Dependence of Blood Biochemical Parameters on the Degree of Microstructural Changes in Chronic Pancreatitis in Dogs

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Abstract

The study investigated the biochemical parameters of blood in comparison to histological findings in dogs with pancreatitis. Tissue samples were collected from the left and right lobes and the body of the pancreas during autopsy, followed by histological examination to assess the degree of inflammation, fibrosis, and necrotic changes. The severity of microstructural damage was classified into three grades based on the progression of fibrotic changes: mild, moderate and severe (characterised by dense pancreatic fibrosis). Histological changes in the pancreas in cases of chronic pancreatitis were compared to those in healthy dog pancreatic tissue obtained post-mortem from animals that died in traffic accidents. For each animal, the histological results were compared with the biochemical parameters of blood serum, measured prior to death during the diagnostic and treatment stages. In cases of mild microstructural changes, connective tissue proliferation occurred alongside minor atrophy of the exocrine component, with the structure of Langerhans-Sobolev islets preserved. Moderate damage involved more pronounced separation of exocrine parenchyma by fibrous tissue. Severe damage was characterised by acinar cell involvement, including vacuolar degeneration and atrophy. Loose fibrosis was typical for mild and moderate damage, while dense fibrosis was indicative of severe microstructural changes. Inflammation in the pancreas was not diffuse but was instead localised. The study established that the development of fibrosis plays a critical role in the pathogenesis of chronic pancreatitis. Blood biochemical parameters were

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correlated with the severity of pathological microstructural changes. Specifically, the activity of alkaline phosphatase increased by 1.3 times. In severe cases, alanine aminotransferase activity showed a significant increase compared to moderate and mild forms. Elevated levels of connective tissue metabolites in blood serum were identified as informative diagnostic markers for chronic pancreatitis of varying degrees of microstructural damage. These markers provide valuable insight into the diagnosis and progression of the disease.

Key words: *pancreatic gland; fibrosis; histological alterations; investigation; degree of lesion; diagnostics*

Introduction

Chronic pancreatitis is a progressive inflammatory disease of the pancreas, and its diagnosis remains challenging (Tomaszewska et al., 2024). The pathology leads to inflammation and fibrosis, potentially resulting in both exocrine and endocrine insufficiency (Kim et al., 2024). Chronic pancreatitis is characterised by prolonged pancreatic inflammation, leading to fibrosis and gradual functional loss of the organ (Afghani et al., 2014). Historically, it was believed that this condition was relatively rare in dogs. However, pathological and clinical studies have demonstrated its high prevalence and significant clinical impact (Watson, 2012). Over the last two decades, the diagnosis of pancreatitis in dogs has become more frequent due to advancements in diagnostic techniques (Cridge et al., 2021).

In clinical practice, an accurate diagnosis or exclusion of pancreatitis in dogs is most reliably achieved through a combination of a thorough anamnesis, serum lipase concentration assessment, abdominal ultrasound, and cytological or histopathological examination of the pancreas (Xenoulis, 2015).

Currently, there is no "gold standard" for antemortem diagnosis of pancreatitis in dogs. Diagnostic approaches often include anamnesis, clinicopathological findings, measurement of pancreatic lipase immunoreactivity, radiography and ultrasound imaging (Lim et al., 2014), as well as advanced modalities such as computed tomography (French et al., 2019), pancreatic lipase concentration determination (Kim et al., 2024), laparoscopy with pancreatic tissue biopsy and subsequent histological examination (Webb and Trott, 2008), and the evaluation of blood biochemical parameters (Bayraktar et al., 2024). These techniques allow for the characterisation and chronicity assessment of the pathological process (Aupperle-Lellbach et al., 2020), although each has its limitations (Lidbury and Suchodolski, 2016).

Histological examination remains the most effective method for diagnosing pancreatitis, but its invasive nature and certain limitations – such as the possibility of missing localised lesions or subclinical pancreatitis – restrict its routine use (Cridge et al., 2020).

There remains a need for deeper investigation into the pathological changes associated with pancreatic diseases in dogs (Santos Pantoja et al., 2023). Currently, serum pancreatic lipase immunoreactivity is considered the most specific biomarker for diagnosing canine pancreatitis (Mawby et al., 2014). In recent years, progress has been made in developing new markers and alternative methods for diagnostic improvement. However, the diagnosis of pancreatitis continues to rely heavily on clinical examination, biochemical data and imaging studies (Dröes and Tappin, 2017).

