Received: 18 October 2021 | Accepted: 18 October 2021

DOI: 10.1111/jvim.16303

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

LETTER TO THE EDITOR

Letter regarding "Utility of the combined use of 3 serologic markers in the diagnosis and monitoring of chronic enteropathies in dogs"

Dear Editors,

We read with interest the paper by Estruch et al "Utility of the combined use of 3 serologic markers in the diagnosis and monitoring of chronic enteropathies in dogs,"[1] the results section of the abstract of which would suggest that an assay based on combined measurements of OmpC (ACA), canine calprotectin (ACNA), and gliadin-derived peptide (AGA) is useful to differentiate chronic enteropathy/inflammatory bowel disease (CE/IBD) and non-IBD gastrointestinal disorders.[1] However, in the materials and methods section, the differentiation of dogs with primary gastrointestinal disease from those with some forms of secondary gastrointestinal disease is described, not the differentiation of dogs with CE/IBD from those with non-IBD chronic gastrointestinal disease as stated in the abstract. The 24 dogs that were labeled as "non-IBD" were diagnosed with pancreatitis (n = 10), hypoadrenocorticism (n = 9), exocrine pancreatic insufficiency (n = 2), lymphoma confirmed by histopathology (n = 1), or with pancreatitis and a suspicion of lymphoma, or other gastrointestinal neoplasia (n = 2).[1] The authors are correct that the definitive diagnosis of dogs with CE is complex and often involves endoscopic collection of intestinal biopsies, and they are also correct that the search for viable serum and fecal markers has been ongoing for many years. However, the diagnostic challenge is not related to the differentiation of dogs with primary and secondary gastrointestinal disorders. Hypoadrenocorticism can easily be excluded by measurement of baseline serum cortisol concentration, and, if needed, an ACTH-stimulation test (as was indeed performed in the dogs here),[2] exocrine pancreatic insufficiency (EPI) can easily be excluded by measurement of serum trypsin-like immunoreactivity concentration, and finally pancreatitis can often be excluded by measurement of serum pancreatic lipase in combination with diagnostic imaging. Thus, there simply is no need for a serologic marker or a combination thereof to differentiate dogs with secondary gastrointestinal disease from those with primary gastrointestinal disease.

Clinically useful biomarkers for CE/IBD would need to reliably differentiate dogs with IBD from those dogs with other forms of chronic primary gastrointestinal disease. Unfortunately, such a cohort of dogs was not included in the study. Dogs with chronic giardiasis, histoplasmosis, or histiocytic ulcerative colitis, among other primary gastrointestinal diseases, may have similar results to the dogs labeled as "CE/IBD" and may have even been part of that group of dogs. Until such cohorts have been studied in detail, the authors' suggestion that a combination of ACA, ACNA, and AGA could aid in IBD diagnosis is unfounded or potentially even detrimental to the dogs diagnosed based on these biomarkers. If a substantial overlap in biomarker concentrations were to exist between these cohorts, the utilization of the 3 reported biomarkers could delay accurate diagnosis and result in immunosuppressive treatment of dogs with underlying infectious disease.

While the 9 dogs with hypoadrenocorticism and the 2 dogs with EPI most likely were correctly definitively diagnosed, the diagnostic data for the remainder of the "non-IBD" dogs are incomplete with no mention of imaging results. More importantly, only 24% of the 157 dogs labeled as "CE/IBD" actually had a histopathologically confirmed diagnosis of IBD. In fact, the clinical signs reported for the 157 dogs with CE/IBD would not all be considered typical of CE or IBD: 50 had signs of abdominal discomfort and 7 had regurgitation. While the work-up mentioned did include a minimum database and fecal examination for endoparasites, a standardized diagnostic work-up, including the outcome of broad-spectrum anthelminthic therapy or dietary trials, or tissue diagnosis, all essential for a diagnosis of IBD, are missing. Thus, all we can conclude from the current study is that the panel of the three markers described has a 90% sensitivity and a 96% specificity in differentiating dogs with a variety of signs of chronic gastrointestinal disease from dogs that have signs of chronic gastrointestinal disease due to hypoadrenocorticism, EPI, possible pancreatitis, possible lymphoma, or healthy control dogs. We would suggest that such a marker would have no clinical usefulness, as such differentiation does not currently present a diagnostic challenge.

Beyond these concerns, another important limitation of the study is that analytical validation data for the three assays used are not presented. The authors do suggest that some performance metrics for the IgA measurements are available in their original publication,[3] but neither the previous nor the current publication report comprehensive analytical validation data.[1,3] A clear description of the development of each assay, with detailed reporting of analytical validation data, is

essential for scientific transparency and to instill confidence that analytical performance of the assays in question is suitable for their intended clinical purpose. At minimum, reporting results of the following analytical validation characteristics for each assay is suggested: specificity for detection of IgA vs other immunoglobulin classes, detection limit, prozone effect, reportable range, linearity, spiking recovery, intra-assay precision, and interassay precision. Also, preanalytical factors can impact biomarker performance and should be acknowledged and controlled.[4] The cohorts in both the former and current studies were not age-, sex-, or breed-matched, and a variety of treatments had been used within each cohort. Evaluating biomarkers in groups of dogs that are not closely matched in their characteristics increases the likelihood that the markers will discriminate between dogs not because of the presence or absence of the disease of interest, but because of these confounding factors.

Furthermore, we advise caution in suggesting a cause and effect relationship between gliadin and CE in dogs. Gluten sensitivity was first described in Irish Setters in the United Kingdom and gluten sensitivity has been suggested as a cause of paroxysmal dyskinesia in Border Terriers.[5,6] However, beyond these two breed-specific examples and despite extensive research in this area, gluten-sensitive enteropathy has not yet been demonstrated to be a clinical entity in dogs. While this may seem a minor point, AGA seropositivity has led to the recommendation of switching the affected dogs to a gluten-free diet, without any evidence to suggest that such dietary alteration is beneficial or at least not detrimental.

Finally, the authors state that the markers described in their study have been used as clinical tools for the diagnosis of gastrointestinal conditions in humans for decades. This statement is misleading. While serum AGA is used as a marker for coeliac disease in humans, the relevance of this condition remains unknown in dogs and the other two markers have not been widely adopted in human gastroenterology.[7,8] Furthermore, American Gastroenterological Association guidelines state "routine use of serological markers of IBD to establish a diagnosis of Crohn's disease is not indicated."

At minimum, we hope that our collective concerns about the Estruch et al study, which also was lacking an appropriate financial conflict of interest statement (ie, the conflict of interest statement notes "no conflict of interest," while Vetica Labs is listed as a private company, with the first author listed as the founder, CEO, and board member), will stimulate further dialogue about the article, as well as the appropriateness of commercial laboratories promoting these biomarkers for the diagnosis and monitoring of "IBD" in dogs without sufficient transparency regarding assay analytical validation or sufficient validation of diagnostic performance. Ideally, we hope the information provided herein will prompt the study authors to consider voluntary retraction of their manuscript, given the flaws in study design, lack of assay validation reporting, misinterpretation and mischaracterization of study results, as well as lack of an appropriate financial conflict of interest statement.

Daniel K. Langlois[1]
M. Katherine Tolbert[2]
Craig B. Webb[3]
Elizabeth M. Lennon[4]
Bente Flatland[5]
Sara A. Jablonski Wennogle[1]
Gary Block[6]
Albert E. Jergens[7]
Romy M. Heilmann[8]
Louise Murray[9]
Michael Wood[10]
Adam Honeckman[11]
Cynthia R. L. Webster[12]
David C. Twedt[3]
Marnin Forman[13]
Sina Marsilio[14]
Yaiza Forcada[15]
Jared A. Jaffey[16]
Keith Richter[17]
Joerg M. Steiner[2]

[1]*Department of Small Animal Clinical Sciences, Michigan State University, College of Veterinary Medicine, East Lansing, Michigan, USA*
[2]*Department of Small Animal Clinical Sciences, Texas A&M University, College of Veterinary Medicine and Biomedical Sciences, College Station, Texas, USA*
[3]*Department of Clinical Sciences, Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, Colorado, USA*
[4]*Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, Pennsylvania, USA*
[5]*Department of Biomedical and Diagnostic Sciences, University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee, USA*
[6]*Ocean State Veterinary Specialists, East Greenwich, Rhode Island, USA*
[7]*Department of Veterinary Clinical Sciences, Iowa State University, College of Veterinary Medicine, Ames, Iowa, USA*
[8]*Department of Small Animal Medicine, University of Leipzig, College of Veterinary Medicine, Leipzig, Germany*
[9]*Internal Medicine Department, Animal Specialty Center, Yonkers, New York, USA*
[10]*Department of Medical Sciences, University of Wisconsin, School of Veterinary Medicine, Madison, Wisconsin, USA*
[11]*Mobile Veterinary Diagnostics, Orlando, Florida, USA*
[12]*Department of Clinical Science, Tufts University, Cummings School of Veterinary Medicine, North Grafton, Massachusetts, USA*
[13]*Cornell University Veterinary Specialists, Stamford, Connecticut, USA*
[14]*Department of Veterinary Medicine and Epidemiology, University of California - Davis, School of Veterinary Medicine, Davis, California, USA*
[15]*Veterinary Information Network, Davis, California, USA*
[16]*Department of Specialty Medicine, Midwestern University, College of Veterinary Medicine, Glendale, Arizona, USA*
[17]*Ethos Veterinary Health, San Diego, California, USA*

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