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: Juana Martin de las Mulas

Reviewer's report:

The work signed by Estrela-Lima and others presents, first, results of characterization of inflammatory response associated with mammary carcinomas of the female dog, including immunophenotyping of lymphocytes, and second, results of correlation of inflammatory response and lymphocyte immunophenotyping with prognostic factors and survival. Lymphocytes were the predominant cell type. Differences between lymphocytes subpopulations were found with respect to histological type of tumour, metastasis and survival.

The background and design of this study is very interesting and the results force debate concerning the protective-promoting effect of inflammatory cells in neoplasia. Thus, an association between inflammation and indicators of worse prognosis has been found. However, methods are incompletely described and the presentation of the results has some pitfalls which should be addressed in order to clarify their content. In addition, discussion is faint and conclusion vague. To my opinion, the histological classification of tumours, the number of tumours studied by flow cytometry and the statistical study used to analyze the prognostic value of the variables under study are points of major concern. Please find below some hints to improve your manuscript.

MAJOR COMPULSORY REVISIONS

Abstract
1) Methods: Indicate precisely which prognostic factors were analyzed.
2) Results:
   a. Indicate precisely the relationship between inflammatory response (intensity, distribution and cellular type) with prognostic factors.
   b. Correct the second sentence where the percentage of T lymphocytes is compared between two different parameters, histological type and metastasis.
3) Conclusion: Be precise with respect to present results.

Methods
1) Create a new section listing the prognostic factors studied.
2) The mitotic index is not a prognostic factor in canine mammary carcinoma. On the contrary, the number of mitosis is one of the parameters evaluated when
establishing the histological grade of malignancy, which is an independent prognostic factor in canine mammary carcinoma (see the chapter written by Lana, Rutteman and Withrow in the 2007 edition of Withrow and MacEwen’s Small Animal Clinical Oncology, Saunders). I suggest calculating the histological grade of the tumours studied.

3) In the morphological and morphometric analysis of the tumour inflammatory infiltrate:

a. Define how intensity was classified into mild, intense or severe.

b. Define the type of staining used and the parameters analysed in the morphometric study of histological fields. As actually presented, it is not clear whether different pictures were taken for the study of eosinophils and the other cell types.

c. Define the “histological groups” mentioned in line 19 of page 8.

d. Define the “histological groups” mentioned in line 8 of page 10.

4) Survival time:

a. In page 10, lines 15-16, please indicate how were the two intervals defined: by nude eye? by morphometry? Do these intervals correlate with your classification of the inflammatory infiltrate into mild, intense or severe?

5) Statistical methods: Although the correlation between any tumour characteristic and well-known prognostic factors and survival is indicative of the prognostic indicator nature of the characteristic under study, multivariate studies are needed to know the dependent or independent nature of such a variable. Taking into account that the authors have data concerning well-established prognostic indicators (clinical stage, metastasis, tumour size) in addition to survival time, it is not clear to this reviewer why a multivariate statistical study has not been used. I strongly recommend reinforcing the power and quality of the results obtained by performing a multivariate statistical analysis.

Results

1) Taking into account the results presented under “Clinico-pathological evaluation” it appears that the histological classification of tumours you have used is not the WHO classification of Misdorp and others (1999) mentioned in the methods section. Complex carcinoma is “relatively common in the dog” (Misdorp et al 2009). Accordingly, it is hard to believe not a single case was presented in your series.

2) Page 12, lines 7-9: The meaning of this sentence is not clear. I suggest writing it down in another way to make it clear what “histopathological diagnosis” correlates with each parameter.

3) Page 12, lines 9-10: Indicate the parameters with respect to which the differences between the groups (MC-BMT and MC) were found.

4) Page 12, lines 15-16: When talking about “distribution of inflammation” clarify which type of those defined in the Methods sections you are referring to (focal, multifocal, or diffuse?)
5) Page 13, lines 10-11: Scores I, II and III have not been defined in the Methods section.

6) What was the reason for the lack of flow cytometry analysis in more than half of the tumours (24 analysed, 51 in total)?

7) Page 15, line 10: Enumerate the “classical prognostic factors analysed”.

Discussion

1) Page 18, second paragraph: To my opinion, there are no data in this work to support this assertion as time between first appearance of symptoms and diagnosis were not recorded. Further, the references are not adequate: This histological type of tumour was first described in literature by Misdorp and others in the last WHO histological classification of tumours of the mammary gland of the dog published up to date (1999).

2) Page 19, second paragraph: Clarify the meaning of the sentence concerning oestrogen synthesis in this context.

3) Page 19, third paragraph: The first two lines should be mentioned in the Methods section also! In addition, when saying “that T lymphocytes represent the predominant fraction in the cellular infiltrate …” the way they were counted should be clarified and also indicated in the Methods section.

4) Page 20, lines 3 and 4: The term “tumour development” when referring to metastasis is confusing. If tumour “development” had been prevented, then the benign mixed tumour would have never become a carcinoma in benign tumour! I suppose you mean “tumour progression”. The same confusing terminology can be found in other parts of the manuscript such as page 20, last paragraph.

5) Page 22, second paragraph: Explain the relationship of this paragraph with your findings.

Conclusion

I suggest you to be more precise in your conclusion on the basis of present results. For example, talk about the favourable or unfavourable prognostic value on the inflammatory reaction in mammary carcinoma of the dog instead of mentioning “important factors”.

References

References to basic, fundamental studies on clinical and morphologic prognostic factors of canine mammary carcinoma are lacking. Most of them are already listed in the main books of clinical (Withrow & MacEwen 2007) and pathological (Meuten 2002) Veterinary Oncology.

MINOR ESSENTIAL REVISIONS

1) Page 6, line 9: “palpitation” is misspelled.

2) Page 7, lines 6-7: Revise the sentence. Do you mean that tumour selected for histomorphometric analysis and immunophenotyping was the larger one in case of multiple tumours?
3) The level of significance of statistical tests is presented as “p=” and “p<” with no defined criterion to do so (see for example the third paragraph in page 15 and the last one in page 17). I suggest unifying.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests