Reviewer's report

Title: Prediction of breast cancer by profiling of urinary RNA metabolites using SVM-based feature selection

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Reviewer: Jean-Luc Ravanat

Reviewer's report:

In the presented work, the authors have evaluated the possibility of using urinary RNA metabolites, using SVM-based feature selection, as a prediction of breast cancer. For that purpose, they have used a mass spectrometry-based approach (LC-ITMS) for measuring concentrations of urinary metabolites in human urines from either healthy controls or breast cancer women. Among the 51 ribosylated nucleosides detected in urine, 35 were selected and their ability to predict breast cancer was evaluated using a bioinformatic approach.

- Major Compulsory Revisions

The article is well written, the main goals clearly defined and the conclusions made by the authors as well as the importance of the work is well discussed. Concerning the experimental approach that has been used, the authors have carefully checked the reproducibility and linearity of the detection methods since such an information is of major importance for the work. Their results clearly indicate that the method is quantitative and reproductive (at least for the selected ribosylated nucleotides) and this is crucial for the work. The reproducively of the method has been determined using one spot urine sample separated into ten different aliquots that were further analysed. To demonstrate the powerfulness of the defined approach, I consider that it is of major importance to evaluate the inter-day variation of the levels of the urinary metabolites. For all the analyses, only one spot urine sample was analysed per patient. In order to demonstrate that the approach is suitable for cancer prediction, the authors should provide experimental evidences that the level of urinary metabolites they measure with their analytical approach does not significantly vary according to the time of urine collection. For a defined woman (from both control and cancer groups) what is the inter-day variation of the urinary metabolite the authors could measure with their analytical approach? If the inter-day variation of the urinary metabolites is too high, the use of such biomarkers as prediction of breast cancer is seriously compromised. Therefore, I consider that such information is of major importance to confirm the conclusions made by the present work.

- Minor Essential Revisions

Page 9 the authors indicate that they generally summarize the corresponding [MH]+, [M+Na]+ and [M+K]+ ions traces due to significant alkali affinity of certain analyze metabolites. However, they also mention that the HPLC separation is
performed using 5 mM ammonium formate buffer at pH 5. Under these separation conditions one would expect that the ammonium adduct of the analyte to be a major ion. Is the ammonium adduct detected? Summarizing the different ions might be a source of variability for the analyses. Could the authors provide additional evidences for the improvement (in term of sensitivity and reproducibility) of using such an approach?

In a previous published work (Ref 13) the authors have identified the ribosylated nucleosides detected using in the present work as potential biomarkers of breast cancer. No information is given in the present work on the method they have used to detect the urinary metabolites. Since the system they have used consisted in an ion trap, the authors have the possibility to detect the ribosylated nucleosides using either single MS or tandem (or even more) mass spectrometry. The only information that is given indicates that the acquisition was performed using the standard enhance scan mode over a mass range of m/z 200-600. Could the authors explain why they have apparently not used ms2 that is considered more sensitive and specific?

- Discretionary Revisions

Several groups are interested in measuring oxidative nucleosides in human urine as a potential biomarker of oxidative stress and potentially cancer. For example, ribosylated 8-oxoguanine has been measured in human urine (Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. Weimann A, Belling D, Poulsen HE. Nucleic Acids Res. 2002 Jan 15;30(2):E7). Such a ribosylated nucleoside should be detected using the analytical approach used in the present work. Could the author provide an explanation for the lack of detection of such RNA degradation product?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests