*Microfluidic sensing of exosomes for ovarian cancer diagnosis*

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Dear Editor,

In the February 25 issue of Nature Biomedical Engineering, Zhang et al describe a microfluidic sensing lab on a chip suitable for analysis of circulating exosomes (1). They claim that this new technology may have significant utility for early diagnosis of cancer, especially ovarian cancer. While we do not question the possible value of this device in bioengineering, we believe that the suggestions for its applicability in cancer diagnosis are questionable. We previously published on the difficulties of newly discovered cancer biomarkers to enter the clinic and attributed these failures to 3 major reasons a) data fabrication, b) false discovery (which means that the discovered biomarkers were due to pre-analytical, analytical or post-analytical errors) or c) exaggerated claims of performance (2-4). We believe that the report of Zhang et al falls into category c, for the following specific reasons.

Exosomes for cancer diagnosis: It is accurate that exosomes represent a relatively newly discovered source of cancer biomarkers. However, there is currently no clinical device or test that is based on exosome utilization. It is anticipated that in the blood of cancer individuals, there will be exosomes derived from normal tissues and exosomes derived from cancer tissues, the latter being a minute fraction (less than 0.1%, depending on tumor size). The molecules that are used for capture and detection of cancer-associated exosomes in this study, namely CD24, EpCAM and FRα, are not cancer-specific and it is highly unlikely that the immunoaffinity process will lead to almost complete separation of cancer-associated exosomes from normal exosomes. This is already admitted by the authors, who mention that CD24 and EpCAM are found on the surface of exosomes that derive from both patients and controls. It is thus questionable that capture of exosomes by using antibodies for these molecules will highly enrich for cancer-associated exosomes.

The authors claim in the abstract that “Our results suggest exosomal FRα as a potential biomarker for early detection and monitoring of progression of ovarian cancer.” However, no data has been presented regarding monitoring or progression of ovarian cancer with this device.

We believe that the major limitation of this paper regarding ovarian cancer diagnosis is related to the quantity and quality of the utilized clinical samples. The authors analyzed 20 serum samples from ovarian cancer patients, of which 14 were from patients with stage III-IV disease and only 6 of stages I-II. Also, the control group consisted of only 10 patients with no cancer, of which only 3 were from benign gynecological conditions. Despite the claim of the authors that “this sample size is sufficiently large to evaluate diagnostic accuracy with desired statistic errors”, these numbers of samples will not support any meaningful statistical analysis. Especially, on certain occasions, the authors break-down this small number to even smaller numbers such as 10 samples from cancer and 5 from controls, to perform various statistics including receiver operating characteristic (ROC) curves. Their ROC curves show areas under the curve of 1.00 in many cases, suggesting perfect performance.

In Figure S18b the authors claim good separation ($p<001$) between controls and ovarian cancer patients by using total protein, a non-specific marker. This unexpected finding requires explanation.

It appears that the most promising biomarker for early ovarian cancer detection in this study is exosomal folate receptor alpha (FRα) which was found to have improved specificity over the other biomarkers. FRα was discovered by our group in 2013 (their reference 43) as a candidate serum ovarian cancer biomarker (5). In our study, we used serum samples from 100 normal women, 100 women with benign gynecological conditions and 100 women with ovarian cancer of various stages. We found that FRα could not outperform the classical ovarian cancer biomarker CA125 for diagnosis of ovarian cancer. We have also shown a good correlation between FRα and CA125 and no complementarity and concluded that this biomarker may not have clinical utility over and above CA125. We speculate that the utility of FRα as an early diagnostic biomarker for ovarian cancer, either in serum or in exosomes, is questionable. The age of the ovarian cancer patients vs the control group is different by at least 10 years and this may have affected the results.

In our previous commentaries with ovarian and other cancer diagnostics, we stress the importance of test's specificity in applications such as early diagnosis and population screening. For ovarian cancer, we and others calculated that a test specificity of >99% is necessary for a successful screening program. The actual specificity of the proposed test has not been calculated.

In a paper that we co-authored in 2011 we provide the most appropriate framework for studying ovarian cancer biomarkers suitable for screening and early cancer detection (6). In that paper we used specimens from the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial and evaluated 180 ovarian cancer cases and 660 benign disease of general population controls. For these patients, we had pre-diagnostic samples (when patients were asymptomatic) and examined the diagnostic power of 49 different biomarkers for cancer detection at asymptomatic stages (the actual scenario for ovarian cancer screening). We found that none of the tested biomarkers was able to outperform the classical cancer biomarker CA125, which was discovered more than 35 years ago.

We take this opportunity to mention that in order to avoid false discovery and other pitfalls when evaluating newly discovered cancer biomarkers, it is mandatory to follow our published recommendations, and those of others, including use of samples of sufficient quality and quantity (7), careful study designs (8) and avoidance of biases (9). It has been documented that one of the most frequent reasons of false discovery is the use of convenient samples which are of either low quality and/or small numbers (7, 9).

While we do not question that the microfluidic device described in this paper may find some important applications, it is premature to claim that it could be useful as a diagnostic tool for early ovarian or other types of cancer. Clearly, more studies are needed to evaluate this device for clinical use.

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