ORIGINAL ARTICLE

Asthma and Rhinitis

WILEY

Efficacy of inhaled salbutamol with and without prednisolone for first acute rhinovirus-induced wheezing episode

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Abstract

Background: Acute rhinovirus-induced wheezing is common in young children and may respond to systemic corticosteroid. There are no trials on the efficacy of inhaled beta$_2$-agonist in this clinical scenario.

Objective: To study *post hoc* the short-term (up to 2 months) efficacy of inhaled beta$_2$-agonist with and without oral corticosteroid in the first acute rhinovirus-induced severe wheezing episode in young hospitalized children.

Methods: The study population came from two randomized controlled trials comparing oral prednisolone (2 mg/kg/d for 3 days) to placebo: Vinku (*n* = 35, NCT00494624) used high-dose regular nebulized salbutamol (0.15 mg/kg 2–4 h intervals) and Vinku2 (*n* = 60, NCT00731575, EudraCT 2006-007100-42) used inhaled salbutamol on-demand. Both studies used identical detailed follow-up assessments. The primary outcome of the former was the duration of hospitalization and of the latter the occurrence of and the time to a new physician-confirmed wheezing episode within 2 months after discharge. Treatment groups included salbutamol high-dose vs. salbutamol on-demand while adjusting for prednisolone status and acknowledging for interactions with exception of the duration of hospitalization in which prednisolone groups could not be fully used due to protocol differences.

Results: Median age of subjects was 13 months, 32% were sensitized and 22% had doctor-diagnosed eczema. In the duration of hospitalization, salbutamol high-dose/placebo versus salbutamol on-demand/placebo groups did not differ (p = .12). In the occurrence of and time to relapse within 2 months, a significant group × treatment interaction was observed (both p = .02), such that high-dose group had less and longer time to relapses than on-demand group in prednisolone arm (both p < .05), but no difference was detected in placebo arm (both p > .26).

Conclusions: In young, hospitalized children with first episode of rhinovirus-induced wheezing, high-dose inhaled salbutamol may interact with oral prednisolone. However, further trials are warranted.

KEYWORDS

Asthma, beta$_2$-agonist, bronchiolitis, corticosteroid, paediatrics, virus
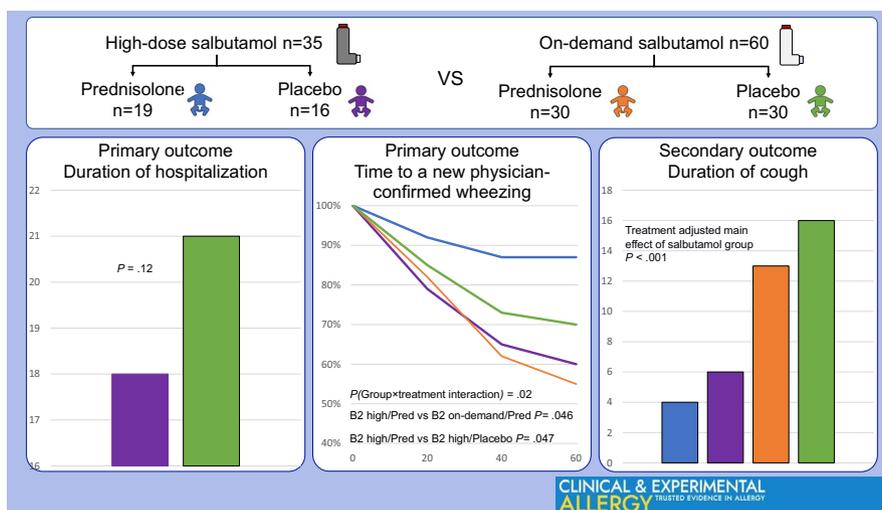
GRAPHICAL ABSTRACT

In young, hospitalized children with first episode of rhinovirus-induced wheezing, high-dose salbutamol may interact with oral prednisolone. However, further trials are warranted.

1 | INTRODUCTION

Bronchiolitis affects up to 20%–30% of children during first two years of life and is the most common reason for hospitalization in children.[1] Respiratory syncytial virus and rhinovirus (RV) are the most common etiologic agents. The former is associated with typical bronchiolitis syndrome whereas RV aetiology starts to dominate at little older age, typically after 12 months of age, and is particularly interesting since it is closely linked to presence of wheezing and has been recognized as a major risk factor of atopic asthma up to the age of 13 years.[2–6] Interestingly, previous *post hoc* studies have found out that RV-affected first-time wheezing children, especially those with high virus genome loads, appear to respond to oral prednisolone

Key Message

- We evaluated high-dose nebulized versus on-demand inhaled salbutamol in children with first rhinovirus-induced wheeze.
- There was an interaction between high-dose salbutamol and prednisolone, but hospitalization duration did not differ.
- Combined prednisolone and high-dose salbutamol for rhinovirus-induced wheeze in young children need further investigation.

in terms of less recurrent wheezing and less need for asthma control medication before school age.[7–9] The potential explanations for the increased risk of asthma include low interferon responses (i.e., impaired viral defence), early airway inflammation (i.e., broken epithelial barrier, T helper$_2$ polarized immune responses), biased host-microbiome and genetic variation at the 17q21 and CDHR3 loci.[1,10,11] Early systemic corticosteroid treatment is likely to down-regulate early inflammatory events.

Bronchodilators (beta$_2$-agonists) are a crucial part of the management of recurrent wheezing and asthma exacerbations. However, all of the current major guidelines (e.g., The Scottish Intercollegiate Guidelines Network [SIGN] 2006, Spanish National Health System [SNHS] 2010, American Academy of Pediatrics [AAP] 2014, Finnish Current Care 2014, National Institute for Health and Care Excellence of UK [NICE] 2015, Australasian Paediatric Research in Emergency Departments International Collaborative [PREDICT] committee 2018 and Canadian Paediatric Society [CPS] 2018) recommend against their routine use in the treatment of bronchiolitis.[12–17] Only SNHS 2010 considers treatment trial with this medication optional while other guidelines do not address this option.[12–17] This is not surprising since only handful of trials has found clinical benefit of beta$_2$-agonists compared to placebo among young first-time wheezing children (main trials are outlined in Table S1, and selection of trials are shown in Figure S1).[18] Moreover, conclusive evidence of their efficacy in subgroups of bronchiolitis remains unavailable.

