Comparison of hypertonic saline and alternate-day or daily recombinant human deoxyribonuclease in children with cystic fibrosis: a randomised trial

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Summary

Background Daily recombinant human deoxyribonuclease (rhDNase) is an established but expensive treatment in cystic fibrosis. Alternate-day treatment, if equally effective, would reduce the drug cost. Hypertonic saline improved lung function to the same degree as rhDNase in short-term studies. We compared the effectiveness of daily rhDNase, hypertonic saline, and alternate-day rhDNase in children with cystic fibrosis.

Methods In an open cross-over trial, 48 children were allocated in random order to 12 weeks of once-daily rhDNase (2·5 mg), alternate-day rhDNase (2·5 mg), and twice-daily 5 mL 7% hypertonic saline. The primary outcome was forced expiratory volume in 1 s (FEV_1). Secondary outcomes were forced vital capacity, number of pulmonary exacerbations, weight gain, quality of life, exercise tolerance, and the total costs of hospital and community care.

Findings Mean FEV_1 increased by 16% (SD 25%), 14% (22%), and 3% (21%) with daily rhDNase, alternate-day rhDNase, and hypertonic saline, respectively. There was no difference between daily and alternate-day rhDNase (2% [95% CI –4 to 9], p=0·55). However, daily rhDNase showed a significantly greater increase in FEV_1 than hypertonic saline (8% [2 to 14], p=0·01). The average difference in 12-week cost between daily and alternate-day rhDNase was £513 (95% CI –546 to 1510) and that between daily rhDNase and hypertonic saline was £1409 (440 to 2318). None of the secondary clinical outcomes showed significant differences between treatments.

Interpretation Hypertonic saline, delivered by jet nebuliser, is not as effective as daily rhDNase, although there is variation in individual response. There is no evidence of a difference between daily and alternate-day rhDNase.

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Introduction

Cystic fibrosis is the most common lethal recessive disorder of white populations. Most of the morbidity and mortality is from pulmonary disease, which is characterised by bronchial and bronchiolar obstruction by thick tenacious secretions that are difficult to clear.[1] Retention of abnormal airway secretions promotes recurrent respiratory infections, cycles of inflammation, and progressive lung damage.

DNA derived from the degeneration of neutrophils is a major contributor to the viscosity of airway secretions and is present in high concentration in the sputum of patients with cystic fibrosis.[2] The human enzyme deoxyribonuclease I was cloned and sequenced in 1990. Clinical trials of recombinant human deoxyribonuclease (rhDNase) have shown improvements in lung function and a reduction in protocol-defined infective exacerbations.[3–5] The enzyme is believed to work by cleaving extracellular DNA in airway secretions, thus aiding expectoration of sputum.[6]

Daily rhDNase is widely used in the treatment of cystic fibrosis, but there are unresolved issues. Although many patients improve on treatment, there is wide variation in individual response.[7] The long-term benefit of rhDNase remains uncertain, and its effect on airway inflammation is still not clear.[8] Moreover, this therapy is expensive, and the long-term cost-effectiveness has not been established. The current dose recommended in children, 2·5 mg once daily, is as effective as two daily doses of 2·5 mg.[5] There have been no studies on the use of alternate-day rhDNase, which if equally effective would halve the drug cost and treatment time.

Nebulised hypertonic saline is a potential alternative mucolytic therapy for cystic fibrosis. Hypertonic saline has been used for decades as an agent to aid airway clearance and sputum induction in various respiratory disorders. In short-term studies in cystic fibrosis, hypertonic saline seemed to have beneficial effects on lung function, mucociliary clearance, and sputum expectoration,[9–12] similar to those of rhDNase. It is much cheaper than rhDNase, but there have been no medium-term or long-term comparisons between hypertonic saline and rhDNase.

We aimed to test the hypothesis that hypertonic saline and alternate-day rhDNase are as effective as daily rhDNase in improving respiratory function in children with cystic fibrosis.

