

# TOPOISOMERASE II ALPHA IMMUNOEXPRESSION AS A POTENTIAL PREDICTOR OF ANTRACYCLINE CHEMOTHERAPY IN CATS WITH INJECTION-SITE SARCOMA

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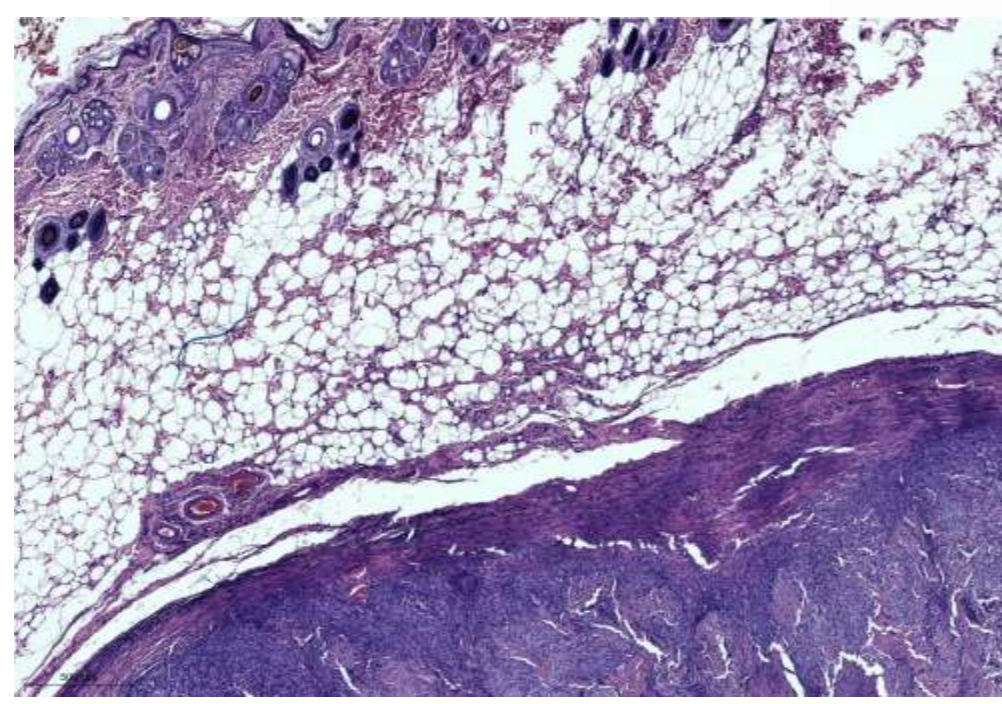


Fig. 1. FISS. A subcutaneous, multilobulated tumour infiltrating adjacent tissues along septa of the panniculus. HE. Bar = 500 µm

