Clinical study on cytomegalovirus-associated haemophago-cytic syndrome following allogeneic hemotoxic stem cell transplantation

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Abstract: **Objective** To study the clinical feature, diagnose and treatment of hematophagocyte lymphohistiocytosis following allogeneic hemotoxic stem cell transplantation. **Methods** We report one case of AML-M2a with HLH after URD-UCBT with review of the literature. **Results** The patient appeared skin, liver and intestinal tract GVHD until day +6, He was treated with corticosteroids, CsA, Zenapax, MMF and FK506 successively. The aGVHD symptoms were ever improved, but appear cytomegalovirus-associated haemophagocytic syndrome on day +26. According to HLH-2004 Therapeutic Guideline for HLH, the patient was treated with VP16, corticosteroids, CsA, antiviral agents, intravenous immunoglobulin and supportive therapy. +61 Days after treatment of serum cytomegalovirus early / late matrix protein (CMV-PP65) negative, +55, +63 days no hemophagocytic bone marrow, bone marrow showed complete remission HLH His clinical situation was ever improved after the therapy, but with positive CMV-PP65 and positive CMV-DNA on day +83, he died for severity pulmonary infection, pulmonary hemorrhage, alimentary tract hemorrhage and multiple organ failure on day +86. **Conclusion** Acute graft versus host disease and immunosuppressive therapy were the risk factors for cytomegalovirus-associated haemophagocytic syndrome following allogeneic hemotoxic stem cell transplantation. HLH-2004 Therapeutic Guideline for HLH did not allow the specificity of HLH after HSCT. To look for appropriated treatment may improve outcome.

**KEYWORDS:** Allogeneic hemotoxic stem cell transplantation; CMV infection; Hemophagocytic lymphohistiocytosis

Hematophagocyte lymphohistiocytosis following allogeneic hemotoxic stem cell transplantation (HSCT-HLH) is a rare complication, and is related to allogeneic immune response between donors and recipients and infection after transplantation, with mortality rate up to 57% [1,2,3,4]. Since HLH may co-exist acute graft-versus-host disease (aGVHD), hepatic veno-occlusive disease (HVOD), pretreatment drug damage, infection and other complications after transplantation, its differential diagnosis and treatment are more difficult. This is the first domestic report of a child's HLH case on AML-URD-UCBT postoperative CMV infection. Also literature was reviewed in order to raise awareness.

