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Histomorphological stratification of stromal types associated with canine mammary tumours#

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Abstract

Cancer associated stroma (CAS) consists mainly of a cellular fraction comprising fibroblasts, myofibroblasts, inflammatory cells, immune cells, endothelial cells, adipocytes and extracellular matrix (ECM). Recent reports have shown that the cancer stroma including the cellular fraction and ECM undergo considerable reprogramming during the process of tumourigenesis. Though there are some studies on CAS of human breast cancers (HBCs), similar studies are very much limited in canine mammary tumours. Hence, the present study was undertaken to classify the stromal types associated with malignant canine mammary tumours. The excisional biopsy samples from 50 numbers of canine mammary tumours presented to Kerala Veterinary and Animal Sciences University hospitals at Mannuthy and Kokkalai during the period from November 2019 to December 2021 formed the study material. Histopathological stratification of cancer stroma was done using qualitative evaluation based on the stromal characteristics suggested for HBCs. Accordingly the cancer associated stroma in different tumour samples were classified as fibrotic stroma, inflammatory stroma and mixed stroma. Fibrotic stroma was further classified as mature/sclerotic, intermediate and immature/desmoplastic types. The grade of tumours in relation to the type of stroma was also analysed and it was identified that with the exception of sclerotic stroma, all other stromal types could be observed in higher grades of canine mammary tumours. Desmoplastic and inflammatory stroma were predominantly seen associated with Grade II and Grade III tumours.

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Recent researches have revealed that the stromal compartment of breast tumours contains more prognostic information than the epithelial component. Cancer stroma including the cellular fraction and ECM undergo considerable reprogramming during the process of tumourigenesis. Based on the principal stromal tissue component, Ahn *et al.* (2012) classified tumour stroma into three types, such as collagen dominant, fibroblast dominant and lymphocyte dominant. They found that this classification could help in stratifying the prognostic outcome of breast cancer. Some other researchers proposed a system for stromal characterization according to the maturity of collagen in ECM (Zhai *et al.*, 2019). Qualitative assessment of ECM especially stromal collagen was used in this type of characterization and classified stromal types into three histological categories *viz*, mature, intermediate and immature. Unlike HBCs, the CAS in certain CMT cases were found to have a predominance of cellular elements, especially infiltrating inflammatory and immune cells. Similarly in many cases, both fibrous tissue components as well as cellular elements were seen in almost equal proportion. Hence, in the present study, a modified method incorporating the stromal features pertinent to CMTs was used and accordingly classified the stroma into fibrotic, inflammatory and mixed types. The histological grades of tumours were also analysed with respect to stromal types.

Materials and methods

The excisional biopsy samples from 50 numbers of suspected canine mammary tumour cases presented to Kerala Veterinary and Animal Sciences University hospitals at Mannuthy and Kokkalai during the period from January 2019 to March 2021 formed the study material. Representative samples of tumour tissues were collected in neutral buffered formalin and processed for histopathological examination. Tissue sections of 4-5 µm thickness were prepared, stained by routine Haematoxylin and Eosin method, mounted with DPX and examined.

The H and E sections were initially assessed under a 10 X objective lens for tumour stromal ratio (TSR), ratio of tumour area to stromal area. Principles of the TSR scoring in this study were applied according to the criteria described by Xu *et al*. (2019) in invasive human breast cancers. A 50% cut off point was selected (TSR=1) to group patients into stroma-low (TSR > 1, proportion of stroma less than 50 %) and stroma-high (TSR < 1 , proportion of stroma more than or equal to 50 %) categories. A TSR value near to one was grouped as moderate stroma. The tumours with TSR ≤ 1, *ie*, with high or moderate stroma only were considered for the study.

From the selected samples, histopathological stratification of cancer stroma was done depending on qualitative evaluation based on the stromal characteristics. Accordingly, the cancer associated stroma in different tumour samples were classified as fibrotic stroma, inflammatory stroma and mixed stroma. Fibrotic stroma was again subdivided into mature/ sclerotic stroma, intermediate stroma and immature/ desmoplastic stroma.

