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Clinical and laboratory criteria for diagnosis of bacterial vaginosis. Literature review

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Abstract

The presence of bacterial vaginosis in women, especially for a long time, increases the risk of developing inflammatory diseases of the pelvic organs, creating serious problems after gynecological surgical interventions. In addition, bacterial vaginosis can be a cause of complications of pregnancy and childbirth, contributing to the miscarriage of pregnancy and premature birth. Bacterial vaginosis can lead to the development of chorioamnionitis, postpartum endometritis, intrauterine fetal infections, peritonitis. In addition, bacterial vaginosis is associated with infection of the urethral tract, cervicitis; is a risk factor for infection with sexually transmitted infections and HIV infection. In conditions of high pH there are favorable conditions for colonization of the genitourinary system by pathogenic microorganisms, as well as exacerbation of the latent course of viral infection of the urogenital tract. Mixed infections or infections developed on the background of severe imbalance in the vaginal microcenosis are observed in 20-30% of cases of clinically pronounced vaginal infections. Bacterial vaginosis negatively affects the quality of life of women. It is shown that in the presence of long-term and abundant discharge from the vagina in patients possible development of psychosomatic disorders, the trinity reduces performance, violates the sexual and childbearing functions, decreases the quality of life. The feature of bacterial vaginosis is its predisposition to relapse after treatment, which is observed in 50% of cases. Bacterial vaginosis is one of the most important factors in the development of

inflammatory diseases of the pelvic organs and complications of pregnancy, in particular - the infection of amniotic fluid, as well as the formation of postoperative complications. Despite the various medical and prophylactic measures conducted in different countries, the reduction of the incidence of bacterial vaginosis is not noted, which makes the problem of finding new ways of effective diagnosis and treatment of bacterial vaginosis.

Key words: bacterial vaginosis, diagnostics, infectious diseases

For the diagnosis of bacterial vaginosis two categories of tests have been developed: clinical criteria and laboratory tests [1]. A preliminary diagnosis of bacterial vaginosis can be posed already during the gynecological examination. The review begins with the external genitalia, which pay attention to the color and edema of the vulvar mucosa, parauurethral moves, the outer opening of the urethra and the ducts of the large glands of the vagina.

Then, using vaginal mirrors, examine the walls of the vagina, paying attention to their puffiness [1, 2]. Differential diagnosis is carried out with candidiasis and trichomoniasis. For candidiasis is characterized by severe hyperemia of the vulva and the vagina, with trichomoniasis, note the superficial focal flushing of the vulva, as well as the appearance of bright red spots on the mucous membranes of the cervix and the walls of the vagina. When looking at the cervix, attention is drawn to the presence of purulent cervicitis, erosion or proliferative changes. During the review, the presence and nature of the allocations. Isolation - dense, abundant, whitish color often accompanied by candidiasis; yellowish or greenish-gray, foamy, often smelly detected with trichomoniasis [1,2].

According to the criteria of Amsel (Amsel) for bacterial vaginosis is characterized by: vaginal pH> 4.5; 20% of the epithelial cells of the vaginal smear are "key cells"; the presence of secretions from the vagina of a specific nature; positive aminotest on a specific odor, which is associated with the release of amine after adding 10% potassium hydroxide solution to vaginal discharge. The presence of three of the four Amsel criteria is sufficient for the diagnosis of bacterial vaginosis [1, 3]. Determine the pH of the vaginal medium, applying an indicator paper strip, directly to the vaginal wall, or collect the vaginal discharge from the probe and determine the pH level by applying the probe to the indicator paper. Uses a universal indicator paper (Lachema) with a reference scale from 0 to 12 or from 4 to 6.5. Determination of the pH level of vaginal contents can also be done using pH meters of various modifications, such as the analyzer ABL-330 (Radiometer). To avoid mistakes, the examination is not conducted during menstruation, within 2-3 days after sexual intercourse (the sperm can change the pH of vaginal discharge), as well as against the use of antibacterial

or hormonal drugs. This method is characterized by high sensitivity (up to 90%) and low specificity. Changing the pH level is common in women after menopause. The presence in the vaginal discharge of cervical mucus, blood, sperm also increases the pH. Normal pH (<4.5) excludes the diagnosis of bacterial vaginosis. If the pH> 4.5, a differential diagnosis is required between bacterial vaginosis, trichomoniasis and mucosal purulent cervicitis, when there is also a change in the level of pH of the vagina in the alkaline side [1, 3, 4].

To detect "key cells", the smear is prepared directly by the gynecological examination, covered with a cover glass and viewed in a light microscope with an increase of x400. Formation of "key" cells occurs in the event of increased colonization of G. vaginalis and their further adhesion to the cells of the vaginal flat epithelium. "Key" cells are rejection of epithelial lining by intact or lithically modified cells colonized by G. vaginalis. G. vaginalis cover the entire surface of the epithelial cells in the form of a cloud or veil, and in most clinically expressed cases, fill the entire intercellular space.

The sensitivity of the microscopic diagnostic method is 93%, and the specificity is 70% [3, 5]. For BV there is a positive aminotest. Vaginal content often has the smell of "rotten fish", which is the result of the production of diamines (putrescine, cadaverin) during the reaction of decarboxylation of amino acids by obligatory anaerobes. The salts of these substances are converted into volatile amines with an alkaline pH value [1, 3]. When carrying out an aminotetest in a drop of vaginal contents deposited on a slide the same amount of 10% of KOH solution is introduced. With positive aminotness, the smell of "rotten fish" is felt. The aminotetest is characterized by high specificity (up to 90%) and low sensitivity [1, 6, 7, 8].

To laboratory studies for the diagnosis of bacterial vaginosis include microscopic examination and analysis of the microflora of the vagina bacteriological method or molecular diagnostic methods; Methods of evaluation of microbial metabolism products and a test for sialidase (BV Blue Test) [1, 8, 9] are also used. The evaluation of the microscopic pattern is carried out according to the Spiegel criteria in the Nugent modifications [1, 9]. To assess the microscopic pattern of the smear also rely on the classification of Kira E.F. and Mawcjutova AR [10, 11, 12]. Microscopic characterization of four types of vaginal biocenosis according to the classification of Kira E.F .: normocenosis - the dominance of lactobacilli, the absence of gram-negative microflora, spores, mycelia, pseudohyphs, the presence of isolated leukocytes and single "pure" epithelial cells in the phase of the menstrual cycle; intermediate type - moderate or insignificant amount of lactobacilli, presence of gram-positive cocci, gram-negative sticks; leukocytes, epithelial cells are detected; BV - insignificant amount or complete absence of lactobacilli, abundant polymorphic gram-negative and gram-positive rod

and coccal microflora; presence of "key" cells; the number of leukocytes varies, the absence or incompleteness of phagocytosis [10, 12]; nonspecific vaginitis - a large number of leukocytes, macrophages, epithelial cells, pronounced phagocytosis, and the morphological landscape of the inflammatory process. When detecting gonococci, trichomonads, mycelium, pseudohyphs, the spores exhibit a corresponding etiological diagnosis - gonorrhea, trichomoniasis, mycotic vaginitis [10, 12].

A. A. Mawtsyutov and co-authors proposed microscopic criteria for differentiation of bacterial vaginosis in three stages: 1st degree - compensated bacterial vaginosis. For this form of bacterial vaginosis, the complete lack of microflora in the investigated material with unchanged epithelial cells is characteristic. The specified condition of the mucous membrane of the vagina is not considered pathological, but the absence of lactobacillary flora indicates the principle of the possibility of settling an empty ecological niche by microorganisms that come from the external genital organs and the subsequent formation of bacterial vaginosis in view of the violation in the absence of lactic acid bacteria of the natural colonization resistance of the mucosa. The described forms can be observed in microscopy as a result of "excessive" preparation of a patient prior to a visit to a doctor or after intensive chemotherapy with antibacterial preparations of a wide spectrum of action (cephalosporins, macrolides, etc.). Second degree - subcompensated bacterial vaginosis: characterized by a quantitative decrease in lactobacilli, comparable to the increase in the number of accompanying grammaribular polymorphic bacterial flora, and the appearance in the field of view of single (1-5) "key" cells with relatively moderate leukocytosis (15-25 in the field view) "Key" cells can be represented as coated with bacterial flora from the outside of the epitheliocytes, as well as those containing intracellular bacteria, due to the non-specific implementation of phagocytosis by the epithelial cells. 3rd degree - decompensated bacterial vaginosis: is clinically expressed in accordance with the symptoms of BV and microscopically characterized by a complete lack of lactobacilli when the entire field of view is filled with "key" cells. Bacterial flora at the same time can be represented by the most diverse (with the exception of lactobacillus) microorganisms, both in monoculture, and in various morpho- and species combinations [12].

Bacteriological research in the diagnosis of bacterial vaginosis is based on the integrated assessment of the microflora, taking into account not only the species composition, but also the quantitative ratios of its representatives. Detection of certain types of obligate anaerobes and G. vaginalis is not equivalent to the microbiological diagnosis of BV, because G. vaginalis and obligatory anaerobic microorganisms can be part of the indigenous microflora [10-12].

In the diagnosis of bacterial vaginosis, the bacteriological method requires proper observance of the technique of cultivation of obligate anaerobic bacteria, which is dominant in bacterial vaginosis. To carry out an adequate bacteriological study on anaerobes and microaerophils, special media, both transport and media for their selection and cultivation, as well as the creation of certain conditions for incubation, are necessary [13].

Normocenosis in the study is characterized by the total number of microorganisms in the vaginal discharge - 106-108 CFU / ml; absolute predominance of lactobacilli; the presence of conditionally pathogenic microorganisms in the low titre - <104 CFU / ml [1, 13].

For BW characterized by massive microbial contamination of vaginal discharge with a total number of microorganisms exceeding 109 CFU / ml; the absence of lactobacilli or a sharp decrease in their number to 104 CFU / ml or less; The polymicrobial nature of the microflora with an absolute predominance of obligatory anaerobic microorganisms and G. vaginalis [1, 13]. According to modern world literature, Anaerobic microorganism Atopobium vaginae is more associated with bacterial vaginosis than other microorganisms [1, 13, 14].

In the examination of patients with complaints of vaginal discharge (n = 100) using smear microscopy, Gram stain and culture methods, 14 different pathogens were identified in comparison with the control group (group III, n = 20), where it was 7 different microorganisms were identified. Among patients with BV (group I, n = 80) with the highest frequency, the microorganism, often associated with BV - G. vaginalis, was identified in 55 (68.7%) cases. Other associated microorganisms were identified with a slightly lower frequency: U. urealyticum in 41 (51.5%) cases, Mobiluncus spp. in 30 (37.5%) cases and M. hominis in 25 (31.2%) cases. With high frequency, the growth of Corynebacterium spp. and Staphylococcus epidermidis in 50 (62.5%) cases. Moreover, in the 2nd and 3rd groups of Corynebacterium spp. also met with high frequency: 15 (75%) and 11 (55%) patients, respectively (p> 0.05). While Staphylococcus epidermidis in the 2nd and 3rd groups was found to be significantly lower in the 6 (30%) and 7 (35%) patients, respectively (p = 0.004). It is also noted that Staphylococcus spp. Was only found in the 1st group. in 8 (10%) patients. Representative of the intestinal microflora E. coli was found to be of relatively similar frequency in all three groups considered (p> 0.05) [15].

In the II group, in addition to the 100% cases of genital mycoplasmas (U. urealyticum and / or M. hominis) detected in a culture study, the representative of the intestinal microflora Enterococcus faecalis was found to be significantly higher in 14 (70%) cases (p = 0.008 when compared with groups I and III). And also with a significantly higher frequency in Group II

compared with Group I, Staphylococcus saprophyticus was detected in 6 (30%) cases (p = 0.001) [15]. Groups II and III showed no microorganisms characteristic of patients with BV (group I): G. vaginalis and Mobiluncus spp. Representatives of the normal microflora of the vagina - Lactobacillus spp., Were found with a relatively low frequency in all of the groups analyzed (p> 0.05) [15].

As a result of conducting a culture study of vaginal content, the spectrum of the most commonly detected opportunistic microorganisms was established in the groups: For group I (n = 80): G. vaginalis, U. urealyticum, Mobiluncus spp., M. hominis, Staphylococcus epidermidis, Staphylococcus spp. For group II (n = 20): U. urealyticum, Enterococcus faecalis, Staphylococcus saprophyticus. Often found in all the groups considered: Corynebacterium spp., E. Coli [15].

