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LETTER TO THE EDITOR

Serendipity and technical considerations for the measurement of serum heat shock protein HSP27 in patients with COPD and lung cancer

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We have read with great interest the work by Cui et al. (2015) and the letter to the Editor by Cappello et al. (2015). Historical knowledge of data acquisition and interpretation is paramount in the field of applied medical research. Our group was the first to describe increased HSP27 serum concentrations in patients with incipient and manifest COPD, in patients with lung cancer (Hacker et al. 2009; Ankersmit et al. 2012; Zimmermann et al. 2012) and patients with end-stage kidney disease (Lebherz-Eichinger et al. 2008). All these information originated from our research dating back to 2006. In this work, our group (a) investigated serum HSP27/60/70/90 content in patients that underwent coronary artery bypass grafting (CABG) surgery (Szerafin et al. 2008) and (b) identified COPD as an autoimmune disease (Lambers et al. 2009). How was this serum HSP27 insight generated in our laboratory? (a) We have collected serum samples of mild and severe COPD and these were stored at our biobank; (b) A Croatian research group had investigated for the first time the role of HSP27/70 in peripheral blood mononuclear cells (PBMC) derived from COPD patients (Lada et al., 2008); (c) Serendipity was in play since the right R&D HSP27/70 ELISA kit, as "left over" from a prior CABG study and was present in our fridge. All these coincidences led to granted patents stating that HSP27 has the potential to be a serum biomarker for early and manifest COPD and lung cancer (EP2141499, EP2652504).

By interpreting the report by Cui et al. (2015) and its comment by Cappello et al. (2015), we want to allude to a recent publication by our research group (Zimmermann et al. 2014). It has to be accepted by the community that an ELISA detection kit for protein XY is sometimes not detecting protein XY (Mueller et al. 2012). In order to investigate this question, we have selected lung cancer patients with known increased serum HSP27 and evaluated five commercially available assays for HSP27 measurement with respect to their capabilities to differentiate non-small cell lung cancer (NSCLC) patients from healthy controls. The following HSP27 ELISA kits were utilized: R&D, Enzo Life Sciences, Invitrogen, Abcam and MyBioSource. We were perplexed by our results! The determined areas under the curve (AUCs) of the receiver operating characteristic (ROC) curves for serum HSP27 to detect NSCLC were published as follows: R&D, 0.834; Enzo Life Sciences, 0.823; Invitrogen, 0.780; Abcam 0.642; and MyBioSource, 0.523. From these results, we concluded that it was *pure coincidence* that we have unintentionally picked the "right" ELISA kit for our investigations in our study period (2007–2015). Abcam or MyBioSource commercial ELISA system would have completely failed to show any difference in our laboratory investigations. We conclude that the results of our clinical method comparison study revealed that commercially available HSP27 assays are not equally useful in detecting increased serum HSP27. By looking at Cui et al. (2015), we observed that a Stressgen Bioreagent HSP27 ELISA was utilized. Since we have not included this commercially available ELISA system in our study, we have difficulty in interpreting the presented results. Since our R&D HSP27

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ELISA has repetitively corroborated our primary data set in COPD/lung cancer (>400 patients), it might be possible that Stressgen is detecting another moiety of HSP27 as compared to the R&D HSP27 system. As far as we are concerned, we believe that scientific statements, discussions and publications dealing with serum HSP27 and COPD/lung cancer are important when comparative methodologies are utilized.

In conclusion, from an epistemological standpoint, it is of great interest to accept that non-hypothesis-driven "luck factor" has helped to gain a better understanding in COPD genesis. The future will show whether the scientific community will be able to present a biomarker to the public that is able to identify patients with risk to develop COPD or lung cancer. We are deeply convinced that only commercial interest will bring this important knowledge into the realm of clinics.

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