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Christmas Disease: Diagnostic Approaches, Treatment Options and Comparison Study with Haemophilia A

Review Article

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ABSTRACT

Haemophilia B or Christmas disease is a genetic bleeding disease defined by a deficiency of factor IX of blood clotting leading to prolonged bleeding and increased risk of hemorrhagic events. Haemophilia A, the more common form involves a deficiency of clotting factor VIII. Traditional management for both types primarily relies on factor replacement therapy which involves administering the respective clotting factors to treat bleeding episodes or for prevention. While effective this approach requires frequent infusions and can lead to the development of inhibitors in some patients. Recent advancements in gene therapy offer promising alternatives aiming to address the underlying genetic defects by delivering functional copies of the factor VIII or IX genes. This innovative approach has the potential for long-lasting effects reducing the need for ongoing factor infusions and improving the quality of life for patients. This review discusses the current management strategies for Haemophilia A and B comparing established factor replacement therapy with emerging gene therapy techniques, highlighting their benefits, limitations and future directions in the treatment landscape for both disorders.

Key words: *Christmas disease, Haemophilia, Factor replacement therapy, Gene therapy, Inhibitors*

Introduction

Haemophilias are the most frequent genetic bleeding diseases. Haemophilia A, often known as classical haemophilia affects roughly one in every 5000 Caucasian males. Haemophilia B, often known as Christmas disease affects around 1 in every 30000 males. The condition was first identified in a guy named Stephen Christmas

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(Brownlee, 1986). Haemophilia A (classical Haemophilia) attacks around a man among each 4000 while Haemophilia B (Christmas disease) is up to seven times fewer in incidence (Larner, n.d.). Both disorders have a sex-linked hereditary dissemination sequence affecting only boys who inherit abnormal bleeding from clinically normal moms who are heterozygous carriers of the genetic abnormality. Daughters of the carrier mothers have a 50% chance of becoming carriers whereas boys have a 50% chance of being afflicted. One-third of cases develop spontaneously from novel mutations with no family history (Larner, n.d.). Haemophilia B is a hereditary bleeding condition characterized by a lack of factor IX. The bleeding frequency is related to the level of insufficiency of factor IX gene. The illness is transmitted as an X-linked ailment although one-third of patients have no family history indicating a novel mutation (Giangrande, 2005). Physical examination and a brief medical history are the main components of a Haemophilia diagnosis. Symptoms of possible bleeding disorders, medication history, family history of bleeding abnormalities and bleeding after prior surgery or trauma should all be included in the medical history. Clinical examinations for Haemophilia B may reveal an extended activated partial thromboplastic time (aPTT), low factor IX levels, normal prothrombin time (PT) and normal bleeding time (BT). In moderate situations the aPTT may still be normal hence a factor IX test is typically necessary. A few people with Haemophilia B have an aberrant factor IX which significantly prolongs the PT (Rose and Mealey, 2015). In 1947, a novel technique known as the prothrombin consumption test demonstrated that blood did not contain free or precursory thromboplastic but rather that it was created when a platelet factor interacted with what was thought to be a single plasma constituent that was absent in Haemophilia. (Quick, 1947). Biopharmaceutical businesses have shown a great deal of interest in the synthesis of innovative therapeutic proteins and a number of techniques have been developed to optimize and guarantee the safety and efficacy of these products (Weatherall, 1986). Therapeutic proteins have been redesigned to improve their clinical potential due to the half-life restrictions of hemostatic medications. Recombinant factor VIII half-life lengthening is only partially successful increasing by around 1·5–1·6 times despite the fact that the half-lives of recombinant factor IX products have been prolonged by 3–6 times (Weatherall, 1986). Therefore, these new medications may primarily streamline the preventive protocols for haemophilia B patients by lowering dose frequency and increasing bleeding protection which would increase treatment compliance and lessen the patient's suffering from the therapy. Longer half-lives of novel recombinant factor VIII products may be advantageous without significantly altering the treatment intervals. Increased trough levels can likely be achieved with somewhat less frequent infusions and smaller product dosages protecting patients against breakthrough bleeding (Larner, n.d.).

Comparison between Haemophilia A and Haemophilia B

Long-term disability may result from the frequent bleeding episodes that people with severe Haemophilia endure. Severe arthropathy, muscle atrophy, pseudo-tumours, persistent discomfort and decreased mobility are all consequences of recurrent joint bleedings which frequently necessitate surgery and arthroplasty to restore joint function (Nagel et al., 2011). Several studies have reported possible differences in bleeding frequency, factor consumption, clinical presentation and the need for orthopaedic surgery despite the fact that the clinical characteristics of Haemophilia A and Haemophilia B are comparable (Nagel et al., 2011) (Castaman and Matino, 2019).

Table 1: Comparison between Haemophilia A and Haemophilia B

Factors	Haemophilia A	Haemophilia B	Reference
Cause	Inadequate levels of factor VIII of blood clotting.	Inadequate levels of factor IX of blood clotting (Christmas disease).	(Larner, n.d.)
Prevalence	More common, impacts around 1 in 4,000 male births.	Less common, affecting about 1 in 30,000 male births.	(Larner, n.d.)
Inheritance	X-linked recessive; predominantly affects males.	Also X-linked recessive, primarily affecting males.	(Ljung et al., 1990)
Symptoms	Easy bruising, joint bleeding, prolonged bleeding, and spontaneous bleeding episodes.	Similar to Haemophilia A, with prolonged bleeding and joint issues.	(Ljung et al., 1990)
Treatment	Factor VIII replacement therapy; options include recombinant factors and extended half-life products.	Factor IX replacement therapy; includes recombinant factors and prothrombin complex concentrates.	(Peyvandi et al., 2016)

Determination process of Haemophilia

Usually, coagulation screening tests show a normal prothrombin time along with a delayed activated partial thromboplastic time. The determination process is made by measuring the clotting activity of factor VIII or factor IX. Haemophilia is categorised into three basic types depending on the blood's residual coagulant material activity (factor VIII or factor IX). severe, moderate, or modest. Haemophilia is classified as severe (factor level < 1 IU/dL), moderate (factor level 1–5 IU/dL), or mild (factor level 5–40 IU/dL) based on the decrease in clotting factor as identified by laboratory tests (Miesbach et al., 2019). Approximately 50% of identified cases are classified as severe when the coagulation factor level is less than 1 IU/dL or less than 1% of normal (50-150 IU/dL) (Kamal et al., 2007).

Early diagnosis of Haemophilia A can occasionally even be made by testing factor VIII activity in the blood just after delivery. Chromogenic assays can be used to measure the plasma activity level of factor VIII . Nevertheless, these assays cannot reliably assess factor VIII levels below 1 IU/dL making it impossible to predict clinical phenotype based only on factor VIII activity (Attard et al., 2013) (Chandler et al., 2003).

A diagnosis of Haemophilia B at birth especially if it is a minor condition, may be deceptive and should be confirmed at six months of age. The well-known and standardized technique for determining the amount of factor IX activity in plasma is the one-stage clotting test. Although established methods for the use of chromogenic tests in clinical homeostasis laboratories are currently awaiting approval, they can still be used (Barrowcliffe, 2003).

Molecular characterization study for detection of Haemophilia

The initial stage in molecular characterization of persons with severe Haemophilia A is the detection of inversions in *VIII* (which encodes factor VIII) introns 22 and 1 which have been found to be inverted in 40-45% of severe patients and 1-6% of severe patients as well (Lakich et al., 1993) (Bagnall et al., 2002). Direct Sanger sequencing is used to screen the whole gene for mutations including all exons, intron-exon boundaries and the

promoter region. The Worldwide Factor VIII Variant Database offers descriptions of approximately 2000 different molecular anomalies in VIII (Oldenburg et al., 2001). Small insertions and deletions account for 25% of all molecular abnormalities recorded whereas point mutations (missense, nonsense and splice site changes) account for 67% (Lakich et al., 1993). Large deletions account for around 6% of all mutations. The multiplex ligation dependent probe amplification test is one possible approach for identifying deletions and duplications (Oldenburg et al., 2001). Between 2 and 18% of peoples with Haemophilia A have no known genetic mutations (Lakich et al., 1993). Full mutation investigation of VIII by direct Sanger sequencing is required in moderate and mild Haemophilia A due to the lack of common gene abnormalities (Oldenburg et al., 2001) (Jayandharan et al., 2005) (Vinciguerra et al., 2006).

