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Effect of iron supplementation in women with chronic cough and iron deficiency

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Summary

Aims. Chronic cough is more frequent and severe in women than in men. Women often have decreased iron stores, because of menses and pregnancies. We investigated if iron deficiency has a role in chronic cough by increasing airway sensitivity to inhaled irritants.
Methods. Twenty-two non-smoking women with chronic unexplained cough and iron deficiency (serum ferritin below 15 ng/ml) were examined in baseline, after 2 months empiric treatment with anti H1-histaminic drug and proton pump inhibitor, and after iron supplementation (330–660 mg iron sulphate tablets daily) for 2 months. Outcome measures were cough visual analogue scale (VAS), and histamine thresholds of the larynx (PC25MIF50, concentration causing 25% in MIF50), bronchi (PC20FEV1) and cough (PC5cough).
Results. Mean serum ferritin was 9.3 ng/ml (95% CI 7.7–10.9), 13 patients had mild anaemia. All the patients had laryngeal and cough hyperresponsiveness, 12 had also bronchial hyperresponsiveness. Empiric treatment produced no significant effect, whereas iron supplementation improved cough VAS from 4.03 (3.6–4.47) to 2.6 (1.9–3.27), $p < 0.0001$, PC20FEV1 from 10.04 mg/ml (5.37–18.77) to 22.2 (11.7–41.8), $p < 0.001$, PC25MIF50 from 3.09 mg/ml (1.9–4.9) to 11.9 (7.3–19.4), $p < 0.001$ and PC5cough from 2.1 mg/ml (1.2–3.6) to 8.8 (5.2–15.1), $p < 0.001$.
Conclusion. In women with unexplained chronic cough unresponsive to targeted treatment, airway and cough hyperresponsiveness may be sustained by iron deficiency. Healthy women with chronic cough should be checked for iron deficiency as iron repletion may resolve such disturbing symptom.

What's known

Chronic cough is more frequent and severe in women than in men. Women often have decreased iron stores, because of menses and pregnancies. Low body iron stores may decrease mucosa defences and impair neural function. Brain areas with great iron-dependent metabolic activity are involved in the neural control of cough.

What's new

Our findings indicate that in women with unexplained chronic cough unresponsive to targeted treatment, cough and airway hyperresponsiveness may be sustained by iron deficiency. Healthy women with chronic cough should be checked for iron deficiency, as iron repletion may resolve cough.

Introduction

Chronic cough is a common condition referred to respiratory physicians. Recent guidelines allow to obtain an accurate diagnosis and to achieve successful treatment in most patients with chronic cough (1,2). Nevertheless, in a consistent percentage of patients (up to 20%) cough remains unexplained (3,4) or does not improve to targeted treatment.

Studies in healthy adult volunteers and in patients with chronic cough have shown that cough is much more frequent and severe in women than in men (5). In a random norwegian population, Voll-Aanerud et al. (6) reported an incidence of chronic cough of 9.2% in women and 7.7% in men. Kastelik et al. (7) demonstrated that women have increased cough sensitivity to inhaled tussigenic stimuli, including capsaicin. Although the reason for the greater prevalence of cough in women remains unknown, the recent observation that in children gender does not influence cough outcome measures (8) suggests that puberty has a role in cough sensitivity (9). This is in analogy with asthma, which becomes more prevalent in girls than in boys only after puberty (10). We reasoned that, besides the different hormonal milieu, decreased iron stores differentiate adult women from men and prepubertal girls. Actually, it is estimated that nearly 20% of childbearing women have iron deficiency (ID), because of menses and pregnancies (11). The mechanisms by which ID may favour cough remain unknown, but the finding of reduced epithelial tongue thickness in subjects with ID (12,13) suggests that cough could be favoured by increased mucosa permeability to irritative stimuli. We previously found that patients with chronic cough often have laryngeal hyperresponsiveness (LHR) (14), which consists in a paradoxical inspiratory vocal cord adduction upon histamine inhalation challenge. Vocal cord adduction can be non-invasively detected by recording inspiratory airflow rates during the challenge, as we found that the decrease in maximum mid-inspiratory airflow rate (MIF50) is closely related to the decrease in glottis area induced by histamine (15).

The aim of this study was to investigate if ID has a role in chronic unexplained cough by contributing to irritable larynx. To this aim, we evaluated laryngeal, bronchial and cough responsiveness to inhaled histamine in 22 women with chronic unexplained cough and ID, before and after iron supplementation.

