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ERS TASK FORCE

ERS guidelines on the assessment of cough

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SUMMARY OF KEY POINTS AND RECOMMENDATIONS

Cough

1) All basic scientific articles should refer to cough as a three-phase motor act. For the purposes of acoustic recordings in clinical studies, however, cough should be described as a forced expulsive manoeuvre or manoeuvres against a closed glottis that are associated with a characteristic sound or sounds.

2) All scientific articles should include a clear definition of what the authors have used as their definition of cough.

Capsaicin and citric acid inhalation cough challenge

1) The methodology for the performance of inhalation cough challenge should be standardised so as to facilitate universal interpretation and comparison of data generated by different laboratories.

2) Comprehensive normal ranges need to be developed using the standardised methodology advocated in the present document.

3) The single-breath concentration–response method using a flow-limited dosimeter is recommended for most experimental protocols.

4) Both C_2 and C_5 should be recorded.

5) Since there is wide inter-individual variation, cough challenge data have no intrinsic significance, but may usefully be used to follow change in cough reflex sensitivity in an individual.

Cough induced by inhalation of aqueous solutions

1) Aerosolised aqueous solutions represent a useful experimental tool in cough research.

2) The cough challenge with ultrasonic distilled water (fog) is difficult to standardise since it is highly dependent upon nebuliser output.

3) Consideration should be given to potential adverse events, such as bronchoconstriction and cross-infection.

Cough monitors

1) No cough monitor is currently the gold standard.

2) Monitors should be developed that are ambulatory, are capable of being digitally processed and permit prolonged (24-h) recording.

3) There is little to commend any particular method of quantifying cough over any other.

Assessment of quality of life of patients with chronic cough

1) Cough can have profound effects on health status, which can be assessed by cough-specific health status questionnaires.

2) Cough visual analogue scale (VAS, 0–100 mm) should be used to assess cough severity in patients with chronic cough.

3) Patients with chronic cough should be assessed with cough-specific quality-of-life questionnaires in clinical studies.

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Animal models of cough

1) The most useful animal model of cough is the conscious guinea pig.

2) Both sound and airflow should be used to define a cough event.

Design and conduct of clinical trials of antitussive drugs

1) The experimental model in which antitussive drugs are tested depends greatly upon the mode of action of the agent.

2) Normal volunteer studies should be designed in the knowledge that a large placebo effect is likely.

3) In acute cough, parallel group studies are required.

4) In chronic cough, the patient population studied should be defined by a diagnostic test.

BACKGROUND

Cough is the most common symptom for which individuals seek medical advice [1, 2]. An acute cough is often the most prominent symptom of the common cold, which itself is the most frequent illness to afflict mankind. In the USA, the direct and indirect costs of the common cold have been estimated at US$40 billion per annum [3]. Chronic cough, as the sole presenting complaint, is known to account for 10–38% of all referrals made to respiratory physicians [4, 5]. Recently, the European Respiratory Society (ERS) published guidelines on the management of cough [6]. The aim of that document, as of guidelines produced by the American College of Chest Physicians, was to provide consensus regarding the diagnosis and treatment of cough in both adults and children [7]. However, little attention has been given to the accurate clinical and scientific assessment of coughing. In 2004, the ERS approved the setting up of a task force with the intention of producing recommendations for cough assessment. The main objective was to produce a practical document, which would provide helpful guidance to researchers, clinicians, the pharmaceutical industry and regulatory authorities. It was agreed that the scope of this document should include recommendations on the following key elements: 1) safe standardised methods of inhalation cough challenge; 2) reliable, reproducible and relevant clinical cough recording and analysis; 3) clinical assessment of cough-related quality of life; 4) appropriate animal models in which to evaluate novel cough treatments; and 5) areas for future research. It is envisaged that an effective document would contribute to improved patient care, enhance the quality of cough research and ultimately assist in the development of effective new therapies.

The need for recommendations regarding the assessment of cough

In addition to the gaps in the existing literature, four major factors justify the need for such a publication.

1) Since the mid-1960s, there has been a sharp increase in the number of published manuscripts concerned with various aspects of cough in human and animal studies. In a PubMed search ranging 1966–2005 and limited to the keyword ''cough'', there were 22,744 citations, more than for other common respiratory symptoms including the term dyspnoea/dyspnea or wheeze. This represents not only an absolute increase but also a two-fold relative increase in cough publications. The incremental rise in citations in the four decades spanning this period is displayed in table 1. The publications are geographically diverse, spanning five continents, and highlight the importance and frequency with which cough presents as a clinical problem.

2) The morbidity of cough is not trivial, and the assessment of cough patients is incomplete without appreciation of the associated clinical impact. A series of cough-specific quality-of-life questionnaires have been designed to capture this, but agreement regarding their interpretation and clinical application is needed [8, 9]. New technologies designed to objectively record and analyse cough events are rapidly being developed. Recommendations regarding clinical and pharmacologically relevant end-points for such devices are required.

3) The value of inhalation cough challenge testing as a research tool in both human and animal studies is established [10, 11]. On review of the existing literature, different methods of challenge have been identified, with wide variation in the choice of tussive agents, delivery device and test end-point employed. Standardisation of cough challenge methodology is required if reliable comparisons of experimental results between laboratories are to be made. In order to ensure continued ethical review board support for cough research, a standardised approved method of safe inhalation challenge testing is required.

4) Although specific therapy directed at the underlying cause of cough is usually successful, there are no particularly effective nonspecific cough treatments. These are desperately needed for patients with idiopathic cough, for those troubled with cough due to pulmonary fibrosis and lung cancer, and when established treatments for asthma and chronic obstructive pulmonary disease (COPD) are ineffective. Drug development is currently hampered by the lack of consensus as to the appropriate animal model for testing of putative novel therapies. Recommendations for the reliable and informative testing of new therapies in animal models would be a major step towards effective cough therapy.

Methods

The task force was composed of a number of invited participants, identified for their particular expertise in the area of cough assessment and treatment. The initial meeting of task force members was held at the 2004 ERS Congress in Glasgow,

TABLE 1 Number of citations identified in PubMed database using the keyword ''cough'' for the period 1966–2005

Period	Citations n
1966–1975	2262
1976–1985	2968
1986–1995	6564
1996–2005	10950

UK, and followed by subsequent meetings in 2005 in Amsterdam, the Netherlands, and later that year at the ERS Congress in Copenhagen, Denmark. At the first meeting, it was agreed that, where possible, recommendations should be based on the peer-reviewed literature (original publications and review articles). However, it was apparent that in many areas of interest, including cough reflex testing, use of quality-of-life questionnaires and new cough recording technologies, only limited data (such as meeting abstracts) existed. Consequently, the opportunity to produce evidence-based guidelines was limited. In such circumstances, recommendations were based on the consensus of experience of task force participants.

Individuals and small groups were allocated specific topics and, after conducting a literature review, asked to produce an article for consideration at the subsequent task force meeting. Based on the group discussion at these meetings, a series of recommendations were agreed upon.

Structure of the document

The present document is prefaced with an executive summary of key points and recommendations. The subsequent sections address the individual components of cough assessment, namely cough challenge in humans, cough event recording, assessment of cough quality of life, animal models of cough, and the design and conduct of clinical trials of antitussive drugs. Each section begins with a summary of the existing literature followed by a concluding statement highlighting areas for future research and finally the task force recommendations.

Defining cough

Although usually defined in textbooks, a clear definition of cough is lacking in the majority of scientific papers concerning cough [12]. For the purpose of the present recommendations, two possible definitions of cough, which have been used elsewhere, are provided [13, 14].

1) Cough is a three-phase expulsive motor act characterised by an inspiratory effort (inspiratory phase), followed by a forced expiratory effort against a closed glottis (compressive phase) and then by opening of the glottis and rapid expiratory airflow (expulsive phase) [13].

2) Cough is a forced expulsive manoeuvre, usually against a closed glottis and which is associated with a characteristic sound [14].

Although some similarities exist, the major discrepancy between these two definitions relates to the respiratory patterns associated with cough. In particular, the preceding inspiratory phase, which constitutes the first definition, is believed to be one of a number of distinguishing features between cough and another airway defensive reflex, the expiration reflex [12].

Furthermore, neither definition adequately deals with the common clinical scenario whereby an initial cough is followed by a series of cough efforts. For the patient, this is often described as a cough ''bout'' or ''attack''. To the researcher, they may represent individual coughs or an extended single cough. Clearly, this is of importance to those concerned with the accurate recording of cough frequency. For the physician and, most critically, the patient, a definition that reflects the sensation associated with an urge to cough, the intensity of the cough and its impact on health status overrides any such debate.

For the purpose of the assessment of cough, the present task force has adopted the following recommendations.

Recommendations

1) All basic scientific articles should refer to cough as a three-phase motor act. For the purposes of acoustic recordings in clinical studies, however, cough should be described as a forced expulsive manoeuvre or manoeuvres against a closed glottis that are associated with a characteristic sound or sounds.

2) All scientific articles should include a clear definition of what the authors have used as their definition of cough.

CAPSAICIN AND CITRIC ACID INHALATION COUGH CHALLENGE

Methodology

The inhalation cough challenge permits measurement of the sensitivity of the cough reflex and assessment of the antitussive effects of specific therapies. In general, inhalation cough challenge testing can be divided into methods that use acid and non-acid tussives. Capsaicin, the most commonly used non-acid tussive to experimentally induce cough in humans, was first described in 1984 [15]. Citric and tartaric acid are the most widely used acid tussigens [16]. Citric acid inhalation cough challenge was described and formally characterised in the mid-1950s [16, 17]. Both citric acid and capsaicin can induce cough in a dose-dependent and reproducible manner [16, 18].

Unfortunately, because of the lack of standardisation of cough challenge methodology in terms of equipment, preparation of solutions, method of administration, nebuliser output, inspiratory flow rate and dose of aerosol per breath, comparisons of cough sensitivity data currently in the literature from different institutions are not valid. In the present section, recommendations that will assist in the standardisation of inhalation cough challenge testing using both acid and non-acid tussigens are proposed.

Preparation and storage of capsaicin solutions

Capsaicin (30.5 mg) is dissolved in 1 mL pure ethanol and 1 mL polyoxyethylene sorbitan (Tween 80) and then further dissolved in 8 mL physiological saline solution to yield a 0.01 M stock solution [19, 20]. Without the detergent Tween 80, a cloudy rather than clear solution results. The solution is subsequently diluted with saline in order to obtain serial doubling concentrations ranging 0.49–1,000 μM. If healthy volunteers are to be tested, the lowest concentration prepared is 0.98 μM, since, in the authors' experience, induction of cough at this concentration is rare.

It is unclear how often fresh stock solution should be prepared. A recent study concluded that capsaicin solutions of $\geqslant 4$ μM are stable for 1 yr if stored at 4°C and protected from light [21].

Preparation of citric acid

Serial dilution of 3 M citric acid stock solution in sterile 0.9% saline solution is performed in order to obtain serial doubling concentrations ranging 1.95–3,000 mM [22–24]. In healthy volunteers, the lowest concentration prepared is 7.8 mM. For both capsaicin and citric acid, fresh dilutions from a stock solution are prepared on each day of testing. The stock solution is maintained at ~ -10°C for capsaicin and 4°C for citric acid. The authors recommend that fresh solutions are prepared for each challenge.

Capsaicin and citric acid are delivered in serial doubling concentrations (the lower concentration is unlikely to influence the higher one), with inhalation of 0.9% saline solution randomly interspersed to increase challenge blindness [22–24].

Administration of capsaicin and citric acid

The two main methods of capsaicin and citric acid delivery during cough challenge testing are the single-dose and dose–response methods [25]. In the former method, a single concentration of capsaicin or citric acid is employed. The dose–response method can involve either the administration of single vital-capacity breaths of incremental concentrations of capsaicin or citric acid *via* a dosimeter-controlled nebuliser or the tidal-breath inhalation of incremental concentrations of tussive agent, each over a fixed time period, usually 15–60 s.

In most experimental circumstances, the single-breath dose–response method is preferred because of the accuracy and reproducibility of the dose delivered and the ease with which a tussive response can be determined. With capsaicin and citric acid inhalation occurring over a prolonged time period, variations in respiratory frequency and tidal volume are likely to cause significant variations in the amount of aerosol delivered from subject to subject, as well as from one concentration to another in an individual subject. This would be of particular concern during the administration of concentrations that induce significant coughing, thereby preventing the subject from inhaling the tussive agent for a significant portion of the fixed time period of aerosol delivery. Nevertheless, a recently published comparison of the tidal breathing and dosimeter methods of capsaicin inhalation challenge demonstrated both to be reproducible, with good agreement between the two methods [26].

Optimisation of reproducibility of capsaicin and citric acid cough challenges

Inspiratory flow rate

The rate of inspiratory flow affects the pattern of deposition of aerosol within the airways. Variations in inspiratory flow rate have been demonstrated to affect the results of capsaicin [27] and citric acid [28] cough challenge. For example, lower inspiratory flow rates (50 *versus* 150 $L \cdot min^{-1}$) result in greater cough response to citric acid [28]. Therefore, the flow rate needs to be constant regardless of effort, since, unless inspiratory flow rate is controlled, variable amounts of tussive agent will be delivered to different subjects, and even breath-to-breath variations may occur in a given subject within the same study. Such potential variability in aerosol delivery may affect the results of studies in which reproducibility of cough challenge results are crucial, such as in pharmacological studies incorporating cough sensitivity measurement before and after drug therapy, and in epidemiological studies comparing different subject populations.

In order to control for inspiratory flow rate, the present authors recommend the use of a compressed air-driven nebuliser (model 646; DeVilbiss Health Care, Inc., Somerset, PA, USA) controlled by a dosimeter (KoKo DigiDoser; nSpire health Inc, Louisville, CO, USA (formerly Ferraris Respiratory Inc) and nSpire health Ltd, Hertford, UK (formerly Ferraris Respiratory Europe) that is modified by the addition of an inspiratory flow regulator valve (RIFR; nSpire health Inc, Louisville, CO, USA, formerly PDS Instrumentation, Inc). The valve limits inspiratory flow rate to 0.5 $L \cdot s^{-1}$ regardless of excessive inspiratory force, thereby guaranteeing a consistent and reproducible inspiratory effort with each breath. Thus, with appropriate instruction to inhale with sufficient force, all subjects achieve an identical inspiratory flow rate during each inhalation of aerosol.

Nebuliser characteristics

Significant variation in the amount of aerosol delivered per inhalation may occur with a standard nebuliser, even in an individual subject who attempts to maintain a constant inspiratory flow rate. The second major determinant of aerosol output is related to the structure of the nebuliser itself. For example, in the DeVilbiss 646 model, the straw and baffle assembly is a removable component of the nebuliser. When this structure is detached for washing and then reattached, variable distances result between the straw and baffle assembly and the source of pressurised air, the jet orifice. This variation in distance, albeit minute, results in variation in nebuliser output. Therefore, in order to optimise reproducibility, two modifications to a nebuliser are suggested. First, an inspiratory flow regulator valve is installed, as described above. Secondly, the straw and baffle assembly of the nebuliser is welded in place, thereby eliminating the variations in nebuliser output that may occur when these components are separated and then reattached with resulting variable distances between the jet orifice and straw. After these modifications are performed, the exact output (in $mL \cdot min^{-1}$) of the nebuliser is determined (characterised nebuliser; nSpire Health Inc, formerly PDS Instrumentation, Inc.). When the exact output of a nebuliser is known, modulation of the duration of aerosol delivery permits the determination of aerosol output per inhalation. For example, a nebuliser with an output of 1.007 $mL \cdot min^{-1}$, programmed to deliver aerosol for 1.2 s, provides 0.02 $mL \cdot breath^{-1}$.

Given the potential variations in nebuliser output, it is essential that research investigations utilise equipment tailored to optimise reproducibility, and that the same nebuliser, or one with identical output, is used in studies incorporating serial cough challenges in individual subjects, or studies comparing distinct subject populations. Given the reality that different types of equipment will continue to be used by cough researchers worldwide, it is recommended that standardisation of cough challenge studies should be attempted by controlling nebuliser output per breath.

Placebo inhalations

In order to increase cough challenge blindness, inhalations of physiological saline (placebo) should be randomly interspersed

between incremental concentrations of capsaicin and citric acid [25, 29]. This strategy may reduce the effects of voluntary suppression or conditioned responses in subjects who would otherwise be anticipating progressively higher concentrations of tussive agent.

Instructions to subjects

Subjects undergoing cough challenge should be specifically instructed not to attempt to suppress any coughs and not to talk immediately after inhalation of tussive agent, since this may potentially suppress cough. The present authors recommend, for example, the following instruction to subjects: ''allow yourself to cough if you need to, and as much as you need to''. Subjects should not be told that the induction of a specific number of coughs is the end-point of the study (see Interpretation of cough challenge data section) [11].

Determination of tussive response to cough challenge

When employing the single-breath method of capsaicin and citric acid administration, the tussive response to each dose of aerosol is immediate and brief. Therefore, only coughs occurring within 15 s of capsaicin and citric acid delivery should be counted [10, 25, 30, 31]. Coughs that occur beyond this time period may not be capsaicin or citric acid induced.

Interpretation of cough challenge data

For each test, the concentrations of capsaicin or citric acid causing two (C_2) and five coughs (C_5) are reported. The C_2 and C_5 can be obtained by determining the first administered concentration that results in two or more and five or more coughs, or by interpolation of logarithmically transformed concentration–response curve data. Overall, there are minor differences between these two methods [32]. Interpolated concentrations are closer to the real C_2 and C_5, but offer no particular advantage over the first administered concentration method. Differing opinions exist among investigators as to which is the preferred primary end-point, C_2 or C_5. Published studies often report both values, but not infrequently C_5 alone is reported. There is evidence that C_5 may be the clinically superior value [11], although other studies have found C_2 to be more reproducible [29]. Until further data are available, the present authors would recommend that both C_2 and C_5 are measured.

A potential problem in serial cough challenges involves the startle phenomenon [11]. A naive subject undergoing cough challenge may cough excessively, a phenomenon described with citric acid in the 1950s [16]. A preliminary familiarisation challenge may be required, or the C_5 may be used, since it is less likely to succumb to this potential pitfall.

A small subgroup of individuals with relatively high cough thresholds may not be able to achieve C_5 despite using the highest concentrations of tussigen. The inhalation of high concentrations of capsaicin is precluded by a strong burning sensation in the upper airway, whereas citric acid inhalation may provoke a choking sensation or pharyngeal discomfort [18]. The present authors recommend that such subjects be excluded from comparative clinical trials because a true C_5 cannot be discerned, and that the C_2 is used in population studies.

Significance of capsaicin and citric acid cough sensitivity measurements

Isolated measurements of capsaicin sensitivity (C_2 or C_5) have no intrinsic significance due to the large variation in cough reflex sensitivity within the normal population (fig. 1). This contrasts with the assessment of bronchial responsiveness, where a provocative concentration of drug causing a 20% fall in forced expiratory volume in one second (FEV_1) outside the normal range is predictive of pathophysiology [34]. Nevertheless, since cough reflex sensitivity to inhaled capsaicin and citric acid is highly reproducible when performed by a individual investigator or laboratory using appropriate methodology [11, 16–18, 25], these challenges have established themselves as an important tool in pharmacological studies incorporating serial challenges, as well as in epidemiological studies comparing distinct populations.

Cleaning of the equipment

The equipment should be sterilised between each subject. Sterilisation of the equipment should be performed in accordance with the individual institution's guidelines. For example, the present authors' protocol includes sterilisation for 15 min in Pera® Safe (Antec International, A DuPont Company, Sudbury, UK). Afterwards, the equipment is washed thoroughly with hot water and air dried.

Tachyphylaxis and reproducibility

Marked tachyphylaxis occurs to repeated cough challenge in the short term. Indeed, continual inhalation over 1 min of citric acid or capsaicin results in a reduction in cough frequency of a third with capsaicin and complete abrogation with citric acid. Repeated single-breath cough challenge at 10-min intervals similarly results in marked tachyphylaxis [35]. The present authors recommend a minimum of 1 h, and preferably 2 h, between cough challenge measurements. The high degree of long-term reproducibility of capsaicin and citric acid cough challenge testing has been reported by numerous investigators

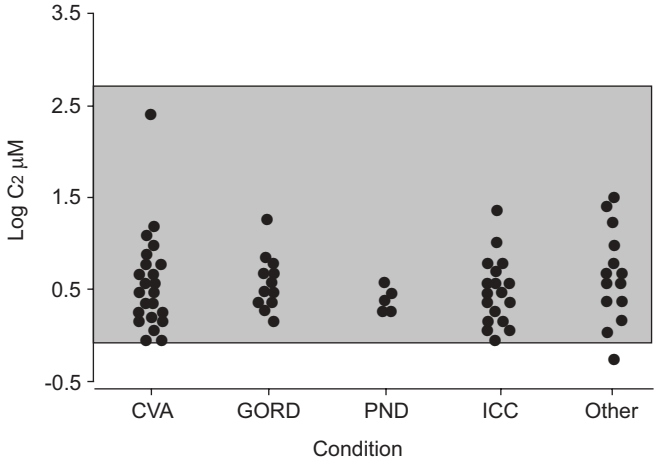

FIGURE 1. Cough reflex sensitivity to capsaicin in patients with chronic cough (▪: normal range (derived from 134 healthy subjects)). C_2: concentration of capsaicin inducing two coughs; CVA: cough variant asthma; GORD: gastro-oesophageal reflux disease; PND: post-nasal drip (rhinitis); ICC: idiopathic chronic cough; Other: chronic obstructive pulmonary disease, sarcoidosis, cryptogenic fibrosing alveolitis, and bronchiectasis. Adapted from [33].

employing the dose–response method and the single dose, with both the single-breath technique [11, 16, 17, 26, 29, 33, 36–41] and a fixed time period of capsaicin and citric acid inhalation [15–18, 26, 35, 42, 43].

Two studies have confirmed the reproducibility of capsaicin cough challenge over 3 months [44] and >6 months [11]. The latter demonstrated good reproducibility of cough challenge in 40 healthy volunteers at a mean interval of 16.7 months. The reproducibility of the recommended citric acid challenge has recently been demonstrated [45].

Safety

A recent review of the 20-yr clinical experience with capsaicin failed to uncover a single serious adverse event associated with capsaicin cough challenge testing in humans [46]. This review included an examination of 122 studies published since 1984, describing 4,833 subjects, including healthy adults and children, as well as patients with pathological cough, asthma, COPD, hypertension, gastro-oesophageal reflux disease, interstitial lung disease, acute upper respiratory tract infection, cervical spinal cord injury, heart–lung transplantation and cystic fibrosis [46]. Side-effects consisted mainly of transient throat irritation in a minority of subjects.

The safety of citric acid inhalation cough challenge was reported in the 1950s [16, 17]. However, inhalation cough challenge using citric acid can result in a small reduction in FEV_1 (<5%), which is unlikely to be of clinical significance [10]. Capsaicin does not induce clinically significant bronchoconstriction in healthy volunteers or asthmatics [15, 47].

However, the present authors would recommend that, when performing inhalation cough challenge, bronchodilator therapy be available.

Female healthy volunteers and female patients with chronic cough exhibit increased cough reflex sensitivity to capsaicin [48, 49] and citric and tartaric acid [22, 50, 51].

The placebo cough response shows a nonlinear increase in cough suppression, which is most pronounced at 4 h [40]. In addition, there are suggestions that females may cough more frequently and exhibit more rapid adaptation of cough than males.

The contact details of investigators experienced in these techniques can be found on the International Society for the Study of Cough website [52].

Further research

At present no data are available regarding the short- and long-term reproducibility of the above-mentioned method of citric acid inhalation cough challenge; however, they are currently being investigated. The amount of information available regarding the reproducibility of all cough challenge methods needs to be increased.

Recommendations

1) The methodology for the performance of inhalation cough challenge should be standardised so as to facilitate universal interpretation and comparison of data generated by different laboratories.

2) Comprehensive normal ranges need to be developed using the standardised methodology advocated in the present document.

3) The single-breath concentration–response method using a flow-limited dosimeter is recommended for most experimental protocols.

4) Both C_2 and C_5 should be recorded.

5) Since there is wide inter-individual variation, cough challenge data have no intrinsic significance, but may usefully be used to follow change in cough reflex sensitivity in an individual.

COUGH INDUCED BY INHALATION OF AQUEOUS SOLUTIONS

In order to evoke cough and other reflex respiratory responses using aerosolised aqueous solutions, the use of an ultrasonic nebuliser is mandatory. There are no reports regarding such responses being obtained using aerosols produced by conventional nebulisers. The technical features and principle of operation of most widely used ultrasonic nebulisers have been reviewed elsewhere [53, 54]. Ultrasonic nebulisers generally produce a much larger solution output per unit volume of air than conventional nebulisers [53]. Since the tussigenic stimulus of any aqueous solution is caused by the reduced concentration of permeant anion, particularly chloride [55], any solution with a low permeant anion concentration can be used as a cough-stimulating agent.

Aerosol delivery

Water aerosols are best delivered during tidal breathing; the use of a two-way valve [55, 56] or an outlet proximal to the subject's airway [57, 58] is required to avoid rebreathing. In order to ensure constant supply, the aerosol should be conveyed to a 1.5–2.0-L reservoir bottle [57].

Dosing schedule

Challenges with nebulised aqueous solutions can be performed according to two different methods, the single-dose and the dose–response method. In the first case, the nebuliser is set at a predetermined power output (usually the maximum attainable) and the nebulised agent is inhaled for a predetermined period (usually 1 min) [35, 59–62]. Dose–response challenges can be performed by the subject inhaling stimuli of progressively higher intensity, obtained by increasing the nebuliser output in steps (fig. 2), each corresponding to a definite fraction of the maximum available output [55, 57]. Alternatively, the stimulus strength can be augmented progressively with constant nebuliser output, using nebulising solutions that are progressively lower in anion concentration [61–63].

Outcome measure/sensitivity

In single-dose challenges, the cough response is assessed in terms of cough frequency [35, 59–62]; however, cough frequency seems to be affected by some degree of stimulus adaptation [35]. In dose–response challenges, cough sensitivity can be evaluated as the cough threshold, *i.e.* the lowest stimulus intensity capable of evoking at least one cough during two dose–response challenges separated by a 30-min interval [56–58]. The reliability of other outcome measures, such as C_2

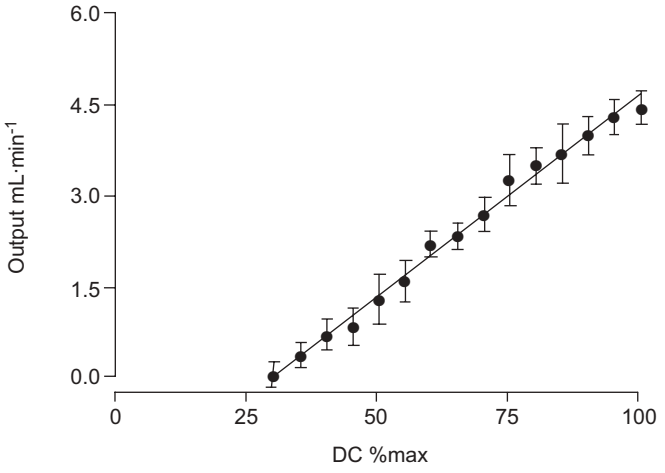

FIGURE 2. Relationship between ultrasonic nebuliser output (*y*) and the corresponding direct current (DC) signal (*x*) analysed using the least squares regression method. Data are presented as mean±SD. The relation fits the linear model ($r=0.95$; $p<0.001$), and mean nebuliser output can be calculated from the following equation (using units shown): $y= -1.90+0.066x$. The range of outputs (means) usually employed is 0.08–4.45 mL·min^{-1}, *i.e.* 30–100% of the maximum DC signal.

and C_5, *i.e.* the lowest stimulus intensity capable of evoking at least two or five coughs, has not been assessed using water aerosols. Quantitative measures, such as cough expiratory flow [57, 64] and the force developed by the expiratory muscles during single cough efforts [56–58, 64], may be useful when the intensity of motor responses needs to be evaluated.

Reproducibility

Several studies have established the reproducibility of cough sensitivity [35, 57, 63–65] and cough intensity [57, 66] in dose–response challenges using ultrasonically nebulised distilled water [35, 57, 66] and other aqueous solutions [63, 65].

Safety

Ultrasonic nebulisers are obviously not disposable, and are expensive. Sterilisation of the equipment is, therefore, recommended at every use. This is potentially a major limitation to the use of ultrasonically nebulised aerosols outside the research field. Furthermore, hypotonic aerosols are potentially bronchoconstrictive in susceptible individuals [55].