Methods

Study population

Children with cystic fibrosis, previously diagnosed on standard criteria, were enrolled from two institutions (Great Ormond Street Hospital for Children NHS Trust and the Royal Brompton and Harefield NHS Trust, London, UK). Inclusion criteria were age between 5 and 18 years; ability to carry out spirometry; and either current treatment with rhDNase or a forced expiratory

volume in 1 s (FEV_1) of less than 70% of predicted.[13] Exclusion criteria were inability to take the trial medication; known hypersensitivity to rhDNase or hypertonic saline; isolation of *Burkholderia cepacia* in the sputum; pregnancy; and breastfeeding. To ensure that patients were enrolled when they were clinically stable, they had to have no lower-respiratory-tract infection requiring a change in antibiotics, steroids, or bronchodilator treatment during the 14 days before randomisation.

The study was approved by the ethics committees of both institutions. Written consent was obtained from the guardian of each child (and the child where appropriate) before entry to the trial.

Study design and procedures

We did a prospective open, randomised cross-over trial. As with previous studies of hypertonic saline, masking was impossible because hypertonic saline can easily be distinguished from rhDNase by its salty taste and timing in relation to physiotherapy.[12,14] Patients already on rhDNase or hypertonic saline discontinued the treatment at least 2 weeks before starting the trial. This period is sufficient for complete washout to occur for both hypertonic saline and rhDNase.[4,12] Each patient was allocated, in random order, consecutive 12-week treatments of once-daily 2·5 mg rhDNase, alternate-day 2·5 mg rhDNase, and twice-daily 5 mL 7% hypertonic saline. Randomisation, by telephone to an independent trials coordinating unit, was stratified by hospital and balanced after each group of 12 children.

The concentration of hypertonic saline chosen was based on previous studies.[10,11] All study treatments were administered with a Sidestream nebuliser and Porta-Neb compressor (Medic-Aid, Bognor Regis, UK). Hypertonic saline was inhaled twice daily immediately before the patient's regular physiotherapy session. rhDNase was administered once a day or once every alternate day, at least 1 h before physiotherapy. There was a 2-week washout period between treatments. Routine medication and physiotherapy were continued throughout the study.

The patients were assessed at the start and end of each 12-week treatment period. Before starting the hypertonic-saline treatment period, each patient received a test dose of hypertonic saline in hospital so that he or she could be monitored for bronchoconstriction. Any child already taking short-acting bronchodilators was asked to have a dose before receiving hypertonic saline. The others were prescribed short-acting bronchodilators if FEV_1 fell by more than 15% after the initial dose of hypertonic saline. If, despite this treatment, there was still a decrease of more than 15% in FEV_1, the patient was not eligible for hypertonic saline. However, the child was still eligible for the two other treatment phases, which were brought forward if they were still to be received. Patients were asked to return all unused bottles of hypertonic saline and empty vials of rhDNase to assess adherence to treatment. Each patient was also given a diary to record the treatment doses taken.

The protocol-defined primary outcome was FEV_1. Secondary outcome measures were forced vital capacity (FVC), number of pulmonary exacerbations, weight gain, exercise tolerance, quality of life, and total health-care cost.

At every visit, lung function was assessed by standard spirometry with a compact spirometer (Vitalograph, Buckingham, UK). FEV_1 and FVC were measured in accordance with the guidelines of the American Thoracic Society.[15] On subsequent clinic visits, lung function was recorded at the same time of day, to within 3 h of the baseline reading, to minimise the effect of diurnal variation. Bronchodilators were withheld for 4 h before each test.

There is no universal definition for a pulmonary exacerbation so a previously outlined protocol for respiratory-tract infections was used.[5] A pulmonary exacerbation was defined as treatment with parenteral antibiotics for any four of the following 12 signs and symptoms: change in sputum; new or increased haemoptysis; increased cough; increased dyspnoea; malaise, fatigue, or lethargy; temperature above 38°C; anorexia or weight loss; sinus pain or tenderness; change in sinus discharge; change in physical examination of the chest; decrease in pulmonary function by 10% or more from a previously recorded value; or radiographic changes indicative of pulmonary infection.

The 3 min step test was done at each visit to assess exercise tolerance.[16] The patients stepped up and down a single 15 cm aerobic step at 30 steps per min for 3 min (regulated by a metronome). Oxygen saturation (SaO_2) was recorded continually during the exercise test (Biox 3700 pulse oximeter, Ohmeda, Boulder, USA). A visual analogue score for dyspnoea was recorded before and after the exercise.[17,18]

The quality of well-being scale self-administered form 1·04 was used to assess quality of life.[19] The questionnaire was filled out by the patient and guardian together at each visit. Five subsections of functioning are combined to produce a well-being score between 0 (death) and 1 (symptom-free full function).

