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Evaluation methods and influencing factors of cough sensitivity

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***Abstract*:** Increased cough sensitivity is an important mechanism of chronic cough, and the evaluation of cough sensitivity helps understand the mechanism of cough and explore better methods to reduce cough. Evaluation methods may be direct or indirect. Direct methods include mechanical stimulation and chemical stimulation, and indirect methods include laryngeal reflex test, questionnaires, and brain functional magnetic resonance imaging (fMRI). Chemical stimulation is the most common method, while the capsaicin cough challenge test is proven and widely used. In this article, we will compare evaluation methods and explore influencing factors of cough sensitivity.

Keywords: assessment, cough sensitivity, standardization, test

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Introduction

Cough is defined as a defensive reflex to clear respiratory secretions and harmful factors. However, chronic cough may lead to physical, psychological, and social impairments, with a substantial impact on quality of life.[1] The severity of cough is closely related to quality of life and treatment satisfaction. Evaluation of the severity of cough is important for assessing the condition of patients and treatment outcomes. In clinical practice, commonly used evaluation methods include subjective visual analogue scales (VAS), cough severity score, cough severity diary, and quality of life assessments, as well as objective monitoring of cough frequency and evaluating of cough sensitivity.[2] For monitoring cough frequency, the Leicester cough monitor has been invented to automatically detect cough from continuous digital audio recording, but the device is not currently commercially available.[3] At present, the evaluation of cough sensitivity is still the most common way to evaluate cough objectively. In recent years, clinical studies have been conducted to investigate how best to evaluate cough sensitivity as researchers start to accept the concept of cough hypersensitivity syndrome (CHS). In this article, we will compare evaluation methods and explore influencing factors of cough sensitivity.

Importance of cough sensitivity assessments

Cough sensitivity is defined as responsiveness of cough reflex to external simuli, which includes chemical stimulation, mechanical stimulation, and thermal stimulation.[4] Increased cough sensitivity indicates that cough receptors are sensitized, resulting in responses to weak stimuli that would not otherwise induce cough or strong responses to the above-threshold stimuli. Most patients with chronic cough have the clinical features of increased cough sensitivity and studies have shown that patients with chronic cough have upper-regulated cough reflex than normal people which indicate increased cough sensitivity is a mechanistic basis of chronic cough.[4,5] In 2009, Morice[6] proposed the concept of CHS and that CHS applies to most cases of chronic cough. Common causes of chronic cough such as cough variant asthma (CVA), eosinophilic bronchitis (EB), gastroesophageal reflux-related cough, and upper airway cough syndrome (UACS) may be different clinical subtypes of CHS. CHS defines chronic cough from a new perspective and provides new ideas for the diagnosis and treatment of chronic cough, especially for refractory cough with unknown cause and no effective treatment. For CHS, the reestablishment of normal cough

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sensitivity may be an important strategy for the treatment of refractory cough in the future.[7]

However, the clinical value of CHS is still controversial. Further research is needed to investigate underlying mechanisms of cough hypersensitivity. No 'gold standard' has been established to detect cough hypersensitivity, and no uniform method has been validated to evaluate treatment outcomes of cough hypersensitivity.[8] The evaluation of cough hypersensitivity plays a critical role in these puzzles. Studies on cough sensitivity in different populations *via* different pathways and on influencing factors will deepen our understanding of the mechanisms of cough hypersensitivity, establish standard evaluation methods and criteria of CHS to facilitate clinical diagnosis, and allow monitoring of changes in cough sensitivity in patients with chronic cough to determine treatment outcomes.

Definition and basic concept of cough sensitivity assessments

Cough sensitivity assessments are narrowly defined as cough challenge tests (chemical stimulation), in which the subject inhales a certain amount of aerosol stimulant particles that stimulate cough receptors in the airway to induce cough.[9] The concentration of cough-inducing stimulant is used to determine cough reflex sensitivity,[9] which mainly reflects the cough reflex mediated by specific peripheral chemoreceptors.[10] In addition to chemical stimulation, mechanical stimulation can also induce cough directly,[11–15] reflecting the cough reflex mediated by mechanoreceptors.[10] Other indirect methods are also used to evaluate cough sensitivity, including different questionnaires, which subjectively and indirectly evaluate cough sensitivity, and brain functional magnetic resonance imaging (fMRI), which objectively and indirectly evaluates cough sensitivity, showing central nervous system activity related to cough reflex. At present, assessment of cough sensitivity is mainly used in studies on efficacy of medication and cough mechanism and has not been applied in clinical practice.[16]

Mechanisms of cough sensitivity assessments

Cough hypersensitivity could result from either an increased sensitivity of cough receptors or from changes in cough central processing.[17]

Cough receptors are sensory nerve endings distributed between tracheobronchial epithelial cells and in the epithelial basal layer. They are divided into two subtypes: myelinated vagal afferent Aδ fibers and nonmyelinated vagal afferent C-fibers.[18] Cough sensitivity mediated by peripheral cough receptors can be evaluated by stimulating these nerve endings.[12,13,19,20]

Vagal afferent C-fiber endings are chemoreceptors that are sensitive to chemical stimulation. Transient receptor potential cation channel subfamily V member 1 (TRPV1) and transient receptor potential cation channel subfamily A member 1 (TRPA1), two members of the TRP family, are expressed on the cell membrane of these nerve fibers. Both TRPV1 and TRPA1 are nonselective cation channel proteins that exist on the cell membrane or intracellular organelle membrane. TRPV1 is sensitive to stimuli such as capsaicin, acid, inflammatory mediators, and higher temperature (>42°C). TRPA1 is sensitive to stimuli such as acrolein, cinnamaldehyde, and lower temperature (<17°C). Once activated, TRPV1 and TRPA1 stimulate vagal afferent C-fiber endings to produce neurogenic inflammation, release neuropeptides, such as substance P (SP) and calcitonin gene–related peptide (CGRP), and upregulate cough sensitivity.[21,22]

Vagal afferent Aδ fiber endings are mechanoreceptors that are sensitive to mechanical stimulation. They are divided into pulmonary stretch receptors and Widdicombe cough receptors.[23,24] Pulmonary stretch receptors include rapid adaptation receptors (RARs) and slow adaptation receptors (SARs). RARs and SARs do not directly participate in the initiation of the cough reflex, but they play roles in regulating and integrating the initiation of the cough reflex. As special RARs located in the extrapulmonary trachea, Widdicombe cough receptors are highly sensitive to slight mechanical changes and acids in the airway but are insensitive to tissue traction, bronchospasms, and chemical stimulation.[23] Studies have shown that TRPV4 may activate Aδ fibers by mediating ATP.[25,26] As with TRPV1 and TRPA1, TRPV4 is another member of the TRP family and is sensitive to myriad physical and chemical stimuli, including shear force, osmotic pressure, citric acid, temperature above 24°C, and arachidonic acid metabolites.[27] Bonvini *et al.* used an *in vivo* animal cough model and calcium imaging and electrophysiological techniques and found that a TRPV4 agonist and hypotonic solution induced the depolarization of all Aδ fibers but had no effect on C-fibers. This TRPV4 channel-mediated effect was blocked by P2X purinoceptor 3 (P2X3R, ATP receptor) antagonist, indicating that ATP plays an important role in the TRPV4-mediated activation of Aδ fibers.[25]

Located in the nucleus tractus solitarius and spinal trigeminal nucleus, the cough center is regulated by the higher cortical center.[28] Brain fMRI and some indirect indicators such as urge to cough and cough suppression (CS) can be used to indirectly evaluate how well the cough centers regulates the cough reflex.[29]

Evaluation methods for cough sensitivity

Cough sensitivity may be assessed directly or indirectly. Direct methods include mechanical stimulation and chemical stimulation; indirect methods include brain fMRI, laryngeal reflex tests, and questionnaires.

Mechanical stimulation

For mechanical stimulation, mechanical vibration is used to stimulate the cough receptors in airways to induce cough.[18,30] An animal study shows that the introduction of a rotating mechanical probe to mechanically stimulate the airway of anesthetized rabbits induces the cough reflex.[11]

Studies of mechanical stimulation to evaluate cough sensitivity in humans have applied mechanical vibration, including laryngeal vibration and thoracic vibration, to different locations of the airway. For laryngeal vibration, mechanical stimulation is applied to the cervical trachea. Lee and Eccles[12] placed a modified electric shaver (the shaving blade was replaced with a finger-like plastic tab) at the level of the jugular notch to mechanically stimulate the cervical trachea. This study has found that the laryngeal vibration can reproducibly induce cough and individuals with upper respiratory tract infection (URTI) are more sensitive to the stimulation than healthy individuals. Kamimura *et al.*[13] designed and used a tracheal compression test (TCT) (using a finger to gently compress the cervical trachea several times), a tracheal stretch test (TST) (the neck was retroflexed and held for 5 seconds), and a tracheal fork test (TFT) (a vibrating fork was placed on the cervical trachea and held for 20 seconds) to mechanically stimulate the cervical trachea of patients with chronic cough. Test responses were categorized as cough, new or worsening airway itching, and no response. In patients with chronic cough, the incidence of cough was 27.7% for the TCT, 39.8% for the TST, and 36.9% for the TFT. Approximately 50% of patients experienced itching with or without cough in each test. Patients with phonation-induced cough (PIC) were more sensitive to the test than the patients without PIC, which supported the hypothesis that transmission of vibrations from the vocal cords to the trachea may be the cause of PIC. Thoracic vibration is a type of stimulation which is applied to the thoracic. Lee and Eccles[14] placed a G5 respiratory percussion device (70 Hz) at sternum to induce cough in URTI individuals and healthy individuals and found that URTI individuals were more sensitive to stimulation. Jones *et al.*[15] further investigated the effect of different frequencies (20, 40, 60 Hz) of percussion stimulation at different thoracic locations (lung base, front chest, and below the sternum) on inducing cough in idiopathic pulmonary fibrosis (IPF) patients. The incidence of cough and cough frequency were also significantly higher in the IPF group than in the healthy group. Besides, the results showed that low-frequency vibration at the base of the lungs was most effective for inducing cough.

These studies indicate that mechanical stimulation can, to some extent, distinguish healthy individuals from patients with cough hypersensitivity. However, there are still some problems need to be explored. There are few studies about mechanical stimulation. No studies have been conducted to establish a threshold for cough hypersensitivity with mechanical stimulation and the validity of mechanical stimulation needs to be further explored. The relevant factors which may influence the incidence of cough reflex in human during the mechanical stimulation are still unknown. In an animal study, the incidence of cough reflex was related to the intensity, duration, and rate of mechanical stimulation.[11] But in human studies, these factors have not been explored. Also, like the study by Jones *et al.*,[15] the effect difference caused by different vibration frequency and vibration position needs to be further studied.

In summary, cough sensitivity assessments with mechanical stimulation are simple, noninvasive, and safe. However, few studies have been conducted in this field, and further researches are needed to investigate the application value and relevant factors for cough sensitivity assessments with mechanical stimulation.

Chemical stimulation

Stimulants and the principle of chemical stimulation. For chemical stimulation, the subject inhales a certain amount of aerosol stimulant particles, which stimulate cough receptors in the airway to induce cough. Although there are different views,[31] cough sensitivity is most commonly assessed by the lowest concentration of stimulations which cause 2 (C2) or 5 (C5) coughs.[9]

Capsaicin is the most commonly used stimulant. Several studies have demonstrated that capsaicin is a useful substance for cough challenge test. The safety,[32] reproducibility,[33] and dose-dependency[34] of capsaicin cough challenge test have been demonstrated. And for patients with chronic cough and sensory hyperreactivity, Pullerits *et al.*[35] have found that capsaicin cough challenge test has an excellent ability to discriminate patients from control subjects. Capsaicin induces cough by activating the ion channel TRPV1 on nonmyelinated afferent C-fibers in the airway.[36] Studies have shown that patients with chronic cough are more sensitive to capsaicin.[5] After treatment, cough sensitivity to inhaled capsaicin is reduced as cough symptoms relieved.[37] The increased cough sensitivity may be associated with elevated TRPV1 expression on airway nerves of patients with chronic cough.[38]

Not every patient with chronic cough shows heightened capsaicin cough sensitivity.[19] In addition to capsaicin, stimulants including TRPA1 stimulants, citric acid, mannitol, and ATP can be used. Allyl isothiocyanate (AITC) and cinnamaldehyde are agonists of the ion channel TRPA1 on afferent C-fibers. Unlike TRPV1, TRPA1 stimulants are more common in daily life and include ozone, nicotine, and acrolein. These substances are widely present in air pollution, vehicle exhaust, and cigarette smoke, suggesting that TRPA1-related cough challenge tests may have broader applications. In addition, studies have shown that AITC and cinnamaldehyde tests are safe and repeatable and can complement the capsaicin challenge test.[19,39]

Followed by capsaicin, citric acid is the second most widely used stimulant. Its mechanism of action is unknown, although TRPV1, TPRA1, and TRPV4 receptors may be involved. Rai *et al.*[40] investigated the effect of pH on citric acid-induced cough and found that after inhaling the citric acid solution, patients with chronic cough coughed more than healthy volunteers. Besides, researchers also found that cough frequency was unrelated to pH value in patients with chronic cough. At a given pH, patients respond in a highly idiosyncratic manner (sometimes not cough to the first few inhalations and then cough excessively to an exactly similar stimulus). Researchers thought that like the response for chronic pain, hypersensitivity is not simply a shift in the dose response curve to citric acid but a fundamental alteration in the pattern of response to a given stimulus.

Mannitol induces cough through osmotic stimulation of the airway. Koskela *et al.*[41] performed mannitol stimulation and found that patients with chronic cough had much higher cough sensitivity than did subjects in other groups and that cough sensitivity was correlated with subjective symptoms.

In a recent study, Fowles *et al.*[20] used ATP and AMP for cough challenge tests and found that ATP was more effective than AMP for inducing cough. Compared with normal subjects, patients with chronic cough coughed more after inhaling a significantly lower concentration of ATP, although the results were similar to those for other cough challenge tests. Among the 40 subjects, the only adverse reaction was urticaria, which may have been an allergic reaction. Studies have shown that under pathological conditions, ATP activates the P2X2/3 receptors located on C and Aδ fibers to stimulate vagal afferent nerve endings in the lungs, leading to the release of local neurogenic inflammatory mediators, bronchoconstriction, and cough.[42,43] At present, gefapixant (also known as AF-219 and MK-7264), a P2X3 receptor antagonist, is the most potential candidate for the treatment of refractory cough, with proven efficacy in improving cough symptoms in clinical studies.[44,45] These data indicate that ATP-P2X plays an important role in chronic cough, suggesting that ATP-related cough challenge tests have broad research value.

Procedures and applications. While many cough sensitivity tests are available, chemical stimulation is still the proven and widely used method. In the section below, we will use the capsaicin test as an example to describe the standard procedures for cough sensitivity assessments with chemical stimulation. Capsaicin may be administrated *via* a tidal-breath inhalation or a dosimeter-controlled inhalation.

The specific procedures for tidal-breath inhalation are described by Fujimura *et al.*[46] A capsaicin stock solution of 0.01 M/L is prepared for later use. Before the test, the stock solution is diluted in normal saline to prepare 12 concentrations (0.49, 0.98, 1.95, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 μM/L). The subject takes a sitting position and wears a nose clip. While breathing calmly, the subject inhales normal saline through a compressed-air nebulizer. After confirming that there is no cough, the subject sequentially inhales different concentrations of aerosol capsaicin solution (15 seconds on, 45 seconds off), starting from the lowest concentration. Cough frequency is recorded for 1 minute, and the lowest concentrations that induce ⩾2 or ⩾5 coughs are recorded (C2 and C5, respectively) as the subject's cough thresholds. Tidal breathing is easy to use and operate. However, tidal volume and respiratory frequency vary from person to person, making it difficult to quantify the dose inhaled. Atomization inhalation duration is long, with a high inhalation volume, and inhalation may be interrupted by coughing, affecting the cough frequency results.[9] Although the safety of capsaicin has been confirmed, the aerosol stimulant particles may irritate both subjects and operators.[32,47]

Dosimeter-controlled inhalation avoids the problems associated with tidal-breath inhalation, it is more conducive to standardization, but the device is complex to operate. The specific procedures for dosimeter-controlled inhalation are described in the ERS (European Respiratory Society) guidelines for the assessment of cough (2007)[9] and the Chinese National Guidelines for the Diagnosis and Management of Cough (2015).[16] To standardize the test, a flow-limited dosimeter-controlled nebulization device is used. With the dosimeter, the inspiration flow rate can be controlled. Also, the structure of nebulizer is modified. With the straw and baffle assembly of the nebuliser welded in place, the variations resulted by the variable distances between the jet orifice and straw can be eliminated. In the ERS guidelines for the assessment of cough (2007), capsaicin and citric acid are used as examples to propose a series of standardization recommendations for cough challenge tests,[9] stating that inhalation cough challenge tests should be standardized to facilitate universal interpretation and comparisons of results from different laboratories. The standardized method is advocated by the guidelines to establish normal ranges. In most cases, the single-breath concentration-response method using a flow-limited dosimeter inhalation test is recommended, and both C2 and C5 should be recorded. Moreover, it is important to explain that cough challenge test results have no inherent meaning due to significant individual variation but can be used to track any changes in cough sensitivity in a given individual.

Based on the inhalation test above, Cho *et al.*[48] Fujiwara *et al.*,[49] and Curtis and Troche[50] also have further improved the processes and devices for cough sensitivity assessments.

Regarding processes, Cho *et al.*[48] developed a CS test. During this dosimeter-controlled inhalation test, the subject is instructed to do his or her best to suppress coughs, and the corresponding concentrations of capsaicin that induce 1, 2, or 5 coughs (CS1, CS2, CS5) are recorded. This study has shown that patients with chronic refractory cough are significantly less able to suppress coughs than healthy subjects, suggesting abnormalities in central nervous pathways in patients with chronic refractory cough. CS5 is highly repeatable. Besides, CS5 is related to 24-hour cough frequency in patients with chronic refractory cough. Moreover, CS5 (threshold: 39 μmol/L) is more sensitive and specific than C5 for the diagnosis of chronic refractory cough.[48]

Regarding devices, researchers designed a device that measures both cough sensitivity and the intensity of the cough reflex. Fujiwara *et al.*[49] invented a modified cough sensitivity assessment device that consists of a mouthpiece, a special double-lumen tube (an inner tube is nested into an outer tube, with 10 ports between the 2 layers of tubes), a nebulizer, and an electronic spirometer. An ultrasonic nebulizer is connected to the outer tube and fills the outer tube with microaerosol stimulant, which enters the mouth and airway through the ports and mouthpiece to induce cough. The electronic spirometer is connected to the inner tube to measure the intensity and duration of the cough reflex. Curtis and Troche[50] invented a hand-held cough sensitivity assessment device that consists of a face mask, a hand-held nebulizer, and an analog peak flow meter connected by a three-way tube. It measures

Table 1. Influencing factors for cough sensitivity.

Factor	First Author	Reference	Year	Methods	Results
TRPV1 polymorphism	Cantero-Recasens G	50	2010	Case-control study Genetic polymorphism analysis Cell functional assay	Asthmatic children with the TRPV1-I585 V genotype have a lower risk of cough Cell assays indicate that Ca^2+ channel activity is decreased in cells transfected with TRPV1-I585V
TRPV1 polymorphism	Smit LA	51	2012	Case-control study	TRPV1 single nucleotide polymorphism is associated with cough in nonasthmatic patients. TRPV1 single nucleotide polymorphism may increase the risk of coughing in smokers and workers with related occupational exposure
TRPV1 polymorphism	Sadofsky LR	52	2017	Capsaicin cough challenge test Genetic polymorphism analysis Cell functional assay	Capsaicin cough sensitivity is significantly lower in 4 pepper-tolerant subjects. Genetic analysis indicates that these subjects harbor the TRPV1-V585 mutation and triple TRPV1-I315-I469-V585 mutations. Cell assays show a similar decrease in Ca^2+ channel activity in cells transfected with TRPV1-V585 versus TRPV1-I315-I469-V585, suggesting that the TRPV1-V585 mutation alone plays a role.
Sex	Fujimura M	55	1990	Tartaric acid cough challenge test	In healthy individuals, tartaric acid cough sensitivity is higher in women than in men
Sex	Fujimura M	45	1996	Capsaicin cough challenge test	In healthy individuals, cough sensitivity is higher in young or middle-aged women than in men
Sex	Long L	19	2019	Capsaicin cough challenge test AITC cough challenge test	In healthy individuals and patients with chronic cough, capsaicin and AITC cough sensitivity is higher in women than in men
Sex	Lai KF	56	2019	Capsaicin cough challenge test	In patients with chronic cough, capsaicin cough sensitivity is higher in women than in men and higher in patients aged 50 years or older than in patients younger than 50 years
Smoking	Millqvist E	61	2001	Capsaicin cough challenge test	Smokers reacted to provocation with significantly fewer coughs than non-smokers
Smoking	Dicpinigaitis PV	62	2003	Capsaicin cough challenge test	In healthy young men, cough sensitivity is lower in smokers than in non-smokers
Smoking	Dicpinigaitis PV	63	2006	Capsaicin cough challenge test	After smoking cessation for 2 weeks, cough sensitivity increases in long-term smokers
Obesity	Laurent Guilleminault	64	2019	Epidemiological investigation Review	Obesity, especially abdominal obesity, is associated with chronic cough
Obesity OSAHS	Jean Guglielminotti	66	2007	Citric acid cough challenge test	Cough sensitivity is reduced in female obese OSA patients

