Short- and long-term efficacy of prednisolone for first acute rhinovirus-induced wheezing episode

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Background: Rhinovirus-induced wheezing is an important risk factor for recurrent wheezing. There are no randomized controlled trials on the effect of systemic corticosteroids in patients with this disease.
Objective: We sought to study the short- and long-term effects of prednisolone treatment of the first acute, moderate-to-severe, rhinovirus-induced wheezing episode in young children.
Methods: After confirming rhinovirus from nasopharyngeal aspirate by using PCR, 79 children with a first wheezing episode at age 3 to 23 months were randomized to receive oral prednisolone (first dose of 2 mg/kg, followed by 2 mg/kg/d in 2 divided doses for 3 days) or placebo. The trial was double blind throughout the 12-month follow-up. The primary outcomes were long term: new physician-confirmed wheezing episode within 2 months, number of physician-confirmed wheezing episodes within 12 months, and initiation of regular controller medication for asthma symptoms within 12 months. The primary interaction analysis examined rhinovirus load.
Results: Seventy-four patients completed the study (mean age, 13 months; 28% atopic). Long-term outcomes did not differ between groups (all $P \geq .30$). For short-term outcomes, the prednisolone group had less cough, rhinitis, noisy breathing, severe breathing difficulties, and nocturnal respiratory symptoms at home within 2 weeks (all $P < .05$). The 25 children with greater than 7000 rhinovirus copies/mL (most sensitive cutoff) benefitted from prednisolone in terms of less risk of physician-confirmed recurrence within 2 and 12 months compared with placebo (both $P < .05$).
Conclusions: Prednisolone cannot be routinely recommended for all young children experiencing their first acute, moderate-to-severe, rhinovirus-induced wheezing episode. Prednisolone might be beneficial in a subgroup of children with high viral loads. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Bronchiolitis, child, corticosteroid, glucocorticoid, treatment, prednisolone, rhinovirus, virus, wheeze, wheezing*

Rhinovirus has been detected in 20% to 40% of wheezing children during the first 2 years of life in both hospital and emergency care settings.[1-3] Rhinovirus-related cause of early wheezing is of particular interest because of its strong association (odds ratios of 3-10 during early life) with recurrent wheezing and doctor-diagnosed asthma up to 13 years of age.[4-9] The suggested explanations for this striking association are low interferon responses (ie, impaired viral defense), early airway inflammation (ie, a broken epithelial barrier), and genetic variation at the 17q21 locus in rhinovirus-affected children (ie, might markedly increase the risk of asthma).[10-13]

Overall, randomized controlled trials (RCTs) on the efficacy of systemic corticosteroids in the treatment of early wheezing have not reported clinical efficacy.[14-16] Virus-specific RCTs on respiratory syncytial virus (RSV)–induced lower airway illness have focused on bronchiolitis and have not found any efficacy of systemic corticosteroids.[17,18] Previously, in the Vinku study we reported a *post hoc* analysis of RCT data showing that oral prednisolone during the first wheezing episode with a rhinovirus-related cause and/or eczema reduced the risk of recurrent wheezing over the next 2 months, 12 months, and 7 years.[4,9,19] Although no prior study has identified a subgroup of young wheezing children who benefit from systemic corticosteroids, they have not focused on rhinovirus as a cause. The high asthma risk associated with rhinovirus-induced wheezing episodes and our earlier results led us to perform the current RCT on the effect of systemic corticosteroids on a patient's first rhinovirus-induced wheezing episode. We hypothesized that prednisolone decreases the risk of relapse in children with their first rhinovirus-induced wheezing episode.

METHODS

Subjects

Recruitment for the Vinku2 trial was carried out in Turku University Hospital from June 2007 to March 2010 (*vinku* means "wheeze" in Finnish). The inclusion criteria were age of 3 to 23 months, delivery at 36 gestational

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Abbreviations used
HR: Hazard ratio
RCT: Randomized controlled trial
RSV: Respiratory syncytial virus

weeks or later, first wheezing episode (based on parental report and confirmed from medical records), rhinovirus detected in a nasopharyngeal aspirate sample by using PCR, ongoing signs of lower respiratory tract symptoms (cough, noisy breathing, or wheezing) at the time when PCR results were available, and written informed consent from a parent or guardian. Exclusion criteria were the presence of a chronic nonatopic illness, previous systemic or inhaled corticosteroid treatment, participation in another study (excluding long-term follow-up studies in childhood), varicella contact in a patient without a previous varicella illness, need for intensive care unit treatment, or poor understanding of Finnish (Fig 1). The study was commenced only after obtaining written informed consent from a parent. The study protocol was approved by the Ethics Committee of Turku University Hospital.

Study protocol

At study entry, the guardian filled out a standard questionnaire on host and environmental risk factors for asthma. Then the child was physically examined, a nasopharyngeal aspirate sample was obtained for viral diagnostics by using a standardized procedure,[2] and a baseline blood sample was drawn. The randomly assigned study drug (prednisolone vs placebo) was initiated as soon as possible for rhinovirus-positive children by a study physician if the child still fulfilled all the study criteria. During the 12-month follow-up period, the guardian was asked to fill out a symptom and medication diary and to bring the child to the study physician each time the child had breathing difficulties. Scheduled follow-up visits were arranged at 2 weeks, 2 months, and 12 months by the study physicians. The study protocol was registered at ClinicalTrials.gov in August 2008 (ClinicalTrials.gov number, NCT00731575). For more details of the protocol, see the Methods section and Figs E1-E5 in this article's Online Repository at www.jacionline.org.

Randomization

Subjects were randomized to receive either oral prednisolone (first dose of 2 mg/kg, followed by 2 mg/kg/d in 2 divided doses for 3 days; maximum, 60 mg/day; Prednisolon 5-mg tablets) or placebo; both were provided by Leiras Takeda (Helsinki, Finland). A double-blind RCT design was used. For more details, see the Methods section in this article's Online Repository.

Definitions

Wheezing refers to expiratory breathing difficulty with a high-pitch sound during expiration. We refer to the concomitant presence of rhinovirus, as detected by means of PCR, as a rhinovirus-induced wheezing episode because this positivity has been linked to the severity of respiratory symptoms, specific rhinovirus genotypes show in most cases relatively short shedding (generally up to 2 weeks), dual-rhinovirus genotype detections are rare, rhinovirus detection has been associated with immune responses *in vivo*, and rhinovirus is able to infect the lower airways.[12,20,21] Atopy was defined as a positive IgE antibody result (≥0.35 kU/L) to any of the following allergens: codfish, cow's milk, egg, peanut, soybean, wheat, cat, dog, horse, birch, mugwort, timothy grass, *Cladosporium herbarum*, and *Dermatophagoides pteronyssinus* (Phadiatop Combi; Phadia, Uppsala, Sweden). Aeroallergen sensitization was defined as positive IgE antibodies to any of the latter 8 allergens. Perennial aeroallergen sensitization was defined as positive IgE antibody results to dog, cat, or *D pteronyssinus.* Birch, mugwort, timothy grass, and *C herbarum* were considered seasonal aeroallergens. Atopic eczema was defined as a physician's diagnosis of eczema according to typical symptoms that included pruritus, typical morphology, and chronicity of disease. Eczema was defined as atopic eczema if a child was atopic. For more details, see the Methods section in this article's Online Repository.

