INTRODUCTION

Inflammatory bowel diseases (IBD), Crohn’s disease (CD) and ulcerative colitis (UC) are chronic, relapsing-remitting inflammatory disorders of the gastrointestinal tract. The exact etiology of IBD is still unknown, but appears multifactorial and influenced by genetics, host immune dysfunction, mucosal barrier defects, and the gut microbiome. Many animal models, particularly mouse models, of IBD have been developed to better understand the pathophysiology of IBD and to pre-clinically explore therapies to be used in humans. Perhaps the most commonly used mouse model of colitis is the administration of dextran sulfate sodium (DSS) to the animals. DSS is a water soluble, negatively charged
sulfated polysaccharide with a highly variable molecular weight. The exact mechanism of DSS-induced colitis is not clearly defined, but its major effect is disruption of the intestinal barrier integrity leading to intestinal inflammation.\textsuperscript{7,9} The severity of the resulting colitis can be altered by many factors including, but not limited to the strain or gender of the animal or the molecular weight of DSS selected.\textsuperscript{10,11} While of significant value in basic scientific studies, the DSS-induced colitis model is acknowledged to be imperfect, and to mostly capture/model the intestinal injury and recovery associated with inflammatory responses of IBD.\textsuperscript{9,12}

DSS colitis most closely resembles human UC,\textsuperscript{13} however, findings from this model are still translated back to IBD in general, which includes CD. Although this difference remains, many therapeutic interventions effective in human CD have been reported to be efficacious against DSS-induced colitis.\textsuperscript{3} Furthermore, the DSS-induced colitis model remains very popular in IBD research particularly due to its simplicity, reliability, and efficiency (relatively short timeframe to obtain results). It can be used as a model for acute, chronic, and relapsing intestinal inflammation simply by adjusting the DSS concentration and frequency of administration. Mice susceptible to DSS typically have a noticeable weight loss, generally about 5%-10%, within 3-4 days of DSS administration.\textsuperscript{14} Weight loss greater than 20% of initial weight is a physiological indicator of animal stress and possibly imminent demise.\textsuperscript{15} Graphing percent change in body weight over time is the standard way to depict disease progression in this colitis model.\textsuperscript{10} Weight loss is almost always used to identify which mice are exhibiting a more severe colitis. In fact our impression, after more than a decade of work with this model, is that weight loss has been a consistent and highly reliable outcome measure of DSS colitis in over 1000 publications. However, much too often, additional redundant testing is performed to further support the weight loss-related conclusion of colitis severity. Supplementary investigations of other measures of colitis are generally costly and time consuming. These include, but are not limited to, histological analysis with scoring by a pathologist, cytokine expression at the mRNA and protein levels, and other indirect measures of colitis activity such as tissue myeloperoxidase (MPO) analysis.

IBD carries significant economic burden worldwide due to its rising prevalence, high morbidity, and costs associated with research and treatment options, particularly biologic agents.\textsuperscript{16-18} It has become increasingly important to practice cost-effective medicine due to rising healthcare costs,\textsuperscript{19,20} which in part is linked to basic research spending.\textsuperscript{21} Numerous studies have analyzed and suggested solutions to reduce the economic burden of IBD targeting healthcare costs,\textsuperscript{22-25} but we propose that scrutinizing research costs can be of indirect benefit as well. Our aim was to highlight the unnecessary excess cost associated with research utilizing a DSS-induced colitis murine model.

2 | MATERIALS AND METHODS

A literature search on “dextran sulfate sodium colitis” was performed using PubMed in October 2018. Over 4000 articles resulted from this search. Twenty\textsuperscript{20} of the most recently published articles at that time were selected (Supplementary Table S1). Articles excluded were those utilizing cancer models of DSS, those not assessing the severity of DSS-induced colitis, or those we did not have access to.

Measures utilized to assess colitis severity were recorded for each article. Testing performed for mechanistic purposes (ie, examining molecular mechanisms involved in DSS colitis) or for other objectives beyond colitis severity assessment were not included. Only measures of colitis severity beyond weight loss were included in the cost estimation. For details of cost estimation, see Supplementary Methods. Additionally, see Supplementary Table S1 for detailed cost analysis breakdown.