Sequence analysis of the eight exons, intron–exon boundaries and the promoter region in *IX* (which codes for factor IX) is used to characterize the molecular makeup of Haemophilia B (Jayandharan et al., 2005). The gene's whole length contains causative genetic variations of which 1095 distinct variants have been identified to date (see the Factor IX Variant Database) (Vinciguerra et al., 2006). About 70% of mutations are missense nonsense and splice site variants with frame shift mutations coming in second at about 17% (Vinciguerra et al., 2006). A multiplex ligation-dependent probe amplification kit can identify deletions and duplications in IX. Approximately 3% and 2% of all known gene variations respectively are significant deletions in IX and promoter region changes which are rather unusual (Casaña et al., 2009). Mutations that spare the androgen-responsive region but impair other transcription factor binding sites (HNF4α, C/EBPα, and HNF6) may cause the unique Haemophilia B Leyden phenotype (Casaña et al., 2009). Mutations may develop in the short area of the proximal promoter (Human Genome Variation Society c.-50 to c.-18; Legacy −27 to +13) (Funnell and Crossley, 2014). These mutations cause a severe phenotype at birth which goes away throughout puberty and results in normal factor IX levels as an adult (Crossley et al., 1992). The human clotting factor IX promoter was studied using DNase I footprinting and gel shifts in vitro. Two cis-acting elements, the X and Y elements and members of the orphan receptor superfamily, HNF4, ARP1, and COUP/Ear3, were compared with a nearby classic HNF4 site at -20 of the human factor IX promoter (Naka and Brownlee, 1996). Less than 3% of individuals with Haemophilia B have no identified causal gene variant (Ljung et al., 2001) (Tagariello et al., 2007) (Goodeve, 2015). No widespread frequent genetic variant such as intron 22 inversion has been identified in Haemophilia B in contrast to Haemophilia A (Tagariello et al., 2007). However, about 10 founder gene variations account for 20–30% of moderate Haemophilia B patients (Jenkins et al., 2008). Improved prospects to define molecular faults in Haemophiliac patients especially those whose molecular defect has not yet been identified are offered by next-generation sequencing (Peyvandi et al., 2013).

Management process of Haemophilia

Direct blood transfusion was the first method of treating Haemophilia in 1840. Fresh frozen plasma was used to treat bleeding episodes throughout the 1950s and a large portion of the 1960s. When Judith Pool identified the cryoprecipitate part of fresh frozen plasma in 1965, modern therapy began (Pool and Shannon, 1965). Later, methods for isolating factor VIII or IX from sizable donor plasma pools produced lyophilised, freeze-dried factor VIII or IX concentrates enabling at-home treatment and significantly enhancing patient quality of life (Pool and Shannon, 1965) (Webster et al., 1965). Factor replacement therapy and gene therapy are the main treatment processes for Haemophilia (Webster et al., 1965).

Table 2: Difference between Factor Replacement Therapy and Gene Therapy

Factors	Factor Replacement Therapy	Gene Therapy	Reference
Definition	Involves administering clotting factors (either factor VIII for Haemophilia A or factor IX for Haemophilia B) to replace the missing or deficient proteins in the blood.	Aims to address the underlying genetic cause of Haemophilia by introducing a copy of the gene that encodes the missing clotting factor directly into the patient's cells.	(Peyvandi et al., 2016)
Administration	Can be given on-demand during bleeding episodes or as prophylactic treatment to prevent bleeding.	Typically involves a one-time infusion using a viral vector to deliver the therapeutic gene.	(Peyvandi et al., 2016)
Duration of Effect	Provides temporary relief, Standard half-life FVIII is around 12 hours and standard half-life FIX is about 18 hours. patients may need regular infusions, often multiple times a week.	Potentially long-lasting, as the body can produce its own clotting factors, reducing the need for regular infusions.	(Nathwani et al., 2014) (Hermans et al., 2021)
Limitations	Patients may develop antibodies (inhibitors) against the factor, reducing treatment efficacy. Requires ongoing management and can be expensive over time.	Still relatively new and may carry risks such as immune reactions. Long-term efficacy and safety are still being studied.	(High et al., 2014)

