Reviewer's report

**Title:** Towards real-time metabolic profiling of biopsy specimen during a surgical operation by 1H HRMAS-NMR: a case report

**Version:** 2 **Date:** 29 September 2011

**Reviewer:** Bernardo Celda Muñoz

Which of the following following best describes what type of case report this is?: Other

If other, please specify:

The proposal of a new potential real-time technique for supporting standard cancerous tissue diagnosis

Has the case been reported coherently?: Yes

Is the case report authentic?: Yes

Is the case report ethical?: No

Is there any missing information that you think must be added before publication?: Yes

Is this case worth reporting?: Yes

Is the case report persuasive?: No

Does the case report have explanatory value?: Yes

Does the case report have diagnostic value?: Yes

Will the case report make a difference to clinical practice?: Yes

Is the anonymity of the patient protected?: Yes

Comments to authors:

The manuscript entitled “Towards real-time metabolic profiling of biopsy specimen during a surgical operation by 1H HRMAS-NMR: a case report” by Piotto et al. describes the potential application of HRMAS NMR as a technique able to provide real time metabolic information during a surgical operation using a single case in a simulated clinical situation. The translation of HRMAS NMR as a potential clinical technique useful for diagnosis purposes could be considered as extremely interesting, because, in principle, NMR is a robust, reproducible and
relatively fast methodology for providing metabolic profiles. However in this manuscript there some important concerns that needed significant revisions as:

i) HRMAS NMR strictly doesn't provide the metabolic profile of “intact” biopsy tissues. It has been shown that a significant number and amount of water soluble metabolites are transferred to the liquid part of the whole sample, D2O, used for locking the magnetic field. On the other hand, some part of the metabolites remains in the tissue. In addition, the tissue suffers some mechanical alterations during the high rate spinning of the HRMAS and is stuck over walls of the rotor.

ii) The histopathological analysis was carried out in parallel, mirror, biopsy samples. Although, this parallel samples could be quite representative of the ones used in metabolic profile analysis, in principle, they are not identical in composition. Therefore, it would be preferable to have done the histological analysis on the same samples after HRMAS measurements. These data would be more directly comparable with the metabolic profiles. This has been quite clearly shown in different previous papers, although by using other type of cancer tissue, as prostate, breast and brain.

iii) The lactate amount in the biopsy samples can not be used as direct biomarker because the anoxic conditions of the surgery process and further biopsy tissue manipulation and measurement in HRMAS technique can alter the concentration of lactate and being not directly correlated to the original one in the tissue.

iv) It would be quite important to know some of the experimental procedure, although it had been described in another paper. For instance, the temperature of HRMAS spectra acquisition. The temperature can play a significant role in the modification of metabolic kinetics and as well in the final total needed time for metabolic profile acquisition.

Therefore, the authors should:

i) To revise the statement of the acquisition of metabolic profiles in “intact” biopsy specimen in the introduction section. It could be advisable to change for “unprocessed” biopsy specimen.

ii) To provide the histological analysis of the biopsy tissue samples after HRMAS measurements, and compare this macroscopic tissue information with the metabolic profiles results.

iii) To revise the role of the Lactate amount, because it is directly related as well with anoxic conditions that can not be identical for all the tissue samples.

iv) To provide some minimum information about experimental conditions, mainly temperature, even if this information is gathered in other papers.

Quality of written English: Acceptable

Declaration of competing interests:
I don't have any competing interest