3 | RESULTS

Among the 20 consecutive articles included, one paper that documented monitoring weights in their methods was unclear about how weight change was incorporated into assessing disease activity.\textsuperscript{26} The remaining 95% of papers had weight loss as a clear and important measure of DSS colitis incorporated into their findings. Figure 1 depicts our highly guarded cost estimate analysis of the 19 articles. The average excess cost beyond weight loss assessment among these papers was approximately $6,700. Two\textsuperscript{2} studies were estimated to have spent over $20,000.\textsuperscript{27,28} The average impact factor (in 2017) of the journals where the manuscripts of this study were published was approximately 3.94. The cost of the added colitis severity measures beyond weight loss did not correlate with the journal of publication’s impact factor ($r = -0.12; P = 0.627$).

Among the 20 studies included, 19 performed histological analysis. If not otherwise documented, studies were assumed to have used one pathologist for assigning histological scores (n = 17). Two studies indicated using two\textsuperscript{29} and three\textsuperscript{30} blinded pathologists. In all cases, histological score was consistent with weight loss (100% consistency for histologic scoring). Disease activity index (DAI) scores were utilized in many of the studies (n = 16) for which weight loss was always a component of the score and DAI always correlated with weight loss alone (100% consistency for DAI). Studies that included cytokine analyses (n = 15) varied in many ways, especially how cytokines were assessed. Samples were from serum or colonic tissue and they were tested via enzyme-linked immunosorbent assay (ELISA), mRNA expression, antigen presenting cell (APC) and/or western blot.
The conclusions from these studies also varied. Most found that their cytokine analysis was consistent with weight loss (n = 10), while one had indeterminate results, one did not correlate and the remaining overlapped with consistent and indeterminate (n = 3) findings (86.7% consistency for cytokine analysis). Those with any form of indeterminate results were all from studies that assessed four or more cytokines. Results of MPO analysis (n = 5) were always consistent with weight loss (100% consistency with MPO).

Additional studies not commonly performed included flow cytometry (n = 3), immunohistochemistry (n = 3), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay for apoptosis (n = 2), short-chain fatty acid analysis SCFA (n = 1), and fecal calprotectin analysis (n = 1). These tests added considerable cost and the findings were all consistent with weight loss (100% consistency with cell-based analyses). Additional tests performed that did not add extra costs included colon length (n = 14) and weight measurements (n = 2) along with spleen weight (n = 4). These were all consistent with weight loss, except one study that found no difference in colon weight, one study that found no difference in colon length and one study that found no difference in spleen weight (85% consistency with objective organ-based assessments).

Therefore, other measures of colitis in the DSS-induced murine model of colitis were found to either support the initial findings (average consistency of additional colitis measures with weight loss = 94%) based on weight loss alone or resulted in indeterminate results.

4 | DISCUSSION

In this work, we examined if weight loss may be a sufficient single outcome measure of DSS colitis in most IBD research. This question was raised based on our impression after more than a decade of work with this model. We found that weight loss is the most consistently used outcome of the model (95% of studies clearly used it, while 5% likely without clear description of the disease activity index used). Several studies (n = 16) included DAI to evaluate the clinical progression of colitis. This is not a standardized tool. Among publications, the components of DAI measurements varied. For instance, some used weight loss, stool consistency, and visual blood in the stool. Other variations included weight loss, stool consistency and visual rectal bleeding or weight loss, stool consistency, general appearance, and visual rectal bleeding. Therefore, the only objectively measured component within these DAs was weight loss. Based on discussions with colleagues in the field, DAI measurements are not blinded, hence the remaining factors assessed beyond weight loss are subjective and prone to bias. Another argument would be to utilize macroscopic evaluation (eg, colon shortening), a variable we reported not to carry an extra cost. However, this measurement is subjective as well and technician dependent. If performed incorrectly, such as by stretching the colon or measuring from the top of the cecum rather than the ileocecal junction, then measurements can be inaccurate. Therefore, we emphasize that the results of weight loss alone always corroborated the final conclusion of the manuscripts studied, which was our subjective conclusion from the prior literature as well. Namely, none of the authors of this publication have been able to identify a single publication on DSS colitis in over 1-10 years of work in the field, where weight loss was inconsistent with the final conclusions on colitis severity.

Nevertheless, all of the consecutive 20 manuscripts examined from 2018 within this work had at least one additional outcome measure investigated. These additional investigations led to a minimum of $6700 average added cost (based on United States of America cost estimates) per publication. In the meantime, the additional measures did not modify the final conclusions of the studies, and in 95% of the cases those corroborated the weight loss findings. Importantly, these extra investigations only addressed colitis severity and did not add any novel conclusions about the biology or pathogenesis of IBD. The extra cost spent did not improve the impact of the papers either, based on the journal citation indices of the publications.

