Unexplained chronic cough and vitamin B-12 deficiency

Caterina B Bucca, Beatrice Culla, Giuseppe Guida, Savino Sciascia, Graziella Bellone, Antonella Moretto, Enrico Heffler, Massimiliano Bugiani, Giovanni Rolla, and Luisa Brusato

ABSTRACT

Background: Chronic cough is characterized by sensory neuropathy. Vitamin B-12 (cobalamin) deficiency (Cbl-D) causes central and peripheral nervous system damage and has been implicated in sensory neuropathy and autonomic nervous system dysfunction.

Objective: We evaluated whether Cbl-D has a role in chronic, unexplained cough.

Design: Laryngeal threshold (histamine concentration that provokes a 25% decrease in the midinspiratory flow), bronchial threshold (histamine concentration that provokes a 20% decrease in the forced expiratory volume in 1 s), and cough threshold (histamine concentration that causes ≥5 coughs) in response to an inhaled histamine were assessed in 42 patients with chronic, unexplained cough [27 Cbl-D patients and 15 patients without Cbl-D (Cbl-N)] before and after intramuscular injections of cobalamin for 2 mo. Laryngeal, bronchial, and cough hyperresponsiveness was diagnosed when histamine concentration thresholds were ≤8 mg/mL. Seven Cbl-D and 3 Cbl-N patients underwent an oropharyngeal biopsy before treatment.

Results: Cbl-D patients had a higher prevalence of laryngeal hyperresponsiveness than did Cbl-N patients (92.6% compared with 66.7%; P = 0.03), a thinner oropharyngeal epithelium [133.7 μm (95% CI: 95, 172 μm) compared with 230.8 μm (95% CI: 224, 237 μm); P = 0.002], a lower number of myelinated nerve fibers [2.25/mm² (95% CI: 1.8, 2.7/mm²) compared with 3.44/mm² (95% CI: 3, 3.8/mm²); P = 0.05], and a higher immunoreactive score for nerve growth factor (NGF) [6.7 (95% CI: 6, 7.3) compared with 2.8 (95% CI: 2.5, 3.1); P = 0.02]. After cobalamin supplementation, symptoms and laryngeal, bronchial, and cough thresholds were significantly improved in Cbl-D but not in Cbl-N patients.

Conclusions: This study suggests that Cbl-D may contribute to chronic cough by favoring sensory neuropathy as indicated by laryngeal hyperresponsiveness and increased NGF expression in pharyngeal biopsies of Cbl-D patients. Cbl-D should be considered among factors that sustain chronic cough, particularly when cough triggers cannot be identified.

INTRODUCTION

Recent guidelines allow for the obtainment of an accurate diagnosis and the achievement of a successful treatment in most patients with chronic cough (1, 2). Nevertheless, despite an exhaustive workup, cough remains unexplained in a consistent percentage of patients (3, 4) and is unresponsive to a variety of empiric treatments. A key feature of unexplained cough is heightened cough sensitivity to subliminal stimuli, such as laughing, an increased rate of breathing, or a strong smell from perfume and cooking (4), which depict a sort of hyperalgesia (5) of upper-airway sensory nerves. These nerves, which are central to protecting the lungs from exogenous noxious agents, consist of unmyelinated, unencapsulated C-fibers and myelinated Aδ-fibers (6) placed beneath and between the laryngeal mucosal epithelium. The stimulation of these endings triggers a nerve impulse that leads to active and passive defensive responses such as cough, airway irritation, vocal cord adduction, reflex airway constriction, and neurogenic inflammation (6–10). Laryngeal sensory neuropathy has been observed by electromyography in patients with chronic cough (11). We previously showed that nonasthmatic patients with chronic cough showed marked laryngeal constriction upon histamine inhalation challenge that is consistent with an irritable larynx (12), which is reflected by a decrease in maximal inspiratory airflow rates.

We reasoned that vitamin B-12 (cobalamin) deficiency (Cbl-D), which is one of the most common nutritional disorders (13) with deleterious effects on the central and peripheral nervous system (13–15), might have a role in chronic cough (16) by inducing sensory neuropathy. Recent experimental observations by Scalabrino et al (17) indicated that Cbl-D increases the expression of nerve growth factor (NGF) in the nervous system, and NGF plays a noxious role in the progression of Cbl-D-induced central neuropathy.

