Diagnostic algorithm for comorbidity of babesiosis and dirofilariasis in dogs, taking into account the correlation of redox homeostasis and morphofunctional disorders of the hepatorenal and cardiopulmonary systems

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Abstract. Activation of lipid peroxidation due to a disorder of redox homeostasis in dogs with comorbidity of babesiosis and dirofilariasis causes the involvement of not only the components of the hepatorenal system, but also the cardiopulmonary system in the pathological process against the background of the development of intoxication and hypoxic phenomena in hepatocytes and cardiomyocytes. As a result of the conducted biochemical studies of the blood of sick animals, it was found that the degree of redox homeostasis disorders directly correlates with the nature of morphofunctional disorders of the hepatorenal and cardiopulmonary systems, and the degree of these changes in the systems has an inverse correlation and is determined by the release of cytosolic enzymes into the blood. The involvement of the components of the hepatobiliary system in the pathological process is accompanied by a disorder of protein, carbohydrate, lipid and pigment metabolism.

1 Introduction

The anatomical and physiological unity of the liver with the kidneys and the heart with the lungs determines not only the commonwealth of their functions, but also some mechanisms of their compensation in pathological processes in the body. Disorders of organ hemodynamics, oxygenation and the development of metabolic disorders are the leading pathogenetic aspects in the development of mixed invasions, this problem is especially acute among vector-borne diseases[1, 2, 3].

In addition, the classical clinical picture of many diseases undergoes changes and gradually loses its typicality, and the issue of comorbidity of diseases is the result of the existence of biological systems[4]. In recent years, the problem of the development mechanism of the association of babesiosis and dirofilariasis remains open, and polymorphism of manifestations of canine babesiosis and the persistence of parasites is an important clinical problem, there are also many unresolved issues in the diagnosis of the
disease, therapy and prevention of this mixed invasion [5]. It has been established that the violation of the homeostatic mechanisms of redox homeostasis of the body [6] is the leading pathogenetic component in the development of morphofunctional disorders in comorbidity of babesiosis and dirofilariasis in dogs.

Thus, the associative course of babesiosis and dirofilariasis complicates both the choice of methods of therapeutic correction [7, 8, 9] and the prediction of the course of this mixed invasion [10, 11, 12], and the chronicity of this pathology and complications associated with intoxication, the development of metabolic disorders cardiopulmonary and hepatorenal systems do not allow to fully implement the complex of therapeutic and prophylactic measures [13, 14, 15].

The aim of our research was to study the nature of the correlation between redox homeostasis and morphofunctional disorders of the cardiopulmonary and hepatorenal systems in dogs with an associative course of babesiosis and dirofilariasis. To achieve this goal, we set the following tasks: to study the clinical status, biochemical blood parameters, data of ultrasonographic studies of the hepatobiliary system in dogs with mixed invasion of dirofilariasis - babesiosis.

2 Methods and Equipment

The work was performed during 2019-2020 at the Department of Therapy and Propedeutics of the Don State Agrarian University (Persianovskiy village) and on the basis of the Animal Clinic veterinary rehabilitation center (Rostov-on-Don).

In order to carry out the research, experimental and control groups of animals were formed. Each group consisted of 10 large breed dogs aged from 3.5 to 6 years old, patients with mixed infestation babesiosis-dirosfilariasis. The groups were formed according to the principle of pairs of analogs as the animals entered the veterinary clinic. Diagnosis was based on history, clinical findings, laboratory blood tests, and microscopy of peripheral blood smears. The clinical study of sick animals was carried out according to the generally accepted method.

In order to confirm the diagnosis of dirofilariasis in dogs, a blood smear was examined using the enriched smear method. For this, 1.0 ml of blood was mixed with 1.5 ml of 5% acetic acid, and then the mixture was centrifuged for 5 minutes at 3000 rpm. From the resulting sediment, smears were prepared, which were stained according to Romanovsky-Giemsa, and then microscopic examination was carried out under a low magnification microscope (10x). For the qualitative detection of the Dirofilaria immitis antigen, the immunochromatographic method (express test VetExpert Heartworm Ag) was used.

To make the final diagnosis of babesiosis, microscopy of capillary blood smears from the ear of dogs stained according to Romanovsky-Giemsa was performed.

