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Probiotic VSL#3 prevents antibiotic-associated diarrhoea in a double-blind, randomized, placebo-controlled clinical trial

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SUMMARY

Background: Antibiotic-associated diarrhoea (AAD) is a frequent complication of systemic antibiotic therapy and *Clostridium difficile*-associated diarrhoea (CDAD) is its most serious form due to associated morbidity and mortality.
Aim: This trial aimed to investigate whether the probiotic VSL#3 prevents AAD and CDAD in average-risk hospital patients.
Methods: Adult hospital inpatients exposed to systemic antibiotics were recruited to this multicentre, randomized, double-blind, placebo-controlled trial. One sachet of VSL#3 or placebo was given twice daily for the length of the antibiotics course and for seven days thereafter. Primary outcomes were AAD and CDAD.
Findings: Patients randomized to active ($N = 117$) and placebo ($N = 112$) groups were well-matched for baseline demographic patient data. No cases of CDAD were detected. The rate of AAD was significantly lower in the active group on per protocol analysis (0% active vs 11.4% placebo; $P = 0.006$). On intention-to-treat analysis the difference in AAD incidence (4.3% active vs 8.9% placebo; $P = 0.19$) was not significant.
Conclusions: VSL#3 is associated with a significant reduction in the incidence of AAD in average-risk hospital inpatients exposed to systemic antibiotics. As the incidence of CDAD has fallen sharply, no cases of CDAD were found. Probiotic administration as prophylaxis for CDAD may not be indicated in average-risk hospital patients.

Introduction

Adverse drug events and nosocomial infections are among the most frequent and potentially serious healthcare-related complications. Systemic antibiotic therapy frequently causes antibiotic-associated diarrhoea (AAD).[1] *Clostridium difficile*-associated diarrhoea (CDAD) — the most serious form of AAD — is associated with significant morbidity and mortality.[2,3] The

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incidence of CDAD has risen significantly since 1995 and the emergence of hypervirulent strains of bacteria associated with increased mortality has been reported.[4–6] In the UK a rapid rise in CDAD incidence led to much media attention in 2008 and subsequently strict government guidance aimed at reducing CDAD.[7]

Whereas targeted antibiotic therapies with metronidazole or oral vancomycin are moderately effective in treating CDAD, prevention of CDAD is the best tactic to avoid the morbidity, mortality and healthcare costs associated with CDAD. Identification of risk factors for CDAD allows detecting patients at high risk. A number of risk factors for CDAD have been proposed; only age, duration of hospital stay, previous CDAD and antibiotic exposure were independent risk factors for the development of CDAD.[5] Other reported risk factors were related to those variables.[5] As these risk factors are mostly not modifiable, they offer no target for intervention.

Prevention of AAD and CDAD by probiotic co-administration during antibiotic exposure is an appealing concept. Probiotic preparations are generally well tolerated and are seen positively by the general public.[8] There is reasonably good evidence that probiotic preparations (especially the strains *Saccharomyces boulardii* or *Lactobacillus* spp.) can prevent AAD in patients exposed to systemic antibiotics.[1,9,10] The evidence for CDAD preventions is, however, inconclusive as the few published trials have shown conflicting results, and are not applicable to the average-risk patient due to strict exclusion criteria.[11,12] This trial aimed to establish whether probiotic preparations can prevent the occurrence of AAD and CDAD in the average-risk hospital patient. To ensure that the study findings are generally applicable, exclusion criteria were kept to a minimum.

Methods

Design

The primary study hypothesis was that the probiotic preparation VSL#3 can prevent the occurrence of AAD and CDAD in the average-risk adult hospital inpatient exposed to systemic antibiotics. The occurrence of AAD and CDAD were the two co-primary endpoints. For the purpose of this trial AAD was defined as more than two liquid stools (Bristol stool chart types 6 and 7) in excess of normal for each patient a day for two or more days.[12] If a patient passed two or more stools of consistency 6 or 7, a stool sample was sent for culture, microscopy and *C. difficile* toxin A and B analysis. Toxin testing was performed with Premier Toxin A+B (Meridian Bioscience, Cincinnati, OH, USA) at the Hull and Bristol sites and C. Diff Quick Chek Complete (Techlab, Blacksburg, VA, USA) at the Weston and Wigan sites. CDAD was defined as meeting the criteria for AAD and a positive *C. difficile* toxin for A and/or B. Secondary outcomes were the length of hospital stay and 30-day mortality. The background incidence of CDAD in the participating hospitals was extracted from data provided by the Health Protection Agency.[13]