The aim of this study was to evaluate if Cbl-D has a role in unexplained chronic cough and if it acts by increasing the expression of the neurotrophic factor NGF in airway mucosa.
SUBJECTS AND METHODS

Patients were selected in the period from January 2002 to June 2008 in outpatients presented to the chronic cough center of the respiratory pathophysiology clinic of the University of Turin, Italy. The selection criteria were as follows: 1) unexplained cough (ie, the absence of a detectable trigger of cough) despite extensive workup into the most common conditions responsible for chronic cough, such as persistent rhinitis, chronic sinusitis, gastroesophageal reflux disease, and asthma; 2) no benefit by empiric treatment with an anti-H1-histaminic drug and proton pump inhibitor, as suggested by cough guidelines (1); 3) normal lung function tests and chest radiography; and 4) no relevant systemic disease, no acute respiratory infection in the past 6 wk, and no pharmacologic treatment in the past 2 wk. In the 60 eligible patients, 27 patients with Cbl-D (vitamin B-12 concentration <200 pmol/L) and increased homocysteine concentration (>13.5 μmol/L) were selected as the study population (Cbl-D group), and 15 patients with a normal nutritional status (without Cbl-D) served as control patients (Cbl-N group); 18 subjects were excluded because of iron or folate deficiencies. Cbl-D patients included 22 women and 5 men (mean age: 43 y; age range: 36–49 y; one smoker; 8 subjects with atopy [immunoglobulin E for a specific allergen >0.10 kUA/L]); Cbl-N patients included 13 women and 2 men (mean age: 42 y; age range: 35–48 y; 2 smokers; 4 subjects with atopy).

The study was approved by the Regional Committee for Medical Research Ethics. Informed consent was obtained from each patient. Because the recruitment began in 2002, the study was not registered in the public trial registry.

Cbl-D and Cbl-N patients underwent a recording of history and symptoms, a physical examination, lung function tests, a histamine inhalation challenge with assessment of bronchial, laryngeal, and cough thresholds, measurements of specific immunoglobulin E for common aeroallergens and fractional exhaled nitric oxide (FENO) as a marker of asthma-related cough (18) in baseline conditions and after 2 mo of vitamin B-12 supplementation. Cough severity was rated on a 5-point visual analog scale with 0 being no cough and 5 being the worst cough ever. Seven patients and 3 control subjects accepted to undergo an oropharyngeal biopsy before vitamin supplementation. The oropharynx was chosen as the site of biopsy because the biopsy is well-tolerated, minimally invasive, and can be easily performed in outpatients in ≤10 min. Although the larynx is more abundantly equipped with cough receptors than is the pharynx, we had to discard a laryngeal biopsy because it requires a general anesthesia.

Lung function tests

Spirometry was performed with the computerized spirometer BAIRES (Biomedin, Padova, Italy) by using the European Respiratory Society guidelines (19). The forced expiratory volume in 1 s (FEV1), FEV1/vital capacity (VC) ratio, maximal mid-expiratory flow (MEF50), and maximal midinspiratory flow (MIF50) were calculated from the curve with the greatest VC and the best maximal inspiratory and expiratory efforts.

Histamine inhalation challenge

The challenge was performed to assess bronchial, laryngeal, and cough thresholds as previously described (20). Histamine was delivered in doubling concentrations starting from 0.5 up to 32 mg/mL by a compressed air nebulizer that was controlled by a breath-actuated dosimeter (MEFAR MB3; Markos, Monza, Italy). Each concentration was inhaled by taking 5 slow VC breaths from the nebulizer. After each set of inhalations, FEV1 and flow-volume loop were recorded, and the best of 3 trials was selected. MIF50 was used as the index of laryngeal narrowing because we previously showed that it reflects the decrease in the cross-sectional glottic area during a histamine challenge (12). The challenge was stopped when a 20% FEV1 drop from baseline was obtained or the highest histamine concentration was reached. The histamine concentration that provoked a 20% drop in FEV1 was the bronchial threshold (PC20FEV1), the histamine concentration that provoked 25% drop in MIF50 was the laryngeal threshold (PC25MIF50), and the histamine concentration that caused ≥5 coughs was the cough threshold (PC5cough). The histamine hyperresponsiveness of the bronchi, larynx (LHR), and cough were defined when the thresholds were equal to or below the histamine concentration of 8 mg/mL.

FENO measurement

FENO was measured according to American Thoracic and European Respiratory Society recommendations (21) with a nitric oxide chemiluminescent analyzer (Sievers NOA 280; Sievers, Boulder, CO) by using a sampling flux of 50 mL/s. FENO values were calculated on an end-expiration plateau ≥3 s long. The mean value of 3 acceptable trials was computed.

Vitamin B-12 assessment and supplementation

Serum concentrations of vitamin B-12 were measured in baseline and after vitamin supplementation with a chemiluminescent microparticle immunoassay (Architect System; Abbott Diagnostic Division, Longford, Ireland). Cbl-D was defined when the serum concentration was <200 pmol/L (22). To confirm the deficiency status, the total plasma homocysteine concentrations, which is a sensitive metabolic marker for Cbl-D (23), was assayed in the serum of patients with low vitamin B-12 concentrations by using HPLC (HPLC Shimadzu; Shimadzu Corp, Kyoto, Japan) and postcolumn fluorescence detection. Vitamin B-12 was administered as an intramuscular injection of 1000 μg cyanocobalamin/d for 5 d followed by 1000 μg cyanocobalamin/wk for 3 wk and 1000 μg cyanocobalamin/mo thereafter (24, 25).

Oropharyngeal biopsy

The oropharynx was anesthetized topically with 2% lidocaine. Biopsy specimens were taken in the middle area of the posterior wall of the oropharynx with a biopsy forceps (fenestrated type FB-20C) introduced in the oral cavity through the mouth. Tissue specimens were fixed in formalin and paraffin embedded, cut into 5-μm-thick sections and mounted onto glass slides for histologic and immunohistochemical analyses.

Hematoxylin and eosin and Luxol fast blue staining

Paraffin sections were dewaxed, hydrated, and processed according to standard procedures for hematoxylin and eosin staining. For the identification of myelinated nerve fibers, rehydrated tissue specimens were fixed in formalin and paraffin embedded, cut into 5-μm-thick sections and mounted onto glass slides for histologic and immunohistochemical analyses.
sections were immersed in a Luxol fast blue 0.1% alcoholic solution at 56°C for 24 h and washed with distilled water, developed with 0.05% lithium carbonate, and dehydrated through graded alcohols before permanent mounting and a light-microscope examination (26).

**Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated in graded alcohol. Heat-induced epitope retrieval was performed with Target Retrieval Solution (Dako Corp, Carpinteria, CA) with an electric pressure cooker for 20 min at 120°C with cooling before immunostaining. To block endogenous peroxidase activity, sections were incubated in a 3% hydrogen peroxide buffer solution for 25 min. Nonspecific antibody binding was blocked by pretreatment with a solution of bovine serum albumin (0.1%) diluted 1:10 in phosphate-buffered saline for 20 min. The sections were incubated overnight with a polyclonal rabbit anti-human NGF diluted at 1:50 (Santa Cruz Biotechnology, Santa Cruz, CA). Immunostaining was done with a peroxidase complex EnVision Plus System kit (Dako Corp) by following the manufacturer’s recommendations. Diaminobenzidine tetrahydrochloride was used as chromogen. The slides were counterstained with Mayer’s hematoxylin for 5 s, dehydrated, and mounted in Clarion medium (Biomeda, Foster City, CA). The negative control was treated with nonimmune rabbit serum. Cerebral tissue was used as a positive control.

**Evaluation of anti-NGF staining**

Sections were blindly evaluated by 2 independent investigators. Images of immunohistochemical stains were captured with a LEICA DMLB light microscope (Leica Microsystems, Wetzlar, Germany). The degree of immunostaining was evaluated by using the immunoreactive score (IRS) proposed by Remmele and Stegner (27), which was computed by multiplying the staining intensity by the average percentage of positive cells in the selected field. The staining intensity was scored as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong; the average percentage of positive cells was scored as 0 = negative, 1 = 1–20% positive cells, 2 = 21–50% positive cells, and 3 = 51–100% positive cells. IRS was calculated as the mean of 10 randomly selected visual fields from different areas of each specimen examined at a magnification of 630×. Dichotomous variables were generated by using mean IRS values ≥2 as the cutoff.