Histological malignancy grading (HMG) was done as described by Clemente *et al*. (2010) which was a modification of Nottingham method of grading human breast cancers. Scoring was based on three main criteria namely tubule formation, nuclear pleomorphism and mitotic count. Tubule formation in the section was assessed semi quantitatively and scores between one and three were given based on the extent of tubule formation. Variation in nuclear size, shape and nature of chromatin were evaluated, based on which another score between one and three was given. Mitotic activity was assessed at a magnification of 400x (high power field, HPF) in a minimum of 10 random fields and based on the mitotic count per 10 HPF, scores between one and three were assigned. After scoring in each of the above aspects, the individual scores were added and the grade was allocated (Table 1)

Results and discussion

Based on histopathology, the samples were initially classified into hyperplastic, benign and malignant. Of the 50 cases observed, five

Table 1. HMG grading of tumour

Tubule formation	Nuclear pleomorphism	Mitotic count	Total score	HMG
Score 1 - If >75% area had tubules	Score 1 - If nuclei were small with uniform chromatin and minimum anisokaryosis	Score 1 - up to nine mitoses per 10 HPF	3 to 5	HMG 1 (well differentiated)
Score 2 – If 10-75% area had tubules	Score 2 - If nuclei were larger with moderate anisokaryosis	Score 2 - 10 to 19 mitoses per 10 HPF	6/7	HMG II (moderately differentiated)
Score 3 - If <10% area had tubules	Score 3 – Vesicular nuclei with marked anisokaryosis and prominent nucleoli	Score 3 - 20 or more mitoses per 10 HPF	8/9	HMG III (Poorly differentiated)

The tumour grades were evaluated in relation to the stromal types.

were hyperplastic samples, six were benign tumours and the rest 39 were malignant. The observation was similar to that of Christy *et al.* (2022), who reported that majority of CMTs were malignant in nature. The TSR evaluation of the malignant tumours identified 13 tumours to be rich in stroma (Fig. 1), 17 were with moderate of stroma (Fig. 2) and nine were stroma poor tumours (Fig. 3). Among the above, total 30 samples of tumours having either rich or moderate stroma formed the cohort for study. According to Vangangelt *et al*. (2018), TSR was a valuable prognostic predictor and an increase in stroma was a feature in tumours that behaved aggressively. The present study identified that not only the quantity of stroma, the type and maturity of the stroma were also factors that influenced the tumour behaviour.

The selected tumour sections were further evaluated for the type of stroma and it was identified that 16 tumours had fibrotic stroma. As per the observations of the present study, fibrous tissue elements constituted the major component of stroma in CMTs. One of the main hallmarks in HBCs was an increase in mammogram density attributed to increased fibroplasia and subsequent collagen deposition (Byrne *et al*. (2000) and Li *et al*. (2005). Hence, comparing the observations in HBCs and CMTs, it could be assumed that fibroplasia/ desmoplasia is protumourigenic in both the species.

Among 16 cases with fibrotic stroma, five tumours had mature sclerotic stroma composed of fine and elongated mature collagen fibres with a few numbers of mature fibrocytes arranged in multiple layers (Fig. 4) which accounted for 16.67 per cent of the total. Immature desmoplastic stroma (Fig. 5) consisting of immature collagen in haphazard patterns with abundance of fibroblasts and myxoid matrix was noticed in four cases (13.33 per cent). Intermediate type (Fig. 6) of fibrotic stroma was observed in seven cases (23.33 per cent). Ueno *et al.* (2021) identified that myxoid stroma was an indicator of desmoplastic microenvironment and desmoplastic stroma at tumour front facilitated increased tumour invasion. According to Gangadhara *et al*. (2012), the CAS enriched with large numbers of activated fibroblasts and myoepithelial cells was an inherent property of certain tumours like breast cancer.

Inflammatory stroma was characterised by the presence of different types of infiltrating inflammatory cells along with vascular events like neovascularisation, congestion and haemorrhage in many of the cases (Fig.7). This type of stroma was typically found in five cases (16.67 per cent). The rest nine tumours examined under study (30 per cent) displayed a mixed stromal pattern characterised by the presence of both fibrous tissue elements and inflammatory cells (Fig. 8).

Further the observations made on comparing the stromal types with grades of tumour are presented in Table 2

The observations revealed that mature/sclerotic stroma was seen associated with Grade I tumour while this type of stroma

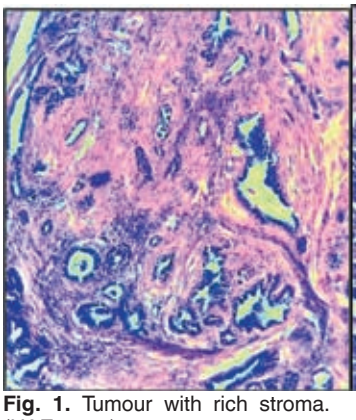

Fig. 1. Tumour with rich stroma. (H&E x 100)

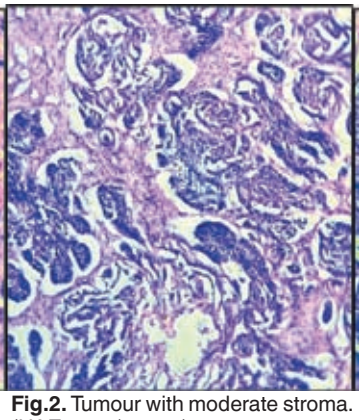

Fig.2. Tumour with moderate stroma. (H&E x 100) x 100)

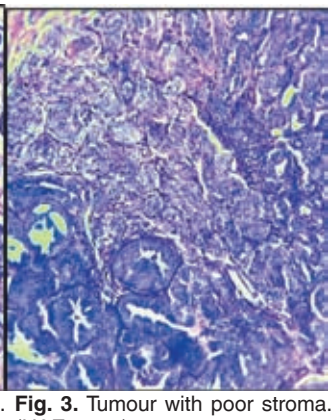

Fig. 3. Tumour with poor stroma. (H&E x 100)

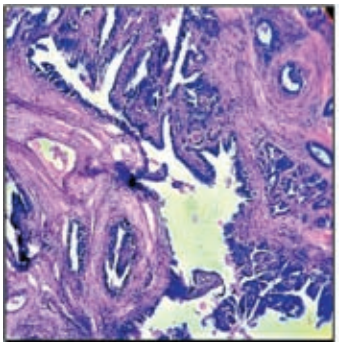

Fig. 4. Sclerotic stroma with mature collagen and less fibroblasts H & E x 100

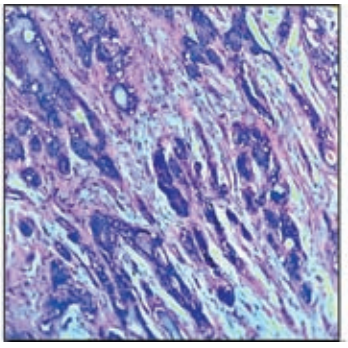

Fig. 5. Desmoplastic stroma with immature collagen and myxoid matrix H & E x 100

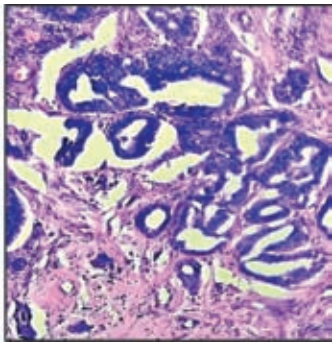

Fig. 6. Intermediate stroma with collagen of intermediate maturity H & E x 100

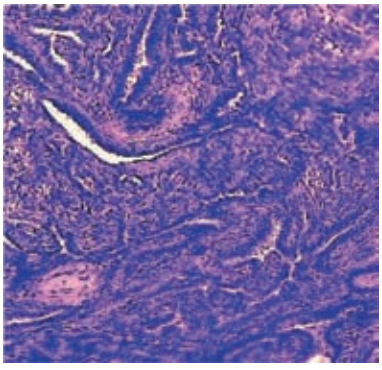

Fig.7. Inflammatory stroma H & E x 100

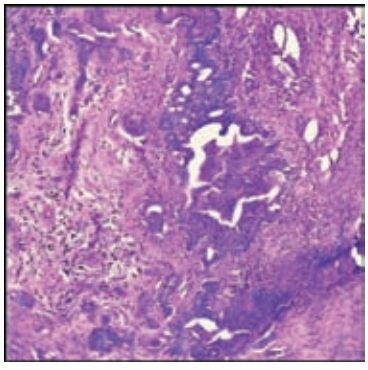

Fig.8. Mixed stroma H & E x 100

was hardly a feature in high grade tumours. Desmoplastic and inflammatory stroma were seen associated with high grade tumours, either Grade II or III and was never a feature of Grade I tumour. The observations were in accordance with that of other researchers in HBCs. A study by Tejeswini *et al*. (2019) also demonstrated that desmoplastic type of stroma in HBC was mostly a feature of Grade III tumours and was often indicative of adverse prognosis and axillary lymph node involvement. The immature stroma with abundant numbers of activated fibroblasts has the potential to secrete several mediators including MMPs, TGFs, SDF and several other cytokines which might contribute to the increase in grade and aggressiveness of these tumours, while the sclerotic stroma which does not have appreciable numbers of active fibroblasts would remain inert. Similarly, the inflammatory stroma associated with CMTs could also trigger the