Methods

The patients were selected among 372 outpatients presented to our clinic for undiagnosed chronic cough who had not yet received a specific diagnosis (14). On the basis of the cough diagnostic workup (1,2), the cause of cough could be established in 311 patients and remained unexplained in 61 (23%). Unexplained cough was defined when: (i) no detectable trigger for chronic cough was identified, such as persistent rhinitis, chronic sinusitis, gastroesophageal reflux disease and asthma, (ii) no benefit was obtained by empiric treatment with anti H1-histaminic drug and proton pump inhibitor, as suggested by cough guidelines (1), (iii) lung function tests and chest radiography were normal, (iv) there were no relevant systemic disease, no acute respiratory infection in the last 8 weeks and no pharmacological treatment with angiotensin converting enzyme inhibitors or beta-adrenergic blocking drugs.

Nutritional assessment in the 61 patients with unexplained cough showed ID, defined as serum ferritin lower than 15 ng/ml (16), in 22 women, who were selected as study group. The patients, all never smoker, were examined in baseline condition, after empiric treatment (1) for 2 months and after iron supplementation for 2 months.

The study was approved by the Regional Committee for Medical Research Ethics. Informed written consent was obtained by each patient.

Study protocol

All the patients underwent clinical examination, lung function tests, histamine inhalation challenge with assessment of bronchial, laryngeal and cough thresholds and measurement of exhaled nitric oxide (FENO), as a marker of asthma-related cough (17). Cough severity was rated on a 5 points visual analogue scale (VAS) with 0 being no cough and 5 being worst cough ever.

Clinical examination and histamine challenge were repeated after empiric treatment with anti H1-histaminic drug and proton pump inhibitor for 2 months, and after iron supplementation at the dose of 1 mg/kg elemental iron, corresponding to one or two 330 mg iron sulphate tablets, for 2 months. FENO measurement was obtained after iron repletion. At least 1 month elapsed between the end of empiric treatment and the start of iron supplementation.

Lung function tests

Spirometry was performed using the computerised spirometer BAIRES (Biomedin, Padova, Italy), using the European Respiratory Society (ERS) guidelines (18). From the curve with the largest vital capacity (VC) and the best maximal inspiratory and expiratory efforts were calculated: forced expiratory volume in 1 s (FEV_1), FEV_1/VC ratio, maximal mid expiratory flow (MEF_{50}) and MIF_{50}.

Histamine inhalation challenge

The challenge was performed to assess bronchial, laryngeal and cough thresholds. Histamine was delivered in doubling concentrations, starting from 0.5 mg/ml up to 32 mg/ml, using a compressed air nebulizer,. controlled using a breath actuated dosimeter (MEFAR MB3; Markos, Monza, Italy). Each concentration was inhaled by taking five slow vital capacity breaths from the nebulizer. After each set of inhalations, FEV1 and flow-volume loop were recorded and the best of three trials was selected. MIF50 was used as the index of laryngeal narrowing, as we previously found that it reflects the decrease in cross-sectional glottic area during histamine challenge (16). The challenge was stopped when 20% FEV1 drop from baseline was obtained or the highest histamine concentration was reached. The bronchial threshold was calculated as the histamine concentration provoking 20% drop in FEV1(PC20FEV1) (19), the laryngeal threshold as the concentration provoking 25% drop in MIF50 (PC25MIF50) (20) and the cough threshold as the concentration causing five coughs (PC5cough). Hyperresponsiveness of the bronchi (BHR), larynx (LHR) and cough (coughHR) was defined when any threshold was equal or below the histamine concentration of 8 mg/ml.

Exhaled nitric oxide measurement

FENO was measured according to American Thoracic and European Respiratory Society (ATS/ERS) recommendations (21), using the NO chemiluminescent analyzer Sievers NOA 280 (Sievers, Boulder, CO), with a sampling flux of 50 ml/s. FENO values were calculated upon an end-expiration plateau 3 s long at least. The mean value of three acceptable trials was computed. The values of Olin et al. (22) were used as reference values.

