How to Diagnose Early 5-Azacytidine-Induced Pneumonitis: A Case Report

Srimanta Chandra Misra1 · Laurence Gabriel2 · Eric Nacoulma1 · Gérard Dine1 · Valentina Guarino2

Abstract Interstitial pneumonitis is a classical complication of many drugs. Pulmonary toxicity due to 5-azacytidine, a deoxyribonucleic acid methyltransferase inhibitor and cytotoxic drug, has rarely been reported. We report a 67-year-old female myelodysplastic syndrome patient treated with 5-azacytidine at the conventional dosage of 75 mg/m² for 7 days. One week after starting she developed moderate fever along with dry cough and subsequently her temperature rose to 39.5 °C. She was placed under broad-spectrum antibiotics based on the protocol for febrile neutropenia, including ciprofloxacin 750 mg twice daily, ceftazidime 1 g three times daily (tid), and sulfamethoxazole/trimethoprim 400 mg/80 mg tid. High-resolution computed tomography of the chest disclosed diffuse bilateral opacities with ground-glass shadowing and pleural effusion bilaterally. Mediastinal and hilar lymph nodes were moderately enlarged. Polymerase chain reaction for Mycobacterium tuberculosis, Pneumocystis jiroveci, and cytomegalovirus were negative. Cultures including viral and fungal were all negative. A diagnosis of drug-induced pneumonitis was considered and, given the negative bronchoalveolar lavage in terms of an infection, corticosteroid therapy was found in our case. An explanation could be a delayed type of hypersensitivity (type IV) with activation of CD8 T cell which could possibly explain most of the symptoms. We have developed a decision algorithm in order to anticipate timely diagnosis of 5-azacitidine-induced pneumonitis, and with the aim to limit antibiotics abuse and to set up emergency treatment.

Introduction

Pneumonitis, often called interstitial lung disease or ILD, is a possible manifestation of many antineoplastic and other drugs, with several ILD subtypes being described in association with drugs. Pulmonary toxicity from 5-azacytidine, a deoxyribonucleic acid (DNA) methyltransferase inhibitor which also exerts cytotoxic effects, has rarely been reported, although the drug has been used since 1982. 5-Azacytidine acts as a hypomethylating agent of the Y globin...
suppressor gene to induce fetal hemoglobin in thalassemia and, since 2000, to treat high-risk myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) with low blast counts. Here, we report a case of 5-azacytidine-associated pneumonitis, review the literature, and develop a diagnostic algorithm for this rare condition to avoid delay in medical care and misuse of antibiotics.

**Case Report**

A 67-year-old woman presented as an outpatient of our Hematology Department in August 2015 for progressive neutropenia, anemia, and fatigue. Peripheral blood examination showed a normochromic normocytic anemia with 9.4 g/dL hemoglobin, 0.350 × 10⁹/L neutrophils and 138 × 10⁹/L platelets. A bone marrow aspirate (BMA) showed hypercellular marrow with trilineage dysplastic features, micromegakaryocytes and 13% myeloblasts. A diagnosis of refractory cytopenia with multilineage dysplasia was given, based on the WHO MDS classification [1]. A trephine biopsy was in accordance with the results from the bone marrow aspirate with 15% myeloblasts displaying dyserythropoiesis and dysmegakaryopoiesis. Karyotype G banding analysis revealed a complex cytogenetic abnormality: 46,XX,del(5)(q14q34) [2]/49,sl,+1,+9,+11 [2]/52,sl,+11,+22,+22 [16].

Based on the above data, high-risk MDS was considered. The patient underwent appropriate tests concerning eligibility for allogenic stem cell transplantation. She received the first cycle of 5-azacytidine at the conventional dosage of 75 mg/m² for 7 days from September 28, 2015. One week after starting 5-azacytidine, she developed moderate fever along with dry cough and, subsequently, her temperature rose to 39.5°C. She was hospitalized on October 11, 2015. Vital signs and pulse oximetry were normal. She was placed under broad-spectrum antibiotics based on the protocol for febrile neutropenia, including ciprofloxacin 750 mg twice daily, ceftazidime 1 g three times daily (tid), and sulfamethoxazole/trimethoprim 400 mg/80 mg tid. Fever did not abate. All routine bacteriological investigations were negative. Procalcitonin levels were within the normal range. The chest and sinus radiographs were normal, as were precipitins against *Aspergillus* and titers against *Cytomegalovirus* (CMV) and Epstein-Barr virus (EBV). CMV antigenemia was negative. An interferon-γ release assay was negative. Marrow re-aspiration revealed a 22% increment of blast number, suggesting a transformation towards acute myeloid leukemia. During her second week in hospital, the patient complained of dyspnea on October 22, 2015. Blood gas showed a PaO₂ of 59 mmHg and PaCO₂ of 29 mmHg. Pulse oxygen saturation was 91% (room air). High-resolution computed tomography (HRCT) of the chest disclosed diffuse bilateral opacities with ground-glass shadowing and pleural effusion bilaterally (Fig. 1). Mediastinal and hilar lymph nodes were moderately enlarged. The patient was transferred to the intensive care unit on October 23 for bronchoalveolar lavage (BAL), which showed 170 red blood cells/mm³ and 10 white blood cells/mm³. Polymerase chain reaction (PCR) for *Mycobacterium tuberculosis, Pneumocystis jiroveci*, and CMV were negative. Immunofluorescence test for *Pneumocystis* was also negative. Cultures including viral and fungal were all negative. The patient was maintained on antibiotics. A diagnosis of drug-induced pneumonitis was considered and, given the negative BAL in terms of an infection, corticosteroid therapy was given at a dose of 1 mg/kg body weight on October 28. Within 4 days, a significant improvement in clinical status and imaging was noted. A repeat chest computed tomography (CT) scan at 1 week also showed significant improvement. Temperature was normal and C-reactive protein returned to normal within 1 week. Following 2 days of quick steroid tapering, the patient again developed fever. Left upper chest pain corresponding to lobulated pleural effusion was noted and 1200 mL of serosanguinous fluid was removed via chest tube. Pleural fluid was a predominantly neutrophilic exudate containing 4 g/dL proteins. Corticosteroids were maintained and antibiotics were discontinued. The patient remained afebrile and was discharged from hospital on November 9. She eventually received a haploidentical bone marrow transplant on December 23, 2015.

**Discussion**

Clinical features of 5-azacytidine-associated ILD include cough, dyspnea, pleuritic chest pain, and hypoxemic respiratory failure [2]. Like many antineoplastic agent-induced lung diseases, prominent imaging findings include...
| Study                     | Disease | Sex | Age | Clinical symptoms                                      | Time of onset of symptoms | Examination                                                                                                           | Treatment                                                                 | Evolution | Rechallenge |
|--------------------------|---------|-----|-----|--------------------------------------------------------|---------------------------|----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------|-------------|
| Adams et al. 2005; USA   | MDS     | M   | 71  | Bilateral crackles and wheezing                        | <7 days                   | Chest radiograph: patchy bilateral, perihilar airspace disease, organizing pneumonitis                               | Cefotaxime, azithromycin, metronidazole                                   | Died      | No          |
|                          |         |     |     |                                                       |                           | Bronchoscopy: scattered petechiae, thin watery secretions, with no lesions or evidence of hemorrhage                  |                                                                           |           |             |
|                          |         |     |     |                                                       |                           | Cultures negative                                                                                                     |                                                                           |           |             |
|                          |         |     |     |                                                       |                           | Biopsy: acute and chronic interstitial and alveolar fibrosis with chronic inflammation, marked atypia of pneumocytes, no pathogens |                                                                           |           |             |
|                          |         |     |     |                                                       |                           |                                                                                                                     |                                                                           |           |             |
| Hueser and Patel 2007; USA | MDS   | F   | 55  | Hyperthermia, hypoxic respiratory failure, acute respiratory distress syndrome | 5 days                   | Chest tomography: bilateral interstitial opacities                                                                 | 1. Antipyretic  
2. Empiric broad-spectrum antibiotics, antifungal drugs and methylprednisolone 100 mg every 12 hours | Recovered | No          |
|                          |         |     |     |                                                       |                           |                                                                                                                     |                                                                           |           |             |
| Pillai et al. 2012; UK   | MDS     | F   | 74  | Fever, dry cough, breathlessness                      | 2 weeks                   | Tomography scan: peribroncholar shadowing  
Cultures negative  
Fever, dry cough, dyspnea  
5 days after 2nd cycle  
Chest X-ray: bilateral patchy shadowing  
CT scan: reticulo-nodular and ground-glass shadowing, pleural effusions | 1. IV antibiotics  
2. Methylprednisolone 1.5 g/day | Recovered | Yes         |
|                          |         |     |     |                                                       |                           |                                                                                                                     |                                                                           |           |             |
| Kotsianidis et al. 2012; Greece | MDS | M   | 55  | Fever, respiratory failure, hypoxemia, hypercapnia    | 27 days                   | NA                                                                                                                  | 1. Broad-spectrum antibiotics  
2. Prednisolone 0.5 mg/kg/day + oxygen | Recovered and died of sepsis after 5 months | No          |
|                          |         |     |     |                                                       |                           |                                                                                                                     |                                                                           |           |             |
| Sekhri et al. 2012; USA  | MDS     | M   | 56  | Dry cough, dyspnea                                   | 7 days                    | Cultures negative  
Cultures negative  
Fever, cough, dyspnea, hypoxia  
7 days after 2nd cycle  
Tomography scan: extensive bilateral airspace disease with nodular opacities  
Biopsy: interstitial lung disease and bronchocentric granulomatous pattern  
BAL negative | 1. Broad-spectrum antibiotics  
2. IV methylprednisolone | Recovered | Yes         |
|                          |         |     |     |                                                       |                           |                                                                                                                     |                                                                           |           |             |
| Study                  | Disease | Sex | Age | Clinical symptoms                              | Time of onset of symptoms | Examination                                                                 | Treatment                                                                                     | Evolution | Rechallenge |
|------------------------|---------|-----|-----|-----------------------------------------------|---------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-----------|-------------|
| Nair et al. 2012; USA [8] | MDS     | M   | 76  | Dyspnea, non-productive cough, fever          | 3 weeks                   | Chest X-ray: bilateral interstitial infiltrates                               | 1. Ceftriaxone + azithromycin IV<br>2. IV methylprednisolone 1 mg/kg twice daily             | Recovered | NA          |
| Hayashi et al. 2012; Japan [9] | MDS     | M   | 74  | Fever, dry cough, worsening shortness of breath | 2 days                    | Chest X-ray: infiltration in the right middle lung field<br>Cultures negative<br>Chest tomography: organizing pneumonia | 1. Cefepime<br>2. Piperacillin/tazobactam<br>3. Meropenem + vancomycin<br>4. Methylprednisolone 1000 mg/day | Recovered | No          |
| Kuroda et al. 2014; Japan [10] | MDS     | M   | 72  | Moderate pyrexia, dyspnea, dry cough, bloody sputum and wheezing, hypoxic respiratory failure | 3 days                    | Chest X-ray: patchy airspace disease<br>Tomography scan: areas of interstitial opacity and ground-glass shadowing<br>No infections in cultures | 1. Oxygen<br>2. Broad-spectrum antibiotics and antifungal agents<br>3. IV methylprednisolone (500 mg) + sulfamethoxazole trimethoprim, vancomycin, micafungin | Died      | No          |
| Verriere et al. 2015; France [11] | AML     | F   | 86  | Grade III skin reaction, nausea, gastric pain, dry cough, hyperthermia, ear pain, asthma, anorexia, hyperthermia | 2nd day of the 3rd cycle  | CT scan: diffuse interstitial opacities and ground-glass shadowing (mediastinal and hilar lymph nodes) | 1. Piperacillin/tazobactam<br>2. Imipenem/cilastatin<br>3. Corticotherapy 0.75 mg/kg per day + oxygen therapy | Recovered | No          |
| Patel et al. 2015; USA [12] | MDS     | M   | 74  | Fever, cough, shortness of breath             | 2 days after 2nd cycle    | Chest radiograph and tomography: bilateral interstitial infiltrates and ground-glass opacities<br>Cultures negative<br>BAL inflammatory | 1. Corticosteroids | Recovered | NA          |
| Study                        | Disease | Sex | Age | Clinical symptoms                                      | Time of onset of symptoms | Examination                                                                                                                                  | Treatment                                                                 | Evolution | Rechallenge |
|------------------------------|---------|-----|-----|--------------------------------------------------------|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------|-------------|
| Ahari et al. 2015; Canada    | MDS     | M   | 73  | Fever, chills, night sweats                           | Start of 3rd cycle        | Blood culture: *Mycobacterium fortuitum*  
Chest radiograph: bilateral hilar enlargement and bilateral perihilar ground-glass opacities  
Chest tomography: bilateral ground-glass opacities with reticulation in the mid- and upper lung zones and patchy peripheral airspace consolidation  
BAL negative | 1. Levofloxacin  
2. Clarithromycin + ciprofloxacin + sulfamethoxazole trimethoprim  
3. High-dose prednisone | Died     | No           |
| Alnimer et al. 2016; USA     | MDS     | M   | 67  | Worsening shortness of breath, mild productive cough  | 2 weeks after 2nd cycle   | CT scan: massive multifocal bilateral pulmonary consolidations, surrounding ground-glass opacities, pleural effusion  
Cultures negative  
Lung biopsy: chronic nonspecific inflammation with macrophages | 4. Levofloxacin + piperacillin/tazobactam  
5. Caspofungin + teicoplanin + oseltamivir + meropenem + levofloxacin  
6. Methylprednisolone 60 mg twice daily | Recovered | No           |

*AML* acute myeloid leukemia, *BAL* bronchoalveolar lavage, *CT* computed tomography, *DILD* drug-induced lung injury, *F* female, *IV* intravenous, *M* male, *MDS* myelodysplastic syndrome, *NA* not available.
Here we review 12 earlier cases of 5-azacytidine-associated pneumonitis (Table 1). Delayed diagnosis following failure of broad-spectrum antibiotic therapy was common [3–14]. Corticosteroids were used depending on severity.

The diagnosis of drug-induced pneumonitis rests on history of drug exposure, clinical imaging, bronchoalveolar lavage, exclusion of other lung conditions, improvement following drug discontinuation, and recurrence of symptoms upon rechallenge with the drug. In the present case, we were reluctant to readminister the drug as the risk of doing so is poorly known. The Naranjo probability score in this case was 6, consistent with probable adverse reaction [15, 16]. In our case, despite steroid use, symptoms relapsed and were characterized as serosanguinous pleural effusion. Serosanguinous pleural exudates with polymorphonuclear leukocyte predominance without

Neutropenic fever

Piperacillin/tazobactam or meropenem (routine blood urine CXR) CRP

Does not subside

CXR characteristic finding and/or dyspnea

ANA and anti-ANCA antibodies dosing

1. Imipenem/cilastatin or meropenem
2. Ag aspergillus, CMV EBV PCR, quantiferon pneumococcal, βDglucan
3. CT scan + BAL

Signs of interstitial pneumonitis

Pleural effusion

Ground glass opacities without cavity

BAL negative:
cytology, gram stain, immnofluorescence

1. Corticosteroid methylprednisolone 1-2 mg/kg
2. Antibiotic coverage as per advice of pulmonary or infectiology consultant

Repeat bacteriological test

CT chest after one week

Improvement:
Continue corticosteroid, limiting antibiotics

Aggravation:
Refer nearest pulmonary referent center

BAL: bronchoalveolar lavage, CRP: C reactive protein, CT: computerized tomography scan, CXR: chest X-rays

Fig. 2 Decision algorithm for 5-azacitidine-induced ILD
bacteriological evidence of infection may be a manifestation of pleurisy such as in lupus erythematosus, which might be induced by the drug in question [17].

