



Supporting Information

Supplementary methods and results

**This appendix was part of the submitted manuscript and has been peer reviewed.
It is posted as supplied by the authors.**

Appendix to: Pretorius CJ, Ungerer JPJ. Assessing angiotensin-converting enzyme (ACE) protein is more appropriate than ACE activity when investigating sarcoidosis. *Med J Aust* 2020; doi: 10.5694/mja2.50620.

Methods

Patients and samples

All test requests were initiated by attending clinicians as part of routine clinical care. We retrieved paired ACE activity and ACE protein results between January 2017 and February 2019 from the laboratory database. This study conformed with the Declaration of Helsinki. As the data were used to assess and set quality standards for routine care, a formal ethics submission was not required by the local ethics committee (Royal Brisbane and Women's Hospital Human Research Ethics Committee; reference, LNR/2019/QRBW/56865).

Diagnostic criteria

The diagnosis of sarcoidosis is based on clinical and radiological criteria, histological evidence of non-caseating granuloma, and exclusion of other causes.¹ For the purposes of this study, sarcoidosis with compatible histology was based on evidence of non-caseating granulomata and exclusion of alternative causes documented in our laboratory database. In instances in which this was not possible, clinically diagnosed sarcoidosis was defined as ophthalmologist-diagnosed uveitis with elevated ACE activity, or long term management for sarcoidosis by a tertiary specialist referral centre.^{2,3} The breakdown of patients by diagnose type in the shaded areas of Box 1 in the main text are provided in the Table below.

Laboratory methods

We measured ACE activity with a Beckman Coulter AU480 analyser, using a kinetic assay (Bühlmann ACE kinetic, Bühlmann Laboratories) with a synthetic substrate (FAPGG, *N*-[3-(2-furyl)acryloyl]-L-phenylalanyl-glycyl-glycine). The limit of the blank is 5 IU/L, precision exceeds 5%, and the reference interval in adults is 20–70 IU/L. ACE protein was measured with a Quantikine ELISA Human ACE Immunoassay (R&D Systems) on a Tecan Evo platform (Tecan Group). The detection limit in serum samples is 0.5 µg/L, precision exceeded 10%, and the reference interval was 40–200 µg/L.

Statistical procedures

Results are presented as means (with standard deviations, SDs), medians (with interquartile ranges, IQRs), or percentages (with 95% confidence intervals, CIs) as appropriate. Regression and difference plot analysis was performed with Analyse-it 5.01 (<https://analyse-it.com/support/release/5.01>).

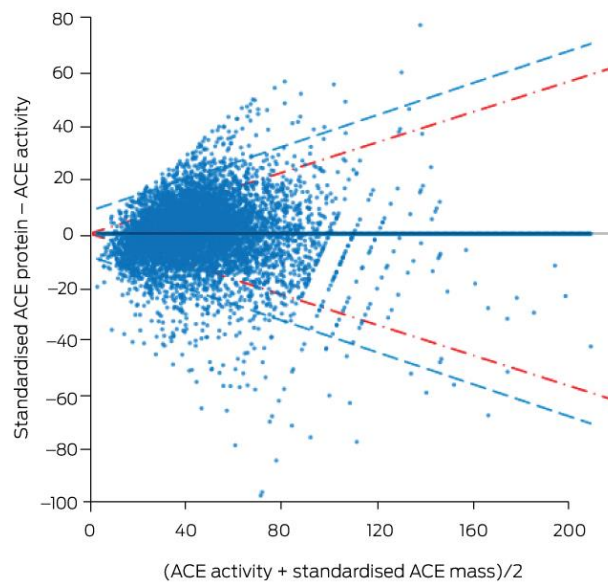
Results

The median age for 4206 women was 53.3 years (IQR, 36.0–65.6 years) and for 4014 men was 55.2 years (42.2–67.3 years). The upper reference limits and regression line nearly intersected (Box 1), which suggested the general biologic equivalence of the two methods and implied that discordant results were not simply due to mismatched reference limits. Correlation of values for the apparently unaffected samples was moderate ($R^2 = 0.71$); further, the differences between the methods were greater than predicted from the respective analytic variances (Figure), indicating that the assays were not interchangeable, even after correcting for scale. There are several plausible explanations for this observation including interferences of either assay type (heterophile antibodies, enzyme co-factors, chemical inhibitors) and genetic variants that affect enzyme activity or antibody binding.

Table. Sarcoidosis prevalence in patients with markedly elevated angiotensin-converting enzyme (ACE) results, by diagnosis type

Sarcoidosis diagnosis	ACE results		
	Activity <70 IU/L Protein > 300 µg/L	Activity > 100 IU/L Protein > 300 µg/L	Activity > 100 IU/L Protein < 200 µg/L
Total clinical audits	50	47	16
Compatible histology	10	10	3
Clinical diagnosis	17	20	1

Figure. Bland–Altman plot of results not affected by angiotensin-converting enzyme (ACE) inhibitors



ACE protein concentration was standardised to emulate ACE activity with the regression equation from Box 1 (main text) to eliminate systematic and constant biases between methods. After standardisation, the mean difference was 0 and the regression equation of the differences v mean concentration was: $y=0.0x + 0.00$, with negligible correlation ($R^2 < 0.001$). The 95% confidence limits are indicated by the dashed blue lines; the predicted 95% limits are indicated by the red lines.

References

1. Ungprasert P, Carmona EM, Crowson CS, Matteson EL. Diagnostic utility of angiotensin-converting enzyme in sarcoidosis: a population-based study. *Lung* 2016; 194: 91-95.
2. Ahmadzai H, Huang S, Steinfurt C, et al. Sarcoidosis: a state of the art review from the Thoracic Society of Australia and New Zealand. *Med J Aust* 2018; 208: 499-504. <https://www.mja.com.au/journal/2018/208/11/sarcoidosis-state-art-review-thoracic-society-australia-and-new-zealand>
3. Niederer RL, Al-Janabi A, Lightman SL, Tomkins-Netzer O. Serum angiotensin-converting enzyme has a high negative predictive value in the investigation for systemic sarcoidosis. *Am J Ophthalmol* 2018; 194: 82-87.