Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point

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Background: Inhaled capsaicin elicits cough reproducibly in human subjects and is widely used in the study of cough and antitussive therapies. However, the traditional end points C2 and C5 (the concentrations of capsaicin inducing at least 2 or 5 coughs, respectively) display extensive overlap between health and disease and therefore might not best reflect clinically relevant mechanisms.

Objectives: We sought to investigate capsaicin dose responses in different disease groups.

Methods: Two novel capsaicin cough challenges were compared in patients with chronic cough (CC; n = 20), asthmatic patients (n = 18), and healthy volunteers (HVs; n = 20). Increasing doubling doses of capsaicin (0.48-1000 μmol/L, 4 inhalations per dose) were administered in challenge 1, whereas the order of the doses was randomized in challenge 2. A nonlinear mixed-effects model compared dose-response parameters by disease group and sex. Parameters were also correlated with objective cough frequency.

Results: The model classified subjects based on maximum cough response evoked by any concentration of capsaicin (E_{max}) and the capsaicin dose inducing half-maximal response (ED_{50}). HVs and asthmatic patients were not statistically different for either parameter and therefore combined for analysis (mean ED_{50}, 38.6 μmol/L [relative SE, 28%]; mean E_{max}, 4.5 coughs [relative SE, 11%]). Compared with HVs/asthmatic patients, patients with CC had lower ED_{50} values (14.7 μmol/L [relative SE, 28%], $P = .008$) and higher E_{max} values (8.6 coughs [relative SE, 11%], $P < .0001$). E_{max} values highly correlated with 24-hour cough frequency ($r = 0.71$, $P < .001$) and were 37% higher in female compared with male subjects, regardless of disease group ($P < .001$).

Conclusions: Nonlinear mixed-effects modeling demonstrates that maximal capsaicin cough responses better discriminate health from disease and predict spontaneous cough frequency and therefore provide important insights into the mechanisms underlying CC. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *Capsaicin, cough, challenge, human, model, nonlinear, reflex, response, sensitivity*

Chronic cough (CC) can be a debilitating symptom experienced by patients with and without underlying respiratory disease. Current treatments are often ineffective or not well tolerated. Objective measures of cough, which improve our understanding of the underlying mechanisms in patients with CC, would be invaluable and might enable the phenotypic characterization of subgroups of patients. Furthermore, in early-phase clinical drug development, such measures could provide a clear scientific rationale for further testing in patients.

Inhaled tussive agents, such as capsaicin and citric acid, evoke coughing reproducibly in healthy volunteers (HVs) and patients. In a standard cough challenge test, single doubling doses are inhaled, and cough sensitivity is arbitrarily defined as the concentration of capsaicin inducing at least 2 or 5 coughs (ie, C2 and C5, respectively).[1] C2 and C5 measurements are reproducible[2] and relatively easy to perform,[1,3] but only weakly correlate with spontaneous cough frequency, as measured based on 24-hour ambulatory cough counts.[4,5] In addition, although patients with CC have a lower C2/C5 value on average compared with healthy control subjects, there is substantial overlap in the data.[6] Given the far higher 24-hour spontaneous cough rates observed in patients with CC[4,7] compared with healthy subjects,[5] this is surprising and might mean that C2/C5 values are not capturing the most relevant pathophysiologic mechanisms.

We hypothesized that formal characterization of the full pharmacodynamic relationship between tussive agent dose and cough response would suggest other parameters that might better differentiate patients with CC from control subjects. First, a leftward shift in the dose-response curve captured by the capsaicin dose inducing half-maximal response (ED_{50}) would imply a reduction in the threshold for initiation of coughing, as has been suggested in HVs taking angiotensin-converting enzyme inhibitors.[8] Second, an increase in the steepness of the slope would indicate an amplification of the number of coughs evoked by any given dose of inhaled tussive agent. Third, the maximum cough response evoked by any concentration of capsaicin (E_{max}) would reflect the maximum capacity of a subject to cough when inhaling increasingly potent stimuli. A plateau at E_{max} implies the presence of a regulatory inhibitory control mechanism limiting the maximum possible number of coughs. To the best of our knowledge, these parameters have never been studied in full

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Abbreviations used

C2: Concentration of capsaicin inducing at least 2 coughs
C5: Concentration of capsaicin inducing at least 5 coughs
CC: Chronic cough
ED_{50}: Capsaicin dose inducing half-maximal response
E_{max}: Maximum cough response evoked by any concentration of capsaicin
FVC: Forced vital capacity
HV: Healthy volunteer
TRPV1: Transient receptor potential vanilloid 1

dose-response curves of cough responses but have been applied to bronchodilator responses to β_2-agonists.[9,10]

The aim of this study was to fully characterize capsaicin dose response in patients with CC, asthmatic patients, and HVs through the application of nonlinear mixed-effects modeling, the gold standard for analysis of dose-response curves in clinical pharmacology.[11] To this end, we have designed and tested new capsaicin cough challenges. Administration of capsaicin by means of tidal breath inhalation seems to amplify the differences in cough responses between health and disease,[12] but the precise dose inhaled is difficult to estimate because it varies with patient minute ventilation. In contrast, standard single-breath inhalation (modified to limit inspiratory flow rate) allows tight control of the dose delivered but poorly differentiates health from disease.

Therefore we designed a challenge to combine the advantages of both methods. Each dose of capsaicin was administered 4 times by using repeated single-breath inhalations from a dosimeter enabling the calculation of an average cough response at each dose. Furthermore, the challenge was continued up to the maximum tolerated dose to determine maximum responses. Compared with the standard cough challenge, this design shows greater similarity to conscious cough challenges in animals[13] and might have greater potential for improved translation of findings from animals to human subjects. Some of these data have been published in abstract form.[14]

METHODS

Subjects

Twenty HVs, 18 patients with mild-to-moderate asthma, and 20 patients with unexplained CC of a duration of greater than 8 weeks were recruited. Asthmatic patients all had documented evidence of bronchial hyperreactivity (positive methacholine challenge result, reversibility in FEV_1 >12%, or both), and those with unexplained CC had undergone full investigation and treatment trials for possible causes of cough in a specialist cough clinic (see the Methods section in this article's Online Repository at www.jacionline.org for further details). Equal numbers of male and female subjects were recruited to each group based on the heightened cough responses observed in female subjects in standard cough challenge protocols.[15,16] The study was approved by the local Research Ethics Committee (REC 09/H1008/119) and registered at www.controlled-trials.com (ISRCTN65122210). All participants provided written informed consent.

A sample size calculation could not be performed for the parameters to be studied because no previous data were available; however, based on the standard C5 end point and assuming an intersubject SD of 0.63 $\mu mol/L^2$, a sample size of 20 control subjects and 20 patients with CC would have 90% power to detect a 2 doubling dose difference in C5 values.

Study design

Subjects attended 2 visits at least 24 hours apart. At visit 1, spirometry (FEV_1 and forced vital capacity [FVC]), height, and weight were measured, and the Hospital Anxiety and Depression Scale the State-Trait Anxiety Index were completed to assess any influence of mood on cough responses.

At both visits, a capsaicin cough challenge test was performed with a dosimeter (Koko Dosimeter; Ferraris Ltd, Hertford, United Kingdom) with a fixed baffle and inspiratory flow rate limitation. After each inhalation, the number of coughs in the first 15 seconds was recorded. Subjects were instructed not to suppress coughing and attached to an ambulatory cough monitor throughout (VitaloJAK; Vitalograph, North Buckinghamshire, United Kingdom), which was later used to verify number of coughs. Spirometry was repeated at the end of each challenge.

Challenge 1: Ascending doubling doses. At visit 1, increasing doubling doses of capsaicin were administered, ranging from 0.48 to 1000 μmol/L capsaicin (12 doses in total). Inhalations were 30 seconds apart, and each dose of capsaicin was inhaled 4 times (Fig 1, *A*).[17]

Challenge 2: Randomized cough challenge. At visit 2, 8 doses were individually determined from the first cough challenge to include the dose that induced an average of 5 coughs (or maximum tolerated dose), 6 doubling doses below this, and a placebo dose. Each dose was again inhaled 4 times, but the order of the doses was randomized to form a single presentation block and then rerandomized for the second, third, and fourth presentation blocks (Fig 1, *B*).[18] The inhalations were separated by 1 minute, and both subject and researcher were blinded to the randomization codes.

Statistical analysis and modeling

Baseline subjects' characteristics, including age, sex, and lung function, were compared by means of ANOVA. Statistical significance of ANOVA was set to the 1% level for consistency with the modeling approach used.[19] Detailed reviews of the concepts underlying the modeling techniques and their applications have been described previously.[19-21]

Model structure. All individual measures of cough from both challenges (4001 observations) were pooled along with capsaicin doses and covariates to constitute the dataset on which a population dose-response model was built. The analysis was performed in NONMEM 7.1 software (ICON Development Solutions, Ellicott City, Md)[22] by using a nonlinear, mixed-effects, maximum likelihood approach[19-21] adapted to the discrete nature of the data (counts) given the Laplace estimation method.[23] To this aim, the response variable (number of coughs) was assumed to follow a Poisson probability density distribution,[24] according to equation 1:

$$P(Y_i = n) = \frac{e^{-\lambda_i} \times \lambda_i^n}{n!}, \quad [1]$$

where $P(Y_i = n)$ corresponds to the probability of subject i having a number of coughs per interval of time equal to n (nonnegative integer values), and λ_i is the individual mean count response expressed as a function of capsaicin dose baseline response and drug specific model parameters, as defined by equation 2 (see Fig E1 in this article's Online Repository at www.jacionline.org for graphical presentation)[25] as follows:

$$\lambda_i = E_0 + \frac{Emax_i \times D^\gamma}{ED50_i^\gamma + D^\gamma}, \quad [2]$$

where E_0 represents the mean count at baseline, is the individual maximum number of coughs, $ED50_i$ corresponds to the potency of capsaicin (dose of capsaicin inducing half of the maximum effect) for the *ith* individual, D is the dose administered, and γ is the steepness factor (Hill factor) of the sigmoid dose response.

If supported by the data, drug-specific model parameters were coded as exemplified for the parameter E_{max} in equation 3:

$$Emax_i = \theta \times e^{\eta_i}, \quad [3]$$

where θ represents the mean estimate in the population, and η_i is the subject-specific random effect assumed log-normally distributed with zero mean and variance Ω.

A tachyphylaxis parameter *(K_i)* was implemented to describe the decrease in E_{max} to repeated capsaicin inhalations, as defined according to equation 4:

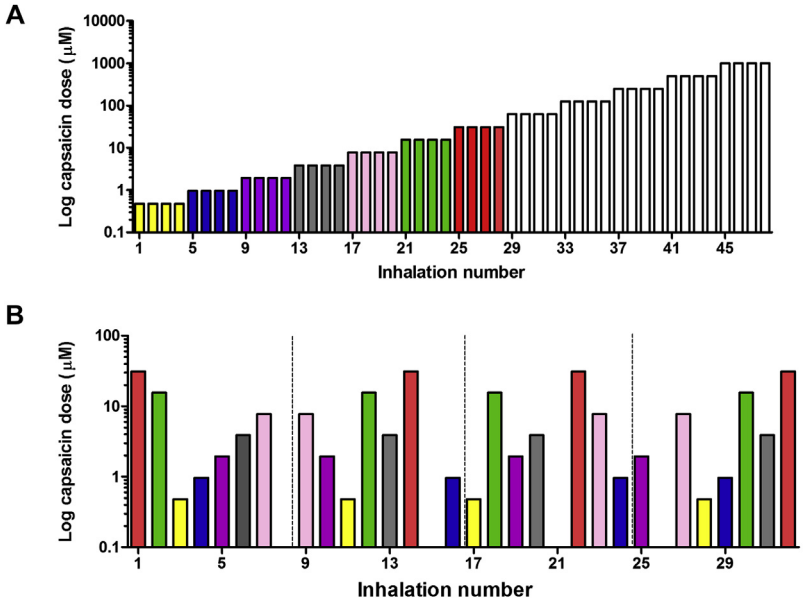

FIG 1. Challenge designs. **A,** Challenge 1: increasing doubling dose challenge. In this example the maximum tolerated dose inhaled was 31.3 μmol/L *(red bars)*, and therefore the *white bars* represent the higher doses not inhaled. **B,** Example of challenge 2: randomized order of doses inhaled in challenge 1. Each *bar* represents a single-breath inhalation of capsaicin, and the *gaps* represent placebo inhalations.

$$E\max_i(j+1) = E\max_i(j) \times e^{-K_i}, \quad [4]$$

where K_i is the tachyphylaxis parameter for subject *i*. For any given single-breath inhalation of capsaicin, *j* was equal to the number of preceding doses that were the same or higher (see Fig E1). Thus tachyphylaxis occurred only for inhalations that were preceded by a higher or equal dose.

