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SUCCESSFUL STEM CELL TRANSPLANTATION IN A CHILD WITH CHRONIC GRANULOMATOUS DISEASE ASSOCIATED WITH CONTIGUOUS GENE DELETION SYNDROME AND COMPLICATED BY MACROPHAGE ACTIVATION SYNDROME.

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LETTER TO THE EDITOR

Successful Stem Cell Transplantation in a Child with Chronic Granulomatous Disease Associated with Contiguous Gene Deletion Syndrome and Complicated by Macrophage Activation Syndrome.

Chronic granulomatous disease (CGD) results from the malfunction of NADPH oxidase subunits in phagocytic cells. Sporadic cases with contiguous gene deletion syndrome (CGS) involving the *CYBB* gene and other genes can be deleted, causing retinitis pigmentosa, Duchenne muscular dystrophy, ornithine transcarbamylase deficiency, and/or the McLeod phenotype (McPh) (1). CGD patients have an unregulated inflammatory response and macrophage activation syndrome (MAS) may occur.(2)

Currently, hematopoietic stem cell transplantation (HSCT) is the only curative option for CGD (3). CGD associated with CGS is a particularly complicated disease in terms of severity and management because Kx antigens are expressed by hematopoietic precursors of red blood cells (RBC); thus, the high risk of allosensitization to the RBC Kx antigen may complicate transfusions and increases the risk of graft failure in HSCT (1,4). We report, for the first time to our knowledge, a patient with CGD-XL, CGS, and MAS successful treated with HSCT. A one-year-old male presented with a neck abscess. He had two male siblings who had died at the ages of 2 and 3 years old due to sepsis. CGD-XL in the patient and the carrier status of the mother were confirmed via dihydrorhodamine 123 assay. At 18 months-old, the patient suffered from sepsis secondary to *Pseudomonas sp.* and *Salmonella sp.* infection; thus, he was treated with broad-spectrum antibiotics and transfusions. MAS was diagnosed based on fever, hepatosplenomegaly, lymphadenopathy, pancytopenia, increased liver enzymes, hyperferritinemia, hypertriglyceridemia, and hemophagocytosis in the bone marrow. Thus, the patient was treated with dexamethasone (6 mg/m2/dose), intravenous immunoglobulin (IVIG), and cyclosporine and exhibited satisfactory improvement; however, there was persistent hemolytic anemia. A McPh was suspected and confirmed based on the absence of the Kx antigen on the RBCs due to hemolytic anemia, previous RBC

transfusions. A deletion of the *CYBB* gene was found, while 22 exons of the *DMD* gene were amplified normally. A comparative genomic hybridization array (CGH, 135 K and 67 K SignatureChipOS®+SNP, Roche NimbleGen) was used and revealed a deletion of 3.4 Mb that involved both the *CYBB* gene and the *XK* gene, which encodes the Kx antigen, and deletions in 10 additional genes (*TMEM47*, *MAGEB16*, *PRRG1, FAM47A*, *FAM47B*, *CXorf22*, *CXorf59*, *CXorf30*, *FAM47C*, and *LANCL3*) that have not been associated with disease.

HSCT was considered for the patient based on the severe infection, the two younger brothers who had died of causes secondary to infection in early childhood, and the availability of an HLA-related compatible Kell-negative donor. He received a dose of 20.25 x10^6 $CD34^+$ cells/kg (peripheral blood stem cells), conditioning regimen consisted of busulphan (16mg/kg) and cyclophosphamide (120mg/kg) and graft-versus-host disease prophylaxis with cyclosporine and methotrexate were administered. Hematological recovery was prompt, and full donor chimerism was achieved on day 11. No graft-versus-host disease was present. After more than 1 year of follow-up, he is currently doing well with full donor chimerism of the leukocytes and normal dihydrorhodamine 123 assay results.

CGS can be diagnosed clinically but CGH array analysis of genomic DNA is a practical approach for documenting additional gene deletions. We recommend assessment of absence of the Kx antigen when a new male patient is diagnosed with CGD (3). Interestingly, retinitis pigmentosa did not seem to be present due to *RPGR* gene deletion. Wang *et al*. reported that different ocular lesions, such as retinitis pigmentosa, are related to systemic infection in CGD, and such infection may be a plausible explanation for the ophthalmic manifestation of our patient (5). MAS is a rare inflammatory complication in CGD patients, only 13 cases of have been reported. The primary triggers of MAS were infections, and the causal microorganism were diverse and included bacteria, fungi and viruses (2). This association is underdiagnosed due to the overlap of some clinical manifestations of sepsis and MAS, parameters such as very high levels of serum ferritin, hemophagocytosis and hypertriglyceridemia can help to differentiate both conditions. The mechanisms of underlying inflammation in CGD include reduced neutrophil apoptosis, unbalanced innate immune receptors, induction of Th17 cells, impaired Nuclear Factor Erythroid 2-related factor, activity and increased inflammasome activation with increased secretion of interleukin-1β (IL-1β) (6). The use of IL-1 receptor antagonist could be considered as part of the