An average lab with modest research activity may complete about 10 DSS experiments per year. Based on our cost estimations, if additional outcome measures beyond weight loss are excluded then the potential annual savings are approximately $67,000 per research lab. To help put this into perspective, private foundation grants in IBD generally offer $100,000 per year for a new project in the USA, so more than half of the grant money could go toward unnecessary excess spending. Hence, the annual savings associated with utilizing weight loss as the sole outcome measure of colitis severity in the DSS colitis model are significant.
Animal models in IBD research are used to address basic science (pathogenesis) questions, evaluate pre-clinical efficacy, study pharmacokinetics, test safety, and identify biomarkers. In the case of the 20 consecutive publications studied, 10 (50%) were in the preclinical efficiency category, 9 (45%) in basic science, and 1 (5%) in biomarker identification. We argue, that in the case of the DSS colitis model (and likely in other animal models of IBD as well) weight loss is a sufficient, practical (real-time, reliable, objective) and cost-effective outcome measure of colitis severity for the basic science and the pre-clinical treatment efficiency studies. As emphasized in the methods section, this conclusion does not pertain to testing performed for mechanistic purposes (ie, examining molecular mechanisms involved in DSS colitis) or for other objectives beyond colitis severity assessment.

We trust that our message from this review work will help to optimize future IBD research utilizing DSS and other murine models toward efficiency and cost-effectiveness.

ACKNOWLEDGEMENTS

RK was supported in part by philanthropic funds from the Wagner Family led Gutsy Kids Fund, and by the Klaasmeyer family funds for PSC research.

CONFLICT OF INTEREST

All authors declare they have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

SB wrote first draft and performed extensive literature search. MK performed extensive literature search. RK conceptual design, literature search, funding, critical revisions, and final formatting. All authors approved the final version of the manuscript for publication.

REFERENCES

1. Kellermayer R. Genetic drift. “Omics” as the filtering gateway between environment and phenotype: the inflammatory bowel diseases example. Am J Med Genet A. 2010;152A:3022-3025.
2. Sartor RB. Key questions to guide a better understanding of host-commensal microbiota interactions in intestinal inflammation. Mucosal Immunol. 2011;4:127-132.
3. Mizoguchi A. Animal models of inflammatory bowel disease. Prog Mol Biol Transl Sci. 2012;105:263-320.
4. Kiesler P, Fuss IJ, Strober W. Experimental models of inflammatory bowel diseases. Cell Mol Gastroenterol Hepatol. 2015;1:154-170.
5. Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. Gastroenterology. 2011;140:1720-1728.
6. Peloquin JM, Nguyen DD. The microbiota and inflammatory bowel disease: insights from animal models. Anaerobe. 2013;24:102-106.
7. Padua D, Vu JP, Germano PM, Pisegna JR. The role of neuropeptides in mouse models of colitis. J Mol Neurosci. 2016;59:203-210.
8. Yan Y, Kolachala V, Dalmasso G, et al. Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis. PLoS ONE. 2009;4:e6073.
9. Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. Drug Des Devel Ther. 2013;7:1341-1357.
10. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol. 2014;104, Unit 15 25.
11. Schneider M. Dextran sodium sulfate-induced murine inflammatory colitis model. Methods Mol Biol. 2013;1031:189-195.
12. DeVoss J, Diehl L. Murine models of inflammatory bowel disease (IBD): challenges of modeling human disease. Toxicol Pathol. 2014;42:99-110.
13. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990;98:694-702.
14. Eichele DD, Kharbanda KK. Dextran sodium sulfate colitis murine model: an indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. World J Gastroenterol. 2017;23:6016-6029.
15. Kim JJ, Shajib MS, Manocha MM, Khan WI. Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp. 2012. https://doi.org/10.3791/3678.
16. Burisch J, Jess T, Martinato M, Lakatos PL. The burden of inflammatory bowel disease in Europe. J Crohns Colitis. 2013;7:322-337.
17. M’Koma AE. Inflammatory bowel disease: an expanding global health problem. Clin Med Insights Gastroenterol. 2013;6:33-47.
18. Stone CD. The economic burden of inflammatory bowel disease: clear problem, unclear solution. Dig Dis Sci. 2012;57:3042-3044.
19. Yu AP, Cabanilla LA, Wu EQ, Mulani PM, Chao J. The costs of Crohn’s disease in the United States and other Western countries: a systematic review. Curr Med Res Opin. 2008;24:319-328.
20. Park KT, Bass D. Inflammatory bowel disease-attributable costs and cost-effective strategies in the United States: a review. Inflamm Bowel Dis. 2011;17:1603-1609.
21. Amadeo K. The rising cost of health care by year and its causes. The Balance. 2019. https://www.thebalance.com/causes-of-rising-healthcare-costs-4064878. Accessed March 5, 2019.
22. Mehta F. Report: economic implications of inflammatory bowel disease and its management. Am J Manag Care. 2016;22:e51-60.
23. Pillai N, Dusheiko M, Burmaband B, Pittet V. A systematic review of cost-effectiveness studies comparing conventional, biological and surgical interventions for inflammatory bowel disease. PLoS ONE. 2017;12:e0185500.
24. Jackson BD, Gray K, Knowles SR, De Cruz P. EHealth technologies in inflammatory bowel disease: a systematic review. J Crohns Colitis. 2016;10:1103-1121.
25. Cronin J, Moore S, Lenihan N, O’Shea M, Woods N. The non-drug costs associated with the administration of an intravenous biologic drug in the hospital setting. Ir J Med Sci. 2018. https://doi.org/10.1007/s11845-018-1925-8 [Epub ahead of print]
26. Fang R, Wu R, Zuo Q, et al. Sophora flavescens Containing-QYJD formula activates Nrf2 anti-oxidant response, blocks cellular transformation and protects against DSS-induced colitis in mouse.
model. Am J Chin Med. 2018;1:15. https://doi.org/10.1142/S0192415X18500829 [Epub ahead of print]