Cough challenges with aqueous aerosols, however, present some features that may potentially be useful in cough research. The stimulus intensity can be increased in small fractional amounts, such that the cough threshold is assessed relatively precisely. In addition, since aqueous aerosols are inhaled during quiet relaxed breathing, it is possible to record several breathing pattern variables simultaneously, and thus investigate the ventilatory adjustments that may be evoked by airway receptor stimulation.

Areas for future research

Sensory receptor stimulation by fog may be due to both the absence of chloride ions (causing cough) and the hypo-osmolarity (causing bronchoconstriction in susceptible individuals). Which of these fog-related stimuli is also responsible for fog-induced changes in the pattern of breathing remains to be established. The feasibility and reproducibility of measures such as C_2 and C_5 for the assessment of cough sensitivity to aqueous aerosols also needs to be determined.

Recommendations

1) Aerosolised aqueous solutions represent a useful experimental tool in cough research.

2) Ultrasonic distilled water (fog) challenge is difficult to standardise since it is highly dependent upon nebuliser output.

3) Consideration should be given to potential adverse events, such as bronchoconstriction and cross-infection.

COUGH MONITORS

Since the 1950s, researchers have been attempting to objectively measure cough [67–69], *i.e.* to quantify the amount of coughing per unit time. Although sound recordings can be made and cough events counted manually, this process is extremely laborious. Currently, no standardised method exists, and there is no adequately validated generic cough monitor that is commercially available and clinically acceptable.

An objective measure of cough would be of use in clinical practice, clinical research and the assessment of novel therapies. It would permit validation of the presence of cough, grading of severity and monitoring of responses to therapeutic trials. This is an exciting area of cough research and progress is being made. Various systems are being developed to automate cough identification and quantification, taking advantage of recent technological advances.

The present guidelines aim to inform both developers and potential users of cough monitors by highlighting the essential features of an ideal device and clarifying the appropriate methods for reporting on the performance of cough monitors.

Nonambulatory methods of cough recording

The very first cough monitoring systems to be developed were nonambulatory; mains-supplied tape recorders with free-field microphones were used to measure cough in hospital inpatients or study subjects overnight [67, 70]. Similar systems can be used for documenting coughing in cough challenge studies.

Ambulatory methods of cough recording

The most useful systems are ambulatory and can count cough over a defined period of time (usually 24 h). Advances in computing and digital storage media have permitted the use of digital sound recordings. Accurate recognition of the cough signal remains the limiting step and has been difficult to achieve. Several groups have applied digital signal processing techniques to cough sound recordings, but with limited success [71–74]. Hence, most systems still use manual counting, which is tedious and limits the size and scope of studies.

To date, more than six different systems have been described that rely on identification of the cough sound [72, 73, 75–79]. One of the first 24-h ambulatory systems used a solid-state multiple-channel recorder to measure the number of coughs [75]. Coughs were identified by the simultaneous occurrence of a digitised cough sound and an electromyographic signal from

the respiratory muscles. The signals were analysed visually and counted manually.

Another system transmitted cough sounds from a microphone to a computer in the subject's home using telemetry [77]. Digitally stored coughs were counted manually, and also the cough latency (period between coughs), cough effort (integral of the acoustic power spectrum), cough intensity (cough effort divided by cough count) and cough wetness were measured.

In one cough recording system, cough was quantified in terms of the amount of time spent coughing, *i.e.* the number of seconds containing at least one explosive cough [78, 80, 81]. This was used to obtain a more encompassing definition of cough rather than just measuring the explosive component that can be heard.

In terms of automation of the cough recognition system, MORICE and WALMSLEY [73] described a probabilistic neural network system for differentiating cough from noncough. Using a Sony Walkman digital audio tape recorder (Sony, Malaysia), it was demonstrated that this system recorded similar numbers of coughs to manual counting.

BIRRING and co-workers [82, 83] developed the Leicester Cough Monitor, which detects coughs in patients with chronic dry cough using a statistical model and quantifies coughs as individual events.

Using an ambulatory cardiorespiratory monitoring system (LifeShirt; Vivometrics, Nahant, MA, USA) with an integrated unidirectional contact microphone, cough was measured in eight COPD patients under laboratory conditions and automatically counted using statistical parameters [79]. Compared with cough counts from video and sound recording, good correlation was obtained.

Defining cough

Coughing produces a characteristic sound [84]. The sound results from rapid changes in airflow generated by the contractions of muscles in the chest wall, abdomen, diaphragm and larynx. Hence a variety of modalities can be used to detect coughing (table 2). The definition of a cough depends upon the signal(s) monitored. Cough counting using sound either alone or in combination with a second signal has been most commonly used [75, 76, 79, 85], and the phases of a typical cough sound are shown in figure 3. It is essential that any cough monitoring device defines exactly what is recognised as a cough, from which signal(s) and, furthermore, how coughing is quantified.

TABLE 2 Signals and sensor types for monitoring cough

	Sensor
Sound	Free-field microphone
	Air-coupled microphone
	Contact microphone
Movement	Electromyography
	Accelerometer
	Induction plethysmography

Quantification of coughing

One of the difficulties in identifying and quantifying cough is that a variety of patterns of coughing occur. If the sound signal is examined, three main patterns have been consistently described in the literature (figs 3–5) [84, 86, 87], but many more exist. Coughing can be quantified in a number of different ways. Figure 6 shows a short sound recording of coughing and four possible methods of quantification as follows. 1) Explosive cough sounds (fig. 6a); counting the characteristic explosive cough sounds is the most intuitive way of counting cough. 2) Cough seconds (fig. 6b); this is a measure of the time spent coughing, *i.e.* the number of seconds per hour containing at least one explosive cough sound. 3) Cough breaths (fig. 6c); these are used in a system that continuously monitors breathing and quantifies cough as the number of breaths which contain at least one explosive cough sound. 4) Cough epochs (fig. 6d); continuous coughing sounds without a 2-s pause are counted.

Whether any of these methods is more valid than any other is not known, but it is fundamental to describe the unit of cough used. There is a tight linear relationship between cough sounds and cough seconds in a variety of conditions (fig. 7).

Other end-points in cough monitoring

Other features of the cough signal apart from the number of coughs are potentially of use as clinical end-points, as follows. 1) The intensity of coughing is likely to be important; subjects with a small number of coughs may still find the symptom very distressing if associated with chest pains, retching or syncope. Both the peak intensity achieved and the overall energy released in coughs may be valuable measures. 2) The pattern of coughing (peals *versus* single coughs) may not only affect the patient's experience but also serve different mechanical purposes. Furthermore, the rates of coughing throughout the day and night may be related to the stimulus to cough [81]. 3) The acoustic properties of the cough sounds are of potential use in identifying the presence of airway secretions or wheeze.

Automated identification of coughs is necessary to facilitate the study of these additional parameters. Ambulatory monitoring over 24 h in the patient's own environment is necessary as cough rates can change from hour to hour, show diurnal variation and may be affected by a nonambulatory setting.

Validation of cough monitors

Validation of cough monitors against a gold standard measure is obligatory. Manual cough counting from video or sound recordings is used, and, although extremely laborious [79, 88], good agreement between observers has been achieved.

Video recordings have the advantage that the movements associated with coughing can be seen, but monitoring has to be restricted to the area in view of the camera. It cannot be assumed that performance is the same in a fully ambulatory subject. Validation of ambulatory cough monitoring is possible using a small digital sound recorder and microphone running simultaneously with the cough monitor [71].

Additionally, the acoustic properties of cough sounds vary in different diseases [87, 89]; thus validity in one patient group is not generalisable. Cough detection should therefore be

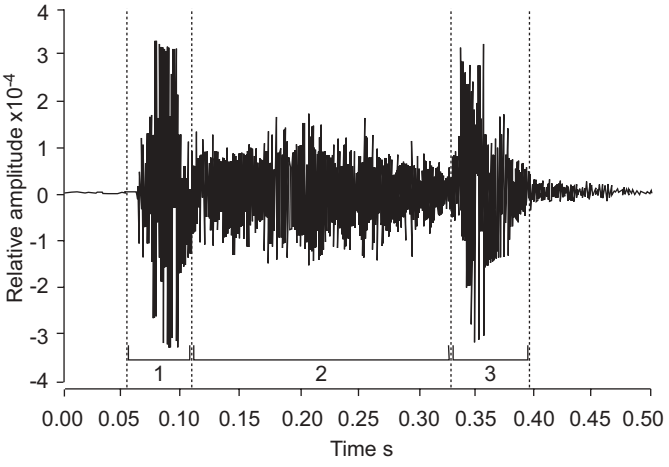

FIGURE 3. Three-phase cough sound (1: explosive phase; 2: intermediate phase; 3: voiced phase).

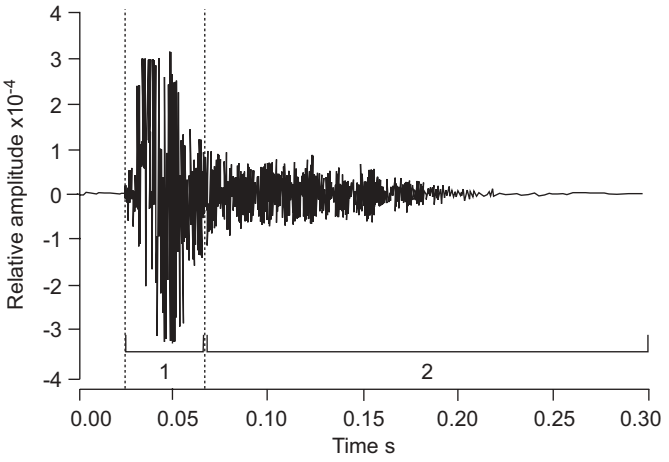

FIGURE 4. Two-phase cough sound (1: explosive phase; 2: intermediate phase).

validated in patients with chronic cough (with a range of aetiologies) and cough in other respiratory conditions.

