REVIEW ARTICLE

# Biotinidase deficiency: clinical and diagnosis aspects and neonatal screening

## *Deficiência de biotinidase: aspectos clínicos, diagnósticos e triagem neonatal*

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### ABSTRACT

Biotinidase deficiency is a hereditary metabolic disease with varied phenotypic expression in which there is a defect in the metabolism of biotin. The symptoms of the classic form are often neurological and in the skin, with possible sequelae such as auditory and visual disorders, and motor development and language delay. These manifestations are usually irreversible, even after treatment, which is simple, of low cost, and based on the oral replacement of biotin, 5 to 20 mg/day, for a lifetime. The treatment, when started within the first months of life, prevents the appearance of the referred symptoms. The combined prevalence of the disease is variable, from 1:60 000 to 1:9000. Biotinidase deficiency meets the World Health Organization criteria for neonatal screening because carriers are asymptomatic during this period of life, the disease's high morbidity rate, and effective treatment with low cost. The objective of this study is to review the national and international literature on relevant aspects of biotinidase deficiency.


**Key words:** Biotin; Biotinidase; Biotinidase Deficiency; Vitamin B Deficiency; Newborn Screening.


### RESUMO

*A deficiência de biotinidase é doença metabólica hereditária com expressão fenotípica variada, na qual há defeito no metabolismo da biotina. A sintomatologia da forma clássica é frequentemente neurológica e cutânea, podendo ocorrer sequelas como: distúrbios auditivos, visuais, atraso motor e de linguagem. Essas manifestações são, geralmente, irreversíveis, mesmo após instituição do tratamento, que é simples e de baixo custo, baseado na reposição oral de biotina, 5 a 20 mg/dia, por toda a vida. O tratamento, quando iniciado nos primeiros meses de vida, evita o aparecimento da sintomatologia referida. A prevalência combinada da doença é variável, de 1:60.000 a 1:9.000. A deficiência de biotinidase preenche critérios da Organização Mundial de Saúde para triagem neonatal devido ao fato dos seus portadores serem assintomáticos nesse período da vida, possuir alta morbidade e tratamento efetivo e de baixo custo. O objetivo deste estudo é o de revisar a literatura nacional e internacional referente aos aspectos relevantes da deficiência de biotinidase.*


***Palavras-chave:*** *Biotina; Biotinidase; Deficiência de Biotinidase; Deficiência de Vitaminas do Complexo B; Triagem Neonatal.*

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## INTRODUCTION

Biotinidase deficiency (BD) is a hereditary metabolic disease with varied phenotypic expression in which there is a defect in the metabolism of biotin. The disease

is classified into two subgroups: deep (DBD) and partial deficiency (PBD), where the serum enzyme activity is less than 10% and between 10 and 30% of the normal average activity, respectively.[1-3]

DBD usually manifests from the 7th week of life with neurological and cutaneous disorders such as seizures, neuropsychomotor development delay, hypotonia, microcephaly, alopecia, and eczematoid dermatitis. Neurological sequelae in patients that are not treated early are auditory and visual disturbances and motor and language delays. Treatment initiated within the first months of life prevents the onset of symptoms, it is simple, of low cost, and based on the oral replacement of biotin at the dose of 5 to 20 mg/day for lifetime.[1,3]

BD fulfills some of the criteria from the World Health Organization (WHO) to be included in neonatal screening: absence of clinical signs in the neonatal period, high morbidity, and effective treatment.[2]

The aim of this study was to review the national and international literature on relevant aspects of biotinidase deficiency.

## METHOD

A bibliographical survey was conducted in articles, national and international journals, handbooks from the Ministry of Health, books, theses and dissertations, in Portuguese, English, and Spanish languages, and between 1999 and 2013. The materials were selected according to relevance to the theme. Some classic articles in the literature, published prior to the referred period, were included due to their historical significance.

The survey was conducted in the databases: Scientific Electronic Library Online (SCIELO), Latin American and Caribbean Health Sciences Literature (LILACS), Medline, Pubmed, and the Cochrane Library.

## DEFINITION

BD is an autosomal recessive hereditary metabolic disease with varied phenotypic expression of biotinidase enzyme deficiency. Its consequence is depletion of endogenous biotin due to organism inability to recycle it or use biotin from proteins provided through diet, resulting in multiple deficiency of carboxylases (MDC) in the youth or late form (Mckusik 253260).[1-3]

## METABOLIC ASPECTS

### Biotin

Biotin is a vitamin in the B complex, water soluble, an essential nutrient for humans,[4] involved in important metabolic processes such as gluconeogenesis, synthesis of fatty acids, and catabolism of various branched-chain amino acids.[3-5]

It has the function to activate enzymes with the property of transporting carboxylic groups: pyruvate-carboxylase, propionyl-CoA carboxylase, beta-methylcrotonyl-Coa carboxylase, and acetyl-CoA carboxylase. Carboxylases (biotin-dependent enzymes) are synthesized as apo-carboxylases and need biotin to be activated.[3, 5,6]

Biotin deficiency can be associated with: total parenteral nutrition; ingestion of avidin (present in raw egg white), protein that prevents its intestinal absorption; serious protein-calorie malnutrition in children; inborn errors of metabolism (biotinidase deficiency); small intestine syndrome; chronic hemodialysis; prolonged administration of some antiepileptic drugs (carbamazepine, phenytoin, primidone), and antibiotics, probably by inhibiting its intestinal absorption or accelerate its catabolism;[3,5,7] decreased sodium-dependent protein multi-vitam transporter (SMVT), which plays an important role in its homeostasis by acting on its transportation in the intestine, liver, peripheral tissues, and renal reabsorption.[5]

The daily requirements of biotin for adults and pregnant women are - 30 μ g; during lactation, 35-30 μg; and infants (0-5 months) – 5 μg[4]

There is a possibility that the lack of biotin is involved in fetal malformations in humans. In animal studies, biotin deficiency is proven to be teratogenic with the most common malformations in rats being cleft palate, micrognathia, micromelia, and syndactyly.[3-5]

Cells that are deficient in biotin exhibit pronounced susceptibility to oxidative damage in response to stress.[3-5]

### Biotinidase

The biotinidase (biotin-starch-hydrolase) (EC 3.5.1.12) is an enzyme of fundamental importance in the cycle of biotin. Its function is to release biotin that is covalently linked to proteins or biotinylated peptides, from diet or endogenous biocitine.[3,7]

## EPIDEMIOLOGY

The global incidence of BD was based on 36 programs of neonatal screening in 1990, involving the examination of 8,532,617 newborns (NBs). The combined incidence of DBD and PBD was 1:60,089 live births (LBs) (1:49,500 and 1:73,100), with 95% confidence interval.[8] In Brazil, studies on the epidemiology of BD are scarce and the results are discordant. In Paraná, the combined prevalence of BD:LBs was 1:62,500, DBD:LBs was 1:121,000, and PDB:LBs was 1:121,000.[9] In 2004, the prevalence of BD, DBD, and PBD observed in 225,136 NBs from several Brazilian regions were 1:9,000, 1:14,192, and 1:9000 LBs, respectively.[10] In 2010, in Minas Gerais, the prevalence of PBD:LBs of 1:18,289 was observed in 182,942 NBs evaluated in the State Neonatal Screening Program (PETN), with no cases of DBD.[11]

## CLINICAL MANIFESTATIONS

### Deep biotinidase deficiency

DBD can clinically be expressed with neurological and skin symptoms in most untreated children between the second and fifth month of life. The symptoms generally appear only after the depletion of the biotin accumulated during the intrauterine life. There are confirmed cases of early onset, in the first week of life, and others in early adolescence.[1-3] Many children not treated prematurely develop developmental delays, hearing loss or deficiency, and vision problems including optic atrophy, usually not reversible with the use of biotin.[1]

The most relevant clinical findings observed in 80 patients[3] are:

- **in more than 50% of the cases:** alopecia, delayed development, muscle hypotonia, seizures, rash and skin infections;
- **between 25 and 50% of the cases:** ataxia, conjunctivitis, hearing impairment, lethargy, respiratory problems, and visual abnormalities;
- **between 10 and 25% of the cases:** feeding difficulties, coma, diarrhea, and fungi infections;
- **in less than 10% of cases:** hepatomegaly, language problems, and splenomegaly. In adult patients: sleepiness, hallucinations, and paresthesias.[3]

### Neurological abnormalities

#### *Epileptic seizures*

The biotinidase activity in normal human brain and liquor is very low. The brain can become unable to recycle biotin and may depend on biotin transported through the blood-brain barrier. The early stages of BD can result in accumulation of lactate in the brain (localized lactic acidosis), which can be associated with epileptic seizures before other clinical symptoms arise.[12]

In 78 BD symptomatic children, 55% had episodes of suggestive epileptic seizures, with the most frequent type being generalized tonic-clonic seizures (GTC), similar to that observed in most inherited metabolic diseases. Infantile spasms and myoclonic seizures were recorded in 16% of the cases and focal crisis were rarely described.[12]

#### *Auditive deficiency*

Hearing loss was observed in 76% of 33 children with DBD from 29 families, identified after the neonatal period or screening, and two thirds of them needed hearing aids. The average age of the hearing loss was 32.5 months, within the critical period of normal language development. High incidence of learning and language problems was recorded in children with hearing loss (78 and 70%, respectively) and without hearing loss (62 and 50%, respectively). The typical audiometry observed showed moderate to severe sensorineural hearing loss. DBD was not progressive in most of the children after the initiation of treatment. Discreet and continuing deterioration in hearing was observed in a few children and one improved over time after the initiation of treatment with biotin.[13,14]

Sensorineural hearing loss, from discreet to severe, was observed in approximately 55% of 20 children with DBD subjected to audiological tests.[15] There is a significant difference in the results of tests of central auditory evoked potential between children diagnosed shortly after birth and siblings diagnosed later. The longer latency was found in the group with late diagnosis compared to that with early diagnosis. Early diagnosis is important to prevent peripheral and central auditory deficiency. The late diagnosis, despite treatment, causes hearing damage to be usually irreversible.[15]

### *Visual disturbances*

In 51% of 78 children with symptomatic BD, ophthalmologic abnormalities were observed including infections (30%), optic neuropathy and visual disturbances (13%), disturbances of ocular motricity (13%), pigmentary retinal alterations (4%), and pupil alterations (1%), with optic atrophy and keratoconjunctivitis being the most common.[3]

### *Compromise of the spinal cord*

The medullary involvement is rare in BD. However, it must be considered in the differential diagnosis of spinal cord demyelination of unknown cause because the immediate treatment of BD can follow its total or partial recovery.[16]

### Dermatological abnormalities

Dermatological alterations are very common in BD and especially characterized by: non-specific macular-papular eruption typically described as rough and dry skin, erythematous especially in moisten and perioricial areas such as around the mouth, nose, and eyes.[6,18] In more severe cases, lichenification, crusting, and open lesions may occur and can get infected by Candida. The hair is sparse and thin, with partial or complete alopecia including the eyebrows. The mechanism by which BD produces cutaneous manifestations is unknown, seeming to be associated to alterations in the metabolism of lipids.[17]

The rashes resolve after treatment with biotin in one or two weeks.[4,17]

### Respiratory abnormalities

In some BD cases, laryngeal stridor, ins and expiratory, are observed and are possibly due to dysfunction of the respiratory center.[16,18]

### Immune dysfunction

BD has adverse effects on cellular and humoral immune functions. Humoral defects can be caused by protein deprivation that affects the synthesis of immunoglobulins.[3]

### Neuropathological aspects

The anatomopathological exam of the brain and spinal cord in a child who has died before the diagnosis of BD revealed necrotic lesions similar to those observed in Leigh disease and Wernicke's encephalopathy. The hippocampus and parahippocampal cortex were also affected. The lesions showed areas of microcavitation, capillary proliferation, and gliosis. A relative preservation of neurons was observed in the grey matter and loss of myelin with relative or moderate axonal damage in the white matter.[19] Chronic cerebellar degeneration, subacute myelopathy, and defective or compromised myelination were recorded in two other cases.[3,19]

### Aspects of neuroimaging

Cerebral atrophy, white matter abnormalities, and fewer common findings include cystic lesions, the radiological appearance of Leigh syndrome, and basal ganglia calcifications. Myelination disorders and other abnormalities of the white matter are prominent in early-onset forms whereas those of late-onset are characterized by abnormalities of the grey matter and atrophy.[3]