After the diagnosis was made, blood sampling and biochemical studies were carried out in the dogs of the experimental and control groups. Blood for biochemical studies was taken from the saphenous vein of the forearm. The level of metabolic disorders in sick animals was studied using a biochemical analyzer BIOBASE-8021A. At the same time, the level of total protein (T-Pro) of blood serum was determined using a biuret reaction, albumin (ALB) - using bromocresolpurine, globulins (GLB) - by electrophoresis on plates with agarose gel, glucose (GLU) - by glucose oxidase method, total bilirubin (BIL-T) - by the Endrassic-Grof method, direct bilirubin (D-BIL) - by the Endrassic-Grof method, urea (UREA) - by the reaction with diacetylmonoxiam in a highly oxidized medium in the presence of thiosemicarbazide and ferric ions, creatinine (CREA) - by the Jaffa kinetic method (IDMS), cholesterol (CHOL) - by the enzymatic method, alanine aminotransferase (ALT) - by the Reitman-Frenkel method, aspartate aminotransferase (AST) - by the Henry method, alkaline phosphatase (ALP) - by the hydrolysis of glycerol cholinesterase (CHS) -
with S-butyrythiocholine iodide, lactate dehydrogenase (LDH) - by the kinetic lactate pyruvate method, creatine kinase (KFK) - by the UV kinetic test, γ-glutamyl transferase (GGT) - by the Shash method. The protein ratio (A/G), the ratio of GGT/AST, GGT/ALT and APRI were calculated.

To clarify the degree of damage to the liver parenchyma and determine the level of involvement of the hepatorenal system in the pathological process, the level of indirect markers of fibrosis was calculated by deriving the following indices: prothrombin index according to Quick (PTI), de Ritis coefficient (de Ritis), APRI - serum test.

Quick prothrombin index (PTI) was calculated according to the formula (Formula 1).

$$PTI = \left( \frac{PT_{\text{healthy animal}}}{PT_{\text{the examined animal}}} \right) \times 100\% \quad (1)$$

where $PT_{\text{healthy animal}}$ - reference value of prothrombin time in sec., $PT_{\text{the examined animal}}$ - prothrombin time of the studied animal sample in sec., 100% - conversion factor of the value into percent.

The calculation of the de Ritis coefficient (de Ritis) was carried out according to the following formula (Formula 2):

$$\text{De Ritis coefficient}(de\text{Ritis}) = \frac{AST}{ALT} \quad (2)$$

where $AST$ – blood aspartate aminotransferase U/l, $ALT$ – blood alanine aminotransferase U/l.

Calculation of APRI - serum test, was carried out according to the formula (Formula 3):

$$\text{APRI} = \left( \frac{AcAT}{(\text{upper limit \ AcAT})} \right) \times 100 / \text{platelets} (10^9/l) \quad (3)$$

where AcAT is the quantitative indicator of aspartate amine transferase in U/l, the upper limit of AsAT is the upper limit of the reference values of aspartate amine transferase in U/l for a given animal species, 100 is the conversion coefficient, platelets is the number of platelets in the studied animal in $10^9/l$.

Ultrasonographic studies of the hepatobiliary system were carried out in sick animals using the MindrayUMT-150 apparatus. At the same time, assessing the size, structure of the liver, gallbladder, as well as the presence of free fluid in the abdominal cavity.

The processing of the research results was carried out by the method of variation statistics using an integrated system for complex statistical analysis and data processing in the Windows STATISTICA system, using the Student's test according to the rules of variation statistics.

### 3 Results

As a result of clinical studies of sick animals, it was found that the manifestation of morphofunctional disorders of the hepatorenal and cardiopulmonary system in comorbidity of babesiosis and dirofilariasis in dogs was characterized by the development of signs of anxiety, general weakness, apathy, dyspnea during exercise, tachycardia (the number of heart contractions - 165 ± 2.0 beats per minute and 167 ± 3.0 beats per minute), tachypnoe (respiratory rate: 39.0 ± 4.0 respiratory movements per minute and 37 ± 2.0 respiratory movements per minute), anorexia and polydipsia. The development of pyretic fever (body temperature: $41.3 \pm 0.4^\circ$ Cand $41.7 \pm 0.6^\circ$ C).