A standard costing method was used to assess the total health-care cost. It incorporated general guidance on measuring costs in economic evaluation.[20,21] We took a hospital and community-health-service perspective, which meant that patients' costs were excluded from analysis. The resources included covered hospital admissions (inpatient, outpatient, and day case), radiological investigations, blood tests, drug use, and the use of community services (visits to general practitioners, district nurses, and physiotherapists). These resources were recorded from medical records, discharge summaries, and patients' diaries. Patients were asked to record in the diary any contacts with health professionals in hospital or the community during the study.

Unit costs (1999–2000) were obtained from the finance departments at the two postgraduate hospitals involved in the study and a district general hospital. These costs included those for health professionals' time, consumables, and overheads. Drug costs were taken from the *British National Formulary* and community-care costs from the publication by Netten and colleagues.[22,23] Total hospital and community costs were calculated by multiplying each patient's resource use by the unit costs.

Statistical analysis

Based on a within-participant SD of FEV_1 in children with cystic fibrosis of 0·13 L,[24] a cross-over trial of 40 patients would have power of more than 90% to detect, as significant at the 5% level, an average difference of 0·1 L (about 8%) in the final measurements between any two treatments. We aimed to recruit 50 patients to allow for withdrawals and non-adherence.

All analyses were done according to a prespecified statistical analysis plan by intention to treat. In keeping with the aims of the study, the analysis focused on two separate pairwise comparisons of the treatments: daily rhDNase versus hypertonic saline, and daily versus alternate-day rhDNase. The analysis compared within-

	Group value
Demography and anthropometry	
Age (years)*	12·6 (2·8; 7·3 to 17·0)
Men/women	19 (40%)/28 (60%)
Weight (kg)*	40·0 (12·6; 18·8 to 77·4)
Spirometry	
FEV_1 (L)*	1·18 (0·47; 0·44 to 2·34)
FEV_1 (% predicted)*	48 (15; 14 to 77)
FVC (% predicted)*	68 (22; 20 to 112)
Exercise tolerance	
Change in oxygen saturation (%) with exercise*†	–2·6 (2·5; –13 to 0)
Change in visual analogue score (cm) with exercise*†‡	2·4 (1·7; 0 to 6·1)
Well-being	
Quality of well-being score*§	0·61 (0·12; 0·35 to 0·84)
Treatment at enrolment	
Hypertonic saline	2 (4%)
rhDNase	39 (83%)
Lung microbiology¶	
Pseudomonas aeruginosa	22 (48%)
Staphylococcus aureus	18 (39%)
Stenotrophomonas maltophilia	1 (2%)

*Mean (SD; range). †Of 42 children tested. Change is calculated as lowest SaO_2 during exercise minus pre-exercise value. ‡10 cm visual analogue score with outcomes "not at all short of breath" and "the most breathless I have ever felt". Change is calculated as post-exercise rating minus pre-exercise rating, positive changes indicating an increase in breathlessness. §Score 0–1 with higher scores indicating greater well-being. ¶Number of children with three positive cultures of the organism in the previous year.

Table 1: **Baseline characteristics of the study population (n=47)**

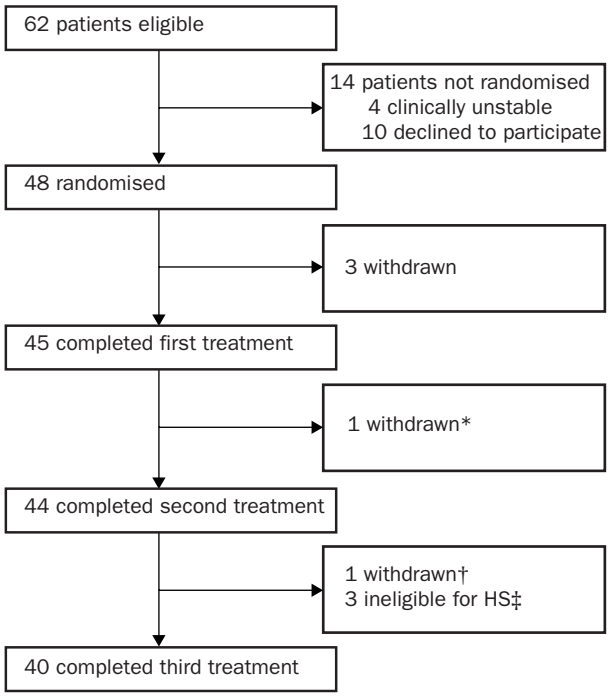

Figure 1: **Trial profile**

HS=hypertonic saline. *Owing to severely deteriorating pulmonary status. †Underwent major liver surgery, while taking HS. ‡Owing to a fall in FEV_1 >15% with the first dose of HS, despite short-acting bronchodilators.

