Methods of Cough Assessment and Objectivization

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Summary

Cough is one of the most important airway defensive reflexes aimed at removing foreign particles or endogenously produced materials from the airways and provides protection against aspiration. Generally considered, cough is a vital physiological defensive mechanism for lung health. However, in case of cough dysregulation this reflex can become pathological and leads to an adverse influence on daily life. Therefore, it is necessary to effectively evaluate the severity of cough for its diagnosis and treatment. There are subjective and objective methods for assessing cough. These methods should help describe the heterogeneity of cough phenotypes and may establish better treatment by monitoring response to nonpharmacological or pharmacological therapies. It is important to keep in mind that the clinical assessment of cough should include both tools that measure the amount and severity of the cough. The importance of a combined subjective and objective evaluation for a comprehensive assessment of cough has been advocated in the guidelines of the European Respiratory Society on cough evaluation. This review article provides an overview of subjective and objective methods for assessing and monitoring cough in children and adults comparing to animal models.

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Introduction

During the last 2 decades, our understanding of cough management and mechanisms has developed substantially through combination of science with clinical practice and the pharmaceutical industry. This helps to develop new diagnostic and therapeutic strategies, and several clinical guidelines have been published on the management of cough. Although many of them led to specific methods of cough evaluation, cough management remains a challenge for the clinician [1]. Many patients suffer from cough sensitivity which is an important mechanism of chronic cough [2]. Cough sensitivity is a pathological intensity reaction of the cough reflex to chemical or mechanical stimuli which can trigger sensitive vagal afferent nerves innervating lungs and airways [3]. Patients with cough sensitivity often complain of excessive sensitivity to inhalation of environmental irritants such as cold air or perfumes which manifests as throat irritation and an urge to cough. Observations of these patients have led to the concept of cough hypersensitivity syndrome as a diagnosis [4]. According to Morice, the cough hypersensitivity syndrome can be applied to most cases of chronic cough [5]. Chronic cough can be caused by cough variant asthma, upper airway cough syndrome, gastroesophageal reflux - related cough or eosinophilic bronchitis. These may be different clinical subtypes of cough hypersensitivity syndrome [2]. Evaluation of cough sensitivity plays a notable role in understanding the mechanism of chronic cough, revealing the underlying cause, and also monitoring response to potential treatment [3].

Assessment of Cough

There are many reasons for measuring and evaluating cough, not only in revealing cough etiology, but also in disease prognosis, respiratory disease recovery monitoring, or clinical evaluation of neurological condition. An accurate assessment of cough can also improve clinical cough management in patients refractory to treatment or those with unknown underlying cause. In patients with cough as a primary disorder, the initial focus is to identify the underlying cause or triggering factors and then provide causal treatment in these patients. The cough assessment can be performed either from the patient perspective or from the clinician or the researcher's perspective. Information obtained from both can be mutually useful and complementary [6]. The clinical assessment of cough should include both tools that measure the amount and severity of the cough. The evaluation of cough severity is important for evaluating the therapy and includes cough frequency, intensity, symptoms severity and impact on life [4]. Due to the clinical importance of cough evaluation, subjective and objective methods have been developed (Table 1). Many of them are applicable and widely used in the clinical practice and research [3].

Subjective Methods for Assessing Cough

Cough is the most common symptom for patients to seek medical advice. About 10% of the global population suffers from chronic cough which is associated with considerable physical and psychological morbidity [6]. In clinical practice, the evaluation of cough should include an evaluation of the severity and subjective impact of chronic cough on the patient. The ideal subjective method for assessing cough should be repeatable, responsive to clinical improvements, but not clinician dependent. In general, it should be self-administered with ease [6,7]. The two main categories of cough subjective evaluation tools are those that focus on the effects of the cough on life quality (cough-related quality of life) and cough severity. The most common and widely used methods for evaluating cough severity are Visual analogue scale (VAS), Cough symptom score (CSS), the Cough Severity Diary (CSD) and for the life quality of patients there are The Leicester Cough Questionnaire (LCQ) and the Cough Quality of Life Questionnaire (CQLQ). A semi-objective cough evaluation method named Twenty-four-hour cough counting is considered to be the best method to quantify cough. However, this method is time-consuming and labour-intensive, thus it is confined only to the research setting [8]. Studies have shown that chronic cough significantly affects patients' quality of life in terms of secondary social, physical, and psychological effects. Psychological and social effects include low mood, anxiety, and excessive self-awareness in public situations whereas physical symptoms include fatigue, syncope, chest pain, urinary incontinence, or sleep disturbances [7].