**Thickness of epithelium and area covered by nerve fibers**

Morphometric analysis was done by computer-assisted light microscopy (Leica Quantimet 500 system; Leica) at a magnification of 40×, which corresponded to a field area of 90,000 μm². Several significant nonoverlapping fields were measured until all of the available area was covered (~15 fields per biopsy). The following variables were measured: 1) the thickness of the epithelium measured at regular intervals of 200 μm in each field and 2) the area covered by nerve fibers in Luxol staining expressed as the percentage of the total field area. All microscope slides were read by a pathologist who was blinded to the clinical and physiologic data of the patients.

**Statistics**

Data were analyzed with the statistical STATA 11 package (StataCorp, College Station, TX). To account for potential differences in patient characteristics, the generalized least-squares random-intercept model was used. This model allowed us to analyze the differences between the 2 groups (Cbl-D and Cbl-N) and the effect of treatment and produced a matrix-weighted average of the between- and within-subjects results. In other terms, the model provided an unbiased estimation of the effect of the vitamin B-12 group, the within-group by between-group random effects, and the fixed effect of grouping and treatment (28).

The analysis of histamine responsiveness had to account for the fact that some subjects did not reach a threshold (ie, were unresponsive), even at the highest concentration (ie, the ceiling effect). To avoid the ceiling effect, a random-effect Tobit model (ie, a censored regression model) was used, which considered the histamine thresholds as right censored. This method fitted a linear model with a fixed censoring value and translated the change in one categorical variable over time into the transition probabilities of a dependent variable (29).

In all models, SEs were estimated by the bootstrap method. All dependent variables were transformed following Box-Cox regression results by using the untransformed value as the dependent variable and a full factorial model with treatment and vitamin B-12 groups as predictors in a pooled ordinary least-squares model (30). Transformation variables were selected with maximum-likelihood criteria. In the case of ≥2 possible variables, the one that normalized the residual distribution and reduced the heteroskedasticity was selected. Statistical significance was set at a 2-sided P < 0.05.

**RESULTS**

The mean cobalamin concentration was 146 pmol/L (95% CI: 128, 164 pmol/L) in Cbl-D patients, which was significantly lower (P < 0.0001) than that of Cbl-N patients (349 pmol/L; 95% CI: 315, 383 pmol/L). The mean homocysteine concentration in Cbl-D patients was 20.8 μmol/L (95% CI: 17.9, 23.7). The cause of Cbl-D was inadequate intake of foods from animal origin in 19 patients (9 patients were strictly vegetarians) and age-related hypochlorhydria in the remaining 8 patients. None of the patients had pernicious anemia.

The age, sex distribution, smoking habits, atopy prevalence (ie, increased serum immunoglobulin E concentration) and lung function tests were similar in the 2 groups. Cbl-D patients had a significantly higher prevalence of LHR than did Cbl-N patients (92.6% compared with 66.7%, respectively; P = 0.03). FeNO was in the normal range in both groups [13.5 ppb, (95% CI: 9.5, 17.6 ppb) in Cbl-D patients and 8.5 ppb (95% CI: 6.0, 11.0) in Cbl-N patients]. Mean (95% CI) values of raw data for Cbl-D and Cbl-N patients before and after cobalamin supplementation are reported in Table 1.

Results of the oropharyngeal biopsy analysis in the 2 groups are reported in Table 2. Compared with Cbl-N patients, Cbl-D patients had a significantly thinner epithelium, lower number of myelinated nerve fibers, and higher NGF IRS. Slides of typical Cbl-D and Cbl-N patients regarding staining for NGF and myelinated nerve fibers are shown in Figure 1. The NGF score was significantly inversely related to the serum concentration of vitamin B-12 (r = −0.68, P = 0.029) (Figure 2).
TABLE 1
Cough visual analog scale (VAS), lung function values, and histamine thresholds before and after vitamin B-12 supplementation in patients with vitamin B-12 (cobalamin) deficiency (Cbl-D) and without Cbl-D (Cbl-N)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cbl-D (n = 27)</th>
<th>Cbl-N (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Cough VAS</td>
<td>4.00 (3.7, 4.3)</td>
<td>1.96 (1.5, 2.4)</td>
</tr>
<tr>
<td>Vitamin B-12 (pmol/L)</td>
<td>146 (128, 164)</td>
<td>426.2 (339, 513)</td>
</tr>
<tr>
<td>FEV1 (mL)</td>
<td>297.5 (265.6, 329.5)</td>
<td>299.7 (267.9, 331.6)</td>
</tr>
<tr>
<td>MEF50 (L/s)</td>
<td>4.04 (3.53, 4.48)</td>
<td>4.06 (3.56, 4.56)</td>
</tr>
<tr>
<td>MIF50 (mg/mL)</td>
<td>4.32 (3.77, 4.87)</td>
<td>4.64 (4.11, 5.16)</td>
</tr>
<tr>
<td>PC20FEV1 (mg/mL)</td>
<td>10.5 (5.9, 15.2)</td>
<td>17.9 (12.8, 23.2)</td>
</tr>
<tr>
<td>PC5cough (mg/mL)</td>
<td>3.30 (1.8, 4.8)</td>
<td>17.2 (11.9, 22.4)</td>
</tr>
<tr>
<td>PC25MIF50 (mg/mL)</td>
<td>2.49 (1.6, 3.4)</td>
<td>15.7 (10.5, 20.8)</td>
</tr>
</tbody>
</table>