Clotting factor replacement therapy for Haemophilia

Any viruses that may have been present in the plasma are eliminated during processing of the concentrate (Berntorp, 2011). In a laboratory, recombinant clotting factors VIII and IX are produced which are not derived from blood. Kogenate FS, ESPEROCT, BeneFIX etc. are some clotting factor replacement therapy that used for Haemophilia management (Srivastava et al., 2020). Recombinant DNA technology is used in their creation. After being condensed into a powder they are combined with sterile water and administered by injection. In order to treat acute hemarthrosis the bleeding must be stopped as quickly as feasible (Aronstam et al., 1983). Ideally, treatment should begin as soon as the patient detects a bleed and before obvious swelling, discomfort and loss of joint function appear (Berntorp, 2011). A dosage of clotting factor concentrate (CFC) should be given right away in order to increase the patient's factor (VIII or IX) level to a level high enough to halt the bleeding (Aronstam et al., 1983) (Hermans et al., 2011) (Mathews et al., 2005). A physical examination, pain assessment and bleeding history assessment should all be part of the acute setting's bleeding evaluation (Hermans et al., 2011). An effective technique to help with the evaluation of early hemarthrosis may be ultrasound (Aronstam et al., 1983). Patients with haemophilia who have severe hemarthrosis should get intravenous clotting factor concentrate replacement therapy as soon as possible until the bleeding stops (Mathews et al., 2005). Patients with haemophilia who experience moderate to mild joint bleeding should get one

intravenous infusion of clotting factor concentrate if necessary, this infusion should be repeated based on how well the bleeding resolves (Mathews et al., 2005). Here a flowchart given below that show the Factor Replacement Therapy steps.

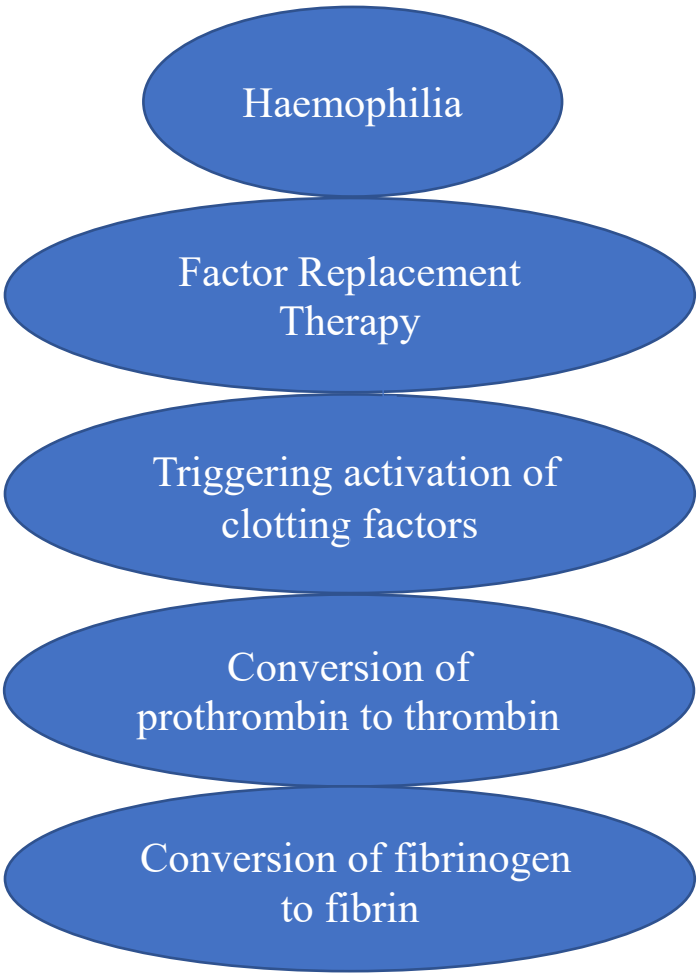

Figure 1: Factor Replacement Therapy steps (Peyvandi et al., 2016)