The question of a potential correlation between blood biochemical parameters

and morphological findings in canine pancreatitis remains highly relevant (Cueni et al., 2023). This correlation could provide critical insights into disease progression and diagnostic strategies.

Materials and methods

The study utilised medical records obtained from private veterinary clinics in the city of Dnipro. The research focused on investigating the features of chronic pancreatitis in dogs by comparing clinical, biochemical and histological data. Tissue samples were collected from the left and right lobes and the body of the pancreas during autopsies, followed by histological examination of the pancreatic tissue. Particular attention was paid to the degree of tissue inflammation, fibrosis and necrotic changes.

During the autopsies of dogs diagnosed with chronic pancreatitis, biopsies were collected from various regions of the pancreas for histological analysis. This analysis determined the extent of fibrotic changes, which were classified into mild, moderate and severe stages, corresponding to the first, second and third stages of fibrosis, respectively. The histological changes observed in cases of chronic pancreatitis were compared to the pancreatic tissues of healthy dogs that had died due to road traffic accidents.

Histological preparations were performed using classical methods. The prepared slides were examined under a microscope to study the microstructural features of pancreatic tissue. The histological diagnosis of pancreatitis was based on the presence of one or more histopathological changes in one or more pancreatic regions, including purulent inflammation, necrosis of acinar cells, peripancreatic fat necrosis, mononuclear inflammation, or fibrosis. Additional features such as haemorrhages, interstitial oedema, atrophy, or cellular degeneration were also considered. The following histological parameters were assessed: neutrophilic and lymphocytic inflammation, extent of necrotic changes, fat necrosis, oedema, fibrosis, atrophy and nodular formations. Particular attention was given to irreversible changes associated with chronic pancreatitis, such as fibrosis and atrophy.

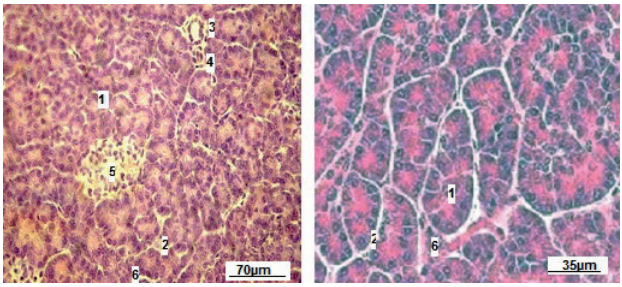
For each animal, a comparative analysis of histological findings was conducted alongside the biochemical parameters of blood serum, measured prior to deaths during diagnostic evaluation and treatment. The results were processed biometrically.

Biochemical blood analyses were performed using a Rayto 1904 Vet semi-automatic biochemical analyser with diagnostic kits manufactured by Felicit Diagnostic. Statistical analysis of the research results was conducted using Microsoft Excel 2021.

Results

Histological studies revealed specific patterns of pathological changes in the pancreas compared to the normal structure. In healthy dogs, the pancreas is enclosed by a capsule composed of dense irregular fibrous connective tissue. The organ's parenchyma exhibits a lobular structure (Figure 1).

As shown in Figure 1, the interlobular spaces of the pancreas are filled with thin connective tissue septa containing small ganglia, blood vessels, and excretory ducts. Analysis of serial sections reveals that the ductal system includes intercalated, intralobular, interlobular and the main pancreatic ducts. Intercalated ducts are difficult to identify as they are compressed between terminal acini and are rarely observed.



A B

Figure 1. Canine Pancreas. Normal Structure. 1 – acinus; 2 – interlobular connective tissue; 3 – blood vessel; 4 – ganglion; 5 – Langerhans-Sobolev islet; 6 – interlobular duct. A – Böhmer's haematoxylin, eosin, ×80; B – Böhmer's haematoxylin, Hart's modified fuchselin, ×100, ×200.

The structural and functional unit of the exocrine part of the pancreas is the acinus. In the canine pancreas, an acinus typically consists of 8–12 acinar cells (acinocytes), which have a pyramidal shape and produce digestive enzymes. The wide base of the acinocytes rests on the basal membrane, forming the intercalated ducts.

The narrow apical portion of the acinocyte contains microvilli projecting into the acinar lumen. Based on their staining properties, acinocytes are divided into two zones: the apical zone (zymogenic zone) and the basal zone (homogeneous zone). The apical zone is acidophilic in response to acidic dyes and contains large secretory enzyme granules in an inactive phase. These granules vary in optical density, indicating different levels of maturity.

In the homogeneous zone, enzyme synthesis occurs, imparting an intense basophilic staining pattern. The lower part of the acinocyte contains the nucleus, which features 1–2 small nucleoli. The endocrine portion of the pancreas is represented by the Langerhans-Sobolev islets (Figure 2).

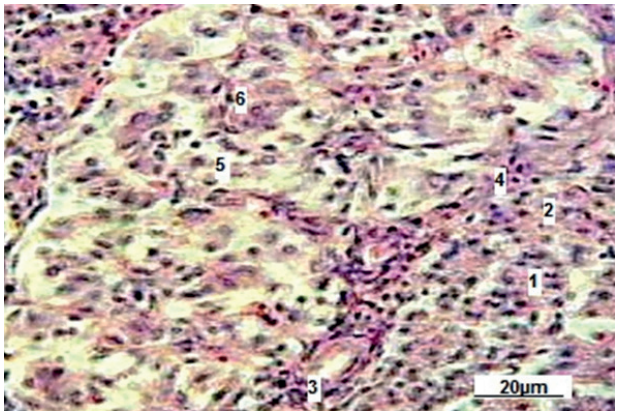
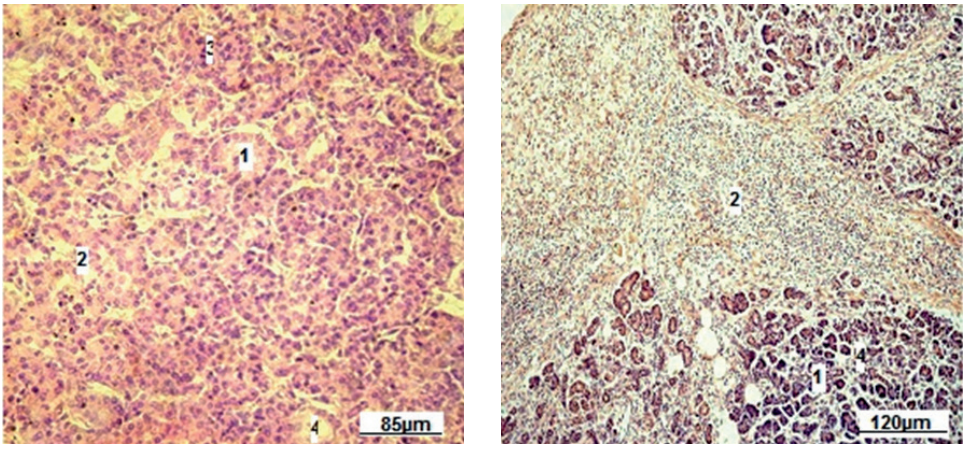


Figure 2. Langerhans-Sobolev islet. Normal Structure. 1 – acinocytes; 2 – interlobular connective tissue; 3 – blood vessel; 4 – ganglion; 5 – A-cells; 6 – B-cells. Böhmer's haematoxylin, Hart's modified fuchselin, ×400.



A B

Figure 3. Mild (A) and moderate (B) fibrosis of the pancreas. Stage 1. 1 – acinus; 2 – fibrous tissue; 3 – Langerhans-Sobolev islet; 4 – interlobular duct. A – Boehmer's haematoxylin, eosin, × 80; B – Boehmer's haematoxylin, Hart's fuchsin (modified), ×50

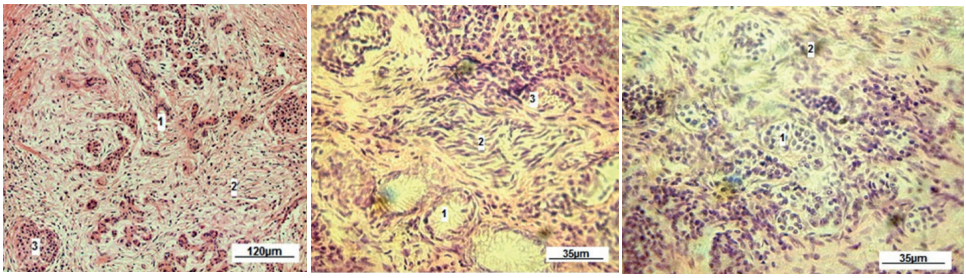
Pancreatic islets are separated from the exocrine region by a thin layer of connective tissue. In dogs, these islets are often round or oval in shape but may also appear in other forms, such as ribbon-like structures, as shown in Figure 2. Within a single histological section, the number, shape, and especially the size of Langerhans-Sobolev islets vary widely, ranging from 70 to 800 μm. The pancreatic islets consist of cell cords with indistinct boundaries, interspersed with a branching network of capillaries typical of endocrine glands.

Insulinocytes are small cells with light cytoplasm and numerous secretory granules. Under light microscopy, A-cells constitute up to 25% of the total population and are relatively easy to identify due to their irregular shape and pronounced acidophilic staining. Their nuclei are spherical and positioned eccentrically. The majority of islet cells are basophilic B-cells, which contain insulin granules.

Chronic pancreatitis is among the most common pancreatic diseases in dogs. Among digestive system disorders, this pathology holds a unique position due to the challenges in both diagnosis and treatment. The main difficulty in diagnosing chronic pancreatitis, particularly in the early stages, lies in the inability to obtain a histological sample for precise diagnosis. This limitation makes histological diagnostics of chronic pancreatitis far from a routine procedure.

Notably, depending on the quality of connective tissue in various forms of chronic pancreatitis in dogs, pancreatic fibrosis can be categorised as loose or dense. Loose fibrosis is characteristic of mild and moderate chronic pancreatitis (Figure 3).

According to Figure 3A, chronic pancreatitis is characterised by a perilobular pattern of connective tissue proliferation occurring against a background of mild atrophy of the exocrine component, with the Langerhans-Sobolev islet structure remaining intact. In the case of moderate fibrosis (Figure 3B), a more pronounced separation of the exocrine parenchyma by fibrous tissue is observed.



A B C

Figure 4. Dense fibrosis of the pancreas. Stage 2. (A) 1 – degenerating acinus; 2 – fibrous tissue; 3 – Langerhans-Sobolev islet. (B) 1 – vacuolar degeneration of acinocytes; 2 – fibrous tissue; 3 – ganglion. (C) 1 – vacuolar degeneration of acinocytes; 2 – fibrous tissue; 3 – ganglion. All staining with Boehmer's haematoxylin, eosin; Magnification A ×50; B, C ×200

A distinctive presentation of dense fibrosis is shown in Figure 4A, which shows that as fibrosis progresses during chronic pancreatitis, there is a gradual reduction in the volume of the exocrine tissue area, an increase in the stromal component, and an expansion of the ductal and endocrine tissue areas. Dense fibrosis is accompanied by a series of microstructural changes (Figure 4B), where the affected acinar tissue exhibits vacuolar degeneration of cells, leading to partial or complete atrophy. Isolated cells undergoing apoptosis were observed against the background of focal vacuolar degeneration of acinocytes. The nerve trunk damage visible in the figure was accompanied by oedema and perineural fibrosis (Figure 4C).

Figure 5 shows that fatty degeneration is accompanied by the appearance of haemorrhagic foci. During this stage, the structure of the pancreatic duct system undergoes pathological changes, including the formation of numerous small proliferating ducts with epithelial dysplasia. Tubular-islet complexes, which are not characteristic of the normal pancreatic structure, are also observed.

Fibrotic changes during chronic pancreatitis lead to sclerotic transformation or necrosis of the glandular tissue. It should be noted that pancreatic inflammation usually does not occur diffusely throughout the organ but is rather localised. Additionally, pancreatitis in dogs is accompanied by the destruction and loss of parenchyma.

The total protein level in animals with chronic pancreatitis tends to increase (Table 1). In cases of severe pancreatic damage, the average total protein level is higher by 3.5 g/L compared to clinically healthy animals, while no significant difference was observed in mild and moderate cases. The causes of hyperproteinemia may include hypovolemia, liver pathology, or inflammation, during which the levels of acute-phase proteins increase.

Changes in cholesterol levels were less pronounced in chronic pancreatitis. For severe cases, cholesterol levels increased slightly by 0.24 mmol/L compared to clinically healthy animals, while no significant differences were observed in other degrees of severity.

According to Table 1, indicators of kidney function (urea and creatinine levels) in dogs with varying degrees of chronic pancreatitis did not differ significantly from those of healthy animals. However, in 20% of severe cases, a high creatinine level (131.5 μmol/L) was recorded.

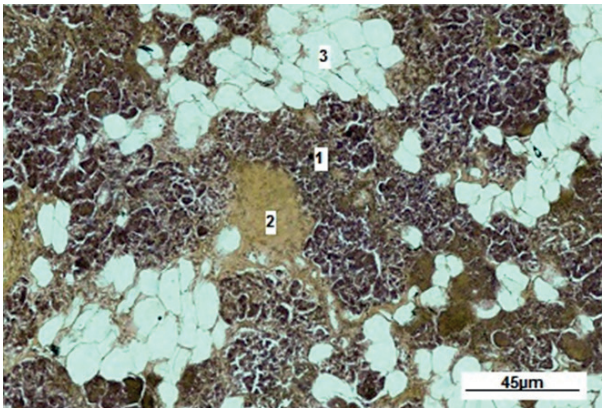


Figure 5. Fatty degeneration of the pancreas. 1 – tubular-islet complexes; 2 – haemorrhagic focus; 3 – adipose tissue. Boehmer's haematoxylin, eosin, ×80

In clinically healthy animals, the activity of α-amylase ranged from 82.4 to 98.8 g/(h×L), with an average of 93.02±3.39 g/(h×L). During chronic pancreatitis of varying severity, α-amylase activity remained within the physiological range or at the lower reference values. Compared to healthy animals, the average α-amylase activity was 87.92±3.54 g/(h×L) in mild cases, 85.26±3.14 g/(h×L) in moderate cases, and 82.42±3.66 g/(h×L) in severe cases (P<0.05). Thus, a slight decrease in α-amylase activity in serum is characteristic of the chronic progression of pancreatic pathology, with no significant differences observed between severity levels.

In mild cases of chronic disease progression, alkaline phosphatase (ALP) activity was 6.81±0.85 Bodansky units, with 40% of affected animals showing elevated levels; however, this increase was not significant compared to healthy dogs. In moderate cases and severe cases, the mean value was significantly higher (P<0.05) at 7.84±0.49 and 8.90±0.55 Bodansky units, respectively.. An increase in ALP activity by 1.3 times may indicate the development of cholestasis caused by impaired digestive enzyme function, leading to insufficient fat emulsification in the intestine. Furthermore, there was a tendency for ALP activity to increase with the severity of microstructural pancreatic lesions.

According to Table 1, the activity of aspartate aminotransferase (AST) in animals with chronic pancreatitis (Groups II–IV) increased on average by 1.3 times compared to Group I (clinically healthy animals, P<0.01). However, despite a slight upward trend, no significant differences were observed between Groups II–IV.

In cases of chronic pancreatitis without signs of exacerbation, alanine aminotransferase (ALT) hyperenzymemia was more pronounced (1.42±0.13 mmol/(h×L), P<0.01 in Group II; 1.74±0.05 mmol/(h×L), P<0.001 in Group III; and 1.80±0.04 mmol/(h×L), P<0.001 in Group IV). The significant increase in ALT activity (P<0.05) in animals with severe microstructural damage can be attributed to pathological alterations in the cytosolic membrane of hepatocytes. The cytosolic membrane becomes more permeable than the mitochondrial membrane, resulting in more intensive ALT release

Table 1. Biochemical blood parameters in dogs depending on the degree of microstructural lesions in chronic pancreatitis (Mean±SE), *n*=5)

		Group			
		I	II	III	IV
Microstructural Lesions		Healthy Animals	Mild Lesions	Moderate Lesions	Severe Lesions
Total Protein (g/L)	Lim	55.4–71.3	59.5–72.9	60.1–73.2	61.5–73.3
	Mean±SE	62.6±2.20	64.4±1.65	65.2±1.77	66.1±1.45
Cholesterol (mmol/L)	Lim	3.7–5.3	3.8–5.4	4.1–5.5	4.0–5.4
	Mean±SE	4.38±0.19	4.48±0.17	4.68±0.19	4.62±0.26
Urea (mmol/L)	Lim	4.5–8.2	4.4–8.4	4.3–8.6	4.5–8.8
	Mean±SE	6.46±0.61	6.60±0.75	6.52±0.73	6.86±0.76
Creatinine (μmol/L)	Lim	51.5–96.8	52.4–97.4	53.2–96.6	58.4–131.5
	Mean±SE	74.4±7.39	75.68±7.53	75.52±7.32	83.26±6.69
α-Amylase (g/(h×L))	Lim	82.4–98.8	76.4–92.6	75.6–90.5	72.4–87.5
	Mean±SE	93.02±3.39	87.92±3.54	85.26±3.14	78.70±3.89*
ALT (mmol/(h×L))	Lim	0.67–0.88	1.10–1.74	1.62–1.84	1.68–1.88
	Mean±SE	0.77±0.04	1.42±0.13**	1.74±0.05***	1.80±0.04***
AST (mmol/(h×L))	Lim	0.64–0.84	0.84–0.94	1.82–1.24	0.84–1.28
	Mean±SE	0.77±0.04	0.90±0.02*	0.97±0.04**	0.99±0.04**
Alkaline Phosphatase (U)	Lim	3.21–6.22	4.52–8.63	6.55–9.42	6.64–9.64
	Mean±SE	4.97±0.61	6.81±0.85	7.84±0.49**	8.90±0.55**
Total Bilirubin (μmol/L)	Lim	4.24–5.56	5.26–6.62	5.68–6.80	5.72–6.64
	Mean±SE	4.92±0.30	5.96±0.29*	6.23±0.25*	6.25±0.20**
Chondroitin Sulfates (g/L)	Lim	0.112–0.184	0.324–0.452	0.374–0.485	0.384–0.496
	Mean±SE	0.156±0.013	0.385±0.021***	0.414±0.015***	0.423±0.018***

