Confounding Case of Hemolysis in a Patient with Acute Leukemia

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CASE DESCRIPTION

A 12-year-old boy with refractory acute myeloid leukemia (AML)2 was transferred to our hospital for compassionate treatment with gemtuzumab ozogamicin (GO; MylotargTM). Before transfer, the patient had received 2 courses of salvage chemotherapy, after which bone marrow biopsy was negative for the disease. A plan for hematopoietic stem cell transplantation during his third cycle was halted because of disease recurrence.

On admission, the patient had leukemic infiltrates in his lungs, skin, distal esophagus, and gastric mucosa. He was profoundly immunocompromised, neutropenic, anemic, and thrombocytopenic. Despite persistent fever, daily blood cultures performed at an outside hospital and on admission at our hospital were negative. The patient was treated with a comprehensive panel of antibiotics without a significant change in his persistent fever.

A day after admission, the patient began a 15-day cycle of GO therapy. On day 5, the laboratory began receiving visibly hemolyzed specimens with increased hemolysis indices. Consequently, results from multiple biochemical tests were suppressed according to laboratory protocol (Table 1). The laboratory was contacted to help determine whether the hemolysis was in vivo or in vitro.

DISCUSSION

During in vivo hemolysis, hemoglobin is released and irreversibly captured by haptoglobin. The haptoglobin–hemoglobin complex exposes a neoepitope that is recognized by the transmembrane CD163 receptor expressed almost exclusively in cells of monocyte lineage (1). The CD163 receptor binds to the haptoglobin–hemoglobin complex at a high affinity and mediates the internalization and subsequent lysosomal degradation of the complex (2). Similarly, another transmembrane receptor, the low-density lipoprotein receptor-related protein, also called CD91, provides an efficient backup mechanism after plasma haptoglobin has been depleted by removing free heme released into the circulation (1). Hemopexin binds free heme circulating in the plasma. The CD91 receptor recognizes the heme–hemopexin complex and mediates the endocytosis of the complex into lysosomes of monocytes and macrophages.

Haptoglobin is an acute-phase protein synthesized primarily in the liver. The primary function of haptoglobin is to scavenge circulating free hemoglobin and ensure prompt clearance by monocytes/macrophages. In addition, the formation of the haptoglobin–hemoglobin complex prevents the peroxidation of hemoglobin and impairs the filtration of the relatively small hemoglobin molecule by the kidney, thereby protecting the kidney tubules from damage (1).

Certain conditions such as trauma, inflammation, intravascular hemolysis, and sickle cell anemia can lead to the saturation of a hemoglobin-scavenging mechanism (1). The half-life of the hemoglobin–haptoglobin complex is several minutes (2, 3). Therefore, because haptoglobin is not recycled, formation of a large amount of hemoglobin–haptoglobin complex rapidly depletes haptoglobin in the plasma. In severe in vivo hemolysis, haptoglobin is typically undetectable (3, 4).

Haptoglobin is often measured to differentiate in vivo from in vitro hemolysis. A suppressed or undetectable concentration of haptoglobin is an indicator of in vivo hemolysis, although it does not exclude coexisting in vitro hemolysis (3). Haptoglobin concentrations are unaffected by in vitro hemolysis. However, the interpreta-

QUESTIONS TO CONSIDER

1. What is the fate of free hemoglobin released in vivo?
2. What laboratory parameters are useful to distinguish in vivo from in vitro hemolysis?
3. Is this patient’s hemolysis likely in vivo or in vitro?
4. How is GO involved with hemoglobin and haptoglobin clearance?
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reference interval</th>
<th>Admission</th>
<th>Days after admission</th>
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<tr>
<td></td>
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<tr>
<td>K+, plasma, mmol/L</td>
<td>3.3-4.9</td>
<td>2.9</td>
<td>2.4</td>
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<td>K+, whole blood, mmol/L</td>
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<td>AST, U/L</td>
<td>10-50</td>
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<td>µmol/L</td>
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<tr>
<td>g/L</td>
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<td>hs-CRP, mg/L</td>
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<td>Ferritin, ng/mL</td>
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<tr>
<td>µg/L</td>
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<td>Hemoglobin, g/dL</td>
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<td>7.4</td>
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<td>g/L</td>
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<td>Hematocrit, %</td>
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<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Urine RBC</td>
<td>None/HPF</td>
<td>—</td>
<td>—</td>
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</table>

*a For simplicity, the final 33 days are grouped into weeks, and ranges of each test are shown for 7 days. GO treatment initiated on day 1 and completed on day 15.
*b Result suppressed owing to hemolysis.

| AST, aspartate aminotransferase; LDH, lactate dehydrogenase; hs-CRP, high sensitivity C-reactive protein; MCV, mean corpuscular volume; WBC, white blood cell; NRBC, nucleated red blood cell count; C, result reported with a “specimen hemolyzed” comment; abs, absolute; RBC, red blood cell; HPF, high power field.
tion of haptoglobin may be confounded by several other clinical factors that negatively or positively affect haptoglobin concentrations (3). For example, low concentrations of haptoglobin are observed in cases of liver cirrhosis, malnutrition, and congenital hypohaptoglobinemia. In contrast, increased haptoglobin is observed with inflammation, hypersplenism, megaloblastic anemia, and medications such as androgens and corticosteroids (2, 3). Because there is no specific test to distinguish in vivo from in vitro hemolysis, a combination of clinical and relevant laboratory evidence should be used when interpreting haptoglobin concentrations (3, 4).

Our patient was treated with GO, a CD33-targeting monoclonal antibody conjugated to a potent cytotoxin, calicheamicin (4). The CD33 receptor is expressed in >90% of patients with AML (4). The CD33 receptor mediates the endocytosis of the drug, after which the drug triggers apoptosis by inflicting DNA damage (4). GO initially received accelerated approval in 2000 for the treatment of older (>60 years of age) patients with CD33+ AML who experienced a relapse. After subsequent confirmatory trials, GO was withdrawn from the US market in 2010 because of safety concerns and a failure to demonstrate clinical benefit over standard treatment (4–6). However, it has been recently reintroduced to the US market for treatment of adults with newly diagnosed CD33+ AML and for patients ≥2 years of age with CD33+ AML who have experienced a relapse or proved refractory to standard treatment (6).

The primary concern in treatment with GO is venoocclusive disease of the liver (6). GO is also known to impair CD163-mediated clearance of free hemoglobin by eradicating cells that coexpress CD163 and CD33 (7). Maniecki et al. reported 2 cases of intravascular hemolysis weeks after the completion of GO therapy. These episodes were characterized by increased haptoglobin and free hemoglobin but normal potassium concentrations. A similar phenomenon of impaired CD163-mediated hemoglobin scavenging in a patient with an acute HIV-1 retroviral syndrome has also been reported (8). A persistent hemolytic-like episode characterized by increased hemolysis index, increased haptoglobin, and normal potassium has also been described in patients treated with chemically modified hemoglobin owing to a reduced or lack of affinity for haptoglobin (9).

Our patient experienced a similar phenomenon characterized by extended hemolysis, increased free plasma hemoglobin, and persistently increased hemolysis index in a setting of low to normal whole blood potassium (Fig. 1 and Table 1). A combination of increased hemolysis index, increased haptoglobin, and increased free plasma hemoglobin would normally suggest in vitro hemolysis. However, the presence of low to normal whole blood potassium and persistent (not sporadic) hemolysis argued against in vitro hemolysis. In addition, the persistent increase in aspartate aminotransferase and hemolysis index with low to normal plasma potassium was indicative of a chronic process rather than a random preanalytical error. The patient’s urine also revealed hemoglobinuria without noteworthy hematuria, which suggested intravascular hemolysis. A positive dipstick for blood without the presence of red blood cells occurs when the amount of free hemoglobin exceeds the concentration of available haptoglobin in the plasma (10). However, it should be noted that increased myoglobin in urine can lead to false positive results that may be confused with hemoglobinuria. Evaluation of creatine kinase and aldolase can help to discriminate between this potential interference and muscle damage in patients with hemoglobinuria.

Despite severe hemolysis, bilirubin concentrations remained within the reference interval throughout the course of the patient’s hospitalization, which is in agreement with the observation noted by Maniecki et al. (7). Typically, in vivo hemolysis is characterized by increased bilirubin. Bilirubin is a breakdown product of free hemoglobin and heme, a mechanism mediated by CD163 and CD91 receptors (2, 7). The CD91 receptor has been shown to coexpress with CD163 and CD33 in monocyte/macrophage cells (7). Thus, the depletion of monocytes/macrophages by the GO therapy may interfere with hemoglobin metabolism and attenuate bilirubin production.

Lactate dehydrogenase activity was markedly increased in the first 3 days after GO initiation (Table 1). Lactate dehydrogenase is a good hemolysis marker but lacks diagnostic specificity because it is a ubiquitous en-
zyme and can be increased in various forms of leukemia independent of hemolysis (10, 11). In addition, blood cell morphology evaluation revealed microcytosis and schistocytes that are commonly seen in intravascular hemolysis (7). Other markers of hemolysis such as hemoglobin concentration, hematocrit, and mean corpuscular volume were decreased. Nucleated red blood cell count was unremarkable, and reticulocyte count was not performed. However, in addition to a lack of diagnostic specificity, these parameters have limited utility when interpreted in the context of severely suppressed bone marrow.

Of note, the hemolytic episode began almost immediately after GO initiation, as opposed to the report described by Manieck et al. (7), and continued after chemotherapy was terminated. Monocytes and macrophages are the primary cells involved in the clearance of the hemoglobin–haptoglobin complex. Therefore, persistent bone marrow suppression due to chemoaiblation may explain why the hemolytic episode continued even after the chemotherapy was stopped (Table 1). It is unclear whether GO elicits hemolysis. A negative direct Coombs test and the presence of some schistocytes in our patient suggested that the cause of hemolysis was unlikely to be immune-mediated. A thorough review of the patient’s medical record did not reveal other potential causes of hemolysis, including G6PD deficiency, hemoglobinopathies, or coadministered drugs.