In 18 animals, the mucous membranes of the oral cavity, the conjunctiva of the eyes were icteric, the urine had a red-brown tint. In sick dogs, an increase in intoxication, the development of dehydration and hypersalivation were recorded.

Bloody sputum was reported in 2 dogs. In this case, auscultation of the heart revealed an increase in the II tone over the pulmonary artery. Also, these animals showed the development of peripheral edema, expansion of the jugular veins, impaired pulmonary
ventilation, the development of obstructive syndrome. Cough and anemic mucous membranes were registered. Auscultation of the chest revealed dry, crackling rales.

Microscopic examination of a drop of blood of sick animals revealed the presence of microfilariae Dirofilaria immitis, their number ranged from 4 to 7 in the field of view of the smear. The results of the immunochromatographic test (express test VetExpert Heartworm Ag) indicated the presence of Dirofilaria immitis antigens in all animals of the experimental and control groups.

As a result of microscopy of capillary blood smears from the ear of sick dogs, the presence of paired pear-shaped forms of Babesia canis canis in erythrocytes was established, while parasitemia reached 1%.

The results of the conducted sonographic studies of the hepatobiliary system in dogs with comorbidity of babesiosis and dirofilariasis indicated the development of diffuse changes in the liver parenchyma. Visualization of the organ was normal, the posterior border of the liver went beyond the costal arch, while its edges were rounded, clear, smoothed, and the contour was even (Fig. 1: a).

![Fig. 1](image)

**Fig. 1.** The result of an ultrasonographic study of the hepatobiliary system in dogs with comorbidity of babesiosis and dirofilariasis: rounded edges and a smooth contour of the liver (a), an increase in liver size (b).

The liver was enlarged (Fig. 1: b), her capsule was well differentiated. The echo structure of the organ was heterogeneous, coarse-grained. There was an increase in the oblique vertical size of the liver and the thickness of its right half with comorbidity of babesiosis and dirofilariasis in dogs. There was a decrease in the general echogenicity of the organ, an increase in the vascular pattern, the bile ducts were dilated. The gallbladder was well visualized, the echo structure of its wall was hyperechoic, and its contents were anechoic.

The spleen was enlarged, and a displacement of its caudal pole was noted. The parenchyma of the organ was hypechoic and homogeneous.

The kidneys were well visualized, their borders were indistinct, there was a thickening of the parenchyma, the echogenicity of the cortical and medullary layers was reduced, and cortical-cerebral differentiation was indistinct.

As a result of the conducted biochemical studies of the blood of sick animals, significant biochemical changes were revealed due to a violation of the metabolic activity of the liver and damage to its parenchyma, which was accompanied by the development of hyperproteinemia ($T$-$Pro - 70.02±1.15$ g/l and $69.18±1.74$ g/l) and dysproteinemia ($ALB - 26.03±0.59$ g/l and $25.70±0.80$ g/l; $GLB - 43.99±0.90$ g/l and $43.48±1.01$ g/l; $A/G - 0.59$ and $0.59$) (Table 1). At the same time, the deviation of the protein-synthetic function of the liver in the experimental group was 6.90% and 5.62% in the control group, compared with the arithmetic mean of the reference values. The extreme elements of the variation series were
presented as follows: T-Pro (max X – 72.10 g/land71.60 g/l; min X –68.85 g/land66.95 g/l), ALB (max X – 26.62 g/land25.57 g/l; min X – 25.47 g/land24.70 g/l), GLB (max X - 44.89 g/land44.59 g/l; min X – 43.01 g/land42.46 g/l)andA/G(max X – 0.59 and0.60; min X – 0.59 and0.55).

In addition, an increase in ESR (8.90±0.70mm/hand9.60±0.64mm/h) andy-glutamyl transferase (GGT - 47.81±2.17 U/l and49.10±1.40 U/l) in sick dogs, it also testified to a violation of the protein-synthetic function of the liver and the development of dysproteinemia (decrease in ALB by 16.03% and 17.09%, increase in GLB by 25.68% and 24.23%).