Note: Lim = range of observed values; Mean±SE), = mean ± standard error of the mean. Statistical significance: *– $P<0.05$; **– $P<0.01$; ***– $P<0.001$ – compared to clinically healthy animals; – $P<0.05$ – compared to animals with mild degrees of microstructural damage

from cells compared to AST, thereby indicating changes in both the cytosolic and mitochondrial structures of hepatocytes.

Total bilirubin was significantly increased in the serum of dogs with chronic pancreatitis. In mild cases (Group II), total bilirubin levels rose by 1.04 μmol/L (P<0.05) compared to clinically healthy animals. For moderate damage (Group III) and severe damage, the same increase was recorded, 1.31 μmol/L (P<0.05). The elevation in total bilirubin levels in chronic pancreatitis occurs as a component of the inflammatory process and is associated with hepatobiliary system damage, liver oedema, cholestasis, and the consequent retention of bilirubin in the bile ducts.

The development of pancreatic fibrosis is a critical component of chronic pancreatitis pathogenesis. This pathological process is characterised by an increase in the serum levels of chondroitin sulfates in affected dogs: by 2.5 times in Group II, 2.7 times in Group III, and 2.8 times in Group IV compared to clinically healthy animals (P<0.001, Table 1). This increase in affected animals is due to characteristic exacerbation-related processes in chronic pancreatitis, including connective tissue proliferation and enhanced glycosaminoglycan synthesis. Thus, the increased serum levels of connective tissue metabolites serve as informative markers for diagnosing chronic pancreatitis with varying degrees of microstructural damage.

Discussion

The diagnosis of chronic pancreatitis is particularly challenging due to the nonspecific and often subtle nature of clinical signs and the relatively low sensitivity of non-invasive diagnostic tests (Watson, 2012). Histologically, the disease is characterised by ductal destruction, interlobular fibrosis, and dense periductular and perivenular lymphocytic aggregates (Coddou et al., 2024). Fibrosis is considered the primary histological hallmark of chronic pancreatitis (Apte et al., 2011) while glandular atrophy is another notable feature of this pathology (Amorim de Lemos et al., 2021). Chronic pancreatitis commonly results in progressive destruction of both exocrine and endocrine components of the pancreas (Westermarck and Wiberg, 2012). In dogs with chronic pancreatitis, irreversible histological changes in pancreatic tissue, such as fibrosis and atrophy, have also been observed (Milastnaia and Dukhnitsky, 2019), consistent with our findings.

The present study corroborates the hypothesis that the pathogenesis of chronic pancreatitis in dogs is fundamentally rooted in pancreatic necrosis. As evidenced by our results, animals with chronic pancreatitis but without pronounced necrosis of the pancreas or surrounding tissues exhibited fewer specific clinical signs and less pronounced changes in certain blood biochemical parameters, reflecting the progression of the pathological process. A correlation was observed between the degree of inflammation and pancreatic fibrosis and enzyme concentrations (Mansfield et al., 2012).

Aupperle-Lellbach et al. (2020) noted that under normal conditions, minimal interstitial lymphocytic infiltration, moderate interstitial fibrosis, and nodular hyperplasia are present. Mild microstructural changes are characterised by moderate infiltration of neutrophils, lymphocytes, and macrophages, with small foci of acute acinar vacuolar degeneration or necrosis affecting less than 10% of tissue. In cases of moderate or severe pancreatitis (affecting 10–40% and over 40% of pancreatic areas, respectively), the authors observed purulent inflammation with signs of necrosis, granulation tissue

formation, and fibrosis. In some cases, severe fibrosis with acinar atrophy was observed without necrosis.