Although bronchiolitis is usually discussed as a single disease, it is a heterogeneous condition, which implies that the treatment should be administered on a more personalized basis, as stated in a recent EAACI Task Force paper.[1] Since RV-induced first wheezing illness has many asthma-like features,[1,2,6,10] we hypothesized that it would respond to beta$_2$-agonists. Therefore, our aim was to investigate post hoc the short-term efficacy of beta$_2$-agonist with and without oral corticosteroid in the first acute RV-induced wheezing episode in children aged less than 2 years.

2 | METHODS

2.1 | Subjects

The study population came from two randomized controlled trials comparing oral prednisolone (2 mg/kg/d for 3 days) to placebo but they differed in regard to the administration of beta$_2$-agonists during hospitalization: Vinku study (n = 35/293 [enrolled/eligible], NCT00494624) used high-dose regular nebulized salbutamol (first 12 h 0.15 mg/kg 2 then 4 h intervals) while Vinku2 study (n = 60/124 [enrolled/eligible], updated version for 7-year follow-up NCT00731575, original version EudraCT 2006-007100-42) used administration of salbutamol via spacer without nebulizer only on-demand. In both studies, administration of salbutamol was given in identical weight-based design (exact dosage in Table 1).[7,9,19] The recruitment for the Vinku study was carried out in 2000–2002, and for the Vinku2 study in 2007–2010 in the Department of Pediatrics,

Turku University Hospital (Turku, Finland). The inclusion criteria for the current analysis were age 3–23 months, delivery at ≥36 gestational weeks, first wheezing episode (parental report and confirmed from medical records), hospitalization, RV detected in nasopharyngeal aspirate sample by polymerase chain reaction (PCR). The exclusion criteria were the use of inhaled corticosteroids before the study entry, chronic non-atopic disease and a need for intensive care. The studies were approved by the Ethics Committee of the Turku University Hospital and commenced only after obtaining written informed consent from the guardians.

2.2 | Study protocol

The need for hospitalization was decided by an on-duty study physician independent of study.

The recruitment to the study was done by study physician. At study entry, the guardian filled in a standard questionnaire on host and environmental risk factors for asthma. Then the child was physically examined by study physician, a nasopharyngeal aspirate sample was obtained for viral diagnostics using a standardized procedure,[20] and a baseline blood sample was drawn. The children were randomized to be given either oral prednisolone or a placebo; at study entry in Vinku, and after positive RV PCR test in Vinku2.[9,19] Both studies used identical sampling and follow-up protocols including daily symptom diaries for the first two months as well as scheduled follow-up visits at 2 weeks and 2 months by the study physicians. In addition, the guardian was asked to bring the child to the study physician each time when the child had breathing difficulties. Of note, Vinku2 study had prospective RCT design whereas Vinku study had post hoc design. Because of this difference, duration of hospitalization was not fully comparable in regards to prednisolone treatment (B2 on-demand/prednisolone group from Vinku2 study) since prednisolone was administered only after positive RV PCR-finding in Vinku2 study which caused a delay in drug administration (45 h) compared to other study groups.

2.3 | Outcomes

Three predefined primary outcomes for this current study were: (1) time until ready for hospital discharge, (2) occurrence of a new physician-confirmed wheezing episode during the 2-month follow-up, as well as (3) time to a new physician-confirmed wheezing episode during the 2-month follow-up.[9,19] Original primary outcome of Vinku study was time until ready for hospital discharge.[19] Original primary outcomes of Vinku2 study were (1) the occurrence of and time to a new physician-confirmed wheezing episode within 2 months after discharge, (2) number of physician-confirmed wheezing episodes during the first 12 months after discharge, (3) diagnoses of asthma during the full study period.[9] The assessment of ready for discharge was based on a clinical scoring, as previously described.[19]

TABLE 1 Patient characteristics at study entry

	Group		
	High-dose salbutamol	On-demand salbutamol	
Characteristic	n = 35	n = 60	p
Age, months	13.1 (6.0)	12.7 (5.8)	.64
Male sex, no.	21 (60)	48 (80)	.03
Weight, kg	10 (1.8)	10.4 (2.1)	.54
Preceding wheezing, days	1 (1–2)	1 (1–2)	.52
Preceding cough, days	3 (2–7)	2 (2–4)	.26
Preceding rhinitis, days	3 (2–7)	2.5 (2–5)	.83
Clinical score, points	7 (5–7)	6 (3–8)	.13
Oxygen saturation, %	97 (94–98)	97 (95–98)	.71
Temperature, C°	37.6 (0.7)	37.6 (0.7)	.66
CRP, mg/L	9.5 (4–19)	12 (6–21)	.40
Antibiotic at entry, no.	19 (54%)	19 (32%)	.03
Otitis media	19 (54%)	19 (32%)	1.0
Pneumonia	1 (3%)	1 (2%)	1.0
Viral coinfection, no.	20 (57%)	21 (35%)	.04
RSV, no (%)	5 (14%)	10 (17%)	.76
Bocavirus, no (%)	9 (26%)	5 (8%)	.02
Parainfluenza, no (%)	2 (6%)	5 (8%)	1.0
Adenovirus, no (%)	4 (11%)	2 (3%)	.19
Dr-dg atopic eczema, no.	6 (18%)	14 (23%)	.53
B-eos >0.4 × 10^9/L	14 (41%)	34 (58%)	.13
Sensitization, no.	9 (26%)	21 (35%)	.39
Food, no.	9 (27%)	19 (32%)	.62
Aero, no.	1 (3%)	12 (20%)	.03
Perennial, no.	1 (3%)	11 (18%)	.050
Parental asthma, no.	3 (9%)	13 (21%)	.10
Parental allergy, no.	14 (40%)	24 (40%)	.18
Parental smoking, no.	21 (60%)	44 (73%)	1.0
S-25-OHD, nmol/L	63 (49–85)	84 (72–98)	.001
S-25-OHD$_2$, nmol/L	17 (5.7–30)	16 (0–31)	.66
S-25-OHD$_3$, nmol/L	40 (24–73)	61 (42–78)	.01
Seasonality of infection			.06
Winter (1.12–28.2)	13 (37%)	19 (32%)	
Spring (1.3–31.5)	7 (20%)	4 (7%)	
Summer (1.6–31.8)	1 (3%)	10 (16%)	
Autumn (1.9–30.11)	14 (40%)	27 (45%)	
Cumulative dose of salbutamol during hospitalization (mg)	15.2 (9.6–20)	9 (5–16)	.001
Cumulative dose of salbutamol during hospitalization per kg (mg)	1.4 (0.9–2.1)	0.8 (0.5–1.4)	.001
Cumulative dose of salbutamol during hospitalization per duration of hospitalization by kg (mg)	0.09 (0.06–0.16)	0.04 (0.02–0.05)	.001

