



compared with patients with COPD or OSA alone, further contributing to inflammatory activation and the potential development and progression of cardiovascular disease and other comorbidities [5,6]. OSA and COPD have also been associated with an increased risk of endocrinal and metabolic conditions, namely insulin resistance, depression of the somatotrophic axis, hypogonadism, imbalance of the adrenal axis, bone loss and hypothyroidism [7–9].

Recently, a link between OSA, COPD and Vitamin D serum concentration was reported in the literature [10–12]. Vitamin D (Vit D) is a fat-soluble vitamin that can be found in some foods and is synthesized in the skin after exposure to sunlight [13]. The classical function of Vit D is the regulation of bone metabolism; however, accumulating evidence suggests that it also possesses anti-inflammatory and immune-modulating properties [13]. Serum 25-hydroxyvitamin D (25(OH)D) concentration is recommended as the best indicator of Vit D status [13]. Vit D insufficiency has exponentially increased over the years, with its prevalence estimated between 13% and 40.4% in European countries, depending on the definition of Vit D insufficiency used [14]. Decreased serum 25(OH)D has been associated with a variety of pulmonary diseases, including respiratory infections, asthma and cancer [13]. Recently, OSA has been associated with hypovitaminosis D, and adequate use of CPAP is likely to augment Vit D levels [10,15]. Similarly, decreased serum 25(OH)D levels have been described in COPD patients, while a protective role against exacerbations has been attributed to Vit D supplementation [11,16]. However, evidence regarding Vit D status in patients with OVS is scarce. Therefore, the aim of the present study is to evaluate Vit D serum levels and to explore the association of those levels with anthropometric, pulmonary function and sleep parameters in patients with OVS.

## 2. Methods

### 2.1. Study Population

The present study protocol was approved by the Alexandroupolis University General Hospital Ethics Committee and all performances were carried out in accordance with the Helsinki Declaration of Human Rights [17]. Participants who enrolled between January of 2019 and December of 2020 from the sleep laboratory of our institution were referred for evaluation of suspected sleep-disordered breathing. Informed consent was provided prior to each participant's enrollment.

Patients diagnosed with both OSA and COPD, as confirmed by overnight polysomnography and pulmonary function testing were included in the study. Exclusion criteria were as follows: refusal to participate, previously diagnosed OSA under continuous positive airway pressure (CPAP) therapy or evidence of exclusively central apneas documented in polysomnography, Vit D supplementation, conditions known to affect calcium, phosphorus and Vit D metabolism and absorption, serious heart diseases, chronic kidney or hepatic disease, cancer, inflammatory diseases, severe cognitive or psychiatric disorders, or any respiratory disorder other than COPD.

### 2.2. Study Variables

Information on previous medical history, current medication use (with an emphasis on Vit D supplementation) and tobacco smoking were obtained from all participants. Anthropometrical data including age, sex, neck, waist and hip circumference were also recorded, and a waist-to-hip ratio (WHR) was calculated. Body mass index (BMI) was calculated using the following formula:  $BMI = \text{Weight (in kilograms)} / \text{Height (in meters)}^2$ . Subjective sleepiness was assessed using the Greek version of the Epworth Sleepiness Scale (ESS), which assesses the tendency to fall asleep during eight typical daytime situations [18]. A score >10 was considered indicative of excessive daytime sleepiness [18].

### 2.3. Polysomnography

All participants underwent an overnight, in-laboratory supervised polysomnography from 22:00 to 06:00 and the data were recorded on a computer system (Alice<sup>®</sup> 4, Philips

Respironics, Murrysville, PA, USA). In each patient, electroencephalogram, electrooculogram, electromyogram (submental and bilateral tibial), and electrocardiogram were recorded. Airflow was detected using combined oronasal thermal sensors. Additionally, continuous pulse oximetry was performed, and impedance belts were used to assess the thoracic and abdomen respiratory effort. Respiratory events (apneas and hypopneas) and electroencephalogram recordings were manually scored according to international guidelines [19]. Apnea was defined as a cessation of airflow or  $\geq 90\%$  of airflow reduction for at least 10 s [19]. Obstructive apneas were defined based on the presence of thoracic efforts. Hypopnea was defined as  $\geq 50\%$  decrement in airflow for at least 10 s in combination with oxyhemoglobin desaturation of at least 4% [19]. The apnea-hypopnea index (AHI) was calculated as the average number of apneas and hypopneas per hour of recorded sleep time [19]. A diagnosis of OSA was made when the AHI in the recorded study was  $>5$  events/h of sleep accompanied by related symptoms (sleepiness, snoring, witnessed apneas) [20]. Subjects with AHI  $<5$ /h of sleep were considered as controls.

#### 2.4. Pulmonary Function Testing

Pulmonary function testing (Screenmate, Erich Jaeger GmbH & Co., Hochberg, Germany) and arterial blood gasses analysis were performed the day before PSG. Forced expiratory volume in the first second (FEV<sub>1</sub>) and forced vital capacity (FVC) were measured and the FEV<sub>1</sub>/FVC ratio was calculated. The diagnosis of COPD was based on the presence of persistent airflow limitation, as defined by a post-bronchodilator FEV<sub>1</sub>/FVC ratio of less than 70%, associated with symptoms such as dyspnea, cough and/or sputum production and a compatible history of exposure to risk factors [21].

#### 2.5. Blood Samples and Analysis

All participants provided a fasting blood sample upon awakening the morning after polysomnography. Blood was then centrifuged (3000 rpm for 10 min) and the serum obtained was conserved frozen at  $-80$  °C for future analysis. Serum 25(OH)D was measured using a commercial radioimmunoassay kit (DiaSorin, Stillwater, MN, USA). Biochemical examinations regarding renal, hepatic function, as well as lipidemic profile were also performed.

#### 2.6. Statistical Analysis

All analyses were performed using the IBM Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA, 2008). Study participants were divided into three groups matched for age, gender and BMI, as controls, OSA and OVS. All variables were presented as median (25th–75th percentile) for continuous variables and proportions for categorical variables. Comparison of baseline characteristics among groups was performed by a chi-square test for categorical variables, or a one-way ANOVA for continuous variables. Post-hoc analysis was performed using the Tukey's post-hoc test when homogeneity of variances was assumed, and the Games-Howell's post-hoc test when the assumption of homogeneity of variances was violated. Multiple linear regression analysis was used to test the association of serum 25(OH)D levels with sleep, anthropometric and respiratory function parameters.

### 3. Results

A total of 90 patients (79 males and 11 females) were included in the analysis. In general, participants were middle-aged (median age 57 years) and obese (median BMI 36.2 kg/m<sup>2</sup>) with a median value of serum 25(OH)D concentration of 18.1 (13.4–24.4) ng/mL. The sample was divided into three groups matched for age, gender and BMI: the control group that included 30 subjects (27 males and 3 females), the OSA group that included 30 patients (26 males and 4 females) and the OVS group that included 30 patients (26 males and 4 females). Neck and waist circumference differed between the groups ( $p = 0.005$  and  $p = 0.023$ , respectively), and patients with OVS were more likely to





#### 4. Discussion

The OVS is characterized by the co-existence of COPD and OSA, both conditions that seem to have an adverse effect on calcium homeostasis. In this study, we found that 25(OH)D serum levels were decreased in patients with OVS, compared with both OSA patients and non-apneic controls. Additionally, AHI and FEV<sub>1</sub> were independently associated with 25(OH)D serum levels in OVS patients.

The definition of Vit D status in OSA patients remained controversial for a long time, with some studies reporting decreased Vit D levels in those patients while others concluded otherwise [22–25]. A meta-analysis conducted by Neighbors et al., [26] including 14 studies with 1513 controls and 3424 OSA patients, reported decreased Vit D serum levels in the latter, with Vit D insufficiency being incrementally exacerbated with increasing severity of OSA (mean differences were  $-2.7\%$  for mild OSA,  $-10.1\%$  for moderate OSA and  $-17.4\%$  for severe OSA). Similarly, results from a more recent meta-analysis conducted by Li et al. [10] showed decreased Vit D levels only in patients with moderate ( $p = 0.002$ ) and severe ( $p < 0.0001$ ) OSA compared with non-apneic controls. OSA treatment with CPAP seems to possess a beneficial effect on Vit D serum levels. Liguori et al. found increased levels of 25(OH)D after 7 nights of CPAP therapy in male OSA patients, and after 1 year of treatment mainly in obese OSA patients [27,28]. Similar conclusions were reached in a randomized sham-controlled trial, where 24 weeks of CPAP treatment improved Vit D serum levels ( $p = 0.045$ ) in patients with severe OSA [29]. In the present study, Vit D serum levels were strongly associated with AHI. These results are in line with those from previous studies. In a study that included 139 OSA patients and 30 controls, OSA patients had lower 25(OH)D levels compared with controls ( $17.8 \pm 7.8$  vs.  $23.9 \pm 12.4$  ng/mL respectively,  $p = 0.019$ ) and there was a significant inverse association between AHI and 25(OH)D levels ( $r = -0.187$ ,  $p = 0.045$ ) [22]. Similarly, in another study that included 75 OSA patients and 31 controls, serum 25(OH)D levels decreased with OSA severity ( $p = 0.003$ ) and were negatively associated with AHI ( $r = -0.40$ ,  $p = 0.0001$ ) [30]. Overall, current evidence suggests that hypovitaminosis D is frequent among OSA patients. Multiple factors such as BMI, gender and disease severity may play a regulatory role in this relationship.

Previous studies have shown that, compared with controls, patients with COPD frequently presented with decreased Vit D levels, with a prevalence that ranged between 33% and 77% according to disease severity [31–33]. In a recent study that included 1609 COPD patients, Vit D deficiency was present in 21% and was associated with a 4.11% decrease in predicted FEV<sub>1</sub> at enrollment (95% CI:  $-6.90\%$  to  $-1.34\%$  predicted FEV<sub>1</sub>,  $p = 0.004$ ), a 1.27% predicted greater rate of FEV<sub>1</sub> decline after 1 year (95% CI:  $-2.32\%$  to  $-0.22\%$  predicted/year,  $p = 0.02$ ), and increased odds of any COPD exacerbation in the prior year (OR: 1.32, 95% CI: 1.00–1.74,  $p = 0.049$ ) [11]. Studies evaluating the effect of Vit D supplementation in lung function produced contradictory results. In a meta-analysis that included 8 studies and 687 COPD patients, Vit D treatment resulted in no significant improvements in FEV<sub>1</sub> ( $p = 0.144$ ), FVC ( $p = 0.299$ ), and FEV<sub>1</sub>/FVC ( $p = 0.995$ ) in COPD patients [34]. On the other hand, another meta-analysis that included 25 studies involving 2670 COPD patients concluded that Vit D supplementation was significantly associated with FEV<sub>1</sub> ( $p < 0.01$ ), FEV<sub>1</sub>/FVC ( $p < 0.01$ ), exacerbations ( $p < 0.01$ ), sputum volume ( $p < 0.01$ ), 6-min walk distance ( $p = 0.02$ ) and CAT score ( $p < 0.01$ ) [35].

Vit D insufficiency in OVS is multifactorial and represents the result of the interaction of individual conditions present in COPD and OSA. In particular, altered cutaneous synthesis due to aging, toxic smoke effects and reduced sun exposure because of disability to perform outdoor activities, sequestration in the adipose tissue, increased Vit D catabolism caused by glucocorticoid therapy (together with impaired liver and renal activation and reduced gastrointestinal absorption) are all factors known to reduce Vit D serum levels in COPD patients [36]. Reduced outdoor activities due to exertional dyspnea may also negatively affect Vit D cutaneous synthesis. In addition, low 25(OH)D levels in COPD correlate with genetic variants of the Vit D-binding gene [32]. Conversely, OSA may represent a risk factor for Vit D insufficiency. Lack of outdoor activity due to obesity and excessive daytime

sleepiness might result in reduced Vit D synthesis because of insufficient sun exposure [37]. Moreover, Vit D is stored in fat tissue, thus reducing the release of Vit D into the circulation and decreasing its bioavailability [37]. Finally, Vit D receptor gene variations affect both 25(OH)D serum levels and disease susceptibility in OSA [38].

