# Immunohistochemistry of nodular dermatofibrosis in a German Shepherd – a case report

Martin Levkut[1], Mária Levkutová[2], Michaela Čechová[1]

University of Veterinary Medicine and Pharmacy, [1]Department of Morphological Disciplines, [2]Department of Epizootology, Parasitology and Protection of One Health, Košice, Slovakia

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**Abstract**

This case report describes nodular dermatofibrosis in an 11-year-old female dog of the German Shepherd breed. Previously, at the age of 6 years (initial stage), a sample from a tumorous nodule on her back was removed. Histological examination of the sample from this period showed hyperplasia of cells with a lobular structure. Immunohistochemistry staining demonstrated focal positivity to pancytokeratin. In the terminal stage (at 11 years of age), clinical examination revealed apathy, uncoordinated movement of the hind limbs, obstipation, anorexia and occasional vomiting with progressive weight loss. Skin inspection found multiple skin ulcerating tumorous lesions localized in the sacral region of the back and intercostally, partly fluctuating around the size 4 cm in diameter. Necropsy revealed an intraabdominal tumour localized among intestinal loops. Nodular lesions were found also in the lung parenchyma, on the dorsal surface of the epiglottis, in the myocardium, the cortex and the medulla of the kidneys, the adrenal gland, and in the intestinal wall. Histological analysis showed systemic production of fibrous nodules and formation of fibrous tissue with atrophy of parenchyma tissue. However, no connection between dermatofibrosis and adenocarcinoma of the kidney was found in this case, which was supplemented with pancytokeratin antibody. CD3 + lymphocytes were observed mainly in the zone of cell proliferation and in the interface towards the fibrous layer. Macrophages were seen mainly in the transitional zone between cellular and fibrous part. This indicated participation of monitored immunocompetent cells in fibroblast degradation.

*Cutaneous nodules, CD3+lymphocytes, macrophages, pancytokeratin, dog*

Nodular dermatofibrosis is a rare hereditary disease in German Shepherd dogs often associated with adenocarcinoma of kidney cells, adenoma, kidney cysts and simultaneously found fibrous cutaneous and subcutaneous nodule (Meuten and Meuten 2017). This syndrome is caused by mutations of the folliculin gene (Lingaas et al. 2003). Inactivation of this tumour suppressor gene is one of the critical steps in the course of this disease (Bønsdorff et al. 2009). This syndrome has also been sporadically found in the Boxer (White et al. 1998) and the Golden Retriever (Marks et al. 1993) breeds.

Clinical signs vary greatly among dogs depending on age and stage of disease at the time of examination (Moe and Lium 1997; Thompson et al. 2019). Lesions generally include numerous cutaneous and subcutaneous nodules, distension of the abdomen, anorexia, fatigue, progressive weight loss, polydipsia, vomiting, and obstipation or diarrhoea (Lium and Moe 1985; Thompson et al. 2019).

Lesions found during necropsy demonstrate firm, well-defined cutaneous and subcutaneous nodules, which may extend throughout the body with predisposition to the limbs, head and back (Suter et al. 1983). Lesions in the kidney are bilateral, multiple, and cystic (Meuten and Meuten 2017; Thompson et al. 2019). Histologically, skin lesions show dermatofibrosis and cystadenocarcinoma in kidneys (Lium and Moe 1985; Souza and Borges 2018).

This case report describes the clinical examination, histological, and immunohistochemical evaluation of nodular dermatofibrosis in a German Shepherd.

**Address for correspondence:**
Mária Levkutová
Department of Epizootology, Parasitology and Protection of One Health
University of Veterinary Medicine and Pharmacy,
Košice, Slovakia

Phone: +421 915 984 652
E-mail: maria.levkutova@uvlf.sk
http://actavet.vfu.cz/

## Case presentation

Multiple cutaneous lesions were found on an 11-year-old female dog of the German Shepherd breed. Retrospectively, dermatofibrosis on the dog's back was examined five years previously and that lesion was surgically removed. The patient then remained in a good condition without clinical signs for a time. Recently, the patient was observed to be suffering from apathy, malcoordination of the hind limbs, constipation, anorexia, occasional vomiting, and progressive weight loss. Upon inspection of the skin, multiple ulcerous, tumorous lesions were localized in the sacral region of the back and intercostally, partly fluctuating around the size 4 cm in diameter.