Note: Values are shown as mean (SD), median (interquartile range) or number (%).

Data were analysed by two-sample *t*-test, Mann-Whitney *U*-test, χ^2 test or Fisher exact test.

Abbreviations: B-eos, blood eosinophil count; Dr-dg, doctor-diagnosed; RSV, respiratory syncytial virus; S-25-OHD, Serum 25-hydroxyvitamin D.

The predefined secondary outcomes for the current analysis included the duration of cough, duration of wheezing, occurrence of a new physician-confirmed wheezing episode within 2-month follow-up as inpatient, and number of bronchodilator puffs during the 2 weeks after discharge, as well as time to a new physician-confirmed wheezing episode. Original secondary outcomes of Vinku study were (1) oxygen saturation during hospital stay, (2) wheeze and cough during 2 weeks after discharge from the hospital, (3) re-admission to the outpatient clinic or hospital for recurrent wheezing during a two-month period after discharge and (4) blood eosinophil counts at discharge.[19] Original secondary outcomes of Vinku2 study included occurrence of symptoms (cough, wheeze, noisy breathing, breathlessness, rhinitis) as well as exact number of bronchodilator puffs used up to 2 months after discharge from the hospital.[9] For the current analysis, study follow-up was limited to 2 months.

2.4 | Definitions

Wheezing refers to expiratory breathing difficulty with bilateral high pitched sounds during expiration. Wheezing episodes accompanied by RV detection by PCR were called RV-induced wheezing episodes which are a subgroup of bronchiolitis.[9] Atopy was defined as positive immunoglobulin (Ig) E antibody (≥.35 kU/L) to any of the following allergens: codfish, cow's milk, egg, peanut, soybean, wheat, cat, dog, horse, birch, mugwort, timothy, *Cladosporium herbarum* and *Dermatophagoides pteronyssinus* (Phadiatop Combi®, Phadia). Aeroallergen sensitization was defined as positive IgE antibodies to any of the latter 8 allergens. Perennial aeroallergen sensitization was defined as positive IgE antibodies to the dog, cat or *Dermatophagoides pteronyssinus*. Birch, mugwort, timothy and *Cladosporium herbarum* were considered as seasonal aeroallergens. Eczema was defined as physician-diagnosis according to typical symptoms that included pruritus, typical morphology and chronicity of disease. The eczema was defined as atopic eczema if a child had atopy (defined above). Monoinfection was defined as sole RV-induced infection, and coinfection was defined as RV-induced infection accompanied with one or more other viruses.

2.5 | Laboratory data

Nasopharyngeal aspirate or swab samples were stored at +4° until analysed within 3 days after collection. Nucleic acids were either extracted with a commercial nucleic acid extraction kit (High Pure Viral Nucleic Acid Kit, Roche diagnostics) or using NucliSens EasyMag automated extractor (bioMerieux). Nucleic acids were stored at −70°C if not analysed immediately.

An *in-house* PCR test was used to detect rhinoviruses. The primers were derived from the highly conserved 5' noncoding region of Picornavirus genome and they virtually detect all rhino- and enterovirus genotypes.[21,22] The forward primers (positive strand) used were 5′-CGGCCCCTGAATGCGGCTAA-3′, and reverse primers (negative strand) were 5′-CGCCCCCTGAATGCGGCTAA-3′.

An RT-PCR hybridization method was used in the Vinku study, as previously described in detail.[22] DNA Thermal Cycler (Perkin-Elmer, Cetus Corp.) was used for PCR cycling. The following instrument settings were used for amplification: incubation +94°C 3 min and then 40 cycles of 94°C 30 s, 53°C 45 s, 72°C 1 min. The discrimination of amplicons between rhino- and enteroviruses was performed by liquid-phase hybridization using entero- and rhinovirus specific probes labelled with lanthanide chelates. Probes with maximum homology between entero-and rhinoviruses, as well as with intra-genus homology and maximum difference between entero- and rhinoviruses were used. The probes were: rhino 5′-TAGTTGGTCCCITCCCG-3′, entero 5′-TAITCGGTTCCGCTGC-3′, entero-rhino 5′-AAAGTAGTIGGTICC-3′.

PCR assay used in the Vinku2 study was upgraded to a real-time format based on the use of SYBR Green as a double –strand DNA dye. The discrimination of entero- and rhinoviruses was done by melting curve analysis, as previously described in detail.[23] Rotogene 3000 instrument (Corbet Reasearch) was used for PCR cycling according to the following procedure: 95°C 15 min, 45 cycles of 95°C 15 min, 65–55°C 30 s, 72°C 40 s.

Adenovirus, human metapneumovirus (hMPV), influenza A and B virus, parainfluenza virus types 1–4, polyomaviruses WU and KI, RSV and RV were detected using PCR, virus culture and time-resolved fluoroimmunoassay antigen detection method in Vinku study.[7,9,20,24] In addition to viruses mentioned ahead, coronaviruses (229E, NL63, OC43 and HKU1) were analysed by PCR in Vinku 2 study.[9,24] Human bocavirus (HBoV) was analysed using PCR and serology as previously described.[24,25] The methodology of these has been previously described.[7,9,19]

Blood eosinophil count and serum levels of allergen-specific IgE were analysed by the routine diagnostics of the Central Laboratory of Turku University Hospital. Serum 25-hydroxyvitamin D measurements were done by liquid chromatography-tandem mass spectrometry at Massachusetts General Hospital (Boston, USA).

