ORIGINAL ARTICLE

CONTENTS

Role of autoimmune processes in the initiation of biliodigestive anastomoses leakage in conditions of cholangitis

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ABSTRACT

Aim: To determine the role of autoimmune reactions on healing of BDA in patients with cholangitis.

Materials and Methods: 166 (100%) non-oncology patients with cholangitis, were divided into three groups. Group I – 92 (55.4%) patients – cholangitis caused by choledocholithiasis. Endoscopic papillotomy with lithoextraction or choledocholithotomy were performed. Group II – 48 (28.9%) patients – cholangitis due to benign strictures of the bile ducts – patients underwent reconstructive interventions. Group III – 26 (15.7%) patients – cholangitis in presence of biliodigestive anastomosis, which required reconstruction.

Results: Autoantibodies to own tissues were found in blood plasma of some patients of II and III groups. Morphological examination of the liver tissue of these patients revealed changes in the form of bridge-like and stair-like necrosis with lymphocyte-macrophage infiltration, which is characteristic of autoimmune damage. In parallel with this, a decrease in the functional condition of the liver was ascertained, which had a decisive effect on the chemical composition of bile, caused sedimentation processes and the appearance of sludge. As a result of reduction of reparative processes in the area of biliodigestive anastomosis, anastomotic leakage developed, then - stenosis.

Conclusions: With a long duration of chronic cholangitis, destructive changes occur in the tissues of the liver and bile ducts, which are caused by autoimmune reactions.

The presence of autoimmune antibodies is a marker of a high probability of deterioration of reparative processes in the area of the biliodigestive anastomosis and contributes to the development of anastomosis leakage.

KEY WORDS: autoimmune reactions, cholangitis, biliodigestive anastomoses

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INTRODUCTION

According to the world literature, the frequency of development of cholangitis after biliodigestive anastomosis (BDA) varies from 4.4% [1] to 13% [2]. Of these, in 57.8% of patients subsequently stricture will develop [3], leading to death in 15% of cases [4]. Therefore, the problem of prevention and treatment of cholangitis after BDA remains relevant.

Among patients with a chronic recurrent course of cholangitis with multiple exacerbations, there is a group of patients, in whom a Roux-en-Y hepaticojejunostomy (HJS) was applied in the past. But this did not bring the improvement, the phenomena of cholangitis and mechanical jaundice did not disappear. Each new reBDA becomes technically more complicated. This increases the likelihood of postoperative complications, including anastomosis leakage. A vicious circle emerges: on the one hand, application of BDA in cholangitis is contraindicated; on the other hand, application of BDA is one of the methods of final treatment of biliary obstruction. Minimally invasive methods of cholangitis treatment (endoscopic papillotomy with stenting, percutaneous transhepatic drainage, as well as laparoscopic interventions) in these patients can be considered as the first stage of treatment [5]. Sometimes these methods could not be used or contraindicated. In these cases, surgeons perform BDA in conditions of cholangitis. It often is complicated by anastomosis leakage [6].

The vast majority of chronic inflammatory processes are accompanied by autoimmune reactions. Cholangitis is no exception. A number of authors suggest that autoimmune processes can play a significant role in the etiology and pathogenesis of cholangitis [7, 8, 9]. In particular, Haiyan Zhang et al. (2018) [10] showed

that autoreactive serum IgA to cells of the biliary epithelium is present in most patients with cholangitis, while these antibodies are practically absent in healthy individuals. Natural autoantibodies under physiological conditions are found in small quantities and do not cause pathological processes, but on the contrary, stimulate tissue regeneration. But an increase in their number can cause the formation of immune autoaggression [11].

AIM

To determine the role of autoimmune reactions on healing of BDA in patients with cholangitis.

MATERIALS AND METHODS

During the treatment of 166 (100%) patients, cholangitis was diagnosed according to the signs of the Tokyo Guidelines (2013). According to the required surgical tactics, we distinguished three groups of patients. Group I – 92 (55.4%) patients – cholangitis due to choledocholithiasis and stenotic papillitis. In patients of this group, following interventions were performed: endoscopic papillotomy with lithoextraction (79 patients), laparotomy with choledocholitotomy (8 patients) and laparoscopic stones extraction (5 patients). Group II – 48 (28.9%) patients – cholangitis due to benign biliary strictures of the main bile ducts, which required reconstructive interventions – BDA. Group III – 26 (15.7%) cases – cholangitis in patients with previously performed BDA which required reoperation.

