Reviewer’s report

Title: Circulating cell-free methylated DNA and cell turnover in colorectal cancer

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Reviewer: Jose Luis Costa

Reviewer’s report:

Philipp et al. aim in this manuscript to correlate the presence of the methylation of the genes NEUROG1, HLTF and HPP1 in serum with tissue breakdown as a candidate mechanism using LDH as a surrogate marker. For this, the authors use pre-therapeutic serum samples from 259 patients of colorectal cancer (CRC). The LDH levels for the patients was previously known.

From the literature: (i) is known that NEUROG1 is frequently hypermethylated in CRC, (ii) HLTF is commonly found to be hypermethylated in CRC and is associated with poor prognosis, and (iii) HPP1 can be found hypermethylated early in the CRC carcinogenesis.

The results obtained are in accordance with the literature. Also, since both HLTF and HPP1 are known as bad prognosis markers it is expected that they would correlate with higher UICC stage, tumor size and metastatic disease. The same is true for high LDH levels. What is surprising is the NEUROG1 results. The authors should discuss this further.

Overall the work does not add any relevant new knowledge to the state of the art and has some methodological issues that need to be clarified.

Minor comments:
1. The authors use throughout the manuscript the terms cell turnover, necrosis and cell breakdown as the biological effect behind the LDH levels. The different biological mechanism are very different and should not be used as synonymous.
2. The threshold for high LDH is not clear. What do the authors mean by “this is the upper limit of normal”? Also it is not clear at what stage the LDH levels were obtained (e.g. at diagnosis?).
3. The title of the manuscript does not reflect the nature of the data in the study.
4. A key aspect in the study of cfDNA is the procedure used for the collection of the blood, in this case serum. It is well described in the literature that variations in results are highly affected by the blood collection procedure. This aspect is not well described in the manuscript. The authors should detail this procedure and provide evidence that variation in the protocol do not influence the end result.
5. Table 2: In the study the authors consider any PFM value above 0 as methylated. The data presented in Table 2 creates confusion to the reader.
6. The study should be reviewed by a statistician.
Level of interest: An article of limited interest

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 I have no competing interests