Table 1. The level of metabolic activity of blood in dogs with morphofunctional disorders of the cardiopulmonary and hepatorenal systems against the background of comorbidity of babesiosis and dirofilariasis.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Test (n = 10)</th>
<th>Control (n = 10)</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X±Sx</td>
<td>max X</td>
<td>minX</td>
</tr>
<tr>
<td>Total protein (T-Pro), g/l</td>
<td>70.02±1.15*</td>
<td>72.10</td>
<td>68.85</td>
</tr>
<tr>
<td>Albumen (ALB), g/l</td>
<td>26.03±0.59**</td>
<td>26.62</td>
<td>25.47</td>
</tr>
<tr>
<td>Globulins (GLB), g/l</td>
<td>43.99±0.90*</td>
<td>44.89</td>
<td>43.01</td>
</tr>
<tr>
<td>Protein coefficient (A/G)</td>
<td>0.59**</td>
<td>0.59</td>
<td>0.59</td>
</tr>
<tr>
<td>Glucose (GLU), mmol/l</td>
<td>4.53±0.15*</td>
<td>4.69</td>
<td>4.40</td>
</tr>
<tr>
<td>Urea (UREA), mmol/l</td>
<td>12.34±0.21**</td>
<td>12.55</td>
<td>12.12</td>
</tr>
<tr>
<td>Creatinine (CREA), µmol/l</td>
<td>116.40±1.66*</td>
<td>118.0</td>
<td>114.6</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP), U/l</td>
<td>177.90±7.30*</td>
<td>186.4</td>
<td>169.8</td>
</tr>
<tr>
<td>Cholinesterase (CHS), mmol/l</td>
<td>214.10±1.26*</td>
<td>215.5</td>
<td>213.8</td>
</tr>
<tr>
<td>Cholesterol (CHOL), mmol/l</td>
<td>7.00±2.00*</td>
<td>9.06</td>
<td>5.10</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT), U/l</td>
<td>215.20±9.07*</td>
<td>225.0</td>
<td>205.9</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST), U/l</td>
<td>173.09±6.07*</td>
<td>181.0</td>
<td>165.9</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH), U/l</td>
<td>172.30±0.30*</td>
<td>172.9</td>
<td>171.8</td>
</tr>
<tr>
<td>Creatine kinase (KFK), U/l</td>
<td>305.80±10.50*</td>
<td>316.7</td>
<td>296.2</td>
</tr>
<tr>
<td>Total bilirubin (BIL-T), µmol/l</td>
<td>13.90±0.25*</td>
<td>14.20</td>
<td>13.05</td>
</tr>
<tr>
<td>Bilirubin direct (D-BIL), mmol/l</td>
<td>6.90±1.81*</td>
<td>8.90</td>
<td>5.01</td>
</tr>
<tr>
<td>Gamma glutamyltransferase (GGT), U/l</td>
<td>47.81±2.17**</td>
<td>50.10</td>
<td>46.05</td>
</tr>
<tr>
<td>De Ritis coefficient (deRitis) (AST/ALT)</td>
<td>0.80±0.10***</td>
<td>0.70</td>
<td>0.90</td>
</tr>
<tr>
<td>GGT/AST</td>
<td>0.28±0.01**</td>
<td>0.29</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Disorder of carbohydrate metabolism in sick animals of both groups was accompanied by the development of hypoglycemia (GLU -4.53±0.15mmol/l and4.46±0.20mmol/l), the deviation of glycosynthesis processes in dogs in the experimental group was3.62 %, and in the control -5.12 % compared to the arithmetic mean of the reference values. The extreme elements of the variation series were left in the experimental group. 4.69 mmol/l (max X) and4.40 mmol/l (min X), and in the control - 4.67 mmol/l (max X) and4.20 mmol/l (min X).

Lipid metabolism was characterized by an increase in cholesterol levels (CHOL -7.00±2.00 mmol/land7.60±1.80 mmol/l) in the blood of sick animals, the extreme elements of the CHOL variation series were9.06 mmol/l(max X) and5.10 mmol/l (min X) in the experimental group, 9.50 mmol/l(max X) and5.70 mmol/l (min X) - in the control (Table 1).