Model building and selection. Comparison between competing nested models was performed by using standard procedures,[26,27] including the likelihood ratio test, evaluation of the precision in parameter estimates, and scientific plausibility. Addition of an extra parameter in the model structure was deemed relevant when reaching a *P* value of .01. The decision to set a stringent *P* value for model selection was made to mitigate the risk of spurious results because of the small number of subjects in this study.[19] The model was considered final when the best fit to the data was obtained under the parsimony principle and after inclusion of statistically meaningful and clinically relevant subjects' covariates.

Statistical analysis of covariates. An initial screening for potential predictors of the intersubject variability in structural model parameters (Ω) was performed given the pool of available subject covariates (group, age, sex, weight, FEV_1, FVC, and questionnaire scores) by using a generalized additive modeling approach[28] incorporated into the software R 2.13.[29] Informative covariates detected by using generalized additive modeling were included within the nonlinear mixed-effects dose response model in NONMEM to identify statistically significant covariates ($P < .01$) based on standard model covariate-building approaches,[27] as well as to quantify their explanatory value on the variability in the population model parameters.

Correlation of parameters with spontaneous cough frequency. A further 20 patients with CC and 20 HVs (see Table E1 in this article's Online Repository at www.jacionline.org) were studied to explore whether ED_{50} and E_{max} values had significant relationships with spontaneous cough frequency. Immediately after a 24-hour period of objective cough monitoring (VitaloJAK), all subjects completed challenge 1. Individual E_{max} values were calculated as the highest average number of coughs induced by any capsaicin dose, and ED_{50} values were calculated as the dose inducing half the maximal response. For details of cough monitoring, see the Methods section in this article's Online Repository.

RESULTS

Subjects' characteristics

Twenty HVs, 18 asthmatic patients, and 20 patients with CC completed the study, with equal numbers of male and female subjects in each group. Subjects' characteristics are summarized in Table I. There were no statistical differences in age, body mass index, lung function, anxiety, or depression scores between groups.

Model structure

The structural model was successfully implemented in NONMEM. Baseline cough frequency *(E_0)* was fixed to zero, which is consistent with the average cough response to inhaled placebo (saline). The mean θ values of the dose-response parameters E_{max}, ED_{50}, K, and γ were precisely estimated by the model, with the relative SE of the means ranging from 10% to 30% (Table II). E_{max}, K, and ED_{50} (but not γ) values could be individualized by the addition of a random effect. Of interest, the ED_{50} value demonstrated a much greater degree of variability between individual subjects (138% to 144% coefficient of variation) when compared with E_{max} (43% to 49% coefficient of variation) and K (72% coefficient of variation) values.

Disease group. HVs were not significantly different from asthmatic patients ($P = .03$ for E_{max} and $P > .8$ for ED_{50}). Therefore subjects were dichotomized into (1) HVs/asthmatic patients (HV/A group) and (2) patients with CC. Subject group explained a moderate proportion of the between-subject variability in E_{max} and ED_{50} values, decreasing the variance Ω by 38% and 12%, respectively. Compared with the HV/A group, patients with CC had significantly higher E_{max} values ($P < .0001$) and significantly lower ED_{50} values ($P = .008$; Fig 2, *A*, and Table II).

Sex effects. Sex did not significantly predict ED_{50} values ($P = .09$). However, sex partially explained the between-subject

TABLE I. Comparison of subjects' characteristics and ANOVA outcomes

Variable	HVs	Asthmatic patients	Patients with CC	*P* value (ANOVA)
Sample size	20	18	20	
Age (y)*	57.1 (15.7)	51.2 (13.5)	58.8 (13.5)	.172
Sex (male/female)	10/10	9/9	10/10	
BMI (kg/m^2)*	25.1 (3.9)	28.5 (3.9)	27.3 (3.4)	.025
Smoking history (pack years)†	0.0 (0.0)	0.0 (0.7)	0.0 (0.5)	
FEV_1 (% predicted)*	110.9 (14.8)	98.3 (18.9)	100.2 (12.7)	.032
FVC (% predicted)*	111.3 (17.7)	110.7 (18.1)	106.8 (11.8)	.643
HADS-depression*	2.7 (3.8)	2.1 (2.17)	4.0 (2.8)	.153
HADS-anxiety*	4.1 (3.0)	5.0 (3.4)	6.6 (4.4)	.099
STAI-state*	26.2 (7.7)	26.6 (7.2)	27.9 (8.3)	.767
STAI-trait*	30.1 (8.2)	35.3 (11.2)	37.3 (10.7)	.075

BMI, Body mass index; *HADS*, Hospital Anxiety and Depression Scale; *STAI*, State-Trait Anxiety Index.
*Mean (SD).
†Median (interquartile range).

TABLE II. Summary of final population model parameters and imprecision estimates

Dose-response parameters	Group	Sex	Mean estimates θ (RSE [%])	Between-subject variability Ω (CV [%])
E_{max} (no. of coughs)	HV/A	Male + female subjects	4.5 (11)	49
		Male subjects	3.5 (17)	43
		Female subjects	5.5 (11)	43
	CC	Male + female subjects	8.6 (11)	49
		Male subjects	6.8 (16)	43
		Female subjects	10.7 (10)	43
ED_{50} (μmol/L; both challenges)	HV/A	Male + female subjects	38.6 (30)	144
	CC	Male + female subjects	14.7 (28)	
ED_{50} (μmol/L; challenge 1)	HV/A	Male + female subjects	25.8 (27)	138
	CC	Male + female subjects	10.8 (27)	
ED_{50} (μmol/L; challenge 2)	HV/A	Male + female subjects	72.0 (30)	138
	CC	Male + female subjects	21.3 (29)	
Tachyphylaxis effect (K)	All groups	Male + female subjects	0.16 (15)	72
Hill factor γ	All groups	Male + female subjects	2.0 (10)	NA

The mean E_{max} value is expressed as the number of coughs, and the mean ED_{50} value is expressed as the capsaicin dose (in micromole per liter). E_{max} values decreased according to K (see text for details). Separate estimates for male and female subjects are shown only where there was a significant sex effect on the dose-response parameter.
CV, Coefficient of variation; *HV/A*, healthy volunteer/asthmatic patient group; *NA*, not applicable; *RSE*, relative standard error.

variability in E_{max} values, decreasing variance Ω by a further 23%. Regardless of disease group, the estimated mean E_{max} value was 37% higher in female compared with male subjects ($P < .001$; Fig 2, *B*, and Table II).

