Amphotericin B (AMB) has been the irreplaceable drug of choice for countless fungal and protozoal infections. One of the lesser-known adverse effects of AMB is Pancytopenia - very rare with very few cases reported - most commonly observed following prolonged administration. We report the case of a patient suffering from visceral leishmaniasis, who developed worsening pancytopenia four to five days after being administered a single bolus dose of Liposomal Amphotericin B (L-AMB). The diagnosis was clinical and management involved supportive care, and granulocyte-macrophage colony-stimulating factor (GM-CSF). AMB is an effective drug, but is also associated with numerous side effects. Physicians are well-versed with the more frequently seen adverse drug reactions and their management. However, pancytopenia, being a rare adverse reaction to AMB, is less known and can be easily overlooked. This case report aims to ensure that the physicians must be aware of such possibilities in the first place to make swift diagnoses and management. The condition itself is seemingly self-limiting, although GM-CSF may be needed in refractory cases. It’s true that few previous case reports have indicated pancytopenia in association with prolonged AMB exposure, but we believe certain conditions may predispose a patient to a more acute presentation - as seen in our case.

**Keywords:** Adverse drug reaction, amphotericin B, pancytopenia, visceral leishmaniasis

**Background**

Amphotericin B (AMB) belongs to the polyene class of antifungals. Its introduction in the late 1950s revolutionized the treatment of invasive fungal infections where cryptococcal meningitis and disseminated fungal infections were almost always fatal. It remained the only effective drug of choice for life-threatening fungal infections in both immune-competent and immune-compromised patients (such as human immunodeficiency virus, HIV) for nearly a decade after that. Apart from fungal infections such as cerebral cryptococcosis, mucormycosis, disseminated candidiasis, histoplasmosis, it is the drug of choice for cutaneous and visceral leishmaniasis – a protozoal infection. It is also used empirically in cases of persistent febrile neutropenia. AMB binds to ergosterol in the fungi’s cell membrane, causing the formation of ion channels that eventually lead to loss of protons and monovalent cations. It is followed by depolarization and concentration-dependent cell killing. AMB also causes oxidative cellular damage through the formation of free radicals, subsequently increasing the membrane permeability. Lastly, AMB has a stimulatory effect on phagocytic cells, which assists in fungal infection clearance.[1]

Fever with chills, nausea, vomiting and venous thrombophlebitis are the most commonly known and reported adverse reactions to AMB and are infusion-related. Other common side effects include hypokalemia, hypomagnesemia, and nephrotoxicity. There are, however, some rare instances of adverse drug effects of AMB that can be fatal, easily overlooked, and hence, imperative that a physician keep them in mind as a possibility. This case report aims to ensure that the physicians must be aware of such possibilities in the first place to make swift diagnoses and management. The condition itself is seemingly self-limiting, although GM-CSF may be needed in refractory cases. It’s true that few previous case reports have indicated pancytopenia in association with prolonged AMB exposure, but we believe certain conditions may predispose a patient to a more acute presentation - as seen in our case.

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Fever with chills, nausea, vomiting and venous thrombophlebitis are the most commonly known and reported adverse reactions to AMB and are infusion-related. Other common side effects include hypokalemia, hypomagnesemia, and nephrotoxicity. There are, however, some rare instances of adverse drug effects of AMB that can be fatal, easily overlooked, and hence, imperative that a physician keep them in mind as a possibility. These include hypersensitivity reactions, myocarditis, and bone marrow suppression. We, hereby, report witnessing another such unique adverse drug reaction (ADR) of AMB in a patient being treated for visceral leishmaniasis at our hospital – pancytopenia.

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**Abstract**

Amphotericin B (AMB) has been the irreplaceable drug of choice for countless fungal and protozoal infections. One of the lesser-known adverse effects of AMB is Pancytopenia - very rare with very few cases reported - most commonly observed following prolonged administration. We report the case of a patient suffering from visceral leishmaniasis, who developed worsening pancytopenia four to five days after being administered a single bolus dose of Liposomal Amphotericin B (L-AMB). The diagnosis was clinical and management involved supportive care, and granulocyte-macrophage colony-stimulating factor (GM-CSF). AMB is an effective drug, but is also associated with numerous side effects. Physicians are well-versed with the more frequently seen adverse drug reactions and their management. However, pancytopenia, being a rare adverse reaction to AMB, is less known and can be easily overlooked. This case report aims to ensure that the physicians must be aware of such possibilities in the first place to make swift diagnoses and management. The condition itself is seemingly self-limiting, although GM-CSF may be needed in refractory cases. It’s true that few previous case reports have indicated pancytopenia in association with prolonged AMB exposure, but we believe certain conditions may predispose a patient to a more acute presentation - as seen in our case.

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Studies have shown that long-term AMB administration could indeed be associated with normochromic, normocytic anemia – likely due to low erythropoietin concentrations. But this patient developed symptoms within days after starting the treatment. With this case report, our goal is to discuss identifying the presentation, ruling out differential diagnoses, and offering possible explanation and treatment plan to manage this ADR. Precedential explanation of such rare side effects would allow a physician to act faster and improve the overall outcome. The patient’s due consent was taken for publishing this case report after explaining its significance and ensuring that no identifiable information shall be used.

Case Presentation

In March 2021, a 48-year-old male was brought to the apex public referral hospital of the most populous province in India with a history of high-grade fever, chills, and weakness for roughly 2 months. He has been a known case of hypertension for 6 years and is a non-vegetarian by diet. At the time of admission, the patient vitals were as follows: blood pressure 126/72 mmHg, pulse rate 88/min. General examination revealed pallor and pedal edema. Icterus and lymphadenopathy were not found. Respiratory, cardiovascular, and nervous system examination revealed bilateral vesicular breath sounds, normal first and second heart sounds (S1 and S2) with no audible murmur, and a Glasgow Coma Scale (GCS) score of 15. Abdominal examination revealed abdominal distension with the liver edge 5 cm and spleen 7–8 cm below the costal margin in the right and left mid-clavicular line, respectively.

(a) Investigations

On admission, routine blood and urine tests were performed and are depicted in the Table 1. The patient was found to be anemic with a hemoglobin level of 9.9 g/dL. His white blood cell (WBC) counts were also low – 1500/mm³ and the platelet count was 0.36 × 10⁹/mm³. The peripheral smear confirmed pancytopenia with normocytic normochromic red blood cells (RBCs). Direct microscopic examination of Giemsa stained thick and thin smears were negative for the malarial and filarial parasite. Tests for scrub typhus, filariasis and leptospirosis were also negative. The urinalysis revealed occasional pus cells; the culture was negative. An ultrasonography of abdomen was performed, which revealed mild hepatomegaly and moderate splenomegaly with minimal ascites. Bone marrow aspiration was performed and revealed normal hematopoiesis. The viral serology for hepatitis B, hepatitis C, and HIV came negative. The indirect Coombs test (ICT) was also negative. Based on the duration of illness, the constellation of presenting clinical symptoms (fever, hepatosplenomegaly, pancytopenia), and the endemicity, visceral leishmaniasis was suspected and a rK39 antigen test performed on day 2 came positive.

(b) Therapeutic Intervention

Once confirmed, the patient was started on the appropriate drug therapy. An injection of 650 mg bolus liposomal amphotericin B (L-AMB) was given stat. This led to a positive outcome as starting day 3, the patient became afebrile and the hemoglobin levels began to improve. Surprisingly, on day 4, the staff noticed a petechial and ecchymotic rash occurring on the patient’s whole body. On day 5, there was also a sudden dip in the hemoglobin levels (8.3 g/dL), along with lower WBCs (1100/mm³) and platelets (0.20 × 10⁹/mm³). Because of lack of any other symptoms (such as jaundice, hemoglobinuria, etc.), the temporal relationship between L-AMB administration and sudden deterioration, L-AMB-induced pancytopenia was suspected – a possible exacerbation of the pancytopenia initially caused by visceral leishmaniasis. L-AMB was not administered thereafter. The patient was managed in a supportive manner and was closely monitored for spontaneous improvement. On day 8, seeing a lack of improvement the patient was administered granulocyte–macrophage colony stimulating factor (GM–CSF) at dose 300 µg, once daily for three days. The counts improved drastically starting day 9, with hemoglobin reaching 10.1 g/dL, WBCs 2500/mm³ and platelets 0.65 × 10⁹/mm³.

(c) Outcome and Follow-Up

The patient was discharged on day 10 with full recovery. His vitals were stable. All the lab values were within normal ranges and his condition was normal during his follow-up visits.

Discussion

In this patient, the differential diagnoses considered include: aplastic anemia, malignancy, autoimmune disease, toxins, vitamin deficiencies, hypersplenism. Aplastic anemia and hematological malignancies were ruled out due to a normal bone marrow result. A negative Coombs’ test and normal vitamin B9 and B12 disqualified autoimmune etiology and vitamin deficiency, respectively. Splenomegaly was detected on abdominal examination and on abdominal ultrasonography but it regressed following treatment whereas the pancytopenia worsened.