Laboratory methods

Rhinovirus species A, B, and C; enteroviruses; and RSV A and B were detected by using "in-house" reverse transcriptase PCR at the Virus Diagnostic Laboratory, Department of Virology, University of Turku.[22,23] A nasal swab (nylon flocked dry swab, 520CS01; Copan, Brescia, Italy) was dipped into the nasopharyngeal aspirate and stored at −70°C. In analysis the swab was diluted in 1 mL of PBS, which was analyzed for viral load (ie, copy number). A multiplex PCR test (Seeplex RV12 ACE Detection; Seegene, Seoul, Korea) was used for detection of rhinovirus A and B, RSV A and B, parainfluenza virus types 1 to 3, human metapneumovirus, adenovirus, coronavirus (229E, NL63, OC43, and HKU1), and influenza A and B virus from frozen samples. Human bocavirus-1 was analyzed by using PCR and serology, as previously described.[24] Blood eosinophil counts and serum levels of allergen-specific IgE were analyzed by using the routine diagnostic procedures of the Central Laboratory of Turku University Hospital. Serum 25-hydroxyvitamin D measurements were done by means of liquid chromatography–tandem mass spectrometry at Massachusetts General Hospital (Boston, Mass). For more details, see the Methods section in this article's Online Repository.

Outcomes

The 3 primary outcomes were the occurrence of a new physician-confirmed wheezing episode during the 2-month follow-up, the number of physician-confirmed wheezing episodes during the 12-month follow-up, and the initiation of regular controller medication for asthma symptoms during the 12-month follow-up. Regular medication was initiated according to 2007 guidelines for initiating daily long-term control therapy for 0- to 4-year-old children.[25] Rhinovirus load was the primary interaction analysis to investigate whether the effects of prednisolone compared with placebo on the 3 primary outcomes were dependent on the rhinovirus copy number.

Secondary outcomes included the occurrence and severity of respiratory symptoms (cough, expiratory breathing difficulty, noisy breathing, rhinitis, and nocturnal wakening for breathing difficulties) on a 4-point scale, medications, and unscheduled doctor's appointments recorded by the parents on a 2-week daily symptom diary. Within 2 months after discharge, outpatient clinic visits, hospitalizations, oral corticosteroid courses, and initiations of inhaled corticosteroids for wheezing were also recorded. As an exploratory outcome, we decided to extend the time to a new physician-confirmed wheezing episode up to the 12-month follow-up with a double-blind design.

Statistics

According to our previous RCT,[19] a sample size of 62 children would be sufficient for 80% power (with a 5% type I error rate) to detect a 34% absolute difference in relapse rate between prednisolone (22%) and placebo (56%) within 2 months after a rhinovirus-induced first wheezing episode. Because the follow-up time was 12 months (ie, 10 months longer) in the current study, with increased risk of dropout, we recruited 79 children.

Baseline differences between groups were analyzed by using 2-sample *t* test for normally distributed and Wilcoxon rank sum tests for nonnormally distributed continuous variables. Categorical variables were analyzed by using χ^2 or Fisher exact tests. The difference between the prednisolone and placebo groups in primary outcomes, new physician-confirmed wheezing episodes within 2 months, and initiation of regular controller medication for asthma symptoms within 12 months was tested with Cox regression. Time to event was defined from baseline to the time of occurrence of the event. Survival times were censored if the event did not occur within 2 months (wheezing episode) or 12 months (asthma symptoms) of follow-up. Poisson regression analysis was used to investigate the difference between the prednisolone and placebo groups in the number of physician-confirmed wheezing episodes within 12 months. The modifying effect of rhinovirus load at study entry on

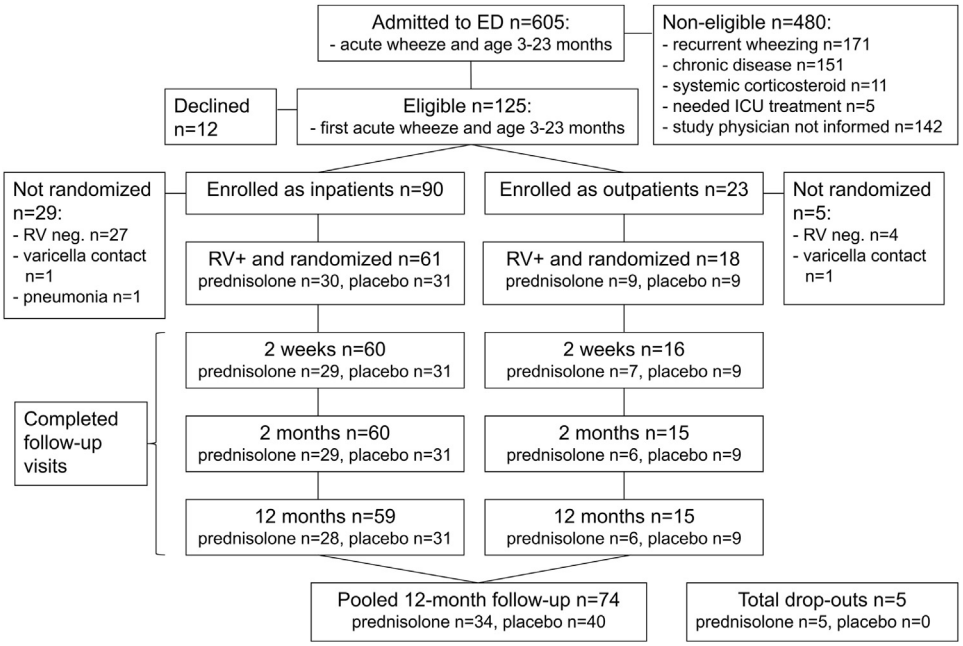

FIG 1. Study flow chart. *ED,* Emergency department; *ICU,* intensive care unit; *neg.,* negative; *RV,* rhinovirus.

the effect of prednisolone (vs placebo) was tested, including a rhinovirus load–group interaction effect in the Cox and Poisson models. Because of highly positively skewed distribution, rhinovirus load was dichotomized. The cutoff for rhinovirus load was identified by testing for different values and selecting the approximate threshold that yielded the lowest P value for rhinovirus load–group interaction effect. Cox or Poisson regression models did not include any covariates because no statistically significant differences in baseline characteristics were found between groups. Proportional hazard assumption in Cox models was checked by using the martingale residual. The 2-sample t test (cough and noisy breathing), Wilcoxon rank sum test with Hodges-Lehmann estimate for median difference (expiratory breathing difficulty, nocturnal symptoms, and bronchodilator at home), and Poisson regression (nocturnal symptoms, outpatient clinic visit, hospitalization, oral corticosteroid, and inhaled corticosteroid) were used to evaluate differences in the secondary outcomes between the groups. In all statistical analyses 2-sided tests were used, and the significance level was set at .05. We used SAS/STAT version 9.1.3 SP4 software for the SAS System for Windows (SAS Institute, Cary, NC).