After receiving an unfavourable prognosis, the owner declined treatment for the dog and opted for euthanasia. The animal was premedicated intramuscularly with xylazine and ketamine (Bioveta, a.s., Ivanovice na Hané, Czech Republic) at a dose of 2 mg/kg and 10 mg/kg, respectively. After induction of anaesthesia, embutramide was administered intravenously at a dose of 400 mg (T-61) (Intervet International B.V., Boxmeer, Netherlands). Pathological morphological changes in the organs were determined post mortem. An intraabdominal tumour located between the proximal internal loops was found during diagnostic autopsy. The formation was multinodular, vascularized, of a size of 18 cm in diameter and weighing 1.35 kg (Plate X, Fig. 1). Cutting the surface of the tumour revealed cystic cavities filled with straw-yellow secretion and numerous haemorrhages. Nodular structures were also observed in the pulmonary parenchyma of the cranial and caudal lobes as well as in the bronchial lymph node, the dorsal area of the epiglottis, and the myocardium in the apex cordis area. Multiple nodules of homogeneous structure of different sizes were found in the cortex and medulla of the kidneys (Plate X, Fig. 2). Nodules were also observed in the adrenal glands and the wall of the intestinal loop.

Samples taken for histopathological and immunohistochemical examination were fixed in 10% neutral buffered formalin and processed using the standard paraffin technique. For histological evaluation haematoxylin-eosin and Masson trichrome were used. The antibodies used for immunohistochemistry are listed in Table 1. Histological examination of the skin and subcutaneous tissue five years previously (initial stage) showed hyperplasia of cells with lobular structure (Plate XI, Fig. 3) Immunohistochemistry staining demonstrated focal positivity to pancytokeratin (Plate XI, Fig. 4). Later, in the terminal stage, histological examination of the skin and subcutaneous nodules demonstrated concentration of collagen fibres on the periphery of the lesions, in variously intertwined bundles with scattered spindle nuclei. The centre of these lesions showed marked cell proliferation with less intercellular mass. The nuclei were hypochromatic with a moderate degree of mitotic activity. Mononuclear cells were observed between the collagen fibres. Hard nodules were spreading from the surface of the skin to the subcutaneous tissue. Macroscopically observed nodes in the lungs, myocardium, intestines, and epiglottis were similar in structure to skin nodes. Fibrous nodules were also seen in the kidneys in which proliferation of fibrous tissue with tubule and glomerular atrophy was observed. Vacuolar degeneration of hepatocytes and proliferation of fibrous tissue arising from sinusoidal capillaries was observed in the liver. Of the examined organs, hyperplasia of fibrous tissue was observed in the myocardium (Plate XII, Fig. 5), liver, pancreas, kidneys, and in the vessel walls, with visible narrowing of their lumen. Collagen staining confirmed a strong density of collagen fibres at the periphery of the lesion (Plate XII, Fig. 6) and cell proliferation around the vessels. CD3 + lymphocytes were observed mainly in the zone of cell proliferation and on the interface towards the fibrous layer (Plate XIII, Fig. 7). Positive cytokeratin staining was seen in the renal tubule epithelium but without changes in malignancy (Plate XIII, Fig. 8). On the other hand, Masson trichrome staining showed proliferation of collagen ligaments originating from the original interstitial tissue

(Plate XIV, Fig. 9). Macrophages were evaluated with CD68, CD163, and HAM 56. Positive reaction in fibrous nodules was found only with CD163. Macrophages stained with this antibody were seen mainly in the transitional zone between cellular and fibrous part (Plate XIV, Fig. 10).