Statistical analysis

Data were analysed using the statistical STATA 11 software package (Stata Press College Station, TX). To account for potential differences in patient characteristics, generalised least squares (GLS) random-intercept (RE) model was used to analyse the effect of treatment on functional variables, producing a matrix-weighted average within subjects. (23). Since in some subjects, the histamine thresholds were at or over the highest concentration (ceiling effect), the results of the challenge were considered as right-censored, and a random effect ‘Tobit model’ (or censored regression model) was used. (24). In all the models, the standard error was estimated using the bootstrap method. All the dependent variables were transformed following the Box-Cox regression results, using untransformed

value as dependent variable and full factorial model with treatment as predictor in pooled Ordinary Least Squares (OLS) model (25).

Results

Iron deficiency was attributable to increased loss because of menses and pregnancies in all the 22 women. Oropharyngeal examination revealed redness, soreness and atrophy of the oral mucosa and tongue papillae; 11 patients had cheilitis angularis. All the patients complained of frequent dysphonia and eight reported episodic choking. None of the patients had ever received a diagnosis of asthma, seven had seasonal atopy. The general characteristics of the patients are shown in Table 1.

Table 1 General characteristics of the study subjects

	Mean	95% CI
Age years	37.5	32.2–42.6
Cough VAS	3.99	3.6–4.47
Blood eosinophils %	4.17	3.0–5.3
Serum ferritin ng/ml	9.34	7.7–10.9
Haemoglobin g/dl	11.58	10.9–12.2
F_{ENO} ppb	18.92	12.4–25.5
FVC baseline, l	3.57	3.33–3.80
FVC baseline, % pred	100.7	95.2–106.3
FEV_1 baseline, l	2.92	2.70–3.15
FEV_1 baseline, % pred	101.82	96.8–106.8
FEV_1/VC %	81.79	79.3–84.3

VAS, visual analogue scale. FVC, forced vital capacity.

Despite the low serum ferritin, only 13 women had a mild degree of anaemia (haemoglobin below 12.5 g/dl) and 11 of them had microcytosis (mean corpuscular volume, MCV, below 80 fl). Lung function tests and FENO values were in the normal range in all the patients, but four. Histamine inhalation challenge showed laryngeal and cough hyperresponsiveness in all the 22 women, whereas bronchial hyperresponsiveness was found only in 12 patients.

Iron supplementation produced a significant increase in the values of haemoglobin, from 11.5 g/dl (95% CI 10.9–12.2) to 13.1 g/dl (95% CI 12.5–13.5) $p < 0.001$, of MCV from 78.8 fl (75.5–82.1) to 83.2 fl (79.3–87.1) $p < 0.001$ and of serum ferritin from 9.33 ng/ml (7.7–10.9) to 42.9 ng/ml (28.9–56.9) $p < 0.001$. The values were normalised in all the patients but two, who had persistent decrease in Hb and MCV caused by thalassaemia trait.

For statistical analysis, all the dependent variables were log-transformed following Box-Cox regression results. The results of the regression analysis on the effect of iron supplementation vs. empiric treatment of cough are reported in Table 2. All the histamine thresholds remained unchanged after empiric treatment with anti H1-histaminic drug and proton pump inhibitor, but were significantly improved with iron supplementation.

Table 2 Effect of empiric treatment and of iron supplementation on bronchial, laryngeal and cough histamine thresholds

	Coefficient	95% CI	p > z
Log$PC_{20}FEV_1$*			
Empiric treatment	0.173	−0.10, 0.44	0.21
Iron supplementation	0.79	0.50, 1.08	< 0.001
Constant	2.31	1.68, 2.93	< 0.001
Log$PC_{25}MIF_{50}$†			
Empiric treatment	0.24	−0.15, 0.62	0.22
Iron supplementation	1.35	0.96, 1.75	< 0.001
Constant	1.13	0.65, 1.61	< 0.001
LogPC_{5cough}‡			
Empiric treatment	0.21	−0.22, 0.63	0.35
Iron supplementation	1.41	0.98, 1.85	< 0.001
Constant	0.77	0.24, 1.29	< 0.001

*$PC_{20}FEV_1$ = histamine concentration causing 20% decrease in FEV1. †$PC_{25}MIF_{50}$ = histamine concentration causing 25% decrease in MIF50. ‡PC_{5cough} = histamine concentration causing 5 coughs. CI, confidence interval.

The mean values with confidence limits of bronchial (PC20FEV1), laryngeal (PC25MIF50) and cough (PC5cough) thresholds in baseline, after empiric treatment and after iron supplementation are reported in Figure 1.