AMB is a marvellous drug whose application has largely been limited by its ADR. The infusion-related reactions can be tamed well with antihistamine and antipyretic drugs. Some of the serious ADR, such as, nephrotoxicity, are controlled well with lipid formulations. These side effects are well known and expected, such that a physician tailors their treatment plan to counter-balance them. However, when it comes to the rarer category of ADR, even the diagnosis can be delayed due to the lack of pre-existing knowledge. This leads to faulty diagnosis, incorrect/delayed treatment, and extended duration of hospitalization. Pancytopenia is one such rare adverse effect. It is, therefore, imperative that the healthcare workers also be aware of previously reported hematological side effects of AMB and monitor for the same.
Previous studies have shown that most patients receiving AMB infusions may develop normocytic normochromic anemia with an 18–35% fall in hemoglobin levels.[2] However, the same studies suggest that this reaction was observed after prolonged administration of AMB (10 weeks or more). This could be a direct drug-induced toxic reaction causing suppression of erythrocytes or erythropoietin[3] or secondary to the nephrotoxicity associated with AMB. Nonetheless, the hematocrit bounces back to normal after several months once the therapy is discontinued.[3] There also have been remote cases of thrombocytopenia associated with AMB.[4,5] Some data suggest that this is a dose-dependent myelosuppression [anemia and thrombocytopenia when patients are receiving AMB at doses of >3.3 mg/kg/day and >3.0 mg/kg/day, respectively].[6]

So far, leukopenia has not been a consistent finding with AMB infusion. However, studies on *in vitro* and animal models suggest that AMB could cause suppression of human lymphocytic function while boosting the number of antibody-producing cells, the cell-mediated immunity, and the macrophagic phagocytic function.[7] A retrospective cohort study by Falci *et al.*[9] concluded that, overall, L-AMB (liposomal AMB) has a safer hematologic profile compared to AMB deoxycholate (d-AMB) and AMB lipid complex (ABLC). Nevertheless, Falci *et al.* also noted that previous hematological disease and the use of other myelotoxic drugs could be important cofactors for the development of this toxicity. Other high-risk factors to be considered include underlying renal disease, autoimmune disease, and so on.

Before hastily diagnosing AMB-induced pancytopenia in a patient, one must not forget that it could be due to some other underlying disease. For instance, visceral leishmaniasis itself presents with the main clinical symptoms of fever, hepatosplenomegaly, and pancytopenia.[10] The diagnosis is made by using a polymerase chain reaction (PCR) of peripheral blood or by direct detection of leishmania in bone marrow aspirates. Notably, from a hematological point of view, some frequent findings reported numerous times in the past are: anemia, leukopenia, thrombocytopenia, bicytopenia, pancytopenia, and high erythrocyte sedimentation rate (ESR).[11]

Table 1: The results of all the blood and urine investigations performed

| Parameter                  | Day 1 | Day 3 | Day 4 | Day 5 | Day 7 | Day 9 | Day 10 |
|----------------------------|-------|-------|-------|-------|-------|-------|--------|
| Hb (g/dL)                  | 9.9   | 9.4   | 9.8   | 8.3   | 8.9   | 10.1  | 10.5   |
| TLC (per mm³)              | 1500  | 1700  | 1400  | 1100  | 1200  | 2500  | 4700   |
| DLC                        | N₁₀₀ L₅₅ | N₁₂₀ L₄₅ | N₁₀₀ L₃₅ | N₁₀₀ L₃₅ | N₁₀₀ L₃₅ | N₁₀₀ L₃₅ | N₁₀₀ L₃₅ |
| PLT (10⁹ /mm³)             | 0.36  | 0.30  | 0.20  | 0.20  | 0.44  | 0.65  | 0.39   |
| MCV (per µm³)              | 79    | 77.5  | 78    | 77    | 79.1  | 81    | 85.1   |
| MCH (pg/cell)              | 27    | 27.9  | 28    | 27.3  | 27.4  | 27    | 28     |
| PT (sec)/INR               | 15.4/1.1 | 130   | 130   | 136   |       |       |        |
| Na                         | 130   | 2.6   |       |       |       |       |        |
| K                          | 6.7   | 3.7   |       |       |       |       |        |
| Mg                         | 1.79  |       |       |       |       |       |        |
| (mEq/L)                    |       |       |       |       |       |       |        |
| BUN (mg/dL)/Cr (mg/dL)     | 31/0.74 | 27.9/0.98 | 49/1.4 |       | 35/1.1 |       |        |
| Bilirubin (mg/dL)          |       |       |       |       |       |       |        |
| Total                      | 1.25  | 2.00  | 0.71  |       |       |       |        |
| Direct                     | 0.73  | 1.18  | 0.39  |       |       |       |        |
| SGOT                       | 35    | 109.4 | 55    |       |       |       |        |
| SGPT                       | 48    | 59.3  | 43    |       |       |       |        |
| ALP                        | 267   | 313.3 | 286   |       |       |       |        |
| (U/L)                      |       |       |       |       |       |       |        |
| s. Protein (g/dL)          | 5.9   | 5     |       |       |       |       |        |
| Total                      | 2.8   | 2.6   |       |       |       |       |        |
| TSH (mU/L)                 | 1.7   |       |       |       |       |       |        |
| T4                         | 3.85  |       |       |       |       |       |        |
| T3                         | 0.44  |       |       |       |       |       |        |
| Vit B12 (pg/mL)            | 183   |       |       |       |       |       |        |
| Vit B9 (ng/mL)             | 12.3  |       |       |       |       |       |        |
| LDH (U/L)                  | 356   |       |       |       |       |       |        |
| Uric acid (mg/dL)          | 2.5   |       |       |       |       |       |        |
| CRP                        | 29    |       |       |       |       |       |        |
| ESR (mm/h)                 | 59    |       |       |       |       |       |        |