A previous study has shown that OVS patients had a similar Vit D status compared to the controls, OSA, or COPD patients and Vit D deficiency as associated with overall mortality among groups [39]. Diagnosis of Vit D insufficiency and correction of Vit D status has several clinical implications in patients with both OSA and COPD. Notably, hypovitaminosis D is highly prevalent in COPD patients and has been associated with worse lung function, increased symptoms and rate of exacerbations, and worse prognosis [11,35]. Moreover, decreased Vit D serum levels were related to COPD severity, and acute exacerbations may be prevented with adequate Vit D supplementation [16,40]. Finally, there is evidence to suggest that a normal Vit D status could improve prognosis in patients with COPD exacerbations caused by respiratory tract infections [41]. Serum 25(OH)D levels were decreased in OSA patients compared with controls, and have been associated with disease severity [10]. On the other hand, with increasing OSA severity serum 25(OH)D levels further decreased, this suggests that serum 25(OH)D might be a risk factor for OSA [10]. Finally, markers of inflammation and HOMA-IR were significantly decreased after Vit D supplementation in a small group of patients with mild OSA [42].

The relationship between an adequate Vit D status and cardiovascular risk is controversial, with data suggesting a negative association between Vit D levels and the risk of cardiovascular disease. Whereas, other studies failed to demonstrate an association between Vit D serum levels and a reduced risk for adverse cardiovascular events [43,44]. In a study conducted in Southern Italy that included 451 participants, the Vit D level did not correlate with cardiovascular risk, as assessed using the Framingham cardiovascular risk charts [45]. On the contrary, the parathyroid hormone was in direct correlation ( $p < 0.001$ ) with cardiovascular risk, and increased parathyroid hormone levels identified a population with a higher risk for cardiovascular events ( $p < 0.001$ ) [45]. Results from this study suggest increased parathyroid hormone levels might be a better predictor of cardiovascular risk in patients with hypovitaminosis D.

Nonetheless, the present study has limitations. First, the study was cross-sectional in design, and consequently, it is not possible to infer any causal relationships. Second, the sample of OVS patients included was rather small and was not representative of the female population. This is largely due to the low prevalence of OVS in the general population and to the disparity in gender-specific estimates of COPD and OSA prevalence [4,46,47]. Thus, larger scale studies are necessary to better assess Vit D status in OVS patients. Third, 25(OH)D was not deseasonalized and the parathyroid hormone was not measured. However, all participants were Caucasian, living in a single region of northern Greece, a relatively small geographical area, with no variations in latitude and with similar conditions regarding cloudiness and air pollution. Moreover, participants had no variations regarding skin pigmentation and in their way of dressing, had similar dietary habits, similar sun exposure and sun-bathing behavior. Furthermore, OVS patients were middle-aged, so caution is warranted in order to extrapolate this data to elderly OVS patients. Finally, only one patient with very a severe air-flow limitation was included in the OVS group. However, it has been shown that Vit D levels decrease with increasing air-flow limitation severity [11]. An analysis of sub-populations based on disease severity in the OSA group was not performed due to the limited sample size.

## 5. Conclusions

In conclusion, lower Vit D levels have been observed in patients with OVS compared with OSA patients and non-apneic controls, indicating an increased risk of hypovitaminosis D in this population. This is expected since both the components of OVS, COPD and OSA are also associated with decreased Vit D levels. Given the protective role of Vit D against FEV<sub>1</sub> decline and COPD exacerbations, as well as the association of low Vit D levels





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