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Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome during treatment with the bivalent CD20xCD3 bispecific antibody glofitamab

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Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) is a life-threatening clinical entity associated with T-cell redirecting treatments (TCRT) and first described after chimeric antigen receptor (CAR)-T cell infusions.[1] More recently, cases of IEC-HS were reported following bispecific antibodies (BsAb), including epcoritamab, mosunetuzumab, and teclistamab.[2–4] A very recent study estimated the incidence rate of BsAb-associated IEC-HS to be 0.7%.[5] Glofitamab is a BsAb with a unique 2:1 structure that binds bivalently to CD20 on B cells and monovalently to CD3 on T cells, offering enhanced avidity and potency compared to other formats.[6,7] Despite its extensive use in treating diffuse large B-cell lymphoma (DLBCL), real-world surveys and database studies have not reported any cases of IEC-HS associated with its administration.[2–4] Here, we present the first documented case of IEC-HS following treatment with glofitamab. Data collection and patient informed consent were obtained according to LYMRO-22 protocol 20/22 OSS, approved by the Ethic Committee of the Istituto Pascale (07-27-2022).

A 62-year-old woman had primary refractory non-germinal center DLBCL unresponsive to frontline Rituximab (R)-CHOP (six cycles, October 2023-February 2024, IPI 2) and to salvage R-Bendamustine (four cycles, August-November 2024). Polatuzumab was not administered upfront due to regulatory restrictions, nor as salvage therapy because of the risk of perforation from extensive gastric transmural lymphoma infiltration. Re-biopsy confirmed DLBCL, and the patient was ineligible for CAR-T cells because of G3 portal vein thrombosis. Therefore, single-agent glofitamab was initiated in December 2024.

On Cycle (C)1 Day (D)1, she received obinutuzumab (1000 mg) followed by glofitamab (2.5 mg, step-up dose 1) on C1D8. On C1D15, after glofitamab step-up dose 2 (10 mg), she developed grade (G)1 cytokine release syndrome (CRS) which resolved with antipyretics. On C2D1, within 24h from full-dose glofitamab (30 mg), she experienced fever and hypotension (G2 CRS), treated with antipyretics and dexamethasone (10 mg twice daily), along with an episode of CRS-related atrial fibrillation that resolved with steroids.

Within 24h from C3D1, the patient developed fever and hypoxia requiring high-flow oxygen (G3 CRS). She received dexamethasone 10 mg (four times daily) and two doses of tocilizumab (8 mg/kg), leading to CRS resolution in 5 days followed by steroid tapering. Concomitantly, she received ganciclovir (5 mg/Kg twice daily) for cytomegalovirus (CMV) reactivation (viral DNA load: 570001 IU/mL), and azithromycin plus meropenem for documented *Campylobacter jejuni* colitis, alongside *Escherichia coli* and *Haemophilus influenzae* isolated from bronchoalveolar lavage (BAL). These exams were prompted by bilateral consolidative lesions with interstitial lung pattern on high-resolution computed tomography (HR-CT). During steroid tapering (dexamethasone 10 mg twice daily), after the resolution of CRS, the patient developed progressive thrombocytopenia, neutropenia, hypofibrinogenemia, hyperferritinemia, hypertriglyceridemia, and new-onset fever (maximum temperature 39°C). On C3D16, platelets were 51x10^9/L, absolute neutrophil counts (ANC) 0.3x10^9/L, fibrinogen levels 75 mg/dL, ferritin levels 604 ng/dL, and triglycerides 433 mg/dL. On the same day (C3D16), bone marrow biopsy was negative for lymphoma involvement, but showed macrophage activation, with large CD68/PGM1+ cells with debris in their cytoplasm (Figure 1A-C). Infections reassessment revealed evidenced colitis resolution and absence of CMV DNA in blood samples. However, due to the persistence of multiple consolidative and ground-glass areas with diffuse interstitial-alveolar pattern, a further BAL was obtained. BAL was negative for infections, while flow cytometry identified an activated T-cell population (CD3+/CD8+/CD38high/HLA-DR+) in BAL fluids and bone marrow, which comprised 11.1% and 12.4% of T-lymphocytes, respectively (Figure 1D-1G). High frequencies of T-cells with this activation profile demonstrated to be a diagnostic biomarker for distinguishing active hemophagocytic lymphohistiocytosis (HLH) patients from those experiencing sepsis[8]. Concurrent PET-CT re-evaluation documented a partial metabolic response (not shown). Taken together, these findings led to the diagnosis of IEC-HS based on the guidelines[1]. In our case, however, creatinine, transaminases, and bilirubin were within normal limits.

Therefore, the patient started the interleukin-1 receptor antagonist anakinra (100 mg twice daily) along with intravenous immunoglobulins (IVIGs). A week later, the fever resolved, and

cytopenias and coagulopathy improved (ANC >1.0x10^9/L; platelets >50x10^9/L; unsupplemented fibrinogen >100 mg/dL). The patient received G-CSF for G4 neutropenia, which resolved only after anakinra improved all other laboratory parameters.

After two days without further improvements, anakinra was increased to 100 mg four times daily. At best response (2 weeks of treatment), we observed a clinical, radiological (Figure 2) and laboratory improvement (hemoglobin 10.5 g/dL, platelets 63x10^9/L, ANC 10.5x10^9/L, ferritin 630 ng/dL, triglycerides 185 mg/dL, fibrinogen 364 mg/dL). Three weeks after anakinra initiation, the patient developed a systemic infection from multidrug-resistant *Pseudomonas aeruginosa*. Despite empirical broad-spectrum antibiotics (meropenem, vancomycin) followed by targeted antimicrobials (ceftolozane/tazobactam), and anakinra dose reductions, the clinical conditions rapidly worsened, requiring ICU admission, and the patient succumbed to septic shock. The clinical timeline of our patient, laboratory parameters and treatments, are summarized in Figure 3.

To the best of our knowledge, this is the first documented case of IEC-HS linked to administration of glofitamab. Secondary HLH after BsAbs, while infrequent, presents several diagnostic and therapeutic challenges.

First, this potentially fatal T cell-mediated hyperinflammatory response, eventually leading to uncontrolled macrophage transactivation, may be triggered by other conditions that frequently coexist in patients receiving BsAbs, such as infections, metabolic disturbances, and the malignancy itself.[1,9] Second, during the initial phases of IEC-HS, clinical and laboratory features, including cytokine abnormalities, may exhibit overlapping characteristics with a severe CRS.[1]

A proposed model for IEC-HS suggests that T-cell activation and proliferation, triggered by tumor antigens, leads to the release of cytotoxic granules and tumor lysis, which in turn results in macrophage transactivation.[1] This process generates a positive feedback loop involving soluble factors such as IFN-γ, sIL-2R, IL-6, IL-10, IL-12, IL-18, IL-1β, TNF-α, IL-33, and ferritin, similarly to primary HLH.[10]

A diagnostic framework for identifying IEC-HS and grading its severity has recently been established.[1] In our patient, the co-occurrence within 72 hours of cytopenias,

hypofibrinogenemia, and hyperferritinemia prompted further investigations, revealing five of six most common manifestations (hyperferritinemia, onset after CRS resolution, hypofibrinogenemia, hemophagocytosis in bone marrow and lungs, newly developed cytopenias) and four of nine accompanying manifestations (lactate dehydrogenase elevations, new fever, pulmonary infiltrates, hypertriglyceridemia) outlined in IEC-HS criteria.[1]

Since secondary HLH can be associated with multiple conditions, IEC-HS diagnosis must include the continuous reassessment for alternative etiologies.[1] Acquired immune disfunction and viral infections commonly trigger HLH.[10] Our patient experienced both viral and bacterial infections prior to the diagnosis of IEC-HS. However, CMV reactivation resolved before HLH development, as did pulmonary and gastrointestinal infections. Accordingly, the reported cases of CMV-related HLH developed during the active infection phase but never after its resolution.[11] Additionally, HLH has been associated with infections caused by intracellular pathogens or during sepsis, neither of which were present in our patient at onset. Other infectious triggers of secondary HLH, such as HIV and influenza, were absent in our patient, as well as EBV which was consistently ruled out by serial DNA testing[10].

While the clinical timeline indicates TCRT as the main cause of HLH in our patient, we must also recognize the predisposing role of the inflammatory environment caused by preceding infections[5]. Similarly, 'malignancy-triggered HLH' usually arises in the context of active progressive disease, PET-CT indicates that our patient was responding to treatment before HLH developed.

Emerging evidence suggests that chronic anti-IL-6 treatment in rheumatologic patients may lead to HLH and that tocilizumab might be linked to CAR-related HLH.[12] This intriguing hypothesis needs to be contextualized in the efficacy-toxicity tradeoff of a critical tool for CRS management such as tocilizumab.