Acinar cells of the exocrine pancreas are crucial for the synthesis of digestive enzymes, while ductal cells secrete bicarbonate-rich fluid to neutralise gastric acid in the duodenum, providing an optimal environment for enzyme activity (Lipovšek et al., 2024). Acini form lobules separated by loose connective tissue, and the secretory acini constitute 80–85% of the pancreatic mass. Each acinus comprises 20–50 exocrine pancreatic cells (acinocytes). Watson (2003) suggested that acinar atrophy is the most common pancreatic alteration in dogs. In cases of severe fibrosis, we also observed damage to acinar tissue, including vacuolar degeneration and partial or complete atrophy.

Chronic pancreatitis results in persistent histological changes and progressive loss of both exocrine and endocrine functions, potentially leading to exocrine pancreatic insufficiency and/or diabetes mellitus if the animal survives long enough. The pancreas has a significant functional reserve, and these conditions develop only after a loss of 80–90% of functional pancreatic mass (Watson et al., 2010; Singh et al., 2018).

Some authors have concluded that pancreatic inflammation is usually localised rather than diffusely distributed throughout the organ, a finding also reflected here. These results indicate that a single biopsy is insufficient to rule out subclinical pancreatitis and that no specific site can reliably serve as a biopsy target unless significant lesions are visible (Newman et al., 2004). While histopathological data are considered definitive for diagnosing pancreatitis, the localised nature of these lesions means that the absence of histopathological evidence cannot exclude the diagnosis (Steiner, 2013).

Researchers suggest that increased liver enzyme activity, or elevated cholesterol and bilirubin concentrations, may result from chronic pancreatitis and are associated with pancreatic necrosis and fat necrosis of the pancreas (Bostrom et al., 2013). Our findings support the hypothesis that the diagnosis of pancreatitis should be based on a combination of diagnostic tests (Xenoulis et al., 2020). The importance and necessity of ultrasound examination have also been emphasised (Cridge et al., 2020; Gori et al., 2021; Pelligra et al., 2022).

Our results confirm that chronic pancreatitis is characterised by irreversible histopathological lesions, potentially leading to the loss of both exocrine and endocrine pancreatic functions, as also reported by Zhang et al. (2021). Histological studies further indicate that chronic pancreatitis exhibits diverse histopathological patterns (Kuzi et al., 2023). It is worth noting that the fatty degeneration of the pancreas observed in this study could be caused by mild degenerative and inflammatory processes.

Currently, three main forms of pancreatitis are recognised in dogs based on the course of the disease: acute, recurrent chronic, and chronic. It is important to emphasise that diagnosing chronic pancreatitis based on microstructural lesions often requires comprehensive clinical, biochemical, histological, and other studies. This poses a significant challenge because chronic pancreatitis, in the absence of recurrence, typically manifests as exocrine pancreatic insufficiency syndrome and progresses over many years.

In the serum of affected dogs, high enzyme levels have been observed, reflecting the functional state of the liver and pancreas, as well as elevated bilirubin levels (Milastnaia and Dukhnitsky, 2019). Histological examinations revealed necrosis of the pancreas and surrounding

connective tissue. Common clinical-biochemical abnormalities included elevated serum transaminase levels and increased bilirubin concentrations. The similarity of clinical signs in dogs with acute and chronic pancreatitis suggests that chronic pancreatitis may result from repeated acute episodes. Biochemical analyses identified diagnostic markers for determining the chronic nature of the disease and differentiating the degree of microstructural lesions. Specifically, these included the absence of acute-phase reactants, stable proteinogram changes, increased aspartate aminotransferase (AST) activity, and more pronounced increases in alanine aminotransferase (ALT) and total bilirubin levels. Serum α-amylase activity remained at the lower end of the reference range. A significant marker of chronic pancreatitis progression was a 2.5–2.8-fold increase in serum chondroitin sulfate levels.

Thus, in diagnosing chronic pancreatitis with microstructural pancreatic lesions in dogs, the most informative laboratory and instrumental criteria can be identified at the time of examination. A 1.3-fold increase in alkaline phosphatase activity indicates cholestasis and impaired digestive enzyme function due to disrupted fat emulsification in the intestine. Alkaline phosphatase activity correlated with the severity of microstructural damage.

