Acta Biomed 2024; Vol. 95, N. 4: e2024104 DOI: 10.23750/abm.v95i4.15527

Case report

Hemophagocytic lymphohistiocytosis and pulmonary embolism caused by bacillus Calmette-Guerin intravesical instillation

Antonio Fabozzi[1*], *Gianluca Paciucci*[1*], *Emma Repaci*[1], *Alessandra Iacovelli*[1], *Luigi Panza*[1], *Ambra Migliarini*[1], *Cristina Santoro*[2], *Paolo Palange*[1]

[1]Department of Public Health and Infectious Diseases, Division of Pneumology, "Sapienza" University of Rome, Italy;
[2]Department of Translational and Precision Medicine, Division of Hematology, "Sapienza" University of Rome, Italy;
*these authors have contributed equally

Abstract. Current international guidelines for bladder carcinoma recommend the use of intravesical instillations of chemotherapeutic agents, including bacillus Calmette-Guérin, for the prevention of recurrences in high-grade non-muscle-invasive bladder carcinomas. Treatment with Bacillus Calmette-Guérin is generally well-tolerated. Usually, adverse effects are mild and reversible with discontinuation of therapy. Severe adverse reactions are rare and may include hemophagocytic lymphohistiocytosis, even only few cases have been described none associated with pulmonary embolism. In this report, we describe the case of a 66-year-old patient who develops low-grade (LG) pulmonary embolism and hemophagocytic lymphohistiocytosis after the third session of intravesical instillation. He was treated with high doses of corticosteroid therapy with a restitutio ad integrum of the clinical and laboratory parameters. (www.actabiomedica.it)

Key words: pulmonary embolism, hemophagocytic lymphohistiocytosis, bacillus Calmette-Guerin, bladder carcinoma, respiratory failure, cytopenia

Introduction

Bladder urothelial cell carcinoma, particularly non-muscle-invasive (NMIBC) types (pTa - Tis - T1), can be differentiated based on the cellular grading level according to the TNM VIII edition (1). The 2016 WHO classification subdivides bladder urothelial cell carcinoma into low grade (LG) or high grade (HG) (2). NMIBC HG presents a recurrence rate within 1 year post-Transurethral Resection of the Bladder (TURB) being 58% for pTa (3) and 71% for T1 (4). Current 2021 ESMO (European Society for Medical Oncology) guidelines recommend full-dose intravesical Bacillus Calmette-Guérin BCG for at least 1-year post-TURB (5). Adverse effects of intravesical BCG are generally local and mild, *e.g.* polyuria, strangury, and macrohematuria with a prevalence of 28.5%, 26.8% and 23.6%, respectively (6). Systemic adverse effects are very rare, mainly gastrointestinal, and musculoskeletal (in 4,9% and 1.6% of cases, respectively) (6). In a review including 2600 patients, pneumonia (0.7%), hepatitis (0.7%), cyptopenia (0.1%) and sepsis (0.4%) have been described as rare adverse events in less than 5% of patients (7). To our knowledge, only four cases of hemophagocytic lymphohistiocytosis (HLH) post-intravesical BCG have been reported in literature (8-11), while no cases of pulmonary embolism (PE) have been reported until today.

Case report

We report a clinical case of a 66-year-old patient who was admitted to the emergency department due to dyspnea and persistent fever following intravesical BCG instillation, administered as post-TURB prophylaxis in NMIBC HG. Past medical history was unremarkable except for dyslipidemia under treatment with statins. The diagnosis of NMIBC-HG was made in 2020, but he presented a recurrency in 2023 and underwent a new TURB procedure. For prevention of further disease recurrence, he started intravesical BCG instillation course. After the third session of treatment, he developed a single episode of hematuria and fever. An antibiotic treatment regimen with ciprofloxacin was prescribed by general physician for seven days. Nevertheless, fever persisted, and, after few days, he developed dyspnea. He was then referred to emergency department.