In assessing the association of various microorganisms detected in bacterial vaginosis in association with A. vaginae, the following results were obtained. Of the 30 (37.5%) cases in patients with bacterial vaginosis, in the microscopy of smears stained with Gram, representatives of Mobiluncus spp. Were identified, in 24 (80%) cases A. vaginae were also identified. Thus, the association Mobiluncus spp. With A. vaginae occurred in 30% of cases of bacterial vaginosis. When comparing the frequency of Mobiluncus spp. and A. vaginae with the duration of the course of bacterial vaginosis, it was found that in patients with prolonged course of bacterial vaginosis, such association was significantly more frequent - in 16 cases (44.4%) than in patients from subgroups with less prolonged course of bacterial vaginosis - in 8 (18.2%; p = 0.02) [15].

Association of Mobiluncus spp., Genital mycoplasma (U. urealyticum and / or M. hominis) and A. vaginae in all patients with bacterial vaginosis occurred in 15 (18.7%) cases. In this case, in the subgroup of patients with prolonged course of bacterial vaginosis, such association was significantly more common (in 11 (30.5%) cases) than in the subgroup of patients with a less long course of bacterial vaginosis - in 4 (9.1%; p = 0, 03). The association of A. vaginae with genital mycoplasma (U. urealyticum and / or M. hominis) was found frequently - in 40 (50%) cases. This association was also more characteristic of prolonged course of bacterial vaginosis and was found in 24 (66.7%) cases, compared to a lower frequency with less prolonged course of bacterial vaginosis - in 16 (36.4% of cases; p = 0.01) [15].

Patients with bacterial vaginosis and duration of the disease for more than 6 months have a higher incidence of detection: A. vaginae (in 100% of cases), Bacteroides spp. (in 75% of cases), genital mycoplasmas (U. urealyticum and / or M. hominis, in 50% of cases),

compared with patients with a duration of disease less than 6 months when A. vaginae, Bacteroides spp. and genital mycoplasmas were detected in 68%, 37% and 5% of cases, respectively ($p < 0.05$).

The high frequency of the association of the pathological process with anaerobic microorganism A. vaginae (82.5%) suggests that it is one of the criteria for diagnosis of bacterial vaginosis, and the detection of A. vaginae in all patients with a long, recurrent course of bacterial vaginosis can serve as a prognostic sign of this course this disease [15].

Rapid and qualitative identification of bacterial vaginosis is possible through genetic diagnostics - DNA-DNA hybridization, qualitative and quantitative polymerase chain reaction (PCR) in real-time, DNA-microchip [16, 17]. The main molecular-biological method of diagnosis of bacterial vaginosis is PCR and its modifications. A distinctive feature of PCR is the universality of the approach for the detection of various microorganisms, as the object of research is a DNA molecule possessing similar chemical properties in all living organisms. PCR is a direct method for the detection of microorganisms and surpasses the sensitivity microscopic and bacteriological methods for the diagnosis of bacterial vaginosis [16, 17].

According to PCR, the study of G. vaginalis was identified in a group of patients with BV in 65 (81.2%) cases. In this case, 58 (89.2%) patients of G. vaginalis were associated with A. vaginae, in part of the examined patients - in 9 (11.2%) women, A. vaginae were identified in the absence of G. vaginalis in the investigated material. The simultaneous presence of A. vaginae and G. vaginalis in the subgroup of patients with prolonged course of bacterial vaginosis was detected in 30 (83.3%) cases, and in the subgroup of patients with a shorter duration of bacterial vaginosis in 28 (63.6%) cases [15]

Association G. vaginalis, A. vaginae and Mobiluncus spp. was noted in 18 (22.5%) patients, of which 11 (13.7%) belonged to a subgroup with prolonged course of bacterial vaginosis and only 7 (8.7%) to a subgroup with a less prolonged course of bacterial vaginosis. The association of all the examined microorganisms (G. vaginalis, A. vaginae, Mobiluncus spp. And genital mycoplasmas) was observed in 13 (16,2%) cases, of which the majority were patients with long-term bacterial vaginosis - 9 (11,2%) and less - patients with less prolonged course BV - 4 (5%). The frequency of detection of A. vaginae in patients with bacterial vaginosis did not depend on its association with other bacterial vaginosis-associated microorganisms (G. vaginalis, Mobiluncus spp., U. urealyticum, M. hominis) and was an independent factor [15].