## Discussion

Rare nodular dermatofibrosis in the German Shepherd showed in this case five years previously in the form of changes to the skin in the sacral region of the dog's back. The initiation of the development of nodular dermatofibrosis in German Shepherds is attributed to an autosomal dominant hereditary syndrome with complete manifestation (Lingaas et al. 2003). An anti-human pancytokeratin antibody mouse, clone AE1/AE3 demonstrate diffuse epithelial staining (Frgelecova et al. 2013). Early histological changes in this patient (initial stage) showed only focal positivity to pancytokeratin in cutaneous tissue which might imply proliferation of cells producing collagen and a decreased number of cells generating cytokeratin. In the terminal stage, the changes in movement coordination, defecation, and occasional vomiting were probably related to the growth of fibrous nodules in the abdominal cavity and in the wall of the digestive tract. The epidermis of the prominent skin may be intact despite ulcerative skin lesions. Similar clinical changes have been described by Suter et al. (1983). Nodular proliferation develops due to overexpression of TGF-β1, which is known to stimulate desmoplasia. The prognosis in patients with nodular dermatofibrosis is unfavourable, as it often leads to renal cancer. In our case, a fibrous nodule and proliferation of fibrous tissue in the intertubular space in the kidney was observed. However, we did not notice any tumour process in the kidneys. The CD3 marker is expressed in the membrane and cytoplasm of normal and tumour T cells (Matter et al. 2012; Levkut et al. 2021). The majority of inflammatory lymphocytes found in chronic diseases are CD3 + lymphocytes, which are mostly CD3 + CD4 + lymphocytes and, to a lesser extent, complementary CD3 + CD8 + cells (Gunasinghe et al. 2021). Higher CD3 + cell counts at the interface between cell proliferation and fibrosis may have been associated with fibroblast degradation. Our conclusion is supported by the finding of macrophages in this zone. Patients diagnosed with primary skin lesions can die within three years of initial signs of uraemia (Lingaas et al. 2003). If we take into account the first diagnosis of collagen proliferation recorded in our patient five years previously, the survival time of the dog was longer than in dogs with renal adenocarcinoma. Collagen lesions in the skin, kidneys, and the formation of fibrous nodules in the lungs, myocardium, intestinal wall, and on the epiglottis are typical of dermatofibrosis in dogs (Meuten and Meuten 2017; Thompson et al. 2019). Collagen is most often produced by fibroblasts, which are specialized cells with this function. We currently distinguish several types of collage. Type I collagen forms fibres and is most commonly detected in connective tissue, bone, vasculature, ligaments, tendons, and skin (Franzke et al. 2005). Type I and III collagen mutations have also been found in dogs with Ehlers-Danlos syndrome (Cho and Kim 2007). Similarly, type I collagen mutations have been found in humans during imperfect osteogenesis (Gajko-Galicka 2002).

In conclusion, nodular dermatofibrosis is one of the rare diseases in dogs with more frequent occurrence in the German Shepherd breed. In this case, clinical examination of the skin revealed the presence of distinct cutaneous partly fluctuating lesions. In the terminal stage, histology showed systemic production of fibrous nodules and formation of fibrous tissue with atrophy of parenchyma tissue. However, no connection between dermatofibrosis and adenocarcinoma of kidney was found in this case, which was supplemented with pancytokeratin antibody. CD3 + lymphocytes were observed mainly in the zone of cell proliferation and on the interface towards the fibrous layer. Macrophages were seen mainly

in the transitional zone between cellular and fibrous parts. This indicates participation of monitored immunocompetent cells in fibroblast degradation.