The distribution of an individual subject's E_{max} and $\log(ED_{50})$ values obtained from the final population model are displayed in each panel of Fig 3 compared by group and sex. A clear shift in the distribution is observed for both parameters when accounting for group, with higher E_{max} and lower $\log(ED_{50})$ individual estimates for patients with CC compared with the HV/A group. When split by sex, both groups reveal a higher E_{max} value for female than male subjects (Fig 3, top panels), whereas no clear trend is visible in the distribution of $\log(ED_{50})$ values, reflecting no significant influence of sex on the potency of capsaicin to elicit cough (Fig 3, bottom panels).

Challenge design. Challenge design had a significant effect on the potency of capsaicin to elicit cough, with an approximately 2-fold higher ED_{50} value for challenge 2 (ie, lower potency with randomized order of doses) compared with challenge 1 (increasing doubling doses, Fig 4 and Table II). E_{max} values were not influenced by challenge design.

Tachyphylaxis effect. The dose-dependent tachyphylaxis effect K on E_{max} cough responses could be estimated, with an average reduction in mean E_{max} cough response of 15% (ie, $1-e^{-K} = 0.15$ for mean K = 0.16) for any capsaicin inhalation that was preceded by an equal or higher dose (Table II and see Figs E2 and E3 in this article's Online Repository at www.jacionline.org). Fig 5 demonstrates that the observed experimental data are accurately predicted by the final model individual mean cough response after the inclusion of the tachyphylaxis parameter.

Other covariates. None of the remaining covariates were found to have a significant effect in the model ($P > .01$), including lung function (FEV_1 and FVC), depression and anxiety questionnaire scores (Hospital Anxiety and Depression Scale and State-Trait Anxiety Index), weight, and age.

Correlation of dose-response parameters with 24-hour spontaneous cough frequency. E_{max} values explained a much greater proportion of the variability in 24-hour

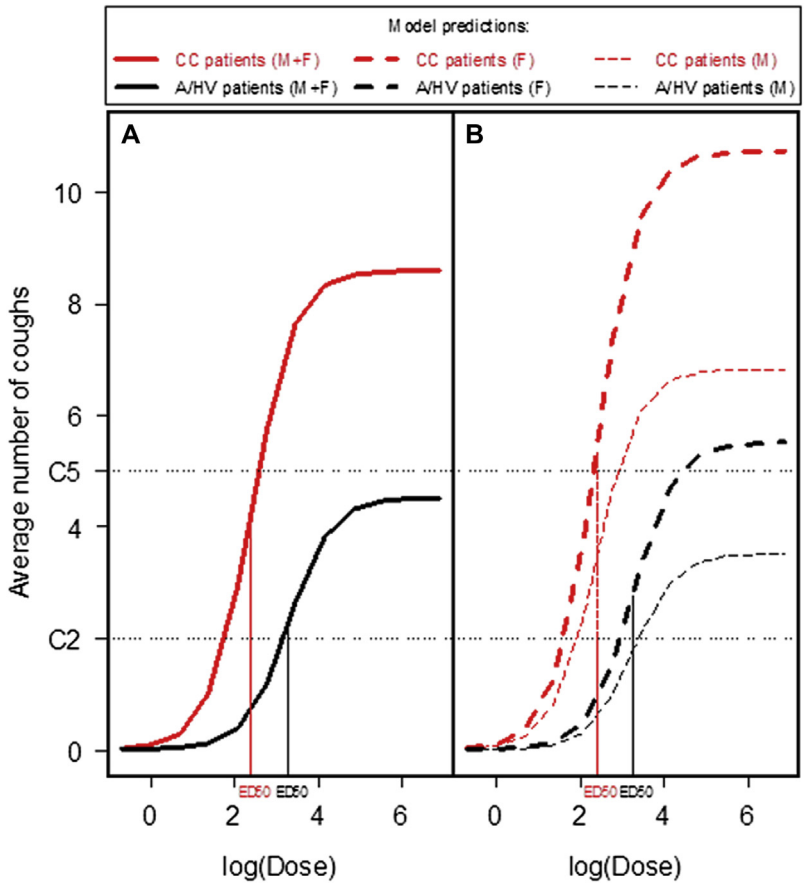

FIG 2. A, Average capsaicin dose-response curves of model predictions for patients with CC and subjects in the HV/asthmatic patient group *(A/HV)*. **B,** Average capsaicin dose-response curves of model predictions for male and female patients with CC and subjects in the HV/A group. Model predictions of cough frequency are averaged at each dose across both challenge designs. Mean log(ED_{50}) estimates are indicated by *vertical lines*. Standard cough challenge end points, C5 and C2, are indicated by *horizontal dotted lines*.

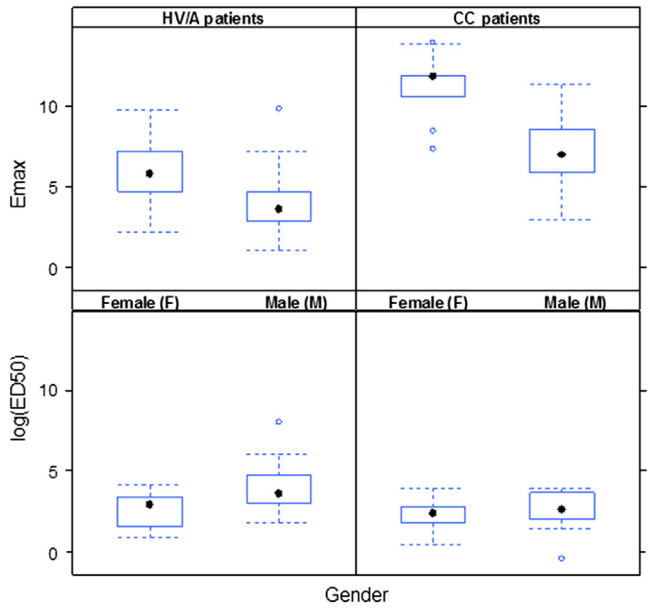

FIG 3. Box and whisker plot of individual E_{max} and log(ED_{50}) estimates stratified by disease group and sex.

cough frequency (50%; $r = 0.711$, $P < .001$) than ED_{50} values (10%; $r = -0.318$, $P = .026$; Fig 6). After controlling for ED_{50} values, E_{max} values continued to explain 45% of the variability in 24-hour cough frequency ($r = 0.672$, $P < .001$), suggesting that E_{max} and ED_{50} values capture separate mechanisms.

DISCUSSION

To the best of our knowledge, this is the first study to apply nonlinear mixed-effects modeling to formally characterize capsaicin dose response in patients with CC, asthmatic patients, and HVs. Dose-response parameters, ED_{50} and E_{max} values, were compared by disease group and sex. In summary, although there were no statistical differences in either parameter between HVs and asthmatic patients, patients with CC had significantly higher E_{max} values and significantly lower ED_{50} values compared with those in the HV/A group, whereas female subjects had significantly higher E_{max} values compared with male subjects regardless of disease group. It should be emphasized that the failure of the model to distinguish between HVs and asthmatic patients could be entirely due to the relatively small sample size and the design of the current study, and no mechanistic or clinical interpretation can be inferred from these results. Furthermore, we also

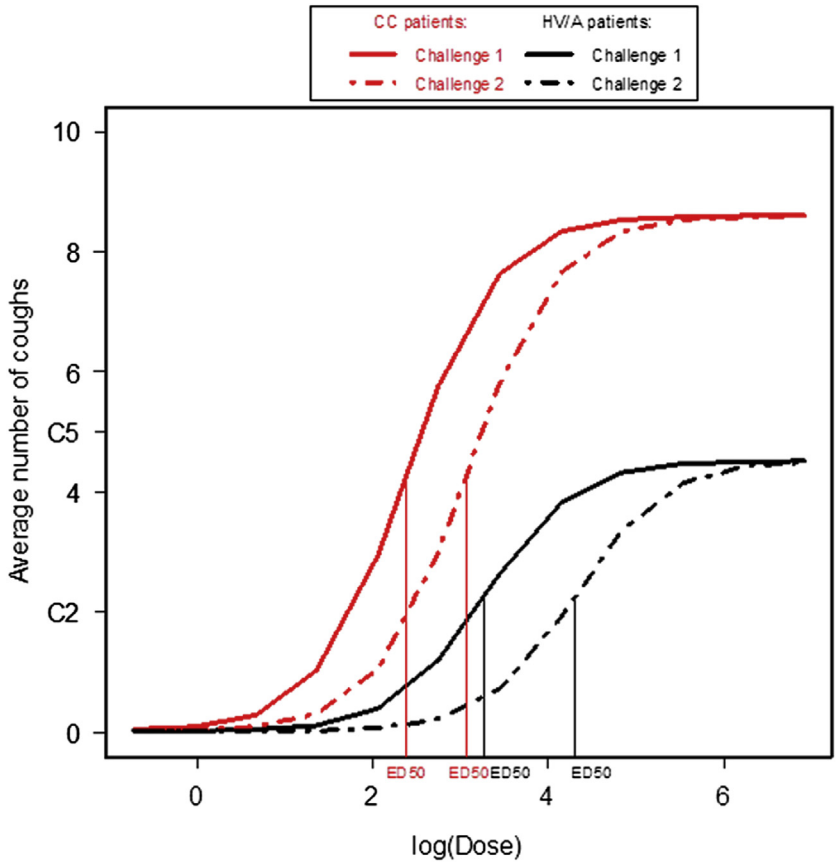

FIG 4. Average dose-response curves of model predictions for female patients with CC and HVs/asthmatic patients *(HV/A)* for challenges 1 and 2. Log(ED_{50}) estimates are indicated.

demonstrated that E_{max} values more strongly predict spontaneous 24-hour cough frequency than ED_{50} values.

Numerous studies have shown that patients with CC are on average more sensitive to inhaled capsaicin than healthy control subjects.[6,30] However, the traditional cough challenge end points vary widely between subjects and discriminate poorly between health and disease,[6] limiting their use to within-subject comparisons. As a possible explanation for this, Figs 2 and 5 clearly demonstrate that the extrapolated estimates of C2 and C5 values do not correspond to the same portion of the dose-response curve in patients with CC compared with the HV/A group or in male and female subjects. For example, in female patients with CC, C5 values are approximately equivalent to the ED_{50} capsaicin dose. In contrast, in male subjects in the HV/A group, the C5 values exceed the E_{max} values. This suggests that C2 and C5 values are hybrid parameters providing a mixed picture of underlying mechanisms depending on the disease and sex studied. In contrast, E_{max}, ED_{50}, and γ values are established pharmacodynamic end points related to the properties of the underlying pharmacologic system, such as receptor expression levels.[31]

Our finding of decreased ED_{50} values to inhaled capsaicin in patients with CC (ED_{50} values approximately half those of control subjects) is therefore in keeping with a hypersensitivity of afferent pathways evoking cough and the recent notion of cough hypersensitivity syndrome.[32-34] A number of possible peripheral neuronal mechanisms could explain this observation, including an increase in the number of capsaicin receptors (transient receptor potential vanilloid 1 [TRPV1]) on nerve terminals,[35-37] decreased thresholds for TRPV1 activation,[38] novel expression of TRPV1 by populations of nerve fibers usually insensitive to capsaicin,[39] or an increase in TRPV1 expressing nerve terminal density in bronchial tissue.[30] Alternatively, decreased activation thresholds of central cough pathways (central sensitization)[40] could produce similar effects. Of note, ED_{50} values were quite variable between subjects with relative SEs of 27% to 30%.