RESULTS

Recruitment and baseline characteristics

Enrollment of the study subjects is shown in Fig 1. Samples from 61 inpatients and 18 outpatients were rhinovirus positive, and the patients were randomized by using separate lists. All 79 patients were able to take at least half of the 3-day course of the study drug. Over the 12-month follow-up, 5 children from the prednisolone group and none from the placebo group dropped out, yielding an analytic sample of 74 patients (59 inpatients and 15 outpatients). Baseline characteristics are shown in Table I.[19] For more details, please see the Results section and Table E1 in this article's Online Repository at www.jacionline.org.

Recurrence data

The medical records of all subjects were reviewed for the entire 12-month study period. In addition, symptom diaries were reviewed; these were returned from 66 (89%) of 74 subjects after the 2-week follow-up, from 72 (97%) of 74 subjects after the 2-month follow-up, and from 38 (51%) of 74 subjects after the 12-month follow-up. Of the first recurrences, 49 (86%) of 57 were seen at the study clinic. Otherwise, recurrences were confirmed from medical records. The median respiratory symptoms score of the first physician-confirmed recurrence was 6 (interquartile range, 5-8). All children with recurrences had expiratory breathing difficulties.

Primary outcomes

The prednisolone and placebo groups did not differ for the 3 primary outcomes: occurrence of a new physician-confirmed wheezing episode within 2 months (P = .30), the number of physician-confirmed wheezing episodes within 12 months (P = .43), and initiation of regular controller medication for asthma symptoms within 12 months (P = .63, Fig 2 and Table II).

Primary interaction analysis

Rhinovirus load at study entry modified the effect of the randomly assigned interventions on the occurrence of a physician-confirmed new wheezing episode during the 2-month follow-up. Rhinovirus load was analyzed as a dichotomous variable by using different cutoff values (Table III). The lowest P value for interaction between study drug and rhinovirus load was found with a cutoff value of 7000 copies/mL (P = .03, Fig 3 and Table III). Among children with a rhinovirus load of greater than 7000 copies/mL, the occurrence of a physician-confirmed new wheezing episode during the 2-month follow-up was lower in the prednisolone group compared with that seen in the placebo group (hazard ratio [HR], 0.2; 95% CI, 0.1-0.7; P = .01), but no difference was found between the randomly assigned groups among children with a rhinovirus load of 7000 copies/mL or less (HR, 1.2; 95% CI, 0.4-3.2; P = .71). In terms of the number needed to treat, treatment of 2 children with a

TABLE I. Baseline characteristics

Characteristic	Prednisolone (n = 34)	Placebo (n = 40)
Age (mo)	13.2 (6.9)	12.2 (5.1)
Male sex, no. (%)	27 (79)	30 (75)
Clinical characteristics at entry		
Respiratory symptoms score*	5.7 (2.5)	5.7 (2.2)
Respiratory rate (breaths/min)	51 (10)	50 (15)
Heart rate (beats/min)	145 (19)	141 (21)
Oxygen saturation (%)	97 (95-98)	97 (94-98)
Temperature (°C)	37.5 (0.6)	37.7 (0.7)
Duration of symptoms before enrollment (d)		
Rhinitis	3 (1-5)	3 (2-6)
Cough	3 (2-5)	3 (2-5)
Dyspnea	1 (1-2)	1 (1-2)
Viral cause		
Rhinovirus load (copies × 10^3/mL)	5.7 (0.2-42)	2.8 (0.01-9.0)
Viral coinfection, no. (%)	11 (32)	15 (38)
RSV, no. (%)	4 (12)	6 (15)
Bocavirus, no. (%)	4 (10)	4 (12)
Parainfluenza virus, no. (%)	1 (3)	4 (10)
Each other virus, no. (%)	≤2 (≤6)	≤2 (≤5)
Delay in study drug initiation (h)†	45 (22)	52 (29)
Atopic characteristics		
Allergic sensitization, no. (%)‡	10 (29)	12/39 (31)
Food sensitization, no. (%)	8 (24)	12/39 (31)
Aeroallergen sensitization, no. (%)	6 (18)	6/39 (15)
Perennial, no. (%)	6 (18)	5/38 (13)
Seasonal, no. (%)	3 (9)	2/38 (5)
Blood eosinophils (×10^9/L)	0.51 (0.45)	0.51 (0.38)
Blood eosinophils (≥0.4 × 10^9/L), no. (%)	17 (50)	21/37 (57)
Doctor-diagnosed eczema, no. (%)	15 (44)	13 (33)
Doctor-diagnosed atopic eczema, no. (%)	9 (26)	6/39 (15)
Parental asthma, no. (%)	8 (24)	9 (23)
Parental allergy, no. (%)	21 (62)	26 (65)
Total IgE (kU/L)	20 (7.5-40)	13 (6.8-49)
No. of children in the family	2 (1-2)	2 (1-2)
Day care, no. (%)		
Home	21 (62)	29 (73)
Small group	6 (18)	5 (13)
Kindergarten	7 (21)	6 (15)
Serum 25-hydroxyvitamin D (nmol/L)	82 (26)	88 (19)
Serum 25-hydroxyvitamin D_2 (nmol/L)	24 (23)	22 (21)
Serum 25-hydroxyvitamin D_3 (nmol/L)	58 (30)	66 (25)
Duration of breast-feeding (mo)	6.4 (4.1)	5.4 (4.3)
Parental smoking, no. (%)	14 (41)	17 (43)

Values are shown as means (SDs), medians (interquartile ranges), or numbers (percentages) of subjects. No statistically significant differences were found between the randomly assigned treatment groups. For the stratification according to inpatients and outpatients, please see Table E1.

*Assessed on a scale of 0 to 12, consisting of the sum of degrees of dyspnea, type of breathing, severity of auscultatory findings on wheezing, and expiratory/inspiratory time.[19]

†Delay was due to rhinovirus PCR diagnostics.

‡Defined as IgE antibodies to any of the common allergens; see the Methods section for details.

rhinovirus load of greater than 7000 copies/mL would prevent 1 relapse within 2 months. Rhinovirus load at study entry also modified the effect of prednisolone on the number of physician-confirmed wheezing episodes during the next 12 months (rhinovirus load–group interaction effect, P = .03).

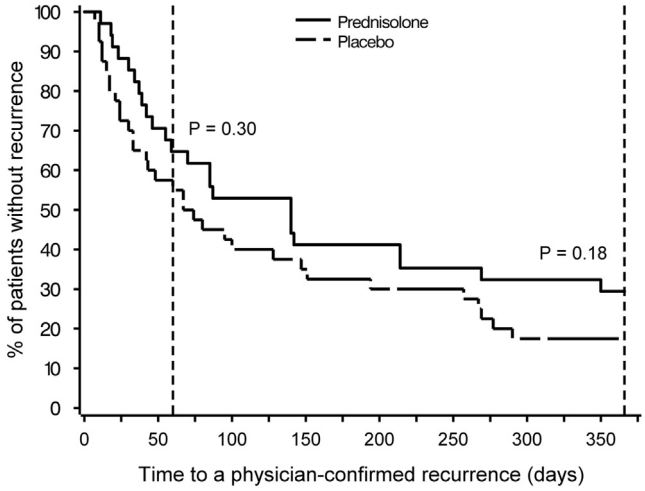

FIG 2. Time to a new physician-confirmed wheezing episode in children randomized to receive prednisolone or placebo for their first rhinovirus-induced wheezing episode. The 2-month time point, which was one of the primary outcomes, has been marked. No difference was found at the 2-month or 12-month time points.

