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LETTER TO THE EDITOR

Response to letter to editor regarding Results of histopathology, immunohistochemistry, and molecular clonality testing of small intestinal biopsy specimens from clinically healthy client-owned cats

Dear Editor,

Thank you for the opportunity to respond to the letter from Dr. Childress regarding our recent publication "Results of histopathology, immunohistochemistry, and molecular clonality testing of small intestinal biopsy specimens from clinically healthy client-owned cats."

We thank Dr. Childress for his letter and for reemphasizing the importance of the interpretation of any laboratory data in the light of the patient's clinical signs, other test results, and would only like to add the importance of clinical outcome as 1 more additional factor. Guidelines by the EuroClonality/BIOMED-2 group on the interpretation of human clonality assays have long highlighted that results of molecular clonality studies should always be interpreted in the context of the clinical, morphological, and immunophenotypic diagnosis.[1,2] However, this approach is not commonly practiced in veterinary medicine, which is reflected in a publication that reclassified cats with signs of chronic enteropathy as having intestinal lymphoma based on results of the clonality assay alone.[3] Hence, with our publication, we intended to reinforce the clinician's responsibility of formulating a final diagnosis based on all available data.

We recognize and agree with Dr. Childress that the positive predictive value (PPV) will be low when the disease prevalence is low. Such low PPV can occur even with a highly specific test when pretest odds are low, and we agree that this contributes to the low PPV in our results.

However, we do not agree that specificity of the test had no role in our results. In our study, 13 of 20 cats were found to have clonal rearrangements in their duodenal biopsy specimens. Based on the histopathology data and the clinical outcome, it appears plausible that 2 cats of those 13 cats (cat No. 10 and 19, table in the supporting information) did have subclinical lymphoma at the time of diagnosis and that the positive clonality test was correct. The remainder of the cats found to be clonal or clonal in a polyclonal background did not develop any signs of chronic enteropathy after a median of 709 days (range, 219–869 days) and thus 1 possible explanation for these results might be that these results represent false-positive results. Six cats were revealed to have polyclonal rearrangements. While a test sensitivity cannot be extrapolated from our data, a test specificity could be calculated as follows:

	PARR results	
	Clonal or clonal in a polyclonal background	Polyclonal
True small-cell lymphoma	2	0
Non-lymphoma	11	6

One cat was found to be pseudoclonal due to insufficient DNA retrieval.

$$Specificity = \frac{True\ negatives}{True\ negatives + False\ positives} = \frac{6}{6+11} = 0.35 = 35\%.$$

Although, our estimate of specificity is based on a small sample of cats, this low specificity contributes to a low PPV.

Dr. Childress cites the sensitivity and specificity for clonality assays in cats from a website of a laboratory in Colorado.[4] However, the laboratory states that these are estimated values. In addition, the referenced laboratory does not perform clonality assays on formalin-fixed, paraffin-embedded tissue samples and thus the cited estimated values are not applicable to most of the clonality assay performed in cats with intestinal small-cell lymphoma. However, there are reports of the sensitivity of clonality assays in cats on formalin-fixed paraffin-embedded tissue in the literature of around 90%.[5,6]

With regard to the disease prevalence, we disagree with Dr. Childress statement that the disease prevalence in the clonality test population is commonly high, even as high as 90%. Clonality testing is usually requested if assessment of H&E-stained tissue biopsies is equivocal for either inflammation or lymphoma. Hence, the authors consider it more realistic to assume a lower disease prevalence in the tested population.

Under the above stated assumptions of a sensitivity of 90%, a specificity of 35%, and a hypothetical disease prevalence of 50% in a population where histopathology revealed ambiguous results, the PPV would be <60%.

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$$PPV = \frac{(Sensitivity \times Prevalence)}{[(Sensitivity \times Prevalence) + ((1 - Specificity) \times (1 - Prevalence))]}$$

$$PPV = \frac{(0.90 \times 0.5)}{[(0.90 \times 0.5) + ((1 - 0.35) \times (1 - 0.5))]} = 0.58 = 58\%.$$

Alternatively, our findings might be explained by true positive results representing benign clonal expansion. As stated in our discussion, clonality is not always equivalent to malignancy and benign clonal expansions have been described in people and dogs with various conditions. However, if this phenomenon were to occur as frequently in the general feline population as it appeared to occur in our study population, it would still substantially reduce the value of a clonality assay.

In summary, the authors realize that a calculation of test specificity on such a small sample size has to be viewed as preliminary. However, our study was the first using a population that is demographically close to the population of cats that is routinely presented for chronic enteropathy. While Dr. Childress is correct about the PPV being of limited value in our study because of the low prevalence of disease, and the very small number of cats, PPV was not the limitation of the test to which we wished to draw attention. Rather, we were pointing out the low estimated specificity of the test from our results (35%). This low specificity has important diagnostic implications that we wanted to bring to the attention of veterinarians. Our results might indicate that a more robust evaluation of the sensitivity and specificity of clonality testing for classifying cats are warranted. In addition, we intended to reemphasize that diagnostic tests should never be interpreted independently of other available patient data.

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