Mechanisms for drug-induced ILD are direct cytotoxicity, hypersensitivity, oxidative stress, release of cytokines and thus pyrogens, and lastly impaired repair by type II pneumocytes. Chronology of events, unexplained fever, and steroid response to clinical and radiological signs constitute a hypersensitivity pneumonitis.

5-Azacytidine is a cytosine analog, a potent inhibitor of DNA methyltransferase, with a hypomethylating effect in vivo and in vitro. Unlike gemcitabine, although cytotoxic at high dose, at low dose it is capable of inducing differentiation and hypomethylation. Hence, profound myelosuppression or direct lung injury like capillary leak syndrome is not encountered during 5-azacytidine toxicity. The role of oxidative stress is still unclear although there are a few reports concerning induction of necrosis in vitro by 5-azacytidine [18]. Oxidative stress could contribute to T-cell response by inhibiting the ERK pathway signaling in T cells. Recently we observed drug-associated ILD in two patients treated with an experimental inhibitor of DNA methyltransferase, suggesting a common class effect [19, 20].

Unlike oxaliplatin, anaphylactic reaction is extremely rare in 5-azacytidine. Few patients develop symptoms during the administration of chemotherapy. Although an elevated IgE level was reported in one case by Nair et al., the evidence is not sufficient to conclude a type I reaction [8]. Most patients develop symptoms within a week to a month after administration of 5-azacytidine. Although the histopathological evidence is rarely possible in immunocompromised patients with hematological malignancy, Sekhri et al. presented a bronchocentric granuloma in their report [7]. Hence, another plausible explanation could be a delayed type of hypersensitivity (type IV) with activation of CD8 T cell, which could explain most of the symptoms. This could possibly occur during a relative immune reconstitution phase of an immunocompromised patient.

The pulmonary fibrosis may be due to DNA hypomethylation causing direct upregulation of type I collagen synthesis. Sanders et al. suggested that the DNA methylation is important in idiopathic pulmonary fibrosis (IPF), as an altered DNA methylation profile has been demonstrated in their experiment [21]. Moreover, there are reports suggesting the epigenetic priming by 5-azacytidine confers transdifferentiating properties to various cells. However, it is difficult to establish a relationship at present [22].

Our diagnostic algorithm is based on that of drug-induced interstitial lung disease (DILD), and is not specific for 5-azacytidine (Fig. 2). Any febrile condition in those patients with worsening pulmonary symptoms despite broad-spectrum antibiotics should arouse suspicion of DILD. HRCT and BAL are crucial as 5-azacytidine-induced pneumonitis remains a diagnosis of exclusion, like many other DILDs. Some nonspecific immunological tests could be helpful, like levels of p-ANCA (antineutrophil cytoplasmic antibody) and ANA (antinuclear antibody). Prompt consultation with a pulmonary care unit is of utmost utility.

Conclusions

A high degree of vigilance is advised to entertain the diagnosis in a timely manner, since the condition can be fatal. We now utilize a decision algorithm in order for timely diagnosis of 5-azacitidine-induced ILD to limit antibiotics abuse and to set up emergency treatment.

Compliance with Ethical Standards

Conflict of interest S.C. Misra, L. Gabriel, E. Nacoulma, G. Dine, and V. Guarino declare that they have no conflict of interest.

Funding No financial support was received for the preparation of this manuscript.

Informed consent Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent may be requested for review from the corresponding author.