participant differences between outcomes at the end of each treatment period, with adjustment for values at the beginning of these periods by ANCOVA. Statistical analyses ignoring these baseline results produced similar results. The primary outcome, FEV_1, was analysed on a log scale, and the results presented as percentage differences. The secondary outcome measures were not transformed.

Adjustment for additional covariates (treatment period, quarterly season of the year, and the child's age at the beginning of the period) was done by multiple regression. A marginal model was used, based on general estimating equations with robust SEs, which allows for the correlation between observations taken on the same person over time.[25] The single prespecified subgroup investigation was whether disease severity (baseline FEV_1) affected the treatment-group differences for FEV_1; this analysis was done as an extension of the regression analysis.

Results

48 children were randomised, eight to each of the six possible treatment orders. One 14-year-old girl withdrew from the study almost immediately after randomisation without starting the first treatment, owing to what became a prolonged illness. Table 1 gives the characteristics of the remaining 47 children as observed at the baseline assessment. Eight children were unable to complete all three treatment periods (figure 1). Four patients had severe declines in their pulmonary status and required long courses of intravenous antibiotics. On clinical grounds, these patients were withdrawn from the trial. Two of them were taking alternate-day rhDNase, one hypertonic saline, and the other daily rhDNase. Therefore, 43 children are included in the comparison of daily and alternate-day rhDNase, and 40 in the comparison of daily rhDNase and hypertonic saline.

On the basis of returned treatment packs, the estimated adherence (average proportion of medication taken) was 84% for both daily and alternate-day rhDNase and 93% for hypertonic saline. Similar or higher proportions were obtained from the diary information.

The mean percentage change from baseline in FEV_1 for each period and each treatment is shown in table 2. The overall increase in FEV_1 was 16% for daily rhDNase, 14% for alternate-day rhDNase, and 3% for hypertonic saline. There was a decrease in FEV_1 for the children who received hypertonic saline in the third treatment period, perhaps because there was a high

Treatment	Treatment period							
	One		Two		Three			
	n	Mean % change	n	Mean % change	n	Mean % change	n	Mean % change (SD)
Daily rhDNase	15	15	14	8	14	25	43	16 (25)
Alternate-day rhDNase	16	14	17	18	10	9	43	14 (22)
Hypertonic saline	12	8	12	12	16	–7	40	3 (21)
All treatments	43	12	43	13	40	8	126	11 (23)

n=number of participants.

Table 2: **Mean percentage change in FEV_1 over each treatment period (final FEV_1 minus initial FEV_1, expressed as a percentage of initial FEV_1)**

	Daily rhDNase *vs* hypertonic saline		Daily rhDNase *vs* alternate day rhDNase	
	n	Mean difference	n	Mean difference
Overall				
Percentage difference	40	8 (2 to 14)	43	2 (–4 to 9)
Percentage difference with adjustment*	40	11 (5 to 17)	43	2 (–5 to 9)
Subgroup analysis†				
Severe illness	20	11 (3 to 21)	22	1% (–10 to 13)
Moderate illness	20	6 (0 to 13)	21	3 (–3 to 9)

*Adjusted for treatment period, age at start of period, and season. †Severe group is children with FEV_1 as % predicted below median (49%); moderate is the other children. p values for difference between subgroups: 0·34 for daily rhDNase *vs* hypertonic saline; 0·78 for daily *vs* alternate-day rhDNase.