All values are means; 95% CIs in parentheses. FEV1, forced expiratory volume in 1 s; MEF50, maximum midexpiratory flow; MIF50, maximum midinspiratory flow; PC20FEV1, histamine concentration that provokes a 20% decrease in FEV1; PC5cough, histamine concentration that provokes a 25% decrease in MIF50; PC25MIF50, histamine concentration that causes ≥5 coughs.

DISCUSSION
The results of this study suggested that Cbl-D favors chronic cough and laryngeal hyperresponsiveness. In patients with an unexplained cough and Cbl-D, histamine LHR was much more common (92.6%) than in Cbl-N patients (66.7%), and cough and LHR resolved after cobalamin supplementation. By contrast, no significant effect of vitamin supplementation was observed in Cbl-N patients, which suggested that cobalamin is devoid of any pharmacologic effect on cough and airway tone. Unfortunately, we found no prior study regarding the role of cobalamin in cough apart from an indirect observation in Colombian children in whom supplementation with nutrient snacks that contained vitamin B-12 produced a decrease in cough prevalence (31).

The association of cough with LHR in Cbl-D patients suggested that Cbl-D acts by favoring sensory neuropathy because sensory nerves are involved in the irritability that characterizes certain airway diseases, particularly cough and airway hyperresponsiveness (32).

Cbl-D induces a number of neurologic complications, including myelopathy, peripheral neuropathy, optic neuropathy, and dementia (13–15, 33). Laryngeal neuropathy because of Cbl-D was described in a patient with hoarseness and laryngoscopic evidence of bilateral vocal-cord paralysis who improved dramatically after cobalamin administration (34).

The hypothesis of neuropathy induced by Cbl-D was sustained by the histopathologic findings in pharyngeal mucosa of our Cbl-D patients, which consisted of a decreased number of myelinated nerve fibers and increased NGF expression. These findings were in agreement with experimental observations in rats fed a Cbl-deficient diet that showed a decreased density of...
myelinated fibers in peripheral nerves (15) and increased NGF expression in the central nervous system (17). In our study, the link between cobalamin and NGF was further supported by the significant indirect relation observed between the vitamin B-12 concentration and NGF-IRS (Figure 2). NGF is a member of the neurotrophin family of proteins which regulate neuronal development and maintenance and are involved in the sensitization of spinal circuits that underlie many forms of hyperalgesia (35). High concentrations of NGF have been observed in the nasal lavage of individuals with increased cough sensitivity and airway symptoms induced by chemicals and odors (36). Experimental NGF administration induced acute and long-lasting hyperalgesia and increased the capsaicin sensitivity of nociceptive neurons (37, 38). These effects depended on the modulatory effect of NGF on the capsaicin (or vanilloid) TRPV1 receptor (39), which is a member of the transient receptor potential (TRP) gene family of ion-channel subunits (40). Capsaicin receptors are known to contribute to chemical hypersensitivity, chronic cough, and airway inflammation (35, 41–43).

The decreased number of myelinated fibers in the Cbl-D group indicated that heightened cough sensitivity was mainly sustained by unmyelinated C-fibers in such patients, and the myelinated sensory nerves with a recognized role in cough physiology, such as Aδ fibers (6–10, 41–43), were not involved. However, the relative role of unmyelinated and myelinated fibers in a cough from laryngeal hypersensitivity deserves further investigation on a greater number of cases.

