Article Type: Letter to the Editor

Title: The reduced Co^{2+}-binding ability of ischaemia-modified albumin is unlikely to be due to oxidative modification of the N-terminus

Authors: Alan J. Stewart[1], Claudia A. Blindauer[2]

Affiliations: *1 School of Medicine, University of St Andrews, St Andrews, KY16 9TF, United Kingdom. 2 Department of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom.*

Correspondence: Dr Alan J. Stewart, School of Medicine, Medical and Biological Sciences Building, University of St Andrews, St Andrews, KY16 9TF, United Kingdom. Tel: +44 (0)1334 463546; Fax: +44 (0)1334 463482; E-mail: ajs21@st-andrews.ac.uk

Word count: 400 words

Figures and Tables: None

Abbreviations: FFA, free fatty acid; IMA, ischaemia-modified albumin

Conflict of interest: The authors do not have any disclosures to report.

Financial support: The authors are supported by grants from the Biotechnology and Biological Sciences Research Council (Grant No. BB/J006467/1 to AJ.S. and C.A.B.) and the British Heart Foundation (Grant No. PG/15/9/31270 to A.J.S.).

To the Editor,

We read with interest the recent article by Giannone *et al.* where the use of ischaemia-modified albumin (IMA) as a molecular marker of bacterial infection in patients with cirrhosis was assessed [1]. IMA corresponds to a "modified" form of albumin that exhibits reduced ability to bind Co^{2+} (with its presence associated with certain medical conditions, including ischaemia). The study revealed a positive correlation between circulating IMA levels and infection in the examined cohort, suggesting that IMA may be a useful marker for screening and monitoring infection in subjects with cirrhosis. Although these findings are indeed interesting, the authors perpetuate a previously held belief that IMA is a modified form of albumin oxidised at the N-terminus, a known binding site for Cu^{2+} and Ni^{2+} ions.

Recent evidence suggests strongly that the basis of IMA is unlikely to be oxidative modification at the N-terminus. It has been shown that plasma IMA levels and N-terminal albumin modification (as probed using N-terminally-directed antibodies) do not correlate in acute coronary syndrome [2]. Also rapid clearance of IMA, observed within hours of an ischaemic event, is incompatible with serum albumin's 19-day circulating half-life [3]. Moreover, the N-terminus is not a preferred Co^{2+}-binding site on albumin [4]. Such findings, together with the observation that elevated plasma free fatty acid (FFA) levels are associated with disease states where high IMA levels are observed (see [5]), led us to examine whether IMA may be FFA-loaded albumin. The effects of FFAs on Co^{2+} binding to albumin were examined *in vitro*, where it was found that physiologically relevant concentrations of the long-chain FFA myristate reduced the Co^{2+}-binding affinity and capacity of albumin at two sites [5], known as sites A and B, which are preferred binding sites for Co^{2+} [4]. This also resulted in a corresponding increase in measured "IMA" using the standard albumin-cobalt binding assay [5].

These observations have important implications for the study by Giannone *et al.,* as it appears that IMA is strictly speaking neither a marker for oxidative stress nor infection but more likely a proxy measurement of plasma FFA levels. Given that the authors discuss the basis of their results exclusively in the context of covalent albumin modifications (which in light of current evidence are unlikely origins for IMA), a re-evaluation of their data considering potential changes in FFA metabolism within their cohort may provide a more consistent explanation for the reported findings.

References

1. Giannone FA, Domenicali M, Baldassarre M *et al.* Ischaemia-modified albumin: A marker of bacterial infection in hospitalized patients with cirrhosis. Liver Int. 2015; doi:10.1111/liv.12860 [Epub ahead of print].
2. Oh BJ, Seo MH, Kim HS. Insignificant role of the N-terminal cobalt-binding site of albumin in the assessment of acute coronary syndrome: discrepancy between the albumin cobalt-binding assay and N-terminal-targeted immunoassay. Biomarkers 2012;17:394-401.
3. Hjortshøj S, Dethlefsen C, Kristensen SR *et al.* Kinetics of ischaemia modified albumin during ongoing severe myocardial ischaemia. Clin Chim Acta 2009;403:114-120.
4. Mothes E, Faller P. Evidence that the principal Co-II-binding site in human serum albumin is not at the N-terminus: Implication on the albumin cobalt binding test for detecting myocardial ischemia. Biochemistry 2007;46:2267-2274.
5. Lu J, Stewart AJ, Sadler PJ *et al.* Allosteric inhibition of cobalt binding to albumin by fatty acids: Implications for the detection of myocardial ischemia. J Med Chem 2012;55:4425-4430.