Hemophagocytic Lymphohistiocytosis/Macrophage-Activation Syndrome (HLH/MAS) Following Treatment with Tisagenlecleucel

Reyes Maria Martin-Rojas[1], Ignacio Gomez-Centurion[1], Rebeca Bailen[1], Mariana Bastos[1], Francisco Diaz-Crespo[1], Diego Carbonell[1], Rafael Correa-Rocha[2], Marjorie Pion[2], Cristina Muñoz[1], Milagros Sancho[1], Isabel Gomez-Fernandez[1], Gillen Oarbeascoa[1], Ana Perez-Corral[1], Carolina Martinez-Laperche[1], Javier Anguita[1], Ismael Buño[1], Javier Menarguez[1], Jose Luis Diez-Martin[1], and Mi Kwon[3]

[1]Hospital General Universitario Gregorio Marañón
[2]Instituto de Investigación Sanitaria Gregorio Marañón
[3]Hospital General Universitario Gregorio Maranon

August 8, 2021

Abstract

CAR-T cell related HLH/MAS is an unusual manifestation of severe cytokine release syndrome (CRS) with high mortality rates and a challenging diagnosis. The establishment of specific diagnosis criteria is essential, and the combination of several techniques for CAR-T cell follow-up, allows a more precise management of this complication.

1. INTRODUCTION

Immunotherapy with T-cells genetically engineered to express CD19-specific chimeric antigen receptor (CAR) has dramatically changed the treatment of aggressive B-cell malignancies[1,2]. Two products- Tisagenlecleucel and Axicabtagene ciloleucel- have been approved by the European Medicines Agency (EMA) for the treatment of relapsed/refractory CD19+ diseases. Tisagenlecleucel (Tisa-Cel) is a CD19-targeted CAR-T cell therapy approved to treat adult patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy and children and adults up to age 25 with relapsed/refractory B-cell acute lymphoblastic leukemia (ALL)[3,4].

Despite CAR-T cells can induce rapid and durable responses, this therapy is associated to specific and severe toxicities, representing an obstacle in its widespread use. Cytokine-release syndrome (CRS) is the most frequent toxicity after infusion[5,6]. This systemic inflammatory response can rarely evolve into a fulminant hemophagocytic lymphohistiocytosis/macrophage-activation syndrome (HLH/MAS) which is associated with high mortality rates[7,8]. Diagnosis criteria for this entity have been recently proposed[7], but discrimination between severe CRS, CAR-T related HLH/MAS and malignancy-associated HLH/MAS can be challenging.

We describe two cases of CAR-T cell related HLH/MAS and the difficulties linked to the diagnosis and management of this unusual complication.

2. CASES REPORT

2.1 CASE 1

A 34-year-old male with refractory T-cell rich DLBCL with associated HLH/MAS at diagnosis, underwent Tisa-Cel therapy in 2019. High tumor burden with progressive disease was documented after bridging therapy.

During lymphodepletion with cyclophosphamide and fludarabine, persistent fever with neutropenia was treated with empiric broad-spectrum antimicrobials agents, without resolution.

On day +1, he presented with grade 1 CRS, managed with tocilizumab on days +15 and +20, achieving partial response. However, high grade fever followed with hyperferritinemia (peak 50,000ng/ml on day +23), severe cytopenias, altered liver function, coagulopathy and high values of IL-15, IL-1β, GM-CSF and IL-6. HLH/MAS was suspected and bone marrow aspiration was performed on day +22, showing hemophagocytosis and no evidence of infiltration by lymphoma.

In order to distinguish between CAR-T cell related and malignancy associated HLH/MAS, a PET-CT was performed on day +25, showing paradoxical response. Concurrently, CAR-T cells expansion in peripheral blood (PB) was detected by flow cytometry and polymerase chain reaction (PCR). Furthermore, a lymph node biopsy was performed where CAR-T cells were detected, with only 4% of neoplastic lymphocytes **(Figure 1).**

Subsequently, high dose corticosteroids, siltuximab, anakinra and cyclophosphamide were administered. Hemoadsorption with extracorporeal cytokine adsorber (CytoSorb®) was initiated on day +35. Etoposide was not considered due to severe liver function impairment.

Patient showed no response to treatment, and died from multiorgan failure on day +36. Tumor necrosis was the predominant finding in necropsy, with an estimated 15% of residual tumor.

2.2 CASE 2

A 22-year old male with relapsed/refractory Philadelphia chromosome negative B-cell ALL underwent Tisa-cel therapy in 2020. After a bridging therapy with cyclophosphamide, vincristine and dexamethasone, he received lymphodepletion with fludarabine and cyclophosphamide. At the time of Tisa-cel infusion the patient was afebrile with a baseline ferritin of 6905 ng/mL. He developed a grade 1 CRS six hours after infusion, progressing to grade 2 on day +2, and was treated with three doses of tocilizumab. Plasma cytokine levels showed high values of IL-15, GM-CSF and IL-6. Both symptoms and cytokine levels rapidly improved after administration of tocilizumab.

On day +11 he presented with a new episode of fever, high flow oxygen therapy requirements, impaired renal and liver function, increased ferritin levels (peak 800,000 ng/mL on day +14) and coagulopathy. CAR-T cells expansion in PB was detected (4,141 CAR-T/mL on day +14). At this point, the patient met criteria of CAR-T related HLH/MAS and further studies were performed, showing high levels of soluble CD25 (>10,000 pg/mL), IL-18 (>10,000 pg/mL), INF-gamma (1,365 pg/mL) and TNF-alpha (241 pg/mL).

The patient was transferred to the ICU and was managed with supportive therapy, dexamethasone 20mg/6h from day +14, etoposide 150mg/m^2 on days +15 and +18 and anakinra 100mg/12h from day +15 to day +17, with progressive improvement. Dexamethasone was slowly tapered and discontinued on day +44 **(Figure 2).**

Despite successful management of CAR-T related HLH, he died on day +47 due to a necrotizing pancreatitis in the context of extramedular ALL progression.

3. METHODS

CAR-T cell expansion in PB was monitored by multiparameter flow cytometry through detection of labeled CD19 in T cells (Human CD19 Protein®) and by quantitative PCR using the HIV /COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche, Switzerland) following manufacturer's instructions.

Tumor samples from core needle biopsies were processed using QIAamp DNA Tissue Kit (Qiagen, Germany) to detect CAR-T cells.

Plasma cytokine levels (IL-6, IL-15, IL-1β, and GM-CSF) were determined with last generation ELISA multiplex (Ella®, ProteinSimple®).

Written informed consent was obtained in compliance with our institutional review board and the Declaration of Helsinki.

4. DISCUSSION

HLH/MAS is an hyperinflammatory syndrome which typically consists on hyperactivation of cytotoxic T and natural killer (NK) cells and macrophages, leading to a massive cytokine production, lymphohistiocytic tissue infiltration and immune-mediated multiorgan failure[7,9]. CAR-T related HLH is rare, with severe and fulminant cases occurring in approximately 1% of patients receiving this treatment. However, this complication is associated with high mortality rates and a prompt diagnosis and early management is mandatory[7].

Given its hyperinflammatory nature, it is considered that CRS and HLH/MAS might belong to a similar spectrum of systemic disorders, which makes HLH/MAS diagnosis difficult, especially in the context of CRS[7]. The traditional diagnosis criteria for secondary HLH/MAS such as HLH-2004[10] and H-Score[11] are not specific, and Neelapu et al. have proposed new criteria for CAR-T related HLH/MAS, considering it is crucial to promptly diagnose this complication[7]. Both our patients met criteria of CAR-T related HLH/MAS according to Neelapu et al, with the second patient showing an optimal response to treatment due to its early management.

Distinction between this entity and malignancy-triggered HLH/MAS can be challenging and their management should be different. Currently, there are no generally accepted criteria for malignancy-triggered HLH/MAS[9]. In Case 1, the presence of previous HLH/MAS at lymphoma diagnosis challenged even more this differential diagnosis. However, detection of CAR-T cells in PB and a lymph node biopsy and the paradoxical response observed in the PET-CT allowed a more specific diagnosis and management in our patient.

So far, very little has been published on CAR-T associated HLH/MAS and no formal guidelines for its management exist. Most authors recommend anti-IL6 therapy and steroids, adding etoposide if no improvement after 48h[7, 16], as etoposide selectively deletes activated T cells and suppresses inflammatory cytokine production[9].

Anakinra, a recombinant IL-1 receptor antagonist is an emerging treatment for CAR-T associated HLH/MAS[16], and was used in our patients. Recent reports and preclinical studies suggest that there may be a benefit in combining Anakinra with other anti-inflammatory agents[17, 18]. However, due to the variability of these studies, the ideal dose schedule for this drug in HLH/MAS is still to be determined[19].

Monoclonal antibodies may have a potential role in the management of this condition in the near future[7]. Emapalumab, an anti-IFN-gamma has been approved by the FDA for patients with refractory primary HLH/MAS and a phase two clinical trial to evaluate its efficacy in secondary HLH/MAS is undergoing, with promising interim results[20]. However, there is still no formal indication of Emapalumab in secondary HLH/MAS[21, 22].

In conclusion, CAR-T cell related HLH/MAS is an unusual manifestation of severe CRS after CAR-T cell therapy, with poor prognosis, high mortality rates and a challenging diagnosis. The establishment of specific diagnosis criteria is essential for a prompt identification of patients suffering from this complication in whom any delay in treatment can be fatal. Also, the combination of diagnosis techniques for CAR-T cell follow-up allows a more precise diagnosis and more accurate distinction between CAR-T cell related or malignancy associated HLH/MAS, therefore granting a better targeted treatment. However, further studies are needed to provide better preventive and treatment strategies to improve the outcome of these patients.

ACKNOWLEDGMENTS

We would like to thank patients and their families for their collaboration towards research.

AUTHOR CONTRIBUTIONS

Conception and design: RMMR, IGC, RB, MK.

Provision of study materials or patients: All authors.

Collection and assembly of data: RMMR, IGC, RB, MK.

Data analysis and interpretation: All authors.

Manuscript writing: RMMR, IGC, RB, MK.

Final approval of manuscript: All authors.

FUNDING

This work was partially supported by the Department of Health, Autonomous Community of Madrid grant COV20/CM, Ministry of Science and Innovation grant PID2019-107545RB-I00, Ministry of Economy and Competitiveness ISCIII-FIS grant FIS PI20/00521 co-financed by ERDF (FEDER) Funds from the European Commission, "A way of making Europe".

CONFLICTS OF INTEREST

None to declare

REFERENCES

1. Kochenderfer JN, Wilson WH, Janik JE, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood. 2010;116(20):4099-4102. doi:10.1182/blood-2010-04-281931
2. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16):1507-1517. doi:10.1056/NEJMoa1407222
3. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. N Engl J Med. 2019;380(1):45-56. doi:10.1056/NEJMoa1804980
4. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-448. doi:10.1056/NEJMoa1709866
5. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188-195. doi:10.1182/blood-2014-05-552729
6. Schuster SJ, Maziarz RT, Rusch ES, et al. Grading and management of cytokine release syndrome in patients treated with tisagenlecleucel in the JULIET trial. Blood Adv. 2020;4(7):1432-1439. doi:10.1182/bloodadvances.2019001304
7. Neelapu SS, Tummala S, Kebriaei P, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. Nat Rev Clin Oncol. 2018;15(1):47-62. doi:10.1038/nrclinonc.2017.148
8. Sandler RD, Tattersall RS, Schoemans H, et al. Diagnosis and Management of Secondary HLH/MAS Following HSCT and CAR-T Cell Therapy in Adults; A Review of the Literature and a Survey of Practice Within EBMT Centres on Behalf of the Autoimmune Diseases Working Party (ADWP) and Transplant Complications Working Party (TCWP). Front Immunol. 2020; 11:524. doi:10.3389/fimmu.2020.00524
9. Lehmberg K, Nichols KE, Henter JI, et al. Consensus recommendations for the diagnosis and management of hemophagocytic lymphohistiocytosis associated with malignancies. Haematologica. 2015;100(8):997-1004. doi: 10.3324/haematol.2015.123562.
10. Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-31. doi: 10.1002/pbc.21039.
11. Fardet L, Galicier L, Lambotte O, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol. 2014;66(9):2613-20. doi: 10.1002/art.38690.
12. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014 Oct 16;371(16):1507-17. doi: 10.1056/NEJMoa1407222.
13. Yamamoto S, Matsumoto SI, Goto A, et al. Quantitative PCR methodology with a volume-based unit for the sophisticated cellular kinetic evaluation of chimeric antigen receptor T cells. Sci Rep. 2020 Oct