At admission, laboratory tests showed elevated inflammatory markers (C-reactive protein [CRP] 14.27 mg/dL, procalcitonin [PCT] 1.65 ng/mL), mild anemia (hemoglobin [Hb] 11.2 g/dL, white blood cells [WBC] 6460 cells/mmc including neutrophils 4850 cells/mmc and lymphocytes 900 cells/mmc, platelets [PLTs] 212000 cells/mmc). Arterial blood gases (ABGs) on room air revealed hypoxemia (PaO2 63 mmHg, PaCO2 36 mmHg, pH 7.48, Lac 1.3 mmol/L, Δ(A-a) O2 32 mmHg, P/F ratio 300). Oxygen therapy was administered with a fraction of inspired oxygen (FiO_2) of 0.31. D-dimer was elevated (2660 μg/L) and a transthoracic echocardiography showed normal findings except for an indirect RV pressure overload with PAPs of 38 mmHg. Pulmonary embolism (PE) was suspected and CT pulmonary angiography (CTPA) was performed demonstrating bilateral thrombotic appositions in lobar pulmonary arteries (Figure 1). Anticoagulant therapy with Enoxaparin was promptly administered. Lower limb CT angiography excluded deep vein thrombosis (DVT).

The patient was then admitted to our pneumology ward for the management of PE. After few days he developed cytopenia (Hb 7.8 g/dL, WBC 2200 cells/mmc [Neutrophils 1610 cells/mmc, Lymphocytes 370 cells/mmc], PLTs 53000 cells/mmc, reticulocytes 2.37%), increase of serum creatinine and elevated liver enzymes (AST 258 IU/L, ALT 154 IU/L, γGT 237 IU/L, alkaline phosphatase 518 IU/L, hyperbilirubinemia: 2.5 mg/dL and conjugated bilirubin: 2.02 mg/dL), and other laboratory abnormalities such as triglycerides 2.67 mmol/L, fibrinogen 102 mg/dL, ferritin >8000 μg/L. Further microbiological investigations were carried out, including HIV-RNA, TB-IGRA test, new blood and urine cultures, resulting all negative. Serological screening for hepatotropic viruses (including CMV, EBV, HSV) were negative.

Both bladder cancer progression and solid neoplasms were excluded by the use of whole-body CT scan and esophagogastroduodenoscopy and colonoscopy. CT scan, however, revealed a condition of hepatosplenomegaly.

Bone marrow aspirate and biopsy (BMB) showed elements of the erythroblastic and granuloblastic series, platelet aggregates, and macrophages with erythro/

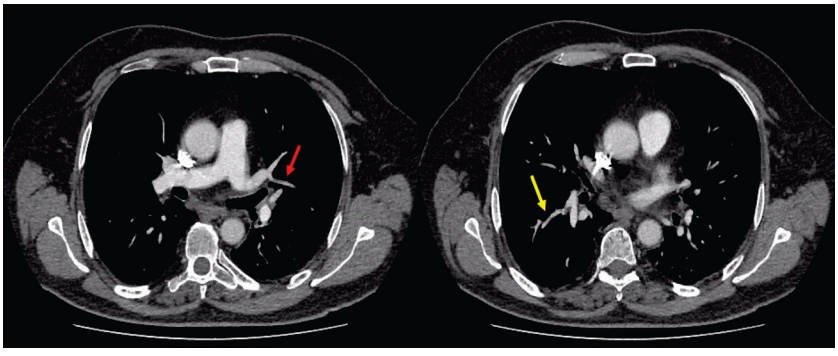

Figure 1. CT pulmonary angiography showed thrombotic appositions in the lower right lobar pulmonary artery (yellow flag) and in the middle left lobar pulmonary artery (red flag).

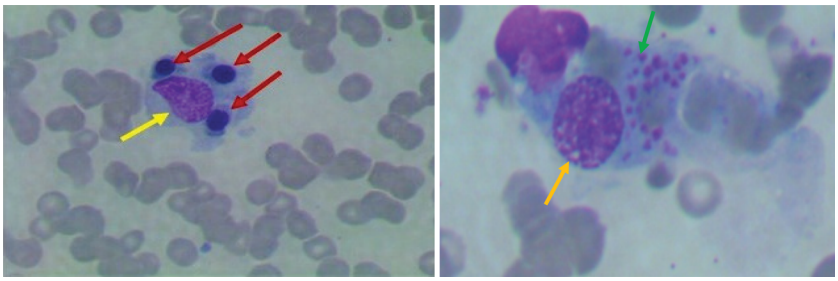

Figure 2. "Hemophagocytosis" on bone marrow aspirate. It is possible to discern the nucleus of the macrophage (yellow and orange flag) and the presence of erythroblasts (red flags) and platelets (green flag) inside its cytoplasm.

hemophagocytosis phenomena, without cellular atypia or pathogenic microorganisms (Figure 2).