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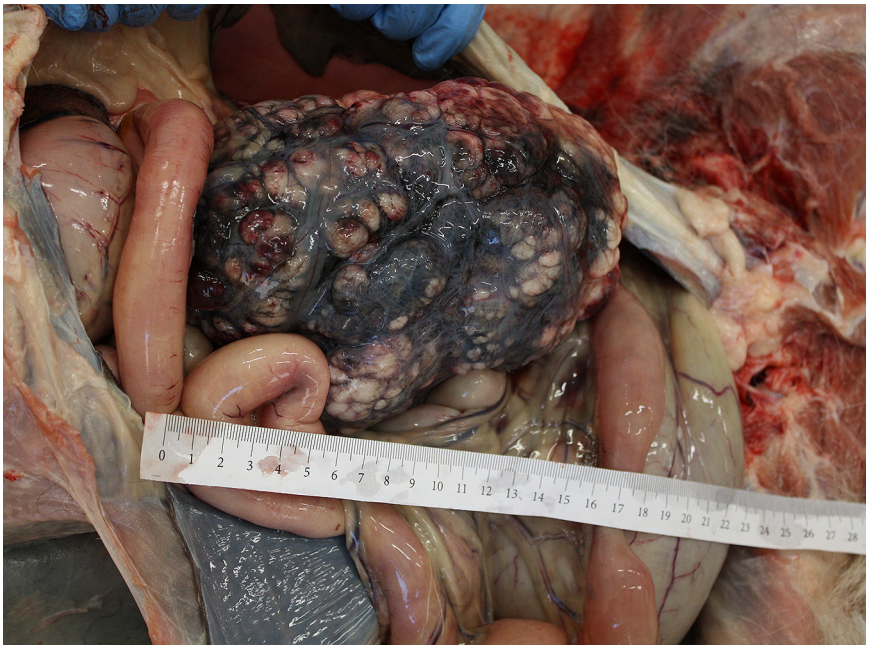

Fig. 1. Intra- abdominally located tumor between proximal intestinal loops

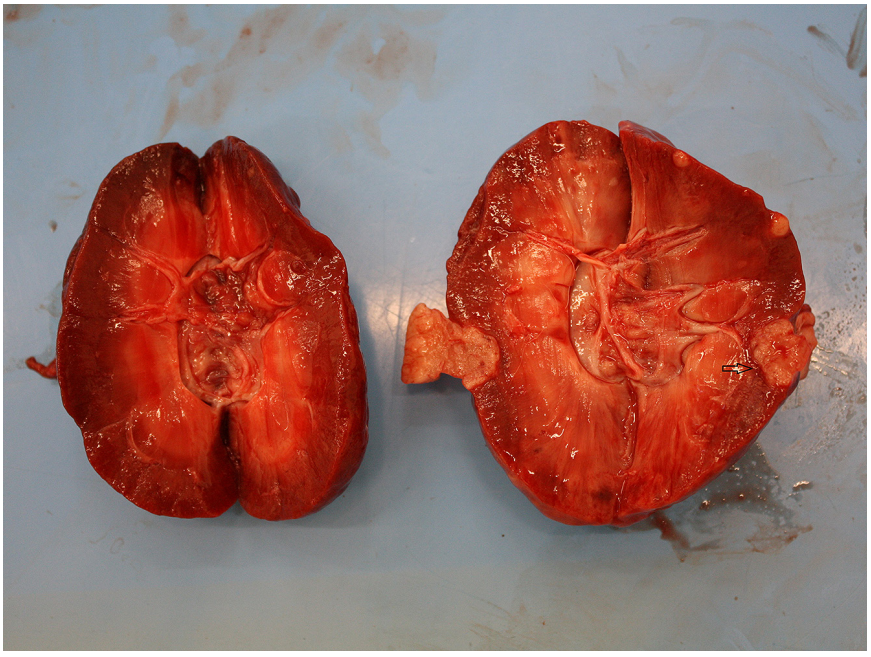

Fig. 2. Fibrous nodules in the renal cortex (arrow)

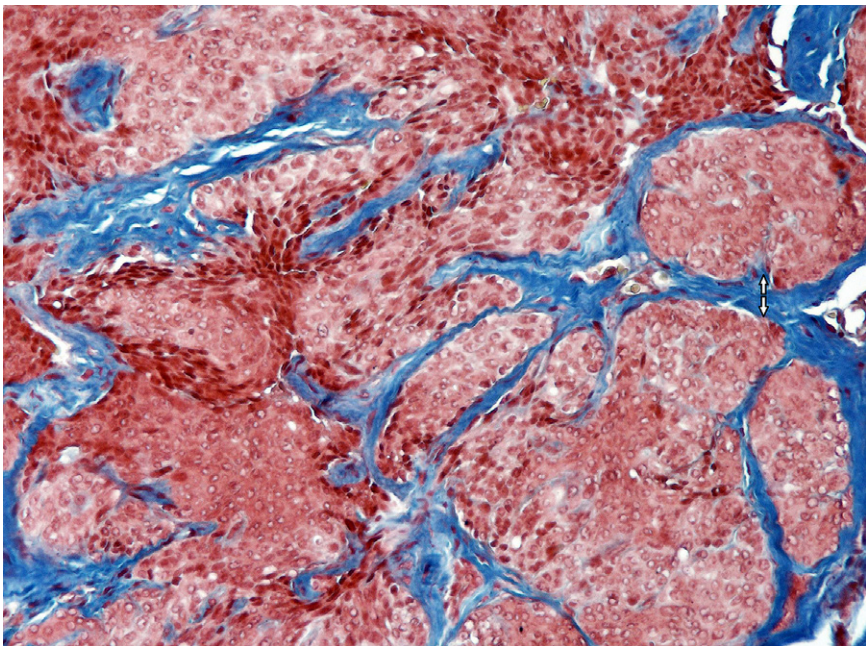

Fig. 3. Hyperplasia of cells forming lobular structure (arrow), (Masson trichrome, × 200)

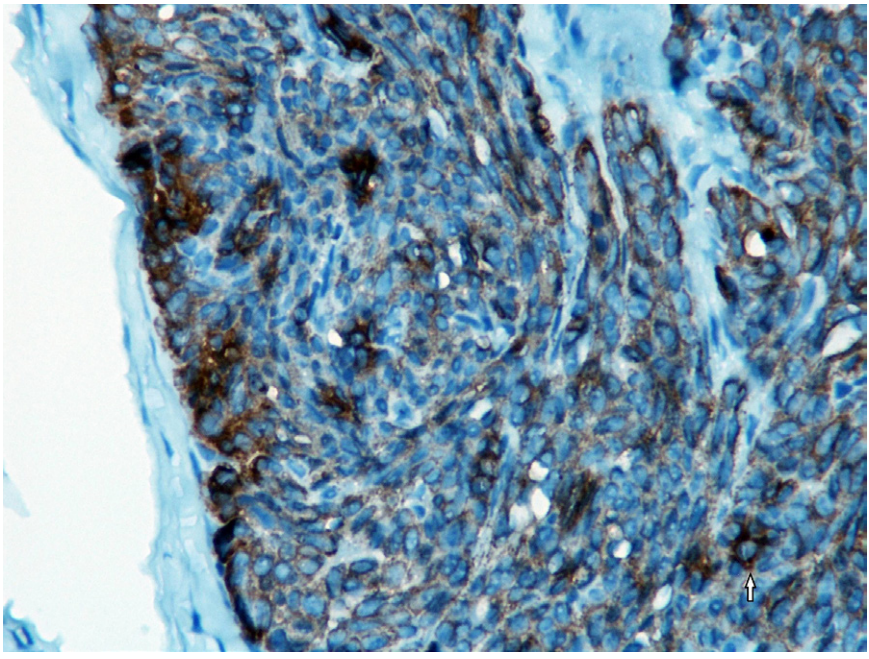

Fig. 4. Focal positivity of cells to pancytokeratin (arrow), (IHC, × 200)

