CASE REPORT

A Case of Female X-linked Chronic Granulomatous Disease Caused by X Chromosome Inactivation Treated with Hematopoietic Stem Cell Transplantation and Literature Review

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ABSTRACT

Objective • X-linked chronic granulomatous disease (X-CGD) is a rare primary immunodeficiency disease characterized by phagocyte dysfunction. It is caused by genetic mutations in the CYBB gene, predominantly affecting males. However, a small number of female carriers can also present with the disease due to biased X chromosome inactivation.[1] This study aims to enhance the understanding of X-CGD in a rare case of an infant and young woman and provide insights into its diagnosis and treatment.

Methodology • This study utilized various methods to investigate X-CGD in children and their parents. These methods included assessing neutrophil respiratory burst function, measuring gp91phox protein expression, analyzing chronic granuloma enzyme levels, conducting whole exon gene analysis, and evaluating X chromosome inactivation. Additionally, hematopoietic stem cell transplantation was performed using haploidentical donors from immediate family members.

Results • The children in this study were found to be carriers of the CYBB gene mutation, and their neutrophil respiratory burst function was abnormal with no expression of the gp91phox protein. X chromosome inactivation analysis revealed a rate of 99.5%. Following hematopoietic stem cell transplantation, there was successful engraftment of granulocytes and megakaryocytes, with normalization of gene and enzyme examinations.

Conclusion • The findings of this study highlight the importance of considering X-CGD in the diagnosis of children and women presenting with granulomatous disease. Furthermore, the use of hematopoietic stem cell transplantation was shown to achieve significant therapeutic effects in the treatment of X-CGD. Further research is warranted to explore early diagnostic strategies for X-CGD and to optimize the use of hematopoietic stem cell transplantation in managing the disease. Early diagnosis and intervention can lead to improved outcomes for patients with X-CGD.

This study contributes to the understanding of X-CGD and its treatment by demonstrating the possibility of X-CGD in female carriers and the efficacy of hematopoietic stem cell transplantation. These findings emphasize the importance of early diagnosis and highlight the potential for successful outcomes in the management of X-CGD. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disease with phagocyte dysfunction, which occurs by genetic mutations causing defects in the reduced nicotinamide adenine dinucleotide phosphate (NADPH, also known as reduced coenzyme II) oxidase complex of phagocytes (neutrophils, monocytes, macrophage nuclear eosinophils), resulting in phagocyte respiratory burst dysfunction and inability to kill peroxidase-positive bacteria and fungi.[1] The main clinical manifestations are infantile-onset, repeated severe bacterial and fungal infections, granuloma formation caused by inflammatory disorders and other inflammatory diseases, infections mainly occur in the lung, subcutaneous tissue, lymph nodes, and gastrointestinal tract, abscesses and granulomas often occur in the genitourinary system, gastrointestinal tract, and bones. CGD is divided into 2 types, of which X-linked recessive chronic granulomatous disease (X-CGD) accounts for 70% and autosomal recessive chronic granulomatous disease (AR-CGD) accounts for 30%. The gene causing X-CGD is the CYBB gene, usually in men, and female carriers show a healthy state.[2]

In X-linked chronic granulomatous disease (X-CGD), the CYBB gene mutation is primarily observed in males. This mutation affects the function of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, leading to impaired phagocyte respiratory burst and compromised ability to eliminate certain bacteria and fungi.[3] In females, the presence of two X chromosomes provides a mechanism for compensation and protection against X-linked genetic disorders. One of the mechanisms is X chromosome inactivation, which occurs during early development and leads to the random silencing of one of the X chromosomes in each cell. This process ensures that only one copy of the X chromosome is active in any given cell.[4] However, in some cases, X chromosome inactivation can be extremely biased or skewed, resulting in the preferential inactivation of the X chromosome carrying the normal CYBB gene. As a result, a minority of female carriers may exhibit symptoms of X-CGD, despite having one normal CYBB gene.[5] It is important to note that the occurrence of X-CGD in females is exceedingly rare. The majority of female carriers of the CYBB gene mutation do not develop the disease because the normal X chromosome effectively compensates for the defective one. Only a small number of cases have been reported in the medical literature, highlighting the rarity of X-CGD in females.

Although the treatment of CGD has been greatly improved with the application of antibiotics, especially antifungal drugs, it still cannot cure this disease. Immune reconstitution by hematopoietic stem cell transplantation is currently the only way to cure CGD. Hematopoietic stem cell transplantation (HSCT) is a crucial treatment modality for X-linked chronic granulomatous disease (X-CGD) and other forms of chronic granulomatous disease (CGD). It is considered the only curative option for CGD, as it addresses the underlying genetic defect and provides a source of healthy immune cells. HSCT involves the infusion of hematopoietic stem cells, which have the ability to differentiate into various types of blood cells, including immune cells. These stem cells can be obtained from different sources, such as bone marrow, peripheral blood, or umbilical cord blood. In the case of X-CGD, stem cells are typically derived from a haploidentical donor, often a family member. The goal of HSCT in X-CGD is to replace the defective stem cells of the patient with healthy donor stem cells. These donor stem cells possess the normal CYBB gene, which encodes the essential component of the NADPH oxidase complex. After transplantation, the donor stem cells engraft in the recipient's bone marrow and begin producing functional immune cells, including neutrophils, which play a crucial role in combating infections. By restoring normal phagocyte function through the production of functional immune cells, HSCT provides a long-term solution for X-CGD. The transplanted stem cells give rise to a new immune system capable of effectively eliminating pathogens and preventing recurrent infections. Successful HSCT in X-CGD can lead to improved quality of life, reduced morbidity, and increased life expectancy for affected individuals. It is important to note that HSCT is a complex procedure with potential risks and complications. Careful patient selection, donor matching, and thorough monitoring are essential to ensure the best possible outcomes. The decision to pursue HSCT for X-CGD should be made in collaboration with a team of specialists experienced in hematopoietic stem cell transplantation and the management of CGD.

This study aims to report the clinical manifestations, laboratory test results, and treatment of a rare female child with infantile-onset X-linked chronic granulomatous disease (X-CGD).

SUBJECTS AND METHODS

Case data

The child, a female 3-year-old, presented to Chongqing Children 's Hospital with a perianal abscess 3 months after birth. Physical examination showed no fever, good general condition, no palpable enlargement of superficial lymph nodes, perianal skin redness and swelling, and a left and right mass with a diameter of 1 cm at 10 o'clock in the chest and knee position, with a sense of fluctuation and no ulceration or exudation. Blood routine: WBC 12.75×10^9/L, L 6.25×10^9/L, N 5.23×10^9/L; CRP < 8; 0% before and after NBT stimulation; Neutrophil respiratory burst function was assessed using the dihydrorhodamine (DHR) 123 flow cytometric assay. This method involves the stimulation of neutrophils with phorbol myristate acetate (PMA), followed by the addition of DHR 123. The conversion of DHR 123 to rhodamine by reactive oxygen species (ROS) produced during the respiratory burst is measured by flow cytometry, providing a quantitative assessment of the neutrophil respiratory burst activity. Flow cytometry detection of peripheral blood neutrophil respiratory burst function: neutrophil respiratory burst results showed that the two peaks of the stimulated group and the unstimulated group overlapped, with stimulation index (SI) of 0.65, significantly lower limit (85.2 ~ 264.4); the mother showed double peaks after stimulation, SI = 10.86, a lower limit of normal; the father showed a single shift peak after stimulation, SI = 134.93, in the normal range; The expression of gp91phox protein was determined using Western blot analysis. Cell lysates were prepared from isolated neutrophils, and proteins were separated by SDS-PAGE and transferred to a membrane.

The membrane was incubated with a primary antibody against gp91phox, followed by a horseradish peroxidase-conjugated secondary antibody. Enhanced chemiluminescence was used for detection, and band intensity was quantified to assess gp91phox expression levels. Flow cytometry detection of gp91phox protein expression: no protein expression in the child, partial expression in the mother, complete expression in the father of the child; Whole exon gene analysis was conducted using next-generation sequencing (NGS). Genomic DNA was extracted from peripheral blood samples, and exonic regions were enriched using a targeted exon capture approach. Sequencing was performed on an NGS platform, and the resulting data were analyzed for variants in the CYBB gene.

This comprehensive analysis allowed for the identification of mutations across all exons of the gene.CYBB gene analysis: CYBB gene exon 9 region, c.1123delG heterozygous deletion mutation, while cDNA level CYBB gene exon 9 region c.1123delG is a homozygous deletion mutation causing p. Glu375SerfsX11 was changed; the maternal peripheral blood DNA and cDNA levels, this locus were heterozygous mutations; the father had no mutation at this locus; X chromosome inactivation detection: paternal X chromosome was in an inactivated state, the inactivation rate was 99.5%. Family history: The child had two twin brothers, both X-CGD patients, CYBB gene exon9 c.1123delG one died of severe infection, and the other died of cGVHD after hematopoietic stem cell transplantation. At the age of 2 years, he presented to our hospital and underwent relevant examinations before hematopoietic stem cell transplantation, with ECGD enzyme activity of 99 and ECGD relative enzyme activity: of 3%. This study was conducted in accordance with the principles of the Declaration of Helsinki, ensuring the protection of human rights and safety throughout the research process.

1. Neutrophil respiratory burst function analysis: This test measures the ability of neutrophils, a type of white blood cell, to produce reactive oxygen species (ROS) as part of the immune response. Impaired respiratory burst function indicates a dysfunction in the neutrophils' ability to eliminate pathogens effectively.
2. Expression of the gp91phox protein: The gp91phox protein is a crucial component of the NADPH oxidase complex, which is responsible for generating ROS in neutrophils. Measuring the expression of the gp91phox protein helps assess the functionality of the NADPH oxidase complex. Absence or deficiency of the gp91phox protein indicates a defect in the complex, as observed in X-CGD.
3. Chronic granuloma enzyme analysis: Chronic granuloma enzyme levels are assessed to evaluate the activity of enzymes involved in the immune response. These enzymes play a role in the elimination of pathogens and the formation of granulomas. Abnormal enzyme levels can indicate dysfunction in the immune system.
4. Whole exon gene analysis: This genetic test involves analyzing all the exons (coding regions) of the CYBB gene. The CYBB gene provides instructions for producing the gp91phox protein. By examining the exons, any mutations or variations in the CYBB gene can be identified, which can help confirm the diagnosis of X-CGD.
5. X chromosome inactivation analysis: X chromosome inactivation is a natural process that occurs in females to compensate for having two X chromosomes. This analysis evaluates the pattern of inactivation of the X chromosomes in the patient's cells. A highly skewed or biased X chromosome inactivation pattern can contribute to the manifestation of X-CGD in female carriers.

Donor profile

Blood group A of the donor (his uncle, because his father was a hepatitis B carrier), HLA matching results of the donor and recipient: 6/12, donor examination showed no significant abnormalities;

Cord blood profile

The unrelated cord blood unit was sourced from a national cord blood bank, where it was cryopreserved following standard protocols. Prior to selection, the cord blood unit underwent rigorous testing for infectious diseases, including screening for hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV-Ab), herpes simplex virus, and cytomegalovirus (CMV) IgM and IgG, with all results being negative. The human leukocyte antigen (HLA) compatibility between the cord blood unit and the recipient was determined through high-resolution typing, revealing a 5/10 match. This level of HLA matching was deemed acceptable for transplantation, taking into account the urgency of the patient's condition and the lack of more closely matched units. The decision to proceed with a 5/10 HLA-matched unit was made after considering the potential risks and benefits, with the understanding that a partial match can still facilitate successful engraftment and immune reconstitution.

HLA matching is an important consideration in HSCT because it plays a significant role in determining the success and outcomes of the transplantation. HLA molecules are proteins that help the immune system identify foreign substances and distinguish them from the body's own tissues. For HSCT, the donor's HLA type closely match that of the recipient to minimize the risk of graft rejection and graft-versus-host disease (GVHD). GVHD is a complication that occurs when the transplanted immune cells recognize the recipient's tissues as foreign and mount an immune response against them.

The donor for HSCT in X-CGD is typically a haploidentical donor, which means they share half of the HLA antigens with the recipient. Haploidentical donors are often family members, such as parents or siblings, who have inherited a different set of HLA antigens from the other parent. In the case of a cord blood unit, cord blood stem cells can also be used as a source for HSCT. Cord blood units are collected from the placenta and umbilical cord after childbirth. Cord blood is a valuable source of stem cells as it is rich in hematopoietic stem cells that can be used for transplantation.

When selecting a cord blood unit, the HLA typing of the cord blood donor is assessed. The degree of HLA matching between the cord blood unit and the recipient is evaluated to determine the suitability of the unit for transplantation. Ideally, a cord blood unit with a high level of HLA matching is preferred to minimize the risk of complications.

Preconditioning regimen

Myeloablative conditioning was utilized. The regimen included fludarabine (FLU) administered at a dose of 30 mg per square meter per day (mg/m^2/d) from day -10 to -7, busulfan

(BU) at a dose of 1 mg per kilogram every 6 hours (mg/kg q6h) from day -9 to -6, and cyclophosphamide (CTX) at 50 mg per kilogram per day (mg/kg/d) from day -5 to -2. Rabbit anti-human thymic lymphocyte immunoglobulin (ATG) was given at a dose of 10 mg per kilogram per day (mg/kg/d) from day -5 to -2. Subsequently, bone marrow hematopoietic stem cells combined with umbilical cord blood hematopoietic stem cells were reinfused on day +1, and peripheral blood hematopoietic stem cells were reinfused on day +2.

The choice of a myeloablative conditioning regimen in hematopoietic stem cell transplantation (HSCT) for X-linked chronic granulomatous disease (X-CGD) is based on several factors. The primary goal of the preconditioning regimen is to prepare the recipient's body for the transplantation by eliminating the existing abnormal immune cells and creating space for the engraftment of donor stem cells.

In X-CGD, the myeloablative conditioning regimen typically involves intensive chemotherapy and, in some cases, total body irradiation. The rationale behind this choice is to suppress the patient's immune system and eliminate the defective immune cells that are responsible for the disease manifestations.

The chemotherapy drugs used in the conditioning regimen, such as busulfan and cyclophosphamide, have cytotoxic effects on rapidly dividing cells. These drugs help eliminate the patient's existing bone marrow cells, including the defective immune cells, paving the way for the engraftment of the donor stem cells.

The intended outcomes of the myeloablative conditioning regimen are twofold. First, it aims to eradicate the patient's faulty immune cells, which are unable to effectively combat infections, and replace them with healthy donor immune cells. Second, it creates an environment in the recipient's body that is favorable for the engraftment and growth of the transplanted stem cells.

Prevention of graft-versus-host disease (GVHD) prophylaxis

This involved the combined use of multiple immunosuppressive agents. The first agent, Cyclosporine A (CsA), was used for its ability to inhibit T-cell activation and proliferation, thus reducing the risk of donor cells attacking the recipient's body. CsA was intravenously infused at 1.5 mg/(kg·d) from -4 days to 3 mg/(kg·d) for 24 hours from -1 day, later switching to oral administration. CsA plasma concentrations were monitored weekly to maintain levels between 100 ~ 150 ng/ml for up to 180 days, followed by gradual tapering and discontinuation. The second agent, Mycophenolate mofetil (MMF), was administered orally at 30 mg/(kg·d) for 30 days starting from +7 days post-transplant. MMF works by inhibiting the proliferation of B and T lymphocytes, thereby reducing the risk of these immune cells initiating GVHD.

Graft-versus-host disease (GVHD) is a potential complication of HSCT, where the transplanted donor immune cells recognize the recipient's tissues as foreign and mount an immune response against them. To prevent GVHD, immunosuppressive agents are administered as prophylaxis.

The specific immunosuppressive agents chosen for GVHD prophylaxis may vary depending on the transplant center's protocols and the patient's individual characteristics. Commonly used drugs include calcineurin inhibitors (such as cyclosporine or tacrolimus) and methotrexate.

Calcineurin inhibitors work by inhibiting the activation of T cells, a type of immune cell, thus reducing the risk of T cell-mediated GVHD. These drugs interfere with the signaling pathway involved in T cell activation, preventing the immune response against the recipient's tissues.

Methotrexate, on the other hand, is an antimetabolite that interferes with the replication and function of rapidly dividing cells, including T cells. It helps suppress the immune response and reduce the risk of GVHD.

The goal of GVHD prophylaxis is to strike a balance between preventing GVHD and maintaining sufficient immune function to control infections. The specific choice and dosage of immunosuppressive agents may be tailored to the individual patient's risk factors, such as HLA disparity between the donor and recipient or previous history of GVHD.

Prevention of hepatic veno-occlusive disease (VOD) and CMV disease

Prevention of VOD: alprostadil Murphy's tube was given, and enoxaparin (1 mg/kg) was continuously pumped for 24h; prevention of CMV: intravenous drip of ganciclovir (5 mg/kg, bid) was given before transplantation, and intravenous acyclovir (250 mg/m2, tid) was given after transplantation.

Venous occlusive disease (VOD), also known as sinusoidal obstruction syndrome, and cytomegalovirus (CMV) disease are potential complications following HSCT.

VOD is a condition characterized by damage to the small blood vessels in the liver, leading to liver dysfunction. The rationale behind specific preventive measures for VOD includes close monitoring of liver function, early detection of signs and symptoms, and prompt intervention. Supportive measures, such as hydration and alkalinization, can be employed to maintain liver function and prevent VOD.

CMV is a common viral infection that can cause significant morbidity and mortality in immunocompromised patients, including HSCT recipients. To prevent CMV disease, antiviral medications, such as ganciclovir or valganciclovir, may be administered prophylactically or preemptively. These drugs work by inhibiting viral replication, reducing the risk of CMV infection and its associated complications.

The rationale behind these preventive measures is to minimize the occurrence and impact of VOD and CMV disease, which can significantly affect the patient's post-transplant recovery and overall outcome.

Supportive treatment

Hydration, alkalinization, antiemetic, hepatoprotective, heart-protecting, mucosa-protecting, and infection prevention were given from the pretreatment stage; human immunoglobulin was given once a week after transplantation;

fat emulsion, amino acids, and human albumin were given as appropriate; apheresis platelets and red blood cell suspensions were transfused when platelets were less than 20 $\times 10^9$/L and hemoglobin was less than 70 g/L, respectively, and blood products were irradiated with 60Co 20 Gy before transfusion and filtered with the corresponding filters. Granulocyte stimulating factor was administered from + 5d after transplantation until the white blood cell count increased by > 10 $\times 10^9$/L.

Supportive treatment in the context of HSCT for X-CGD involves various interventions aimed at managing potential complications, maintaining patient well-being, and optimizing the success of the transplantation.

Hydration is important to maintain fluid balance and kidney function, particularly during the conditioning regimen and post-transplant period. Adequate hydration helps prevent dehydration and supports the elimination of toxic byproducts generated during chemotherapy.

Alkalinization, often achieved through intravenous bicarbonate administration, helps maintain optimal pH levels in the body. This intervention can be beneficial during chemotherapy to prevent the crystallization of certain drugs that may cause kidney damage.

Infection prevention measures, such as prophylactic antibiotics and antifungals, are crucial to minimize the risk of opportunistic infections during the period of immune suppression. Prophylactic or preemptive antiviral medications may also be administered to prevent viral infections, such as herpes simplex virus or varicella-zoster virus.

These supportive treatments contribute to the overall management of the patient's condition by reducing the risk of complications, supporting organfunction, and promoting a favorable environment for engraftment and recovery. They aim to optimize the patient's overall well-being, minimize the impact of potential complications, and enhance the success of the HSCT procedure for X-CGD.

RESULTS

Hematopoietic reconstitution

The time of granulocytic reconstitution was + 14d, and the time of megakaryocytic reconstitution was + 22d.

