Reviewer’s report

Title: Validation of previously identified serum biomarkers for breast cancer with SELDI-TOF MS: a case control study

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Reviewer: Dalibor Valik

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The manuscript by AWJ van Winden (first author) presents an interesting material relevant to the field of clinical cancer proteomic studies using technology SELDI TOF. Unfortunately, this technology has been more questioned than explored before having been sufficiently tested in various fields in clinical research to fully assess its potential. Saying this, I am aware that all the possible objections that have been raised against the use of this technique could also be brought up concerning this manuscript as well. However, since me and my collaborators have some level of experience with this instrumentation and technology and are well acquainted with its pros and cons, I believe that this manuscript has the potential to pass interesting information the readers of BMC.

My comments, which are in principle minor, follow:

As the authors point out significant preanalytical differences have been observed in SELDI TOF studies. Furthermore, peaks they four significant are located within a suspicious mass range 3500 – 5000 amu. Since they very probably reflect some kind of concomitant epiphenomenon the authors may also have looked at different blood-derived material such as various kinds of plasma to assess the relevance of their results. Since this is not possible in the retrospective study, authors may choose to discuss this point and may consider planning future prospective work using not only serum as probably – in my opinion – the least suitable material. We have previously shown that clot-activator collecting devices may significantly contribute to the low-molecular peak spectrum using this technology (Valik et al., Clin Chem.2006). In line with this authors shall describe and discuss specimen sampling in more particular – specifically, what kind of collection devices were used and subsequent possible preanalytical influence thereof....

“Serum samples of the cases and controls, which were collected between January 2003 and June 2005, were obtained from a serum bank at The Netherlands Cancer Institute (NKI), Amsterdam, The Netherlands. These serum samples were collected (using what ?) after receiving individuals’ informed consent under approval of the Institutional Review Board Control. Serum samples of the cases were obtained after diagnosis of breast cancer and before surgery or any other kind of treatment. Blood collection, processing and storage of the serum samples was performed under strictly defined conditions which were the same for cases and controls. After collection, blood samples were allowed to clot for 30 minutes at room temperature and were subsequently centrifuged for 15 minutes at 3000 rpm at room temperature. Thereafter, samples were aliquoted
and stored at -30°C. Cases and controls were frequency matched regarding their age and the storage duration of their serum sample as much as possible.

Furthermore, I would suggest to replace the words “up-regulated and down-regulated” using clear words “increased and decreased”, respectively, as being more relevant for serum, plasma or blood-utilizing studies and do not confound medical readers with terms originating from more experimentally structured, yet not always clear, molecular biology and cell biology studies. Logically – here the relation between breast cancer and i.e., ITIH4 or C3adesArg#8 is not “up/downregulation” implying causation but merely increase/decrease.

Analytical and statistical parts of the study are in my opinion well performed, trustful, robust and reproducible for those interested. Minor correction of English will also improve quality of the material presented.

Taken together, after minor correction I suggest that this manuscript is acceptable for publication and believe that it becomes a very interesting piece of information clinical cancer research area. I was pleased to be chosen by BMC as a reviewer of this manuscript.