Open Access This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits any noncommercial use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Eclache V. Classification of myelodysplastic syndromes 2015 [Internet]. 2015 [cited 2016 Feb 2]. Available from: http://atlasgeneticsoncology.org/Anomalies/ClassifMDSID1058.html
2. European Medicines Agency-Find medicine-Vidaza [Internet]. [cited 2016 Jan 22]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000978/WC500050239.pdf
3. Adams CD, Szumita PM, Baroletti SA, Lilly CM. Azacitidine-induced interstitial and alveolar fibrosis in a patient with myelodysplastic syndrome. Pharmacotherapy. 2005;25:765-8.
4. Hueser CN, Patel AJ. Azacitidine-associated hyperthermia and interstitial pneumonitis in a patient with myelodysplastic syndrome. Pharmacotherapy. 2007;27:1759–62.
5. Pillai AR, Sadik W, Jones PAH, Thachil J. Interstitial pneumonitis—an important differential diagnosis for pulmonary sepsis in haematology patients. Leuk Res. 2012;36:e39–40.
6. Kotsianidis I, Spanoudakis E, Nakou E, Miliades P, Margaritis D, Tsatalas C, et al. Hypomethylating therapy and autoimmunity
7. Sekhri A, Palaniswamy C, Kurmayagari K, Kalra A, Selvaraj DR. Interstitial lung disease associated with azacitidine use: a case report. Am J Ther. 2012;19:e98–100.

8. Nair GB, Charles M, Ogden L, Spiegler P. Eosinophilic pneumonia associated with azacitidine in a patient with myelodysplastic syndrome. Respir Care. 2012;57:631–3.

9. Hayashi M, Takayasu H, Tada M, Yamazaki Y, Tateno H, Tazawa S, et al. Azacitidine-induced pneumonitis in a patient with myelodysplastic syndrome: first case report in Japan. Intern Med. 2012;51:2411–5.

10. Kuroda J, Shimura Y, Mizutani S, Nagoshi H, Kiyota M, Chinen Y, et al. Azacitidine-associated acute interstitial pneumonitis. Intern Med. 2014;53:1165–9.

11. Verriere B, Ferreira V, Denis E, Zahreddine K, Deletie E, Quinsat D, et al. Azacitidine-induced interstitial pneumonitis. Ther: Am J; 2015.

12. Patel V, Sarkar S, Cervellione KL. A case of azacitidine induced interstitial pneumonitis in a patient with myelodysplastic syndrome (MDS) (ATS Journals). Abstr: Am Thorac Soc Int Conf Meet; 2015.

13. Ahrari A, Sabloff M, Bredeson C, Pakhale S, Souza C, Zwicker J, et al. Rare respiratory and neurologic adverse reactions to azacitidine in the treatment of myelodysplastic syndrome of patients treated at the Ottawa Hospital. J Hematol. 2015;4:231–4.

14. Alnimer Y, Salah S, Abuqayas B, Airabi K. Azacitidine-induced cryptogenic organizing pneumonia: a case report and review of the literature. J Med Case Rep. 2016;10:15.

15. Naranjo CA. A clinical pharmacologic perspective on the detection and assessment of adverse drug reactions. Drug Inf J. 1986;20:387–93.

16. Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther. 1981;30:239–45.

17. Dail and Hammar’s Pulmonary Pathology—Volume I: Joseph F. Tomashefski | Springer [Internet]. [cited 2016 Feb 19]. Available from: http://www.springer.com/9780387721392

18. Tian E, Tang H, Xu R, Liu C, Deng H, Wang Q. Azacytidine induces necrosis of multiple myeloma cells through oxidative stress. Proteome Sci BioMed Central. 2013;11:24.

19. Vasu TS, Cavallazzi R, Hirani A, Marik PE. A 64-year-old male with fever and persistent lung infiltrate. Respir Care. 2009;54:1263–5.

20. Molina M, Yellapragada S, Mims M, Rahman E, Rivero G. Pulmonary complications of azanucleoside therapy in patients with myelodysplastic syndrome and acute myelogenous leukemia. Case Rep Hematol. 2015;2015:357461.

21. Sanders YY, Ambalavanan N, Halloran B, Zhang X, Liu H, Crossman DK, et al. Altered DNA methylation profile in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186:525–35.

22. Mirakhori F, Zeynali B, Kiani S, Baharvand H. Brief azacytidine step allows the conversion of suspension human fibroblasts into neural progenitor-like cells. Cell J. 2015;17:153–8.