The diagnosis of IEC-HS was supported by flow cytometry, which identified a distinct population of activated T-cells with a typical CD8+ CD38high/HLA-DR+ phenotype. The evidence of >7% CD38high/HLA-DR+ cells among CD8+ T cells differentiated HLH from sepsis, with positive predictive value of 96% and a negative predicting value of 100%, both in pediatric and adult

populations[7,8,13] This case is unique in documenting these T-cell findings, both in bone marrow and BAL.

Our patient displayed an atypical IEC-HS dynamics. There were no cytopenias in the preceding cycles, and previous episodes of mild (≤ G3) CRS (step-up dosing and first full dose) resolved with standard management, which did not contraindicate further delivery of glofitamab. This strengthens the connection between the risk of developing IEC-HS and the severity of the nearest CRS episode, rather than the order of cycle administration.

The established first-line treatment of IEC-HS is anakinra with or without corticosteroids.[1] Since our patient was tapering dexamethasone, we introduced anakinra (2-10 mg/kg per day) along with IVIGs. Although not explicitly recommended, IVIGs have demonstrated clinical benefits[14] and are suggested for managing secondary HLH due to their anti-inflammatory properties, ability to inhibit complement activation, blocking antibody Fc fragments and macrophage Fc receptors, and neutralize cytokines.[9] We increased the dosage of anakinra after the initial response, leading to further clinical and laboratory improvements; unfortunately, it did not prevent the fatal bloodstream infection[1].