Diagnosis of hemophagocytic lymphohistiocytosis (HLH) was confirmed and treatment with methylprednisolone 1 mg/kg/day promptly was started. Within few days the patient improved clinically as well as laboratory tests. 30-day after the diagnosis the following laboratory changes were observed: Hb 11.2 g/dL, WBC 5300 cells/mmc (neutrophils 4270 cells/mmc, lymphocytes 730 cells/mmc), PLTs 128000 cells/mmc, creatinine 0.92 mg/dL, AST 19 IU/L, ALT 46 IU/L, γGT 56 IU/L, total Bilirubin 1.46 mg/dL (of which direct was 0.65 mg/dL), triglycerides 1.25 mmol/L, ferritin 659 µg/L, fibrinogen 1.96 g/L (Figure 3). Patient was discharged with a diagnosis of PE and HLH syndrome in good clinical conditions.

Discussion

Currently, intravesical BCG instillations are considered the most effective treatment in preventing recurrences in NMIBC HG compared to intravesical mitomycin (12). The procedure is considered safe and generally well-tolerated. However, adverse effects, both locally and systemic, even serious, have been described in literature (7). In our patient, intravesical BCG instillation was associated to the onset of HLH. HLH is a systemic hyperinflammatory state due to anomalous immune activation. HLH is classified as primary (genetic) and secondary to malignancies, autoimmune diseases, or systemic infections. The pathophysiological mechanism of HLH remains poorly understood, but in both primary and secondary forms, an abnormal

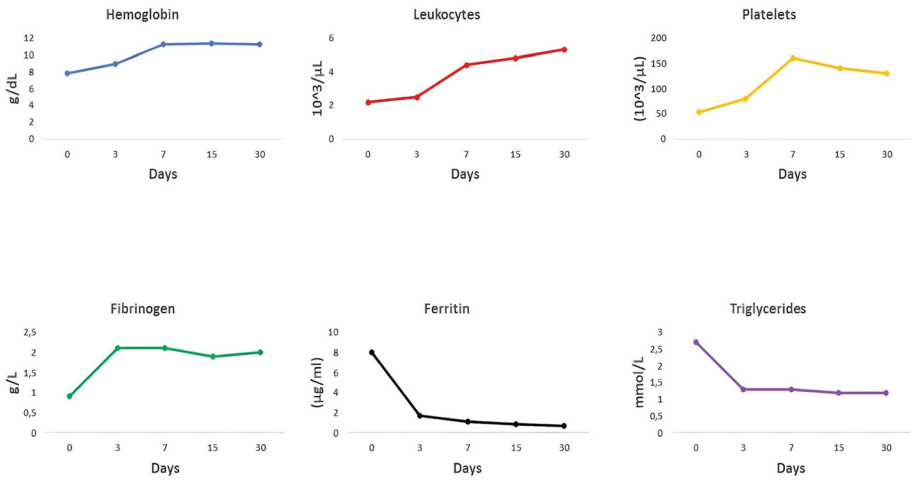

Figure 3. Laboratory exams' trends before and after treatment. Day 0 corresponds to the beginning of high dose steroid treatment.

activation of CD8+ T lymphocytes and Natural Killer (NK) cells, promoting macrophage activation and the release of an abnormal number of pro-inflammatory cytokines (cytokine cascade) has been described (13,14). The phenomenon "hemophagocytosis" refers to macrophages that phagocytose blood cells (15). In our case, HLH was initially suspected based on clinical manifestations (fever, hepatosplenomegaly) and biochemical findings (cytopenia, hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia), subsequently confirmed by bone marrow aspirate and biopsy. Our case met 6 out of 8 HLH-2004 diagnostic criteria (cut-off ≥5) and had an HScore of 250 points as described in literature (16).

Due to the close temporal relationship, we suspected the intravesical BCG instillation as the cause of HLH.