However, the most striking differences between disease groups and sexes were seen for the maximal cough responses (ie, the E_{max} parameter). The E_{max} parameter was approximately doubled in patients with CC compared with those in the A/HV group and better discriminated between these groups than ED_{50} values because the variability between subjects was less, with a relative SE of 10% to 17%. Current cough challenge protocols terminating at C5 might never achieve maximal responses for patients with CC and therefore do not capture the parameter that best explains between-subject differences. This finding also has important mechanistic implications, suggesting cough hyperresponsiveness might be just as important a phenomenon as hypersensitivity. An increase in E_{max} values might seem initially difficult to explain in neurophysiologic terms because if all directly stimulated afferent nerves were firing at maximum action potential frequency and amplitude, then the maximal response that could be achieved would be expected to reach a similar ceiling in all subjects that is limited by the number of axons present.

We speculate that there are 2 possible explanations for the effect we observed in patients with CC. First, previously silent afferent fibers can become activated by capsaicin as a consequence of a

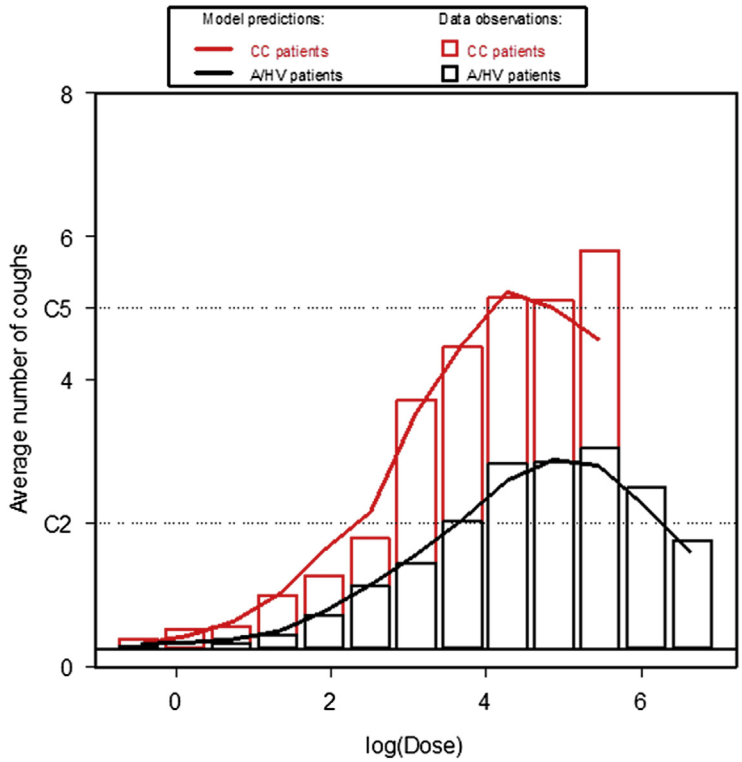

FIG 5. Average capsaicin dose-response curves for experimental data *(bars)* and individual model predictions *(lines)* for patients with CC and HVs/asthmatic patients *(HV/A)*. Model predictions of cough frequency are averaged at each dose across both challenge designs after adjusting for tachyphylaxis. Standard cough challenge end points, C5 and C2, are indicated by *horizontal dotted lines*.

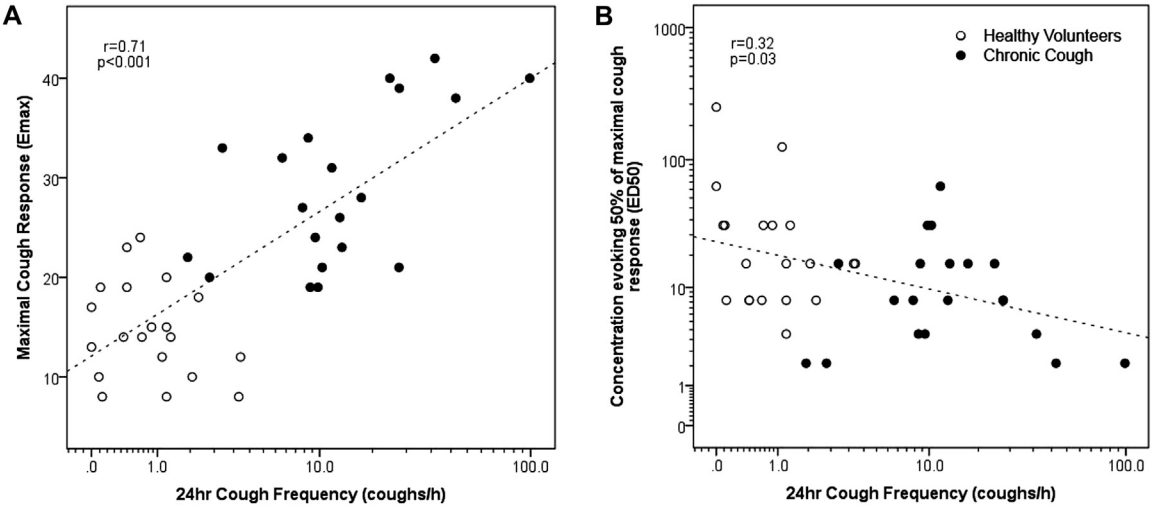

FIG 6. Correlation of E_{max} **(A)** and ED_{50} **(B)** values with 24-hour spontaneous cough frequency. ED_{50} value and cough frequency are plotted on log_{10} scales. Note the extensive overlap in individual ED_{50} values in HVs and patients with CC but not with E_{max} values.

phenotypic switching. Recent animal work has shown that tracheal vagal Aδ-fibers can be induced to express TRPV1 when exposed to brain-derived neurotrophic factor and also allergen.[41] Alternatively, an increase in maximal cough responses might arise because of a failure of descending inhibitory pathways. A failure of top-down inhibitory influences in patients with CC would have profound effects on the ability of the subject to control or terminate coughing bouts, leading to high cough frequencies. By analogy, less effective inhibitory pain mechanisms are thought to predispose female subjects to the development of chronic pain conditions, such as irritable bowel syndrome and fibromyalgia.[39,42-45] If female subjects also lacked effective inhibitory cough mechanisms, this could explain their predisposition to the development of CC. Sex effects on capsaicin cough responses were unlikely to be due to lung function differences because FEV_1 and FVC had no significant effect in the model. This is supported by previous studies showing that lung

function also does not predict or significantly correlate with C2/C5 values.[15,16]