**Medical record**

One child patient, 10 year-old boy, was hospitalized because he had fever for 40 days and skin ecchymosis for 10 days. He was diagnosed as AML-M2a through bone marrow cell morphology and immunohistochemical examination, as well as flow cytometry immunophenotyping examination. He got complete relief after NAE chemotherapy. Following two courses of HD AraC + NVT and HD AraC + VP16 consolidation therapy, he received unrelated HLA5 / 6 matched cord blood transplantation with $6.1 \times 10^7$/kg nucleated cells and...
36.6 × 10^9/kg on Jan 6, 2005. CD34 cells conditioning regimen: busulfan (Bu) 14mg/kg + horse anti-thymocyte globulin (ATG) 90mg/kg + fludarabine 150mg/m²; GVHD prevention programs: CsA2mg/kg was taken daily starting from day four. There was no liver and spleen enlargement before transplantation. The test results of Hepatitis viruses, CMV-PP65, CMV-DNA, EBVCA-IgM, EBV-DNA and HIV antibody were negative. On day +6, the patient started to have the gradual emergence of fever, rash, flushing face, red ears, hands and feet, and miliary rash with itching; patient's liver enlarged to 2.5cm below ribs and spleen did not enlarge; transaminase elevated (highest value AST942u / L, ALT2826u / L), and total bilirubin increased (highest value 36umol / L). On day +22, the patient had paroxysmal abdominal pain, especially around cullen and xiphoid area. The amount of drainage stools was 600-1500ml per day.In consideration of skin, liver, and intestinal aGVHD, CsA was increased to 2.5mg/kg per day on day +6. The patient was given methyl prednisone 5mg / kg per day for 6 days and 2.5mg/kg per day for 2 days. On day +15, due to increased liver aGVHD, the Methylprednisolone "shock" treatment was performed with 20mg/kg.d × 1 day, 10mg/kg.d × 2 days, 5mg/kg.d × 3 days, 2.5mg/kg.d × 3 days, 2mg/kg.d, followed by oral administration with reduced amount until +29 days to a stop. After the treatment, fever, rash and jaundice gradually disappeared, but liver enlarged to 5cm below ribs. Transaminases became slightly better but still was abnormal. From 23 day, Daclizumab (CD25 mAb) 1mg/kg per day was given on 1,4,7,14,21 day, with MMF0.25 q8h × 30 days.After treatment, transaminase gradually decreased to normal level and liver returned to 1cm below ribs. However, on +26 and +46 days for testing of serum CMV-PP65 and CMV-DNA (+), there were gradually fever, enlarged liver, increased transaminases, ascites, hypoproteinemia, low high-density lipoproteins, low-hypernatremia, abdominal pain and diarrhea. On 29, +33, +37, +42 days, in bone marrow macrophages Hemophagocytic was visible (up 1.5%) (see Figure 1). At the same time, the numbers and proportions of abnormal lymphocytes (up to 3%), reticular cells (up 5.5%) and monocytes cells (up to 7%) increased; peripheral blood three-line decreased accompanied by increased proportion of mononuclear cells in leukocytes (up to 87.8%); triglycerides (up to 3.42mmol / L) and serum ferritin (highest value 1015ug / L) increased; hypofibrinogenemia (minimum 0.96g / L) and lymphocyte subsets testing showed Th (21%) decreased, Ts (39%) increased, Th / Ts (0.54) decreased, NK cells (8%) decreased, Th1 (0.46%) decreased, Th2 (11.26%) increased, Tc1 (3.73%) decreased, and Tc2 (8.73%) increased. According to HLH-2004 diagnostic criteria, this cases met 6 diagnostic criteria out of 8, so he was diagnosed as AML-UCBT postoperative HLH. Because HLH is associated with CMV infection, starting on day +29, the patient was given IVIG (0.3g/kg.d, 2 to 3 days per week × 7 weeks), acyclovir (ACV) (0.125 q8h × 8 days), and Ganciclovir (GCV) (5 mg / kg. bid × 14 days, 5 mg / kg. qd × 28 days) to treat CM. Red blood cells, platelets, plasma, albumin, fibrinogen, and prothrombin complex were transfused as supportive treatment. Referring to HLH-2004 program and grafting factors, we adjusted HLH treatment as follows: In order to get favorable implant and reduce donor stem cell damage and bone marrow suppression, we reduced the amount of VP16 (50mg / m².d × 2 days); in order to avoid excessively strong immunosuppressive therapy, we changed dexamethasone reduction from once two weeks to once a week. The amount of dexamethasone was 10mg/ m².d × 7 days, 5 mg/m².d × 7 days, 2.5 mg/m².d × 7 days, 1.25 mg/m².d × 7 days, reduced amount to 0 × 7 days. Intestinal aGVHD caused diarrhea, and due to poor oral drug absorption, oral CsA 5mg/kg.d was replaced by intravenous administration of 1.5 mg / kg.d × 38 days. On day +61, the test result of serum CMV-PP65 was negative. On day +55 and +63, there was no hemophagocytic bone marrow; bone marrow showed HLH complete remission; in peripheral blood the proportion of mononuclear cells in leukocytes was back to normal level; white blood cells and hemoglobin recovered (WBC highest value 7.89 × 109 / L, hemoglobin highest value 106g / L), but platelets were still low and serum ferritin level fell. Hypoproteinemia improved and the ratios of serum transaminases, triglycerides, fibrinogen and NK cells returned to normal. Abdominal pain and diarrhea had improved and then got
worse again. On day +70, because HLH was completely relieved and intestinal aGVHD was uncontrolled, patient was given ATG 1.8mg/kg.d × 3 days and methylprednisolone shock therapy was performed. However, there was no improvement in diarrhea, and lung infections occurred. On day +73, anteroposterior chest film showed reduction of the brightness of both lungs, with ground glass changes, and a lung infection was proposed. To clarify pathogen infection, bacterial and fungal cultures of the blood, stool, urine and ascites were repeated throughout the course, and the results were negative; EBV, HIV, parvovirus B19, adenovirus, hepatitis virus, herpes simplex virus, respiratory syncytial virus, parainfluenza virus, mycoplasma, chlamydia and parasites were all negative. Although anti-bacterial, anti-fungal, and anti-viral treatments were performed, the patient situation continued to deteriorate. On day +83, the test results of serum CMV-PP65 and CMV-DNA were positive. On day +86, the patient died from severe lung infection, pulmonary hemorrhage, gastrointestinal bleeding and multi-organ failure.