Cough Severity Visual Analogue Scale (VAS) and Numeric Rating Scales (NRS)

The visual analogue scale (VAS) is probably the most widely used method for subjective evaluation of cough due to its simplicity, easy accessibility, and responsiveness to symptom changes. It is often used as an indicator in comparative studies of therapeutic effects [9]. VAS is very simple to administer and consists of a linear scale from 0 to 100 mm on which higher scores indicate higher severity [6]. It can also be used with a calibration of 0, 1, 2 to 10 cm where 0 indicates asymptomatic and 10 represents the most serious cough [7]. The patient marks a point on a straight line based on his self-perception. The distance between the mark point and the starting point is measured to give a score reflecting the severity of cough in this patient [10]. As a responsive outcome, VAS reduction $\geq$ 30 mm was estimated as a clinically meaningful change threshold [11]. A minimal important difference (MID) has been reported for acute cough (17 mm), but not for chronic cough. Intraclass correlation coefficient (ICC) is a widely used reliability index. It is a value between 0 and 1, where values below 0.5 indicate poor reliability, between 0.5 and 0.75 moderate reliability, between 0.75 and 0.9 good reliability, and any value above 0.9 indicates excellent reliability [11]. A good reliability of VAS in chronic cough (ICC=0.604) and chronic obstructive pulmonary disease (COPD) (ICC=0.87) was reported. On the other hand, its correlation with cough frequency varied in different studies and different timepoints [8]. In clinical practice, VAS is widely used in the subjective evaluation and longitudinal assessment of acute or chronic cough [12]. It is an easy and simple method which is less affected by language than other methods. However, the evaluation may not be accurate due to strong subjective patient influence [10]. Another recommended cough severity assessment method is the NRS (0-10 point). When using VAS or NRS a standardized consensus must be made [13].

Cough symptom score (CSS)

CSS was first proposed by Hsu et al. in 1994, and its reliability and treatment response have been confirmed [14]. The CSS is a simple, short, and practical tool which has been adapted and widely used after translation and amendment in many countries, such as Korea and China [15]. It consists of two-part referring to daytime and night-time symptoms. Each question score ranges from 0 to 5, and the total score ranges from 0 (no cough) to 10 (most severe cough). Scoring is based on the frequency, intensity and influence of cough on daily activities and sleep [8]. According to the Guidelines for the Diagnosis and Treatment of Cough developed by the Respiratory Branch of Chinese Medical Association the simplified cough score (SCS) was recommended as a tool for evaluating the severity of cough [10]. The SCS has evolved from the CSS and makes it simpler to use due to grading cough symptoms from 0 to 3, and the total scores are 0 to 6. It is highly correlated with CSS with high repeatability over 3 days (day: ICC=0.90; night: ICC=0.89). Thus, the SCS and CSS can be used as effective tools for the clinical assessment of cough severity [10].

Cough Severity Diary (CSD)

The cough severity diary is a simple 7-item daily diary with an 11-point Likert scale for each item [6]. The CSD is used for recording the scores of items that can quantify cough severity, response to treatment, and also progression of subacute and chronic cough. Patients are asked to rate their cough severity in three domains: cough frequency (three items), cough intensity (two items), and impact of cough on life and sleep (two items). Responses to these items are recorded on an 11-point scale ranging from 0 to 10. Patients recall their cough experience within the last 24 hours and then score their cough on a scale. The total score of the CSD is calculated by averaging the seven items. Higher scores indicate a higher severity [16]. Compared to VAS, the CSD has more validation data available, and unlike a single-item VAS, the CSD can be used to determine which aspect of cough severity was affected by treatment [4]. MID definition for CSD is a threshold of ≥1.3-point reduction on the total and subscale scores. CSD was also utilized and proven to be a meaningful endpoint in investigating the antitussive efficacy of gefapixant [17].

Cough Severity Index (CSI)

The CSI consists of 10 simplified questions statistically developed from a 49-item cough-specific questionnaire. It has been validated and proven to be a responsive outcome measure with high internal consistency (0.928) and test-retest repeatability (r=0.83). A moderate correlation (r=0.60) was found between CSI and CQLQ [8]. CSI has been used in evaluation of the treatment efficacy in several cough studies [8,18].

Multidimensional Cough Index (MCI)

The MCI is a simple and practical scale composed of nine items to measure cough intensity, frequency, physical impact, psychosocial impacts, and sputum characteristics. The first four components were scaled with a range of 0 to 20 [8]. The MCI has been validated and significantly correlated with VAS frequency (r=0.651), VAS intensity (r=0.543), and LCQ (r=−0.824). Its reliability was reported (ICC=0.779). The MCI ≥4 could distinguish respiratory patients from healthy subjects, with a sensitivity of 80% and a specificity of 85% [19].

Cough Evaluation Test (CET)

The CET is a newly developed, short, simple, patient-completed 5-item test that involves the dimensions of cough severity, social impact and psychological effect. Each item is scaled using a 1–5 points Likert scale. CET has been verified as a reliable, valid, and responsive tool with good repeatability (ICC =0.84), MID definition 2.0, and strong correlation with LCQ (r=−0.74), CSS (r=0.71) and VAS (r=0.70) [8,20].

McMaster Cough Severity Questionnaire

The McMaster cough severity questionnaire is a newly developed cough symptom severity questionnaire for patients with refractory chronic cough. This method provides 43 items addressing the following domains: urge to cough sensations (subdomains: frequency and intensity) and cough symptoms (subdomains: frequency, control, bout duration, intensity, and associated features) [21]. Currently, this method is a conceptual framework and further studies are required to simplify this questionnaire [8].

