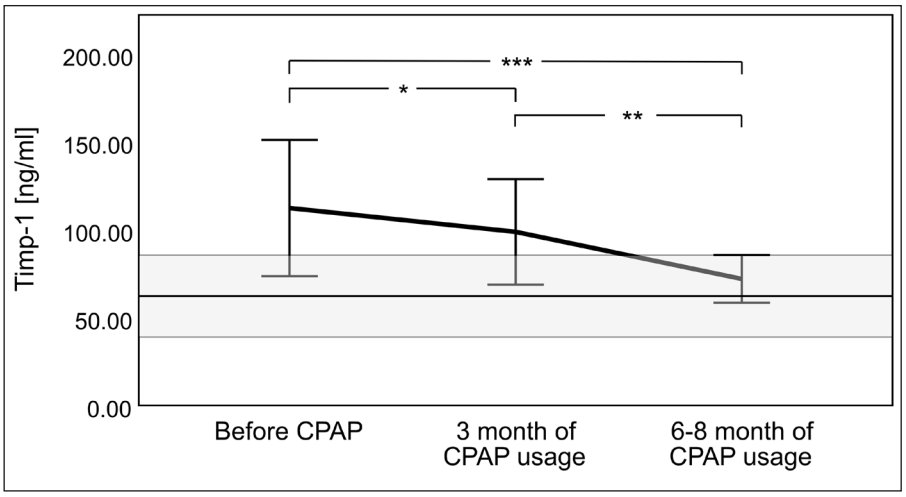

Figure 4. Effect of CPAP-usage on TIMP-1-levels in the medium- (3 months) and long-term (6-8 months). Trend to decreasing after 3 months and a significant decrease after 6-8 months of CPAP-usage. The elevated TIMP-1 serum values of OSA patients approach the serum values of the healthy controls (=Baseline) in the course of CPAP therapy.
Baseline: the grey-shaded area represents the mean value ± 1 SD of the TIMP-1-levels of healthy controls (51.15 ± 25.36 ng/ml). $^{*}p<0.038$, $^{**}p<0.008$, $^{***}p<0.002$.

ture course of the disease and opportunities for a more targeted therapeutic approach as a means to achieving an individualized precision medicine.

If a patient could be identified as being at high risk of subsequent myocardial infarction, more aggressive management of OSA and other cardiac risk factors (e.g., hypertension) could be considered. Also, the importance of managing a suspected but still undiagnosed sleep apnea in the perioperative environment must not be underestimated. Regarding surgical procedures, OSA patients have an increased risk for various and sometimes serious perioperative complications[20]. The possibility of an easily available (even in the short term) preoperative screening using a serum-based biomarker could reduce the occurrence of perioperative complications.

Levels of circulating biomarkers may provide such information[21]. Nevertheless, critics of serum-based biomarkers have a fundamental concern regarding extrapolating from markers derived from the circulation because they represent a pool emanating from several different cells and therefore often lack specificity. Thus, it is of high relevance to adjust potential confounders (e.g., cardio-or cerebrovascular and metabolic comorbidities).

Of the investigated potential biomarkers for OSA, MMP-9, IL-6, IL-10, ICAM-1, VCAM-1, thioredoxin and endocan particularly stand out so far[10, 22-25]. A study on 44 patients with OSA and 18 obese control patients has shown that serum-levels of MMP-9 were significantly elevated in patients with OSA ($p<0.03$). It was, however, surprising that in the same study, serum-levels of TIMP-1 did not appear to change significantly in patients with OSA[10]. Two main effects related to our study could explain these contradictory findings. Firstly, different definitions of OSA severity were used and, secondly, the two studies also differ significantly in terms of the investigated group sizes (n=62 *vs.* n= 273).