Discussion

1 Risk factors of HLH occurrence after transplantation. For this case, the patients received non-blood-related HLA mismatched hematopoietic stem cell transplantation. The incidence of severe aGVHD after transplantation is that only CsA was used for GVHD prevention programs, without MTX. Some studies show that serious allogeneic immune response between donor and recipient, such as aGVHD or graft rejection, activates donor’s T lymphocytes and recipient epithelial cells to have excessive secretion of cytokines, causing macrophage excessive activation. This may lead to occurrence of HLH after transplantation. In order to control severe aGVHD, active immunosuppressive therapy was performed. aGVHD symptoms had improved after treatment, but CMV viremia and active CMV infection combination with HLH occurred. Meanwhile, laboratory tests showed immune disorders of T cells, monocytes and NK cells as well as elevated serum ferritin. Some studies showed that T cells infected by CMV caused T-cell immune disorders, released cytokines, activated macrophages, and led to hemophagocytic lymphohistiocytosis histiocytosis and high serum ferritin hyperlipidemia. The HLH incidence of this case suggests that patients receiving allogeneic hematopoietic stem cell transplantation have immunodeficiency because of unrecovered immune system and immunosuppressive drug for aGVHD. This caused him to be susceptible for infections, particularly CMV infection which is the risk factor for HLH after transplantation. This is consistent with the conclusions of studies of Abdelkefi [11,12,13,14].

2 HLH prevention, treatment and lessons learned. In the future, during hematopoietic stem cell transplantation, how to prevent CMV infection associated HLH from happening after transplantation? (1) clear as much as possible CMV, EB and other viruses that can induce HLH before transplant; (2) try to choose blood related HLA-match donor, and avoid serious allogeneic immune response between donor and recipient, such as severe GVHD and graft rejection; this will be beneficial to reduce post-transplant CMV infection and HLH incidence; (3) balance between implanted and aGVHD selection; in HLA match URD-UCBT, GVHD prevention program can include removing MTX to facilitate implantation; in HLA mismatched URD-UCBT, low (micro) amount of MTX (5mg/m²) should be adopted to prevent aGVHD; (4) actively prevent CMV infection. For patients with high risk factors of CMV infection after transplantation, such as the presence of aGVHD, in addition to using ACV and GCV to prevent CMV infection, it is recommended to stick with IVIG 0.5g/kg.w (5) strengthen the monitoring of CMV infection. Recommend sticking to weekly monitoring of CMV infection (including blood CMV-PP65 and CMV-DNA). With reference to HLH-2004 program, after VP16, glucocorticoids, CsA, anti-CMV and other support therapy, patient fever, abdominal pain, diarrhea, energy, and appetite improved; CMV-PP65 turned to negative, bone marrow showed complete HLH relief; the ratio of peripheral blood mononuclear cells in white blood cells was back to normal; white blood cells and hemoglobin improved; serum ferritin level fell, hypoalbuminemia improved, the ratios of blood transaminase, triglycerides, fibrinogen and NK cells were normal. These indicate that the treatment was once valid although the child eventually died. The reason why referring to HLH-2004 program failed to save the life of the HLH patient after transplantation may be that HLH-2004 program is not directed at post-transplant HLH treatment programs, and it does not take into account HLH patient’s special post-transplant factors. Treatment of HLH patients need to handle the following specific areas after transplant: (1) balance between post-transplant HLH therapy and implant. When GVHD and HLH occurred, VP16 amount should be reduced to prevent the secondary implant failure; (2) should not abandon the "traditional" GVHD treatment method and use so-called "new therapy": for example, in this case if MTX 8mg/m².w was given first, the chance of secondary immunocompromise after HD-MP / ATG treatment may be less, and the "resurgence" under the basic condition of CMV infection may be reduced after transplantation, patients was in immunodeficiency states because the immune function was not fully restored; this was one of possible reasons why CMV infection recurred after it was inhibited through active anti-CMV therapy. (4) immunosup-
pressive drug for aGVHD caused immune deficiencies and made the child susceptible to CMV and other opportunistic infections; the opportunistic infections can induce or aggravate aGVHD. Experience in the treatment of this case showed that HLH-2004 program had failed to break above vicious cycle. After transplantation, HLH patient may coexist pretreatment drug damage, HVOD, implantation syndrome, graft rejection, GVHD and opportunistic infections and other complications. In order to confirm the diagnosis, the post-transplant HLH patient must be given a positive differential diagnosis. Then we can develop specific treatment program which is not only for treating HLH, but also other post-transplant complications. For example, this HLH patient had serious aGVHD and active CMV infection at the same time; during the treatment of HLH and control of aGVHD, sodium phosphate and other treatment should be actively applied for active CMV infection. Only after the correct diagnosis is made and targeted therapy is performed, it may improve the therapeutic effect. Currently, there have been foreign literature reports on applying gamma globulin, ganciclovir, blood transfusion and other supportive therapy to cure CMV infection after transplantation associated HLH, but no large prospective multi-center clinical study available.

In summary, aGVHD and anti aGVHD immunosuppressive therapy after allogeneic hematopoietic stem cell transplantation are risk factors that cause active CMV infection associated with HLH after transplantation. Because HLH-2004 program does not take into account the particularity of HLH children after transplantation, it failed to save the life of this child. HLH special treatment plan for post allogeneic hematopoietic stem cell transplantation needs to be studied further in order to improve its efficacy.

References