Abbreviations: Hb=Hemoglobin; TLC=Total leukocyte count; DLC=Differential leukocyte count; PLT=Platelet count; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; PT=Prothrombin time; INR=International normalized ratio; BUN=Blood urea nitrogen; Cr=Creatinine; SGOT=Serum glutamic oxaloacetic acid; SGPT=Serum glutamic pyruvic acid; ALP=Alkaline phosphatase; TSH=Thyroid stimulating hormone; LDH=Lactate dehydrogenase; CRP=C reactive protein; ESR=Erythrocyte sedimentation rate. (Note: The parameters that came normal after first work-up for pancytopenia were not repeated, such as, LDH, s. Bilirubin, and so on)
Our patient already had pre-existing pancytopenia at presentation, possibly due to the visceral leishmaniasis, that seemingly exacerbated after L-AMB administration. We also noted that unlike previously reported cases, wherein pancytopenia occurred after prolonged exposure (weeks) to AMB, our patient developed pancytopenia within a few days of exposure. Again, we believe this is because the pre-existing pancytopenia must have rendered our patient to more vulnerable to the toxic effects of the drug. In the absence of other risk factors, exposures or symptoms that could point towards some other etiology, and the temporal relationship between drug administration and hematologic deterioration, the diagnosis of L-AMB-induced pancytopenia became most likely.

While we understand that there are no standard guidelines to address this complication, we recommend getting a pretreatment complete blood count (CBC), close monitoring following drug administration, and stopping the exposure to L-AMB plus supportive management if pancytopenia becomes evident. This will lead to improvement in most asymptomatic or mildly symptomatic cases. In case of no response, GM-CSF can help improve the cell count speedily. Regular follow-up of the patient is advisable.

Different countries have different treatment regimens for treatment of visceral leishmaniasis with AMB. In India, we favour a single dose of 10 mg/kg L-AMB. Knowing the possible serious side effects of AMB, it is important to also be acquainted with alternative treatment regimens for visceral leishmaniasis. The regimens preferred in India are mentioned in the Table 2 along with their common side effects. Our patient’s visceral leishmaniasis showed significant improvement following the single dose of L-AMB administered (as the patient became afibrile and splenomegalgy resolved). Hence, no further treatment was sought.

For all of us, the take-home points from this case we witnessed can be summarized as follows:

• Pancytopenia is a rare side effect of prolonged (weeks) AMB infusion.
• It can cause accelerated pancytopenia (within a few days) in patients with Leishmaniasis and other such conditions associated with reduced blood cell counts.
• It can resolve spontaneously following prompt diagnosis and drug withdrawal.

| Drug | Regimen | Side Effect |
|------|---------|-------------|
| Sodium stibogluconate/ meglumine antimoniate* | 20 mg/kg IV or IM, daily for 28-30 days | Arthralgia, myalgia, elevated serum aminotransferases, QT prolongation, chemical pancreatitis |
| Paromomycin | 11 mg/kg IM, daily for 21 days | Hepatotoxicity, reversible ototoxicity |
| Miltefosine | 50 mg (for adults) oral, twice daily for 28 days | Mild-moderate vomiting, diarrhoea |

*Even though sodium stibogluconate and meglumine antimoniate are popular worldwide, significant resistance is witnessed in Indian population.

GM–CSF administration can help improve blood cell counts in patients not improving with supportive care alone.

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**Patient’s consent**

Patient consent was not recorded since no identifiable information is present in the main manuscript. Complete anonymity is guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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