2.6 | Statistics

Differences in baseline characteristics between groups were analysed by using two-sample *t* test for normally distributed and Mann-Whitney *U*-test for non-normally distributed data. The normality of distribution was assessed by Kolmogorov-Smirnov test. Categorical variables were analysed using chi-squared test or Fisher's exact test. The effects of group (salbutamol high-dose vs. on-demand) and treatment (prednisolone vs. placebo) on a new physician-confirmed wheezing episode, and a new physician-confirmed wheezing as inpatient were analysed with logistic regression. Negative binominal regression was used to analyse the effects of group and treatment on duration of cough, duration of wheezing, and number of beta2-agonist puffs used during 2 weeks after discharge. The difference in duration of hospitalization was analysed in two phases with

negative binomial regression. First, in stringent analysis, between placebo study groups (high-dose/placebo and on-demand/placebo). Second, in loose analysis, between high-dose/prednisolone and placebo groups. This approach was selected, because salbutamol on-demand/prednisolone and on-demand/prednisolone groups were not fully comparable to each other since in Vinku2 study the study drug was initiated only after positive RV PCR. The effects of group and treatment on the time to a new physician-confirmed wheezing episode within 2 months were analysed with Cox regression. Time to event was defined from study entry to the time of occurrence of the event. Survival times were censored if the event (new wheezing episode) did not occur within 2 months. Group×treatment interaction effect was included in models and if a statistically significant interaction was found, group effect (salbutamol high-dose vs. on-demand) was estimated separately in Prednisolone and Placebo groups. If the interaction was not statistically significant, the effects for group and treatment were estimated from the main effects model. A two-sided p value < .05 was considered statistically significant. Data analyses were made using JMP software (version 13.1.0, SAS Institute) and SAS System for Windows (version 9.4, SAS Institute).

3 | RESULTS

3.1 | Study population

Originally, 417 children were enrolled on the two trials (Figure 1). Of these 322 children did not fulfil criteria for the current analysis. The main reasons for exclusion were age ≥2 years (n = 118), non-RV aetiology (n = 107), and previous wheezing episode (n = 63). Together, 95 children were included in the current analysis: 35 (37%) were from

Vinku study (high-dose salbutamol) and 60 (63%) from Vinku2 study (salbutamol on-demand). Two-month follow-up was completed in 88 (93%) of children.

3.2 | Patient characteristics

At study entry, the median age was 13 months (interquartile range 8 to 17 months), 73% of the children were boys, 32% had atopy, 22% had atopic eczema, and 43% had virus coinfection. Type of antibiotics varied markedly and only two subjects used macrolides (data not shown). High-dose and on-demand salbutamol groups differed according to sex, antibiotic use, viral coinfection, bocavirus, aeroallergen sensitization, perennial sensitization and serum 25-hydroxyvitamin D and D_3 (Table 1). Further analysis showed that only viral coinfection was associated with outcomes (new physician-confirmed wheezing episode and time to new physician-confirmed wheezing episode) (both p > .05). Of note, all patient characteristics were equally distributed between the prednisolone and placebo groups (Table S2).

3.3 | Primary outcomes

In the stringent analysis, when excluding prednisolone treatment salbutamol high-dose versus salbutamol on-demand did not show a difference in the duration of hospitalization (RR 0.71, 95% CI 0.46–1.09, p = .12) (Figure 2). The loose analysis showed that salbutamol high-dose/prednisolone group had shorter duration of hospitalization than salbutamol on-demand/placebo group (RR 0.58, 95% CI 0.38–0.87, p = .008) (Figure S2). However, interaction could not be evaluated due to the exclusion of study groups.
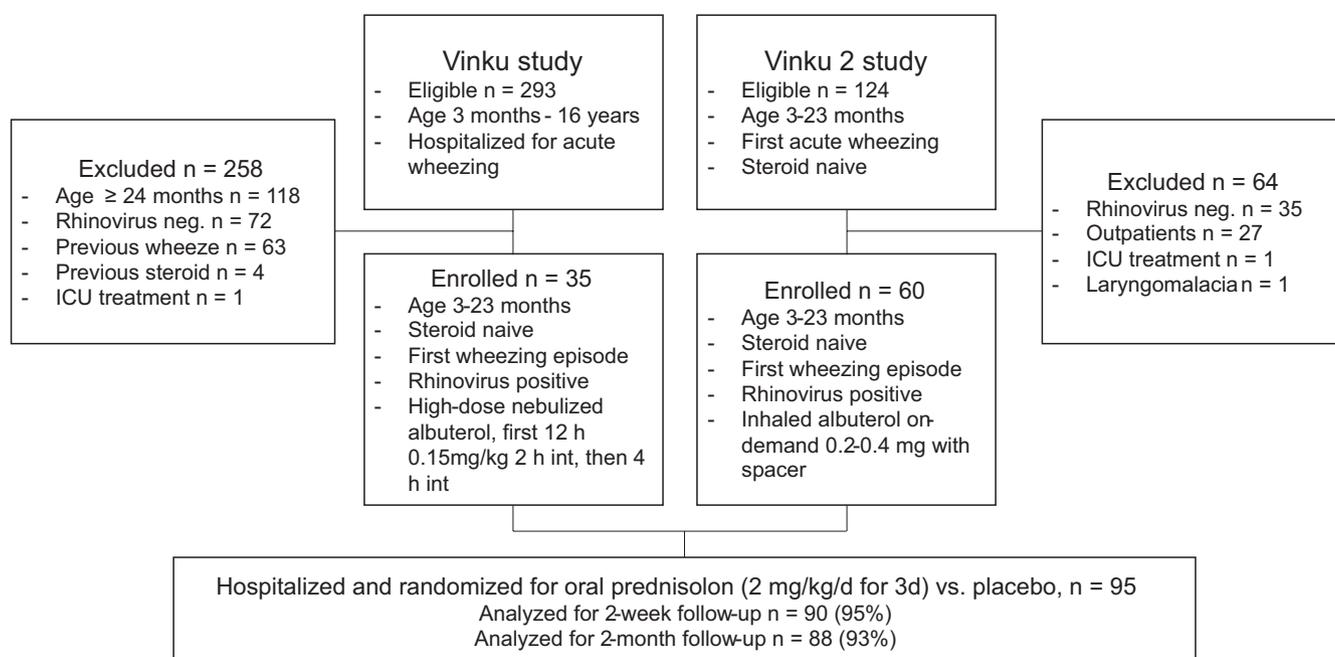