Gene Therapy for Haemophilia management

The current approach to gene therapy is preventative rather than curative. Gene therapy by constantly manufacturing factor VIII or IX after a single gene vector infusion can lower illness severity from severe to moderate or mild (Nathwani et al., 2011). This is especially intriguing because even a little increase in circulating coagulant proteins to at least 1% of normal levels can greatly improve the bleeding phenotype (Nathwani et al., 2011). A significant advancement in a gene therapy study for haemophilia B employing a self-complementary adenoassociated virus serotype 8 has been reported by Nathwani and associates. Patients who were monitored for 1·0–4·5 years showed a steady rise in factor IX activity to 5–7%

(Nathwani et al., 2014). With the exception of elevated liver enzymes which were treated with prednisolone in four individuals receiving the high vector dosage no severe adverse effects have been documented (Nathwani et al., 2014). There are currently active clinical studies for haemophilia B using several approaches (McIntosh et al., 2013). ROCTAVIAN (for haemophilia A) and HEMGENIX (for haemophilia B) are FDA approved gene therapy which used for Haemophilia (Ozelo et al., 2022) (Pipe et al., 2023). Gene therapy for haemophilia The VIII cDNA's size of 7.0 kb exceeds the packing capacity of adenoassociated viral vectors, posing a considerable challenge to developing effective viral gene delivery systems. (High et al., 2014). The creation of human VIII cDNAs with codon optimisation has shown that shorter VIII constructs can be more efficient and might lead to successful gene therapy (McIntosh et al., 2013) (Wang et al., 2014). A number of teams are working to get around the packing restriction by employing lentiviral vectors for gene transfer or by creating dual recombinant adenoassociated viral vectors for VIII delivery (Matsui et al., 2011). Here a flowchart given below that show the Gene Therapy process steps.

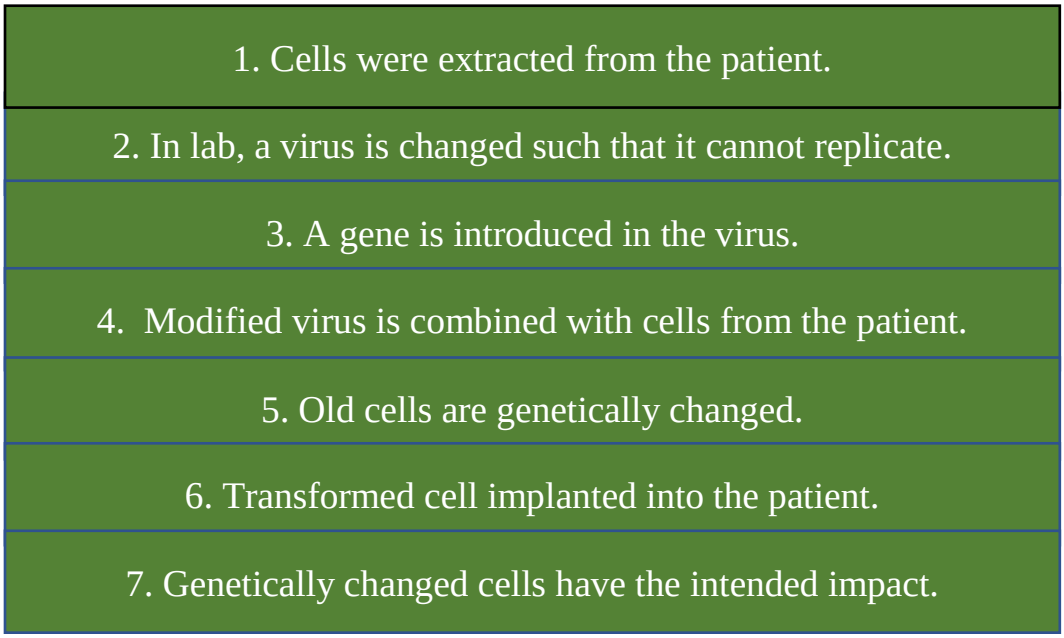

Figure 2: Gene Therapy process steps (High et al., 2014)