Among children with a rhinovirus load of greater than 7000 copies/mL, the number of physician-confirmed new wheezing episodes during the 12-month follow-up was lower in the prednisolone group compared with that seen in the placebo group (relative risk, 0.6; 95% CI, 0.4-0.98; P = .04), but no difference was found between the groups among children with a rhinovirus load of 7000 copies/mL or less (relative risk, 1.3; 95% CI, 0.8-2.1; P = .33). Among children with a rhinovirus load of greater than 7000 copies/mL, initiation of regular controlled medication for asthma symptoms within 12 months was nonsignificantly lower in the prednisolone group compared with that seen in the placebo group (HR, 0.5; 95% CI, 0.2-1.2; P = .13), but no difference was found between the groups among children with a rhinovirus load of 7000 copies/mL or less (HR, 1.2; 95% CI, 0.5-3.3; P = .70). For more details of the primary interaction analysis, see the Results section in this article's Online Repository.

Secondary outcomes

Short-term outcomes differed between the 2 groups (Table IV). The prednisolone group had less days of cough, rhinitis, and noisy breathing during the 2-week follow-up compared with the placebo group (all P < .05). With regard to severity of symptoms during these first 2 weeks, 2 (6%) children had severe expiratory breathing difficulties in the prednisolone group compared with 9 (23%) children in the placebo group (P = .045). Moreover, 5 (15%) children had nights with 2 or more awakenings for breathing difficulties in the prednisolone group compared with 17 (43%) children in the placebo group (P = .009). We did not observe differences for the other secondary outcomes (Table IV).

Exploratory outcome

Extending the time to a new physician-confirmed wheezing episode up to the 12-month follow-up did not show an overall difference between the prednisolone and placebo groups (HR, 0.7; 95% CI, 0.4-1.2; P = .18; Fig 2). Again, however, rhinovirus load at study entry modified the effect of the randomly assigned

TABLE II. Primary outcomes

Outcome	Prednisolone (n = 34)	Placebo (n = 40)	HR or relative risk (95% CI)	*P* value
A new physician-confirmed wheezing episode within 2 mo after discharge	12 (35%)	18 (45%)	0.7 (0.3-1.4)*	.30
No. of physician-confirmed wheezing episodes within 12 mo after discharge	58	83	0.9 (0.6-1.2)†	.43
Initiation of regular controller medication for asthma symptoms within 12 mo after discharge	15 (44%)	20 (50%)	0.8 (0.4-1.7)*	.63

*Cox regression analysis: HR for the difference between the prednisolone and placebo groups.
†Poisson regression analysis: relative risk for the difference between the prednisolone and placebo groups.

TABLE III. Primary interaction analysis of the occurrence of a new physician-confirmed wheezing episode at the 2-month follow-up according to different cutoffs of rhinovirus load and the study drug

Cutoff of rhinovirus load/mL	No. (%) of patients with >cutoff in prednisolone/placebo group	*P* value for interaction*	Patient with ≤cutoff value, prednisolone vs placebo, HR (95% CI)†	Patient with >cutoff value, prednisolone vs placebo, HR (95% CI)†
3,000	18 (60)/17 (47)	.29	1.0 (0.3-3.2)	0.5 (0.2-1.2)
5,000	17 (57)/13 (36)	.049	1.3 (0.4-3.6)	0.3 (0.1-0.8)
7,000	14 (47)/11 (31)	.03	1.2 (0.4-3.2)	0.2 (0.1-0.7)
9,000	13 (43)/9 (25)	.13	0.9 (0.4-2.4)	0.3 (0.1-0.9)
11,000	13 (43)/8 (22)	.37	0.8 (0.3-2.0)	0.4 (0.1-1.3)

Available rhinovirus load data: n = 30 for the prednisolone group and n = 36 for the placebo group.
*Cox regression model included the main effects of dichotomized rhinovirus load and group and the interaction effect of rhinovirus load by group.
†The Cox regression model included the group effect.

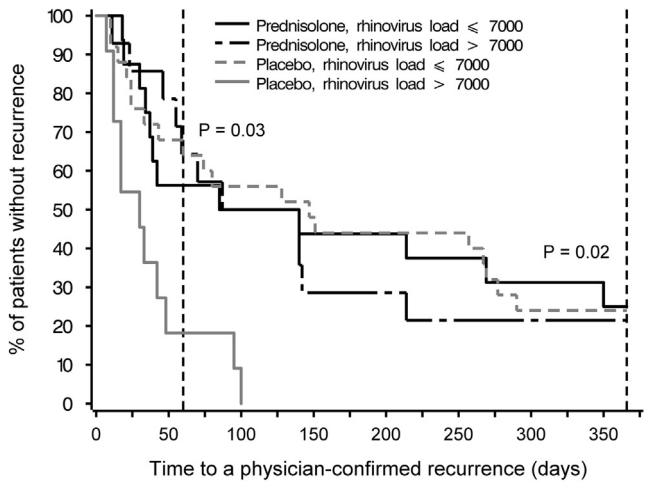

FIG 3. Time to a new physician-confirmed wheezing episode in children randomized to receive prednisolone or placebo for their first rhinovirus-induced wheezing episode. Data are represented according to rhinovirus load. *P* values for interaction between the study drug and rhinovirus load are shown at the 2-month and 12-month follow-ups. Children with a rhinovirus load of greater than 7000 copies/mL had significantly less recurrence at both time points in the prednisolone group compared with the placebo group (see text for details). All children with a high rhinovirus load in the placebo group had a relapse within 100 days.

interventions on the occurrence of a physician-confirmed new wheezing episode during the 12-month follow-up (rhinovirus load–group interaction effect, $P = .02$; Fig 3). Among children with a rhinovirus load of greater than 7000 copies/mL, occurrence of a physician-confirmed new wheezing episode during the 12-month follow-up was lower in the prednisolone group compared with that seen in the placebo group (HR, 0.2; 95% CI, 0.1-0.6; $P = .004$), but no difference was found between the groups among children with a rhinovirus load of 7000 copies/mL or less (HR, 1.0; 95% CI, 0.5-2.1; $P = .99$). Prednisolone treatment of 5 children with a rhinovirus load of greater than 7000 copies/mL would prevent 1 recurrence within 12 months.

Adverse events

There were no differences in the incidence of adverse events between the prednisolone and placebo groups (results not shown). No clinically significant adverse events were reported.

DISCUSSION

This is the first randomized, double-blind, placebo-controlled trial to investigate the effect of prednisolone in the first acute, moderate-to-severe, rhinovirus-induced wheezing episode. Although the study did not support a beneficial effect of prednisolone for the long-term primary outcomes, the prespecified interaction analysis showed that the effect of prednisolone was closely linked to rhinovirus load. To be more specific, children with a rhinovirus load of greater than 7000 copies/mL benefitted from prednisolone in terms of longer time to a physician-confirmed relapse during the 2- or 12-month follow-up and less physician-confirmed wheezing episodes within 12 months; they also tended to need less regular controlled medication for asthma symptoms within 12 months. With regard to the short-term (secondary) outcomes, in the total sample the prednisolone group appeared to have benefitted, with less cough, rhinitis, noisy breathing, severe expiratory breathing difficulties, and nocturnal respiratory symptoms at home within 2 weeks compared with the placebo group.