Sensitivity and specificity

Sensitivity, specificity and positive and negative predictive values contribute to a description of cough monitor performance. As sounds that could be mistaken for cough (*e.g.* speech, throat clearing, laughing and sneezing) may occur more often than coughs, the number and nature of false positive results should also be reported. Agreement between manual and automated cough counts [90] gives a clear representation as to how much the two measures differ and whether this difference is related to the magnitude of the measurement. The reproducibility of the performance of the cough monitor and responsiveness to change are also important attributes. The precision of all of these measures (*i.e.* 95% confidence intervals) permits assessment of the consistency of performance.

Future work in cough monitoring

There has been very significant progress towards fully automated cough counting in recent years, but testing of the performance of systems needs to be rigorous. Accurate cough counters will add a new dimension to cough assessment. Simple cough counts are, however, only one dimension of cough, and probably explain only part of the patient's experiences of the symptom. Further end-points, such as cough intensity, coughing patterns over both the short and long term, and acoustic parameters (representing wheeze and airway secretions), are wide open for development and investigation.

Recommendations

1) No cough monitor is currently the gold standard.

2) Monitors should be developed that are ambulatory, capable of being digitally processed and permit prolonged (24-h) recording.

3) There is little to commend any particular method of quantifying cough over any other.

ASSESSMENT OF QUALITY OF LIFE OF PATIENTS WITH CHRONIC COUGH

Chronic cough is often perceived as a trivial problem, but can be a disabling symptom associated with significantly impaired quality of life [8, 9]. Until recently, there have been no tools for measuring cough-specific quality of life. Indeed, there is a striking paucity of well-validated outcome measures in chronic cough. There is no consensus regarding the definition of health-related quality of life, but the World Health Organization definition of health as ''a state of complete physical, mental and social well-being, and not merely the absence of disease'' is widely quoted [91]. Health status or health-related quality-of-life measurement is a means of quantifying the impact of disease or symptoms on patients' daily life and general well-being in a standardised and objective manner. Quality-of-life questionnaires are widely used in clinical studies and are standard end-points in most randomised controlled trials. This section focuses on the effects of chronic cough on health status and the measurement of quality of life in patients with chronic cough.

Adverse impact of cough on health status

Cough has wide-ranging effects on health status. The reasons why patients with chronic cough seek medical advice are poorly understood, but may relate to worry about the cough, embarrassment, self-consciousness and the presence of associated symptoms, such as nausea and exhaustion [92]. In acute cough, health status is impaired transiently. The impact of chronic cough on health status is varied, being minimal in some patients who do not seek medical attention to disabling in others, associated with impairment of quality of life comparable to that in other chronic respiratory disorders, such as COPD. The physical, psychological and social domains of health are commonly affected [9]. Patients with chronic cough frequently report musculoskeletal chest pains, sleep disturbance and hoarse voice. More marked symptoms, such as blackouts, stress incontinence and vomiting can occur. Some patients report adverse symptoms related to the urge and sensation to cough and the act of cough suppression. The psychological aspects of health status affected often include worry about serious underlying diseases, such as cancer and

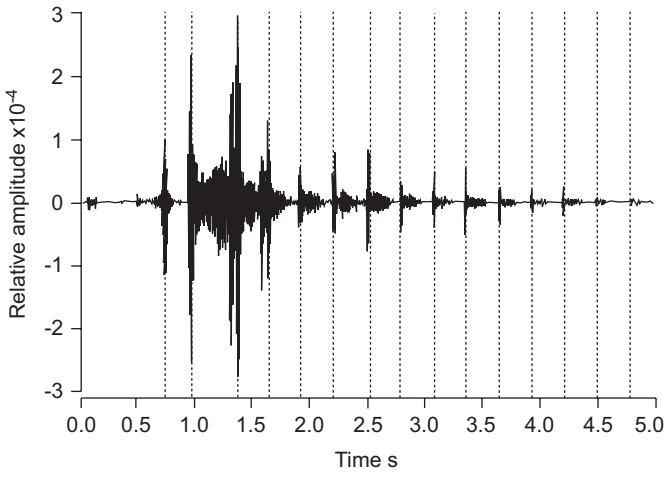

FIGURE 5. Peal of coughs after a single inspiration with repeated explosive phases (·····).

tuberculosis. The impact of cough on social well-being depends upon individual circumstances. The cough may result in difficulty in relationships, avoidance of public places, disruption at work and, in severe cases, time off work. The wide-ranging and potentially profound effects of cough on health status highlight the importance of a detailed history of associated symptoms and concerns when assessing a patient with chronic cough.

Assessment of health status

Cough scores, diaries and visual analogue scales

Cough scores [93], diaries [27, 94, 95], symptom questionnaires [96, 97] and visual analogue scales (VASs) [98] are commonly used in clinical studies to evaluate cough severity, but all lack thorough validation for this purpose. Most symptom-based tools include questions relating to cough frequency, but probably measure a combination of aspects of cough severity that encompass cough frequency and intensity, mood and quality of life. Very little is known about their relationship to other parameters of cough severity, such as objective cough frequency and cough reflex sensitivity. Unlike quality-of-life questionnaires, the different components of health status cannot be identified from cough diaries and VASs.

Cough diaries comprise questions relating to cough frequency [75]. They correlate weakly with objectively measured daytime cough frequency and there is no relationship with nocturnal cough counts [75]. The reproducibility and responsiveness of cough diaries has not been reported and their relationship to other parameters of cough severity, such as cough reflex sensitivity, is not known for patients with chronic cough. In children, the reproducibility of parent-completed cough symptom questionnaires is poor [97]. Self-completed cough diaries for children show good responsiveness and correlate better with objective cough frequency [94]. Interestingly, in children, self-completed cough diaries relate better to objective cough frequency than those completed by their parents [94].

Cough VASs are 100-mm linear scales on which patients indicate the severity of their cough; 0 mm represents no cough and 100 mm the worst cough ever (fig. 8). It is important that patients receive clear instructions regarding the time period during which cough severity is being assessed. Cough VAS score is highly repeatable over a 2-week period in patients with cough due to COPD (within-subject SD 7.8 mm; intra-class correlation coefficient 0.87) [99]. Cough VAS score is also highly responsive when used as an outcome measure in clinical studies of patients with a chronic cough [9, 100, 101]. Cough VAS score relates well to cough-specific quality of life, but not to cough reflex sensitivity [9]. Its relationship to objective cough frequency is not known.

Quality-of-life questionnaires

Quality-of-life measures can be used to facilitate communication with patients and establish information regarding the range of problems affecting them. The impact of illness on health and treatment preferences often differ between patient and physician, and, therefore, quality-of-life considerations should take the patient's perspective into account. The simplest method of assessing quality of life is to ask the patient [102]. Drawbacks to this are that some observers are poor judges of patients' opinions. Assessment of patients using quality-of-life instruments is essentially similar to a structured clinical history, although the outcome parameter is an objective, validated and quantifiable measurement. Quality-of-life domains are usually measured separately to assess emotional and psychological well-being, as well as the physical and practical aspects of daily life. Questionnaires can be generic or disease-specific. Generic instruments are intended for general use, irrespective of illness. Quality-of-life scores from patients can be compared with those in other conditions and even healthy subjects [103]. However, generic instruments lack specificity, do not focus on issues related to patients' conditions and are less responsive to specific interventions compared with disease-specific tools. This has led to the development of three cough-specific quality-of-life questionnaires for use in patients with chronic cough, the cough-specific quality-of-life questionnaire (CQLQ), Leicester Cough Questionnaire (LCQ) and Chronic Cough Impact Questionnaire (CCIQ) [8, 9, 104].

Cough-specific quality-of-life questionnaire

The CQLQ is a recently published 28-item questionnaire that has been developed and tested in North America [8]. It is intended for use in adults with acute and chronic cough. The questionnaire is self-completed and contains a four-point Likert response scale. The items are divided into six domains: physical complaints, extreme physical complaints, psychosocial issues, emotional well-being, personal safety fears and functional abilities. Items for this questionnaire were selected by the investigators and allocated to domains by factor analysis. Factor analysis is a psychometric method that is used to select and allocate items to domains and is based largely on the structure of correlations between items, although the investigators must make a number of subjective decisions throughout the process. Although commonly used in the development of quality-of-life questionnaires, a weakness of factor analysis is that it does not take into account the perception of the clinical relevance of items by the intended population. Concurrent validity, the comparison of an instrument against other standards that provide an indication of the true value for measurement, was assessed for a preliminary