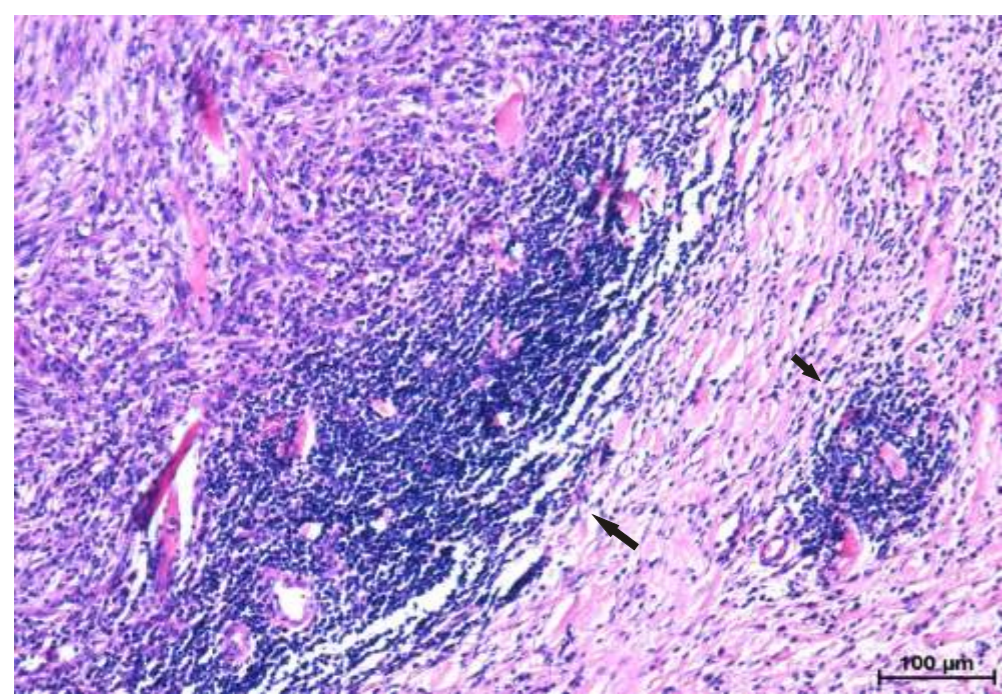


Fig. 2. FISS. Perivascular (small arrow) and peripheral (long arrow) lymphocytic infiltrates. HE. Bar = 100 µm

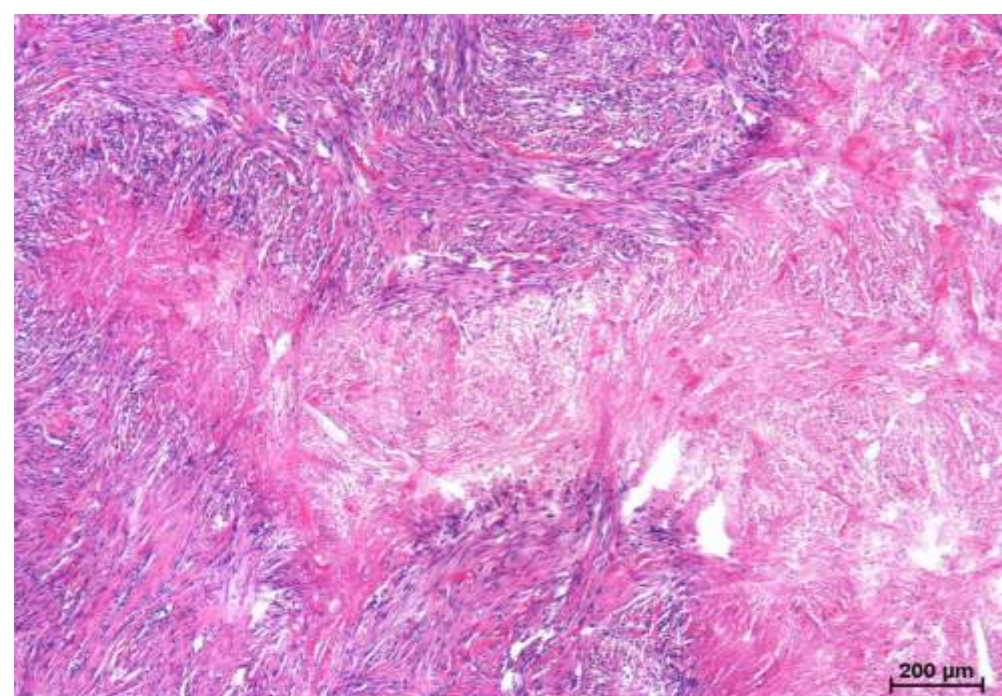


Fig. 3. FISS. An extensive area of central necrosis. HE. Bar = 200 µm.

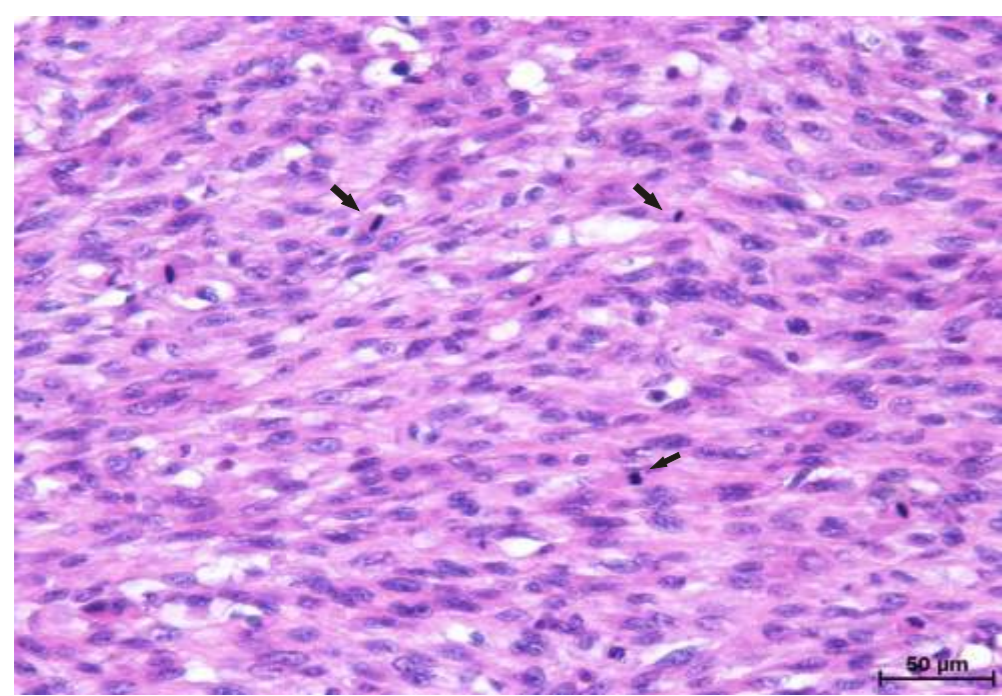


Fig. 4. FISS. Bundles of highly atypical spindle cells with large nuclei and frequent mitotic figures (arrows). HE. Bar = 50 µm

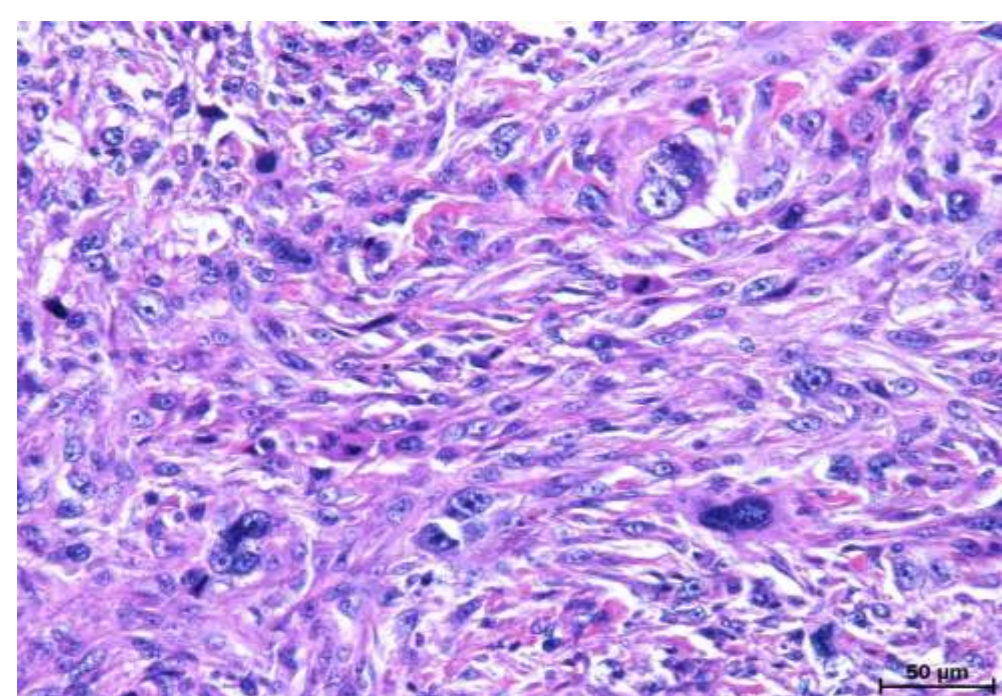


Fig. 5. FISS. Highly pleomorphic population of spindle cells with marked anisokaryosis. Scattered, large multinucleated cells are visible. HE. Bar = 50 µm.

**Introduction:** Feline injection-site sarcomas (FISSs) are mesenchymal tumours that develop in cats following vaccination or injection with various medications. FISSs appear to be moderately chemosensitive, and the use of adjuvant or neoadjuvant anthracycline chemotherapy has been proposed to complement surgery. Unfortunately, there are no specific indications for the use of anthracyclines in individual patients. Topoisomerase II (TOPII) is a key enzyme in DNA replication and a molecular target for TOPII inhibitors, including the most commonly used anthracyclines such as doxorubicin and epirubicin. This study aimed to evaluate the expression of TOPII in FISSs, considering the suitability of this assessment for the selection of patients for the treatment with TOPII inhibitors.

**Materials and Methods:** Samples of formalin-fixed paraffin-embedded FISSs were immunohistochemically labeled with anti-TOPII antibody. The number of positive cells and the intensity of the reaction were taken into account in order to assess TOPII immunorexpression (Tab.1)

**Table 1. Scoring system by Remmele and Stegner (IRS, Immunoreactive Score) taking into account the percentage of cells (PP) and the intensity of the staining (IS).** (Remmele, W., Stegner, H.E., 1987. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 8(3):138-40.

Percentage of positive cells	x Intensity of staining	= IRS (0-12)	IRS - classification
0 = no positive cells	0 = no colour reaction	0 - 1 = negative	0 = negative
1 = < 10% of positive cells	1 = mild reaction	2 - 3 = mild	1 = positive - weak expression
2 = 10 - 50% positive cells	2 = moderate reaction	4 - 8 = moderate	2 = positive - moderate expression
3 = 51 - 80% positive cells	3 = intense reaction	9 - 12 = strong	3 = positive - strong expression
4 = > 80% positive cells			

**Results:** Nuclear, granular TOPII immunorexpression was evident in all cases, although differences in the number of positive cells and the intensity of the reaction were shown (Fig. 6-10, tab. 2). Moreover, differences were observed between individual cases. TOPII expression tended to increase with increasing histological malignancy grade. These were independent of the anatomical location of tumours.

**Conclusions:** It can be assumed that FISSs with elevated level of TOPII immunorexpression might respond better to anthracycline chemotherapy than tumours with low immunorexpression of this enzyme. Therefore, it can be hypothesized that the assessment of TOPII immunoreactivity in FISSs may represent a predicting factor for adjuvant or neoadjuvant treatment with drugs interfering with TOPII function, including doxorubicin and epirubicin.

**Table 2. Clinical, histological and immunohistochemical characterisation of 18 cases of feline injection-site sarcoma included in the study.**

Breed	Sex	Age (years)	Location	Size (mm)	Histopathology	Grading	TOPII? expression(IRS score)/classification
Mixed	M	11	Right thigh	15	Fibrosarcoma	II	4 / 2
Mixed	M	10	Dorsal neck	35	Fibrosarcoma	III	6 / 2
Mixed	F	5	Back	12	Fibrosarcoma	I	2 / 1
Ragdoll	M	14	Interscapula	44	Chondrosarcoma	II	4 / 2
Mixed	F	10	Back	24	Fibrosarcoma	III	6 / 2
Mixed	F	9	Back	20	Fibrosarcoma	II	4 / 2
Persian	M	3	Interscapula	16	Fibrosarcoma	II	6 / 2
Mixed	F	13	Interscapula	26	Chondrosarcoma	II	4 / 2
Mixed	M	12	Back	32	Fibrosarcoma	III	9 / 3
Mixed	M	5	Back	18	Fibrosarcoma	I	4 / 2
Persian	F	4	Dorsal neck	28	Fibrosarcoma	II	6 / 2
Mixed	M	15	Right thigh	14	Fibrosarcoma	I	3 / 1
Mixed	M	4	Right scapula	14	Undifferentiated sarcoma	III	6 / 2
Mixed	F	5	Interscapula	25	Fibrosarcoma	II	9 / 3
Mixed	F	6	Interscapula	37	Fibrosarcoma	III	6 / 2
Devon Rex	M	7	Dorsal neck	18	Undifferentiated sarcoma	III	9 / 3
Mixed	M	10	Left scapula	30	Fibrosarcoma	II	6 / 2
Mixed	F	11	Back	43	Fibrosarcoma	III	9 / 3

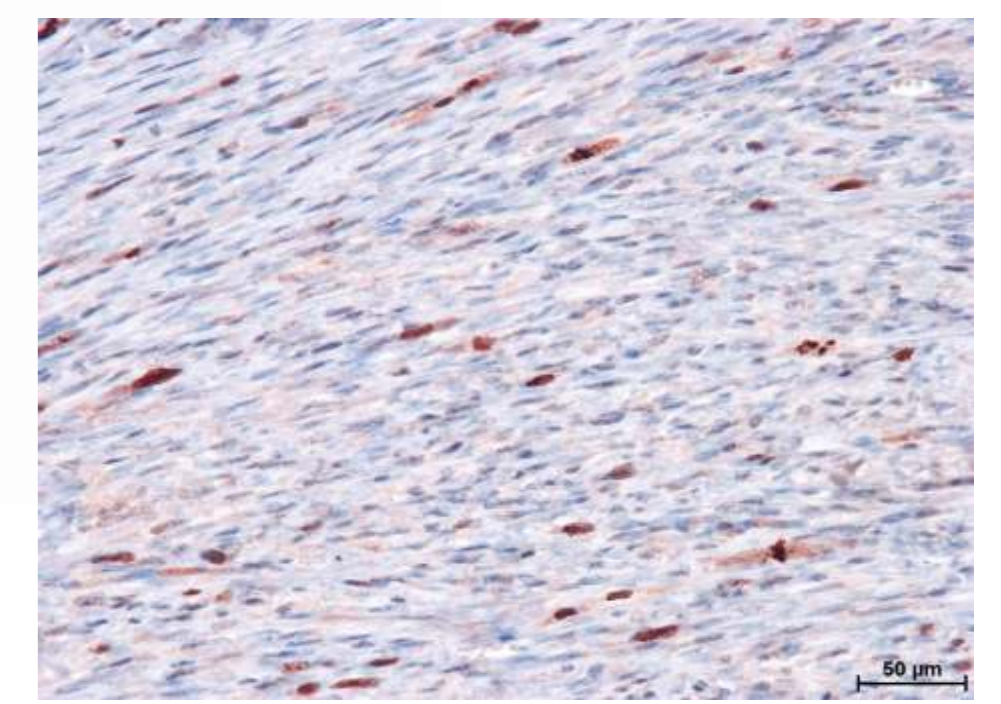


Fig. 6. FISS. A small number of cells showing a moderate to intense immunohistochemical reaction for TOPII. IHC staining, Mayer's haematoxylin counterstain. Bar = 50 µm

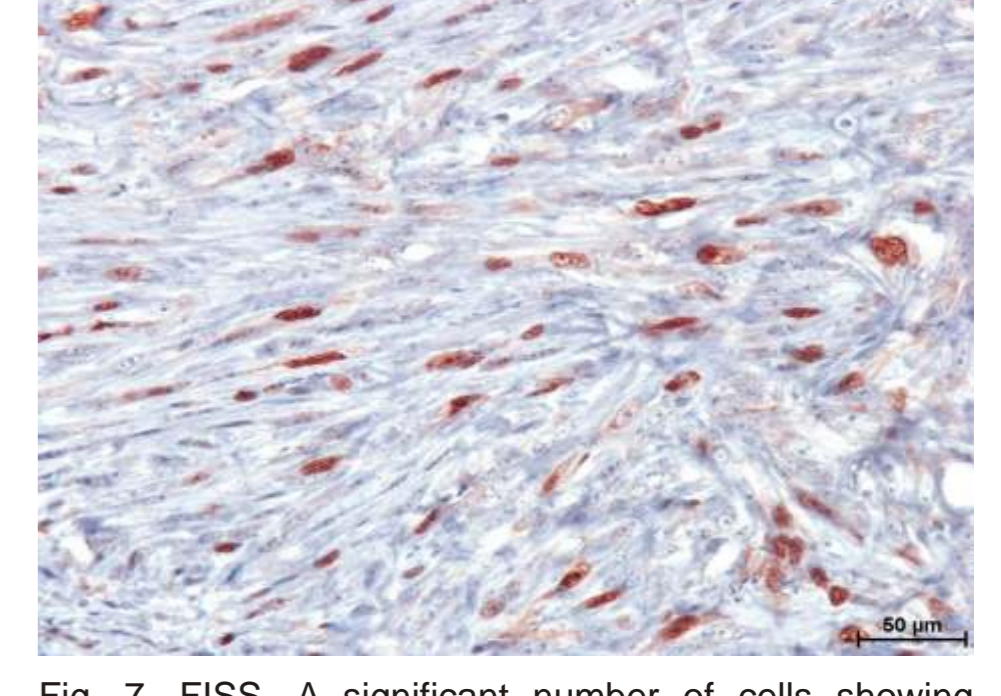


Fig. 7. FISS. A significant number of cells showing a moderate immunohistochemical reaction for TOPII. IHC staining, Mayer's haematoxylin counterstain. Bar = 50 µm

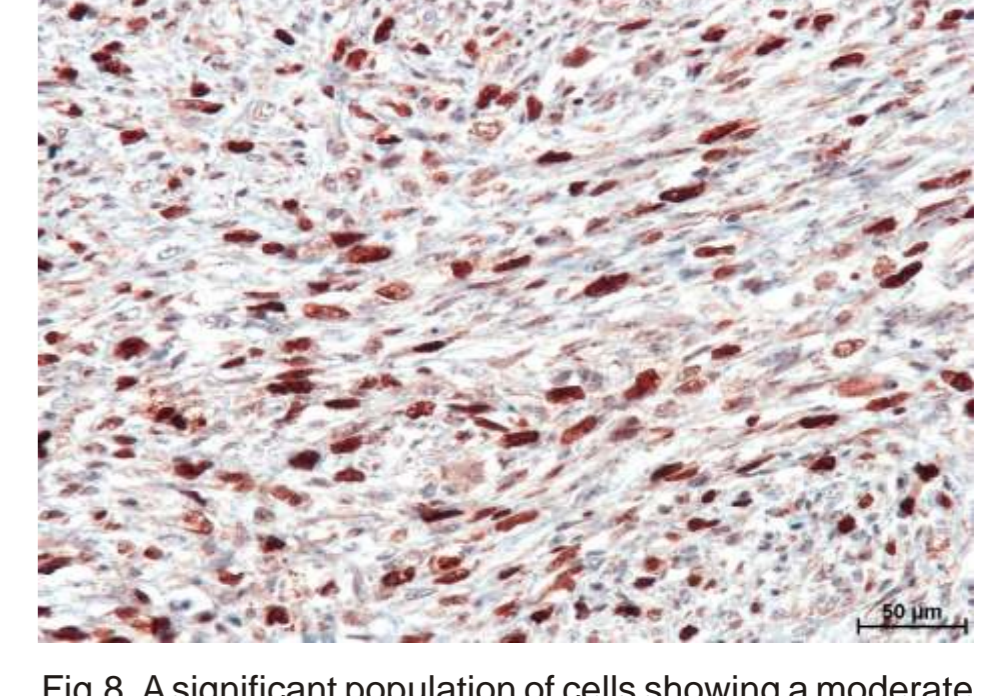


Fig. 8. A significant population of cells showing a moderate to intense immunohistochemical reaction for TOPII. IHC staining, Mayer's haematoxylin counterstain. Bar = 50 µm

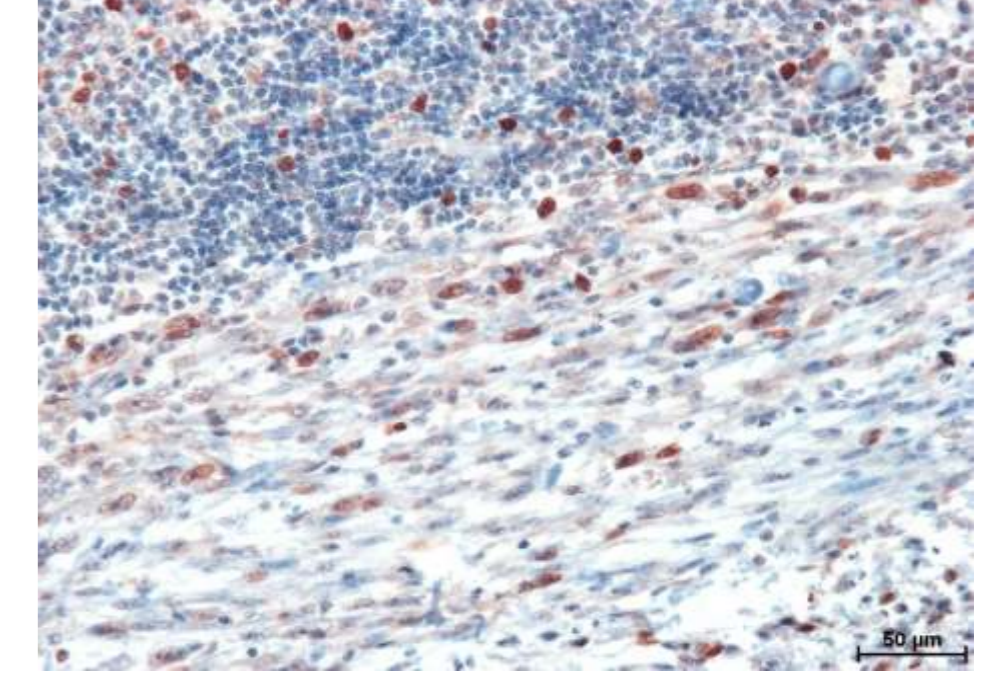


Fig. 9. Positive immunohistochemical reaction for TOPII in spindle-shaped nuclei of tumour cells and nuclei of some lymphocytes in the periphery of the tumour. IHC staining, Mayer's haematoxylin counterstain. Bar = 50 µm