In this study we also explored the influence of the order of capsaicin doses, comparing ascending doses (challenge 1) with randomized order (challenge 2). We are unable to conclude from a statistical standpoint which of these challenge designs is preferable, but randomized challenges were practically more complex to design/perform and were associated with decreased cough sensitivity (increased ED_{50} values), probably because of significant tachyphylaxis. Indeed, it is well established in animal studies that airway C-fibers desensitize on exposure to high doses of capsaicin and might become completely unresponsive with prolonged application (10-20 minutes).[46] In challenge 1 repeat inhalation of the same capsaicin dose also resulted in attenuated cough responses, which is consistent with a previous human study showing that tidal inhalation of a single capsaicin concentration produces both short- and long-term (minutes to hours) reductions in cough responses.[47] Consequently, we incorporated a tachyphylaxis parameter in our model that was estimated based on the number of preceding capsaicin doses that were either the same or higher (see Fig E1); this produced the model that most closely predicted our observed data (Fig 5).

These novel cough challenges do have some limitations: they are more time-consuming to perform than standard challenges, and further validation is needed to assess repeatability and relationships with other cough assessment tools. However, preliminary data with challenge 1 suggest that the E_{max} value demonstrates a stronger correlation with 24-hour spontaneous cough frequency than the ED_{50} value or values observed with existing cough challenge end points (C2 or C5 values).[4,5,7] This suggests that the E_{max} value captures important disease mechanisms of the patient population, whereas ED_{50} values are more related to the potency of the tussive agent used to elicit cough. Furthermore, the between-subject variability of E_{max} values is much smaller than that of ED_{50} values and thus, if used as a primary end point in intervention studies, would require fewer patients to detect a significant change.

In conclusion, we have shown that nonlinear mixed-effects dose-response modeling provides a new tool for the design, analysis, and interpretation of cough challenge tests. Our results call into question the utility of the traditional C2/C5 end points. We propose that continuing challenges beyond these end points allows calculations of pharmacodynamic parameters, such as E_{max} and ED_{50}, which provide more meaningful information about the mechanisms underlying patient phenotypes and therefore a more scientific basis for the development and testing of novel treatments. Further development of these challenges might provide a screening tool equivalent to methacholine challenge for identifying patients with cough hyperresponsiveness.

Key messages

- **Pharmacodynamic modeling of capsaicin dose responses suggests the maximal capsaicin cough response (E_{max}) discriminates health from disease and correlates well with spontaneous cough frequency.**
- **The E_{max} value also provides important insights into the mechanisms underlying CC, suggesting either a failure of inhibitory control pathways or a phenotypic switch in airway neurons not usually responsive to capsaicin.**

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METHODS

Subjects

Twenty HVs with normal lung function and no current or past history of respiratory disease were recruited by means of poster advertisements displayed around the hospital.

Eighteen patients with physician-diagnosed asthma with documented evidence of bronchial hyperreactivity (positive methacholine challenge results, reversibility in FEV_1 >12%, or both) were also studied. These patients had well-controlled mild-to-moderate asthma (FEV_1 >75% of predicted value, use of short-acting bronchodilators as required and inhaled corticosteroids, and no courses of oral steroids within the previous 4 weeks) and were able to omit any long-acting bronchodilators before participation.

Twenty patients with unexplained CC with a duration of greater than 8 weeks were recruited from a tertiary referral specialist cough clinic (University Hospital of South Manchester, Manchester, United Kingdom). These patients had undergone full investigation (lung function, bronchial challenge testing, nasendoscopy, computed tomographic scan, bronchoscopy, and 24-hour impedance/pH monitoring) and did not improve with treatment trials for gastroesophageal reflux disease, asthma, eosinophilic bronchitis, and/or nasal disease, as indicated.

Exclusion criteria for all subjects were as follows: recent (<4 weeks) upper respiratory tract infection, current smoker or exsmoker with a greater than 10 pack year smoking history, use of angiotensin-converting enzyme inhibitors or any medications that could alter the sensitivity of the cough reflex, and a history of drug or alcohol abuse.

Twenty-four-hour objective cough monitoring

Ambulatory cough sound monitoring was performed by using a custom-built device attached to a lapel microphone and a chest wall sensor positioned over the sternum (VitaloJAK, Vitalograph). Recordings were transferred to a personal computer; silences/background noise were removed by using validated, custom-written software; and cough sounds were counted by a trained observer using an audio editing package (Cool Edit 2000, Syntrillium). Previous studies found excellent agreement between trained observers.

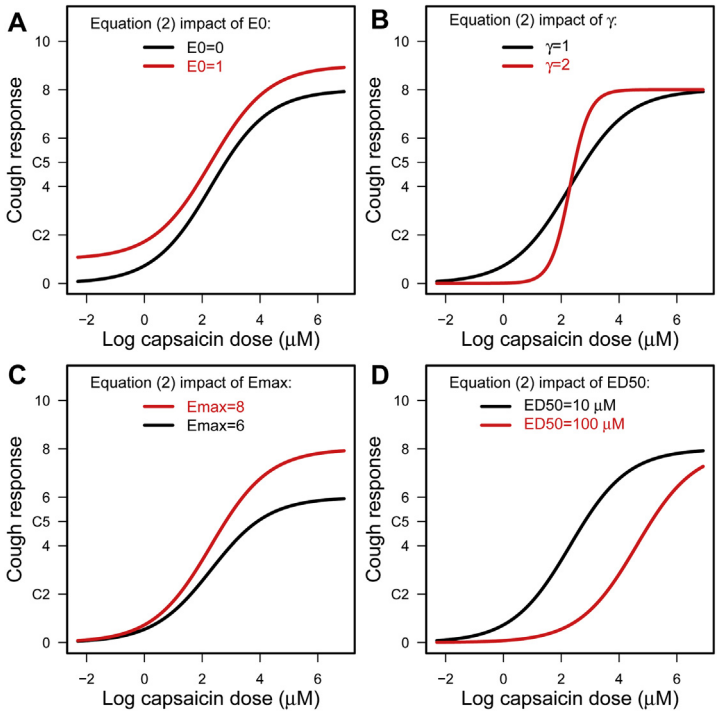

FIG E1. This figure shows the sigmoid curve representing equation 2, and the effect on this curve of increases in the intercept E0 **(A)**, the slope γ **(B)**, the E_{max} value **(C)**, and the ED_{50} value **(D)**.