Contrary to our primary hypothesis, prednisolone showed no overall effect on the primary outcomes. The hypothesis was justified because long-term cohort studies had demonstrated that a rhinovirus-induced wheezing episode is an important early risk factor for recurrent wheezing and doctor-diagnosed asthma in children.[4-9] Moreover, susceptibility to rhinovirus-induced

TABLE IV. Secondary outcomes

Variable	Prednisolone (n = 34)	Placebo (n = 40)	Mean/median difference or RR (95% CI)	*P* value
Within 2 wk after discharge				
Cough (d)	6.2 (3.6)	8.9 (3.7)	−2.8 (−4.5 to −1.0)	.002
Expiratory breathing difficulty (d)	2 (0 to 4)	3 (1 to 5)	−1 (−2 to 0)	.17
Noisy breathing (d)	5.3 (3.3)	7.3 (3.9)	−2.0 (−3.6 to −0.29)	.02
Rhinitis	5.5 (2 to 9)	8.5 (6 to 11.5)	−3 (−5 to −1)	.008
Nocturnal symptoms	19 (56)	24 (60)	0.9 (0.5 to 1.7)	.82
Use of bronchodilator (puffs/14 d)	9.5 (1 to 22)	18 (3.5 to 29.5)	−4 (−12 to 1)	.095
Within 2 mo after discharge				
Outpatient clinic visit, no. (%)*	16 (47)	15 (38)	1.3 (0.6 to 2.5)	.53
Hospitalization, no. (%)*	2 (5.9)	5/39 (13)	0.5 (0.1 to 2.4)	.37
Oral corticosteroid, no. (%)*	10 (29)	17 (43)	0.7 (0.3 to 1.5)	.36
Inhaled corticosteroid, no. (%)*	3 (8.8)	1 (2.5)	3.5 (0.4 to 33.9)	.27

Values are shown as means (SDs), medians (interquartile ranges), or numbers (percentages) of subjects. Data were analyzed by using the 2-sample *t* test (cough and noisy breathing), the Wilcoxon rank sum test with a Hodges-Lehmann estimate for median difference (expiratory breathing difficulty, nocturnal symptoms, and bronchodilator at home), or Poisson regression analysis (nocturnal symptoms, outpatient clinic visit, hospitalization, oral corticosteroid, and inhaled corticosteroid). Nocturnal symptoms are shown as a categorical variable because of the low frequency of symptoms.
RR, Relative risk.
*For acute expiratory breathing difficulty.

wheezing has been associated with many asthma-related factors, such as allergic sensitization, eczema, increased blood eosinophil counts, increased exhaled nitric oxide levels, decreased lung function, illness severity, and maternal atopic asthma; increased IL-4, IL-5, and IL-13 responses and decreased IFN-α/β/γ/λ and IL-10 responses in airway secretions; and broken airway epithelium *in vitro*.[1,6,8,10-12,19,26-30] In line with these extensive epidemiologic and mechanistic data, in a *post hoc* analysis we previously found that prednisolone reduced recurrent wheezing at 2-month, 12-month, and 7-year follow-ups in children with rhinovirus, eczema, or both affected by first-time wheezing at the ages of 3 to 35 months.[4,9,19]

The short-term benefits of prednisolone that we observed (eg, less respiratory symptoms during the 2-week follow-up) are in line with some,[19,31,32] but not all,[14-16] previous studies. In our previous review[14] we found that 7 (44%) of 16 trials showed beneficial effects of systemic corticosteroids on short-term outcomes, whereas 9 (56%) of 16 did not. More recently, researchers have focused on which subgroup might benefit the most or on the role of combined treatments. Alansari et al[32] found that dexamethasone effectively decreased the time to readiness for discharge in infants with bronchiolitis and increased risk of asthma (as determined based on eczema or a family history of asthma) by 31%. Also, use of systemic corticosteroids has effectively reduced the occurrence of relapses resulting in a clinic visit during a 2-month follow-up in hospitalized first- or second-time wheezing children aged 3 to 35 months with associated rhinovirus infection or blood eosinophil counts of 0.2×10^9/L or greater.[19] Thus systemic corticosteroids might have a beneficial effect on short-term outcomes when targeted for children at high risk for asthma, as defined by rhinovirus positivity, eczema, family history of asthma, and/or increased blood eosinophil counts. Moreover, systemic corticosteroids, when combined with nebulized epinephrine, were of borderline significance in reducing the need for hospitalization in infants with bronchiolitis, and the effect was not affected by positive RSV status (rhinovirus was not examined), a personal or family history of atopy, presentation early in the course of illness, or severe illness.[31] Four studies have not shown an effect in children with atopic characteristics, but they have been underpowered to fully address the atopy issue, or the prednisolone was parent initiated.[15,17,31,33]

When looking at longer-term outcomes (ie, ≥2 months), prednisolone made a difference only for those with high rhinovirus loads. This finding is supported by our previous trial, with 1- and 7-year follow-ups showing long-term effect of prednisolone in rhinovirus-positive children with first-time wheezing.[4,9] During our previous study, conventional PCR followed by liquid hybridization was used in rhinovirus diagnostics. In the current study this method was replaced by real-time PCR, which more efficiently detects rhinoviruses.[34] Quantitative PCR was not done in our previous study. The most likely explanation for the effect of prednisolone in the group with high copy numbers is that the high rhinovirus load is associated with more severe airway inflammation.[1,6,10-12,19,26,29,30] Airway inflammation is typically associated with low interferon expression, leading to decreased viral clearance, increased virus replication, and eventually more severe airway inflammation. Epithelial cell cultures with a broken surface are more susceptible to rhinovirus replication than those with an intact surface.[27] In agreement, previous studies have shown a positive association between rhinovirus load and illness severity, as did we with cough severity.[35,36]

With regard to the other factors, the baseline characteristics of rhinovirus-affected participants in our current trial and a previous trial[3] were similar: median age of 0.9 versus 1.2 years, prevalence of atopy of 30% versus 20%, mean serum 25-hydroxyvitamin D level of 84 versus 75 nmol/L, and index hospitalization of 80% versus 100%, respectively. Although 25-hydroxyvitamin D is thought to protect against acute respiratory tract infections,[37] both samples had average levels that would be considered sufficient based on current international standards. The susceptibility to viral infections appears to markedly increase at a 25-hydroxyvitamin D level of less than 50 nmol/L in wheezing children.[38] Atopic characteristics, such as eczema, might have importance in first-time wheezing children when considering responsiveness to systemic corticosteroids.[4,9] Importantly, aeroallergen sensitization, which is a very specific risk factor for asthma among wheezing children, develops rather slowly, is usually not present during infancy,

and is present in only 5% to 10% of children during the second year of life.[39]

The strengths of the study include the randomized, double-blind, placebo-controlled study design with a 12-month follow-up and careful characterization of the subjects. With regard to limitations, we recognize that the study was underpowered to detect a less than 34% absolute difference. Even 10% absolute differences in relapse rates have recently been considered meaningful in wheezing children.[40] The results might not be generalizable to outpatients because 80% of our subjects were enrolled at hospitalization; the sample size was too small to permit meaningful analysis of inpatient versus outpatient interactions. There was a delay in the initiation of study drug in the current trial compared with our previous trial (45 vs 0 hours, respectively) because of completion of rhinovirus PCR. This is why our primary outcome was the time to recurrence and not the short-term (secondary) outcomes. However, we found no association between the delay and primary or secondary outcomes. We checked medical records and interviewed a parent using a standard questionnaire to confirm that there was no previous wheezing episode. Bacterial coinfections were not investigated, and rhinovirus species were not typed.

In summary, prednisolone treatment cannot be routinely recommended for all children experiencing their first acute, moderate-to-severe, rhinovirus-induced wheezing episode because we were not able to show an effect in our primary outcomes. However, our results suggest that prednisolone treatment is beneficial in a subgroup of young children with high viral loads at presentation. The apparent effect of prednisolone on long-term outcomes among children with high rhinovirus loads and its benefits on most short-term outcomes warrant further study. Clinically, the challenge is early identification of children at high risk of asthma and identification of an effective intervention to reduce respiratory morbidity. A rhinovirus-related cause of early wheezing is a promising new marker for these high-risk children,[4-9] and clearly, there is a need for a bedside quantitative rhinovirus detection test. Our RCT findings support a role for high rhinovirus load as an important marker in those children with early pulmonary inflammation who might benefit from acute treatment with prednisolone.

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Key messages

- **Despite some benefits in secondary short-term outcomes, prednisolone cannot be routinely recommended for all children with their first acute rhinovirus-induced wheezing episode because it did not affect primary long-term outcomes.**
- **Interestingly, prednisolone decreased relapses in those with a high rhinovirus load, suggesting early pulmonary inflammation in these children.**

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METHODS

Study protocol

Hospital staff contacted study physicians when eligible children were admitted to the emergency department or hospital ward. The need for hospitalization was decided by an on-duty physician outside of the study team. After interviewing the guardian, the study physician asked him or her to fill in a standard questionnaire on host and environmental risk factors for asthma (Fig E1). A nasopharyngeal aspirate sample was obtained at study entry for viral diagnostics by using a standardized procedure.[E1] If the rhinovirus PCR result was positive, a study physician was notified immediately by telephone. Consequently, the study physician contacted the guardian and also the hospital ward if the child was still hospitalized and verified the child's symptoms.

Every new patient was examined between 8 AM and 10 PM 7 days per week. During hospitalization, patients were examined every morning by the study physician (Fig E2). The median respiratory symptoms score was assessed on a scale of 0 to 12 daily at the hospital and each study visit after discharge.[E2] Ward patients received nebulized albuterol as needed. After discharge, parents were advised to use a β_2-agonist as needed.

The guardian was instructed to fill out daily symptom and medication diaries for 2 months (the first 2 weeks also assessing symptom severity on a 4-point graded scale) and thereafter to fill in the dates of expiratory breathing difficulties, respiratory medications, and visits to health care providers (Figs E3-E5).

All data were collected on paper and continuously entered in an electronic database by the study physicians. After completion of the 12-month follow-up, the electronic database was double checked by 2 independent persons. Thereafter, the study code was revealed.

The study protocol was checked on a yearly basis by the Finnish Medicines Agency (Fimea; www.fimea.fi) and checked on site by Fimea authorities in May 2009. The results of the trial are published according to the CONSORT statement.[E3,E4]

Randomization

Separate computer-generated randomization sequences in random permuted blocks of 4 were used for inpatients and outpatients. They were prepared by and stored in the hospital pharmacy and were revealed only after all children had completed the 12-month follow-up. Prednisolone and placebo were provided in identical containers labeled with the patient's number only. The placebo tablets and packages were indistinguishable in appearance from those of the active drug. The recommended administration of all tablets was to crush the tablet and then mix it with jelly or yogurt. The study drugs were administered by hospital nurses independent of the study or by a guardian at home. Study drug administration was recorded on follow-up forms.

Definitions

Physician-confirmed wheezing episodes were scored on a standard sheet at the study clinic and considered a positive outcome only if expiratory breathing difficulty with wheeze was present. If the recurrence was treated outside the study clinic, a copy of the outside medical record was ordered and reviewed, and the episode was considered a positive outcome if expiratory breathing difficulty was present.

Laboratory methods

After obtaining nasopharyngeal aspirates, a sterile flocked swab (Copan) was dipped into the aspirate, placed into a new dry tube, and stored at −70°C until analysis. The nucleic acids for RT-PCR were extracted by eluting the swab in PBS and lysis buffer. After this, they were extracted with the NucliSens EasyMag automated extractor (bioMèrieux, Boxtel, The Netherlands), according to the manufacturer's instructions. Quantitative RT-PCR was done for rhinovirus by using known concentrations of rhinovirus 14 plasmid as a positive control. The plasmid was a gift from Glyn Stanway at the University of Colchester (Essex, United Kingdom).

RESULTS

The mean age of the 74 children was 12.7 months (SD, 6.0 months); 11 children were aged 3 to 5 months, 22 were aged 6 to 11 months, and 41 were aged 12 to 23 months. Fifty-seven (77%) children were male, 22 (30%) of 73 had allergen-specific IgE sensitization, 28 (38%) had eczema, 15 (21%) of 72 had atopic eczema, 17 (23%) had parental asthma, and 18 (24%) had virus codetection, and the mean level of 25-hydroxyvitamin D was 85 nmol/L (SD, 23 nmol/L; see Table E1). The 2 randomly assigned study groups (prednisolone versus placebo) did not differ in any of the patient characteristics. Compared with outpatients, inpatients had lower oxygen saturation (median, 97% [interquartile range, 95% to 98%] vs 98% [interquartile range, 96% to 99%]; $P = .02$), higher respiratory rate (mean, 52 [SD, 13] vs 45 [SD, 10] breaths/min, $P = .03$), and higher body temperature (37.7°C [0.7°C] vs 37.2°C [0.4°C]; $P = .002$) at study entry. The study groups did not differ with respect to use of nonstudy medications from the index hospitalization or emergency department visit through day 14 (data not shown).

No difference was found between the treatment groups in the delay of prednisolone initiation at study entry (prednisolone vs placebo: mean, 45 [SD, 22] vs 52 [SD, 29] hours; $P = .27$; Table I and Table E1). In addition, the delay was not associated with the primary or secondary outcomes (results not shown).