## Partial biotinidase deficiency

PBD was unknown before the implementation of neonatal screening for BD. All 16 children with PBD identified in a neonatal screening in the US were healthy at the time of diagnosis. These children with PBD, with the exception of one who developed symptoms, remained asymptomatic or presented discreet and nonspecific symptoms. The diagnosis of PBD in these children would probably not happen if it were not for the neonatal screening.[20]

Seven were children with PBD were identified in Hungary between 1989 and 2001. Most of them displayed mild symptoms such as hypotonia and face dermatitis at the time of diagnosis. The ages of the children ranged from several weeks to months. These symptoms resolved after starting treatment with biotin.[21]

In Minesotta, USA, 26 children were identified with PBD, at two weeks of life, all were asymptomatic and received treatment immediately.[22]

## ASYMPTOMATIC CASES

There are many BD cases, in both forms, identified or followed into adulthood that remained asymptomatic. This suggests that there may be enough residual enzymatic activity required as a substrate, and the presence of a stressor, such as a severe infection, can trigger symptoms. There are factors related to dietary differences or epigenetic factors that may protect some asymptomatic individuals.[1,3] The factors that precipitate symptoms in asymptomatic BD are unknown and can be triggered at any age.[23]

## DIAGNOSTIC METHODS

The BD diagnosis consists in determining the biotinidase enzyme activity. It can be suspected at the neonatal screening, confirmed by the quantitative serum biotinidase enzyme dosing and, more rarely nowadays, in the culture of peripheral blood leukocytes or fibroblasts obtained by skin biopsy.

One of the most widely used tests for neonatal screening is the colorimetric test, which measures the ability to release the artificial substrate N-biotinil p-aminobenzoate (BPABA). There are methods that use biocitine and/or other derivatives of biotin as natural substrates, such as biotin-6-aminoquinoline.[3]

The use of biotin by the patient does not interfere with the biotinidase level whose activity is correlated with various aspects including age, gender, and capacity of hepatic protein synthesis.[19] The molecular study identifying mutations in probands, although costly, is currently the gold standard for the diagnosis of BD.

The same adult values are considered in children over 30 days old. In the neonatal period, low enzyme activity is noted, especially in the first few days after birth, with values between 50 and 70% of the normal average activity in adults.[2]

## BIOCHEMICAL ASPECTS

The following were reported in 80 DBD cases in 2003 and at some point in their clinical evolution: organic aciduria (80%), metabolic ketoacidosis (75%), lactacidemia, and hyperammonemia.[1]

Biochemical abnormalities occur due to MDC and consist of lactic acidosis, increased urinary excretion of lactate, ß-hydroxy-isovaleric acid (most often observed), ß-hydroxypropionate, methylcitrates, and beta- methylcrotonyl glycine. The possible normality of urinary organic acids does not eliminate the possibility of disease. Moderately increased quantities of 3 hydroxyvaleric acid and discreet increase in levels of methylcitric acid are strong indicators of possible BD.[2-4]

## GENETIC ASPECTS

BD, as most of the inborn errors of metabolism, presents autosomal recessive inheritance affecting both genders indiscriminately. The prevalence of heterozygotes is estimated as one per 123 individuals.[3]

The genomic structure of the biotinidase gene has been determined;[1,3] the human biotinidase gene (BTD: 609019) consists of four exons. The biotinidase gene is mapped on the short arm of chromosome 3(3p25). The complementary DNA that deciphers the human normal serum biotinidase has already been cloned and encoded. Northern blot analysis has demonstrated the expression of biotinidase in the liver, kidneys, pancreas, lungs, skeletal muscle, heart, brain, and human placenta. About 140 pathogenic mutations are recognized in association with DBD.[7,22,24]Most individuals with PBD has the c. 1330G > C (p. D444H) mutation in one of the alleles, in combination with the second mutation for DBD on the other allele.[3] This mutation alone causes 48-52% of activity loss in the aberrant enzyme of that allele. The frequency of this allele in the general population is estimated around 0.039.