(Continued)

Table 1. (Continued)

Factor	First Author	Reference	Year	Methods	Results
OSAHS	Shi CQ	65	2019	Capsaicin cough challenge test Induced sputum cytology	OSAHS patients with a normal BMI tend to have cough hypersensitive, which is related to airway inflammation
Obesity	Pecova R	67	2020	Capsaicin cough challenge test	In children with chronic cough, BMI is weakly correlated with decreased cough sensitivity (C5; $R^2 = 0.03$)
Capsaicin exposure	Blanc P	46	1991	Capsaicin cough challenge test	In workers with long-term pepper exposure, their capsaicin cough thresholds present a bimodal distribution pattern
Capsaicin exposure	Slovarp LJ	54	2019	Capsaicin cough challenge test	Using cough suppression strategies with inhalation of aerosolized capsaicin given in gradually increasing doses, C5 was significantly increased at both 1 and 3 weeks post-treatment
Capsaicin exposure	Ternesten-Hasséus, E.	53	2015	Capsaicin cough challenge test	After treatment with capsaicin, the thresholds for C2 were higher. Among patients, the concentration needed to reach C2 and C5 increased after the period with the active substance compared to cough thresholds at baseline. The cough symptom scores improved after 4 weeks of active treatment compared to the baseline scores.
Allergic inflammation	Weinfeld D	68	2002	Capsaicin cough challenge test	Before the season, the cough sensitivity was similar to that of healthy control subjects, but during the pollen season, the sensitivity was significantly increased.
Allergic inflammation	Ternesten-Hasséus E	69	2002	Capsaicin cough challenge test	The patients with multiple chemical sensitivity coughed more than the control subjects at each dose of capsaicin. The capsaicin provocation also induced significantly more symptoms in patients.
Chemosensory regulation	Paul M. Wise	70	2012	Capsaicin cough challenge test	Oral rinse with sucrose and inhalation of menthol vapor significantly increase the cough threshold
Chemosensory regulation	Millqvist E	71	2013	Capsaicin cough challenge test	In patients with chronic cough, pre-inhalation of menthol reduces cough sensitivity to inhaled capsaicin and influences inspiratory flows.
Other	Pecova R	72	2008	Capsaicin cough challenge test	Capsaicin cough sensitivity is significantly higher in patients with allergic rhinitis (no cough) than in normal volunteers
Other	Huang Y	73	2011	Capsaicin cough challenge test Induced sputum cytology	CVA may be associated with normal or increased cough sensitivity, which may be related to neutrophil infiltration and activation

AITC, allyl isothiocyanate; BMI, body mass index; CVA, cough variant asthma; OSA, obstructive sleep apnea; OSAHS, obstructive sleep apnea hypopnea syndrome; TRPV1: transient receptor potential cation channel subfamily V member 1.

the intensity of the cough reflex. After each cough reflex, the subject completes a modified Borg scale to indicate his or her urge to cough. Further research is still needed to evaluate specific clinical applications for these and similar devices.

Criteria for cough sensitivity assessments with chemical stimulation and influencing factors for cough sensitivity. No uniform normal ranges have been established for cough sensitivity. Some studies have shown that cough sensitivity assessments with chemical stimulation may be subject to many factors, such as TRPV1 polymorphisms, capsaicin exposure, sex, smoking, obesity, allergic inflammation, and chemosensory regulation (Table 1), with major individual and population variations.

Studies have shown that the TRPV1-V585 mutation may be related to decreased cough sensitivity to capsaicin.[51–53] Capsaicin exposure may also reduce cough sensitivity. Blanc *et al.*[47] found that compared with those in workers without pepper exposure, workers with long-term pepper exposure showed a bimodal distribution pattern, including both a higher cough threshold (3*10-6 M capsaicin) and a lower cough threshold (3*10-7 M capsaicin). Within the exposed group, a higher cough threshold was significantly related to male gender and was associated to a lesser extent with dietary preference for hot food and cumulative cigarette smoking. The researchers believed that capsaicin exposure and other factors contributed to decreased cough sensitivity. In a study by Ternesten-Hasséus *et al.*,[54] they found that after taking capsules with pure capsaicin for 4 weeks, capsaicin cough sensitivity of cough patients decreased and cough symptoms and cough scores improved. The researchers thought that may related to the desensitization of TRPV1. Moreover, Slovarp and Bozarth[55] paired CS strategies with inhalation of aerosolized capsaicin given in gradually increasing doses in 5 healthy people and found that C5 was significantly increased at both 1 and 3 weeks post-treatment. However, the sample size of these three studies were small, and further research is needed to investigate the relationship between capsaicin exposure and decreased cough sensitivity.

Several studies have shown that women have higher cough sensitivity than men during cough challenge tests,[19,46,56,57] a finding that may be related to hormones, as estrogen and progesterone affect TRPV1 activation and expression,[58,59] eosinophils and mast cell activation,[60] and the onset of certain conditions, such as gastroesophageal reflux disease (GERD),[61] all of which may increase cough sensitivity.

Millqvist and Bende[62] and Dicpinigaitis,[63] Dicpinigaitis *et al.*[64] proved that long-term smokers have decreased cough sensitivity. Millqvist *et al.* thought the decreased cough sensitivity of smokers may support the hypothesis that nicotine either inhibits the receptors of the C-fibers of the sensory nerves in the respiratory epithelium, or induces a depletion of neuropeptides and this explained why increasing airway symptoms were often seen after cessation of smoking.[62] Research by Dicpinigaitis *et al.*[64] further illustrated this point because even after many years of smoking, cough sensitivity enhanced as early as 2 weeks after smoking cessation. Furthermore, Researchers thought the decreased cough sensitivity of smokers may also be related to the desensitization of cough receptors in the airway epithelium and increased airway mucus secretions that cover cough receptors due to long-term smoking.[63,64]

Obesity may also affect cough sensitivity. Epidemiological surveys show that obesity, especially abdominal obesity, is a risk factor for chronic cough and certain conditions,[65] suggesting that obese individuals may have higher cough sensitivity. In our study, Shi *et al.*[66] capsaicin cough challenge test results indicate that patients with normal weight and obstructive sleep apnea hypopnea syndrome (OSAHS) have higher cough sensitivity than do normal subjects, but in a study by Guglielminotti *et al.*,[67] citric acid cough challenge test results show that female obese OSAHS patients have lower cough sensitivity than normal subjects. The discrepancy may be related to the study population and indicate that obesity may play a critical role in impaired cough sensitivity. Pecova *et al.*[68] investigated the relationship between capsaicin cough sensitivity and body mass index (BMI) in children with chronic cough and found that in these children, BMI was weakly correlated with decreased cough sensitivity (C5) and a low coefficient of determination (R2 = 0.03). Increase of body mass index in one unit is associated with -34.959 mmol/l decrease of C5. Further research is needed to investigate the effect of obesity on cough sensitivity.