Specific Cough Questionnaires

Cough questionnaires are mainly used to monitor the impact of cough on quality of life. Compared to cough scales, questionnaires provide better capture of cough impact on patients' life, thus a structured and standardised approach to quantifying health status is provided. They are also well validated and highly responsive to change [4]. There are various types of cough questionnaires. In this

review, the most widely used questionnaires for adult patients are mentioned.

Leicester Cough Questionnaire (LCQ)

One of the most widely used, validated, reproducible, and accurate questionnaire is LCQ. It has also been recommended by ERS cough guidelines [22]. The LCQ consists of 19 items including 8 physical, 7 psychological, and 4 social items with a 7-point Likert scale for each item. Evaluation of the regional score includes a sum of items score in each area and the total score is the sum of regional score [10]. The total possible scores range from 3 to 21, where lower scores indicate a serious impact of cough on the health status of the patient [7]. Thus, higher scores indicate better quality of life [8]. LCQ has been translated into a wide range of languages [10] and validated separately in acute cough, COPD, sarcoidosis, cystic fibrosis, noncystic fibrosis bronchiectasis, idiopathic pulmonary fibrosis, and tuberculosis. Furthermore, LCQ is now routinely used in assessing the effectiveness of cough interventions, such as proton pumps inhibitors, neuromodulators or behavioural cough suppression therapy, and physiotherapy, speech and language therapy [8]. In patients with chronic cough, cough frequency significantly correlated with LCQ scores. The LCQ also strongly correlates with VAS (r=−0.72). The MID of the total LCQ score has been established to be 2.0 in acute cough and 1.3 for chronic cough, where the MIDs for domains are as follows: physical 0.2 (0.8), social 0.2 (1.1) and psychological 0.8 (1.5) [8,23].

Cough Quality of Life Questionnaire (CQLQ)

The ERS cough guidelines also recommended the CQLQ [22]. The CQLQ is a 28-item questionnaire with a 4-point Likert scale for each item in six domains: physical complaints, extreme physical complaints, psychosocial issues, personal safety fears, emotional state, and functional abilities [6]. The sum of the scores for 28 items is the total score, where lower scores represent a better quality of life [10]. The CQLQ has been validated in acute and chronic cough, COPD, and idiopathic pulmonary fibrosis with high internal consistency, repeatability over 2 weeks, responsiveness in acute and chronic cough, and MID 13 units for chronic cough [7].

Chronic Cough Impact Questionnaire (CCIQ)

The CCIQ is a 16-items questionnaire with structure of four dimensions: sleep/concentration, social relationship, mood, and daily life impact. Each item is self-evaluated on a 5-point Likert scale. Numeric values for responses to the CCIQ items are converted to 0–100 points, where 100 represents the worse quality of life [24]. CCIQ has been a valid tool for chronic cough with high satisfactory level of internal consistency for sleep/concentration and relationship, and acceptable levels for mood and for daily life impact. The CCIQ also has good reliability (r=0.67 to 0.88) and responsiveness to treatment improvement [8,24].

Cough Assessment Test (COAT)

The COAT is a short, patient-completed questionnaire. It consists of five items, including cough frequency, daily activity, sleep disturbance, fatigue, and cough hypersensitivity. Each item scores on a 0–4 scale and higher scores indicate worse impact on life quality. COAT has been validated in the population of South Korea and showed good repeatability, reliability and validity. COAT also well correlated with LCQ (r=−0.71 to −0.81) and NRS (r=0,62 to 0,82) and MID has been established to 2.0 of 20 [25].

Advantages and Disadvantages of Subjective Methods for Cough Evaluation

There are various subjective methods for cough evaluation with quite different clinical application. Cough scales are simple, easy to use and responsive to symptom changes in patients. They are also less affected by language than other methods [10]. Compared to cough scales, questionnaires provide better capture of cough impact on patients' life, thus a structured and standardised approach to quantifying health status is provided [8]. However, all subjective methods can be easily affected by subjective factors such as emotions, expectations or attention to symptoms which often leads to evaluation error. In clinical practice, the severity of cough cannot be evaluated only with subjective methods, but objective methods are also needed to monitor cough and its response to treatment [8,10].

Objective Methods for Assessing Cough

Objective methods of cough assessment are mainly focused on monitoring the cough frequency and cough intensity. Another objective method for cough evaluation can be an assessment of the cough reflex sensitivity [8,26]. Currently, the assessment of cough frequency is considered as the gold standard for the objective assessment of cough [22]. However, cough

frequency is a semi-objective measure because of the impossibility to differentiate between spontaneous and voluntary cough [26]. The addition of cough intensity monitoring may be valuable as another determinant of cough severity, but in clinical practice it has been limited by the lack of validation data against physiological measures of cough intensity in the ambulatory setting [26]. Moreover, there are other tools for objective assessment of cough in addition to those listed above (Table 2). These tools are intended for understanding the underlying cause and are used mainly in research rather than clinical evaluation. Examples include analysis of neuronal mechanisms from animal models, functional magnetic resonance imaging for investigating central neurological pathways, and tests of potential genetic polymorphism on cough. Analysis of the bronchoalveolar lavage sample, induced sputum cells counting or fraction of exhaled nitric oxide are tests used in the evaluation of airway inflammation [6].