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The key questions

To be filled by study physician at parental interview

Name: _____
Social security number: _____
Names of the parents / guardians: _____
_____
Address: _____
Phone: _____
Email: _____

Does the child fulfill inclusion criteria of the study: age 3-23 months, ≥h37+0, first episode of breathing difficulty and written informed consent from the parents? Yes ☐

Does the child fulfill inclusion criteria of the intervention trial: rhinovirus PCR positive and still signs of lower respiratory infection (breathing difficulty, noisy breathing or cough)?
Yes ☐ No ☐

Randomized to receive the study drug: Yes ☐ No ☐
If yes, when (day, time) _____

Any exclusion criteria: chronic other than atopy related illness, previous systemic or inhaled corticosteroid treatment, participation to another study (excluding long-term follow-up studies in childhood), varicella contact if previously intact, need for intensive care unit treatment, or poor understanding of Finnish No ☐

Parents / guardians have received routine hospital wheezy questionnaires (2 forms) and symptom diaries (3 forms):
Yes ☐

Height _____ cm and weight _____ kg
Still breastfeeding Yes ☐ No ☐
Duration of breastfeeding ___ months
Duration of exclusive breastfeeding ___ months
Does the child have doctor-diagnosed atopic eczema: Yes ☐ No ☐

	Mother	Father
Doctor-diagnosed asthma:	Yes ☐ No ☐	Yes ☐ No ☐
Allergic rhinitis:	Yes ☐ No ☐	Yes ☐ No ☐
Smoking:	Yes ☐ No ☐	Yes ☐ No ☐
Furry pets:	Yes ☐ No ☐	
Number of children in the family:	___ children	
Daycare:	Home ☐ Small group ☐ Kindergarden ☐	

FIG E1. The key questions of parental interview and wheezy questionnaire.

Wheezy questionnaire

To be filled by a parent/guardian

1. Does your child have a family doctor?
   No □ Yes □, Dr _____ practicing in _____
2. Type of daycare?
   1) Home □ 2) Family day care □ 3) Day care center □ 4) Other □, what? _____
3. Type of home?
   1) Apartment building □ 2) House □ 3) Row house □ 4) Farm □ 5) Other □, what? _____
4. Number of children in the family? ____
5. Parental smoking? No □ Yes □, if yes, smoking:
   1) inside No □ Yes □
   2) in the car No □ Yes □
6. Pets at home?
   dog No □ Yes □
   cat No □ Yes □
   other animals No □ Yes □, what? _____
7. Other allergen sources at home?
   feather pillows/blankets No □ Yes □
   fitted carpet No □ Yes □
8. At day care
   pets/animals? No □ Yes □, what? _____
   smoking? No □ Yes □
9. At other places, weekly exposure to
   animals? No □ Yes □
   smoking? No □ Yes □
10. Are there allergic symptoms in the family?
    eczema No □ Yes □, underline: mother / father / sibling
    rhinitis No □ Yes □, underline: mother / father / sibling
    asthma No □ Yes □, underline: mother / father / sibling
11. Does the child have allergic symptoms? Please, mark the suspected source on the reverse side.
    eczema No □ Yes □
    rhinitis No □ Yes □
    asthma No □ Yes □
12. Does your child have an "allergy diet"?
    No □ Yes □. Please, specify the diet to the study nurse.
13. Has your child ever undergone skin prick tests?
    No □ Yes □, when ___/___(month/year), where _____
14. Information about **allergies** (please circle the suspected sources):
    1) Dietary: chocolate, cocoa, citrus, egg, fish, tomato, strawberry, pea, apple, carrot, nuts, pear, peach, cow´s milk, breast milk substitute, rye, barley, oats, wheat, other _____
    2) Animals: dog, cat, horse, cow, guinea pig, feather, other _____
    3) Pollen: birch, alder, conifer, hay, mugworth, other _____
    4) Other causes: room dust, fungal spore, other _____

FIG E1. *(Continued)*

15. Information about the child´s **respiratory infections**:
During the last 12 months:
1) "common cold" ___ times
2) antibiotic prescription ___ times
3) pneumonias ___ times
4) bronchitis ___ times
5) otitis ___ times
6) parasenthesis ___ times
7) other, what? _____
16. Adenoidectomy
No ☐ Yes ☐, when ___/___ (month/year), where _____
17. Maxillary sinus puncture
No ☐ Yes ☐, when ___/___ (month/year), where _____
18. Information about breathing difficulty symptoms:
Were there "common cold" symptoms during the current difficulty in breathing?
No ☐ Yes ☐ I can't say ☐
If you suspect other causes, please name them: _____
19. The duration of respiratory symptoms before study entry?
1) rhinitis _____ days
2) cough _____ days
3) rhinitis _____ days
20. Have other family members had "common cold" symptoms?
No ☐ Yes
21. Is this your child's first episode of breathing difficulties?
No ☐ Yes
22. Does your child have any regular medication?
No ☐ Yes , what? _____

*The key questions are directly translated from Finnish study form. The wheezy questionnaire contains selected questions from 2 page standard wheezy questionnaire and 7 page standard allergy questionnaire used at Turku University Hospital.

FIG E1. *(Continued)*

Name _____ **Social security number** _____

(After study entry, to be filled in the mornings 2 hours after bronchodilator).

	An example				
Date, time	7.9.2007 8.00				
Heart rate/min	112				
Breathing frequency/min	55				
Oxygen saturation	98				
Body temperature	37.6				
Inspiratory:expiratory time: 0 (2:1), 1 (1:1), 2 (1:2), 3 (1:3)	1:2				
Degree of dyspnea: 0 (normal), 1 (mild), 2 (moderate), 3 (severe)	2				
Auscultatory findings on wheezing: 0 (none), 1 (expiratory), 2 (inspiratory and expiratory), 3 (audible without stethoscope)	2				
Type of breathing: 0 (normal), 1 (use of stomach muscles), 2 (use of intercostal muscles), 3 (nasal flaring)	1				
Pneumonic crackles: 0 (no), 1 (mild), 2 (moderate), 3 (severe)	0				
Otitis media: 0 (no), 1 (yes)	0				
Antibiotic: 0 (no), 1 (yes), what and when initiated	0				
Other symptoms +/++/+++ and their connection to study medication: 1 (no), 2 (maybe), 3 (yes)					
Cough	++/1				
Rhinitis	+/1				
Eye symptoms	-				
Eczema	-				
Any other symptoms/signs (report everything)	Restlesness ++/1				
Other notes, e.g. the cause of symptoms	flu				
Study drug given (tally)	lll				
Mediacation (drug name, dosage) before physical examination (tally)	ER: albuterol 1.2 mg x2, ward: albuterol 1.5 mg x2				
…and before blood draw	+ albuterol 1.5 mg x2				
Oxygen (hour) or i.v. or nasogastric hydration (volume/time)	Oxygen 5 h i.v. fluid 700 ml				
Exact times: Study entry at ER/ward (1), study drug initiation (2), ready for discharge considering breathing difficulties (3), actual discharge (4).	(1) 15.30				

FIG E2. Clinical score sheet for hospitalized patients.

Name _____ **Social security number** _____

Daily symptom and medications until 2-week visit.

Date (fill in one column per day)	An example 1.1.07								
Hospitalization for expiratory breathing difficulty, yes or no	Yes								
Cough, 0 (no) - 3 (severe)	2								
Expiratory breathing difficulty, 0 (no) - 3 (severe)	1								
Noisy breathing, 0 (no) - 3 (loud)	0								
Rhinitis, 0 (no) - 3 (severe)	0								
Night wakening for breathing difficulties, 0 (no), 1 (once), 2 (often), 3 (continuously)	1								
Temperature, exact or on scale 0 (no) - 3 (high)	Fever 37.9, or 1								
Any other symptom (report any deviation from normal)	Fell in stairs, tearful								
Other notes (e.g. cause of symptom)	scute otitis media, playing with a cat								
Study drug taken (tally)	111								
Bronchodilator (name, dose, number of doses; tally)	Ventoline 0.1 mg, puffs 1111								
Other medication	Naprosyn mixt. 5 mg/ml 3 ml/dose, 111 Amorion mixt 80 mg/ml 3.7 ml x2								
Doctor's appointment: where, why, name of the doctor, and treatment	Healthcenter Mäntymäki 1, fever, cough, tearful, Naprosyn and Amorion for acute otitis media								

If any questions, do not hesitate to contact study physician by phone.

FIG E3. Symptom and medication diary for 2 weeks after discharge.

Name _____ **Social security number** _____

Daily symptoms and medications for 2 months. Check a box if yes.

Month _____ (fill in one column per day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Rhinitis																															
Cough																															
Expiratory breathing difficulty																															
Noisy breathing																															
Nocturnal symptoms																															
Bronchodilator																															
Oral corticosteroid																															
Inhaled corticosteroid																															
Antibiotic																															
Doctor's visit for expiratory breathing difficulty																															
Hospitalization for expiratory breathing difficulty																															

Month _____ (fill in one column per day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Rhinitis																															
Cough																															
Expiratory breathing difficulty																															
Noisy breathing																															
Nocturnal symptoms																															
Bronchodilator																															
Oral corticosteroid																															
Inhaled corticosteroid																															
Antibiotic																															
Doctor's visit for expiratory breathing difficulty																															
Hospitalization for expiratory breathing difficulty																															

Month _____ (fill in one column per day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Rhinitis																															
Cough																															
Expiratory breathing difficulty																															
Noisy breathing																															
Nocturnal symptoms																															
Bronchodilator																															
Oral corticosteroid																															
Inhaled corticosteroid																															
Antibiotic																															
Doctor's visit for expiratory breathing difficulty																															
Hospitalization for expiratory breathing difficulty																															

If any questions, do not hesitate to contact study physician by phone.

FIG E4. Symptom and medication diary from 2 weeks to 2 months after discharge.

Name _____ **Social security number** _____

Report every episode of expiratory breathing difficulty during the 12 month follow-up period (one episode may last more than a day) and check also whether the patient was treated solely at home, as outpatient or as inpatient.

Episode number	Date	Treated solely at home	Doctor's appointment	Hospitalization	Corticosteroid	
					Oral	Inhaled
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

If you have any questions, do not hesitate to contact study physician by phone.

FIG E5. Symptom and medication diary from 2 months to 12 months after discharge.

TABLE E1. Baseline characteristics

Characteristic	Inpatients		Outpatients	
	Prednisolone (n = 28)	Placebo (n = 31)	Prednisolone (n = 6)	Placebo (n = 9)
Age (mo)	14 (7.2)	12.3 (5.0)	10.7 (4.3)	11.9 (5.8)
Male sex, no. (%)	22 (79)	25 (81)	5 (83)	5 (56)
Clinical characteristics at entry				
Respiratory symptoms score*	5.9 (2.6)	5.9 (2.3)	5.0 (2.0)	4.8 (1.9)
Respiratory rate (breaths/min)	52 (10)	53 (15)	50 (8.9)	41 (10)
Heart rate (beats/min)	145 (17)	144 (18)	145 (25)	128 (26)
Oxygen saturation (%)	97 (95-98)	96 (94-98)	99 (96-100)	98 (96-99)
Temperature (°C)	37.5 (0.6)	37.8 (0.7)	37.2 (0.3)	37.1 (0.5)
Duration of symptoms before enrollment (d)				
Rhinitis	3 (1-5)	3 (2-6)	5 (1-15)	4 (3-7)
Cough	3 (2-5)	2 (1-5)	4 (2-10)	4 (2-6)
Dyspnea	1 (1-2)	1 (1-2)	2 (1-4)	1 (1-2)
Viral cause				
Rhinovirus load (copies $\times 10^3$/mL)	5.7 (0.3-34)	2.9 (0.1-7.5)	8.4 (0.09-113)	2.7 (0.0001-9.9)
Viral coinfection, no. (%)	8 (29)	14 (45)	3 (50)	1 (11)
RSV, no. (%)	4 (14)	6 (19)	0 (0)	0 (0)
Bocavirus, no. (%)	2 (7)	4 (13)	2 (33)	0 (0)
Parainfluenza virus, no. (%)	1 (4)	4 (13)	0 (0)	0 (0)
Each other virus, no. (%)	≤2 (≤7)	≤2 (≤7)	≤1 (≤17)	≤1 (≤11)
Delay in study drug initiation (h)†	43 (21)	55 (29)	52 (35)	43 (27)
Atopic characteristics				
Allergic sensitization, no. (%)‡	9 (32)	12/31 (39)	1 (17)	0/8 (0)
Food sensitization, no. (%)	7 (25)	12 (39)	1 (17)	0/8 (0)
Aeroallergen sensitization, no. (%)	6 (21)	6 (19)	0 (0)	0/8 (0)
Perennial, no. (%)	6 (21)	5/30 (17)	0 (0)	0/8 (0)
Seasonal, no. (%)	3 (11)	2 (6)	0 (0)	0/8 (0)
Blood eosinophils ($\times 10^9$/L)	0.55 (0.48)	0.51 (0.41)	0.30 (0.13)	0.52 (0.31)
Blood eosinophils ($\geq 0.4 \times 10^9$/L), no. (%)	16 (57)	16/29 (55)	1 (17)	5/8 (63)
Doctor-diagnosed eczema, no. (%)	12 (43)	10 (32)	3 (50)	3 (33)
Doctor-diagnosed atopic eczema, no. (%)	8 (29)	6/30 (20)	1 (17)	0 (0)
Parental asthma, no. (%)	6 (21)	7 (23)	2 (33)	2 (22)
Parental allergy, no. (%)	18 (64)	25 (80.6)	3 (50.0)	1 (11.1)
Total IgE (kU/L)	22 (8.8-45)	16 (6-51)	13 (5.8-22)	13 (9-27)
No. of children in the family	2 (1-2)	2 (1-2)	2 (1-2)	2 (1-4)
Day care, no. (%)				
Home	17 (61)	23 (74)	4 (67)	6 (67)
Small group	5 (18)	4 (13)	1 (17)	1 (11)
Kindergarten	6 (21)	4 (13)	1 (17)	2 (22)
Serum 25-hydroxyvitamin D (nmol/L)	81 (27)	87 (19)	89 (25)	91 (22)
Serum 25-hydroxyvitamin D_2 (nmol/L)	22 (23)	21 (22)	35 (25)	25 (18)
Serum 25-hydroxyvitamin D_3 (nmol/L)	59 (30)	66 (27)	54 (34)	66 (18)
Duration of breast-feeding (mo)	6.6 (4.5)	5.5 (4.5)	5.6 (2.1)	5.3 (3.7)
Parental smoking, no. (%)	11 (39)	12 (39)	3 (50)	5 (56)

Values are shown as means (SDs), medians (interquartile ranges), or numbers (percentages) of subjects. No statistically significant differences were found between the treatment groups.

*Assessed on scale of 0 to 12, consisting of sum of degrees of dyspnea, type of breathing, severity of auscultatory findings on wheezing, and expiratory/inspiratory time.[E2]

†Delay was due to rhinovirus PCR diagnostics.

‡Defined as IgE antibodies to any of the common allergens; see the Methods section for details.