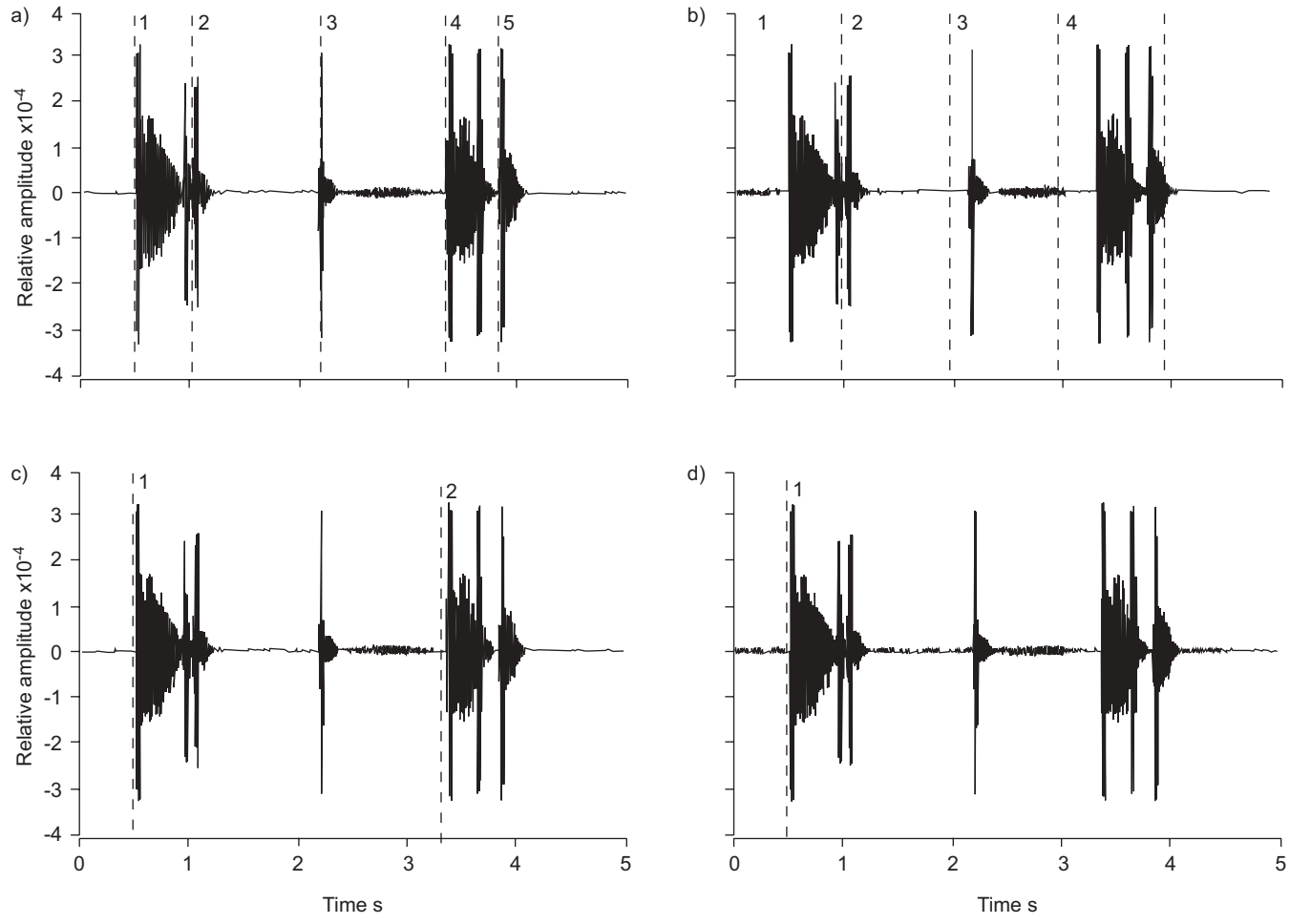

FIGURE 6. Various methods of quantifying coughing: a) explosive cough sounds; b) cough seconds; c) cough breaths; and d) cough epochs (– – –: units of cough counting; numbers represent cough count).

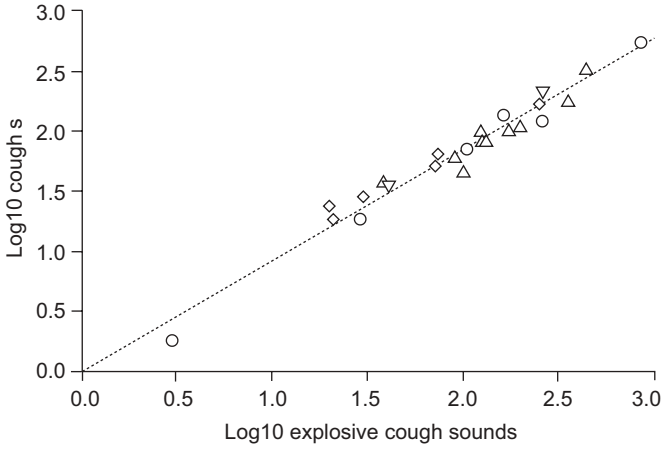

FIGURE 7. A comparison of cough quantification using cough seconds and explosive cough sounds in different diseases. △: cystic fibrosis; ◇: idiopathic pulmonary fibrosis; ▽: chronic obstructive pulmonary disease; ○: asthma. ·····: overall line of best fit.

version of the CQLQ, called the Adverse Cough Outcome Survey (ACOS) [92]. The ACOS correlated moderately with a generic quality-of-life questionnaire (Sickness Impact Profile). The CQLQ is both repeatable and responsive to change in patients with chronic cough, but this was not tested for patients with acute cough. The relationship between CQLQ, cough reflex sensitivity and objective cough frequency is not known. Studies to determine the minimal important clinical difference are under way.

Leicester Cough Questionnaire

The LCQ is a brief, easy to administer and well-validated chronic cough health-related quality-of-life questionnaire developed in the UK [9]. The LCQ comprises 19 items and three domains (physical, psychological and social). The questionnaire is self-completed and contains a seven-point Likert response scale. One of the key differences between the LCQ and the CQLQ is that items for the LCQ were chosen using the clinical impact factor method. This method chooses items that patients label as a problem and ranks them by the importance associated to them. Items were categorised into domains using clinical sensibility. The LCQ was extensively validated against other quality-of-life questionnaires and

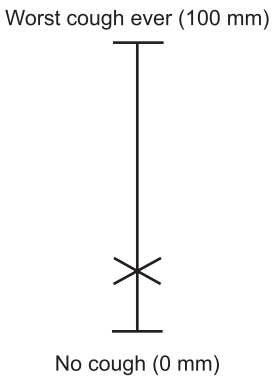

FIGURE 8. Cough visual analogue scale. Please put a cross on the line to indicate the severity of your cough in the last 2 weeks. (Not to scale.)

measures of cough severity [9]. The LCQ has been shown to be repeatable and responsive in patients with chronic cough and responsive in acute cough [9, 105]. Preliminary data suggest that the LCQ correlates with objectively measured cough frequency in patients with chronic cough [82]. The minimal important clinical difference for patients with chronic cough is LCQ total score 1.3 [106]. A study to determine the minimal important clinical difference for patients with acute cough is under way. The LCQ has been a responsive tool when used as a primary outcome parameter in clinical trials of antitussive therapy for patients with chronic cough [107]. The LCQ can be obtained from the online version of [9].

Chronic Cough Impact Questionnaire

The CCIQ is the most recently described cough-specific quality-of-life measure, developed in Italy [104]. It comprises a 21-item questionnaire divided into four domains (sleep/concentration, impact on relationship, impact on daily life and mood) and contains a five-point Likert response scale. The CCIQ is brief, simple to administer and validity has been tested in large numbers of patients. The published report describing the development of the CCIQ lacks important validation data [104]. The repeatability and responsiveness of the questionnaire are not reported, and the minimal important clinical difference and relationship to other markers of cough severity are not known.

Health status in chronic cough

Little is known about the effects of cough on health status. However, preliminary data from studies using cough-specific quality-of-life questionnaires afford an insight. Quality of life is significantly impaired in acute cough; this impairment affects males and females equally [108]. In patients with chronic cough, quality of life is impaired, to a greater extent in female patients compared with males [109] and not related to age, duration or aetiology [92, 110]. The psychological aspects of health status are particularly affected in patients with chronic cough, and there is a high prevalence of depressive symptoms [109–111]. There is good evidence that health status improves significantly after specific therapy for the cough [8, 9].

A recent study comparing a translated LCQ and CQLQ in Turkish patients with chronic cough found a moderate correlation between the questionnaires [112]. Both translated questionnaires showed good concurrent validity and responsiveness, which suggests that the LCQ and CQLQ may be adaptable for patients from a different cultural background.

Conclusions

Chronic cough can have profound effects on quality of life. Its management should include an assessment of health status. The LCQ, CQLQ and CCIQ are intended for use in adult patients with chronic cough. Quality-of-life questionnaires can be used to assess longitudinal changes in patients with chronic cough and identify the specific health domains affected. Quality-of-life questionnaires should be used to supplement objective markers of disease severity and assess the effectiveness of therapeutic interventions in the clinic and clinical trials, and be integral to cost utility analysis.

Future research

Further work is required to determine the minimal clinically important difference for cough health status questionnaires and investigate the relationship between quality of life and other markers of cough severity. Further clinical trials using cough-specific quality of life as outcome measure are awaited.

Recommendations

1) Cough can have profound effects on health status, which can be assessed by cough-specific health status questionnaires.

2) Cough VASs (0–100 mm) should be used to assess cough severity in patients with chronic cough.

3) Patients with chronic cough should be assessed with cough-specific quality-of-life questionnaires in clinical studies.

ANIMAL MODELS OF COUGH

Cough is a reflex defence mechanism and is a most common symptom of many inflammatory diseases of the airways [113]. At present, there are no satisfactory treatments for acute cough, as outlined in a recent review in which over-the-counter cough medicines were assessed [114]. Therefore, the search is on for possible novel antitussive therapies. However, prior to evaluation of such agents in humans, they must be tested in appropriate animal models. The ultimate goal of an animal model is to provide a system in which to elucidate mechanisms and test putative drug candidates. The model needs to be reliable, robust and reproducible and accurately reflect the disease in humans as closely as is possible.

Species

Most pre-clinical studies of neural pathways involved in the cough reflex and the pharmacological regulation of such pathways have been conducted in mice, rats, guinea pigs, rabbits, cats and dogs [115], as well as, more recently, in conscious pigs [116].