treatment of refractory CGD-MAS. There are no descriptions of the association between CGD, McPh and MAS in the literature, but separate cases of CGD-MAS and CGD-McPh have been described. In CGD-McPh, the anemia worsens after the patient receives a Kx-positive transfusion, in such situations transfusions of Kx-negative RBCs are necessary to avoid complications. Currently, there are only 6 cases of McPh reported to have received HSCT to date.(table 1) (4, 7-10). No defined protocol neither special indication for HSCT in CGD patients with McPh exists; the indication of the five previous reported patients were severe infections and refractory chronic inflammatory lung disease (Table 1) . The ideal donor for HSCT is an identical Kx-negative HLA, but it is difficult to find it. Kx-negative donors were available in only one out of the five reported cases. Seger *et al*. reported the following precautions that should be considered when patients with CGD and the Mcleod phenotype undergo transplantation: 1) pretransplant erythropoietin administration, 2) cryopreservation of the Kx-negative patient's RBCs, 3) B cell depletion with anti-CD20, 4) complete myeloablation and immune ablation to avoid mixed chimerism with persistent Kx- and Kell-sensitized host B and T cells (e.g., with TBI 12 Gy TD, fludarabine, 160 mg/m2 TD, and rabbit ATG, 10 mg/kg TD), 5) RBC depletion of Kx-positive transplants, 6) transfusion with Kell-negative packed RBCs posttransplantation, and 7) posttransplant donor lymphocyte infusions for alloimmune hemolysis (3).

As remarkable aspects we have to consider that CGD-XL patients with severe hemolytic anemia and retinitis pigmentosa can be complicated by CGS, which worsen the prognosis and specials therapeutics strategies are required for a successful HSCT. MAS that must be differentiated from sepsis in the presence of a systemic inflammatory response syndrome.

Table 1 Reports of CGD patients with the McLeod phenotype who have received a HTSC.

Patients	Age at HTSC	Known disease-causing deleted genes	Detection method	Association with macrophage activation syndrome	Indication of transplant	Donor
P1	3 years	*XK, CYBB*	Array CGH	Absent	Liver abscess and pulmonary aspergillosis	HLA-matched unrelated replete Bone Marrow
P2	After 3 years	*XK, CYBB*	Array CGH	Absent	Pulmonary aspergillosis, chronic inflammatory lung disease, pulmonary and cerebral granuloma.	HLA-matched unrelated replete bone marrow
P3	20 years	*XK, CYBB*	Array CGH and PCR	Absent	Refractory invasive aspergillosis	Unrelated-donor umbilical cord blood
P4	4 years	*XK, CYBB*	Not described	Absent	Pulmonary aspergillosis, chronic pulmonary disease.	K-antigen-negative, peripheral-blood stem cells from an HLA-identical unrelated donor
P5	7 years	*XK, CYBB*	Not described	Absent	Not described	Matched unrelated donor bone marrow
P6	2 years	*XK, CYBB*	Array CGH	Present	Sepsis associated to mechanical ventilation and neurological deterioration.	Matched related donor bone marrow

Preparative regimen	Additional HSCT measures in McLeod phenotype	Graft-versus-host disease prophylaxis	GvHD and treatment	Outcome	Author
BU and CY	None	CsA and MTX	At day 945 skin GvHD treated with donor-lymphocyte infusion mycophenolate mofetil and CsA.	At day 100, he had severe hemolytic anemia, he received azathioprine, rituximab, vincristine, CsA and donor-lymphocyte infusion. Also splenectomy. Successful	Kordes [7]
BU and CY, rabbit anti-thymocyte globulin	Erythropoietin for 1 week prior to conditioning	CsA and MTX	Mild GvHD of the skin. He ameliorated with steroids.	Successful	Schuetz [10]
Reduced-intensity: CY Fludarabine, total body irradiation	None	CsA and mycophenolate mofetil	Grade 1 acute GvHD, treated with methylprednisolone	Successful	Suzuki N [8]
Total body irradiation, Fludarabine, anti-thymocyte-globulin	Rituximab	CsA and MTX	None	Successful	Honig [9]
Submyeloablative conditioning	Rituximab, splenectomy	CsA and MTX	None	Successful	Héritier [4]
BU and CY	None	CsA and MTX	None	Successful	Studied case

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