Evaluation methods and influencing factors of cough sensitivity

Hao Mei*, Wenhua Gu*, Linxin Ran*, Siwan Wen, Li Yu and Xianghuai Xu

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

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 stimuli, 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, Morice6 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
sensitivity may be an important strategy for the treatment of refractory cough in the future.\textsuperscript{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.\textsuperscript{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.\textsuperscript{9} The concentration of cough-inducing stimulant is used to determine cough reflex sensitivity,\textsuperscript{9} which mainly reflects the cough reflex mediated by specific peripheral chemoreceptors.\textsuperscript{10} In addition to chemical stimulation, mechanical stimulation can also induce cough directly,\textsuperscript{11–15} reflecting the cough reflex mediated by mechanoreceptors.\textsuperscript{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.\textsuperscript{16}

**Mechanisms of cough sensitivity assessments**

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 A\textsubscript{\textdelta} fibers and nonmyelinated vagal afferent C-fibers.\textsuperscript{18} Cough sensitivity mediated by peripheral cough receptors can be evaluated by stimulating these nerve endings.\textsuperscript{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.\textsuperscript{21,22}

Vagal afferent A\textsubscript{\textdelta} fiber endings are mechanoreceptors that are sensitive to mechanical stimulation. They are divided into pulmonary stretch receptors and Widdicombe cough receptors.\textsuperscript{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.\textsuperscript{23} Studies have shown that TRPV4 may activate A\textsubscript{\textdelta} fibers by mediating ATP.\textsuperscript{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.\textsuperscript{27} Bonvini et al. used an \textit{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 regulate 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 Eccles12 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 Eccles14 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, cough sensitivity is most commonly assessed by the lowest concentration of stimulations which cause 2 (C2) or 5 (C5) coughs. Capsaicin is the most commonly used stimulant. Several studies have demonstrated that capsaicin is a useful substance for cough challenge test. The safety, reproducibility, and dose-dependency of capsaicin cough challenge test have been demonstrated. And for patients with chronic cough and sensory hyperreactivity, Pullerits et al. 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. Studies have shown that patients with chronic cough are more sensitive to capsaicin. After treatment, cough sensitivity to inhaled capsaicin is reduced as cough symptoms relieved. The increased cough sensitivity may be associated with elevated TRPV1 expression on airway nerves of patients with chronic cough.

Mannitol induces cough through osmotic stimulation of the airway. Koskela et al. 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. 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. 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. 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. 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. Although the safety of capsaicin has been confirmed, the aerosol stimulant particles may irritate both subjects and operators.

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, 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 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 processes, Cho et al. developed a CS 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 Troche50 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                           | 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. |
|                        |                        |           |      | Genetic polymorphism analysis                |                                                                          |
|                        |                        |           |      | Cell functional assay                        |                                                                          |
| 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 single nucleotide polymorphism analysis |                                                                          |
|                        |                        |           |      | Cell functional assay                        |                                                                          |
| TRPV1 polymorphism      | Sadofsky LR            | 52        | 2017 | Capsaicin cough challenge test               | 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. |
|                        |                        |           |      | Genetic polymorphism analysis                |                                                                          |
|                        |                        |           |      | Cell functional assay                        |                                                                          |
| 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               | In healthy individuals and patients with chronic cough, capsaicin and AITC cough sensitivity is higher in women than in men.          |
|                        |                        |           |      | AITC cough challenge test                    |                                                                          |
| 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                 | Jean Guglielminotti    | 66        | 2007 | Citric acid cough challenge test             | Cough sensitivity is reduced in female obese OSAH patients.                                                                         |

(Continued)
| Factor                | First Author | Reference | Year | Methods                                      | Results                                                                                                                                                                                                 |
|----------------------|--------------|-----------|------|----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| OSAHS                | Shi CQ       | 65        | 2019 | Capsaicin cough challenge test               | 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               | CVA may be associated with normal or increased cough sensitivity, which may be related to neutrophil infiltration and activation.                                                                                                                        |

AITE, 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. Capsaicin exposure may also reduce cough sensitivity. Blanc et al. 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 (10^-6 M capsaicin) and a lower cough threshold (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., they found that after taking capsules with capsaicin, cough sensitivity. In a study by Ternesten-Hasséus et al., exposure and other factors contributed to decreased smoking. The researchers believed that capsaicin might reduce cough sensitivity. Blanc et al. thought that 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. Research by Dicpinigaitis et al. 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.

OBesity may also affect cough sensitivity. Epidemiological surveys show that obesity, especially abdominal obesity, is a risk factor for chronic cough and certain conditions, suggesting that obese individuals may have higher cough sensitivity. In our study, Shi et al. capsaicin cough challenge test results indicate that patients with normal weight and obstructive sleep apnea syndrome (OSAHS) have higher cough sensitivity than do normal subjects, but in a study by Guglielminotti et al., 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. 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. For patients with multiple chemical sensitivity, their capsaicin cough sensitivity also increased. 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 and inhalation of menthol vapor 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.75 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) 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. 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 > 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. 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. 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.26 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. Therefore, laryngeal hypersensitivity is an indirect indicator of cough hypersensitivity.

Bucca et al. 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. 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. 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. 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. The high intra- and inter-rater reliability and accuracy of LPEER for evaluating laryngopharyngeal sensitivity have been proved. Studies have shown that in patients with laryngeal irritation, the LART is slightly increased and the CRT and GRT are decreased. 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. 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. 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. 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. further demonstrate that the HARQ is a valuable tool for predicting GERD and is more effective when combined with the GERD questionnaire (GerdQ). Also, Johansson et al. 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. Gabapentin has been shown to reduce severity of cough, providing further evidence of central sensitization. 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, 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
Hao Mei: Conceptualization; Investigation; Writing – original draft; Writing – review & editing.
Wenhua Gu: Conceptualization; Writing – original draft; Writing – review & editing.
Linxin Ran: Conceptualization; Writing – original draft; Writing – review & editing.
Siwan Wen: Conceptualization; Writing – review & editing.
Xianghuai Xu: Conceptualization; Funding acquisition; Project administration; Supervision; Writing – original draft; Writing – review & editing.
Li Yu: Conceptualization; Funding acquisition; Project administration; Supervision; Writing – original draft; Writing – review & editing.

Conflict of interest statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the National Natural Science Foundation of China (grant nos. 82070102 and 81770097); the Project of Science and Technology Commission of Shanghai Municipality (grant no. 20ZR1451500); the Fund of Shanghai Youth Talent Support Program; the Fund of Shanghai Municipal Health Commission for Excellent Young Scholars (grant no. 2018YQ01); the Shanghai Sailing Program (grant no. 19YF1444100).
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