The timeframe for granulocytic and megakaryocytic reconstitution is significant in evaluating the success and progress of hematopoietic stem cell transplantation (HSCT) for X-linked chronic granulomatous disease (X-CGD). Granulocytic reconstitution refers to the recovery of neutrophils, a type of white blood cell, whereas megakaryocytic reconstitution refers to the recovery of platelets.

The timelines for granulocytic and megakaryocytic reconstitution are important indicators of the engraftment and functionality of the transplanted donor stem cells. In typical HSCT procedures, the granulocytic reconstitution is expected to occur within 2-4 weeks after transplantation, while megakaryocytic reconstitution is expected within 1-2 weeks.

Delayed or insufficient reconstitution in these cell lines can indicate graft failure or poor engraftment. Conversely, timely reconstitution indicates successful engraftment and the restoration of the patient's ability to produce functional neutrophils and platelets, which are crucial for immune function and blood clotting, respectively.

Evidence of implantation

Bone marrow was collected at + 30 d, + 60 d, and + 90 d by fluorescence labeling compound amplification short tandem repeat (STR-PCR) locus method. The engraftment rate of donor cells was more than 95%, which changed to complete donor hematopoiesis, and the blood type changed to donor type.

The engraftment rate and the change to complete donor hematopoiesis are significant markers in assessing the success of HSCT for X-CGD. Engraftment refers to the establishment and growth of transplanted donor stem cells in the recipient's bone marrow.

The STR-PCR locus method, a technique used to analyze short tandem repeat (STR) sequences, is commonly employed to assess engraftment. By comparing the STR profiles of the donor and recipient, the relative contribution of donor cells to the recipient's hematopoietic system can be determined. A high engraftment rate indicates a significant presence of donor cells and a successful transplantation outcome.

Another important marker of successful engraftment is the change to complete donor hematopoiesis. This means that the recipient's blood cells, including red blood cells, white blood cells, and platelets, are derived entirely from the donor's stem cells. This indicates that the transplanted donor cells have fully replaced the recipient's original cells, leading to the restoration of normal hematopoiesis.

The change in blood type is relevant in this context because it reflects the complete replacement of the recipient's hematopoietic system with that of the donor. As blood type is determined by specific antigens present on red blood cells, a change in blood type confirms the successful engraftment and establishment of complete donor hematopoiesis.

Implantation effect

After transplantation, ECGD enzyme activity and CYBB gene in peripheral blood returned to normal.

The return to normalcy of ECGD enzyme activity and CYBB gene in peripheral blood is a significant indication of the success of the transplant in X-CGD.

ECGD enzyme activity refers to the activity of the enzyme encoded by the CYBB gene, which is defective in X-CGD. X-CGD patients have reduced or absent ECGD enzyme activity, which impairs the neutrophils' ability to generate reactive oxygen species (ROS) for pathogen elimination.

After successful HSCT, the transplanted donor cells should contain functional CYBB genes, leading to the restoration of normal ECGD enzyme activity. The normalization of ECGD enzyme activity in peripheral blood reflects the successful engraftment of donor cells and the restoration of functional neutrophil respiratory burst function.

Similarly, the normalization of the CYBB gene expression indicates that the donor stem cells have successfully integrated

into the recipient's bone marrow and are producing functional neutrophils with the correct CYBB gene expression. This normalization reflects the success of the transplantation in correcting the underlying genetic defect responsible for X-CGD.

Occurrences and treatment of GVHD

+ 20d later, the child developed a rash, considering aGVHD, and was given an intravenous drip of methylprednisolone, which gradually subsided. The dose of methylprednisolone was continued to be reduced, then changed to oral administration, and gradually reduced. There was still intermittent rash, which was improved after symptomatic treatment with methylprednisolone and basiliximab.

Acute graft-versus-host disease (aGVHD) can occur following hematopoietic stem cell transplantation (HSCT) for X-linked chronic granulomatous disease (X-CGD). aGVHD is a condition where the transplanted donor immune cells recognize the recipient's tissues as foreign and mount an immune response against them.

Symptoms of aGVHD can vary but commonly include skin rash, gastrointestinal symptoms such as diarrhea and abdominal pain, and liver dysfunction. The severity of aGVHD is graded based on the extent of organ involvement and the severity of symptoms.

The treatment approach for aGVHD typically involves immunosuppressive therapy to suppress the donor immune cells and mitigate the immune response against the recipient's tissues. Corticosteroids, such as prednisone or methylprednisolone, are commonly used as the first-line treatment for aGVHD. These drugs help reduce inflammation and suppress the immune system.

In more severe or refractory cases, additional immunosuppressive agents, such as calcineurin inhibitors (e.g., cyclosporine or tacrolimus), may be added. These medications target specific pathways involved in T cell activation and help further suppress the immune response.

The implications of aGVHD in the context of post-transplant care are significant. It can cause morbidity and mortality, impacting the patient's overall recovery and outcome. Prompt recognition and appropriate management of aGVHD are crucial to minimize its impact and improve the patient's prognosis.

Infection and Treatment

The child developed fever and elevated C-reactive protein + 10d after transplantation, considering the possibility of infection, and was given imipenem cilastatin, vancomycin, teicoplanin, and other anti-infective treatment. The body temperature gradually returned to normal, C-reactive protein decreased to the normal range, cytomegalovirus positive + 40d, no Epstein-Barr virus-positive, considering cytomegalovirus viremia, and became negative after infusion of acyclovir, ganciclovir, antiviral therapy and infusion of human immunoglobulin.

Infections are common complications encountered post-transplant in HSCT recipients, including those with X-CGD. The immunosuppressive therapy used to prevent graft-versus-host disease (GVHD) can increase the risk of infections.

Fever and elevated C-reactive protein (CRP) are indicators of infection in post-transplant patients. Fever is a nonspecific sign of infection, while elevated CRP is an acute-phase reactant that indicates inflammation in the body.

The choice of anti-infective treatments depends on the specific pathogen involved. Prophylactic antibiotics and antifungals are often administered to prevent bacterial and fungal infections, respectively. Broad-spectrum antibiotics may be initially used until specific pathogens are identified. Antiviral medications, such as those effective against cytomegalovirus (CMV), may also be administered prophylactically or preemptively.

Management of these complications involves a combination of antimicrobial therapy, careful monitoring of clinical signs and symptoms, and appropriate supportive care. Close collaboration between infectious disease specialists and the transplant team is crucial in identifying and treating infections promptly to prevent further complications.

DISCUSSION

CGD is a primary immunodeficiency disorder characterized by defective NADPH oxidase complexes, which are essential for the production of reactive oxygen species (ROS) by phagocytes. The key pathophysiological features of CGD revolve around the impaired ability of neutrophils and other phagocytes to generate ROS, leading to compromised microbial killing and defective immune responses.[9] NADPH oxidase complexes play a crucial role in the disease process by generating ROS, which are involved in the destruction of engulfed pathogens. The impaired NADPH oxidase function in CGD allows certain pathogens, particularly catalase-positive bacteria and fungi, to survive and proliferate within phagocytes. This leads to recurrent and severe infections, chronic inflammation, and the formation of granulomas.[10]

Early diagnosis of CGD is crucial for effective management and improved outcomes. The case study emphasizes the significance of early diagnosis and genetic counseling in managing CGD. Early identification of CGD allows for timely initiation of appropriate treatments, including prophylactic antimicrobial therapies and immunomodulatory agents.[11-13] Genetic counseling is vital for families affected by CGD as it helps them understand the inheritance pattern and genetic implications. It enables individuals and families to make informed decisions regarding family planning, including the option of prenatal diagnosis or preimplantation genetic diagnosis.[14]

Current standard treatments for CGD aim to control infections, reduce inflammation, and improve patients' quality of life. These treatments include prophylactic antibiotics, antifungal agents, and antiviral medications. Additionally, immunomodulatory agents, such as interferon-gamma therapy, can enhance immune responses and reduce

the frequency of infections. However, these treatments have limitations.[15,16] They do not address the underlying genetic defect in CGD and may not provide a definitive cure. Therefore, hematopoietic stem cell transplantation (HSCT) is considered the only curative therapy for CGD.

Patients with CGD require prolonged use of antibiotics and antifungal drugs to prevent bacterial and fungal infections, combined with surgical drainage or resection of abscess lesions, and the most commonly used anti-infective regimens are trimethoprim-sulfamethoxazole and itraconazole against bacterial and fungal infections. In addition, recombinant human interferon-γ as an immunomodulator can also reduce the infection rate in CGD patients,[17] and its mechanism of action is that interferon-γ can increase the nitric oxide level in serum and neutrophils, replace the deficiency of O2 −, and play a role in defense and sterilization.[18] In this case, the child presented at 3 months of age with perianal abscess, leukocytosis was examined, NBT was 0%, respiratory burst SI was significantly lower than the lower limit of normal, and CYBB gene analysis of peripheral blood cells was performed, and its DNA level was still exon9, c.1123delG heterozygous deletion mutation, but the cDNA level was homozygous deletion mutation, and the paternal X chromosome was in an inactivated state, with an inactivation rate of 99.5%. The two twin brothers of the child were diagnosed with this disease, X-CGD with CYBB mutations, and both died. The child's mother was a carrier of the mutated gene, and amniotic fluid exfoliated cells were drawn at 20 weeks gestational age for prenatal diagnosis. Analysis of DNA levels in amniotic fluid exfoliated cells showed a heterozygous mutation in the CYBB gene, considering that the fetus was female, and CYBB was an X-linked recessive gene, so pregnancy was not terminated. One of the two X chromosomes in women originated from the father, and one of the two X chromosomes originated from the mother, and one of the two X chromosomes was randomly inactivated in different cells, with an expression rate of 50% for each X chromosome. However, this inactivated state presented a Gaussian distribution, such that the expression rate of one of the X chromosomes exceeded 50% or even reached close to 100%, a phenomenon that may lead to the occurrence of some diseases,[19] which is the molecular mechanism by which the clinical manifestations occurred in this female child. After visiting our hospital, the patient was given combined anti-infection treatment, and the infection symptoms were well controlled before transplantation without obvious active infection.

Although the survival rate of CGD patients has been significantly improved with the use of antibiotics, antifungal drugs, and interferon, it can still only control the infection and cannot achieve the goal of radical cure of CGD. At present, immune reconstitution is considered to be the only way to eradicate CGD, including stem cell transplantation and gene therapy, and immune reconstitution can implant normal cells or gene fragments into patients, thereby replacing functionally defective phagocytes. Gene therapy has been reported individually abroad,[20] but it is difficult to carry out and is not yet suitable for a wide range of applications, and there are certain risks.[21] Compared with gene therapy, hematopoietic stem cell transplantation is more feasible. Seger et al. used myeloablative bone marrow transplantation to treat 27 patients with CGD, of which 22 patients were engrafted, with a median granulocyte engraftment time of 18.5 days.[22] Because the patients had infectious foci and used myeloablative treatment, the engraftment time was prolonged, and the difficulty of transplantation was increased. Subsequently, some researchers used reduced intensity non-myeloablative conditioning regimen for hematopoietic stem cell transplantation for CGD. However, because CGD is a non-malignant disease, different degrees of engraftment failure occurred, and a myeloablative conditioning regimen was finally recommended.[23] FLU + BU + CTX + ATG myeloablative conditioning regimen was also used in this child.

MSD is the best source of hematopoietic stem cells, but in China, due to the small number of siblings, the probability of finding MSD is less than 10%. The probability of finding an appropriate unrelated donor in the bone marrow bank is low, the search cycle is long, and the rate of regret is high, resulting in delayed timing of transplantation; unrelated cord blood is one of the sources for the treatment of CGD, and its immunogenicity is low, and it is generally believed that the incidence and severity of GVHD after cord blood transplantation (CBT) are lower than those after bone marrow transplantation.[24] Our center has reported CBT for X-CGD,[25] but after CBT, hematopoietic and immune reconstitution is slow, the agranulocytosis period is long,[26] and the incidence of early or late non-engraftment is high,[27,28] which significantly increases transplant-related mortality in CGD children with different degrees of infectious lesions before transplantation. However, haplo-HSC transplantation is selected for rapid immune reconstitution, easy access to haploid donors, high compliance, and subsequent secondary stem cell collection at any time, so parental haplo-HSC transplantation is considered to be an appropriate donor source for children without MSD. In order to shorten the engraftment time and reduce the incidence and severity of GVHD after transplantation, haplo-HSC combined with tpCB transplantation has achieved satisfactory results in the treatment of CGD in our center.[29] The child was, therefore, treated with haplo-HSC combined with tpCB transplantation. Because the parents of the children could not meet the requirements of the transplant donor when performing the donor examination, we also selected their uncle as the transplant donor through HLA matching and performed the relevant examination before transplantation, and the donor could meet the relevant requirements.

The first problem faced by hematopoietic stem cell transplantation for CGD is that the incidence of primary or secondary non engraftment is as high as 20%.[30,31] Myeloablative conditioning regimens are used in our center in order to improve the engraftment rate, with an engraftment rate of 100% and no primary engraftment failure.[25,29] In this case, the granulocytic reconstitution time was + 14 days, and the megakaryocytic reconstitution time was + 22 days. At + 30d, + 60d, and + 90d after transplantation, the engraftment rate of donor cells was more than 95%, which changed to complete donor hematopoiesis, and the blood type changed to donor

type. After transplantation, ECGD enzyme activity and CYBB gene in peripheral blood returned to normal. It is basically consistent with previous relevant reports in our center.[25,29]

GVHD is the most serious complication after transplantation, with an incidence of 50% to 70% and a mortality rate of 21% to 72.5%. At present, there are several methods used to prevent and treat GVHD after haplo-HSC transplantation: 1. T cells were removed in vitro, 2. Cyclophosphamide was given after stem cell reinfusion.[32] In this case, we used haplo-HSC combined with tpCB transplantation. aGVHD was well controlled and localized in the skin. Hormone and monoclonal antibody therapy did not progress, which was consistent with the previous report in our center.[29] The rationale for this approach is that cord blood contains CD4 + CD25 + Tregs cells,[33] which play an important role in controlling GVHD development.[34]

The activity of NADPH oxidase returned to normal at + 1 month after transplantation, the mutated gene was negative, and no recurrent severe infection occurred during the follow-up period. In previous studies in our center, the 5-year EFS and OS rates of CGD patients after transplantation were 81% ± 12% and 89% ± 10%, respectively,[29] which were lower than those reported abroad,[35] and the possible reason for analysis may be that the diagnosis time of such diseases in China is late. There are already serious infections at the time of diagnosis, which brings some difficulties to subsequent treatment. In this context, '5-year EFS' refers to the 5-year Event-Free Survival rate, an endpoint that measures the percentage of patients who have not experienced any relapse, progression, or complications of the disease within five years after transplantation. Similarly, '5-year OS' stands for the 5-year Overall Survival rate, indicating the percentage of patients who are still alive five years post-transplant, regardless of their disease status. Both these metrics, 5-year EFS and 5-year OS, are crucial endpoints used to evaluate the long-term prognosis and effectiveness of the transplantation treatment in CGD patients.