21;10(1):17884. doi: 10.1038/s41598-020-74927-8.
14. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. J Clin Invest. 2016 Jun 1;126(6):2123-38. doi: 10.1172/JCI85309.
15. Demaret J, Varlet P, Trauet J, et al. Monitoring CAR T-cells using flow cytometry. Cytometry B Clin Cytom. 2021 Mar;100(2):218-224. doi: 10.1002/cyto.b.21941.
16. La Rosée P. Treatment of hemophagocytic lymphohistiocytosis in adults. Hematology Am Soc Hematol Educ Program. 2015;2015:190-6. doi: 10.1182/asheducation-2015.1.190.
17. Wohlfarth P, Agis H, Gualdoni GA, et al. Interleukin 1 Receptor Antagonist Anakinra, Intravenous Immunoglobulin, and Corticosteroids in the Management of Critically Ill Adult Patients With Hemophagocytic Lymphohistiocytosis. J Intensive Care Med. 2019;34(9):723-731. doi: 10.1177/0885066617711386.
18. Strati P, Ahmed S, Kebriaei P, et al. Clinical efficacy of anakinra to mitigate CAR T-cell therapy-associated toxicity in large B-cell lymphoma. Blood Adv. 2020;4(13):3123-3127. doi: 10.1182/bloodadvances.2020002328.
19. Major A, Collins J, Craney C, et al. Management of hemophagocytic lymphohistiocytosis (HLH) associated with chimeric antigen receptor T-cell (CAR-T) therapy using anti-cytokine therapy: an illustrative case and review of the literature. Leuk Lymphoma. 2021 :1-7. doi: 10.1080/10428194.2021.1881507.
20. ClinicalTrials.gov Identifier: NCT03311854; De Benedetti et al. (EULAR 2019).
21. Locatelli F, Jordan MB, Allen C, et al. Emapalumab in Children with Primary Hemophagocytic Lymphohistiocytosis. N Engl J Med. 2020 May 7;382(19):1811-1822. doi: 10.1056/NEJMoa1911326.
22. Vallurupalli M, Berliner N. Emapalumab for the treatment of relapsed/refractory hemophagocytic lymphohistiocytosis. Blood. 2019 Nov 21;134(21):1783-1786. doi: 10.1182/blood.2019002289.

FIGURES LEGENDS

Figure 1. Evolution of CAR-T cells detection in peripheral blood by multiparameter flow cytometry (MFC) and quantitative PCR (qPCR), cytokines measurements and patient clinical management (Case 1). CAR-T cells detection is showed in cells/mL and cytokines in pg/mL. Normal levels: IL-6 0.16-37.7 pg/mL; IL-1 0.17-24pg/mL; IL-15 1.25-13.1pg/mL; GM-CSF 0.5-728.1pg/mL.

G1 CRS: Grade 1 cytokines release syndrome; BMA: Bone marrow aspirate; Dex: Dexamethasone; Cy: Cyclophosphamide.

Figure 2. Evolution of CAR-T cells detection in peripheral blood by multiparameter flow cytometry (MFC) and quantitative PCR (qPCR), cytokines measurements and patient clinical management (Case 2). CAR-T cells detection is showed in cells/mL and cytokines in pg/mL. Normal levels: IL-6 0.16-37.7 pg/mL; IL-1 0.17-24pg/mL; IL-15 1.25-13.1pg/mL; GM-CSF 0.5-728.1pg/mL. G1 CRS: Grade 1 cytokines release syndrome; Dex: Dexamethasone.