Cough Frequency Monitors

There is a necessity of recording cough events over a time period for objective evaluation of cough associated with different diseases and for the assessment of the treatment efficacy for chronic cough [22]. Cough frequency can be assessed by specific cough monitor systems. These monitors can assess cough frequency during daytime, nighttime or 24 hours, and it is recommended that measurements should last for at least 24 hours due to diurnal variations in cough frequency [4,6]. The ideal cough monitor system should be small, compact, minimally intrusive to the patient, and capable of recording for at least 24-hour period. The ideal device should also have a high sensitivity and specificity in order to detect all cough sounds and distinguish them from other noise or respiratory movements [8]. There is currently no commercially available cough frequency monitor for clinical use that analyzes cough in real time and without technician input, thus, the measurement of cough frequency has remained predominately a research tool [6]. In this review, we discuss only two most widely used cough monitor systems – the Leicester Cough Monitor and VitaloJAK™. Both systems are now well established in clinical research with good validation demonstrated [26]. Other cough monitors include the Hull Automatic Cough Counter, the Cayetano Cough Monitor and the LEOSound-system (Table 1). Smartphone-based cough detection system is an advanced technique of cough counting, used mainly during coronavirus disease pandemic [8].

Leicester Cough Monitor (LCM)

The Leicester Cough Monitor is an automated sound-based ambulatory cough monitor comprising a commercially available portable battery-operated digital sound recording device and a flip-collar microphone. It was developed and initially validated by Birring et al. in 2008 [27]. The LCM records sounds for 24 hours or longer, and the recorded data are subsequently analyzed by an automated algorithm to identify cough sounds and distinguish them from noncough noises [27,28]. Human operator input takes approximately 5 minutes per 24-hour recording and is required only for calibration to improve the specificity of the device [29]. Cough was defined as an individual explosive sound no matter whether it occurred as a single event or in a cluster. The reported sensitivity and specificity of LCM were 91% and 99% for the classification of cough events with a false positive rate of 2.5 events/hour. The accuracy of manual and automated cough counts appeared similar [8]. The Leicester Cough Monitor can record consecutive data for more than 24 hours (up to 4 days) and has been successfully used in a number of clinical and pharmacological studies. The LCM is effective in measuring cough frequency in acute cough, COPD, sarcoidosis, bronchiectasis, or tuberculosis including the investigation of healthcare use and costs in chronic cough, post-exercise cough in asthma and cough patterns in asthma and non-asthma, and chronic cough in patients with vocal cord dysfunction [6,8]. A retrospective study from 2021 provided the prospects of the feasibility and clinical utility of the LCM in the outpatient clinical setting, in which cough monitor was responsive to intervention and claimed to identify different diseases by the cough frequency and pattern [30].

VitaloJAK™

The VitaloJAK™ is a semi-automated cough monitoring system, developed by the collaboration between clinical academics and Vitalogragh, and is currently the only system approved for use in clinical trials of investigational medicinal products [31]. It consists of digital sound recording device (with a free field lapel microphone and a contact microphone attached to the upper sternum of chest wall), a web-based portal for data transfer, tracking, storage, and a digital signal processing algorithm to filter from a 24-hour recording and only retain possible cough sounds [8,29]. The system is able to record for 24 hours, and the resulting audio recordings are processed and compressed by the VitaloJAK™ software algorithm via removing noncough noises and all silent

periods. These recordings are analyzed and coughs are counted by experienced human operators listening to the recordings. Each record lasts approximately 1.5 hours per 24-hour monitoring period and audio-visual display is used to cough detection [29]. A recent study supported the sensitivity and efficiency of VitaloJAK™ to measure cough frequency across a range of diagnoses and age groups without being influenced by cough numbers [31]. The VitaloJAK™ has been also used in many clinical studies including asthma, COPD, chronic cough, pulmonary and cystic fibrosis [29]. The usefulness of this tool has been proven in the antitussive efficacy evaluation of GSK2339345 (a novel sodium channel inhibitor), GSK2798745 (the selective TRPV4 channel blocker), lesogaberan (a novel peripherally acting GABA$_B$ agonist), eliapixant (a P2X3 receptor antagonist) and gefapixant (a P2X2/3 receptor antagonist) in refractory chronic cough [8].

