Reviewer's report

Title: Inflammatory response associated with mammary carcinomas in female dogs: immunophenotyping of lymphocytes and the relationships between prognostic factors and survival rates

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Reviewer: Giuseppe Sarli

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The paper, by using proper techniques, describes the role of the inflammatory/immune response in the progression of canine mammary tumours, allowing to get prognostic indications from the cell types present and their distributions. The paper is clear in the aims but both the introduction and the discussion are too much devoted to human results and lack of the information available on this topic in the veterinary field. Some points of the text in the material and methods and results sections needs to be improved as follows.

Major Compulsory Revisions

Abstract
1. What does “pure carcinoma” means? Simple carcinoma or complex carcinoma?

Background
2. Rewrite the sentence “It is believed that, in certain situations, the cells responsible for modulation of the inflammatory response release autocrine and paracrine factors that stimulate cellular proliferation and angiogenesis, as well as inhibiting apoptosis, thus altering the immune response to aggression [7;13].” It is not comprehensible.

Methods
3. Change palpitation with palpation
4. Why only chest radiographs were used to follow up the animals?
5. “In cases with multiple nodules, the tumour was classified as a larger tumour for the histomorphometric analysis and immunophenotyping.” This way is not correct because in case of multiple nodules you should consider only the larger, otherwise a bias is referred to all the smaller nodules if they are not related to the largest. In the dog often the mammary tumours consist of multiple nodules not related each other.
6. How where WHO and Cassali et al classification systems merged or integrated?

7. It is correct to refer mitotic index to 10 fields, each with the same area, but the data is not corrected for cellularity. Four mitoses in a field of 0.239 mm$^2$ of a solid carcinoma are lower than four mitoses in a field of 0.239 mm$^2$ of a papillary carcinoma. Correction of the data for cellularity must be considered, otherwise the data are not comparable between different types of tumours.

8. The objective used to capture the images needs to be reported.

9. Was only for eosinophil counts the chromotope 2R stain used? Were all the other cell types counted on H&E stained sections? Please specify.

10. For the quantitative variables the test used to identify normal or not normal distributions of the data must be reported.

11. The Kaplan Meyer are curves and the COX is a test. Did you use the log rank test or the COX test to compare the curves? 0.05 was the alpha level used for all the test or only for the log rank test? Which was the alpha level for the other comparisons?

Results

12. “The carcinoma group consisted of, in increasing degree of malignancy, three tubular carcinomas, five papillary, three tubular-papillary, four solid (Fig. 1B), two micropapillary, two anaplastic and one special type called mucinous carcinoma.” The sentence does not respect the tumour groups reported in the WHO classification. It is considered a tubular-papillary carcinoma group and not 3 different types (tubular, papillary and tubular-papillary), the increasing malignancy is in the progression tubuloar-papillary, solid and anaplastic carcinomas. How many were the in situ carcinomas?

13. “The histopathological diagnosis showed a strong inverse correlation with the mitotic index ($p = 0.005$), survival ($p = 0.009$), and range of total inflammatory infiltrate ($p = 0.025$).” The sentence is not correct because the histopathological diagnosis is a qualitative variable. Which is the explanation of an inverse correlation between a qualitative and a quantitative variable?

14. “There was significant difference between the groups (MC-BMT and MC) (Fig. 2).” The sentence is not clear. The difference was significant for which values?

15. “Tumour size presented a strong positive correlation with stage ($p < 0.001$), ……….”. The result is expected because the size of the tumour give a great contribution to the final clinical stage in the TNM system, so this analysis should be erased.

16. What are score I and II? They are not presented in materials and methods.

17. “The mitotic index presented a strong correlation with ………., histological diagnosis ($p = 0.005$) and ……….” How was the correlation calculated and what does the result mean?

18. Change mononuclear lymphocytes in lymphocytes.
19. “The proportion of lymphocytes showed a significant and positive correlation with the distribution (p = 0.042) and type of inflammatory response (p = 0.004), and ……………”. Explain what does it mean or which distribution and type of inflammatory response was associated with the proportion of lymphocytes.

20. Please refer (or give a table of) the median values for each group before starting the presentation of the results of the survival time paragraph.

21. What are intervals 1 and 2? They are not presented in materials and methods.

22. “The survival rate showed a significant and inverse correlation with ……… histological diagnosis (p = 0.009), ………………………” Please explain.

Discussion

23. Explain the difference of results obtained in this study with other in the veterinary field on the same topic.

24. The mechanisms of B, CD4+ and CD8+ cells in the control of tumour should be briefly reported.

25. “This finding probably results from the extensive areas of ulceration associated with the larger tumours, mainly occurring in this group, which results in bacterial infection and neutrophil recruitment. These cells are also attracted to the tumour site by cancer cells secretion of GM-CSF. The neutrophils also produce oncostatin M (IL-6 cytokine family). When neoplastic cells bind to this cytokine, this induces the secretion of VEGF from the neoplastic cells, which, in turn, increases tumour invasiveness and indicates a poorer prognosis [44].” Erase these sentences because the cell type is not important.

26. “Macrophages are cells that belong to the mononuclear phagocytic system, and are derived from circulating monocytes [45]. These cells migrate into tissues, where they undergo differentiation into two cell types, M1 and M2, with distinct features. The M1 macrophages are activated by INF-_, and are potent effector cells in fighting tumours. This is very distinct from M2 macrophages, which promote proliferation by producing growth factors related to angiogenesis, tissue repair and remodelling [46].” Erase these sentences because they do not discuss the results presented.

Conclusion

27. Refer a short conclusion also on the role of the B cells.

Minor Essential Revisions

28. Due to the presence of misspelled words, the text needs the editing of an English mother tongue.

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

**Quality of written English:** Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: 
I declare that I have no competing interests