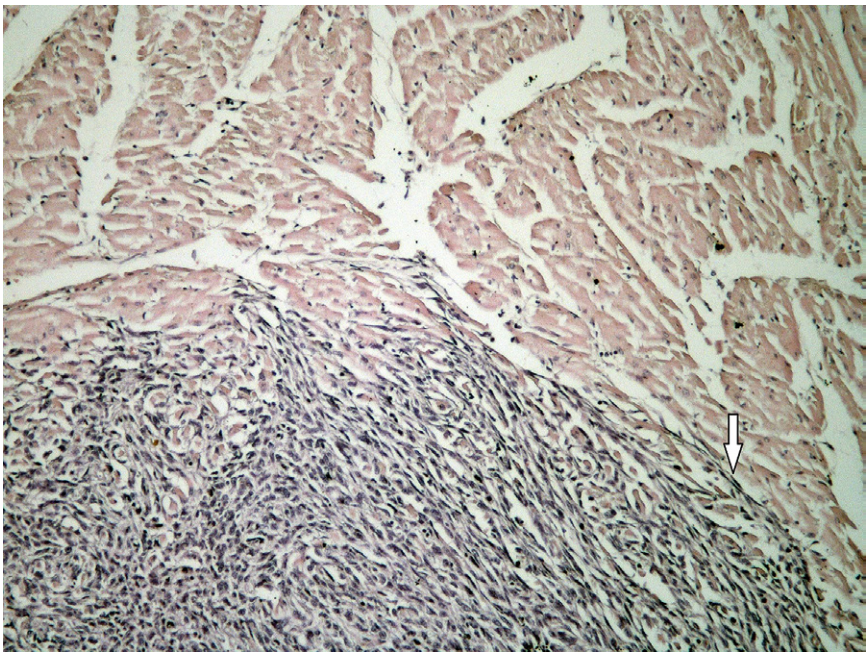

Fig. 5. Expansive growth of nodule in the myocardium (arrow), (haematoxylin-eosin, × 100)

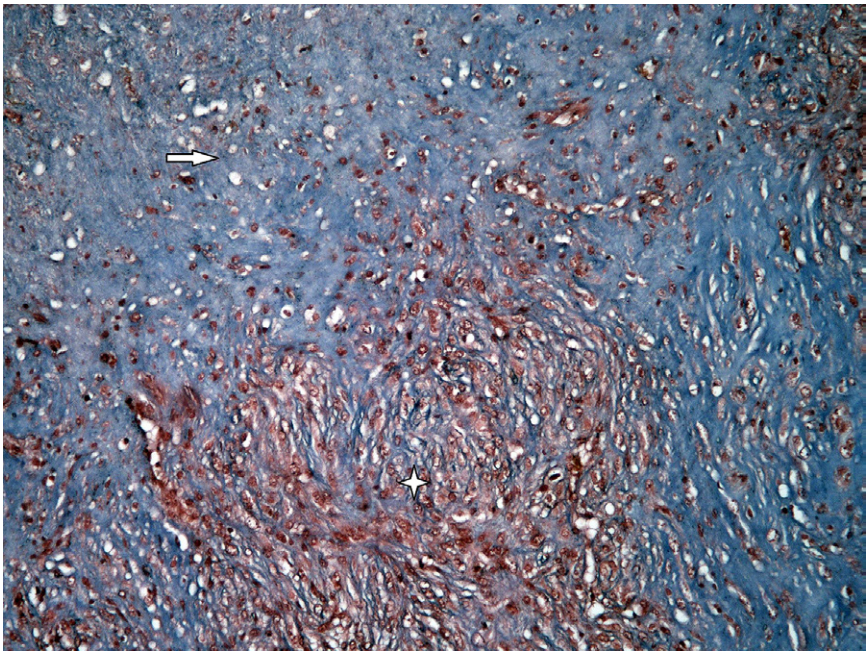

Fig. 6. Central cell proliferation (star) and density of collagen fibers at the periphery of the lesion (arrow), (Masson trichrome, × 100)