## PRENATAL DIAGNOSIS

The BD prenatal diagnosis is possible from the determination of the enzyme's activity in cell extracts from the amniotic fluid or in molecular analysis. Considering that the NB affected by BD is asymptomatic and the procedures listed previously are not risk-free, the quantitation of serum biotinidase activity shortly after birth when the family history is positive is suggested.[3]

## DIFFERENTIAL DIAGNOSIS

The main BD differential diagnosis is the holocarboxylase synthetase deficiency (HCS), which is a rare innate error of metabolism that interferes with the metabolism of biotin leading to MDC. HCS defi-

ciency is confirmed by enzyme dosing in culture of fibroblasts from skin.[3]

Other possible BD differential diagnoses are: isolated deficiency of carboxylase (which does not respond to treatment with biotin); chronic use of parenteral nutrition in children without supplementation of biotin or from diets containing scarce amount of biotin.[1,3] In older children, biotin-responsive basal ganglia disease, a rare neurological disorder of undetermined etiology, can be present with pyramidal and extrapyramidal symptoms and bilateral lesions in the head of the caudate nucleus and putamen observed at MRI, with dramatic response to biotin.[25]

## TREATMENT

The dosage of biotin for treating BD varies between 5 and 20 mg/day, orally, regardless of age. The amount of biotin needed will decrease with increasing age. The only method to monitor if the biotin dose is suitable is tracking if the dosing of organic acids in urine normalizes. However, about 20% of children with BD do not show abnormalities in urinary organic acids dosing, even when symptomatic.[3]

There is consensus on the treatment of DBD, being controversial for PBD; however, the treatment of both forms of BD is recommended.[23]

## NEONATAL SCREENING FOR BIOTINIDASE DEFICIENCY

BD was incorporated into neonatal screening since 1984 in the State of Virginia in the USA, when a simple colorimetric method was developed and used to detect the absence or presence of biotinidase activity in blood specimens from NBs, dried on paper filter (PF) and using an artificial substrate B-PABA[2].

Subsequently, several countries initiated screening pilot programs for the disease (Table 1).

Between October of 2004 and May of 2008, in Minnesota, the neonatal screening of 264,727 NBs showed combined incidences for BD:LBs of :8,540; DBD:LBs of 1:10,181; and PBD:LBs of 1:52,945.[22]

Table 2 shows data published in 2007 regarding BD neonatal screening in European countries.

**Table 1** - Statistical results from neonatal screening for biotinidase deficiency in national, and international studies, at various periods.[8,9,10,11]

| | Worldwide | Paraná | Porto Alegre | Minas Gerais |
|---|---|---|---|---|
| Period | Jan 1984 to Dec 1990 | Mar 1994 to Nov 1994 | Oct 1995 to Nov 1999 | Sep 2007 to Jun 2008 |
| Test used | Heard *et al.* | Heard *et al.* | Heard *et al.* | Umtest Blotinidasa |
| N° of cases identified | BD = 76<br>PBD = 66 | BD = 1<br>PBD = 1 | BD = 3<br>PBD = 11 | BD = 0<br>PBD = 10 |
| N° NBs sorted | 8,532,617 | 125,000 | 225,136 | 182,942 |
| Co. Prevalence with LB | 8,532,617 | 125,000 | 225,136 | 182,942 |
| Sensitivity % | NR | 100 | 100 | 100 |
| Specificity % | NR | 99.88 | 99.88 | 99.94 |

International and national studies, at various periods. 8-11 LB = live births; BD= Biotinidase Deficiency; PBD = Partial Biotinidase Deficiency; NR = not reported; Co = combined.

In Brazil, neonatal screening became known as 'the little foot test' and was incorporated into the Unified Health System (SUS) by Ordinance GM/MS No. 22 from January 15, 1992, with legislation that stipulated it as mandatory to all LBs including the evaluation for phenylketonuria and congenital primary hypothyroidism.[27] In the second phase that started in March of 1998, sickle cell anemia and other hemoglobinopathies became part of the neonatal screening. In 2001, the Ministry of Health issued the Ministerial Decree No. 822 of June 6, 2001, creating the National Program for Neonatal Screening (PNTN) and also the Reference Services in Neonatal Screening (SRTN) in each state.[27] The third phase was implemented in 2003 with the inclusion of cystic fibrosis. Currently, the fourth phase has been implemented including congenital adrenal hyperplasia and biotinidase deficiency in the neonatal screening.[28]