In rodents, the cough reflex is difficult to study in anaesthetised animals, since anaesthesia suppresses neuronal conduction and activity in the central nervous system (CNS). However, several investigators have used a conscious rat model of cough to study the effect of potential antitussive therapies. Although many studies have been performed in conscious rats and cough sounds recorded [117], there is much scepticism regarding the ability of these animals to produce a cough that resembles the reflex seen in humans. Indeed, it is

thought that, if cough can be elicited in rats, the main reflexogenic origin of the cough is the larynx rather than the tracheobronchial tree [13]. Indeed, expulsive events originating from the larynx can include expiration reflexes, which are difficult to differentiate from cough. Furthermore, the two reflexes are regulated differently [118]. Other studies have described a murine model of cough [119], but again, certain reservations exist regarding the use of this model given that mice do not have rapidly adapting receptors (thought, along with C-fibre afferents, to play an important role in the cough reflex) and have been found to be lacking in intra-epithelial nerve endings and thus are thought to be without a cough reflex [119]. It has also been shown that mice cannot cough [13], as they cannot generate the energy needed to cough. It is, therefore, probable that investigators using the model are measuring an expiration reflex rather than a true cough.

The use of large animals, such as cats, dogs and pigs, involves a cost element with regard to not only their purchase price but also their feeding and housing and the production of large quantities of drug substance for screening purposes. Although the use of these animals is thus precluded for routine screening, they may be of value in tertiary screening.

The most useful and commonly used model for cough studies in recent years has been the conscious guinea pig [120, 121]. Much information has been gathered using this model regarding both the physiological [122] and the pharmacological modulation of the cough reflex. In these experiments, cough can be detected by putting the guinea pig in a transparent Perspex chamber, exposing it to aerosols of tussive stimuli and measuring changes in airflow, observing the characteristic posture of an animal about to cough and recording the cough sound [123–125].

The guinea pig model of cough

As stated above, the guinea pig is the most useful laboratory animal for experimental studies of chemically induced cough, compared with the rat and rabbit [126]. This animal has been utilised extensively, with cough being induced in conscious animals by inhalation of aerosols of either capsaicin or low pH solutions such as citric acid [10, 123, 127–129]. The guinea pig provides a good model of the human cough reflex, and this has been confirmed in a study showing the similarity in response to both citric acid and capsaicin in humans and the guinea pig [10]. Furthermore, recent *in vitro* data suggest that the isolated guinea pig vagus nerve depolarises in response to tussive stimuli in a similar manner to the isolated human vagus [130].

However, the guinea pig model differs from humans in certain respects. Although both guinea pigs and humans respond to capsaicin in a similar fashion in eliciting cough, capsaicin (and to a lesser extent citric acid) produces tachypnoea in guinea pigs [10]. Furthermore, if the local release of substance P from C-fibre nerve endings contributes in any way to the cough reflex, this may lead to differences in the cough reflex given the sparse population of neuropeptide-containing nerves in human airways. Furthermore, the guinea pig is an obligate nose breather, which may also introduce species differences in the cough reflex. Lastly, the demonstrated activity of certain development molecules with no demonstrated efficacy in humans in animal models has led to the suggestion that data generated from these systems may not be predictive of the situation in humans.

Conscious versus *anaesthetised animals*

In rodents, the cough reflex is difficult to study in anaesthetised animals since anaesthesia suppresses neuronal conduction and activity in the CNS. However, in some species, a suitable depth of anaesthesia, with essentially intact respiratory reflexes, can be obtained and a tussive response easily measured [115, 131, 132]. An example of this is the anaesthetised cat, which has been utilised to analyse both the central effects of antitussives administered intracerebroventricularly [133] and the peripheral effects of compounds administered intravenously [134]. In these experiments, cough in response to mechanical and chemical stimuli is characterised by a deep inspiration followed by an active expiratory effort. In other experiments, cough has been defined, in anaesthetised animals, as a large burst of electromyographic activity in the diaphragm immediately followed by a burst of activity in the rectus abdominus muscle [135]. Interestingly, data recently presented by CANNING *et al.* [131] demonstrated that capsaicin and bradykinin (C-fibre stimulants) are totally ineffective at initiating the cough reflex in anaesthetised guinea pigs even though the cough reflex initiated by mechanical stimuli (largely rapidly adapting receptor-selective) is entirely preserved in the anaesthetised state. Consistent with this data, TATAR and PECOVA [136] showed that, in urethane-anaesthetised guinea pigs, cough could be regularly elicited by mechanical stimulation of the larynx and tracheobronchial tree. Inhalation of a capsaicin aerosol did not change the respiratory pattern and did not elicit any cough. However, these chemical agents elicit a cough reflex in conscious guinea pigs. This suggests that anaesthesia is perhaps best avoided when studying the cough reflex in animal models.

Methodology

A procedure for measuring cough in conscious guinea pigs has been described [10, 120, 124–127]. The guinea pig is placed in a small Perspex box (~1 L in volume) that allows free movement during exposure to aerosols. Some investigators have used a double-chambered body plethysmograph with some success [137]. Airflow through the box is provided by compressed medical air *via* a flow regulator at ~600 mL·min^{-1}, with changes in airflow induced by respiration and coughing detected by a pneumotachograph, amplified *via* a pressure transducer and recorded on a chart recorder or computer. Cough sounds are amplified and recorded *via* a microphone sited in the cough chamber and recorded concurrently on the computer. Tussive agents (capsaicin, citric acid, *etc.*) are delivered by aerosol using an ultrasonic nebuliser, with an output of ~0.4 mL·min^{-1} and delivering a median particle diameter of ~1 μm, connected to the airflow port. The animal is exposed for a defined period, usually $\leqslant$10 min, depending on the tussive agent used. A dose–response curve to the chosen stimulus should be constructed, and a submaximal dose chosen for further studies. Other protocols include using citric acid aerosol in gradually increasing concentrations (0.05–1.6 M), each for 30 s. Cough is recorded during the 30-s inhalation of each concentration of the tussigen and during the subsequent 60-s observation time. Therefore, the interval between exposures is 1.5 min. 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 [138].

It can be extremely difficult to differentiate cough from other upper airway reflexes. Therefore, coughs should be assessed and counted by a trained observer using three different methods in order to ensure that only coughs are counted and that sneezes and augmented breaths are excluded. The three methods are as follows. 1) Observation (or video recording) by an observer trained to differentiate between coughs and sneezes and to recognise the changes in posture (splaying of the front feet and forward stretching of the neck) and characteristic opening of the mouth associated with cough. 2) Pressure or airflow changes reflecting the deep inspiration and explosive expiration occurring during cough. 3) Sound, *i.e.* the characteristic sounds of a guinea pig cough. Results can be expressed as coughs per minute or coughs per 10 minutes, and comparisons made with vehicle-control-treated animals.

Tussive stimuli

The cough reflex can be elicited by electrical, mechanical (in anaesthetised animals) or chemical stimulation, as well as by changes in ion concentration or osmolarity in the mucosal surface fluid, of sensory afferents (in the larynx, trachea or bronchial mucosa) or by stimulation of the CNS. More recent studies have utilised the irritant capsaicin and low pH solutions (*e.g.* citric acid) to study the cough response. Citric acid confers the advantage of allowing repeated cough measurements without the occurrence of tachyphylaxis, whereas repeated exposure to capsaicin is known to result in tachyphylaxis, preventing the production of a reproducible cough response in the same animal [25, 138]. Different methods of stimulation may involve different populations of sensory afferent, and there has been much discussion in the literature regarding the selectivity of agents for different fibre types, *e.g.* the use of capsaicin as a selective C-fibre stimulant [124, 139].

Experimental design

Animals should be housed under controlled conditions with frequent changes of bedding, as the build-up of ammonia in cages has been shown to influence the cough response to citric acid [140]. M.G. Belvisi and co-workers (unpublished data) have shown that the cough response to a given stimulus varies greatly from guinea pig to guinea pig, but that repeated assessments within the same animal are fairly reproducible with citric acid but not with capsaicin. Therefore, it is probably wise to perform experiments in separate animals with test group data compared with vehicle-treated animals rather than in the same animal before and after vehicle/drug treatment. However the inherent variability in conscious models suggests that large numbers, of between eight and 12, should be utilised in studies. The same authors have not found it necessary to precondition guinea pigs to accept aerosol exposure in the challenging box.

Disease models

It is now recognised that many pulmonary disorders are associated with enhanced cough. However, the mechanisms involved in these exaggerated responses are not known.

Allergic models

Most models have been configured in the guinea pig and have demonstrated increased coughing in response to capsaicin $\geqslant$1 day after antigen challenge in sensitised animals [141, 142]. The increased tussive response is associated with eosinophilia. Tachykinins appear to play a role in the augmented cough response in allergic guinea pigs, since enhanced cough response to capsaicin, following antigen challenge, has been shown to be suppressed by neurokinin $(NK)_1$ and NK_2 and dual (NK_1/NK_2) receptor antagonists [141]. An enhanced cough response to mechanical stimulation of the trachea has also been seen in anaesthetised dogs sensitised and challenged with ragweed antigen [143].

Models of post-nasal drip

Chronic disorders of the nose and sinuses (*e.g.* allergic and nonallergic rhinitis, sinusitis and vasomotor rhinitis) are also common causes of chronic cough [7]. This phenomenon has been demonstrated in animal models in that stimulation of nasal afferents with capsaicin has been shown to enhance experimentally induced cough in cats and guinea pigs following nasal antigen challenge in sensitised animals [137, 138].

Angiotensin-converting enzyme inhibitors

Chronic systemic treatment with several angiotensin-converting enzyme inhibitors potentiates the irritant-induced and spontaneous cough in guinea pigs [124, 144, 145].