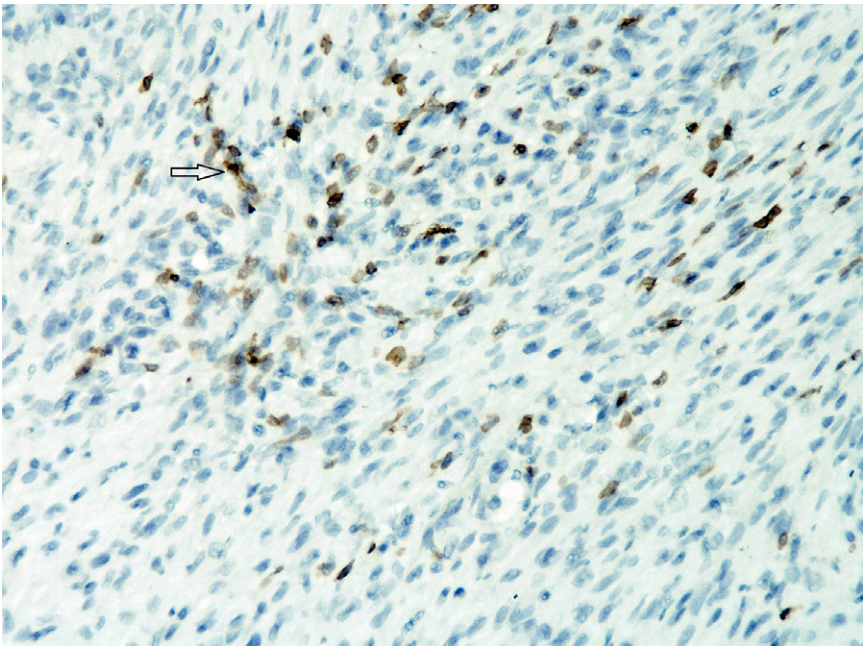

Fig. 7. CD3 + lymphocytes located mainly in the zone of cell proliferation and in the interface towards the fibrous layer (arrow), (IHC, × 200)

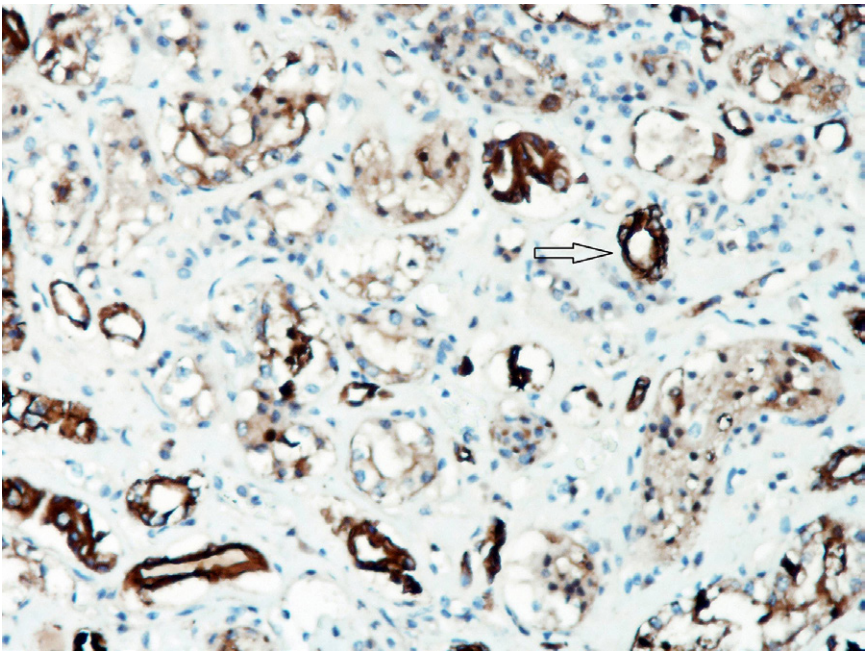

Fig. 8. Positive cytokeratin cells in the renal tubules but without changes in malignancy (arrow), (IHC, × 200)