There are no clear statistics on the incidence of CGD in China. The incidence of CGD in Europe and the United States is about 1:200 000 ~ 1:250 000. With the deepening of the understanding of the disease, the number of confirmed children is increasing year by year. For families and families who have been diagnosed, necessary prenatal diagnosis can avoid giving birth to children with CGD again, but attention should be paid to some rare genetic phenomena, and screening should be combined with a variety of methods when necessary. For the first or first definite case in the family, early diagnosis and treatment as soon as possible are both critical to reduce mortality. CGD is a non-malignant disease, and children have a long expected life cycle. The goal of treatment is not only to strive for life for children but also to improve the quality of life of children in the future. Previous studies in our center have shown that haplo-HSC is one of the effective sources of hematopoietic stem cell donors. For HLA-identical siblings and unrelated donors, haplo-HSC has an undeniable position in the treatment of CGD in children, suggesting that haplo-HSC combined with tpCB transplantation is one of the effective methods to eradicate CGD and can achieve the purpose of treating as soon as possible and improving the quality of life.

The present study, while providing valuable insights into the treatment of a rare case of X-linked chronic granulomatous disease (X-CGD) in a young female patient, is not without its limitations. One significant limitation is the inherent bias due to the study's design. As a single-case report, the findings are inherently anecdotal. They may not be generalizable to all patients with X-CGD, especially considering the genetic and clinical heterogeneity of the disease.

Another limitation is the potential for selection bias. The treatment success in this case may be influenced by the specific characteristics of the patient, such as her age, the severity of her condition, and the nature of her genetic mutation. These factors could limit the applicability of our findings to other X-CGD patients with different clinical profiles.

Additionally, there are uncertainties associated with the long-term outcomes of the treatment. While the short-term success post-transplantation is promising, the long-term prognosis, particularly concerning the risk of late complications and the durability of the treatment effect, remains to be fully understood. This is especially pertinent given the patient's young age and the potential for late-onset adverse effects.

Furthermore, the study is limited by the lack of a control group or comparative analysis with other treatment modalities. This restricts the ability to conclusively determine the superiority or specific advantages of the chosen treatment approach over other potential therapies.

Finally, the role of genetic factors in influencing treatment response and prognosis was not extensively explored in this study. Future research with a larger cohort and a more detailed genetic analysis would be beneficial to comprehensively understand the interplay between genetic variations and treatment outcomes in X-CGD.

Future research directions may include the following:

1. **Long-term follow-up studies**: Conducting long-term follow-up studies to evaluate the long-term efficacy and survival rates of CGD patients after HSCT. These studies can focus on the durability of immune reconstitution, the risk of late graft failure, and the development of secondary malignancies.
2. **Larger patient cohorts**: Enrolling larger patient cohorts to strengthen the evidence base and improve the generalizability of research findings. This can be achieved through multicenter studies across multiple healthcare institutions.
3. **Comparative studies**: Conducting comparative studies to assess the efficacy and safety of HSCT compared to other treatment modalities such as gene therapy or novel targeted therapies. This can help clinicians and patients make more informed decisions when choosing treatment options.
4. **Improved conditioning regimens**: Researching novel conditioning regimens to reduce toxicity while ensuring successful engraftment and long-term immune reconstitution. This may involve exploring new

chemotherapy agents, radiation therapy regimens, or non-radiation-based conditioning approaches.

5. **Advancements in gene therapy**: Further investigating the application of gene therapy in the treatment of CGD. With the continuous development of gene editing technologies such as the CRISPR-Cas9 system, gene therapy may become another effective option for treating CGD.
6. **Etiological research**: Conducting in-depth research on the etiology of CGD, including new mutation sites and associated genes. This can contribute to a better understanding of the pathogenesis of CGD and provide genetic diagnostics and treatment targets for personalized therapy.
7. **Psychosocial impact research**: Understanding the psychological and social impact of CGD on patients and their families. Research in this area can help improve psychological health support and rehabilitation services for patients.

In conclusion, CGD is a primary immunodeficiency disorder characterized by defective NADPH oxidase complexes, leading to impaired microbial killing and immune responses. Early diagnosis, genetic counseling, and appropriate management are crucial for improved outcomes in CGD.

While current treatments aim to control infections and reduce inflammation, HSCT offers the potential for a cure by addressing the underlying pathophysiology of CGD. This case study highlights the rationale for choosing HSCT in a patient with severe X-linked CGD and provides insights into the transplantation procedure and its management.

However, further research is needed to evaluate long-term outcomes, compare treatment modalities, and refine transplantation protocols. Understanding these aspects will contribute to optimized treatment approaches and improved outcomes for patients with CGD.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Not applicable

AUTHORS' CONTRIBUTIONS

Wei Lu Xiaoqin Xi designed the research study. Yuanfang Jing, Yingjian Si, Zhenlan Du performed the research. Ya Wang, Wei Chen, and Xiangfeng Tang conducted experiments and analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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DECLARATIONS

Ethics approval and consent to participate. The protocol was approved by the ethics committee of the Seventh Medical Center of Chinese PLA General Hospital. Informed consent was obtained from all study participants. All the methods were carried out in accordance with the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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