Relationship between PCNA Proliferating Marker and Angiogenesis in Bitch Mammary Cancer

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SUMMARY

The goal of the study was to evaluate the prognosis significance of intratumor angiogenesis that was compared with PCNA proliferating marker, histology grade, mitotic index, tumor size, and histology type. The study had been realized using different histology types of bitch mammary tumors according to WHO classifications. There were evaluated some potential angiogenesis markers, such as intratumor microvessel density, total microvascular area and perimeter according to microscopic tumor area, and average vessel area and perimeter in different benign and malign mammary tumors.

The study was realized in 8 bitch mammary tumors represented by two benign lesions (simple adenoma and fibroadenoma) and 6 carcinomas (grade I, II and III tumors). From all tumors had been harvested fragments represented by 5 mm slices that were fixed in 10% buffered formalin and proceeded by paraffin technique. The slides were stained by usual methods (HE and TM). To realize immunohistochemistry reaction paraffin embedded slices were attached onto silanized glass slides (Dako). PCNA proliferating marker (clone PC10, izotype IgG2a kappa - Dako) was evaluated by counting by 3 evaluators 8-10 high power magnified fields (400x), about 1000 cells (immunomarked nuclei). There was established average PCNA percentage for each tumor type. Intratumor angiogenesis had been established using CD31 marker (clone JC70A, izotype IgG1 kappa - Dako) and evaluated using semiautomatic image analysis method. Both immunohistochemical reactions utilized LSAB technique.

To evaluate new methods necessary to establish bitch mammary tumors malignancy, which is important for mammary cancer prognosis, we utilized several malignancy markers. Also, because intratumor angiogenesis quantification provided contradictory results obtained by many authors, this parameter was related and compared with classic and reliable markers (PCNA antigen, mitotic index, histology grade, tumor size, and tumors’ characteristics).

PCNA marker was increased in poorly differentiated tumors (cases 2, 3, 5, and 8 – grade II and III mammary tumors) comparatively with differentiated one (cases 6, 7 – grade I cancers) and benign tumors (cases 1 and 4).

Intratumor microvessel density (IMD) was closely related with tumors malignancy but our dates weren’t statistically significant. Also, this parameter should be associated with reliable parameters (Ki-67 and tumor histology grade). Average vascular area and perimeter is reduced in grade II and III carcinomas comparatively with differentiated one due to increased angiogenesis.

Concluding, our dates indicate a correlation of PCNA malignancy marker with IMD according to microscopic image area, but there is not a rule regarding IMD reliability. The others studied parameters (vascular area and perimeters) hadn’t had prognosis significance.