In severe microstructural lesions, a significant increase in ALT activity was observed compared to moderate and mild forms ($P<0.05$), unlike AST activity, which also increased in CP but showed no intergroup differences. As fibrotic lesions progress, affecting the pancreas and hepatobiliary system, bile retention occurs in the biliary tract. We established a significant 1.2-fold increase in this parameter in mild and moderate cases ($P<0.05$) and a 1.3-fold increase in severe cases ($P<0.01$). Elevated connective tissue metabolite levels in serum proved informative for diagnosing degrees of microstructural damage in chronic pancreatitis, with increases ranging from 2.5-fold in mild cases to 2.8-fold in severe cases ($P<0.001$). Increased ALT and AST activity indicates the potential development of cytolytic syndrome in pancreatic parenchyma and concomitant liver damage, as well as potential myocardial dystrophic changes due to intoxication in affected dogs. Acute pancreatitis is also characterised by increased cholesterol levels linked to elevated lipase activity, which contributes to free fatty acid formation.

Conclusions

Chronic pancreatitis in dogs is characterised by specific pathological microstructural changes in the pancreas. Depending on the severity of these changes, both loose and dense fibrosis may be observed. Dense fibrosis is accompanied by several microstructural alterations, including degeneration and atrophy of acini, and perineural lymphocytic infiltration. Inflammation is not diffuse but occurs in discrete regions of the organ, progressing with the destruction and loss of parenchyma. Loose fibrosis is typical of mild to moderate degrees of microstructural lesions in chronic pancreatitis.

Several informative laboratory diagnostic criteria for pancreatitis have been identified. A 1.3-fold increase in alkaline phosphatase activity has been observed, correlating with the severity of microstructural lesions. Severe forms are associated with a marked increase in alanine aminotransferase (ALT) activity compared to mild and moderate forms. Elevated levels of connective tissue metabolites in blood serum are a valuable diagnostic indicator

of chronic pancreatitis at various degrees of microstructural damage and may serve as a marker for the condition.

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Ovisnost biokemijskih parametara krvi o stupnju mikrostrukturnih promjena kod kroničnog pankreatitisa u pasa

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Studija je istražila biokemijske parametre krvi u usporedbi s histološkim nalazima u pasa s pankreatitisom. Prikupljeni su uzorci tkiva iz lijevih i desnih režnjeva i tijela gušterače tijekom obdukcije, nakon čega je uslijedio histološki pregled u svrhu procjene stupnja upale, fibroze i nekrotskih promjena. Ozbiljnost mikrostrukturnog oštećenja je klasificirana u tri stupnja, na temelju progresije fibroznih promjena: blaga, umjerena i ozbiljna (okarakterizirana gustom pankreatskom fibrozom). Histološke promjene u gušterači u slučajevima kroničnog pankreatitisa uspoređene su s onima pankreatskog tkiva zdravih pasa dobivenog nakon smrti životinja koje su stradale u prometnim nesrećama. Za svaku životinju, rezultati histoloških pregleda su uspoređeni s biokemijskim parametrima krvnog seruma, izmjerenima prije smrti tijekom faza dijagnoze i liječenja. U slučajevima blagih mikrostrukturnih promjena, došlo je do proliferacije veznog tkiva uz manju atrofiju egzokrine komponente, s očuvanim strukturama Langerhansovih-Sobolevovih otočića. Umjereno oštećenje uključivalo je izraženije odvajanje egzokrinog parenhima fibroznim tkivom. Ozbiljno oštećenje je okarakterizirano uključenošću acinarne stanice, uključujući vakuolarnu degeneraciju i atrofiju. Sporadična fibroza bila je tipična za blago i umjereno oštećenje, dok je gusta fibroza ukazivala na ozbiljne mikrostrukturne promjene. Upala u gušterači nije bila difuzna, već lokalizirana u specifičnim područjima. Studija je utvrdila da razvoj fibroze igra ključnu ulogu u patogenezi kroničnog pankreatitisa. Otkriveno je da su biokemijski parametri krvi korelirani s patološkim mikrostrukturnim promjenama. Preciznije, aktivnost alkalne fosfataze se povećala za 1,3 puta. U teškim slučajevima, aktivnost alanin-aminotransferaze je pokazala značajno povećanje u usporedbi s umjerenim i blagim oblicima. Povećane razine metabolita vezivnog tkiva u krvnom serumu su prepoznate kao informativni dijagnostički markeri za kronični pankreatitis raznih stupnjeva mikrostrukturnog oštećenja. Ti markeri pružaju vrijedan uvid u dijagnozu i napredak bolesti.

Ključne riječi: *žlijezda gušterača; fibroza; histološke promjene; istraga; stupanj lezije; dijagnostika*