Three pathophysiological mechanisms have been described to explain the etiology of systemic adverse events of intravesical BCG immunotherapy. First, an infectious mechanism linked to hematogenous BCG spread (7, 17); second, an autoimmune mechanism linked to cross-reactivity between BCG antigens and self-proteins (17, 18); and third, a lymphocyte-Th1-mediated hypersensitivity mechanism (8, 19).

The precise mechanism of action of intravesical immunotherapy with BCG is still poorly clear. When BCG attaches to the urothelium, it is internalized by urothelial cells and immune cells (20). Internalization stimulates a local and systemic immune response, which results in the release of multiple chemokines and cytokines, including Interleukin-6 (IL-6), Interleukin-8 (IL-8), Granulocyte-macrophage colony-stimulating factor (GM-CSF) and Tumor Necrosis Factor (TNF) (21,22). This cytokine cascade stimulates immune-mediated cytotoxicity of bladder cancer cells by recruiting neutrophils, monocytes, CD4+ and CD8+ T lymphocytes, B lymphocytes, macrophages and natural killer (NK) cells (20). Intravesical immunotherapy with BCG also works via adaptive immunity. BCG antigens are presented on the surface of antigen-presenting cells (APCs). APCs, interact with CD4+ T-cell receptors, through the major histocompatibility complex class II (MHC II), resulting in the activation and differentiation of an immune response mediated mainly by T helper 1 cells (20).

Both innate and adaptive immune system responses result in long-lasting protection against tumor recurrence and progression (20). Intravesical immunotherapy with BCG can confer characteristics of adaptive immunity cells to circulating monocytes through epigenetic reconfiguration triggered by monocytic phagocytosis of BCG (23). These epigenetic modifications increase access to promoter regions of genes related to inflammatory pathways, such as cytokines and inflammatory pattern recognition receptors (24). Consequently, BCG-stimulated innate immune cells express higher levels of inflammatory pattern recognition receptors and produce higher levels of pro-inflammatory cytokines (25).

In our case, HLH is likely the result of a hypersensitivity and autoimmune mechanism, as microbiological investigations did not reveal hematogenous BCG spread, and there was a rapid clinical and laboratory response to immunosuppressive therapy.

The heterogeneity of HLH in adults precludes a universally valid treatment protocol. The HLH-94 protocol designed for the pediatric population is also currently indicated for adults. It consists of corticosteroids, usually dexamethasone, etoposide, cyclosporine A (indicated after the first 8 weeks of therapy in case of primary treatment ineffectiveness), and intrathecal therapy with methotrexate (in case of neurological involvement) (16). Currently, etoposide is clearly indicated in cases of severe HLH with imminent severe organ failure (26).

In our case report, methylprednisolone 1 mg/kg/day for 2 weeks was used, followed by a tapering regimen, halving the dose every 2 weeks for 8 weeks, following the latest HLH-2004 guidelines (16). We decided not to administer etoposide because our patient presented a moderate clinical picture and immediately improved after starting corticosteroid treatment.

The etiopathogenesis of PE in our patient is not entirely clear. Two possible hypotheses have been considered. Firstly, PE could be attributed to a thrombo-inflammatory mechanism related to HLH, mainly due to activated neutrophils and neutrophil extracellular traps (NETs). NETs role in stimulating platelet aggregation and fibrin plug formation is well known (27).

Secondly, PE could be explained by a paraneoplastic process. Indeed, an increased risk of venous

thromboembolism has been observed in patients with NMIBC (28). The high prevalence of PE in cancers is due to multiple factors, such as venous stasis from compression by neoplastic cells, endothelial damage caused by malignant cell invasion, and hypercoagulability caused by over-expression of Tissue Factor (TF) (29).

In conclusion, our clinical case illustrates that HLH is a rare but potentially life-threatening complication of intravesical BCG therapy. In case of severe adverse effects, including HLH, we believe the continuation of antitumor prophylaxis through intravesical BCG instillation should be avoided. Treatment of HLH consists in high-dose immunosuppressive agents for an extended period, eventually in association with cytostatic drugs, cyclosporine A, plasma exchange or intravenous immunoglobulins, and anti-TNFα (30). Clear evidence in term of length and management of treatment are lacking.

Acknowledgements: All authors would thank all the pneumologist and haematologists of Policlinico Umberto I of Rome for their care to the patient involved in this clinical case.