The involvement of the hepatorenal system components in the pathological process in dogs with comorbidity of babesiosis and dirofilariasis was accompanied by a disorder of nitrogen metabolism, which was manifested by an increase in the level of urea (UREA -12.34±0.21 μmol/l and11.97±0.19 μmol/l) and creatinine (CREA - 116.40±1.66μmol/l and119.70±1.73 μmol/l), which exceeded the level of the arithmetic mean of the reference values in the experimental group by 105.67% and 38.57%, and in the control group by 99.50% and 42.50%, respectively. The extreme elements of the UREA variation series in animals of the experimental and control groups were max X – 12.55 μmol/landmin X - 12.12 μmol/l, in the control - 12.16 μmol/land11.74 μmol/l, a CREA in the experimental group maxX – 118.05 μmol/landmin X - 114.62 μmol/l, and in the experimental one - 121.45 μmol/l and117.90 μmol/l respectively.

Pigment metabolism in animals of both groups was accompanied by a significant increase in the level of total (BIL-T - 13.90 ± 0.25 μmol / l and 15.05 ± 0.30 μmol / l) and direct bilirubin (D-BIL - 6.90 ± 1.81mmol / l and 6.55 ± 1.49 μmol / l).

Activation of lipid peroxidation processes in babesiosis-dirofilariasis mixed invasion in dogs promoted the release of cytosolic enzymes into the blood: ALT, AST, LDH, GGTand the enzyme of the biliary pole of hepatocyte membranes - ALP, which was manifested by an increase in the level ALT (215.20±0.07 U/l and227.10±8.30 U/l), AST (173.09±6.07U/l and190.30±5.90 U/l), LDH (172.30±0.30 U/l and 180.90±0.40 U/l), GGT (47.81±2.17 U/l and 49.10±1.40 U/l) andALP (177.90±7.30 U/l and 181.30±7.90 U/l). At the same time, the level of ALT in the experimental group was higher than the arithmetic mean of the reference values by 481.62 % and505.60 % in the control group, AST- at835.62 % and898.92 %, LDH – at 89.34 % and 98.79 %, GGT – at 1285.79 % and 1323.19 %, ALP – at4.64 % and6.65 % respectively. The extreme elements of the variation series were presented as follows: ALT (max X – 225.07 U/l and236.40 U/l; min X – 205.90 U/l and218.90 U/l ), AST (max X – 181.05 U/l and196.30 U/l; min X – 165.90 U/l and184.80 U/l), LDH (max X – 172.90 U/l and 181.37 U/l; min X – 171.87 U/l and180.23U/l), GGT (max X – 50.10 U/l and50.60 U/l; min X –46.05U/l and47.70 U/l) andALP (max X – 186.40 U/l and188.60 U/l; min X –169.80U/l and173.05 U/l).

An increase in the GGT / ALT coefficient in animals of the experimental group to 0.22 ± 0.02 and to 0.21 ± 0.03 in the control group indicated damage to hepatocytes against the background of the development of oxidative stress.

An increase in the level of alkaline phosphatase (ALP) against the background of an increase in the level of lactate dehydrogenase (LDH) indicates the development of cytolytic
syndrome and damage to the small bile ducts, and an increase in the level of γ-glutamyl transferase (GGT) against the background of an increase in the level of alkaline phosphatase (ALP) indicates damage to hepatocytes, leading to an increase in the GGT / ALP ratio (0.22 ± 0.02 and 0.21 ± 0.03) by 300.00% in the experimental group and by 281.82 % in the control compared to the arithmetic mean of this indicator.

Decreased cholinesterase levels (CHS - 214.10±1.26 mmol/l and 221.80±1.15 mmol/l) in dogs of both groups indicated the involvement of the liver parenchyma in the pathological process and the development of mass death of hepatocytes in the comorbidity of babesiosis and dirofilariasis. The extreme elements of the CHS range in the experimental group were 215.50 mmol/l (max X) and 213.80 mmol/l (min X), in the control group - 223.05 mmol/l and 220.60 mmol/l, respectively.