Allergic inflammation may increase cough sensitivity. For patients with allergic asthma, capsaicin cough sensitivity increased during the birch pollen season, while the response was similar to that of healthy control subjects before the season.[69] For patients with multiple chemical sensitivity, their capsaicin cough sensitivity also increased.[70] Researchers thought that allergic inflammation in the lower and/or upper airways may trigger neurogenic mechanisms of significant clinical importance.

Chemosensory regulation affects cough sensitivity, as an oral rinse with sucrose[71] and inhalation of menthol vapor[71,72] significantly increase the cough threshold, which explains to a certain extent why cough syrup without active ingredients has some antitussive effects and adding menthol to cigarettes might make smokers more tolerable to tobacco smoke.

Moreover, individuals with high capsaicin cough sensitivity may not complain about cough. Pecova *et al.*[73] report that patients with seasonal allergic rhinitis do not cough but that they have high capsaicin cough sensitivity. On the contrary, patients who cough may or may not have high capsaicin sensitivity, such as in some CVA patients. In our study, Huang *et al.*[74] analyzed the clinical manifestations, airway inflammation, and types and levels of inflammatory mediators in CVA patients with high or normal cough sensitivity. The results showed that CVA patients with high cough sensitivity had more severe cough and more significant neutrophil infiltration and activation in the airway, suggesting that high cough sensitivity and normal cough sensitivity may be 2 subtypes of CVA that differ in neutrophil activation and infiltration in the airway.

Different cough challenge tests have been used in China and abroad, making it difficult to analyze results across studies. The ERS guidelines for the assessment of cough (2007)[9] propose a series of standardization recommendations for solution preparation and storage, inhalation methods, inspiratory flow, nebulizers, inhalation of placebo, cough measurement, and data interpretation of capsaicin and citric acid cough challenge tests. The guidelines stated that isolated measurements of capsaicin sensitivity (C2 or C5) had no intrinsic significance due to the large variation in cough reflex sensitivity within the normal population but are important tools in pharmacological studies incorporating serial challenges, as well as in epidemiological studies comparing distinct populations. There is no recommendation regarding normal reference of C2 and C5 values for cough sensitivity in ERS guidelines. In 2013, Chen *et al.*[75] conducted a study in 110 healthy volunteers from Guangzhou, China, to establish a normal reference value for capsaicin cough sensitivity in the Chinese population. The initial result was $C5 \geqslant 125$ μmol/L. However, the sample size was small, and the subjects were from a single area (Guangzhou, China). Further research is needed to validate the reference value, especially given the potential factors mentioned above, such as capsaicin exposure.

In summary, some standard procedures for cough challenge test have already been established according to the ERS guidelines and researchers are further exploring the device and process to improve cough challenge test. Capsaicin is proved to be a valid and useful tool, and new stimulant like ATP is under investigation. Also further researches are needed to explore the influencing factors of cough sensitivity and to compare the cough sensitivity among distinct population.

fMRI. Cough central processing dysfunction plays an important role in increased cough sensitivity.[28] However, no tests are available to directly measure central cough sensitivity. fMRI can be used to visualize the activity of the central nervous system while coughing and thus indirectly assess central cough sensitivity. During fMRI, a specific sensory organ is stimulated to cause neural activity (activation of a functional area) in the corresponding cerebral cortex, which is detected on fMRI because the local cortical blood oxygen level increases during neural activity, resulting in high T2 signals in the corresponding area.[76] Researchers have investigated neural activity after the inhalation of capsaicin to analyze central cough sensitivity in patients with chronic cough *versus* other populations. Ando *et al.*[77] show that after the inhalation of capsaicin, compared with the control group, in CHS patients, the signals are significantly increased in brain areas related to the regulation of the pain system, such as the cuneiform nucleus of the midbrain, the gray matter around the midbrain aqueduct, and the dorsal raphe nucleus, and are significantly decreased in brain areas related to CS, such as the dorsal medial prefrontal lobe and the anterior cingulate cortex. Morice *et al.*[29] show that healthy women are more sensitive to the capsaicin cough challenge tests and that their somatosensory cortex is twice as activated as that in men after the inhalation of capsaicin.

The use of fMRI in CHS patients may help in understanding cough hypersensitivity at the central level and to develop new antitussive therapies.

Laryngeal reflex text. Laryngeal hypersensitivity refers to an excessive laryngeal response to external stimuli; this response is related to the contraction of laryngeal muscles and the extrathoracic airway. Laryngeal dysfunction can cause chronic cough, and most patients with chronic cough have laryngeal irritation; besides, their laryngeal hypersensitivity improves after treatment for chronic cough.[78] Therefore, laryngeal hypersensitivity is an indirect indicator of cough hypersensitivity.

Bucca *et al.*[79] used a histamine inhalation test to assess the laryngeal sensitivity. A reduction in maximum inspiratory flow (MIF50) by 25% was used as the criterion for laryngeal stenosis, and laryngeal hypersensitivity was considered if the corresponding histamine concentration was ⩽8 mg/mL. The study showed that 66% to 93% of patients with chronic cough had varying degrees of laryngeal hypersensitivity, which was resolved or alleviated after cough symptoms relieved.

Tatar *et al.*[80] performed laryngeal electromyography (EMG) and laryngoscopy in 20 patients with idiopathic cough and a history of upper airway virus infection and found that EMG score was significantly positively related to the quality of life score in patients with chronic cough. The researchers believed that post-viral vagal neuropathy (PVVN) resulted in a lower discharge threshold of the vagus nerve, triggering laryngeal hypersensitivity and chronic cough. These data suggest that laryngeal EMG may be used to diagnose PVVN-related chronic cough. However, the sample size was small; therefore, large prospective studies are needed to validate the results.

Giraldo-Cadavid *et al.*[81–83] designed and developed a novel laryngopharyngeal endoscopic esthesiometer and rangefinder (LPEER) composed of a high precision air pulse generator, an endoscopic laser rangefinder. The device uses endoscopic air pulses to generate pressure on the mucosa of the laryngopharyngeal tube; increasing pulse intensity triggers the laryngeal adductor reflex, cough reflex, and gag reflex. The intensity of the air pulse that triggers the reflex reflects laryngopharyngeal sensitivity and the laser rangefinder helps to precisely control the location and angle of the air pulse.[81] The laryngeal adductor reflex threshold (LART) and cough reflex threshold (CRT) were measured on the laryngeal mucosa at a point between the corniculate and cuneiform cartilages and the gag reflex threshold (GRT) was explored at the lateral wall of the pharynx at a point lateral to the epiglottis.[81] The high intra- and inter-rater reliability and accuracy of LPEER for evaluating laryngopharyngeal sensitivity have been proved.[82,83] Studies have shown that in patients with laryngeal irritation, the LART is slightly increased and the CRT and GRT are decreased.[81] At present, studies are focused on the application of this device in the evaluation of laryngopharyngeal sensitivity in patients with difficult swallowing, while no related studies have been conducted in patients with chronic cough.