Participants

Adult inpatients receiving systemic antibiotics for infections at four acute National Health Service (NHS) hospitals [Royal Albert Edward Infirmary (Wigan), North Bristol NHS Trust, Hull Royal Infirmary, and Weston General Hospital] were eligible for participation. Informed written consent was obtained from competent individuals. The trial was permitted to recruit patients lacking capacity in accordance with section 30 of the Mental Capacity Act 2005 provided next of kin agreed. Patients having taken antibiotics within four weeks prior to admission, patients on high-risk antibiotics, and patients with bowel pathology (provided there was no diarrhoea on presentation) were all eligible for participation. Patients were excluded from participation if they were unable to take the study medication (persistent vomiting, no enteral feeding possible, etc.), required admission to an intensive care unit, had diarrhoea at presentation (endpoint), suffered from acute pancreatitis (adverse outcome data), had taken probiotic preparations within a week of hospital admission (confounder) or were at theoretical risk of probiotic-induced infection (severe immunosuppression or risk for endocarditis). Antibiotic exposure was classified as high risk in accordance with Dial *et al*. if the patient was exposed to multiple antibiotics or to a single antibiotic seen as high risk for CDAD (clindamycin, cephalosporins and broad-spectrum penicillins).[14]

Intervention

The active group was allocated to receive the probiotic preparation VSL#3 twice daily for the duration of the antibiotic course and for a further seven days thereafter. The placebo group received a similar-appearing placebo (containing maltose and silicon oxide) in the same fashion, thus guaranteeing effective blinding of patients, nursing staff, medical staff and all investigators. VSL#3 contains *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus* in a concentration of 450 billion live bacteria per sachet. VSL#3 was chosen as its content closely matches the bacterial species and strains, which have previously shown promise in the prevention of AAD and CDAD.[1,12] Furthermore VSL#3 has the highest concentration of all commercially available probiotic preparations. The dosing regimen was based on that of a pilot study in critically ill patients on intensive care units demonstrating that two sachets of VSL#3 reduced the number of liquid stools.[15] Adherence was monitored using patient diaries and non-adherence was defined as taking less than 80% of the prescribed doses.

Study plan

Potential participants were identified and approached by the research nurses within 48 h of first hospital antibiotic administration. After obtaining informed consent (or completion of the procedure for patients lacking capacity) baseline data were recorded and participants were randomized by the hospital pharmacy. The first study medication was administered within 48 h of first hospital antibiotic administration. Participants and ward nursing staff filled in the patient diary and daily Bristol stool charts until the end of follow-up, which occurred for 28 days after the last antibiotic dose. Those patients receiving repeat antibiotics within the 28-day follow-up period were restarted on the study medication and 28-day follow-up was again initiated after antibiotics were stopped.

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When participants were discharged from hospital, they were provided with the required amount of study medication, patient diaries, stool charts and stool specimen containers. The research nurses then arranged for weekly telephone follow-up.

Sample size

Power and sample size calculations were based on the most up-to-date AAD and CDAD incidence data at trial design provided by the trial from Hickson *et al.*[12] Hence incidences of 30% for AAD and 15% for CDAD were expected for the placebo group. The investigators aimed to detect a 50% reduction in incidence of AAD and, assuming a 10% drop-out, 389 patients were needed to achieve 90% power using Fisher's exact test and 5% significance level. To detect a reduction in CDAD incidence from 15% to 5%, 445 patients were required to achieve 90% power. The trial was therefore powered for two co-primary endpoints.

Randomization

Participants were randomized 1:1 to active or placebo group using computer-generated random-permuted blocks stratified by centre, which were supplied by an independent statistician. Allocation of participants was performed by the pharmacies at each site, which remained blinded throughout the trial.

