Comments on: Preliminary study of high mobility group box chromosomal protein 1(HMGB1) in ankylosing spondylitis patients

Sirs,

Chen *et al*. recently reported that serum HMGB1 levels were significantly higher in ankylosing spondylitis (AS) patients than healthy controls detected with a commercial ELISA kit (USCNLife Science Inc, Wuhan) (1). In this study they test the serum HMGB1 levels in 71 AS patients and 40 healthy controls, and the results were 1056.10±1033.05 ng/ml and 27.05±21.50 ng/ml, respectively. However, we wish to point out that the results may shift from the actual values.

The values of HMGB1 in this study were much higher than the previous report using the same ELISA kit: Oktayoglu *et al*. reported that serum HMGB1 levels were 0.86±0.37 ng/ml and 0.65±0.39 ng/ml in 30 AS patients and 29 health controls, respectively (2); Albayrak *et al*. used the same ELISA kit to detected the serum HMGB1 levels in 60 patients with acute appendicitis, and the results were 36.92±15.43 ng/ml (3); Fraisier *et al*. used the same ELISA kit to detected the serum HMGB1 levels in 49 patients with West Nile neuroinvasive disease, and the results were149.5±88.4 ng/mL (4). Some other studies that involved testing the serum HMGB1 levels are summarised in Table I, and the mean values range from 1.61 to 184.9ng/ml. According to the manufacture's protocol, the test principle applied in the HMGB1 ELISA kit of USCN Life Science is sandwich enzyme immunoassay, and the detection range is from 62.5 to 4000pg/ml. That means the serum samples from AS patients were diluted at least at a ratio of 1:250, and the kit did not provide a dilution buffer. The linearity of the kit was assayed by testing samples spiked with appropriate concentration of HMGB1 and their serial dilutions, and the highest dilution was 1:16. It seems that the kit was not supposed to be used in this situation. Therefore, we do not think that Chen *et al's*. view is convincing regarding the fact that the higher results are attributed to the quality control standards discrepancy of different ELISA kits. Yamada *et al*. discussed the detection limits in they study about setting up an HMGB1 ELISA test, and it does not seem reliable to detect HMGB1 at a concentration over 1μg/ml (5).

The authors mentions that HMGB1 acts as an endogenous DAMP and plays proinflammatory roles in the progress of chronicle inflammatory response and autoimmune diseases. As a cytokine like DAMP molecules, we think its hard to accumulate to mg/L level in the serum.

The researchers should give out the detail ed procedures of the test including the dilution strategy and linearity of samples that were diluted over 1:16. Perhaps a comparison with standard concentration of HMGB1 using quantitative Immunoblot Assay is a good choice to confirm the results.

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Table I. Summary of some studies that involve testing serum HMGB1 levels.

Study	Condition	Method	Patients (ng/ml)	Controls (ng/ml)
Andrassy *et al*., 2011 (*Journal of Internal Medicine* 270:245-253)	Myocardial infarction with type 2 diabetes mellitus	ELISA (Shino-Test Corp)	Myocardial infarction with type 2 Diabetes mellitus: 9.0±4.4 (n=27) Myocardial infarction: 4.4±3.6 (n=68)	–
Angus *et al*., 2007 (*Critical Care Medicine* 35:1061-1067)	Community-acquired pneumonia	Quantitative Immunoblot Assay	184.9±105.9 (n=122)	11.2±20.8 (n=38)
Hu *et al*., 2009 (*Clinica Chimica Acta* 406:139-142)	Coronary artery stenosis	ELISA (Shino-Test Corp)	Stable angina pectoris: 5.23 (3.65–7.65) (n=35) Unstable angina pectoris: 8.85 6.12–10.75) (n=37)	1.49 (0.71–2.57) (n=32)
Karlsson *et al*., 2008 (*Intensive Care Medicine* 34:1046-1053)	Severe sepsis	ELISA	3.6 (1.9–6.5) (n=170)	0.65 (0.51–1.0) (n=10)
Naumnik *et al*., (*Folia Histochemica et Cytobiologica* 47:703-709)	Non-small cell lung cancer	ELISA (Shino-Test Corp)	2.75±0.7 (n=40)	2.08±0.3 (n=15)
Qiu *et al*., 2014 (*Medical Oncology* 31:316)	laryngeal squamous cell carcinoma	ELISA (Shino-Test Corp)	4.81±2.33 (n=71)	3.21±1.08(n=50)
Tabata *et al*., 2013 (*Journal of Clinical Gastroenterology* 47:684-688)	Malignant Peritoneal Mesothelioma	ELISA (Shino-Test Corp)	13.5±12.7 (n=13)	5.3±1.7 (n=45)*
Tseng *et al*., 2014 (*Disease Markers* 2014: 804654)	Severe Pneumonia and Acute Respiratory Distress Syndrome	ELISA (R&D Systems)	Survivors: 1.61±0.64 (n=40) Non-survivors: 2.11±0. 57 (n=16)	–
Wu *et al*., 2013 (*The Journal of International Medical Research* 41:1796-1802)	Atrial fibrillation	ELISA (Shino-Test Corp)	Persistent: 9.12±2.52 (n=33) Paroxysmal: 6.89±1.43 (n=53)	3.18±0.91 (n=30)
Xu *et al*., 2014 (*World Journal of Emergency Surgery* 9:61)	Severe acute pancreatitis	ELISA (Shino-Test Corp)	6.02±2.42 (n=80)	1.87±0.63 (n=10)

*include 26 patients with benign asbestos-related diseases.