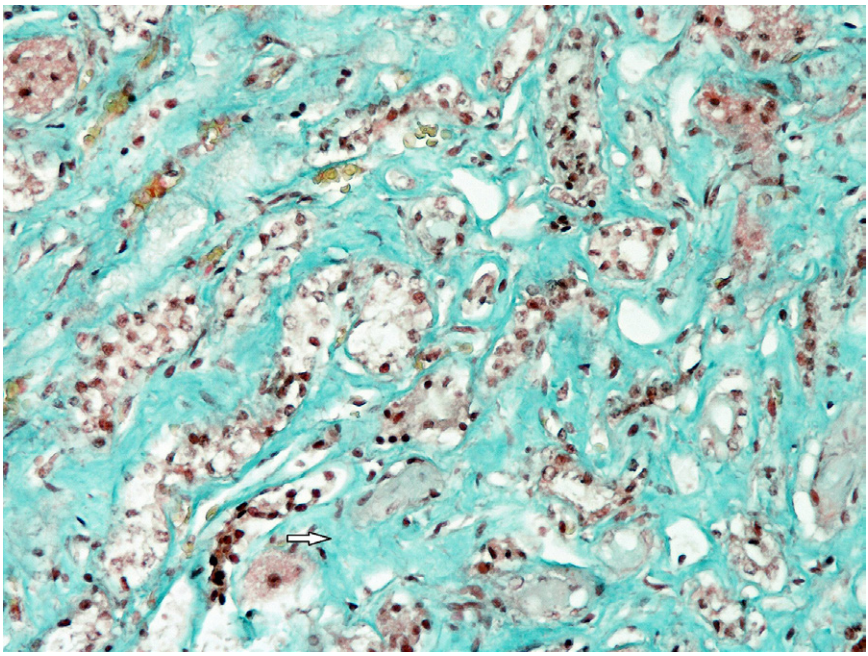

Fig. 9. Severe density of fibrous tissue among the renal tubules (arrow)

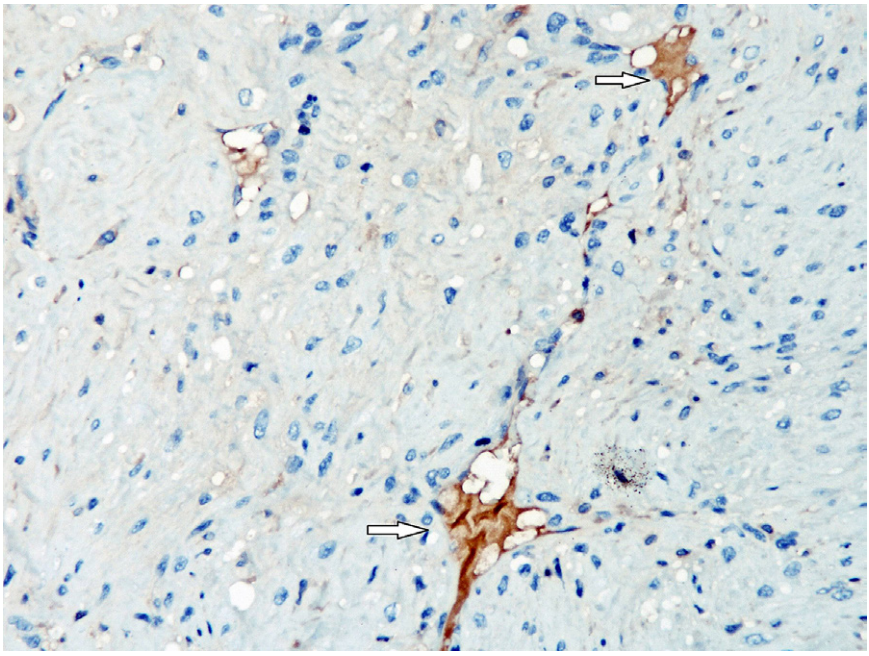

Fig. 10. Macrophages CD 163 seen mainly in the transitional zone between cellular and fibrous part (arrow), (IHC, × 200)