Another factor that contributed to laryngeal hypersensitivity induced by Cbl-D could have been the decreased defensive barrier of the mucosa. Disordered oral epithelium maturation has been described in Cbl-D subjects (44), and in our Cbl-D patients, the pharyngeal epithelium was significantly thinner than it was in Cbl-N patients.

There are some points of weakness of the current study that should be acknowledged. The first point regards pharyngeal biopsy. The pharynx is not directly involved in cough mediation, so it could not accurately reflect lesions that putatively occurred at the level of tussigenic areas such as the larynx or the tracheobronchial tree. Nevertheless, we chose the oropharyngeal biopsy because it was minimally invasive and well tolerated, whereas a laryngeal biopsy would have required a general anesthesia. Unfortunately, only a few patients accepted to undergo a biopsy (7 Cbl-D compared with 3 Cbl-N patients), so that our results deserve confirmation in a wider number of cases.

Because patients were assessed after 2 mo of cobalamin treatment, it is possible to argue that cough improvement was due to spontaneous resolution of an undisclosed cause. However, a 2-mo treatment is the minimum time required to obtain a neurologic improvement from vitamin B-12 supplementation (45). In contrast, because none of the Cbl-N patients improved, the hypothesis of spontaneous cough resolution seems untenable.
Finally, it is also possible to argue that Cbl-D was too mild to induce neurologic damage because none of our Cbl-D patients had pernicious anemia. However, it has been observed that the neurologic symptoms of Cbl-D neuropathy may appear long before hematologic abnormalities (46). According to Carmel (24), there is even a “subclinical cobalamin deficiency” that is an asymptomatic state in which metabolic insufficiency is demonstrable in patients and seemingly healthy nonpatients who do not have megaloblastic anemia.

In conclusion, our results indicated that Cbl-D may contribute to chronic cough and must be investigated in all patients in whom a cough remains undiagnosed or is unresponsive to treatment.

The authors’ responsibilities were as follows—CBB, GR, and LB: conceived of the study, participated in the interpretation of results, and drafted the manuscript; GB, GG, AM, and EH: performed the selection of patients and performed diagnostic tests; SS and GB: performed analyses of oropharyngeal biopsy and participated in the interpretation of results; MB: performed statistical analyses and participated in the interpretation of results; and all.

### TABLE 3

| Effect of 2-mo supplementation with cobalamin on vitamin B-12 concentrations, the cough visual analog scale (VAS), and lung function tests in patients with chronic, unexplained cough with or without vitamin B-12 deficiency |
|-----------------|-----------------|-----------------|-----------------|
|                  | Log VAS         | Log FEV1        | Log MIF50       |
| Pre-post difference | −0.173 (−0.404, 0.059) | 0.144           | 0.545           |
| Cobalamin deficiency | 0.080 (−0.180, 0.340) |               |               |
| Interaction | −0.657 (−0.949, −0.364) | <0.0001       | <0.0001       |
| Constant | 1.300 (1.093, 1.507) |               |               |

### TABLE 4

| Effect of 2-mo supplementation with cobalamin on bronchial, laryngeal, and cough histamine thresholds in patients with chronic, unexplained cough with or without vitamin B-12 deficiency |
|-----------------|-----------------|-----------------|-----------------|
|                  | Log PC20FEV1    | Log PC20MIF50   | Log PC20MIF50   |
| Pre-post difference | 0.055 (−0.170, 0.280) | 0.633           | 0.350           |
| Cobalamin deficiency | −0.462 (−1.432, 0.508) |               |               |
| Interaction | 1.042 (0.746, 1.338) | <0.0001       | <0.0001       |
| Constant | 2.187 (1.402, 2.973) |               |               |

1 All values are coefficients; 95% CIs in parentheses. Pre-post difference, difference between pre- and posttreatment values; Interaction, interaction between pre-post difference and cobalamin deficiency; FEV1, forced expiratory volume in 1 s; MIF50, maximum inspiratory airflow rate; MIF50, maximum midinspiratory airflow rate. Data were analyzed with regression analysis. All dependent variables were log transformed following Box-Cox regression results. Data were analyzed with the Tobit random effect regression model for right censored data. There were significant interactions of the pre-post treatment difference with group (patients with vitamin B-12 deficiency compared with patients without vitamin B-12 deficiency) on improvement of all histamine thresholds. PCScough was also slightly improved in patients without vitamin B-12 deficiency after cobalamin supplementation.

### REFERENCES