

6. SUMMARY

DETECTION AND POSSIBLE SIGNIFICANCE OF THROMBUS FORMATION AND EXTRAVASCULAR FIBRIN DEPOSITS IN MAMMARY TUMOURS IN THE BITCH

Many types of neoplasia interact with the host's blood coagulation and fibrinolysis system in various ways. Extravascular fibrin deposits in tumours are thought to facilitate tumour growth, while intratumoural thrombi can theoretically lead to a suppression in tumour growth. As mammary tumours are the most common neoplastic disease in the bitch, the aim of this study is to test these tumours for the presence of extravascular fibrin deposits and thrombus formation and to determine the possible significance of the findings.

As current literature lacks reports about the specific detection of extravascular fibrin deposits and thrombi in formalin fixed, paraffin-wax embedded canine tissue, an appropriate method has to be found.

The histological methods for the detection of fibrin described by LADEWIG (1938), LENDRUM *et al.* (1962), modified by PUSEY und EDWARDS (1978), SHOOBRIDGE (1983) and GARVEY *et al.* (1987) are tested, as well as the immunohistochemical detection of fibrin, using the alkaline phosphatase-anti alkaline phosphatase (APAAP) method (CORDELL 1984, modified by WALTER 1999). For this, a monoclonal antibody raised against a highly conserved epitope of human fibrin (antibody no. 350; American diagnostica, Greenwich, CT, USA) is used. Various dilutions for the primary antibody between 1 in 20 and 1 in 200 and three methods for the demasking of the antigene (predigestion in protease, preheating in an autoclave or a microwave oven) are tested on canine tissue sections.

Best results are obtained by using the APAAP-method, with an optimum dilution for the primary antibody of 1 in 50, used on canine tissue sections preheated in citrate citrate buffer i a microwave oven at 600 W for 2x5 min. This method leads to a specific labelling of even small amounts of fibrin. Sometimes it cannot be determined if fibrin lies intra- or extravascular. In these cases, the APAAP double labelling method is performed on a parallel section, for the simultaneous labelling of vessel endothelium and fibrin, the former using an antibody raised against the von Willebrand factor. Fast Blue and Fast Red, in this order, are used as chromogenes and Weigert's Ferrohaematoxylin as counterstain. After mounting, the coverplates are framed with nail polish to reduce air invasion under the coverplates.

Histological methods, on the contrary, are unsuitable for the detection of fibrin, particularly if trying to detect small quantities. There is a risk to mistake other structures, stained in a similar colour, for fibrin. Nevertheless, the SHOOBRIDGE (1983) method can be used to determine the age of fibrin in cases where its position in the specimen is known. Optimum results are achieved by using saturated aqueous picric acid at room temperature for 5 minutes as primer instead of picro-formol-primer at 50° centigrade for 30 minutes.

Tissue specimens from 84 dogs with either one or more total or partial mastectomies were available for this study. A total of 269 tumours of the mammary gland of 79 of these animals are studied, as well as tumours of other or unclear origin, normal or non-neoplastically altered mammary tissue, or the inguinal lymphnodes of some animals. On average, the dogs are supervised for two years after their first operation. The tissues are routinely stained with H.E. and the immunohistochemical fibrin detection is performed as described above. A parallel section of each fibrin positive tumour is double labelled for simultaneous vessel detection. In cases where a postoperative thrombogenesis seems possible, a second parallel section is stained with the modified SHOUBRIDGE method.

The majority of tumours (60.97%) do not have extravascular fibrin deposits and thrombi. Extravascular fibrin deposits could be detected in 31.60% of the tumours, one or more thrombi in 36.43%. In normal or non-neoplastically altered mammary tissue of this study, extravascular fibrin is a very rare finding, and no thrombi can be detected. The results of statistical tests indicate a correlation of the following characteristics of the tumours studied:

- Increasing amount of extravascular fibrin deposits and increasing size of tumour, presence of ulceration, presence of abundant purulent inflammation, increasing degree of necrosis, increasing degree of bleeding, presence of / suspected angiosarcoma.
Extravascular fibrin deposits do not occur in benign tumours. Malign tumours without extravascular fibrin deposits are often of a relatively low degree of malignancy, as indicated by the tumour's sum of points.
Tumours with low vessel density seldom have extravascular fibrin deposits.
Extravascular fibrin deposits occur often in tumours from mammary chains, the ipsilateral inguinal lymphnode of which showed metastases and in tumours from dogs with fatal outcome of the disease.
On average, tumours without extravascular fibrin deposits grew in animals of comparatively young age.
Spayed bitches show extravascular fibrin deposits in tumours more often than intact bitches.
- Increasing number of thrombi and increasing size of tumour, presence of ulceration, presence of abundant purulent inflammation, increasing degree of necrosis, increasing degree of bleeding, presence of or suspected angiosarcoma.
Thrombus formation in benign tumours is a very rare finding. Malign tumours without thrombi are often of a relatively low degree of malignancy, as indicated by the tumour's sum of points.
Extravascular fibrin deposits occur often in tumours from mammary chains, the ipsilateral inguinal lymphnode of which showed metastases and in tumours from dogs with fatal outcome of the disease.
Thrombi occur often in tumours from mammary chains, the ipsilateral inguinal lymphnode of which showed metastases.
On average, tumours without thrombi grew in animals of comparatively young age.
Spayed bitches show intratumoural thrombus formation more often than intact bitches.
- Necrosis-free tumours often are of small size and low vessel density.

No correlation could be proven with the following characteristics of the tumours studied:

- Extravascular fibrin deposits and location of tumour, count of operation (first respectively only or additional operation), intratumoural macrophages with fibrin positive labelling.
- Thrombi and location of tumour, count of operation (first respectively only or additional operation), intratumoural macrophages with fibrin positive labelling, vessel density, outcome of the disease.

Thus, in this study, there is a correlation of characteristics of the tumour that indicate an increase in malignancy with an increase in the number of extravascular fibrin deposits or thrombi. However, because of the wide range of results and the comparatively impractical detection method, thrombi or extravascular fibrin deposits are neither suitable independent predicting factors nor useful additional aids for tumour classification.

Extravascular fibrin deposits in canine mammary tumours probably are nothing more than necrosis- or bleeding-associated epiphenomena. The amount of extravascular fibrin seems to be too small to exert an influence on tumour growth.

Thrombi on the other hand probably do account for the development of necrosis in some of the tumours studied, as a high degree of necrosis often occurs in tumours with many thrombi in the tumour and not in tumours of low vessel density. Possible reasons for intravascular clotting are vessel abnormalities as well as clotting influencing substances that are generated by tumour cells. However, thrombi may indirectly promote tumour growth and invasion. The question is, do mammary tumours in dogs influence the tumour's, or the host's fate, in a positive or in a negative way. Further studies on the causes and effects of intratumoural thrombus formation should be pursued.