Table 3: **Percentage difference in FEV_1, adjusted for baseline (95% CI)**

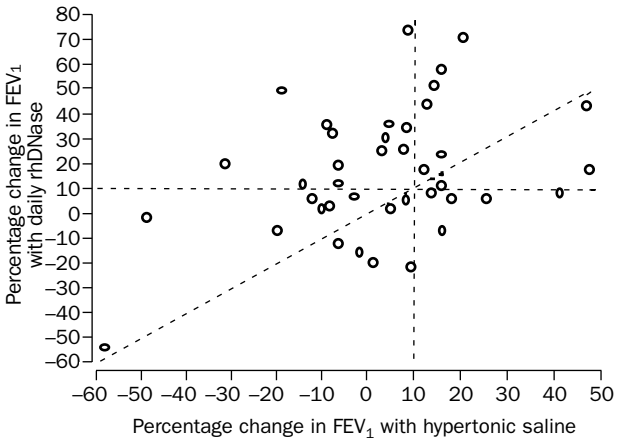

Figure 2: **Individual patients' responses to daily rhDNase and hypertonic saline**

An improvement of at least 10% in FEV_1 is judged clinically significant. Every point on the graph represents one patient.

mean FEV_1 at the beginning of that treatment period which could have masked a beneficial response to hypertonic saline. Table 3 provides estimates of the treatment differences—an 8% (95% CI 2 to 14, p=0·01) advantage for daily rhDNase over hypertonic saline but none for daily compared with alternate-day rhDNase (2% [–4% to 9], p=0·55). Adjustment for additional covariates confirmed these results, and there was no evidence that these treatment effects varied according to severity of illness (table 3).

Figure 2 shows the percentage change in FEV_1 with daily rhDNase and hypertonic saline for individual children. 26 of the 40 children responded better with daily rhDNase than with hypertonic saline. 22 children had an improvement in FEV_1 of 10% or more on daily rhDNase compared with 14 on hypertonic saline.

There was no evidence of treatment differences for any of the secondary clinical outcomes (data are available on request from *The Lancet* offices), nor in the number of adverse events occurring. During the hypertonic saline, daily rhDNase, and alternate-day rhDNase treatment periods 15, 18, and 17 children had one or more pulmonary exacerbations; there was therefore no evidence of differences between treatments.

Lung microbiology was examined for all the children who completed a treatment period with hypertonic saline. 18 of these children began their hypertonic saline treatment without *Pseudomonas aeruginosa* infection; the organism was not isolated from any of them at the end of that treatment period.

The total medical costs incurred were greater on daily rhDNase than on hypertonic saline owing to the higher drug costs (table 4).[26] The lower drug costs during the alternate-day rhDNase regimen than during daily rhDNase led to lower mean total costs during this period, though this difference was not significant.[26]

Discussion

This randomised trial compared the effects of hypertonic saline and rhDNase in cystic fibrosis patients over 12 weeks. This duration was used because the response to rhDNase at 12 weeks is strongly predictive of response at 1 year.[7] We found that treatment with daily rhDNase results in a significantly greater increase in FEV_1 than hypertonic saline over 12 weeks and that daily and alternate-day rhDNase result in similar increases in FEV_1. The mean difference in cost for 12 weeks' treatment was £1409 between daily rhDNase and hypertonic saline, and £513 between daily and alternate-day rhDNase. None of the other secondary clinical outcomes differed significantly between the treatments.

Our study was open-label and the participants knew that rhDNase is very expensive, which might have affected our results. However, masking of hypertonic saline was impossible to achieve, as previously reported.[12,14] We used an objective measure for the primary clinical outcome (spirometry) and standard forms for collection of data on health-care resource use to protect against bias.

Results of studies have shown that single doses of hypertonic saline improve mucociliary clearance and sputum expectoration in patients with cystic fibrosis.[9–11] However, there have been only two short-term randomised trials that have assessed lung-function changes with hypertonic saline used over several days.[12,14] Eng and colleagues[12] compared 2 weeks of 6% hypertonic saline with isotonic saline by ultrasonic nebuliser, in a parallel trial. They noted a significant improvement in mean FEV_1 of 15% with hypertonic saline compared with 3% with isotonic saline. In the

	Daily rhDNase *vs* hypertonic saline			Daily rhDNase *vs* alternate-day rhDNase		
	Daily (n=40)	Saline (n=40)	Mean difference (95% CI)*	Daily (n=43)	Alternate (n=43)	Mean difference (95% CI)*
Intervention	1755	37		1749	857	
Other drugs	2271	2364		2367	2349	
Hospital and community care	1668	1883		1595	1992	
Total	5694	4285	1409 (440 to 2318)	5711	5198	513 (–546 to 1510)

*Calculated by percentile non-parametric bootstrap methods.[26]

Table 4: **Mean costs (UK£) over each 12-week treatment period, and differences between treatment groups**

second study, Ballmann and colleagues[14] did a pilot cross-over trial comparing 3 weeks of daily rhDNase with 5·85% hypertonic saline in 14 patients. Both drugs were administered by jet nebuliser. Increases in mean FEV_1 were 8% and 9% for hypertonic saline and rhDNase, respectively. Statistical testing was not done owing to small numbers.

These short-term studies reported greater improvements in mean FEV_1 with hypertonic saline than our study did, perhaps because an initial increase in FEV_1 with hypertonic saline might not be sustained long term by all patients. This feature occurs with rhDNase. Fuchs and colleagues[5] found that the initial improvement in FEV_1 of about 9% with rhDNase declined over the first month and remained stable at between 5% and 6% thereafter.

Both previous studies administered a larger volume (10 mL) of hypertonic saline than we did. However, the time taken to deliver a nebulised drug is important for patients' adherence.[27] Ultrasonic nebulisers tend to deliver a larger volume over a shorter period but are not recommended for rhDNase,[28] and they are generally not used for domiciliary therapy in cystic fibrosis. Ballmann and colleagues[14] reported that administration of 10 mL hypertonic saline nebulised twice a day by jet nebuliser took about 84 min. This long inhalation time was unacceptable to the patients, and the investigators suggested that if this regimen were instituted as permanent therapy, there would be problems with adherence. Our trial needed to be pragmatic, so we used a volume of 5 mL 7% hypertonic saline, which takes about 10 min to administer by jet nebuliser.

Hypertonic saline was well tolerated by the patients; only three had significant bronchospasm with the initial dose. There was no difference in adverse events compared with rhDNase. Only five patients complained of the salty taste, which was not severe enough for them to discontinue treatment. Hypertonic saline tended to make the patients cough during administration. Despite the need for twice daily administration, adherence was good.

We found a pronounced variation in individual response to the treatments, which has also been seen in previous studies of rhDNase.[5,7] About 50% of the patients on daily rhDNase and about 35% on hypertonic saline had an improvement in FEV_1 of more than 10%. With any treatment regimen there will be a distribution in response, but most clinicians would call an increase in FEV_1 of more than 10% a significant response. In clinical practice, patients who do not respond to rhDNase might show benefit from a trial of hypertonic saline.

The effect of hypertonic saline on airway inflammation and defences remains unclear.[29] Although there was no increased risk of pulmonary exacerbation with hypertonic saline or alternate-day rhDNase compared with daily rhDNase, the study might not have been long enough (or large enough) to detect any significant differences. Fuchs and colleagues[5] have shown that daily rhDNase significantly reduces risk of pulmonary infection compared with placebo over 6 months, but these changes were of little clinical significance.

Our results show that rhDNase given on alternate days has similar efficacy to a daily regimen. Both treatments led to significant improvements in FEV_1. This finding suggests that the effect of rhDNase lasts for at least 48 h. The drug could therefore be given less frequently, which would mean fewer time-consuming nebulisations and lower costs to the patient if rhDNase is taken on alternate days, but the inclusion of those costs was outside the scope of our analysis. The total hospital and community-care costs during the alternate-day than with the daily rhDNase period were lower because of the lower rhDNase costs. This difference was not significant. The costs of hospital and community care were higher during the alternate-day rhDNase period, but these costs had a high variance, which is commonly observed with total cost data;[26] the sample size was insufficient to test whether there was a difference in total cost between the treatment groups.

In summary, we have shown no evidence of a difference in effectiveness between daily and alternate-day rhDNase. However, hypertonic saline delivered by jet nebuliser is not as effective as daily rhDNase, although there is variation in individual response.

Contributors

Ranjan Suri designed the protocol, recruited and followed the patients through the trial, and analysed results. Colin Wallis and Andrew Bush conceived the project, obtained the funding, oversaw the study, and acted as guarantors for the report. Christopher Metcalfe and Simon Thompson participated in study design and statistical analysis. Belinda Lees and Marcus Flather participated in design, data management, and analysis of the study. Richard Grieve and Charles Normand participated in the design and analysis of the costing aspect of the study. Ranjan Suri wrote the report in collaboration with the other authors.

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