Statistical analysis

To test the primary hypothesis that VSL#3 can prevent AAD and CDAD, categorical outcome data were compared using Fisher's exact test and continuous variables were analysed using Student's *t*-test. Patients with poor adherence (<80%) and those with major protocol breaches were excluded from per protocol analysis. Intention-to-treat (ITT) analysis was performed on the basis of the last observation carried forward. Interim outcome and safety analyses were performed annually using SPSS, Inc. (Chicago, IL, USA) software.

Study registration and approval

The study was approved by the Medicine and Healthcare Products Regulatory Authority (EUDRACT: 2008-005244-16); ethical approval was granted by the North Staffordshire Local Research Ethics Committee (08/H1201/147); clinical trial registration number: NCT00973908.

Results

In all, 3151 patients were screened for participation and 231 (7.3%) were recruited to the study from April 2010 to February 2012 (Figure 1). Major causes (Table I) for non-recruitment were the patients' unwillingness to participate (34.2%) and patients feeling too unwell to consider participation (14.3%). Only three of 353 screened patients lacking capacity were recruited to the trial.

There were no differences in baseline patient data between active and placebo groups (Table II). There was no difference in high-risk antibiotic regimen exposure between the active and placebo groups (82.1% vs 73.0%; $P = 0.74$; Table III). Repeat courses of systemic antibiotics were given to 26 (14 in the active group) patients, of whom 12 received a repeat course of active drug (seven patients) or placebo (five patients), while 14 were withdrawn from the trial. Non-adherence to the study medication (40.2% vs 37.5%; $P = 0.69$) and the proportion of patients finishing the trial as per protocol (52.1% vs 54.4%; $P = 0.79$) did not differ between active and placebo groups. Main protocol breaches were refusal to take the study medication ($N = 10$), poor adherence ($N = 10$), lost to follow-up after discharge from hospital ($N = 12$), patients becoming too unwell ($N = 11$), and longer-term courses of antibiotics ($N = 9$). The study medication was well tolerated and no drug-related adverse events occurred (Table III). AAD occurred in 15 cases. Six patients (four of them after hospital discharge), who developed AAD, failed to provide stool specimens for analysis. All cases of AAD were self-limiting and no cases of CDAD were detected. There were no differences in adverse event rates between active and placebo groups (Table IV).

The rate of AAD was significantly lower in the active group on per protocol analysis [11.4% (seven cases) placebo vs no cases active; $P = 0.006$]. On ITT analysis the detected difference in AAD incidence [8.9% (10 cases) placebo vs 4.3% (five cases) active; $P = 0.19$] was not significant. A reduction in length of hospital stay [6.7 (active) vs 8.7 (placebo) days; $P = 0.09$] on per protocol analysis was not significant (Table V). The incidence of CDAD per 100,000 bed-days for the participating hospitals was 136.7 in 2007, 51.3 in 2008, 36.7 in 2009, and 20.5 in 2010.

The trial was stopped prematurely on advice by the independent data-monitoring committee as significance on the AAD co-primary endpoint had been achieved. It was deemed futile to continue the trial as the low incidence of CDAD made it very unlikely that significance could be shown.

Discussion

Nosocomial infections and especially CDAD pose a significant risk to patients due to associated morbidity and mortality. Prevention of CDAD has therefore become a key goal of UK hospital policy and strict national monitoring has been implemented.[7] Prevention of CDAD by probiotic co-administration is seen as an appealing concept, but the evidence is not robust enough to recommend widespread prophylactic use.[16] This large, multicentre, double blind, randomized, placebo-controlled trial aimed to establish whether prophylactic administration of the probiotic preparation VSL#3 can prevent the occurrence of AAD and CDAD in average-risk hospital inpatients. The authors aimed to ensure general applicability of the trial findings and deliberately set wide inclusion criteria; all adult hospital patients on systemic antibiotics were eligible for participation. Exclusion criteria were kept to a minimum required for safety purposes. By contrast with previous trials, patients with a history of gastrointestinal disease or previous gastrointestinal surgery were included as these patients are often seen as at higher risk of CDAD.[12] More importantly patients on antibiotics putting them at high risk of CDAD were specifically included, again by contrast with previous trials.[12] Effective blinding of participants and staff was achieved and the multicentre design incorporated a mixture of teaching and district general hospitals. Recruitment proved difficult as previously experienced by other investigators and only 7.3% of screened patients were recruited.[11,12] The majority of screen