Comparison of Leicester Cough Monitor and VitaloJAK™

The LCM and VitaloJAK™ can record data continuously for 24 hours with the LCM capable of doing so for up to 4 days [6]. However, these systems differ in their approach to cough detection. The Leicester cough monitor is largely automated, thus it requires significantly less operator time. On the other hand, VitaloJAK™ may have greater accuracy due to manual assessment of cough recordings by experienced operators, but this claim is difficult to quantify from published data [4]. The accuracy of automated cough monitors is established by comparison with manually counted recordings [27]. Furthermore, VitaloJAK™ has been tested and used successfully in children, although the LCM also could probably undergo the validation process to demonstrate its use in children [32]. The VitaloJAK™ recording system was designed specifically for cough sounds, whereas the audio recording device of the LCM was developed primarily for recording speech. The LCM contact microphone has the disadvantage of being highly sensitive to noise from movement artefact, thus it is more likely to overestimate cough counts if other individuals in the patient´s own environment are also coughing [29]. The LCM is smaller and lighter, making it potentially more practical and acceptable to the wearer. However, it is also easier to remove and replace than VitaloJAK™, so there is an assumption that wearer might potentially remove the system during a recording period. This can be more easily recognised with the VitaloJAK™ because of lack of recorded data from the contact microphone and with recording analysis by human operators [29].

Cough Intensity Assessment

Assessment of cough intensity is as important as cough frequency to evaluate the impact on the patients' quality of life. Although the assessment of cough frequency is valuable, it is also unable to differentiate the cough characteristics of each patient [33]. There is a possibility that measuring the intensity of cough might help to identify patients with the most infectious diseases, such as tuberculosis and other respiratory infections [34]. Cough intensity assessment methods include cough exspiratory flow, electromyography, and cough sound amplitudes. The standart for measuring cough exspiratory air flow is limited by the requirement of pneumotachography. However, this method is restricted to the laboratory use which caused interest in portable spirometers or peak exspiratory flow meters [35]. Nowadays, there is no standardized portable device or method for measuring the intensity of cough which could be very valuable for routine clinical applications [6]. Electromyograph (EMG) studies focus on cough intensity assessment by using noninvasive surface electrodes attached to exspiratory respiratory muscles of the abdominal wall. According to 1997 study, EMG data of the abdominal wall appear to correlate with cough expiratory flow rates, expiratory volume, and cough sound amplitude [36]. The disadvantages of the EMG method are equipment size, and cardiac or other muscles contamination of EMG signals which make electromyography only a research tool [37]. The other approaches for measuring cough intensity could be optoelectronic plethysmography, cough sound amplitude, or a combination of esophageal and gastric pressure transducers to measure transdiaphragmatic pressures. However, these methods require further validation and for now remain restricted to the research setting [6].

Assessment of the Cough Reflex Sensitivity (CRS)

The evaluation of cough reflex sensitivity is a widely and commonly used technique in research and clinical practice. Cough can be elicited by chemical or mechanical stimulation of cough receptors and then compared by stimulus intensity or response to irritants [3]. Mechanical stimulation consists of airway vibrations, chest percussion, and stimulation of the Arnold nerve reflex (Fig. 1). However, only a few studies deal with the assessment of cough reflex sensitivity by mechanical stimulation. In general, there is no established threshold

for this method and the mechanical stimulation validity needs to be explored further, too [2]. On the other hand, chemical stimulation is well described and commonly used. It is based on inhalation of a tussigenic aerosol that is easily delivered to the central airways, where the sensory nerve terminals are denser and cough is elicited by inhalation. This method is also known as the cough challenge test [4]. The two most widely used tussive agents in cough challenge testing are capsaicin and citric acid [38]. Others include tartaric acid, ultrasonically nebulised distilled water, mannitol or acetic acid (Fig. 1). A 2017 study has shown that inhaled adenosine triphosphate can also be classified as a tussive agent suggesting that ATP-related cough challenge tests may have a broad research value [2,39]. Differences between capsaicin and citric acid are mentioned in Table 3.
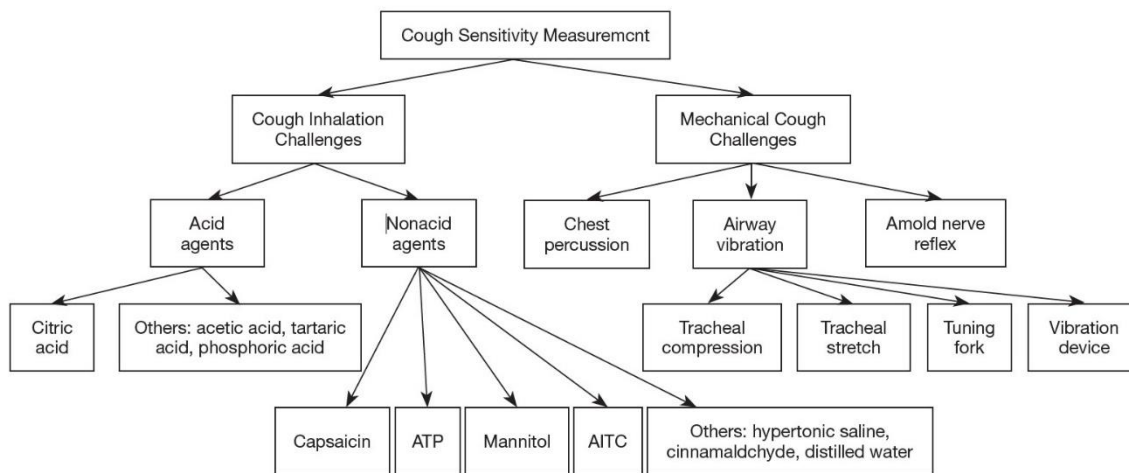