Bile collection for research was obtained during duodenoscopy and papillotomy (comparison group), during intervention or postoperatively from external biliary drainage. The amount of primary bile acids was determined by an immunoenzymatic analyzer "StatFax 3200" (USA).

The concentration of immunoglobulins IgA, IgM, IgG in blood serum was determined by the enzyme immunoassay method; the presence of specific antinuclear autoantibodies (ANA), antibodies to smooth muscle actin (ASMA), antibodies to liver and kidney microsomal antigen (LKM) was assessed by indirect immunofluorescence.

Statistical data processing was performed using the Statistica 5.0 for Windows package. The sample mean (M) and standard deviation of the mean (σ) were determined. The reliability of the differences in indicators was determined according to the Student's test with a selected confidence probability of 95% ($p \leq 0.05$).

The Pearson test ($\chi 2$) was used to analyze qualitative data.

RESULTS

Detection of autoantibodies is one of the main criteria in the diagnosis of autoimmune processes of the hepatobiliary system [10]. ANA, ASMA and LKM antibodies, as well as IgA, are the most important. The formation of autoimmune reactions was detected in some patients with cholangitis, which was confirmed by the presence of specific autoantibodies in the blood serum. We previously reported that in patients with chronic cholangitis, a significant increase in class A antibodies was detected in patients with biliary strictures and in patients after reconstructive interventions [12].

Table 1 shows the number of cases of autoantibodies in the studied groups of patients. In groups II and III, chronic recurrent cholangitis lasted more than 6 months.

In short-term cholangitis (group I), autoantibodies were never detected. A significant increase in the content of membranotropic cytotoxic factors, including autoantibodies to own lymphocytes, was found in groups with a chronic course of cholangitis, and this indirectly indicates the formation of autoimmune reactions. In group II, autoantibodies (any or their combination) were detected in 8 (16.7%) patients, in group III - in 14 (53.8%) patients.

The amount of bile acids in bile was studied in patients who had already undergone reconstructive interventions on the biliary tract, but still had attacks of cholangitis (group III). This group of patients was divided into two subgroups. Subgroup "a" - patients in whom autoimmune antibodies were not found, and subgroup "b" - patients with positive reactions to autoimmune antibodies (Table 2).

As can be seen from the indicators, in the presence of autoantibodies caused by long-term cholangitis, in the biochemical analysis of bile amount of primary bile acids was critically reduced. So the autoimmune destruction of hepatocytes reduced the concentration of bile acids in bile and thus facilitated the processes of sedimentation in it.

Morphological examination of liver biopsies in patients of the III group, who had autoantibodies, revealed changes typical for cholestatic and autoimmune hepatitis of various degrees of severity. With a mild and moderate degree of severity, these processes manifested mainly in the form of portal hepatitis with weakly or moderately pronounced lympho-macrophagic infiltration of the portal tracts, often with an admixture of plasma cells and eosinophils, with the spread of the infiltrate to the adjacent parts of the liver lobules (Fig. 1).

With a severe course, the process corresponded to active (aggressive) hepatitis. There was a dense lymphocytic-macrophagic infiltration with admixtures of eosin-

Table 1. The presence of autoantibodies in the blood plasma of patients with cholangitis

Specificity of autoantibodies	Patients examined for the presence of autoantibodies		
	I (choledocholithiasis, cholangitis) n = 36	II (stricture, cholangitis) n = 48	III (HJA, cholangitis) n = 26
ASMA	-	4	6
ANA (antibodies to enzymatic complexes of mitochondria)	-	4	9
anti-LKM	-	3	5
The total number of patients with any autoantibodies or their combination	-	8	14

Table 2. The amount of primary bile acids in bile depending on the presence or absence of autoimmune antibodies