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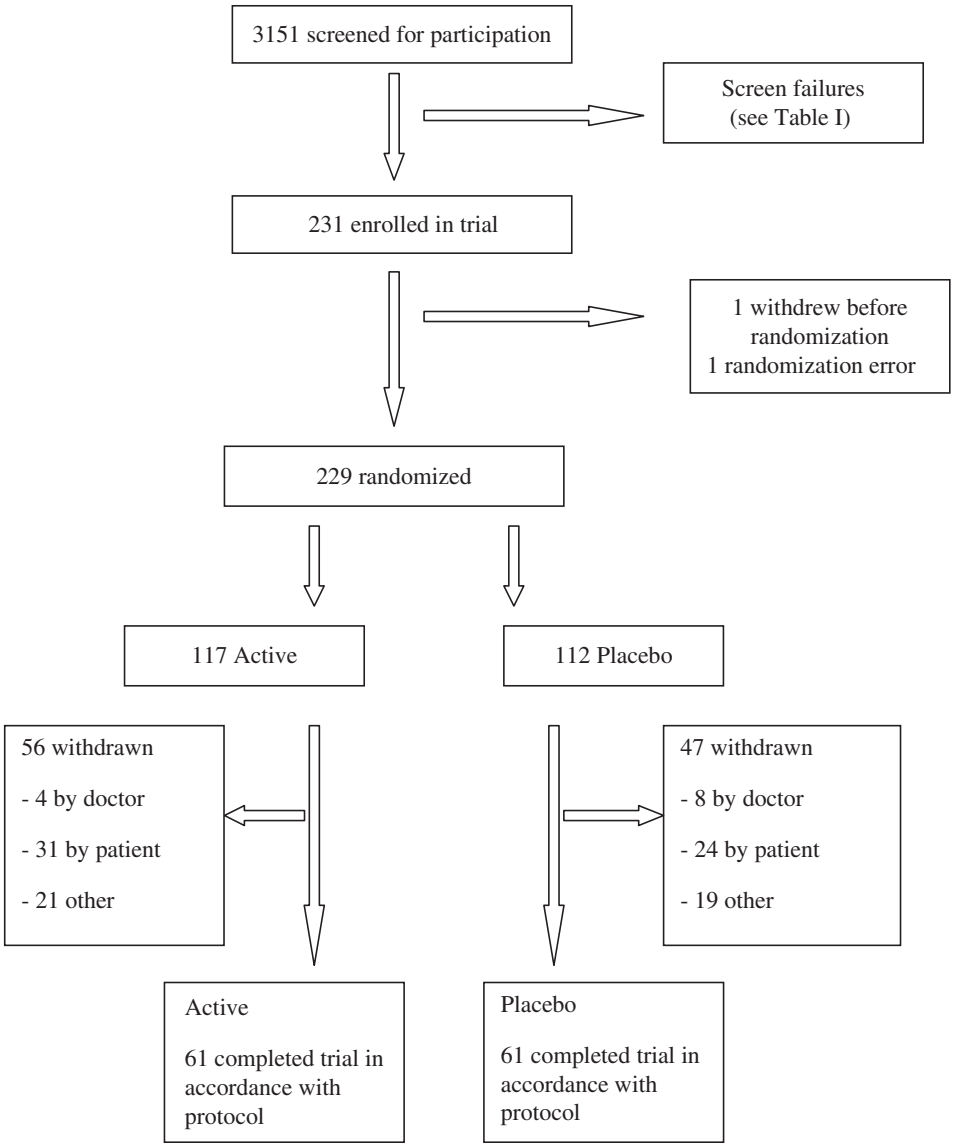

Figure 1. Patient flow.

Table I
Screening results

Result	Total
Recruited	231 (7.3%)
Vomiting or otherwise unable to take oral medications	251 (8.0%)
Diarrhoea at screening	236 (7.5%)
Previous probiotic consumption	60 (1.9%)
Patients too unwell	452 (14.3%)
Acute pancreatitis	16 (0.5%)
Severely immunocompromised	84 (2.7%)
Risk of endocarditis	69 (2.2%)
No capacity to consent and no relative or friend contactable	353 (11.2%)
Patient unwilling to participate	1079 (34.2%)
Others	320 (10.2%)
Total	3151 (100%)

failures were due to reluctance to participate rather than medical conditions requiring exclusion.