## FACTORS RESPONSIBLE FOR FALSE-POSITIVES AND FALSE-NEGATIVES IN NEONATAL SCREENING TESTS FOR BD

### Factors responsible for false positive results

#### Jaundice

Low biotinidase activity is observed in term and premature NBs and fetus that are small for the gestational age with jaundice, being attributed to impair-

ment of the hepatic function. The high levels of total bilirubin (TB) can have an inhibitor role for the enzyme. The gestational age (GA) and BT levels should therefore, be written on Guthrie cards for the correct evaluation of biotinidase activity.[29]

### Prematurity

In Brazil, in 1999, biotinidase activity showed a direct correlation with gestational age in 86 premature babies.[6]

## Factors responsible for false negative results

The neonatal screening tests that use the colorimetric method may show very high biotinidase activity in children under treatment with sulfonamides,[2] which are primarily aromatic amines and react with the chemicals used in the test, thus, developing color. In the case of procaine/benzylpenicillin, the color is due to the 4-aminobenzoic group in the procaine structure. False negatives can appear in these two conditions. It is recommended that these two determinations, with and without substrate, are conducted simultaneously on the test to eliminate diagnostic errors.[2,3]

This is described in the case of two children with suggestive BD symptoms, however, with very high serum biotinidase activity. Glycogenosis type Ia (GSD) was diagnosed in both. This type of disease should be considered in children with very high serum biotinidase activity levels. The real reason of this elevation is unknown.[30]

## CONCLUSION

BD is a hereditary metabolic disease with an autosomal recessive inheritance pattern in which there is a deficiency of the biotinidase enzyme. The combined worldwide incidence of BD is estimated around 1:60,000, with variability in incidence in different countries, highlighting the importance of regional studies.

BPD manifests itself with neurological and skin symptoms and the possibility of irreversible neurological sequelae in patients not treated early. BD fulfils the WHO criteria for inclusion in neonatal screening, an important public health measure already adopted in various countries and regions worldwide, including the SUS since 2013.

The BD diagnosis can be performed from the quantitative dosing of the biotinidase enzyme, which is not free of false positive results. Therefore, the DNA molecular study assumes great importance and is considered the gold standard for the confirmation of BD.

Currently, there are approximately 140 recognized pathogenic mutations associated with BD. So far, it is not possible to infer which patient will fully develop or not symptoms based only on the genotype or even on enzyme dosing.

The treatment consists in the use of biotin in patients with DBD. There is significant controversy in relation to PBD treatment. The favorable factor for the treatment of these patients is that the real consequences of partial deficiency of biotin, when associated with other triggering factors, have not been fully elucidated; the brain could be the first organ affected in this situation.

**Table 2** - Data on the prevalence, recall rate, and cut off points for used methods in neonatal screening program for BD in European countries in 2004[26]

| Country | NB * | Lab. | Methods | CO % | RR % | CD | Prevalence |
|---|---|---|---|---|---|---|---|
| Austria | 79.022 | 1 | (C) | Visual | 0.014 | 2 | 1:39.511 |
| Belgium Flanders | 33.324 | 1 | Wolf | n.d. | n.d. | 1 | 1:33.324 |
| Belgium | 44.651 | 2 | (C) | 10 | 0.04 | _ | _ |
| Germany | 726.973 | 13 | F, C | 30 | 0.05 | 16 | 1:45.436 |
| Wallonia | NR | NR | NR | NR | 0,21 | NR | NR |
| Italy | 105.471 | 2 | (C) | n.d. | 0,03 | 1 | 1:105.471 |
| Spain | 20.420 | 1 | (C) | n.d. | n.d. | 1 | 1:20.420 |
| Sweden | 101.471 | 1 | AND | 20 | 0.004 | 3 | 1:33.817 |
| Switzerland | 75.842 | 2 | Wolf | n.d. | n.d. | 1 | 1:75.842 |
| Total | 1.321.989 | | | | | 25 | 1:47.486 |

RR = recall rate: percentage of children referred for diagnoses confirmation; CO = cut off point, C = colorimetric; E = enzymatic; F = fluorimetric; W = Method of Wolf, NR = not referred, Lab = laboratory; ND = no data, * screened; CD = confirmed diagnosis.

More research on the BD is needed, especially in the identification and evolution of symptomatic and asymptomatic cases, and biochemical and molecular studies. Such data may bring contributions to a deeper understanding of the clinical variability in BD.

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