Measurement terms		Group I (antibodies are absent) n = 32 M ± σ	Group III (n = 26)	
			Subgroup "a" (antibodies are absent) n = 12 M ± σ	Subgroup "b" (antibodies are present) n = 14 M ± σ
Cholic acid (nmol/ml)	Initial	3,59±1,15	1,57±0,21	1,32±0,21
	After the intervention (3rd day)	3,62±1,17	1,62±0,22	1,41±0,22
	After intervention (10 days)	-	2,33±0,14*	1,02±0,13*
Chenodeoxycholic acid (nmol/ml)	Initial	17,31±1,5	4,61±0,33	3,92±0,31
	After the intervention (3rd day)	17,02±1,5	5,23±0,38*	4,25±0,32*
	After intervention (10 days)	-	9,98±0,41*	6,04±0,38*

The sign * indicates reliable differences in indicators between groups, obtained at the same time

ophils and neutrophils in the portal tracts. In the liver lobules, the infiltrate captured not only their peripheral parts, but also the middle and even central zones. At the same time, necrosis of some hepatocytes or small groups of them was observed, and not only in the zone of the "limiting plate", but bridge-like and step-like porto-central and porto-portal types of necrosis occurred most often (Fig. 2, Fig. 3). In their place, the formation of porto-central and porto-portal fibrosis zones was detected. The surviving hepatocytes showed severe alterative changes, often with the formation of Mallory bodies, which are known to have antigenic properties.

In the bile capillaries and ducts, phenomena of cholestasis were observed, often with the destruction of hepatocytes and epithelial cells of the ducts.

A very similar picture was found when examining the walls of the bile ducts. Their degeneration and focal destruction were established.

All patients of the II and III groups underwent Roux-en-Y hepaticojejunostomy (or bihepaticojejunostomy) and Praderi drainage. The presence of BDA leakage was determined as bile discharge through the abdominal drainage or presence of bile collections. In group II (48 patients), anastomotic leakage occurred in 17 (28.2%) patients: in 12 without autoantibodies and in 5 with the presence of autoantibodies. In group III (26 patients), anastomotic leakage occurred in 7 (37.1%): in 6 without autoantibodies and in 1 case with the presence of autoantibodies (Fig. 4). According to the Pearson test: the presence of autoantibodies increases the probability of the development of BDA leakage both during primary reconstruction ($\chi 2 = 3.076$; $p < 0.1$) and during reconstruction ($\chi 2 = 3.914$; $p < 0.05$).

DISCUSSION

According to obtained morphological, physicochemical, bacteriological and immunological data, we concluded that there is a close causal relationship between the start and manifestation of autoimmune processes and poor results of treatment of patients with chronic cholangitis. Based on these results, we created a chain of probable pathophysiological processes that trigger the autoimmune component of inflammation (Fig. 5).

With long-term chronic inflammation in the bile ducts, a moment occurs when during the destruction of own cells (cholangiocytes and hepatocytes) and their autolysis by macrophages (antigen-presenting cells), antigenic high-molecular polypeptides or their fragments are formed. They are not recognized as "host" for some reason. As a result, an immune response is triggered and autoimmune reactions are

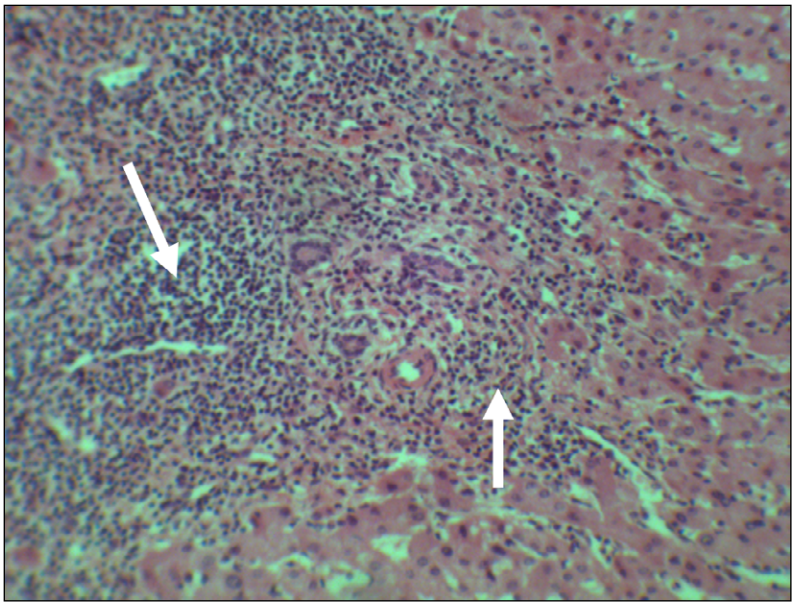

Fig. 1. Lymph- and macrophage infiltration of the stroma of the portal tracts. Preparing with hematoxylin and eosin. x 40

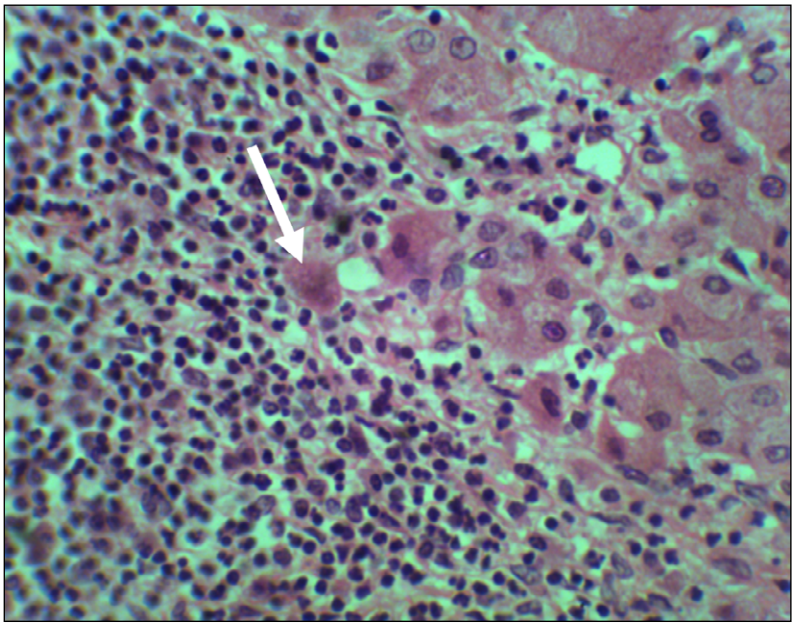

Fig. 2. Step-like necrosis of hepatocytes on the periphery of the liver lobules (staining with hematoxylin and eosin, x100)

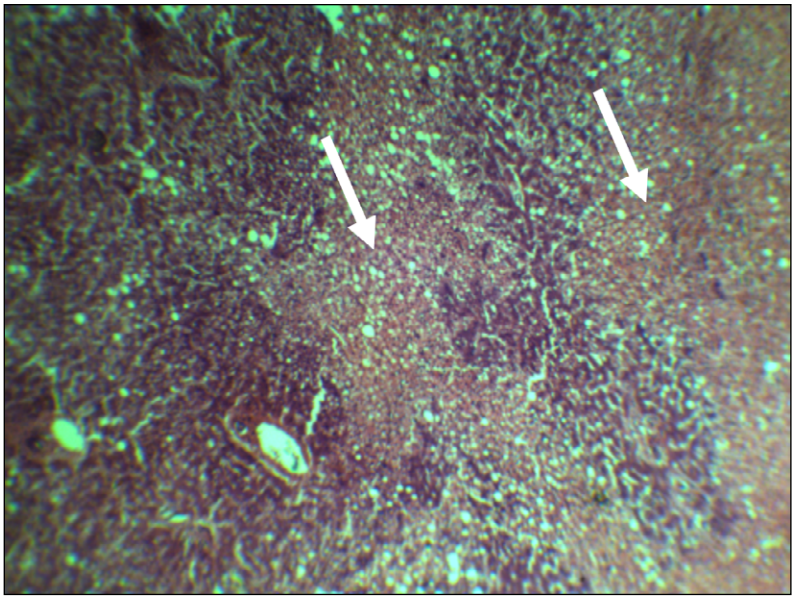

Fig. 3. Bridge-like necrosis (staining with hematoxylin and eosin, x40)

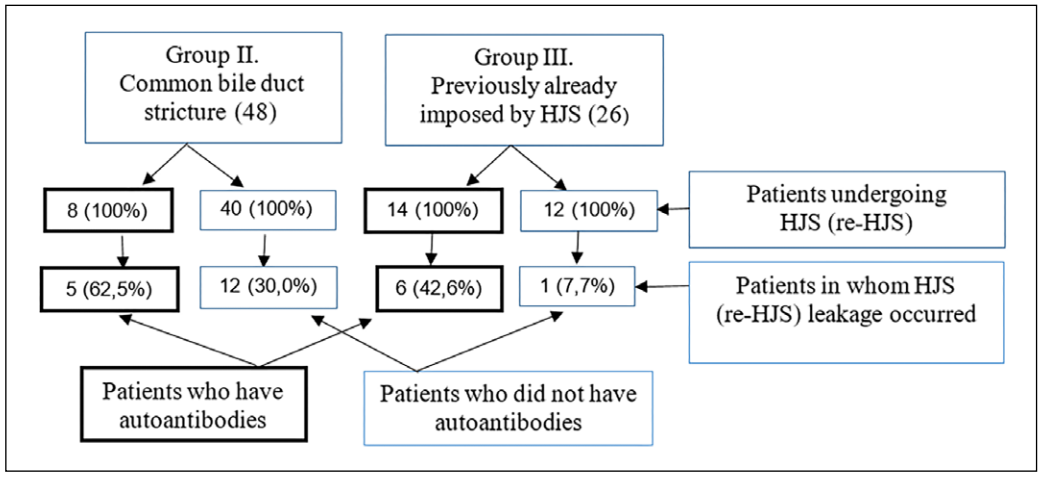

Fig. 4. The frequency of occurrence of hepaticojejunoanastomosis leakage (rehepaticojejunoanastomosis) depending on the presence of autoantibodies in the patient's blood