We report a case of prolonged in vivo hemolysis presumably caused by the eradication of CD163-expressing cells by GO therapy. We believe that the persistent and marked increase in haptoglobin concentration was because of, at least in part, impaired clearance of the hemoglobin–haptoglobin complex (Table 1). As an acute-phase reactant, it is also reasonable to argue that the haptoglobin was increased because of inflammation, especially with markedly increased C-reactive protein and ferritin concentrations (Table 1). However, Kormoczi et al. argued that haptoglobin is a reliable marker of in vivo hemolysis, even in the presence of concomitant inflammation (3). This study showed that most patients with hemolysis and concomitant acute-phase reaction had suppressed or undetectable haptoglobin. This observation is not consistent with increased haptoglobin concentrations detected throughout our patient’s clinical course.

The present report serves not only to bring to light a case of a prolonged hemolytic episode of a relatively underrecognized mechanism (7–9) but also to highlight the importance of combining clinical and relevant laboratory evidence when interpreting haptoglobin concentrations (2, 3). GO therapy is likely to be a new standard of care for CD33+ AML patients (4, 6, 7).

### Points to Remember

- During in vivo hemolysis, circulating free hemoglobin is cleared from blood by forming a complex with haptoglobin, which is then internalized by cells of monocyte/macrophage lineage via the CD163 receptor.
- There is no single laboratory test to distinguish in vivo from in vitro hemolysis. In confusing cases, multiple markers of hemolysis should be used to differentiate in vivo from in vitro hemolysis.
- Decreased haptoglobin concentration is an indicator of in vivo hemolysis. However, results should be interpreted along with clinical and relevant laboratory findings.
- Haptoglobin concentrations can be increased owing to inflammation, hypersplenism, megaloblastic anemia, or medications such as androgens and corticosteroids. Decreased haptoglobin concentrations are observed in those with liver cirrhosis, malnutrition, and congenital hypohaptoglobinemia.

### CASE FOLLOW-UP

The patient remained critically ill and profoundly immunocompromised, pancytopenic, and anemic during his hospitalization. The patient’s course was complicated by prolonged sepsis, multiorgan failure including acute kidney injury requiring continuous renal replacement therapy, persistent methemoglobinemia, and hypertension. He also developed persistent vancomycin-resistant Enterococcus faecium and Trichosporon asahii infections during the remainder of his hospitalization. Because of the persistent infection, he did not qualify for bone marrow transplantation or further chemotherapy. The patient did not recover from the hemolytic incidence and died 40 days after GO initiation.

GO impairs the clearance of hemoglobin–haptoglobin complex by eradicating CD163/CD33 coexpressing cells, prolonging haptoglobin clearance and confounding the assessment of hemolysis detected during laboratory testing.

### Author Contributions

All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

### Authors’ Disclosures or Potential Conflicts of Interest

Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

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References


Commentary

Alina G. Sofronescu*

Unfortunately, there is no specific laboratory test available that can fully differentiate between in vivo and in vitro hemolysis. Often underestimated in frequency, in vitro hemolysis is a preanalytical artifact occurring during specimen collection and/or processing. In contrast, in vivo hemolysis is a pathologic process with a multitude of causes, requiring clinical and laboratory investigations and, depending on severity, medical intervention.

Increased hemolysis index and K⁺⁺, in the absence of symptoms, is pathognomonic for in vitro hemolysis. On the other hand, increased lactic acid dehydrogenase together with depletion of haptoglobin and increased total and direct bilirubin, as well as anemia and reticulocytosis, are good indicators of in vivo hemolysis (1). Furthermore, administered drugs can induce hemolysis or can interfere with the process of clearance of free hemoglobin post hemolysis. In these circumstances, a patient’s list of medications and the side effects of each should be carefully reviewed.

Gemtuzumab ozogamicin (GO), a potent chemotherapy drug, is often used for the treatment of patients with acute myeloid leukemia. It is known that GO impairs the clearance of hemoglobin–haptoglobin complexes post hemolysis by targeting CD 163—expressing macrophages and interfering with their role in the process of scavenging and clearance of the resulting hemoglobin complexes (2). Therefore, haptoglobin concentrations in patients treated with GO can be deceiving, and prolonged hemolytic episodes can be masqueraded by normal/increased haptoglobin values concurrent with normal K⁺⁺ concentrations. In these circumstances, haptoglobin laboratory results should be interpreted with caution, and the possibility of hemolysis should not be excluded even in the context of normal haptoglobin.

With the likely increase in the utilization of GO as part of the clinical management for patients with acute myeloid leukemia, these kinds of challenging laboratory results might be seen more often in practice. Therefore, a deep understanding of the hemolytic mechanism and its laboratory assessment becomes more important than ever.

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