For refractory cases, alternatives like ruxolitinib, emapalumab, and etoposide may be considered based on clinical and laboratory presentations.[1,15] The main 'primary' concern remains the risk of infection, necessitating ongoing monitoring and prompt intervention[1]. Despite therapeutics advancements[1], IEC HS mortality rates can reach 58%,[2–4] highlighting the need for further studies to improve screening, diagnosis, and management.

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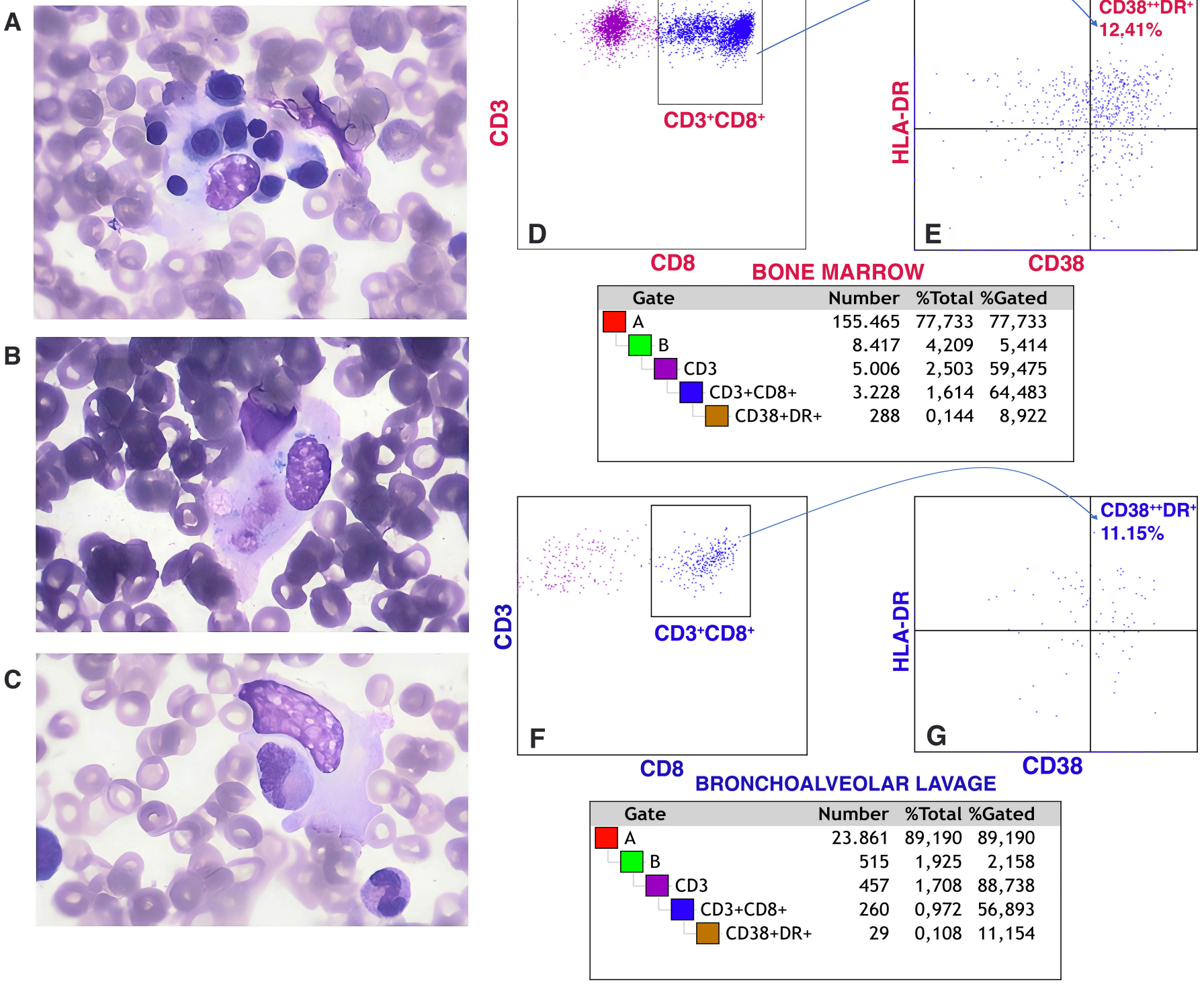
Figure 1. Macrophage activation and hemophagocytosis in bone marrow aspirate, and flow cytometric analysis of CD3+CD8+ T cells and activation markers in bone marrow (BM) and bronchoalveolar lavage (BAL). In the bone marrow aspirate, evidence of macrophage activation with erythrophagocytosis (1A), macrophages with cellular debris (1B), and initial hemophagocytosis (1C). In the flow cytometric analysis, total lymphocytes were initially selected based on forward scatter (FSC) and side scatter (SSC) characteristics and CD45 expression. From this gate, cells were plotted for CD3 versus CD8 to identify the CD3+CD8+ T cell subset both in BM (1D, blue population) and BAL (1F, blue population). Within the CD3+CD8+ populations defined in 1D and 1C, expression of activation markers CD38 and HLA-DR was assessed. The CD38++HLA-DR+ activated subset is gated in the upper right quadrant both for the bone marrow (1E) and the BAL (1G) analyses. A subset of $CD38^{++}HLA\text{-}DR^{+}$ cells exceeding 7% was considered indicative of pathological T-cell activation, as reported in HLH.

Figure 2. High-resolution computed tomography (HR-CT) scans at diagnosis of IEC-HS (3A) and after 3 weeks of treatment with anakinra (3B). Bronchoalveolar lavage was performed after the first CT scan acquisition.

Figure 3. Trends of cytopenias, hypofibrinogenemia, hypertriglyceridemia and hyperferritinemia since glofitamab administration (C3D1), during the development and resolution of CRS, the onset of IEC-HS and after the start of treatment with anakinra. BAL: bronchoalveolar lavage; BM: bone marrow biopsy; C3D1: Cycle 3 Day 1; CRS: Cytokine Release Syndrome; Dex: dexamethasone; G-CSF: growth stimulating factor; IEC-HS: Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome; IVIG: intravenous immunoglobulin.


A
B
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CD3
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Gate Number %Total %Gated
A 155.465 77,733 77,733
B 8.417 4,209 5,414
CD3 5.006 2,503 59,475
CD3+CD8+ 3.228 1,614 64,483
CD38+DR+ 288 0,144 8,922
CD3
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F
CD8
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HLA-DR
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BRONCHOALVEOLAR LAVAGE
Gate Number %Total %Gated
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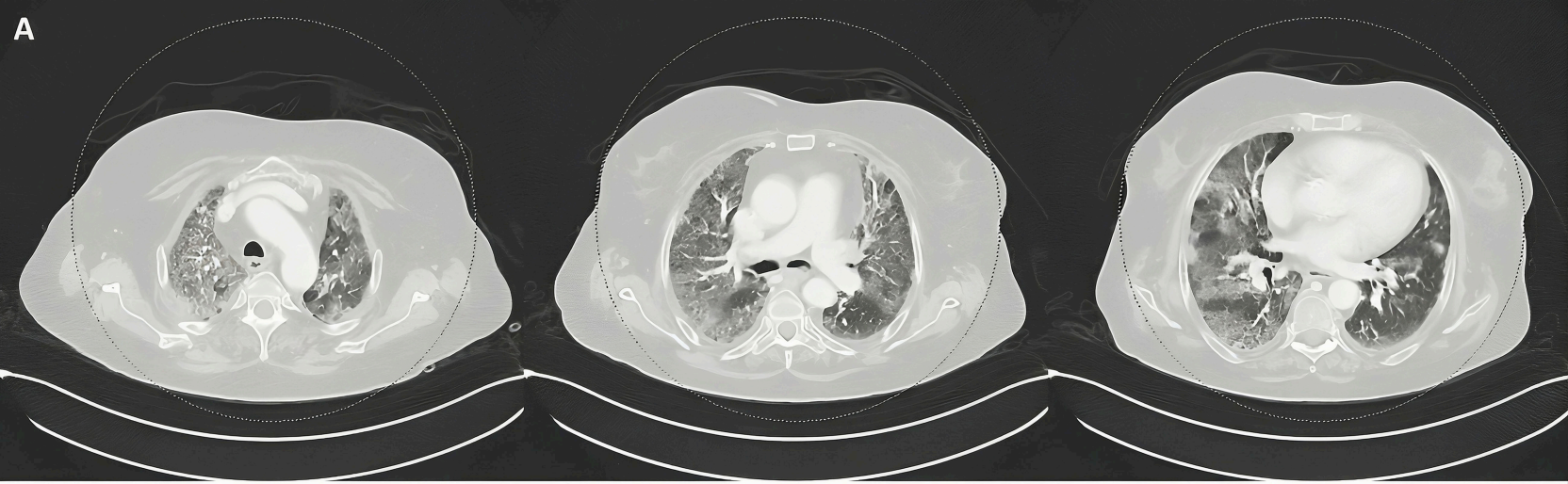
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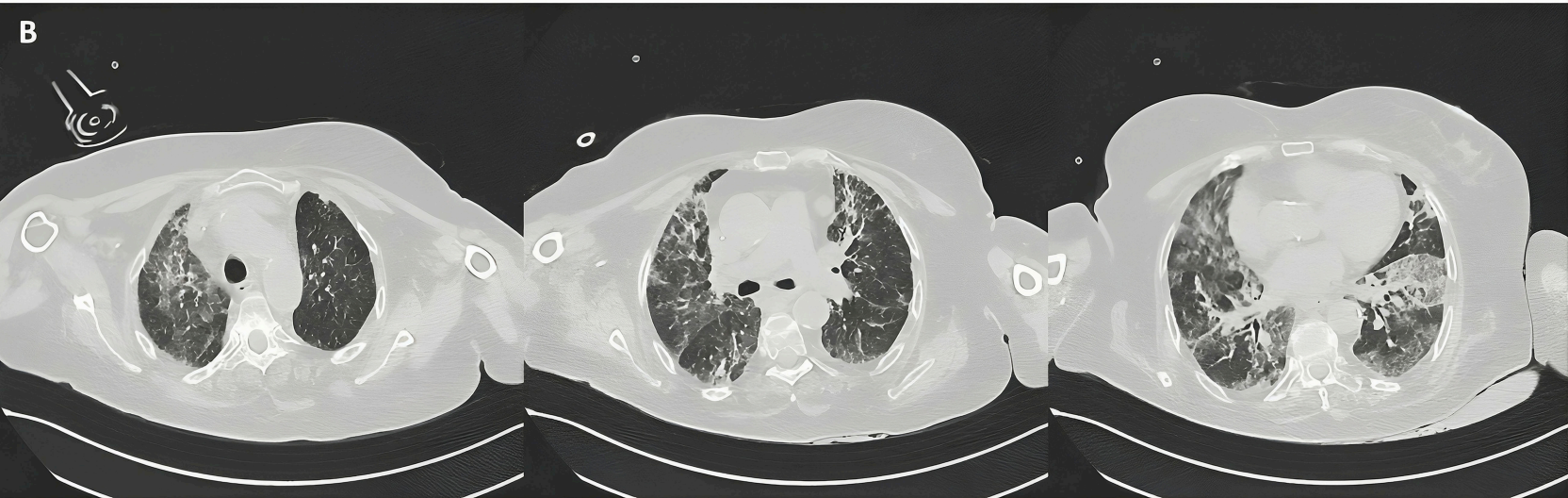
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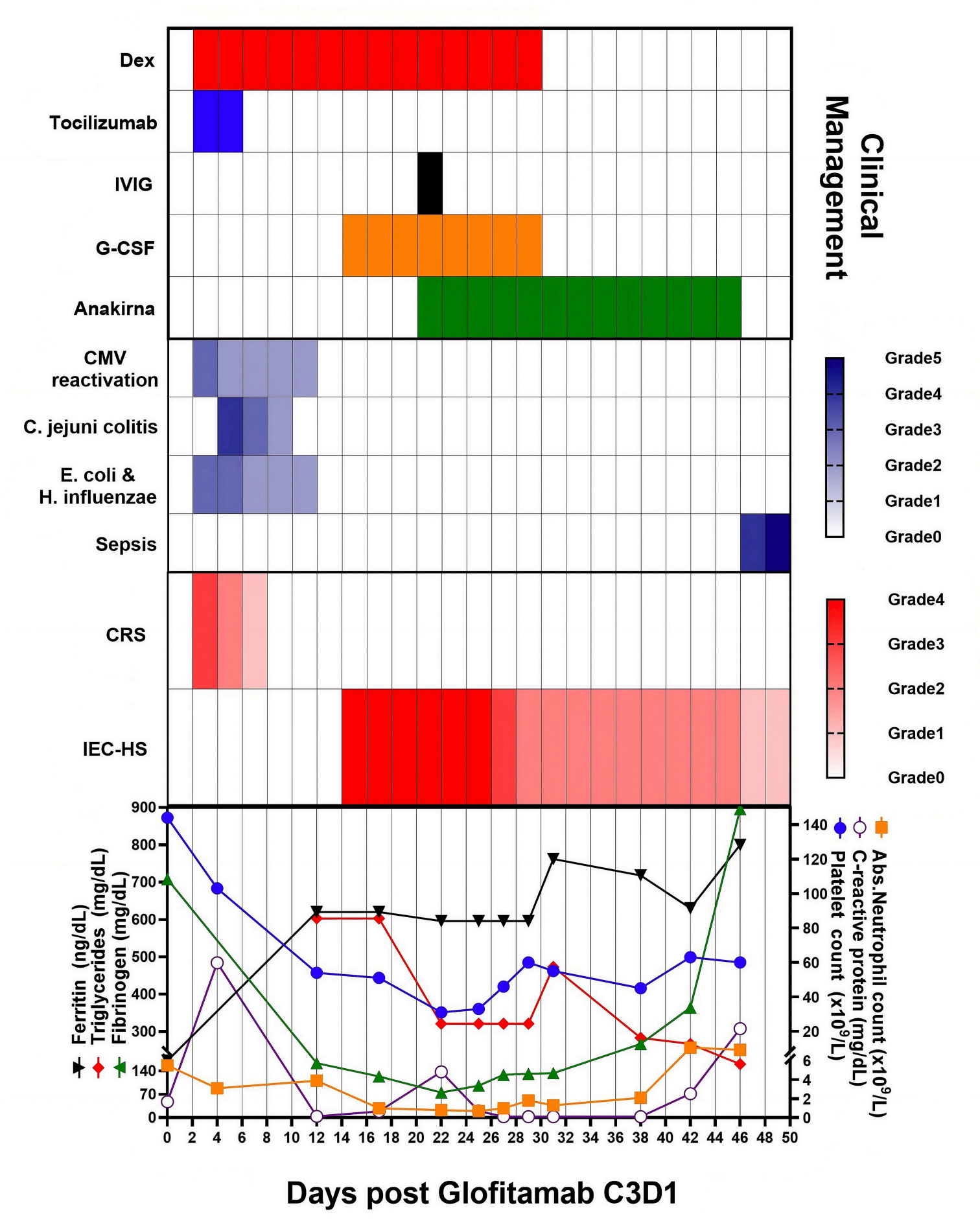
Clinical Management
Dex
Tocilizumab
IVIG
G-CSF
Anakirna
CMV reactivation
C. jejuni colitis
E. coli & H. influenzae
Sepsis
CRS
IEC-HS
Grade5
Grade4
Grade3
Grade2
Grade1
Grade0
Grade4
Grade3
Grade2
Grade1
Grade0
Ferritin (ng/dL)
Triglycerides (mg/dL)
Fibrinogen (mg/dL)
Platelet count (x10⁹/L)
C-reactive protein (mg/dL)
Abs.Neutrophil count (x10⁹/L)
Days post Glofitamab C3D1