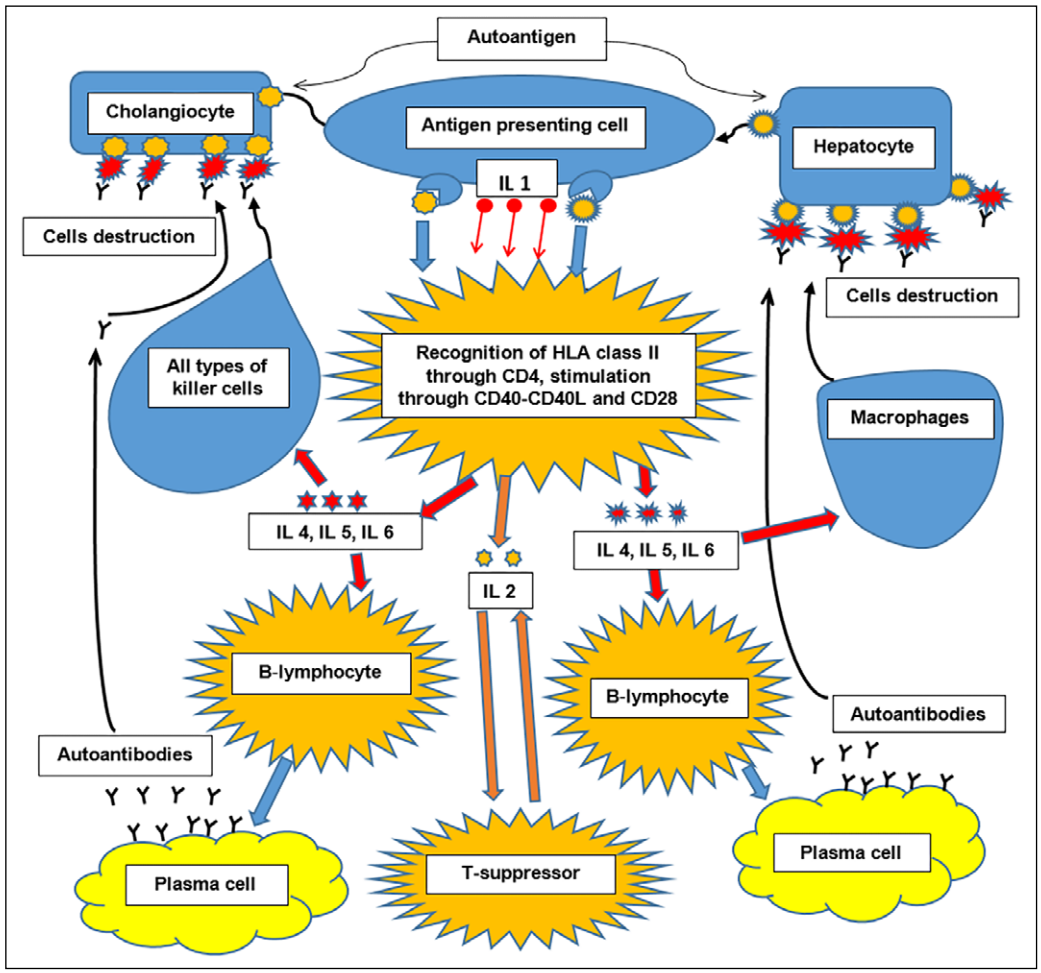

Fig. 5. Scheme of the probable mechanism of autoimmune reactions in chronic cholangitis

formed. The receptor of T-helper recognizes an antigenic determinant (epitope) expressed together with the molecule of the major histocompatibility complex II (MHC-II) on the surface of the macrophage. As a result of this interaction, T-helper 2 triggers the synthesis of a number of cytokines (IL-4, IL-5, IL-6), which stimulate the transformation of B-lymphocytes into plasma cells. The plasma cell then begins to produce antibodies, including to autoantigens – high-molecular compounds of hepatocytes and cholangiocytes. Cytokine stimulation of CD8+ T-suppressor receptors causes activation of effector cells (destroyer cells, killer cells). Violation of the regulatory action of T-suppressors in relation to various immunocytes, including T-helpers, B-lymphocytes, T- and B-effectors, can lead to the production of an unlimited number of antibodies, in particular, in relation to self-antigens. This leads to the formation of autoimmune reactions aimed at the destruction of hepatocytes and cholangiocytes by autoantibodies, killer cells and complement. Due to the fact that the targets of autoantibodies are the own tissues of the biliary system, immune-mediated

destruction of hepatocytes and cholangiocytes is triggered. Further, the course of chronic cholangitis turns into a completely different state - a state with a complex of aggressive autoimmune reactions. At some stage, the consequences of these autoimmune processes begin to have a decisive effect on the reparative processes in the area of formed BDA and on the functional condition of the liver. As a result, there is a decrease in the concentration of bile acids in the bile, the appearance of sludge, problems with patency of BDA and a recurrence of cholangitis.

We did not find any direct data on the negative impact of autoantibodies on BDA repair in the available literature. However, there are studies on the mechanisms of development of cholangiopathies with chronic inflammation in the biliary system. According to C. Pinto et al. (2018), cholangiopathies are genetic, infectious, and immune-mediated [13]. For the latter, the leading role belongs to disorders of immune processes at the level of cholangiocytes. In chronic bacterial inflammation, the expressed cholangiocyte releases an excessive amount of pro-inflammatory mediators. Self-directed autoimmune processes occur, causing massive apoptosis of cholangiocytes. The regulation of the balance between the cessation of inflammation and the continuation of inflammation is disturbed. This can affect reparative processes in the BDA zone.

According to T. Cargill et al. (2021), a significant role in the development of autoimmune reactions in chronic inflammation of the bile ducts belongs to the excessive activity of B cells, which have transformed into plasma cells. They secrete excessive amounts of IgA and IgG. At the same time, autoantibodies levels are correlated with biochemical and pathomorphological changes in the biliary system [14].

It is difficult to predict the course of a chronic inflammatory process in the biliary system, especially in cases of autoimmune reactions that damage one's own tissues. The inclusion of immunosuppressants into treatment is highly questionable. Nevertheless, the use of drugs for symptomatic therapy (salts of ursodeoxycholic acid) with constant intake in maximum therapeutic doses is sometimes enough to obtain a positive effect.

Surgical tactics and medical treatment of chronic cholangitis, complicated by autoimmune processes, with application of BDA in conditions of persistent inflammation of the biliary system, require further careful study and development of new approaches.

CONCLUSIONS

With a long duration of chronic cholangitis, destructive changes occur in the tissues of the liver and bile ducts, which are caused by autoimmune reactions.

The presence of autoimmune antibodies is a marker of a high probability of deterioration of reparative processes in the area of the biliodigestive anastomosis and contributes to the development of anastomosis leakage.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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