CARTA AL EDITOR

UNRAVELING THE CAUSES OF NEGATIVE STUDIES: A CASE OF *S BOULARDII* FOR THE PREVENTION OF ANTIBIOTIC-ASSOCIATED DIARRHEA

To the Editor: Bravo et al. published their study testing *Saccharomyces boulardii* for the prevention of antibiotic-associated diarrhea (AAD) in adults[1]. They enrolled 86 outpatients receiving amoxicillin for various acute infections and randomized patients to either *S boulardii* or placebo. They did not find a significant difference in AAD in the group (7.3%) compared to the placebo (11.1%). However, this result conflicts with earlier studies that did find a significant protective effect of *S boulardii* in adults receiving antibiotics[2]. Negative studies (or studies that do not find a significant efficacy of an investigational treatment) may result from several factors not related to the efficacy of the treatment itself. Bravo's study suffers from several of these factors.

The first limitation was a lack of power. Unfortunately, many clinical trials testing probiotics enroll insufficient numbers of patients, resulting in a negative study result. Because of the small study size, it is impossible to determine if the treatment was truly ineffective or if the negative result is due to low power. In the study of Bravo et al, although the rate of AAD was lower in the *S boulardii* group (3/41, 7.3%) compared to the placebo group (5/45, 11.1%), there was only a 4% power to detect a significant difference. A larger study may have detected a significant protective effect. Previous clinical trials finding a significant protective effect of *S boulardii* for AAD have typically had larger study sizes (151-389 enrolled subjects)[3-7].

The second limitation in their paper is in the study design. This study gave the probiotic or placebo for 12 days and then followed subjects for only an additional nine days. Insufficient follow-up time after the antibiotic has been discontinued does not allow the detection of delayed onset AAD. Delayed onset AAD, which may occur 2-8 weeks after antibiotics have been stopped[8], has been cited previously in trials of other types of probiotics finding no significant efficacy for AAD[9,10] and for one study testing *S boulardii*[11]. Clinical trials finding a significant efficacy for probiotics in preventing AAD followed subjects for a longer time than Bravo et al's study. Beausoleil et al followed subjects 21 days post-antibiotics[12] and Cremonini et al followed subjects for four weeks[13]. McFarland et al followed patients for seven weeks and found cases of delayed onset AAD up to 46 days post-antibiotics. All three of these studies with longer follow-up periods found significant efficacy of probiotics for the prevention of AAD.

A third limitation is an uncertainty about the strain of *S boulardii* used in the trial. As not all probiotic strains or strains of *Saccharomyces* are effective as probiotics and many probiotics that are commercially available do not contain the probiotic strain listed on their label[14], it is important that clinical trials document not only the strain of probiotic used, but the source of manufacturer. Neither of these two were done in the study by Bravo et al. Perhaps the strain of *S boulardii* used was not the same strain that was found to be significantly protective for AAD in other trials.

In addition to the limitations listed above, there are other concerning errors and inconsistencies in the study by Bravo et al. The frequency of AAD for the *S boulardii* group is reported in the abstract as 4/41, but in the text, it is reported as 3/41. In the discussion section, the authors state that the study of McFarland et al. did not find efficacy for *S boulardii* and AAD. This is incorrect, as AAD was significantly less frequent in the *S boulardii* group (7.2%) compared to the placebo group (14.6%, $P < 0.05$)[4]. The authors also incorrectly quote that a meta-analysis by D'Souza et al found 3 or 4 trials of *S boulardii* did not show clinical benefits for preventing AAD. This is incorrect, as this meta-analysis found that 3 of 4 trials did have significant benefits, and only one trial did not find any benefit[15]. Other meta-analyses have confirmed this finding. Szajews-

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ka et al pooled five randomized clinical trials of *S boulardii* and found a significantly protective effect for AAD [pooled Odds Ratio (OR) =0.43, 95% CI 0.23, 0.78][16]. I also found this protective effect in the pooled odds ratios from six trials of *S boulardii* and AAD [pooled OR =0.37, 95% CI 0.26, 0.52][2]. Overall, there are seven randomized controlled trials that found significant protective efficacy of *S boulardii* for the prevention of AAD[3-7,13,17] and one that failed to find efficacy[11].

In conclusion, the publication of clinical trials with negative findings adds value to our body of scientific knowledge; however, trials may be prone to factors that may mask true efficacy of investigational treatments. It is important that authors address these other variables in their studies. It is imperative that studies have sufficient power to detect a significant efficacy if it is present, use proper study design, and thoroughly evaluate other reasons for a lack of an effect before concluding that a treatment has no efficacy.

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