Table 2. Stromal types with respect to histological grades of tumour

Stromal type	No. of cases			
	Histological Grade I	Histological Grade II	Histological Grade III	Total
Mature/sclerotic stroma	5	0	0	**5**
Intermediate stroma	2	4	1	**7**
Desmoplastic/Immature stroma	0	1	3	**4**
Inflammatory stroma		2	3	**5**
Mixed stroma	3	5	1	**9**
Total	**10**	**12**	**8**	**30**

release of several inflammatory cytokines like interleukins, TNF-α *etc*., thereby increasing the malignancy potential of tumours (Liubomirski *et al.*, 2019).

Conclusion

From the inferences of this study, it can be concluded that stroma associated with cancers could contain several prognostic cues which are of immense application in formulating treatment protocols in CMT cases. The findings of the study thus support the recommendation of Xu *et al.* (2019) that the existing system of Tumour-Node-Metastasis staging should be revised by incorporating the TSR component.

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References

Ahn, S., Cho, J., Sung, J., Lee, J. E., Nam, S. J., Kim, K. M. and Cho, E. Y. 2012. The prognostic significance of tumor-associated stroma in invasive breast carcinoma. *Tumour Biol*. **33**:1573-80.

Byrne, C., Colditz, G. A, Willett, W. C, Speizer, F. E., Pollak, M. and Hankinson, S. E. 2000. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. *Cancer Res*. **60**(14): 3744-3748.

Christy, M. J., Sajitha, I. S., Devi, S. S., Dhanush, K. B. and Nair, S. N. 2022. Study on histopathological subtypes and grading of canine mammary tumours. *J. Vet. Anim. Sci*. **53**: 85-88.

Clemente, M., Perez-Alenza, M. D., Illera, J. C. and Pena, L. 2010. Histological, immunohistological, and ultrastructural description of vasculogenic mimicry in canine mammary cancer. *Vet. Path*. **47**: 265-274.

Gangadhara, S., Barrett-Lee, P., Nicholson, R. I. and Hiscox, S. 2012. Pro-metastatic tumour–stroma interactions in breast cancer. *Future oncol.* **8***:* 1427-1442.

Li, T., Sun, L., Miller, N., Nicklee, T., Woo, J., Hulse-Smith, L., Tsao, M. S, Khokha, R., Martin, L. and Boyd, N. 2005. The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. *Cancer Epidemiol. Biomarkers Prev*. **14**(2): 343-349.

Liubomirski, Y., Lerrer, S., Meshel, T., Rubinstein-Achiasaf, L., Morein, D., Wiemann, S., Korner, C. and Ben-Baruch, A. 2019. Tumor-stroma-inflammation networks promote pro-metastatic chemokines and aggressiveness characteristics in triple-negative breast cancer. *Front. Immunol*. 10:757-785.

Tejeswini, V., Chaitra, B., Premalatha, P., Renuka, I.V., Vaishnavi, R. and manasa, B. 2019. Tumor desmoplastic stroma - Its utility as a prognostic marker for breast

carcinoma. *Annals pathol. lab. Med*. 6 (10):496-501.

Ueno, H., Kajiwara, Y., Ajioka, Y., Sugai, T., Sekine, S., Ishiguro, M. and Kanemitsu, Y. 2021. Histopathological atlas of desmoplastic reaction characterization in colorectal cancer. *Jpn. J. Clin. Oncol*. **51:** 1004-1012.

Vangangelt, K. M. H., Van Pelt, G. W., Engels, C. C., Putter, H., Liefers, G. J. , Smit, V. T. H. B. M, Tollenaar, R. A. E. M., Kuppen, P. J. K., Mesker, W. E. 2018. Prognostic value of tumor-stroma ratio combined with the immune status of tumours in invasive breast carcinoma. *Breast Cancer Res. Treat*. **168:** 601-612.

Xu, S*.,* Xu, H*.,* Wang, W., Li, *S.*, Li, *H.*, Li, T., Zhang, *W.*, Yu, X., Liu, L.2019. The role of collagen in cancer: from bench to bedside. *J. Transl. Med.* **17**: 309 – 331.

Zhai, Q., Fan, J., Lin, Q., Liu, X., Li, J. and Hong R. 2019. Tumor stromal type is associated with stromal PD-L1 expression and predicts outcomes in breast cancer. *PLoS One* [on line]. **14**(10)**:** e0223325