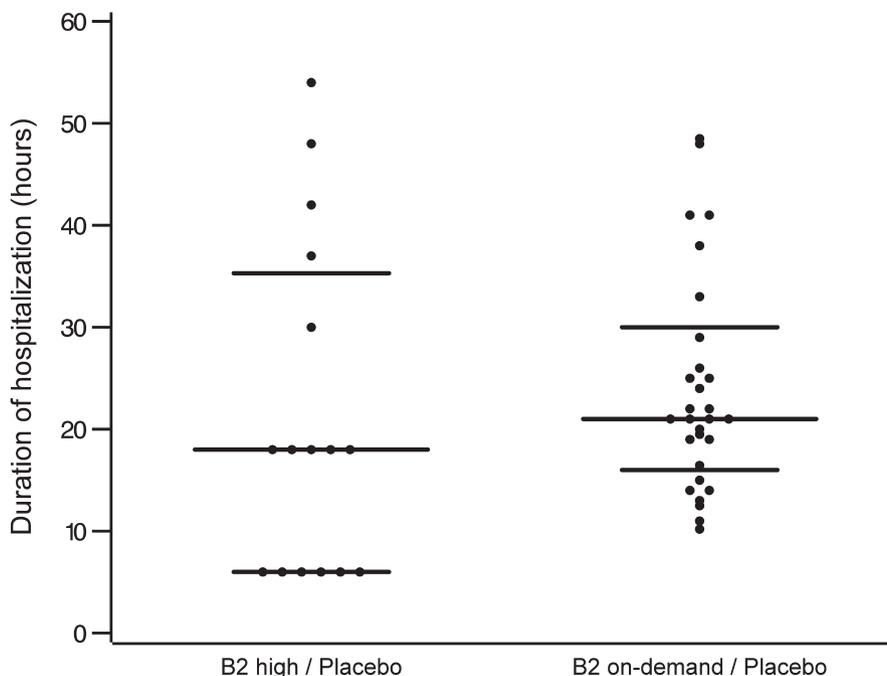**FIGURE 1** Study flow chart. ICU, Intensive care unit; int, interval; d, day

FIGURE 2 Duration of hospitalization. Data are presented as median (interquartile range). In duration of hospitalization, salbutamol high-dose/placebo (n = 16) 18 h vs. salbutamol on-demand/placebo (n = 30) 21 h did not show a difference (p = .12). Prednisolone treatment groups (Salbutamol on-demand/prednisolone group from Vinku2 study) could not be used for this specific analysis since the study drug was initiated only after positive RV PCR-finding in Vinku2 study which caused a delay in drug administration (45h) compared to other study groups. One outlying value (204 h) in B2 on-demand/Placebo group was excluded from figure (included in the analyses). In B2 high / Pred -group median is equal to upper quartile. Pred, prednisolone; B2 high, high-dose salbutamol; On-demand, on-demand salbutamol

FIGURE 3 A new physician-confirmed wheezing episode. The interaction between salbutamol group and prednisolone treatment on new physician-confirmed wheezing episode was significant (group × treatment p = .02) (black). Salbutamol high-dose group had fewer wheezing episodes than on-demand group in prednisolone arm (p = .03), but no difference was seen in placebo treatment arm (p = .29). The group × treatment interaction effect on new physician-confirmed wheezing as inpatient was not significant (p = .30) (grey). Prednisolone treatment arm had less new physician-confirmed wheezing as inpatient than placebo arm (salbutamol group adjusted main effect of treatment p = .03). Pred, prednisolone; B2 high, high-dose salbutamol; On-demand, on-demand salbutamol
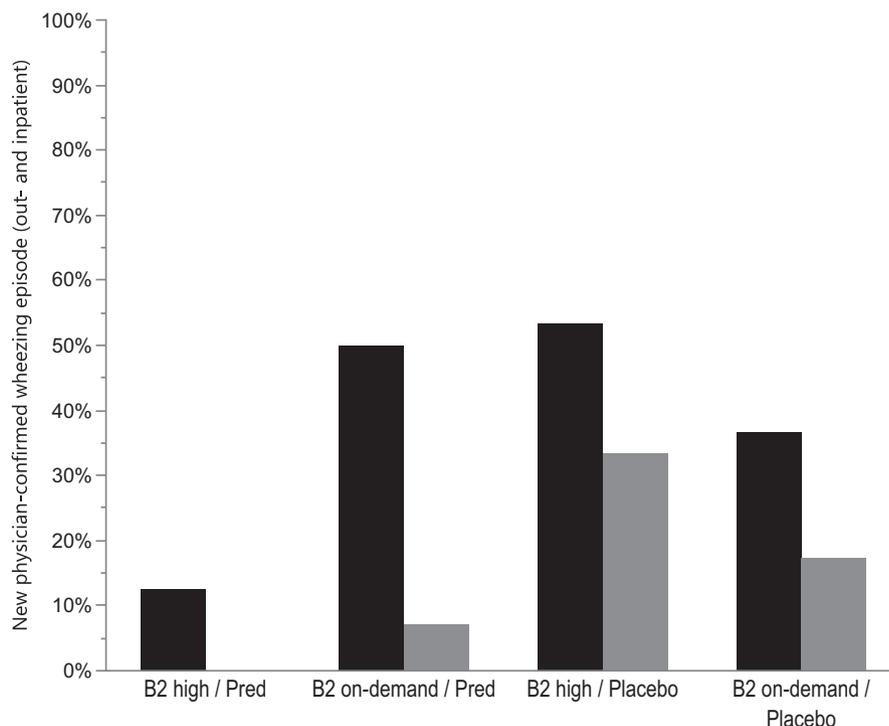
The interaction between salbutamol group and prednisolone treatment on new physician-confirmed wheezing episode was significant (group × treatment p = .02), indicating that the effect of salbutamol was different in prednisolone and placebo groups. Salbutamol high-dose group had fewer wheezing episodes than on-demand group in prednisolone arm (OR 0.15, 95% CI 0.03–0.87, p = .03), but no difference was seen in placebo treatment arm (OR 1.97, 95% CI 0.56–6.94, p = .29) (Figure 3A).