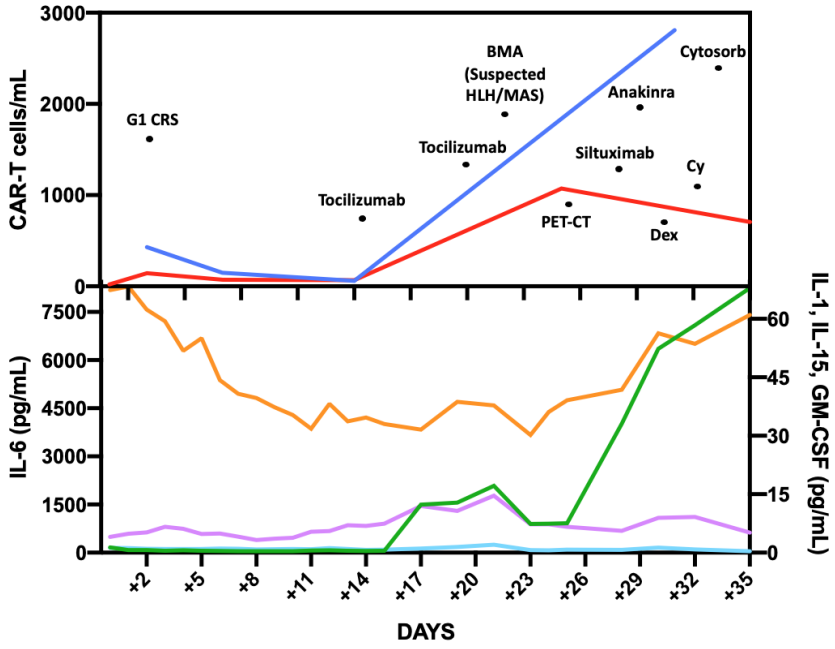

FIGURE 1. CASE 1 CAR-T CELLS AND INTERLEUKINS DYNAMICS

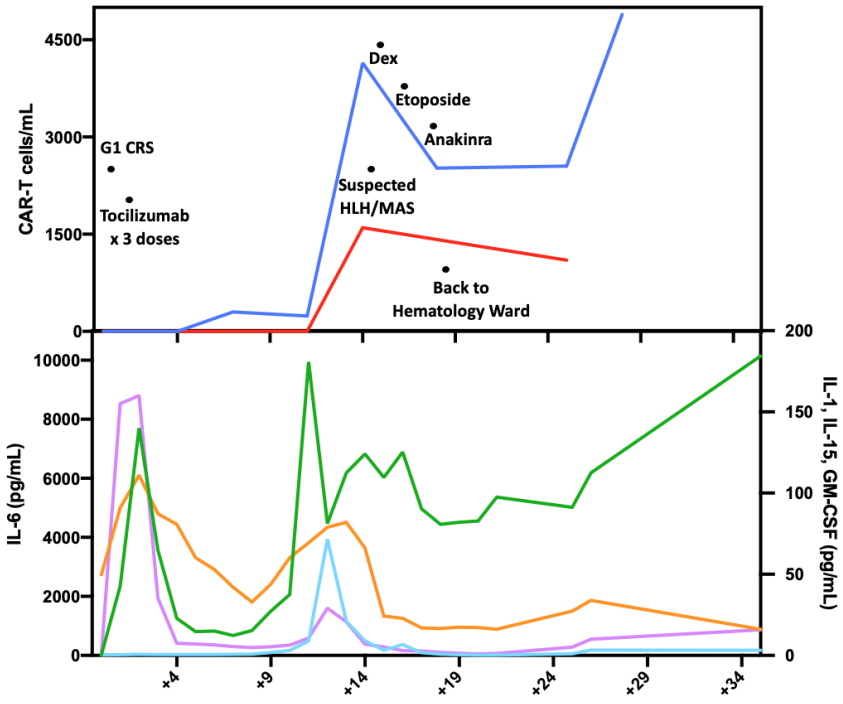

FIGURE 2. CASE 2 CAR-T CELLS AND INTERLEUKINS DYNAMICS