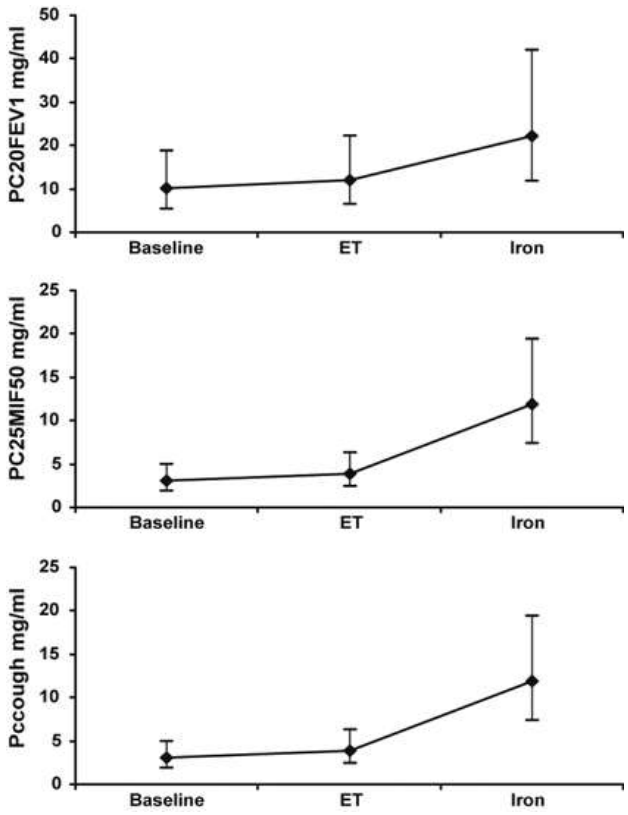

Figure 1. Mean values with 95% confidence intervals of bronchial (PC20FEV1), laryngeal (PC25MIF50) and cough (PC5cough) histamine thresholds in baseline, after empiric treatment (ET) and after iron supplementation.

The mean values with 95% confidence limits of cough VAS in baseline and after iron intake decreased from 4.03 (3.6–4.47) to 2.6 (1.9–3.27), $p < 0.0001$; those of PC20FEV1 increased from 10.04 mg/ml (5.37–18.77) to 22.2 (11.7–41.8), $p < 0.001$; those of PC25MIF50 from 3.09 mg/ml (1.9–4.9) to 11.9 (7.3–19.4), $p < 0.001$; and those of PC5cough from 2.1 mg/ml (1.2–3.6) to 8.8 (5.2–15.1), $p < 0.001$. No significant relationship was found among the improvement in cough VAS and in histamine thresholds and the improvement in ferritin, haemoglobin and MCV; the results of the linear regression analyses are reported in Table 3.

Table 3 Results of the linear regression analysis (r coefficient and p-value) among the changes after iron supplementation (Δ) of the histamine thresholds and cough VAS and the changes of ferritin, haemoglobin (Hb) and MCV. No significant relationship was found.

	Δ-Ferritin	p	Δ-Hb	p	Δ-MCV	p
Δ-$PC_{20}FEV_1$	0.137	0.542	0.007	0.976	0.153	0.495
Δ-$PC_{25}MIF_{50}$	0.042	0.852	0.056	0.805	0.212	0.344
Δ-PC_{5cough}	0.116	0.607	0.133	0.554	0.211	0.347
Δ-cough VAS	0.345	0.116	0.270	0.224	0.327	0.137

VAS, visual analogue scale.

F_{ENO} measurement was not affected by iron supplementation, being 18.92 (SEM 3.15) ppb in baseline and 17.68 (SEM 2.42) ppb after iron, p = 0.249. Oropharyngeal examination after treatment showed regression of cheilitis angularis and of mucosa redness in all the women.

Discussion

The results of this study indicate that ID may contribute to chronic unexplained cough and to increased sensitivity of airway receptors, particularly of those sited in the extrathoracic airway. Actually, in baseline conditions, all the patients had laryngeal hyperresponsiveness to inhaled histamine, in agreement with our prior finding that chronic cough is associated with an irritable larynx (14). Half of the patients had bronchial hyperresponsiveness, mainly of mild degree, despite negative history of asthma and normal lung function tests. The strongest evidence for the role of ID in cough is the dramatic improvement of cough and histamine hyperresponsiveness observed in all the patients after iron repletion, in face of the absence of any beneficial effect with anti H1-histaminic and proton pump inhibitor drugs. Only two patients had still BHR, despite absence of symptoms. In case of cough recurrence, these patients could be assumed to have cough variant asthma.