Inhibitors used in Haemophilia management

One of the most serious issues that haemophilia patients encounter is the development of coagulation factor inhibitors. Following therapy with factor concentrates up to one-third of patients acquire an antibody (inhibitor) to that factor making it inactive and putting the patient at danger for life-threatening haemorrhage (Saint-Remy et al., 2004). The procoagulant action of the relevant clotting factor is particularly neutralized by an inhibitor which is a polyclonal high-affinity IgG antibody that makes bleeding treatment challenging. Two characteristics of inhibitors are the titre and the anamnestic reaction (Giles et al., 1998). The patient's plasma's ability to neutralize clotting factor

in normal plasma is referred to as the titre (Giles et al.,1998). The International Society of Thrombosis and Homeostasis's F VIII/IX Subcommittee of Scientific Standardization Committee recommends using the Nijmegen Bethesda test to quantify inhibitors activity (Giles et al., 1998). A low-titre inhibitor is defined as one that falls between a cutoff value (often 0–6 BU) and 5 BU whereas a high-titre inhibitor is defined as having 5 BU or more. Patients with titres below 5 BU are classified as high responders or those who experience a quick anamnestic reaction to factor injection (Key, 2004). This distinction is significant because traditional replacement medication even at greater dosages to overwhelm the inhibitor can be utilised to treat persons with low titre and low responding inhibitors (Giles et al., 1998). Unless the inhibitor is completely eliminated patients with high titre or high response inhibitors can only be effectively treated with bypassing medicines (Key, 2004). Although significantly superior to prior therapies such as plasmapheresis, bypassing medicines have been associated with uneven predictability since the first usage of prothrombin complex concentrates (PCC), activated PCC (APCC), and recombinant factor VIIa (rFVIIa) (Shapiro et al., 2018). When exposed to severe haemophilia A, factor VIII inhibitors are formed. Percent of patients often in the initial 20 to 30 days of inhibitor development immunology is intricate and poorly understood (Giles et al., 1998). A multicausal immune response involving elements relevant to the patient and the therapy causes such a development (panel). Genetic factors include the kind of causal *VIII* mutation (Miller et al., 2012). Single-nucleotide polymorphisms in the 6 HLA (Human Leukocyte Antigen) locus and other immune regulatory genes and ethnic background have been implicated in studies on the genetic drivers of inhibitor development (Aledort and Dimichele, 1998). In non-severe types of haemophilia A inhibitors often appear when the immune system is highly stimulated or when there is an abnormally high level of concentrate exposure (such as during the postoperative phase) (Sharathkumar et al., 2003). Mutations that result in a persistent aberrant conformation of factor VIII especially those grouped in the A2 and C2 domains (Arg593Cys and Arg2150His) are linked to a high incidence of inhibitor development in mild haemophilia (Thompson et al., 1997). Although the cumulative prevalence might reach 4-5 percent the development of inhibitors is less common in severe haemophilia B than in severe haemophilia A (Thompson et al., 1997). Over 80% of these individuals react well to treatment. Inhibitors are extremely uncommon in patients with non-severe illness (0·05 per 100 therapy years according to one study (Fischer et al., 2015)). Recombinant factor IX is the same as that of plasma-derived factor IX and inhibitors in haemophilia B likewise show up after a median of just 9–11 exposure day (Poon et al., 2002). The tendency for individuals to experience anaphylactic responses at the moment of inhibitor production is a distinctive characteristic of factor IX inhibitors. For this reason it is advised that the first 10 factor (blood clotting factor) infusions be administered under close physician supervision (Giles et al., 1998).

Conclusion

The diagnosis and management of Haemophilia have significantly improved by the last 20 years. Haemophilia (A and B) are mainly treated by clotting factor replacement therapy and gene therapy. Gene therapy for haemophilia offers the potential of becoming free of haemorrhage without the requirement for ongoing pharmacological treatment. The many processes involved in gene therapy should be coordinated in a graded and partially overlapping integrated care paradigm (known as the hub-and-spoke model). In comparison to earlier therapies, gene therapy for haemophilia has advanced dramatically over the past ten years raising the possibility of much reduced bleeding rates and to a large part hemorrhage-free living. Since the liver is still maturing in youngsters it is possible that various concepts are required for gene therapy, therefore,

one cannot assume a steady response to gene therapy. To get above these restrictions more creative gene therapy ideas are needed. Treating the bleeding propensity without running the danger of developing inhibitors and creating new treatment choices for people who already have inhibitors will be the most significant advancement in the management of haemophilia in the future. Expanded access to treatment choices would be a significant advancement in many regions of the world. A variety of novel treatment approaches are being developed and in order to evaluate their safety and effectiveness sufficient and standardized post-registration surveillance procedures must be in place.

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