27. Nunes NS, Kim S, Sundby M, et al. Temporal clinical, proteomic, histological and cellular immune responses of dextran sulfate sodium-induced acute colitis. World J Gastroenterol. 2018;24:4341-4355.

28. McAlpine W, Wang KW, Choi JH, et al. The class I myosin MYO1D binds to lipid and protects against colitis. Dis Model Mech. 2018;11.

29. Kinoshita Y, Arita S, Murazoe H, Kitamura K, Ashizuka S, Inagaki-Ohara K. Subcutaneously administered adrenomedullin exerts a potent therapeutic effect in a murine model of ulcerative colitis. Hum Cell. 2019;32:12-21.

30. Chi JH, Kim YH, Sohn DH, Seo GS, Lee SH. Ameliorative effect of Alnus japonica ethanol extract on colitis through the inhibition of inflammatory responses and attenuation of intestinal barrier disruption in vivo and in vitro. Biomed Pharmacother. 2018;108:1767-1774.

31. Kellermayer R, Balasa A, Zhang W, et al. Epigenetic maturation in colonic mucosa continues beyond infancy in mice. Hum Mol Genet. 2010;19:2168-2176.

32. Ihekweazu FD, Fofanova TY, Queliza K, et al. Bacteroides ovatus ATCC 8483 monotherapy is superior to traditional fecal transplant and multi-strain bacteriotherapy in a murine colitis model. Gut Microbes. 2019;1:17. https://doi.org/10.1080/19490976.2018.1560753 [Epub ahead of print]

33. Kim MS, Kim JY. Ginger attenuates inflammation in a mouse model of dextran sulfate sodium-induced colitis. Food Sci Biotechnol. 2018;27:1493-1501.

34. Wasilewska E, Zlotkowska D, Wroblewska B. Yogurt starter cultures of Streptococcus thermophilus and Lactobacillus bulgaricus ameliorate symptoms and modulate the immune response in a mouse model of dextran sulfate sodium-induced colitis. J Dairy Sci. 2019;102:37-53.

35. Xie X, Ni Q, Zhou D, Wan Y. Rab32-related antimicrobial pathway is involved in the progression of dextran sodium sulfate-induced colitis. FEBS Open Bio. 2018;8:1658-1668.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Britto SL, Krishna M, Kellermayer R. Weight loss is a sufficient and economical single outcome measure of murine dextran sulfate sodium colitis. FASEB BioAdvances. 2019;1:493–497. https://doi.org/10.1096/fba.2019-00035