The effectiveness of VSL#3 in preventing AAD has been demonstrated clearly by significance ($P = 0.006$) on per protocol analysis. As a large number of protocol breaches were due to poor adherence or complete refusal to take the study medication, it is probably not surprising that the reduction of AAD on ITT analysis (8.9% to 4.3%) was not of the same magnitude and hence did not reach significance ($P = 0.19$). The study medication was well tolerated as adverse events and protocol breaches were equally common in active and placebo groups. Many protocol breaches were due to the demanding nature of the study protocol on the patient (need to keep stool charts, long courses of antibiotics and length of follow-up) rather than the tolerability of VSL#3 or placebo. Probiotic species and strains vary significantly between the commercially available products, therefore the trial results cannot be extrapolated to other probiotic products with different species or strains, as demonstrated by a recent

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Table II
Participants' baseline data

	Active group ($N = 117$)	Placebo group ($N = 112$)	*P*-value
Male	67 (57%)	53 (47%)	0.15[a]
Average age (years)	57.9	57.0	0.72[b]
Capacity to consent	116 (99%)	110 (98%)	0.62[a]
Dementia	0	2 (2%)	0.24[a]
Malignancy	6 (5%)	7 (6%)	0.78[a]
Cardiovascular disease	34 (29%)	25 (22%)	0.29[a]
Respiratory disease	45 (38%)	30 (27%)	0.07[a]
Immobility	13 (11%)	12 (11%)	1.0[a]
Hospital admission within eight weeks prior to enrolment	24 (21%)	27 (24%)	0.63[a]
Nursing or residential home resident	0	3 (3%)	0.12[a]
Previous CDAD	2 (2%)	1 (1%)	1.0[a]
Proton pump inhibitor	32 (27%)	33 (29%)	0.77[a]
Antibiotics within four weeks prior to enrolment	40 (34%)	44 (39%)	0.49[a]
Gastrointestinal disease	21 (18%)	24 (21%)	0.51[a]
Previous gastrointestinal surgery	7 (6%)	10 (9%)	0.46[a]

CDAD, *Clostridium difficile*-associated diarrhoea.
[a] Fisher's exact test.
[b] Student's *t*-test.

trial in which *S. boulardii* as a single agent failed to prevent AAD.[17]

To judge the effectiveness of VSL#3 in preventing CDAD, a minimum incidence is required. However, no cases of CDAD were detected in this trial. The trial was designed and powered in 2007, which coincided, as we now know, with the peak incidence of CDAD in the UK. Department of Health guidance has since led to considerable changes in infection control policy.[7] Hand-wash policies were tightened, a 'bare below the elbow' policy was introduced for all clinical staff, isolation efforts for infected patients intensified and antibiotic policies were revised to reduce the use of high-risk antibiotics.[7] Subsequently the rate of CDAD infections per 100,000 bed–days fell sharply in the four participating hospitals from 136.7 in 2007 to 20.5 in 2010. Furthermore, the positive effects of the 'cleanyourhands' campaign on CDAD incidence have recently been demonstrated at national level.[18] The reduced CDAD incidence in the participating centres rendered the original power calculation incorrect and the trial was therefore technically underpowered to detect an effect on CDAD. Even if a larger trial were to show an effect on CDAD incidence, the effect would be small and the number needed to treat to prevent one case of CDAD rather large. In light of such reductions in background CDAD incidence and the findings of this trial, probiotic prophylaxis to prevent CDAD in the average-risk hospital patient appears not to be indicated. Such a measure would not derive significant clinical benefit and would not be cost-effective.

The reduction in AAD incidence was associated with a shorter hospital stay, but this fell just short of significance. By contrast with CDAD, AAD is rarely associated with considerable morbidity and mortality. VSL#3 is efficacious in preventing AAD in the average-risk hospital patient. In the absence of a clear effect on hospital stay, it remains questionable whether widespread AAD prophylaxis would be cost-effective.