With regard to cytokines, the strong correlation of AHI and IL-6 and IL-10 in exhaled breath condensate and serum seems impressive. However, it is critically noted by the authors themselves that the discriminative power of the two markers is more due to the choice of the cut-off point at an AHI of 20 / h than to the high correlation coefficient[22]. Also, thioredoxin (n=63), which is considered to be a potent protein disulfide reductase in the antioxidant defense, shows a significant correlation with the AHI, revealing a respectable sensitivity and specificity of 0.91 and 0.78 respectively; however, it had only some value for identifying and assessing OSA severity. In addition, the control group in this study consisted of only 9 subjects and concomitant hypertension was also significantly associated with elevated thioredoxin levels[23]. For endocan –another potential OSA biomarker – a positive correlation between serum levels and AHI could also be demonstrated[25]. However, patients with mild OSA were not included in the study, so at least healthy controls could be distinguished from patients with moderate or severe OSA (sensitivity 82.5%, specificity 77.5%). Regarding CPAP-treatment, the authors were able to show that in 40 patients the initially pathologically elevated serum endocan decreased significantly (p <0.001) after 3 months. In a further study[22] on 39 patients with moderate and severe OSA and 34 healthy volunteers, it was shown that serum levels of ICAM-1 and VCAM-1 correlated significantly with AHI (regardless of age, gender, BMI, smoking status, coronary heart disease and hypertension). However, the sensitivities and specificities of 0.69 and 0.82 respectively (ICAM-1) and of 0.74

and 0.65 (VCAM-1) were only moderate. Regarding the necessity and the possibility of risk stratification in OSA treatment, TIMP-1 is suitable in many respects. TIMP-1 is already known to inhibit most active MMPs, which are expressed in atherosclerotic plaques and for which there is strong experimental evidence of a direct proatherosclerotic role[26-29]. In addition, TIMP-1 has already been shown to be an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction and to be elevated in patients with acute myocardial infarction[29]. With TIMP-1, we may have found a way to identify patients, who are at high risk for future cardiovascular events and consequently require more aggressive OSA management. This, in turn, would be the next fundamental step towards precision medicine.

According to Peres et al[21], the negative results from the SAVE trial, where CPAP therapy, after a mean follow-up of 3.7 years, did not prevent cardiovascular events in unselected patients with OSA, support the initiative to develop novel strategies to treat patients with OSA, in order to prevent future complications[30]. OSA patients at high risk of subsequent myocardial infarction may need more aggressive treatment of OSA. The screening and monitoring of specific biomarkers may help to target therapy more precisely in future. In this regard, TIMP-1 fulfills all the requirements to become an important component on the way to precision medicine in the future.

Limitations

However, our study has potential limitations. The healthy controls and patients with OSA are not meticulously matched by age, gender, and BMI. Since TIMP-1 seems to be an independent predictor of OSA, no significant additional effect on TIMP-1 levels due to the lack of matching is expected in regression analyses. Secondly, the effect of CPAP-treatment on TIMP1 was not analyzed with a randomized and placebo-controlled (sham-CPAP) design. In a next step, TIMP-1 should be investigated in a prospective randomized clinical trial to verify its validity with regard to a therapy success control.

Conclusions

TIMP-1 serum levels are significantly related to OSA as well as to disease severity and are not influenced by age, gender, BMI, or cardiovascular comorbidities. In the clinical routine, TIMP-1 may help to stratify the individual OSA-associated cardiovascular risk and to monitor the treatment response to CPAP-therapy as a further step on the way to a personalized therapy. Furthermore, TIMP-1 is the first biomarker for OSA that is defined exactly according to ICSD-3 criteria by AHI and complaints / comorbid diagnoses. Taking this into account, TIMP-1 seems to be meeting the specified requirements for a reliable biomarker: easily accessible, disease-specific with a mandatory presence in all affected patients (high sensitivity and specificity), involved in the main physiological pathway, provides new information, reversible on proper treatment, reflects the severity of the disease and provides a cut-off value between the healthy state and disease[6]. Besides the possibility of OSA-screening in primary diagnostics and cardiovascular risk stratification, TIMP-1 also makes it possible to monitor the current cardiovascular risk-status, in the sense of the therapeutic response, as well as therapy compliance.

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Conflicts of Interest

The authors declare no competing interests.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Ethics Committee of the Friedrich-Alexander-University Erlangen-Nuremberg (FAU) (Ref-Nr. 103_18 B) approved the study.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Authors' Contributions

MT is responsible for the conception of the work; MT, JB and OW are responsible for the design of the study; MT and JH have written the manuscript; MT, JB, MEM and JH were responsible for data acquisition; MT, JB and OW performed the data analyses; MT, JB, SKM, KM, AOG and HI carried out the interpretation of the data and substantively revised the manuscript.

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