The study of vaginal microflora in bacterial vaginosis by PCR method allows to exclude the presence of unconditionally pathogenic microflora (Chlamydia trachomatis,

Neisseria gonorrhoeae and Trichmonas vaginalis) [13, 14]. There are other approaches to the identification of the presence of various microorganisms, such as DNA chips, based on the principle of complementary hybridization of single-stranded polynucleotides. The basis of all types of biochips with immobilized DNA is the exact correspondence between the direct and complementary DNA strand according to the Watson-Creek rule. Hybridization allows for the analysis of unknown nucleotide sequences using known probes. Probes can be oligonucleotides, fragments of genomic DNA, RNA [15, 16].

The technology of DNA chips has a significant potential for use in microbiology: it allows for one experiment to determine up to a thousand microbial species or genera, depending on the purpose of the study [15, 16]. Using a DNA chip and a standard PCR, a material (vaginal discharge) was obtained from 45 patients with complaints of vaginal discharge. As a control group, 8 healthy people were screened for the results of a clinical and laboratory examination of women, no complaints were filed at the time of treatment. In the material obtained from patients with vaginal discharge complaints (n = 45), DNA chip identified 29 different microorganisms out of 42 possible, including difficult-to-cultivate anaerobic bacteria. The spectrum of the detected microflora consisted of both opportunistic microorganisms and microorganisms that are part of the normal composition of the microbiocenosis of the vagina. In the DNA chip used in this study, along with pathogenic microorganisms, three pathogens were included: N. gonorrhoeae, C. trachomatis, M. genitalium.

Pathogenic microorganisms were not detected in the analysis of the microbial composition using the DNA chip, because the presence of sexually transmitted infections was a criterion for exclusion from the study. Among the 29 microorganisms detected by chips, different groups of bacteria were identified, including: microaerophilic bacteria (G. vaginalis in 30 (66.7%) cases), obligatory anaerobic gram-positive and gram-negative bacteria (Bacteroides spp. In 37 (82.2 %) cases, A. vaginae in 34 (75.5%) cases, Lactobacillus spp. in 25 (55.5%) cases, Clostridium spp. in 9 (20%) cases, Prevotella spp. in 9 (20%) Porphyromonas spp. in 8 (17.8%) cases, Fusobacterium spp. in 7 (15.5%) cases, Peptostreptococcus spp. in 5 (11.1%) cases, Mobiluncus spp. in 4 (8.8 %) cases of Veilonella spp. in 3 (6.6%) cases) and facultative anaerobic gram-positive bacteria (M. hominis, U. urealyticum in 15 (33.3%) cases, Streptococcus spp. in 3 (6.7%) cases, Staphylococcus spp. in 1 (2.2%) case).

The identified spectrum of microorganisms is largely due to the composition of the study group, in which out of 45 patients in 39 (86.7%) was diagnosed with bacterial vaginosis

(according to Amtal criteria). In 6 (13.3%) patients who had no signs of BV, genital mycoplasmas (U. urealyticum and / or M. hominis) were identified as the only possible cause of pathological secretions from the vagina in a culture study [15, 16].

At the examination of the control group (the material was obtained from the posterior vagina, n = 8), only 5 different microorganisms were detected using the DNA chip, among which the most common (in 5 cases) was the representative of the normal microflora of the vagina Lactobacillus fermentus [15, 16]. The use of DNA chips allowed not only to diagnose bacterial vaginosis, but also to establish the factors of prolonged course of bacterial vaginosis - microorganisms, which may indicate a long, recurrent course of the disease: A. vaginae with prolonged course of bacterial vaginosis was detected in 100%; Bacteroides spp. with prolonged course of bacterial vaginosis detected in 75%; Genital mycoplasma (U. urealyticum and / or M. hominis) was detected in 50% with prolonged course of bacterial vaginosis [15, 16].

Some facultative-anaerobic bacteria and aerobic cultures have encountered a slightly higher frequency than DNA-chip studies. This may be due to the established threshold of diagnostic sensitivity for these microorganisms on the DNA chip, when the result was considered positive, corresponding to the result of "abundant growth" in the culture study. Higher detection frequency of representatives of Mobiluncus spp. In the microscopy of the smear stained for Gram, in comparison with the results of the study using a DNA chip, in addition to the threshold for diagnostic sensitivity, it could be due to the fact that smear microscopy allowed to determine the morphotip without the specific identification of the microorganism. While identifying using the DNA chip allows the detection of two types of Mobiluncus curtisii and Mobiluncus mulieris [15, 16].

For the diagnosis of bacterial vaginosis, the methods of identification in the vaginal contents of metabolic products of microorganisms living in the vagina have been developed: a test for sialidase (detection of the enzyme sialidase in vaginal contents), which is used in BV as a method of express diagnosis [10, 11]. It is possible to determine the nature of the changes in the microflora of the vagina in terms of changes in the qualitative and quantitative composition of carboxylic acids (lactic, amber, acetic, isopropionic, propionic, isosalic, oleic, isovaleric, valeric, isocapronic, kapron) in vaginal contents [1, 10, 11]. According to Ardatsky M.D. and co-authors, the state of the microflora of the vagina can be estimated by the value of the total number of short fatty acids (normally 0.08-0.16 mg / g), and the verification of the species composition of the vagina microflora, to determine the percentage content of individual acids. Content of acetic acid 69-83%, propionic acid 10-18% and butyric acid 7,0-

13,0% on the profile of acetic, propionic and butyric acids; The content of isomers of fatty acids 9,9-14,9% of the total acid content indicates the normal state of the microflora of the vagina. Changes in the acid content within 1-2% of the normal values indicate minimal changes in the vaginal microflora. An increase in the percentage of acetic acid and isomers of fatty acids indicates an increased number of aerobic populations of microorganisms and reduced obligate populations of bifidobacteria and lactobacilli. An increase in the percentage of propionic and butyric acid indicates an increase in the amount of vaginal anaerobic populations of microorganisms and a decrease in obligatory populations of bifidobacteria and lactobacilli. The content of propionic acid in 22,0-39,0% and acetic acid to 55-65% corresponds to an increase in the vaginal amount of bacterial genera Bacteroides spp., Veillonella spp., Propionibacterium spp. Detection of the percentage of butyric acid up to 22-41%, acetic acid up to 46-65% and propionic acid up to 7-15% correspond to an increase in bacteria of the genus Clostridium spp. and Fusobacterium spp .; propionic acid - 20-31%, butyric acid - 15-25% and acetic acid - 47-60% corresponds to an increase in the vagina of the mixed anaerobic flora. The content of propionic acid at the level of 21-29%, butyric acid at 17-32% and isoacids at 16.2-18.4% indicates an increase in the anaerobic flora in the vagina, which has proteolytic and hemolytic activity. The content of acetic acid at the level of 85-93%, propionic acid at the level of 4,0-10,0%, butyric acid at 3,0-5,0% indicates an increase in E. coli, aerobic streptococci and staphylococci; The content of acetic acid at the level of 87-93% and isomers of short fatty acids at the level of 18,5-25,4% indicates an increase in the vagina of aerobic populations of microorganisms possessing proteolytic activity [18].

The long-term relapsing course of bacterial vaginosis is most often associated with bond-anaerobic gram-positive A. vaginae, both independently and in association with bond-anaerobic gram-positive Mobiluncus spp. and facultative-anaerobic gram-positive genital mycoplasmas (U. urealyticum and / or M. hominis) [19, 20]. The bacteria G. vaginalis and A. vaginae are frequent satellites of bacterial vaginosis. However, while G. vaginalis occurs equally frequently for different duration of bacterial vaginosis, detection of A. vaginae is a prognostic factor in the long-term relapse of bacterial vaginosis [18, 19, 20].

It is interesting to develop new approaches to assessing the state of the vaginal ecosystem, which allowed a more objective assessment of the norm and objectively signal the emerging pathology. In this plan, environmental parameters that can be used to describe the structure and functioning of biocenoses of higher organisms - plants and animals using a number of environmental criteria may be useful: the species species (Pi), the dominant index (d), the Simpson index (C). This allowed us to propose a classification of the microflora of the

vaginal biocenosis: dominant species - d more than 1%, subdominant - d from 0.1% to 1.0%, insignificant - d less than 0.1% [1, 16, 20].

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