Cigarette smoke exposure

Chronic mainstream or sidestream cigarette smoke exposure can lead to an enhanced cough response to tussive stimuli in a guinea pig model [146–148]. In some cases, this is associated with airway eosinophilia and neutrophilia [146]. In one study, environmental tobacco smoke increased citric-acid-induced cough and bronchoconstriction, and this was blocked, in part, by an NK_1 receptor antagonist injected into the nucleus *tractus solitarius* [147].

The inflammatory processes present in some of these models have been suggested to alter the phenotype and/or the excitability of sensory airway afferents [149-151], which may lead to increased sensory input into the central control mechanisms that elicit cough. The mechanisms involved in this increased responsiveness have not yet been clearly defined, but the elucidation of specific pathways that sensitise this reflex may lead to the development of more effective therapeutics.

Conclusion

Cough, irrespective of which airways disease it is associated with, represents an unmet clinical need. There are no effective treatments available for cough, and those that are available have been shown to be ineffective [114]. It is, therefore, essential to identify and develop new treatments for cough. In order to achieve this end, it is necessary to develop and utilise an animal model of cough that accurately reflects the condition in humans. The guinea pig shows distinct advantages over other small rodents in that it coughs in response to given stimuli in the conscious state and that the physiology of the cough response reflects that in humans, and, for these reasons,

the present task force recommends the use of this species as an investigational tool.

Recommendations

1) The most useful animal model of cough is the conscious guinea pig.

2) Both sound and airflow should be used to define a cough event.

DESIGN AND CONDUCT OF CLINICAL TRIALS OF ANTITUSSIVE DRUGS

Since acute cough is so common and chronic cough such a distressing and disabling symptom, it is unsurprising that novel antitussive treatments, both specific and nonspecific, are currently undergoing development. Cough in humans differs from that in animals, and compounds that may be highly effective in animal models, *e.g.* NK receptor antagonists, may fail in the clinic [152]. The demonstration of efficacy or lack of efficacy in humans is fraught with difficulties, including the choice of model, recruitment of subjects, choice of end-point, applicability to clinical research and overcoming the placebo effect. This section provides guidance in order to maximise the chance of providing a genuine result from any clinical pharmacology study.

Choice of model

Cough in clinical scenarios in which drug therapy is required differs from evoked cough in normal volunteers because the cough reflex is sensitised in disease. This is true of both acute [29] and chronic cough [22, 24], in which cough challenge dose–response curves are shifted to the left and return towards normal after either spontaneous resolution [29] or drug treatment [101, 153, 154]. The molecular cause of this cough reflex hypersensitivity is unknown. Airway inflammation leading to peripheral hypersensitivity of cough receptors would appear to be a reasonable explanation when the airways are inflamed, such as in acute viral bronchitis. A proliferation of nerves containing the putative cough receptor transient receptor potential vallinoid (TRPV)1 has been demonstrated [155], although expression of this receptor may not be confined to the sensory neurons [156] Animal work supports the hypothesis that sensitisation occurs in the vagal ganglia located in the relay stations of the nodose and jugular parasympathetic ganglia [157]. Central modulation and hypersensitivity of the cough reflex certainly occurs [31]. Thus, in designing studies to test for antitussive activity, knowledge of the putative mode of action is vital. For example, leukotriene antagonists have been demonstrated to be effective in cough variant asthma [153], and are one of the mainstays of clinical treatment when inhaled steroids alone prove ineffective. When tested in cough challenge models in classic asthma, however, they are ineffective [158]. This is because the heightened cough reflex is due to the asthmatic inflammation, particularly the clustering of mast cells around airway nerves [159]. In the absence of inflammation, no beneficial effect is seen from these agents. In contrast, in the development of a TRPV1 capsaicin receptor antagonist, it would not be unreasonable to assume that an effective agent should block capsaicin cough challenge in normal volunteers.

Cough is a vital protective reflex. Conditions with a demonstrably reduced cough reflex, such as stroke and Parkinson's disease, are associated with an increased incidence of aspiration and pneumonia. Care is required, therefore, in the design of clinical studies looking at nonspecific cough suppression. Thus, although the suppression of cough due to viral bronchitis is unlikely to have adverse consequences, suppression of cough in studies in which the patient population has a chronic productive cough may lead to inspiration of secretions and clinical worsening. However, even in situations in which there is increased sputum production, cough reflex sensitivity may be exaggerated. Whether the suppression of this abnormal reflex sensitivity to normal levels results in an improvement or deterioration in clinical status remains to be determined from future studies.

Normal volunteer studies

Studies in normal volunteers are almost invariably performed using cough challenge methodologies. Two challenges are commonly used, citric acid and capsaicin [25]. The challenges do not measure the same reflex sensitivity since there is little correlation between the sensitivities of the two challenges in an individual [23]. There is little to choose between the two modalities of challenge, and both have been used to demonstrate antitussive effects. Lack of knowledge regarding how these responses are related to what occurs in the clinic is a major weakness of cough challenge in normal subjects.

Subject selection should include a screening visit during which challenge is performed. There is a strong argument for excluding subjects who show cough responses only at high challenge concentrations. In these subjects, it is difficult to demonstrate cough suppression because they are already approaching the maximum tolerable dose, and nonspecific effects, such as a burning sensation with capsaicin or a choking sensation with citric acid, mask any therapeutic effect [18].

The placebo response

In normal volunteer challenge studies, the placebo response is a major factor. A reduction in cough challenge of >30% can be seen with placebo, and this effect may continue for several hours [160]. Indeed, the pharmacokinetics of placebo activity in cough challenge have been modelled [39]. A number of strategies have been used in an attempt to minimise this, *e.g.* performing a screening experiment with a placebo described as an antitussive and excluding responders, randomisation of challenge administration and using subjects previously known to exhibit a small response to placebo. None of these strategies appear to be effective. Studies should be designed with the probability that a considerable placebo response may occur whatever precautions are taken.

Sex

Females exhibit a heightened cough reflex [49, 51]. On average, females cough twice as much as males to any given cough challenge, or, conversely, C_2 and C_5 are consistently lower in female subjects. The cause of this sex-related difference is unknown, but it also occurs in patients attending the cough clinic [22] and those receiving angiotensin-converting enzyme inhibitors [161]. Provided this fact is understood in the design of exclusion criteria, there is no reason for differential treatment of males and females within normal volunteer studies.

Normal volunteer studies with airway inflammation

In an attempt to obtain a model that has some of the elements of clinically relevant cough in normal subjects, smokers with cough have been recruited to clinical studies [162]. These subjects provide another end-point, *i.e.* their smoker's cough, and also represent a state in which there is airway inflammation. In the conduct of such studies it should be remembered that there is a profound effect of cigarette smoking on the cough reflex [163]. Indeed, smoking reduces cough reflex sensitivity and approximately halves the number of coughing episodes per hour. Thus, the key time for observing drug effects is after overnight abstinence, with the subject refraining from smoking for the duration of the study.

Although giving a model of ''natural'' cough, the use of smokers may confound the study of certain groups of agents. In particular, the negative effect of smoking on inhaled steroid efficacy has been well described [164].

Studies in patients

Perhaps the major difficulty in the study of clinically important cough is defining the study population.

Acute cough

Acute cough is a benign self-limiting condition. Having recruited a study population, there is an inevitable marked spontaneous regression back to normality. This, coupled with a particularly powerful placebo effect [165, 166], makes the study of subjects with acute cough highly challenging. These factors, but particularly the daily variation in baseline, make crossover studies virtually impossible. Since there is individual variability in response, any parallel group study must be of a large size in order to convincingly show efficacy. Indeed, the only robust study demonstrating antitussive efficacy in acute cough is a meta-analysis of >300 subjects [167]. This study undertook laborious aural analysis of cough data. More recently, digital cough recorders offer the opportunity for objective cough frequency measurement, which may become the gold standard for such studies in future [73, 80, 83, 160]. Until then, subjective cough scores, VASs and, again more recently, quality-of-life measurements may be used as end-points [8, 9].

Post-viral cough

Cough which persists after an arbitrarily defined 2-week period may be termed post-viral cough [6]. Again, spontaneous remission of symptoms is the rule, but this group of patients have been successfully used to demonstrate, for example, the effect of inhaled steroids on post-viral cough. Recruitment is obviously quite seasonal and can be very slow.

Chronic cough

Confusion exists in the categorisation of patients with chronic cough. This, in part, explains the wide variation in the proportion of patients diagnosed with the various cough syndromes in reports from cough clinics [168]. It is thus perhaps better to define the study population according to the presence or absence of a particular diagnostic result rather than use diagnostic labels about which there is disagreement. Patients with reflux disease may, through aspiration, show bronchial hyperresponsiveness [169]. Similarly, patients with asthma may also exhibit reflux symptoms [170]. In the study of asthmatic cough, it is better to define the study population on the basis of showing methacholine bronchial hyperresponsiveness or sputum eosinophilia. Similarly, patients with reflux disease should have a proven diagnosis from objective testing in the knowledge that many other patients will be excluded who also have reflux disease, but at least this is a certain entry criterion. A similar situation exists in studies of classic asthma where entry into a study is predicated by reversibility testing, even though only a small percentage of asthmatics fit into these reversibility criteria. One interesting group of patients for study are those who fail to meet the diagnostic criteria for a cough syndrome or fail multiple trials of appropriate therapy. They have been termed idiopathic cough patients and are frequently found in cough clinics. Unlike the situation in acute cough, these patients do not show placebo effects, and, since cough is reproducible, crossover studies are possible, limiting the number of subjects required [107].

Recommendations

1) The experimental model in which antitussive drugs are tested depends greatly upon the mode of action of the agent.

2) Normal volunteer studies should be designed in the knowledge that a large placebo effect is likely.

3) In acute cough, parallel group studies are required.

4) In chronic cough, the patient population studied should be defined by a diagnostic test.

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