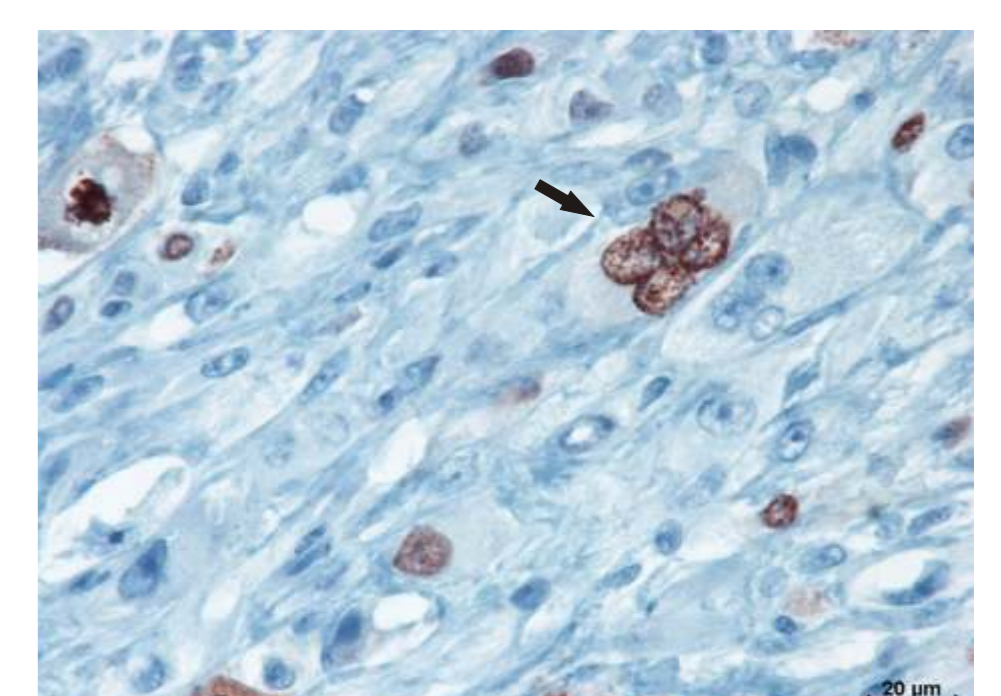


Fig. 10. A multinucleated giant cell with intense nuclear reaction for TOPII (arrow). IHC staining, Mayer's haematoxylin counterstain. Bar = 20 µm

## References:

1. Barber LG, Sorenmo KU, Cronin KL, Shofer FS. Combined doxorubicin and cyclophosphamide chemotherapy for nonresectable feline fibrosarcoma. J Am Anim Hosp Assoc. 2000;36(5):416-421; 2. Bray J, Polton G. Neoadjuvant and adjuvant chemotherapy combined with anatomical resection of feline injection-site sarcoma: results in 21 cats. Vet Comp Oncol. 2016;14(2):147-60; 3. Bregazzi VS, LaRue SM, McNeil E, Macy DW, Dernel WS, Powers BE, et al. Treatment with a combination of doxorubicin, surgery, and radiation versus surgery and radiation alone for cats with vaccine-associated sarcomas: 25 cases (1995-2000). J Am Vet Med Assoc 2001; 218: 547-550; 4. Martano M, Morello E, Ughetto M, Iussich S, Petterino C, Cascio P, et al. Surgery alone versus surgery and doxorubicin for the treatment of feline injection-site sarcomas: a report on 69 cases. Vet J 2005; 170: 84-90; 5. Poirier VJ, Thamm DH, Kurzman ID, et al. Liposome-encapsulated doxorubicin (Doxil) and doxorubicin in the treatment of vaccine-associated sarcoma in cats. J Vet Intern Med. 2002;16(6):726-731. 6. Torrigiani F, Romanelli G, Roccabianca P, Treggiari E. Neoadjuvant and adjuvant doxorubicin chemotherapy in a case of feline soft tissue sarcoma. JFMS Open Rep. 2019;1;5(2):2055116919857870.