The interaction between salbutamol group and prednisolone treatment on a time to new physician-confirmed wheezing episode was significant (group × treatment p = .02), indicating that the effect of salbutamol was different in prednisolone and placebo groups. Salbutamol on-demand group had shorter time to new physician-confirmed wheezing episode than high-dose group (HR = 0.22, 95% CI 0.05–0.98, p = .047), but no difference was seen in placebo treatment arm (HR = 1.72, 95% CI 0.68–4.35, p = 0.26) (Figure 4).

The vast majority (32/35, 91%) of post-hospitalization recurrences were confirmed at the study clinic.

3.4 | Secondary outcomes

No significant interactions were detected in secondary outcomes. High-dose group had shorter duration of cough than on-demand group (treatment adjusted main effect of salbutamol group p < .001) (Figure 5). Prednisolone treatment arm had less new physician-confirmed wheezing episodes as inpatient than placebo arm (salbutamol group adjusted main effect of treatment p = .03) (Figure 3B) (Table 2).

3.5 | Adjusted analyses for interactions

The interaction between group and treatment on new physician-confirmed wheezing episode remained significant after adjustment for viral coinfection (p = .02), but no difference was detected in prednisolone arm (OR 0.24, 95% CI 0.04–1.48, p = .12) or in placebo arm (OR 2.22, 95% CI 0.61–8.16, p = .23). The interaction between group and treatment on a time to new physician-confirmed wheezing episode remained significant after adjustment for viral coinfection (p = .04), but no difference was detected in prednisolone arm

(HR 0.39, 95% CI 0.09–1.78, p = .23) or in placebo arm (HR 1.77, 95% CI 0.70–4.53, p = .23) (Table S3).

3.6 | Adverse events

No clinically significant (severe or serious) adverse events were reported.

4 | DISCUSSION

Rhinovirus-induced early wheezing has been recognized a major risk factor for subsequent asthma in many studies.[4-6] This *post hoc* analysis is the first to investigate and to demonstrate the efficacy of beta$_2$ agonist medication in the first RV-induced early wheezing episode. In predefined primary outcomes, we were able to show that (1) high-dose regularly administered beta$_2$-agonist was more effective compared to on-demand administered salbutamol in terms of less relapses within two months. (2) We also observed a significant interaction between the treatments indicating that effect of salbutamol treatment was further improved with concomitant prednisolone treatment. (3) In another primary outcome, the duration of hospitalization, we were only able to show the benefit of combined high-dose salbutamol and prednisolone in the loose analysis. Here protocol differences complicated the analysis. (4) In secondary outcomes, the high-dose beta$_2$-agonist effectively decreased the duration of cough. However, no further benefit was found in the other secondary outcomes in terms of duration of wheezing, relapses necessitating rehospitalization within 2 months, and number of beta$_2$-agonist puffs used during 2 weeks after discharge.
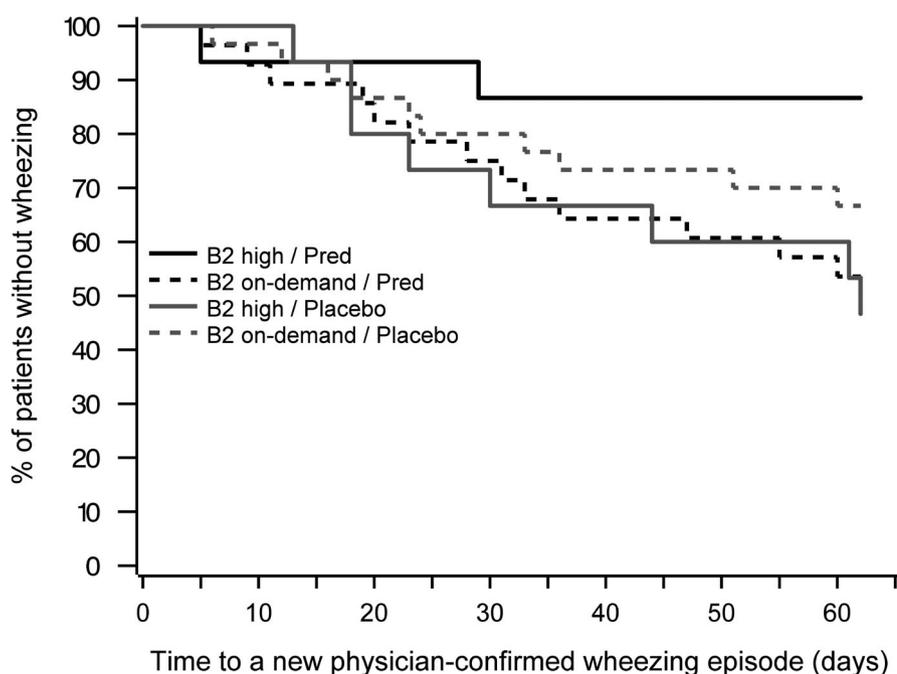

FIGURE 4 Time to a new physician-confirmed wheezing. The interaction between salbutamol group and treatment on a time to new physician-confirmed wheezing episode during the 2-month follow-up was significant (group × treatment p = .02). Salbutamol on-demand group had shorter time to new physician-confirmed wheezing episode than high-dose group (HR = 0.22, 95% CI 0.05–0.98, p = .047), but no difference was seen in placebo treatment arm (HR = 1.72, 95% CI 0.68–4.35, p = 0.26). Pred, prednisolone; B2 high, high-dose salbutamol; On-demand, on-demand salbutamol

FIGURE 5 Duration of cough. Data are presented as medians (interquartile ranges). The group × treatment interaction effect on duration of cough was not significant (p = .46). High-dose group had shorter duration of cough than on-demand group (treatment adjusted main effect of salbutamol group p < .001). Pred, prednisolone; B2 high, high-dose salbutamol; On-demand, on-demand salbutamol
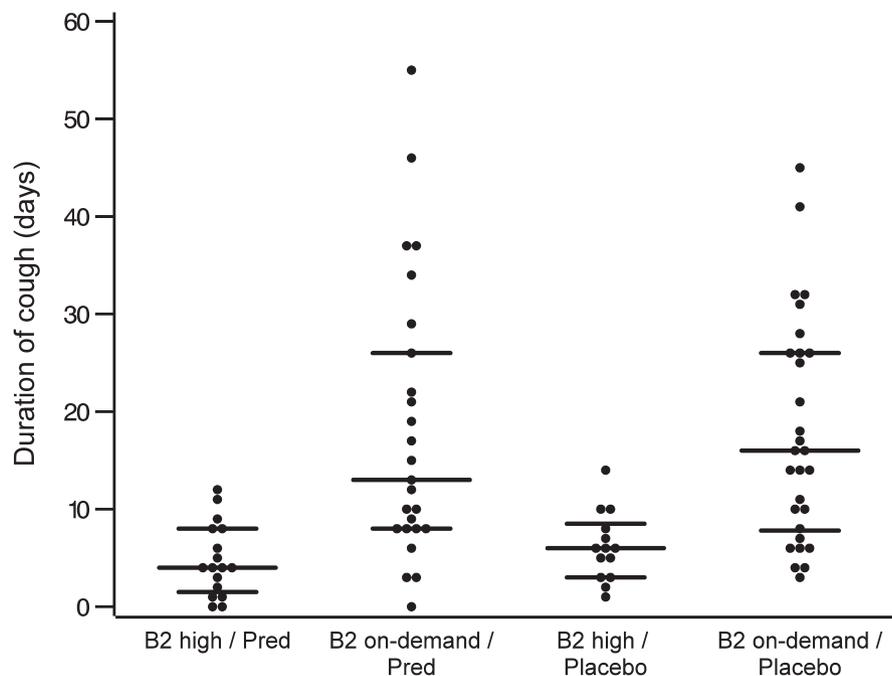

TABLE 2 Primary and secondary outcomes

Outcome	Group effect		Treatment effect		Group×treatment interaction effect
	High-dose vs. on-demand		Prednisolone vs. placebo		
	Estimate (95% CI)	p	Estimate (95% CI)	p	p
Primary					
Duration of hospitalization (h)[g]	0.71[f] (0.46–1.09)	.12			-
New physician-confirmed wheezing episode	0.15[a] (0.03–0.81)	.03[d]			.02
	1.97[a] (0.56–6.94)	.29[e]			
Time to a new physician-confirmed wheezing episode (days)	0.22[c] (0.05–0.98)	.047[d]			.02
	1.72[c] (0.68–4.35)	.26[e]			
Secondary					
Duration of cough (days)	0.30[b] (0.22–0.42)	<.001	0.93[b] (0.69–1.26)	.64	.46
Duration of wheezing (days)	0.82[b] (0.49–1.38)	.46	0.70[b] (0.43–1.14)	.15	.75
A new physician-confirmed wheezing as inpatient	1.15[a] (0.41–5.52)	.53	0.17[a] (0.04–0.83)	.03	.30
Number of beta$_2$-agonist puffs used during 2 weeks after discharge	0.97[b] (0.55–1.71)	.91	0.75[b] (0.44–1.29)	.30	.17

Note: Values are shown as medians (interquartile range) or numbers of subjects (%).

Abbreviation: CI, Confidence interval.

[a]Odds ratio; binary logistic regression.

[b]Relative risk; negative binomial regression.

[c]Hazard ratio; Cox regression.

[d]Group effect in prednisolone treatment arm.

[e]Group effect in placebo treatment arm[f] Relative risk; negative binomial regression; Salbutamol high dose/prednisolone vs. salbutamol on-demand/placebo.

[f]Relative risk; negative binomial regression; Salbutamol high-dose/placebo vs. salbutamol on-demand/placebo.

[g]Prednisolone treatment groups (B2 on-demand/prednisolone group from Vinku2 study) could not be used since prednisolone was administered only after positive RV PCR-finding in Vinku2 study which caused a delay in drug administration (45 h) compared to other study groups.

Most previous trials investigating the efficacy of beta₂-agonists in children with bronchiolitis and/or first wheeze did not demonstrate clinical benefit (Table S1). These discouraging results have led most clinical guidelines do not recommend the use of beta₂-agonists in bronchiolitis.[12-17] Only one recent study has investigated subgroups, namely comparing atopic and non-atopic children and found better clinical efficacy in atopic compared to non-atopic children.[26] This finding is in line with our study. In recent years, we have learned that there are different clinical entities of bronchiolitis, with main clusters being RSV or RV dominant illnesses.[1,2,10] The latter has more asthma-like characteristics, both clinically (dry cough, wheezing) and pathophysiologically (T-helper₂-polarized immune responses and pronounced atopic characteristics).[1,2] Thus, it seems logical that those with RV-induced severe first-time wheezing would benefit from bronchodilators and RV aetiology could serve as an important clinical marker in this regard. Currently, RV PCR testing takes about one working day. Our study adds an important patient group not investigated before, young first-time wheezing children who suffer from severe RV infection and shows clinical efficacy in them up to two months. Our findings support on recently published medical algorithm on diagnosis and treatment of pre-school asthma in children aged 1–5 years.[27]