The mechanisms by which ID favoured cough remain unknown. To our knowledge, the only observation in the literature regarding iron and cough is in angiotensin converting enzyme (ACE) inhibitor induced cough, where Lee et al. (26) demonstrated a beneficial effect of iron supplementation. The authors attributed this effect to decreased ACE inhibitors induced generation of NO in bronchial epithelial cells, as iron is able to inhibit the activity of inducible NO synthase, the enzyme involved in NO production. However, this explanation seems unlikely in our study, as F_{ENO} was normal in most of the patients and did not change after iron repletion, despite cough improvement.

The possibility that ID favoured cough through anaemia seems improbable, as 41% of our women had normal haemoglobin levels. In this regard, ID has been shown to induce morphological, physiological and biochemical changes in many organs before there is any drop in haemoglobin concentration (27) and iron repletion has been found to improve several functions regardless of increasing haemoglobin level (28).

A plausible explanation is that ID acted by impairing the defence mechanisms of airway mucosa. Oral mucosa atrophy with ID has long been recognised at a descriptive level (29). In our patients, mucosa thinning was testified by direct pharyngeal inspection, showing oral mucosa atrophy and cheilitis angularis. Moreover, ID weakens the defensive response to injuries by impairing blastogenesis, mitogenesis and several aspects of immune function, such as T lymphocyte number, bactericidal activity of macrophages, neutrophil myeloperoxidase and cell migration (30) and impairs the production of interleukin-2, a cytokine, which plays a pivotal role in maintaining the normal immune response (30).

The presence of histamine hyperresponsiveness and of throat irritative symptoms, suggests that ID induced sensory neuropathy in addition to mucosa atrophy. Iron is absolutely necessary for normal neuronal and glial energy metabolism, neurotransmitter production and myelination, and its deficiency has been shown to impair central and peripheral neural function (31). The negative effect of ID is particularly relevant in brain areas with great iron-dependent metabolic activity, such as hippocampus, prefrontal cortex and anterior cingulated cortex (32), areas which are involved in the neural control of cough (33). The influence of ID on sensory system is suggested by a recent experimental observation that ID increases acute pain threshold and sensitivity to C-fibre–mediated chronic pain (34). There are important analogies between pain and cough, as several stimulants known to selectively stimulate nociceptive C-fibres (e.g. capsaicin, bradykinin) also evoke cough in laboratory animals and in humans as well (35). Moreover, iron is required for proper myelination of

the spinal cord (36). We recently found that deficiency of vitamin B-12, a nutrient involved in myelin synthesis, may cause cough and laryngeal hyperresponsiveness through sensory neuropathy (37).

We would acknowledge some weaknesses of the study. First, it is a series study without a control group, because it would have been unethical to administer iron to patients without ID. Second, it is an open study, as both patients and physicians were not blinded to the treatment assignment. Nevertheless, our observation may be relevant to clinical and public health practice. Respiratory specialists and general practitioners do inevitably come across women who suffer from a chronic, dry, tickly cough, in whom conventional causes have been investigated and excluded, and therapeutic empiric approaches have failed. Our findings suggest that ID should be investigated in such patients, before prescribing further expensive and invasive investigations for cough assessment.

Author contributions

Concept/design: Bucca, Rolla. Data analysis/interpretation: Bugiani, Cicolin, Ricciardolo, Bucca, Rolla. Drafting article: Bucca, Brussino, Bugiani, Cicolin, Culla. Critical revision of article: Ricciardolo, Bugiani, Cicolin. Approval of article: all the authors. Funding secured by: Bucca. Statistics: Bugiani. Data collection: Brussino, Bucca, Culla.

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Disclosures

None. The authors deny any potential conflicts of interest, including all relevant financial interests, in any company or institution that might benefit from the publication.

All listed authors meet ICMJE http://www.icmje.org/authorship criteria. Nobody who qualifies for authorship has been excluded. Credit for authorship are based on: (i) substantial contributions to research design, or the acquisition, analysis or interpretation of data; (ii) drafting the paper or revising it critically; (iii) approval of the submitted and final versions.

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