Blurring the picture in leaky gut research: how shortcomings of zonulin as a biomarker mislead the field of intestinal permeability

With great interest we read the work by Talley *et al*[1] reporting the inadequacy of zonulin as a biomarker due to its failure to identify the irritable bowel syndrome, functional dyspepsia and non-coeliac wheat sensitivity. Zonulin as a biomarker is highly disputed.[2] A recent study showed that zonulin-mediated intestinal barrier integrity is an important mechanism by which gut microbial dysbiosis affects the transition from asymptotic autoimmunity to inflammatory disease associated with increased circulating zonulin in patients with arthritis.[3] In all of these studies, zonulin measurements are based on commercial ELISA.

There is no doubt about the clinical relevance of studies addressing the relation between intestinal permeability and inflammatory diseases. Zonulin, precisely pre-haptoglobin 2 (preHP2), was identified as a human homologue to a second *Vibrio cholerae* enterotoxin regulating tight junction permeability and subsequently has gained much attention as a potential biomarker for intestinal permeability.[4] However, the commercial ELISAs very frequently used to measure zonulin were produced using the first published sequence, which later has been shown to be unrelated to the zonulin protein.[4] These developments have resulted in the following two major critical yet widely overlooked issues.

COMMERCIALLY AVAILABLE ELISAS DO NOT MEASURE ZONULIN

The shortcomings of the commercial ELISA have been demonstrated in independent work and have been discussed previously.[5 6] Measurements using these commercial ELISA do not reflect actual zonulin levels, but concentrations of unknown proteins. Consequently, this has to preclude scientists from drawing conclusions on the role and importance of zonulin in the context of intestinal permeability and related diseases based on these ELISA measurements, both positive and negative. This, also retrospectively, applies to numerous studies reporting findings relying on the commercial ELISA kits.[6] Furthermore, these zonulin ELISA measurements only poorly correlate with functional gut permeability as assessed by, for example, lactulose mannitol test (table 1).

Importantly, this does not take away from zonulin/preHP2 as a regulator of intestinal permeability and does not rule out correlations of zonulin levels with intestinal barrier function.

ZONULIN AS PRE-HAPTOGLOBIN2 IS NOT EXPRESSED IN MICE

Animal models of intestinal barrier dysfunction are highly useful for translational research, yet zonulin as preHP2 is not naturally expressed in mice. While haptoglobin is conserved in most mammals, the HP2 genotype is unique to humans. This renders measurements of serum zonulin in rodent models highly questionable and potentially misleading.[3] Along these lines, differential ELISA signals obtained in mouse sera further indicate detection of unspecific and unknown proteins by the ELISA.[3] For translational research, assessing zonulin levels in mouse models does only become relevant when using zonulin-specific assays in 'humanised mice' genetically modified to express human HP2, as has been previously described.[7]

CONCLUSION

Together, it has become obvious that using the commercial zonulin ELISA is neither adequate to measure intestinal permeability nor the postulated biomarker zonulin. Even more important, previously published results based on zonulin ELISA measurements have to be seen with great caution and do not establish a relation to the function of the protein zonulin/preHP2. New and specific detection methods and assays for zonulin/preHP2 are urgently needed to address the usefulness of zonulin as a biomarker for intestinal permeability. Until then, researchers are strongly encouraged to circumvent the unspecific measurement of zonulin and instead apply rigorous tests of intestinal permeability such as dual-sugar assays, and use immunohistochemistry and expression profiles of zonula occludens proteins.[3]

Table 1 Studies using zonulin ELISA and correlations with intestinal permeability

Study	Year	Zonulin kit	N	Correlation	Citation
Halasa *et al*	2019	IDK	38	R=0.11, p>0.05	8
Linsalata *et al*	2018	IDK	71	R=0.17, p>0.05	9
Kuzma *et al*	2020	IDK(distributed by ALPCO)	24	R=0.033, p=0.79	10

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