We have previously investigated the short- and long-term efficacy of prednisolone in young first-time wheezing children in two trials which, in fact, form the screening cohort of the current analysis. These RCT and *post hoc* trials have shown that prednisolone decreases the duration of hospitalization and respiratory symptoms, new wheezing episodes, and most importantly, the initiation of regular asthma control medication by 30% in the subsequent 4 to 7 years.[4,7-9,19] Due to study design, the current trial partly repeats these findings regarding the efficacy of prednisolone, but interestingly, results suggests that most beneficial response will be achieved with the combination of nebulized salbutamol and prednisolone. While many previous trials as, for example, in Cochrane review suggest that oral corticosteroids have no role in treatment of bronchiolitis, they fail to separate different bronchiolitis phenotypes.[28] In agreement, other RCT studies have also shown that best treatment response may be achieved by combining systemic corticosteroid with adrenergic agonists in children with bronchiolitis.[29,30] Moreover, in our data the short-term main outcome, time until discharge, supports the possibility of clinical benefit of high-dose beta₂-agonist treatment (loose analysis), the longer-term primary outcomes, relapse and time to relapse due to wheezing within 2 months, support the benefit of high-dose salbutamol as well as oral corticosteroid and the 'medium-term' secondary outcome, duration of cough within 2 weeks supports the benefit of both. In addition, in our data, a discrepancy was found when comparing different relapse types (in- and outpatient relapses). This inconsistency was found only in salbutamol on-demand/prednisolone groups, and it indicates that sole high-dose beta₂-agonist without prednisolone might not necessarily be optimal approach, because of the increase in relapses as shown in Figure 3. Exact mechanism is not clear, but it may be due to increased bronchial hyperreactivity.[31] Thus, combination

of systemic corticosteroid and adrenergic agonist in this scenario would appear to have most beneficial efficacy.

Benefit of beta₂-agonists in young children suffering from RV-induced wheezing was expected since this illness has many asthma-like characteristics.[1] Many of them have T-helper2-polarized immune responses, higher level of atopy and eczema, increased exhaled NO levels, their parents are likely to have asthma or atopy, and they seem to respond to systemic corticosteroid trials especially if the episode is severe and RV genomic load is high.[5,7-9,24] Also, RV-induced bronchiolitis/early wheezing illness has been highly associated the development of asthma.[2,4-6] It is generally accepted that RV aetiology of wheezing is an indicator of these biased immune responses rather than a cause. Thus, the treatment responses of beta₂-agonists and systemic corticosteroids should be interpreted that this early asthma-like inflammatory response in the airways is responsive to these medications rather than the virus infection itself. This synergistic effect may be partly due to the stimulation of beta₂-receptor transcription by prednisolone, but the exact mechanism is not clear.[32,33]

The strengths of the current study include careful characterization of the subjects and same detailed prospective follow-up in both original trials. The study design of combining data from two previous prednisolone intervention trials provided greater statistical power. However, the beta₂-agonist treatment regimens differed between the two studies. There are also weaknesses. Statistical power analysis for the salbutamol intervention was not done, and rather small sample size did not permit optimal analyses in multivariable model. However, the significant interactions persisted in adjusted analyses, and results of many outcomes were in line arguing against false-positive finding. Moreover, the results may not be generalizable to outpatients, since all our subjects were enrolled in the hospital ward; the sample size was too small to permit meaningful analysis of inpatient *versus* outpatient interactions. Also, because the differences in study protocols (Vinku2 study had prospective RCT design whereas Vinku study had *post hoc* design) duration of hospitalization was not fully comparable in regards with prednisolone treatment (B2 on-demand/prednisolone group from Vinku2 study) since prednisolone was administered only after positive RV PCR-finding in Vinku2 study which caused a delay in drug administration compared to other study groups. In other outcomes, the 45 h time delay in the administration of prednisolone is statistically taken to into account by the group effect (study effect). One can question whether these were truly the first wheezy episodes. To address this issue, we checked medical records and interviewed a parent using a standard questionnaire to confirm that there was no previous wheezing episode. Although some patients received additional medication after discharge during 2-month follow-up period, no differences were found between the use of salbutamol or antibiotics. Use of inhaled and oral corticosteroids seemed to be linked to relapse outcomes (data are not shown).

Although RVs are the most commonly detected respiratory viruses during symptomatic infections, they are also the most commonly found respiratory viruses in asymptomatic children. However,

previous studies have found that RV reinfections are in nearly all cases caused by different RV genotype, and that time period for a new RV infection is relative short.[34] Due to this finding we can assume positive RT-PCR sample shows an incipient, ongoing or passing RV infection which can be symptomatic or asymptomatic.

In summary, while previous studies have shown that beta$_2$-agonists are not efficacious for the treatment of bronchiolitis, our data suggest that high-dose salbutamol and prednisolone may be beneficial for a subgroup of young children with an initial severe wheezing episode caused by RV. It is probable that some of these children have early asthma-like airway inflammation, which may explain the observed efficacy. Our data support the use of a short course of systemic corticosteroid and at least a therapeutic trial with high-dose short-acting beta$_2$-agonists in these selected, RV-induced cases of bronchiolitis. However, our results are partly inconsistent and should be considered as hypothesis-generating. Prospective clinical trials are warranted. More broadly speaking, bronchiolitis is a heterogeneous condition[1,2,10] and our data suggest that its treatment should be administered on a more personalized basis than is recommended by current clinical guidelines.

CONFLICT OF INTEREST

The authors have no conflict of interest in connection with this paper.

AUTHOR CONTRIBUTIONS

The study protocol and manuscript were written by the investigators. Data were collected by study physicians (T.J., P.L., R.T.) and analyzed by investigators (P.H. and T.J.) and statistician (T.V.). K.H. and P.H. performed literature search and reviewed previous trials. Viral analyses were supervised by T.V. J.G. participated in drafting the original study protocol, providing primers for rhinovirus detection and writing the manuscript. C.C. initiated and organized the vitamin D analyses and was involved in writing the manuscript. The study was supervised by T.J. Leiras Takeda (Helsinki, Finland) provided prednisolone and placebo preparations, but did not offer any financial support nor required any confidentiality agreements. The granting agencies covered all costs and played no role in study design, data analysis or manuscript preparation.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.