Funding: None

Ethic Committee: This study was conducted by the ethical standards of the Declaration of Helsinki. The patient provided written informed consent before treatment and gave approval for the publication of his clinical data, figures, and photographs.

Conflict of Interest: Each author declares no conflict of interest.

Authors Contribution: Conception and design of the manuscript: GP and AF. Provider of photograph: CS. Drafting and revising the manuscript: GP, AF, AI, LP, ER, AM, CS, PP. All authors approved the final version of the manuscript.

References

1. Paner GP, Stadler WM, Hansel DE, Montironi R, Lin DW, Amin MB. Updates in the Eighth Edition of the Tumor-Node-Metastasis Staging Classification for Urologic Cancers. Eur Urol. 2018;73(4):560-9. doi: 10.1016/j.eururo.2017.12.018.
2. Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and Bladder Tumours. Eur Urol. 2016;70(1):106-19. doi: 10.1016/j.eururo.2016.02.028.
3. Herr HW. Restaging transurethral resection of high risk superficial bladder cancer improves the initial response to bacillus Calmette-Guerin therapy. J Urol. 2005;174(6):2134-7. doi: 10.1097/01.ju.0000181799.81119.fc.
4. Hollenbeck BK, Montie JE. Early cystectomy for clinical stage T1 bladder cancer. Nat Clin Pract Urol. 2004;1(1):4-5. doi: 10.1038/ncpuro0008.
5. Powles T, Bellmunt J, Comperat E, et al; ESMO Guidelines Committee. Bladder cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2022;33(3):244-58. doi: 10.1016/j.annonc.2021.11.012.
6. Koga H, Kuroda M, Kudo S, et al. Adverse drug reactions of intravesical bacillus Calmette-Guerin instillation and risk factors of the development of adverse drug reactions in superficial cancer and carcinoma in situ of the bladder. Int J Urol. 2005;12(2):145-51. doi: 10.1111/j.1442-2042.2005.01000.x.
7. Lamm DL, van der Meijden PM, Morales A, et al. Incidence and treatment of complications of bacillus Calmette-Guerin intravesical therapy in superficial bladder cancer. J Urol. 1992;147(3):596-600. doi: 10.1016/s0022-5347(17)37316-0.
8. Thevenot T, Di Martino V, Lagrange A, et al. Granulomatous hepatitis and hemophagocytic syndrome after bacillus Calmette-Guerin bladder instillation. Gastroenterol Clin Biol. 2006;30(3):480-2. doi: 10.1016/s0399-8320(06)73208-0.
9. Schleinitz N, Bernit E, Harle JR. Severe hemophagocytic syndrome after intravesical BCG instillation. Am J Med. 2002;112(7):593-4. doi: 10.1016/s0002-9343(02)01066-5.
10. González MJ, Franco AG, Alvaro CG. Hemophagocytic lymphohistiocytosis secondary to Calmette-Guèrin bacilli infection. Eur J Intern Med. 2008;19(2):150. doi: 10.1016/j.ejim.2007.05.007.
11. Misra S, Gupta A, Symes A, Duncan J. Haemophagocytic syndrome after intravesical bacille Calmette-Guérin instillation. Scand J Urol. 2014;48(3):328-30. doi: 10.3109/21681805.2013.836724.
12. Shelley MD, Wilt TJ, Court J, Coles B, Kynaston H, Mason MD. Intravesical bacillus Calmette–Guerin is superior to mitomycin C in reducing tumour recurrence in high-risk superficial bladder cancer: a meta-analysis of randomized trials. BJU Int, 93 (2004), pp. 485-90. doi: 10.1111/j.1464-410x.2003.04655.x.
13. Egeler RM, Shapiro R, Loechelt B, Filipovich A. Characteristic immune abnormalities in hemophagocytic lymphohistiocytosis. J Pediatr Hematol Oncol. 1996;18(4):340-5. doi: 10.1097/00043426-199611000-00002.
14. Ishii E, Ueda I, Shirakawa R, et al. Genetic subtypes of familial hemophagocytic lymphohistiocytosis: correlations with clinical features and cytotoxic T lymphocyte/natural killer

