Reviewer's report

Title: Clinical significance of preoperative serum vascular endothelial growth factor, interleukin-6, and C-reactive protein level in colorectal cancer.

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Reviewer: Gennaro Galizia

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The Authors have determined the basal serum levels of vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), and C-reactin protein (CRP) in 143 colorectal cancer patients undergoing surgery. Such markers were found to be significantly higher in cancer patients than in non-cancer patients, and they did not correlate with other important tumor prognostic factors (such as TNM stage). Overall survival (OS) rate [but not disease-free survival (DFS) rate] was reduced in patients showing high preoperative VEGF values. Conversely, basal IL-6 and CRP serum level were neither correlated to OS nor DFS rates.

The Authors concluded that basal VEGF serum level could be proposed as a prognostic factor for overall survival in colorectal cancer patients.

Although, the Authors' conclusions are in agreement with several previous reports, to my own opinion, some very important issues have to be considered and manuscript requires a complete revision.

Major comments:

# Common limitations of all studies evaluating serum level of any substance are cut point selection. In many reports and very large variety of human tumors, the basal VEGF and IL-6 serum levels have been reported with a very wide range. Of course, for statistical purposes it is necessary that continuous variables, such as serum levels, are clearly divided in groups thus requiring to select defined cutoff values. Usually, mean or median values have been used.

In this article, the Authors selected the median values. Unfortunately, basal VEGF serum levels were not only very skewed (620 + 438 in colorectal cancer, and 334 + 219 in non colorectal cancer patients, respectively), but even a clear overlap between two groups should be supposed. This issue was particularly noticeable in IL-6 analysis. The median value selected to unequivocally separate low to high IL-6 serum level patients was 5.74 pg/mL that was virtually equal to mean value in control group (5.65 pg/mL). Thus, statistical analyses related to OS and DFS rates are unacceptable because colorectal cancer patients are not correctly grouped.

In continuous variables (such as basal VEGF, IL-6, and CRP serum levels) the best method to individuate the better cutoff value is a statistical analysis called Receiver Operating Characteristic (ROC) Curve. This method allows to compare the performance of different values of a continuous variable versus a known
outcome, providing predictive accuracy value for any specific level of the continuous variable. Thus, it may be used to identify the cutoff value that shows the better predictive accuracy.

# After a correct cut-point selection, and well-defined sub-groups of patients (i.e. low and high basal serum levels) a major concern should arise: are the two sub-groups well-matched? In short, are clinical, pathological, and surgical characteristics of the two sub-groups statistically not significant? Particularly, is tumor progression similar? Are the rates of curative surgery not different between two groups?

# The most important limitation of this study is the lack of data concerning postoperative VEGF, IL-6, and CRP serum levels. As it is, and giving a correct statistical analysis (see above), this study shows that high VEGF serum level patients could have worse prognosis. To date, it is unclear how it is likely to change the management of colorectal cancer patients undergoing surgery.

On the contrary, determination of postoperative serum levels would have many advantages:

1. serum level normalization could suggest radical surgery;
2. serum level decrement would show that marker is secreted by the tumor;
3. patients undergoing potentially curative surgery who doesn't show serum level normalization of the marker should be treated with chemotherapy regardless of the TNM stage
4. slight or rapid increase of the marker during the follow-up period could be very useful to suspect cancer recurrence.

Minor comments:

# In the paragraph Methods, subparagraph Patients, it is stated that patients were followed by computed tomography every 3 months for the first 2 years, and then every 6 months for a total of 5 years. It means 14 CT/5 years for each patient and appears offline respect to current method of follow-up for colorectal cancer patients undergoing surgery;

# No data are supported regarding the control group;

# Table 1 shows many typographical errors, at least in the provided copy. Particularly, depth of tumor invasion is not clear. Moreover, several continuous variables (such as tumor size, CEA serum levels, and lymph node ratio) are grouped according to a not-specified method to identify the cut-off values;

# Table 3 showing correlations between tumor markers and other clinicopathological parameters is redundant. It should adequate to report in the text only significant values.

In conclusion, this study doesn't seem to add anything to what is currently already known.
A fundamental bias in statistical analysis could have lead to conclusions that are not supported by data, thus requiring substantial re-analysis. The lack of postoperative surveys needs to be corrected by providing data and reconsidering results.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.