Table III
Antibiotic exposure

	Active group ($N = 117$)	Placebo group ($N = 112$)	*P*-value
Median length of antibiotic course in days	7	7	0.28[a]
Intravenous antibiotics	79 (67.5%)	78 (69.6%)	0.74[b]
High-risk antibiotic regimen	96 (82.1%)	89 (73.0%)	0.78[b]
Penicillins and broad-spectrum penicillins	93 (79.5%)	84 (75.0%)	0.78[b]
Cephalosporins	4 (3.4%)	7 (6.3%)	0.36[b]
Quinolones	7 (6.0%)	4 (3.6%)	0.54[b]
Macrolides	28 (23.9%)	18 (16.1%)	0.19[b]
Aminoglycosides	15 (12.8%)	24 (20.3%)	0.11[b]
Imidazoles	8 (6.8%)	12 (10.7%)	0.35[b]
Others	15 (12.8%)	18 (16.1%)	0.57[b]

[a] Mann–Whitney *U*-test.
[b] Fisher's exact test.

Table IV
Adverse events

	Active group ($N = 117$)	Placebo group ($N = 112$)	*P*-value
Non-serious adverse events	8 (6.8%)	10 (8.9%)	0.63[a]
Nausea	4	2	
Abdominal pain	0	1	
Heart burn	0	2	
Pregnancy	0	1	
Rash	0	1	
Leg swelling	0	1	
Tachycardia	0	1	
Fall	0	1	
Sore mouth	1	0	
Pulmonary oedema	1	0	
Nose bleed	1	0	
Pleural effusion			
Serious adverse events	6 (5.1%)	6 (5.4%)	1.0[a]
Hospital readmission	2	3	
Same condition	2	2	
Oesophageal bleed	0	1	
Deaths	2	3	
Pneumonia	1	1	
Cancer	1	1	
Pulmonary embolus	0	1	
Prolonged hospital stay	2	0	
Myocardial infarct	1	0	
Severe diarrhoea and vomiting	1	0	
Suspected unexpected serious adverse reaction	0	0	1.0[a]

[a] Fisher's exact test.

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Table V
Study outcomes

	Intention to treat			Per protocol		
	Active group ($N = 117$)	Placebo group ($N = 112$)	*P*	Active group ($N = 61$)	Placebo group ($N = 61$)	*P*
AAD	5 (4.3)	10 (8.9%)	0.19	0	7 (11.4%)	0.006
CDAD	0	0	1.0	0	0	1.0
LOS (days)	8.6	8.5	0.96	6.7	8.7	0.09
30-day mortality	2 (1.7%)	4 (3.6%)	0.44	0	0	1.0

AAD, antibiotic-associated diarrhoea; CDAD, *Clostridium difficile*-associated diarrhoea; LOS, length of stay.

CDAD prophylaxis is not required for the average-risk patient, but high-risk patients could still benefit from prophylactic probiotic administration. Recruiting very high-risk patients to this trial proved difficult, but this is common in CDAD prevention trials.[11,12,17] Due to the lack of any CDAD cases in this trial, the question whether VSL#3 can prevent CDAD in principle remains unanswered. Patients at extremely high risk of CDAD may benefit from probiotic preparations as demonstrated by a reduction in CDAD (18.6% vs 5.8%, $P = 0.02$) in a randomized trial of 138 ventilated patients on an intensive care unit.[19] Hence there may be a role for probiotic preparations in high- and extremely high-risk patients. Furthermore the risk of developing CDAD for average-risk patients may rise during pandemics. Any effect of probiotic preparations in preventing CDAD will therefore depend on the background incidence of CDAD.

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Conflict of interest statement
C.P.S., A.B., A.C., M.L., S.S. and N.H. have support from Ferring Pharmaceuticals Ltd for the submitted work; C.P.S., A.C., M.L., S.S. and N.H. have relationships with Abbott, Dr Falk, Ferring Pharmaceuticals Ltd, Merck Smith Klyne, Procter & Gamble, Shire, and Warner Chillcott that might have an interest in the submitted work in the previous three years. Ferring Pharmaceuticals Ltd was not involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. Ferring Pharmaceuticals Ltd received the final version of the manuscript prior to submission for publication.

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