Questionnaires

There are also questionnaires that reflect cough sensitivity from different perspectives. Vertigan *et al.*[84] developed a questionnaire to assess laryngeal paresthesia in patients with laryngeal dysfunction syndrome (including chronic cough, vocal cord dysfunction, dystonia, muscle tension dysphonia, and globus pharyngeus). The questionnaire includes 14 questions to assess laryngeal paresthesia (obstruction, pain or heat, and irritation) on a 1–7 scale (1: *always*; 7: *never*). The questionnaire can successfully distinguish patients from healthy subjects, as in the initial study, the score for each question was significantly higher in the healthy control group than in the clinical group. However, the questionnaire cannot distinguish clinical subgroups, such as chronic cough, vocal cord dysfunction, muscle tension dysphonia, and globus pharyngeus.

Morice *et al.*[85] developed the Hull airway reflux questionnaire (HARQ) to evaluate cough sensitivity and diagnose CHS. It is a self-assessment questionnaire that includes 14 items, with a maximum score of 70. In a study, 185 patients with chronic cough and 70 normal volunteers completed the questionnaire. The results showed that the HARQ score was significantly higher in patients with chronic cough than in normal volunteers. In normal volunteers, the 95% confidence interval (CI) for the HARQ score was 0–13. When using 13 as the threshold, the sensitivity was 94.1%, the specificity was 95%, and the area under the receiver operating characteristic (ROC) curve was 0.99 for the diagnosis of cough hypersensitivity with the HARQ, suggesting that the HARQ has a high diagnostic value for cough hypersensitivity. Moreover, for patients with chronic cough, the score significantly decreased after treatment, indicating that the HARQ score can be used to evaluate treatment response. The study showed that women had higher HARQ scores than did men. The cause is unknown. Moreover, age was unrelated to HARQ score in patients with chronic cough.

In our study, Huang *et al.*[86] evaluated the effectiveness, repeatability, treatment response, and clinical value of the Chinese version of the HARQ. The results show that the HARQ effectively identifies cough hypersensitivity and is repeatable with good treatment responses. Our study shows that for the HARQ, the upper limit of normal is 12.75 and that the HARQ score is significantly higher in patients with GERD than in patients with other diseases or compound diseases, suggesting that the HARQ can be used to predict GERD. Wen *et al.*[87] further demonstrate that the HARQ is a valuable tool for predicting GERD and is more effective when combined with the GERD questionnaire (GerdQ).[88] Also, Johansson *et al.*[89] translated the questionnaire into Swedish version (HARQ-S) and found that the HARQ-S had good reliability and validity to diagnose chronic cough.

Moreover, Zhang *et al.* show that the HARQ has moderate value in predicting the treatment response of gabapentin, while C2 and C5 values from the capsaicin cough test are useless in this regard.[90] Gabapentin has been shown to reduce severity of cough, providing further evidence of central sensitization.[91] The predictive value of the HARQ on the efficacy of gabapentin indicates that certain items in the HARQ (such as laryngeal itching or obstruction, cough when eating, and morning cough) indirectly reflect central cough hypersensitivity,[90] further confirming that the HARQ can be used to indirectly evaluate cough sensitivity. The HARQ provides a subjective evaluation of cough sensitivity. Combined, the objective capsaicin challenge test and the HARQ provide a comprehensive evaluation of cough sensitivity.

Conclusion

Mechanical stimulation and chemical stimulation can be used to directly evaluate cough sensitivity. Mechanical stimulation is safe and easy to conduct, while there are few relevant studies. The validity of mechanical stimulation needs to be further confirmed. Chemical stimulation is more commonly used in research. While the capsaicin cough challenge test has been extensively studied, other cough challenge tests, such as AITC, ATP can be used as needed. A series of standardization recommendations are available for the capsaicin cough challenge test. However, chemical stimulation is subject to many factors, and further research is needed to establish normal reference values for cough sensitivity and investigate specific clinical applications of cough challenge tests. In addition, further research is needed to investigate indirect evaluation methods such as brain fMRI, laryngeal reflex responses, and the patient-report questionnaires.

Author contributions

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Conflict of interest statement

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References

1. Ma W, Yu L, Wang Y*, et al.* Changes in health-related quality of life and clinical implications in Chinese patients with chronic cough. *Cough* 2009; 5: 7.
2. Birring SS and Spinou A. How best to measure cough clinically. *Curr Opin Pharmacol* 2015; 22: 37–40.
3. Birring SS, Fleming T, Matos S, *et al.* The Leicester Cough Monitor: preliminary validation of an automated cough detection system in chronic cough. *Eur Respir J* 2008; 31: 1013–1018.
4. McGarvey L, McKeagney P, Polley L, *et al.* Are there clinical features of a sensitized cough reflex? *Pulm Pharmacol Ther* 2009; 22: 59–64.
5. Choudry NB and Fuller RW. Sensitivity of the cough reflex in patients with chronic cough. *Eur Respir J* 1992; 5: 296–300.
6. Morice AH. The cough hypersensitivity syndrome: a novel paradigm for understanding cough. *Lung* 2010; 188(Suppl. 1): S87–S90.
7. Chung KF. Chronic 'cough hypersensitivity syndrome': a more precise label for chronic cough. *Pulm Pharmacol Ther* 2011; 24: 267–271.
8. Morice AH, McGarvey LP and Dicpinigaitis PV. Cough hypersensitivity syndrome is an important clinical concept: a pro/con debate. *Lung* 2012; 190: 3–9.
9. Morice AH, Fontana GA, Belvisi MG, *et al.* ERS guidelines on the assessment of cough. *Eur Respir J* 2007; 29: 1256–1276.
10. Canning BJ, Chang AB, Bolser DC, *et al.* Anatomy and neurophysiology of cough: CHEST Guideline and Expert Panel report. *Chest* 2014; 146: 1633–1648.
11. Demoulin B, Coutier-Marie L, Ioan I, *et al.* In vivo documentation of stimulus velocity tuning of mechanically induced reflex cough. *Physiol Res* 2020; 69: S139–S145.
12. Lee P and Eccles R. Cough induced by mechanical stimulation of the upper airway in humans. *Acta Otolaryngol* 2004; 124: 720–725.
13. Kamimura M, Mouri A, Takayama K, *et al.* Cough challenge tests involving mechanical stimulation of the cervical trachea in patients with cough as a leading symptom. *Respirology* 2010; 15: 1244–1251.
14. Lee PC and Eccles R. Cough induction by high-frequency chest percussion in healthy volunteers and patients with common cold. *Respir Med* 2004; 98: 771–776.
15. Jones RM, Hilldrup S, Hope-Gill BD*, et al.* Mechanical induction of cough in idiopathic pulmonary fibrosis. *Cough* 2011; 7: 2.
16. Chinese Thoracic Society Asthma Consortium. Guidelines for the diagnosis and treatment of cough (2015). *Chin J Tuberc Respir Dis* 2016; 39: 323–354.
17. Mazzone SB, Chung KF and McGarvey L. The heterogeneity of chronic cough: a case for endotypes of cough hypersensitivity. *Lancet Respir Med* 2018; 6: 636–646.
18. Lavinka PC and Dong X. Molecular signaling and targets from itch: lessons for cough. *Cough* 2013; 9: 8.
19. Long L, Yao H, Tian J*, et al.* Heterogeneity of cough hypersensitivity mediated by TRPV1 and TRPA1 in patients with chronic refractory cough. *Respir Res* 2019; 20: 112.
20. Fowles HE, Rowland T, Wright C*, et al.* Tussive challenge with ATP and AMP: does it reveal cough hypersensitivity? *Eur Respir J* 2017; 49: 1601452.
21. Lee LY, Hsu CC, Lin YJ*, et al.* Interaction between TRPA1 and TRPV1: synergy on pulmonary sensory nerves. *Pulm Pharmacol Ther* 2015; 35: 87–93.
22. Xu X, Chen Q, Qiu Z*, et al.* Association of cough hypersensitivity with tracheal TRPV1 activation and neurogenic inflammation in a novel guinea pig model of citric acid-induced chronic cough. *J Int Med Res* 2018; 46: 2913–2924.
23. Canning BJ, Mazzone SB, Meeker SN*, et al.* Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs. *J Physiol* 2004; 557: 543–558.
24. Widdicombe J. Functional morphology and physiology of pulmonary rapidly adapting receptors (RARs). *Anat Rec A Discov Mol Cell Evol Biol* 2003; 270: 2–10.
25. Bonvini SJ, Birrell MA, Grace MS*, et al.* Transient receptor potential cation channel, subfamily V, member 4 and airway sensory afferent activation: role of adenosine triphosphate. *J Allergy Clin Immunol* 2016; 138: 249–261.

26. Zhang M, Wang S, Yu L, *et al.* The role of ATP in cough hypersensitivity syndrome: new targets for treatment. *J Thorac Dis* 2020; 12: 2781–2790.

27. Grace MS, Bonvini SJ, Belvisi MG, *et al.* Modulation of the TRPV4 ion channel as a therapeutic target for disease. *Pharmacol Ther* 2017; 177: 9–22.

28. Driessen AK, McGovern AE, Narula M, *et al.* Central mechanisms of airway sensation and cough hypersensitivity. *Pulm Pharmacol Ther* 2017; 47: 9–15.

29. Morice AH, Jakes AD, Faruqi S, *et al.* A worldwide survey of chronic cough: a manifestation of enhanced somatosensory response. *Eur Respir J* 2014; 44: 1149–1155.

30. Widdicombe JG. Afferent receptors in the airways and cough. *Respir Physiol* 1998; 114: 5–15.

31. Hilton EC, Baverel PG, Woodcock A, *et al.* Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point. *J Allergy Clin Immunol* 2013; 132: 847–855.

32. Dicpinigaitis PV and Alva RV. Safety of capsaicin cough challenge testing. *Chest* 2005; 128: 196–202.

33. Dicpinigaitis PV. Short- and long-term reproducibility of capsaicin cough challenge testing. *Pulm Pharmacol Ther* 2003; 16: 61–65.

34. Johansson A, Löwhagen O, Millqvist E, *et al.* Capsaicin inhalation test for identification of sensory hyperreactivity. *Respir Med* 2002; 96: 731–735.

35. Pullerits T, Ternesten-Hasséus E, Johansson EL, *et al.* Capsaicin cough threshold test in diagnostics. *Respir Med* 2014; 108: 1371–1376.

36. Lee LY, Ni D, Hayes D Jr, *et al.* TRPV1 as a cough sensor and its temperature-sensitive properties. *Pulm Pharmacol Ther* 2011; 24: 280–285.

37. O'Connell F, Thomas VE, Pride NB, *et al.* Capsaicin cough sensitivity decreases with successful treatment of chronic cough. *Am J Respir Crit Care Med* 1994; 150: 374–380.

38. Groneberg DA, Niimi A, Dinh QT, *et al.* Increased expression of transient receptor potential vanilloid-1 in airway nerves of chronic cough. *Am J Respir Crit Care Med* 2004; 170: 1276–1280.

39. Birrell MA, Belvisi MG, Grace M, *et al.* TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 2009; 180: 1042–1047.

40. Rai ZL, Fowles HE, Wright C, *et al.* The effect of pH on citric acid cough challenge: a randomised control trial in chronic cough and healthy volunteers. *Respir Physiol Neurobiol* 2018; 257: 51–54.

41. Koskela HO, Lake C, Wong K, *et al.* Cough sensitivity to mannitol inhalation challenge identifies subjects with chronic cough. *Eur Respir J* 2018; 51: 1800294.

42. Pelleg A and Hurt CM. Mechanism of action of ATP on canine pulmonary vagal C fibre nerve terminals. *J Physiol* 1996; 490: 265–275.

43. Weigand LA, Ford AP and Undem BJ. A role for ATP in bronchoconstriction-induced activation of guinea pig vagal intrapulmonary C-fibres. *J Physiol* 2012; 590: 4109–4120.

44. Abdulqawi R, Dockry R, Holt K, *et al.* P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet* 2015; 385: 1198–1205.

45. Smith JA, Kitt MM, Morice AH, *et al.* Gefapixant, a P2X3 receptor antagonist, for the treatment of refractory or unexplained chronic cough: a randomised, double-blind, controlled, parallel-group, phase 2b trial. *Lancet Respir Med* 2020; 8: 775–785.

46. Fujimura M, Kasahara K, Kamio Y, *et al.* Female gender as a determinant of cough threshold to inhaled capsaicin. *Eur Respir J* 1996; 9: 1624–1626.

47. Blanc P, Liu D, Juarez C, *et al.* Cough in hot pepper workers. *Chest* 1991; 99: 27–32.

48. Cho PSP, Fletcher HV, Turner RD, *et al.* Impaired cough suppression in chronic refractory cough. *Eur Respir J* 2019; 53: 1802203.

49. Fujiwara K, Kawamoto K, Shimizu Y, *et al.* A novel reflex cough testing device. *BMC Pulm Med* 2017; 17: 19.

50. Curtis JA and Troche MS. Handheld cough testing: a novel tool for cough assessment and dysphagia screening. *Dysphagia* 2020; 35: 993–1000.

51. Cantero-Recasens G, Gonzalez JR, Fandos C, *et al.* Loss of function of transient receptor potential vanilloid 1 (TRPV1) genetic variant is associated with lower risk of active childhood asthma. *J Biol Chem* 2010; 285: 27532–27535.

52. Smit LA, Kogevinas M, Antó JM, *et al.* Transient receptor potential genes, smoking, occupational exposures and cough in adults. *Respir Res* 2012; 13: 26.

53. Sadofsky LR, Cantero-Recasens G, Wright C, *et al.* TRPV1 polymorphisms influence capsaicin

cough sensitivity in men. *J Thorac Dis* 2017; 9: 839–840.

54. Ternesten-Hasséus E, Johansson EL and Millqvist E. Cough reduction using capsaicin. *Respir Med* 2015; 109: 27–37.

55. Slovarp LJ and Bozarth E. Altering cough reflex sensitivity with aerosolized capsaicin paired with behavioral cough suppression: a proof-of-concept study. *Ann Transl Med* 2019; 7: 7.

56. Fujimura M, Sakamoto S, Kamio Y, *et al.* Sex difference in the inhaled tartaric acid cough threshold in non-atopic healthy subjects. *Thorax* 1990; 45: 633–634.

57. Lai K, Long L, Yi F, *et al.* Age and sex distribution of Chinese chronic cough patients and their relationship with capsaicin cough sensitivity. *Allergy Asthma Immunol Res* 2019; 11: 871–884.

58. Patberg KW. The female preponderance to cough hypersensitivity syndrome: another clue pointing to the role of TRPV1 in cough. *Lung* 2011; 189: 257–258.

59. Xu S, Cheng Y, Keast JR, *et al.* 17beta-estradiol activates estrogen receptor beta-signalling and inhibits transient receptor potential vanilloid receptor 1 activation by capsaicin in adult rat nociceptor neurons. *Endocrinology* 2008; 149: 5540–5548.

60. Chen C, Gong X, Yang X, *et al.* The roles of estrogen and estrogen receptors in gastrointestinal disease. *Oncol Lett* 2019; 18: 5673–5680.

61. Jensen-Jarolim E and Untersmayr E. Gender-medicine aspects in allergology. *Allergy* 2008; 63: 610–615.

62. Millqvist E and Bende M. Capsaicin cough sensitivity is decreased in smokers. *Respir Med* 2001; 95: 19–21.

63. Dicpinigaitis PV. Cough reflex sensitivity in cigarette smokers. *Chest* 2003; 123: 685–688.

64. Dicpinigaitis PV, Sitkauskiene B, Stravinskaite K, *et al.* Effect of smoking cessation on cough reflex sensitivity. *Eur Respir J* 2006; 28: 786–790.

65. Guilleminault L. Chronic cough and obesity. *Pulm Pharmacol Ther* 2019; 55: 84–88.

66. Shi C, Liang S, Xu X, *et al.* Cough hypersensitivity in patients with obstructive sleep apnea hypopnea syndrome. *Sleep Breath* 2019; 23: 33–39.

67. Guglielminotti J, Tesniere A, Rackelboom T, *et al.* Cough reflex sensitivity is decreased in female obese patients with obstructive sleep apnea. *Respir Physiol Neurobiol* 2007; 158: 83–87.

68. Pecova R, Sojak J, Durdik P, *et al.* Relationship between cough reflex sensitivity and body mass index in children with chronic cough – a pilot study. *Physiol Res* 2020; 69: S463–S470.

69. Weinfeld D, Ternesten-Hasséus E, Löwhagen O, *et al.* Capsaicin cough sensitivity in allergic asthmatic patients increases during the birch pollen season. *Ann Allergy Asthma Immunol* 2002; 89: 419–424.

70. Ternesten-Hasséus E, Bende M and Millqvist E. Increased capsaicin cough sensitivity in patients with multiple chemical sensitivity. *J Occup Environ Med* 2002; 44: 1012–1017.

71. Wise PM, Breslin PA and Dalton P. Sweet taste and menthol increase cough reflex thresholds. *Pulm Pharmacol Ther* 2012; 25: 236–241.

72. Millqvist E, Ternesten-Hasséus E and Bende M. Inhalation of menthol reduces capsaicin cough sensitivity and influences inspiratory flows in chronic cough. *Respir Med* 2013; 107: 433–438.

73. Pecova R, Zucha J, Pec M, *et al.* Cough reflex sensitivity testing in in seasonal allergic rhinitis patients and healthy volunteers. *J Physiol Pharmacol* 2008; 59(Suppl. 6): 557–564.

74. Huang Y, Qiu Z, Wei W, *et al.* [Comparative study of cough variant asthma and cough reflex sensitivity]. *J Tongji Univ Med Sci* 2011; 32: 68–72.

75. Chen R, Luo W, Liu C, *et al.* A pilot study on normal reference value of capsaicin cough sensitivity. *Int J Respir* 2013; 33: 1334–1337.

76. Glover GH. Overview of functional magnetic resonance imaging. *Neurosurg Clin N Am* 2011; 22: 133–139, vii.

77. Ando A, Smallwood D, McMahon M, *et al.* Neural correlates of cough hypersensitivity in humans: evidence for central sensitisation and dysfunctional inhibitory control. *Thorax* 2016; 71: 323–329.

78. Ryan NM, Vertigan AE and Gibson PG. Chronic cough and laryngeal dysfunction improve with specific treatment of cough and paradoxical vocal fold movement. *Cough* 2009; 5: 4.

79. Bucca CB, Bugiani M, Culla B, *et al.* Chronic cough and irritable larynx. *J Allergy Clin Immunol* 2011; 127: 412–419.

80. Tatar E, Öcal B, Korkmaz H, *et al.* Postviral vagal neuropathy: what is the role of laryngeal electromyography in improving diagnostic accuracy? *J Voice* 2015; 29: 595–599.

81. Giraldo-Cadavid LF, Agudelo-Otalora LM, Burguete J, *et al.* Design, development

and validation of a new laryngo-pharyngeal endoscopic esthesiometer and range-finder based on the assessment of air-pulse variability determinants. *Biomed Eng Online* 2016; 15: 52.

82. Giraldo-Cadavid LF, Burguete J, Rueda F, *et al.* Accuracy of a Laryngopharyngeal Endoscopic Esthesiometer (LPEER) for evaluating laryngopharyngeal mechanosensitivity: a validation study in a prospectively recruited cohort of patients. *Dysphagia* 2018; 33: 15–25.

83. Giraldo-Cadavid LF, Burguete J, Rueda F, *et al.* Reliability of a laryngo-pharyngeal esthesiometer and a method for measuring laryngo-pharyngeal mechano-sensitivity in a prospectively recruited cohort of patients. *Eur Arch Otorhinolaryngol* 2017; 274: 2861–2870.

84. Vertigan AE, Bone SL and Gibson PG. Development and validation of the Newcastle laryngeal hypersensitivity questionnaire. *Cough* 2014; 10: 1.

85. Morice AH, Faruqi S, Wright CE, *et al.* Cough hypersensitivity syndrome: a distinct clinical entity. *Lung* 2011; 189: 73–79.

86. Huang Y, Yu L, Xu XH, *et al.* [Validation of the Chinese version of Hull airway reflux questionnaire and its application in the evaluation of chronic cough]. *Zhonghua Jie He He Hu Xi Za Zhi* 2016; 39: 355–361.

87. Wen S, Wang S, Niu S, *et al.* Sensitivity and specificity of combination of Hull airway reflux questionnaire and gastroesophageal reflux disease questionnaire in identifying patients with gastroesophageal reflux-induced chronic cough. *Ann Transl Med* 2020; 8: 1564.

88. Jones R, Junghard O, Dent J, *et al.* Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. *Aliment Pharmacol Ther* 2009; 30: 1030–1038.

89. Johansson EL and Ternesten-Hasséus E. Reliability and validity of the Swedish Version of the Hull Airway Reflux Questionnaire (HARQ-S). *Lung* 2016; 194: 997–1005.

90. Zhang M, Chen Q, Dong R, *et al.* Prediction of therapeutic efficacy of gabapentin by Hull Airway Reflux Questionnaire in chronic refractory cough. *Ther Adv Chronic Dis* 2020; 11: 2040622320982463.

91. Ryan NM, Birring SS and Gibson PG. Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial. *Lancet* 2012; 380: 1583–1589.