cell functions. Blood. 2005;105(9):3442-8. doi: 10.1182/blood-2004-08-3296.
15. Risma K, Jordan MB. Hemophagocytic lymphohistiocytosis: updates and evolving concepts. Curr Opin Pediatr. 2012;24(1):9-15. doi: 10.1097/MOP.0b013e32834ec9c1.
16. La Rosée P, Horne A, Hines M, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. Blood. 2019;133(23):2465-77. doi: 10.1182/blood.2018894618.
17. Pérez-Jacoiste Asín MA, Fernández-Ruiz M, López-Medrano F, et al. Bacillus Calmette-Guérin (BCG) infection following intravesical BCG administration as adjunctive therapy for bladder cancer: incidence, risk factors, and outcome in a single-institution series and review of the literature. Medicine (Baltimore). 2014;93(17):236-54. doi: 10.1097/MD.0000000000000119.
18. Prescott S, Jackson AM, Hawkyard SJ, Alexandroff AB, James K. Mechanisms of action of intravesical bacille calmette-guérin: local immune mechanisms. Clinical Infectious Diseases. 2000;31(3):S91–3. doi: 10.1086/314066.
19. Abid H, Figuigui M, Adil Ibrahimi S, et al. Acute Hepatitis Induced by Intravesical BCG Therapy: A Rare but Serious Complication. Case Reports Hepatol. 2021;2021:4574879. doi: 10.1155/2021/4574879.
20. Pettenati C, Ingersoll MA. Mechanisms of BCG immunotherapy and its outlook for bladder cancer. Nat Rev Urol. 2018;15(10):615-25. doi: 10.1038/s41585-018-0055-4.
21. El-Demiry MI, Smith G, Ritchie AW, et al. Local immune responses after intravesical BCG treatment for carcinoma in situ. Br J Urol. 1987;60(6):543-8. doi: 10.1111/j.1464-410x.1987.tb05039.x.
22. Prescott S, James K, Busuttil A, Hargreave TB, Chisholm GD, Smyth JF. HLA-DR expression by high grade superficial bladder cancer treated with BCG. Br J Urol. 1989;63(3):264-9. doi: 10.1111/j.1464-410x.1989.tb05187.x.
23. Kaufmann E, Sanz J, Dunn JL, et al. BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis. Cell. 2018;172(1-2):176-90. doi: 10.1016/j.cell.2017.12.031.
24. Kleinnijenhuis J, Quintin J, Preijers F, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A. 2012;109(43):17537-42. doi: 10.1073/pnas.1202870109.
25. Arts RJW, Moorlag SJCFM, Novakovic B, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. Cell Host Microbe. 2018;23(1):89-100. doi: 10.1016/j.chom.2017.12.010.
26. Arca M, Fardet L, Galicier L, Rivière S, et al. Prognostic factors of early death in a cohort of 162 adult haemophagocytic syndrome: impact of triggering disease and early treatment with etoposide. Br J Haematol. 2015;168(1):63-8. doi: 10.1111/bjh.13102.
27. Carminita E, Crescence L, Panicot-Dubois L, Dubois C. Role of Neutrophils and NETs in Animal Models of Thrombosis. Int J Mol Sci. 2022;23(3):1411. doi: 10.3390/ijms23031411.
28. Balan D, Vartolomei MD, Magdás A, Balan-Bernstein N, Voidăzan ST, Mártha O. Inflammatory Markers and Thromboembolic Risk in Patients with Non-Muscle-Invasive Bladder Cancer. J Clin Med. 2021;10(22):5270. doi: 10.3390/jcm10225270.
29. Kacimi SEO, Moeinafshar A, Haghighi SS, Saghazadeh A, Rezaei N. Venous thromboembolism in cancer and cancer immunotherapy. Crit Rev Oncol Hematol. 2022;178:103782. doi: 10.1016/j.critrevonc.2022.103782.
30. Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C. Infections associated with haemophagocytic syndrome. Lancet Infect Dis. 2007;7(12):814-22. doi: 10.1016/S1473-3099(07)70290-6.

Correspondence:
Received: 15 December 2023
Accepted: 26 March 2024
Antonio Fabozzi, MD
Pulmonology Division, Department of Public Health and Infectious Diseases, Policlinico Umberto I, "Sapienza" University of Rome
Viale del Policlinico, 155, Rome, 00161 Italy
E-mail: antonio.fabozzi@uniroma1.it
ORCID ID: https://orcid.org/0009-0006-6070-428X