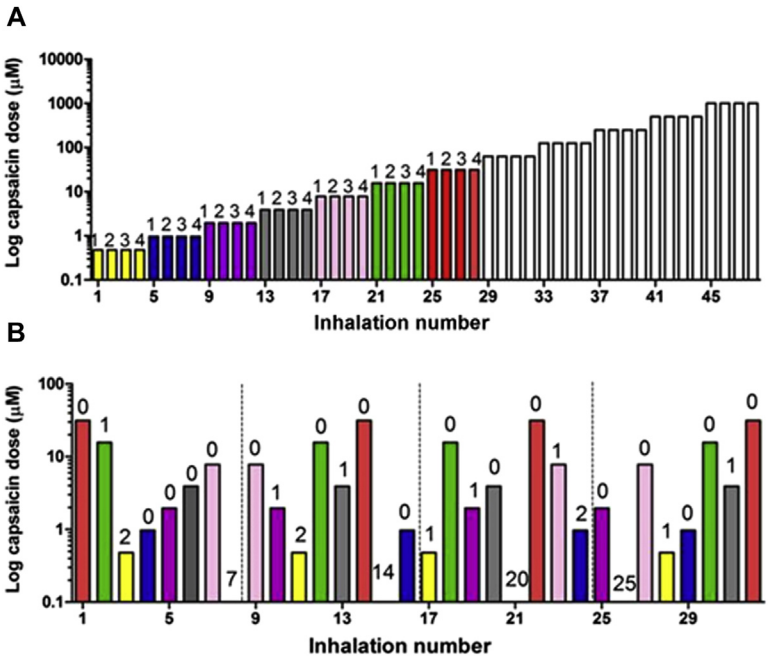

FIG E2. Calculation of the j-value for challenge 1 **(A)** and an example for challenge 2 **(B)**. Each *bar* represents a single-breath inhalation of capsaicin. A dose-dependent tachyphylaxis effect was estimated according to j, which was defined as the number of preceding capsaicin doses that were equal or higher (indicated above the *bars*).

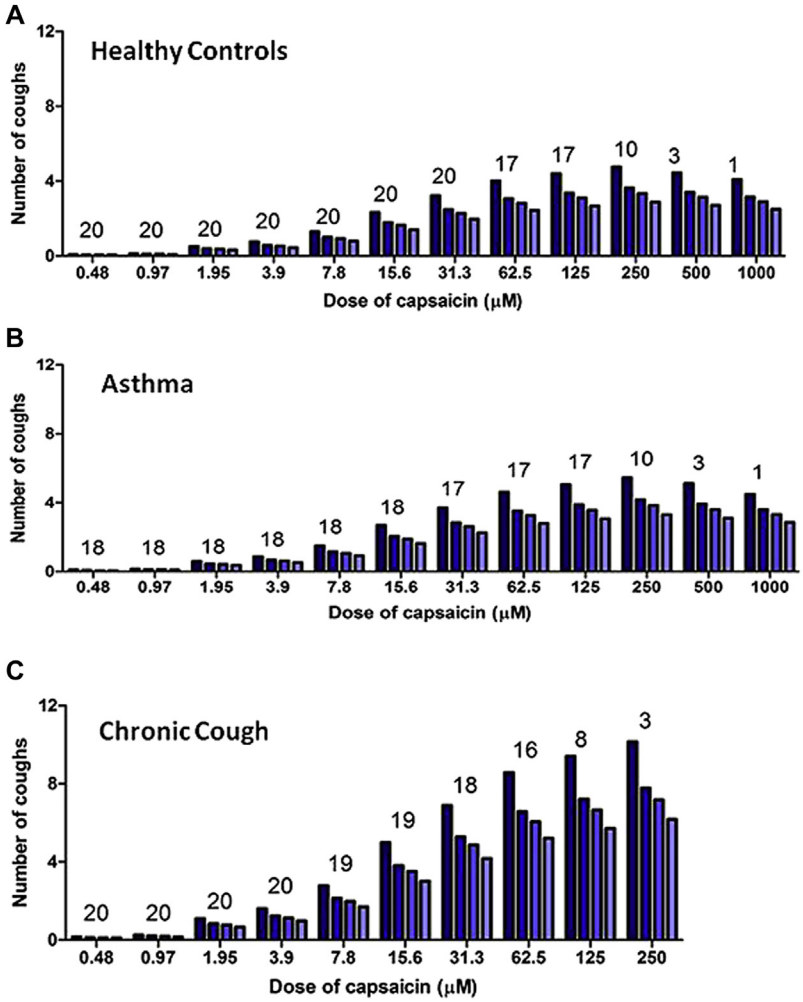

FIG E3. Mean number of coughs with each inhalation for challenge 1 in all patient groups, demonstrating the effect of tachyphylaxis when repeated inhalations of the same concentration of capsaicin are inhaled. The numbers above each concentration represent the number of subjects inhaling each concentration.

TABLE E1. Characteristics of subjects included in the correlation analysis of 24-hour spontaneous cough frequency and capsaicin dose-response parameters

Variable	HVs	Patients with CC	*P* value (ANOVA)
Sample size	20	20	
Age (y)*	55.0 (14.21)	60.5 (13.2)	.217
Sex (male/female)	10/10	9/11	
BMI (kg/m^2)*	26.6 (4.36)	28.1 (3.59)	.245
Smoking history (pack years)†	0.0 (0.00)	0.0 (1.81)	.444
FEV_1 (% predicted)*	105.7 (13.79)	98.8 (16.45)	.157
FVC (% predicted)*	107.8 (15.95)	102.7 (15.48)	.309

BMI, Body mass index.
*Mean (SD).
†Median (interquartile range).