Reviewer's report

Title: Sialyl Lewis x expression in canine malignant mammary tumours: correlation with clinicopathological features and E-Cadherin expression

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Reviewer: Juana Martin de las Mulas

Reviewer's report:

General
The purpose of the original manuscript written by Pinho and co-workers is very interesting for those interested in both veterinary and comparative pathology. On the one hand, the finding of new markers of malignancy in canine mammary tumours is strongly needed taken into account the highly heterogeneous histological nature of these tumours and the poor correlation between histological diagnosis of malignancy and biological behaviour. On the other hand, finding of new similarities between canine and human mammary cancer enhances the utility of the model. The authors analyse the immunohistochemical (IHC) expression of sialyl Lewis x (sLex) in 53 canine mammary tumours. Results show some level of sLex expression in all malignant tumours analysed. Further, a correlation between the absence of lymph node metastases and the lower levels of sLex expression (<25% of stained tumour cells) is also found. On the basis of these results, the authors conclude that sLex contributes to the malignant phenotype and tumour progression of canine mammary tumours and can be used as prognostic factor in canine malignant mammary tumours. The relationship between E-cadherin and sLex expression is also analysed. To my opinion, the study has some interesting results, but also important drawbacks.

Major Compulsory Revisions

1. Benign proliferative lesions have not been analysed. - The contribution of sLex to the malignant phenotype of canine mammary tumours cannot be assessed without the study of benign lesions for comparison.

2. Immunohistochemical findings are incompletely described. – There are 2 histological types of tumours, carcinoma in situ (n=2) and carcinoma in benign tumour (n=2) (page 12, second paragraph) which represent different stages in the progression of malignant tumours. Accordingly, they are excellent controls to study the contribution of sLex to the malignant phenotype of canine mammary tumours. However, no mention is made about the expression of sLex in those tumours. Neither is mentioned which cellular component of complex and mixed tumours expresses sLex.

3. Follow-up data are incompletely reported.- Concerning both lymph node and distant metastases, no mention is made about the time of detection, the duration of the disease free-period after surgery, cause and time of death, necropsy findings, etc. And concerning the follow-up period, differences in sLex status with regard to the disease free period after surgery? (i.e. 3 months vs 22 months) are not mentioned either.

4. There is not indication of the type of lesions found in dogs with more than one tumour. From the results shown, some dogs had MORE than one malignant tumour (35 dogs, 53 malignant tumours). However, the pathological findings do not mention the location of those malignant tumours (within the same mammary gland or in different glands?), nor the presence or absence of other type of lesions in the same and/or different glands of the same female dog with multiple tumours. The co-existence of different types of dysplasia as well as the benign tumours with malignant tumours is a very common finding in the mammary glands of dogs. Adjacent benign lesions are excellent internal controls of the immunohistochemical expression of sLex study.

5. The value of sLex as a prognostic tumour marker: The relationship between the IHC of sLex expression in canine mammary carcinomas and any of the well recognised prognostic indicators of these tumours has not been proved (page 16, first paragraph; and page 19, first paragraph of Conclusions). The disease free period after surgery, survival time, tumour size, and histological grade of malignancy, all accessible to the authors on view of the Materials and Methods of their work, have not been shown or analyzed (see the chapter by Prof. G. Rutteman and others in Withrow and MacEwen's Small Animal Clinical Oncology, Saunders, 2001).

6. The basis for the classification of the IHC expression of sLex: Indicate the basis for this classification as <25% and ? 25% of stained tumour cells.

7. Relationship between sLex and E-cadherin expression: Address how a change in one cell (i.e. loss of E-cadherin) can condition the overexpression of sLex in another cell.
8. Figures: In figure 1, both pictures A and B should have the same magnification in order to be comparable.
In Figure 2, immunostaining is very weak. Some marks on the pictures would help interpretation.

Minor Essential Revisions
-Page 6, last paragraph: 53 canine malignant mammary tumours is not a “large” series of tumours in the
dog unless the animals belong to a country/region where ovariectomy is routinely practiced. Was this the
case?
-Page 12, third paragraph: There is an important mistake, as it says that “lymph node metastases were
diagnosed in 50 tumours”(out of 53!)

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major
compulsory revisions

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

Quality of written English: Needs some language corrections before being published

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