Fig. 1. Methodologies of cough sensitivity measurement [3]

Table 1. Subjective and objective methods for cough assessment [4,8]

Subjective Methods		Objective Methods
Cough Scores	**Specific Cough Questionnaires**	**(Cough Counting)**
Visual Analogue Scale (VAS) and Numeric Rating Scales (NRS)	Leicester Cough Questionnaire (LCQ)	Leicester Cough Monitor (LCM)
	Cough Quality of Life Questionnaire (CQLQ)	The VitaloJAK™
Cough Symptom Score (CSS)	Chronic Cough Impact Questionnaire (CCIQ)	The Hull Automatic Cough Counter (HACC)
Cough Severity Index (CSI)	Cough Assessment Test (COAT)	The Cayetano Cough Monitor (CayeCoM)
Cough Severity Diary (CSD)		The LEOSound-system
Multidimensional Cough Index (MCI)		Mobile device technologies

Table 2. Other tools for objective cough assessment [6]

| Parameters	Common instrument
Airway inflammation	Bronchoalveolar lavage
	Induced sputum cell counts
	Exhaled nitric oxide fraction
Peripheral neural pathways	Animal models
Central pathways	Functional neuroimaging
Genetic predisposition	Genotyping (peripheral blood or saliva)

Table 3. Comparison of capsaicin and citric acid [3]

	Capsaicin	**Citric Acid**
Origin	Non-acid agent	Acid agent
Receptors	TRPV1	Unknown (TRPV1, TRPA1, TRPV4)
Main Utilization	Cough inhalation challenge	Pharmacological studies of potential antitussive medication

Inhalation methods in cough challenge testing are divided into single-dose and dose-response challenge. A single concentration of tussive agents is administered to the airways in the single-dose inhalation challenge which is simple and time-saving and has no severe side effects. On the other hand, it is necessary to determine the cough threshold in dose-response challenge. The cough threshold can be defined as the lowest concentration of an agent capable of eliciting cough in two challenges with a 30-minute interval. The most commonly used cough challenge end points are C2 and C5 determined by the lowest concentration of tussive agent inducing at least two or five coughs, respectively [38]. C5 is the preferred end point in the cough challenge test due to its better short-term reproducibility and lower susceptibility to subjective factors. Furthermore, C5 better reflects the involuntary sensitivity of cough to tussive agents than C2 [3].

Cough challenge testing can be potentially used as a biomarker of cough hypersensitivity. CRS has been higher (with a reduction in C5) during upper respiratory tract infection than during subsequent recovery in healthy individuals, correlating with self-reported cough symptoms. Also, during COPD exacerbations CRS increases in comparison to periods of stable disease. Furthermore, cough challenge tests have given support to the concept of cough phenotypes, whereby excessive cough in different contexts or diseases may be associated with different patterns of response to a panel of tussive agents [40].

Disadvantages of CRS as it is currently measured are wide variability amongst individuals with chronic cough, and overlap with measurements from healthy subjects. However, recent re-analysis of data from a previous study of capsaicin challenges in patients with refractory chronic cough and healthy subjects suggested a specific threshold for C5 of 29 mmol/L as providing relatively high sensitivity and specificity (of 72% and 88%, respectively) for separating the two groups [41]. On the other hand, this analysis needs confirmation with new data, perhaps involving variations in the methods or equipment currently employed for measuring CRS [40].
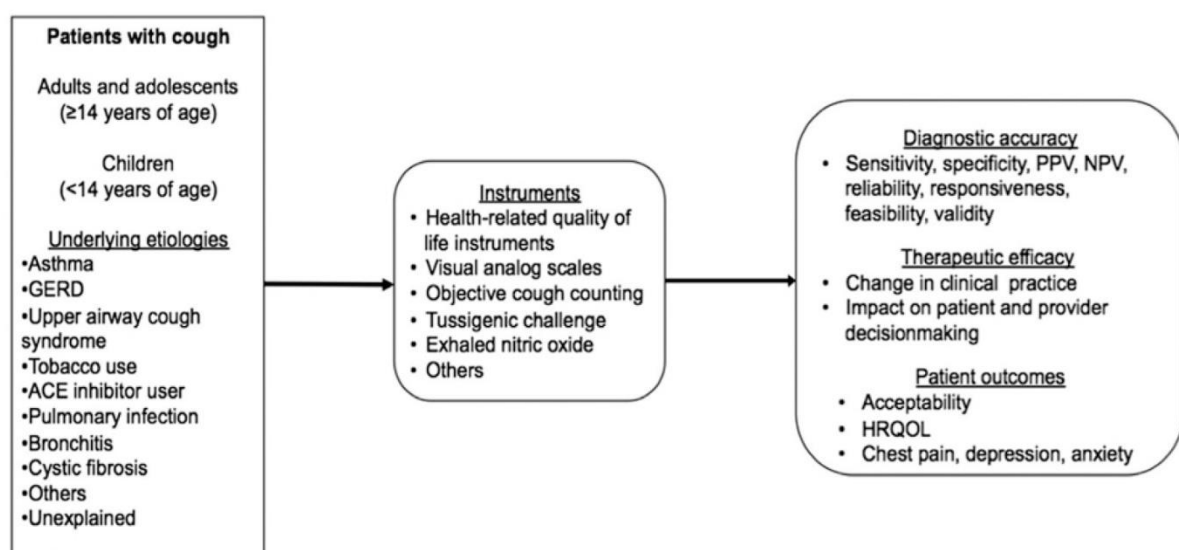
Fig. 2. Cough Assessment Methods in Children and Adults [13].
HRQOL = health-related quality of life; NPV = negative predictive value; PPV = positive predictive value

Methods of Cough Objectivization in Children

Cough is a natural process that protects the airway. However, when it is dysregulated it can become chronic and lead to patient distress and increased healthcare utilization [42]. According to recent guidelines, cough is considered chronic when it is present longer than 8 weeks in adults and 4 weeks in children [22]. In comparison, chronic cough in children is different from that in adults due to differences in the airway morphology, a higher degree of vulnerability to noxious insults, reduced control of the cough reflex and differences in maturation of the neurological and immunological system in the different paediatric age groups [43]. Chronic cough in children is best considered as a symptom of an underlying disease. Therefore, the disease and its accompanying symptoms can be influenced by the quality of the health care system as well as health care independent factors such as age range, gender, and indoor and outdoor air pollution [44]. Common etiologies of pediatric chronic cough include asthma, eosinophilic bronchitis, gastroesophageal reflux disease (GERD), upper airway cough syndrome, protracted bacterial bronchitis, tracheomalacia, habit cough, and various systemic disorders [22,42]. Chronic cough in older children (> 14 years) and adults can be also elicited by tobacco smoking and taking antihypertensive medicaments, such as angiotensin-converting enzyme (ACE) inhibitors (Fig. 2).

There are many tools available for cough assessment in children, capturing different concepts which are used to evaluate various aspects of the symptom (Fig. 2). Subjective methods of cough evaluation include health-related quality of life (QoL) questionnaires which have been shown to be valid and reliable in adults and adolescents ($\geq$ 14 years of age) and in pediatric populations [13]. The recommended QoL questionnaires for adults and adolescents are The Cough Quality of Life Questionnaire (CQLQ) and Leicester Cough Questionnaire (LCQ) because of their good validation, reliability and responsive measures of the impact of chronic cough on adults and adolescents. In these patients, QoL questionnaires are based on self-report. In the pediatric population, the questionnaires are completed by the parents, not the child. According to this, the results reflect the parents' perception of the impact of cough on their child's QoL which can lead to an evaluation error [13]. In children under 14 years of age, Parent Cough-Specific Quality of Life Questionnaire has been found to be a reliable and valid instrument of

measuring parental perception of the impact of chronic cough on their child [45]. Cough Severity Visual Analogue Scale (VAS) and Numeric Rating Scales (NRS) can be also used in standard manner in all patients [13].

The objective methods include inhalation cough challenges and cough counting. In patients of all ages, cough frequency can be assessed by acoustic cough counting. On the other hand, it is not a reliable way to assess the cough severity [13]. Because of limited and insufficient evidence of tussigenic challenges, these are not recommended as primary outcome measures for determining cough severity or the impact of cough. However, tussigenic challenges may be useful to investigate the mechanisms of cough, thus they can be utilized in research settings [13,45].

Cough Assessment and Objectivizaton in Experimental Conditions

Pathological excessive cough is a serious clinical problem in many patients. It is no doubt that an increased activation of airway vagal nociceptors in disease conditions results from dysregulation of the neural pathway that control cough. Because current antitussives have limited efficacy and unwanted side effects there is still a continual demand for a development of a novel more effective antitussive. Therefore, inhibiting the increased activity of airway vagal nodose Aδ and jugular C-fibres in disease conditions represents a rational approach to development of effective antitussive drugs [46]. However, prior to evaluation of such agents in human, they must to be tested in appropriate animal models for testing putative drug candidates. The cough model needs to be reliable, robust, reproducible and to accurately reflect the disease in human [47]. The efficacy, feasibility and potency are other very important and necessary properties. Undoubtedly, animals must meet specific criteria and be appropriate for the research goal to be used as a model. Although there are some concerns about clinical validity and application from animal data, the utilization and research value of animal models is unquestionable [48,49].

Among most pre-clinical studies of neural pathway involved in the cough reflex and the pharmacological regulation of these pathways, the most useful and commonly used model for cough studies in recent years has been the conscious guinea pig [46,47,50]. The afferent vagal innervation, the immune response, neuropharmacologic response and cough response to standard tussigens such as citric acid and capsaicin in

guinea pig model are comparable to human [46,50]. In addition, these animals are small, easily accessible and not too expensive.