Increased lactate dehydrogenase levels (LDH – 172.30±0.30 U/l and 180.90±0.40 U/l) and creatine kinase (KFK – 305.80±10.50 U/l and 297.09±12.40 U/l) in dogs of both groups, evidence of the involvement of the cardiopulmonary system in the pathological process in the comorbidity of babesiosis and dirofilariasis due to parasitism of Dirofilariaimmitis. The extreme elements of the KFK range in the experimental group were 316.70 U/l (max X) and 296.20 U/l (min X), and in the test group and max X - 301.93 U/l and 285.10 U/l, respectively.

The level of indirect markers of liver fibrosis in dogs with comorbidity of babesiosis and dirofilariasis was characterized by an increase in APRI (0.99 ± 0.03 and 1.01 ± 0.01) and a GGT/AST ratio (0.28 ± 0.01 and 0.26 ± 0.02), a decrease in the de Ritis coefficient (AST/ALT - 0.80 ± 0.10 and 0.84 ± 0.15), which indicated the development of functional liver failure. At the same time, the APRI level in the experimental group was higher than the arithmetic mean of the reference values by 182.86% and 188.57% in the control group, the GGT/AST ratio - by 250.00% and 225.00%, the de Ritis coefficient was lower by 38.46% and 36.38%, respectively.

4 Discussion

Disorder of redox homeostasis in dogs with comorbidity of babesiosis and dirofilariasis was characterized by the involvement of the components of the hepatorenal and cardiopulmonary systems in the pathological process, which was accompanied by the release of cytosolic enzymes and the biliary pole of hepatocyte membranes into the blood. Morphofunctional disorders of the components of the hepatobiliary system were accompanied by a disorder of protein, carbohydrate, lipid and pigment metabolism, an increase in the GGT/ALT and GGT/ALP coefficient, a decrease in the level of cholinesterase (CHS), which confirmed the involvement of the liver parenchyma in the pathological process and massive death of hepatocytes due to parasitization of Babesiacanis. An increase in the level of alkaline phosphatase (ALP) against the background of an increase in the level of lactate dehydrogenase LDH confirmed the development of cytolytic syndrome and damage to small bile ducts, and an increase in the level of γ-glutamyltransferase (GGT) against the background of an increase in the level of alkaline phosphatase (ALP) indicates damage to hepatocytes. The development of functional liver failure in dogs with comorbidity of babesiosis and dirofilariasis was confirmed by an increase in the APRI and GGT/AST ratio, and a decrease in the de Ritis coefficient (AST/ALT).

Disorder of nitrogen metabolism was revealed, accompanied by an increase in creatinine and urea levels due to impaired renal filtration and the development of acute renal failure in dogs with mixed infestation babesiosis-dirofilariasis. In the ultrasonographic structure, these functional changes led to the development of severe damage to hepatocytes...
of the type of acute inflammation, bile outflow disorder, splenomegaly and changes in the morphological picture of the kidneys.

An increase in the level of lactate dehydrogenase (LDH) confirmed the involvement of the myocardium in the pathological process due to the parasitization of Dirofilaria immitis in babesiosis-dirofilariaisis mixed invasion in dogs.

5 Conclusion

Thus, with the comorbidity of babesiosis and dirofilariaisis in dogs, there is a direct correlation between the level of morphofunctional disorders of the hepatorenal system and the nature of redox homeostasis disorders, which appears as a disorder of the metabolic activity of the liver, an increase in the catalytic activity of serum enzymes due to the activation of lipid peroxidation processes, as well as a disorder of nitrogenous exchange. A direct correlation was also found between the level of oxidative stress and morphofunctional disorders of the cardiopulmonary system in dogs; in addition, an inverse correlation was established between the degree of functional disorders of the hepatorenal and cardiopulmonary systems.

Consequently, the diagnostic algorithm for comorbidity of babesiosis and dirofilariaisis in dogs should be carried out taking into account the correlation between the level of redox homeostasis and morphofunctional disorders of the hepatorenal and cardiopulmonary systems, based on the data of biochemical blood tests and the results of ultrasonographic studies. The combination of such diagnostic criteria makes it possible to more holistically represent not only the nature of the lesion of the cardiopulmonary system and the involvement of the components of the hepatorenal system in the pathological process, but also to further predict the pathological process, taking into account the commonality of the systems in the body of sick dogs.

Conflict of Interest

The authors have no conflict of interest to declare.

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