The method for measuring cough involves bodyplethysmograph box where animals are placed. Cough number and sound have been used to assess cough sensitivity and intensity. In our experimental design, we use a double-chamber bodyplethysmograph in which airflow and sound are recorded. The head chamber is connected to the compressed air-driven nebulizer. A suction device set to balance the nebulizer output is also connected to the head chamber to maintain constant airflow. Respiratory changes in the airflow are recorded using a pneumotachograph Fleisch head connected to the head chamber and recorded and analyzed by the Biopack system. Data are analyzed with the acquisition system ACQ Knowledge software installed on the computer. Respiratory sounds including cough are recorded with a microphone placed in the head chamber connected to a preamplifier and MP3 recorder. The pneumotachograph and microphone output are simultaneously recorded for off-line analysis and the cough has been identified visually and detected as the expiratory airflow accompanied by the cough sound using the software Sonic Visualizer [46,50,51,52].

We usually use a standard method for cough provocation using an aerosol of capsaicin (25µM - 50µM) or citric acid (0.4M) in the threshold concentrations for 5 min [46,53-56]. A submaximal dose was chosen based on our previous study using dose-response curve [52]. Prior to the beginning of the experiment, guinea pigs are adapted to laboratory conditions by inhalation of saline aerosol for 5 min at least twice on different days of the week which is very important to avoid a stress response and movement. On the base of our experiences, the cough response is quite variable in the guinea pigs. According to this variability, it is recommended to check the cough responsiveness of every single animal if two or more groups are being compared. Then it is necessary to divide them into hypo-, normo- and hyper-responders. Each group should consist of the same proportion of animals with decreased, normal and increased responsiveness [51,57]. Cough response in the guinea pigs is significant to the majority of the following aerosols: capsaicin, citric acid, bradykinin, and TRPA1 agonists [47,58]. To evaluate the sensitivity of cough reflex, animals in cough research are most often exposed to citric acid aerosol in gradually increasing concentrations from 0.05 to 1.6 M each for 30 s followed by subsequent 60 s observation time. The number of coughs elicited by each concentration is compared with a control group. When there are significant differences in cough numbers during inhalation of lower concentrations, it can be concluded that the cough sensitivity is changed [52,59].

Detection of cough is based on characteristic airflow changes, posture change, visual observation and cough sound [57,60-62]. Recordings from pneumotachograph are analyzed together with the visual input from the records during the entire cough challenge and the cough sound. Cough differentiation from sneezes or artefacts is performed by various softwares [50].

Cough provocation tests using capsaicin or citric acid in humans act via two distinct pathways. While capsaicin is a selective C-fibre stimulant, citric acid commonly stimulates C-fibres as well as Aδ-fibres. Nevertheless, citric acid is the preferred tussigen in animal models because of its lower tachyphylaxis compared to capsaicin [46].

Recently, a new whole bodyplethysmograph by EMKA technologies has been developed. This plethysmograph provides non-invasive measurements for longitudinal studies with conscious and freely moving animals. The aerosol is generated via a jet nebulizer and delivered to the bodyplethysmograph at an output of 0.5 mL/min, with a median diameter of the particles between 0.5 and 2 mm. The cough module of the EMKA system, a respiratory flow analyzer is used to monitor the number of coughs during the inhalation of citric acid, by detecting the changes in the pressure waveform and sound waveform.

A typical cough response in conscious animals can be identified by appearance of the following three key signals: (1) a transient and great change in the respiratory flow; (2) a cough sound; and (3) a cough-related body posture (splaying of the front feet and/or forward stretching of the neck with an opening mouth) [63]. Zhuang et al. in their paper demonstrated several specific disadvantages in the current system. First, cough numbers were only counted manually in the system, and such counting is time consuming. Second, use of this system may lead to an inaccuracy of cough count. A cough is a transient event. The cough sound was recorded and analyzed in studies using guinea pigs to distinguishing a cough [with the peak of power spectral density (PSD) at ~1.5 kHz] from a sneeze sound (with PSD peak at 3.5–6.5 kHz) [51]. However, this analysis and subsequent cough count were considered only in an offline manner. Thus, the risk of subjective judgement exists [63].

Conclusion

There are many methods for cough assessment either clinical or experimental. However, there are some limitations in the developed evaluation methods of cough. Subjective methods of cough assessment can be easily affected by emotions, expectations or attention to symptoms which often leads to evaluation error. On the other hand, the assessment of cough frequency is considered as the gold standard for the objective assessment of cough. It requires specific cough monitor system, such as the Leicester cough monitor and VitaloJAK™. Both systems are well established in clinical research with good validation demonstrated. However, the most commonly and widely used technique is cough challenge test. It can be used in research and clinical practice and can be potentially used also as a biomarker of cough hypersensitivity. Cough assessment based on characteristic airflow changes and cough sound are objective methods in the guinea pig model of citric acid-induced cough and is beneficial for assessing the efficacy of putative antitussive drugs in animal without and with respiratory diseases.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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