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CASE REPORT

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The influence of methyldopa on immunohematological tests during pregnancy

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Summary

Over the years, it has been proven that methyldopa can cause production of autoantibodies against red blood cells leading to immune hemolytic anemia. Currently, methyldopa is used to treat gestational hypertension due to demonstrated safety for use during pregnancy and multiple clinical trials. Positive antiglobulin tests observed in some pregnant women taking methyldopa.

Keywords: methyldopa, drug-induced hemolytic anemia, antiglobulin test, hypertension in pregnancy

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Introduction

Drug-induced immune hemolytic anemia (DIIHA) is considered rare and is often confused with autoimmune hemolytic anemia (AIHA). The incidence of related to drug use has been estimated at 1 case per 1,000,000. In comparison, AIHA occurs in approximately 1 in 80,000 individuals in the general population. The number of drugs and the hypotheses regarding mechanisms involved in drug-induced immune hemolytic anemias have evolved over the past few decades [1]. Currently, second- and third-generation cephalosporins are primarily recognized as causes of drug-related hemolytic anemia [2]. In the 1970s, methyldopa was the most commonly associated drug with hemolytic anemia [3]. It has been shown to be safe during pregnancy and is currently used to treat gestational hypertension [4].

Hypertension during pregnancy is a significant condition that poses risks to both maternal and fetal health, impacting approximately 6–10% of pregnant women [5]. Its rising incidence is being linked to factors such as older maternal age [6], obesity, diabetes, dyslipidemia and genetic predisposition. The involvement of genes related to coagulation pathways and endothelial functions, as well as dysregulation of the renin-angiotensin-aldosterone system, has been suggested [7–9]. According to current guidelines, hypertension in pregnancy is diagnosed when systolic blood pressure (SBP) is ≥ 140 mm Hg and/or diastolic blood pressure (DBP) is ≥ 90 mm Hg [5, 7, 10]. The choice of antihypertensive medication during pregnancy is influenced by disease severity and drug efficacy, but primarily, the selected medication should be safe for both the mother and the developing fetus. The selection of antihypertensive drugs is not straightforward due to the limited number of clinical studies [6]. Methyldopa, having the most extensive safety data [11], remains a first-line medication that can be administered as monotherapy or, depending on

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the patient's clinical condition, in combination with other drugs [5, 7, 10].

Drug-induced immune hemolytic anemia (DIIHA) arises from interactions between the drug, antibodies, and components of the erythrocyte membrane [3]. The *in vivo* mechanisms by which drugs interact with erythrocyte antigens remain poorly understood, and it is likely that more than one mechanism is involved in drug-induced hemolysis [2]. Several immunological mechanisms contribute to red blood cell destruction [11]. Antibodies responsible for drug-induced DIIHA can be classified as either drug-dependent or drug--independent. Drug-dependent antibodies react *in vitro* only in the presence of the drug e.g., with cefotetan and ceftriaxone [2]. Two mechanisms have been identified for drug-dependent antibodies: the hapten mechanism and immune complex formation [12]. Drug-independent antibodies are reactive *in vitro* without any drug presence. The mechanisms through which drugs interact with erythrocyte antigens are still not fully understood, and it remains unknown why and how particular drugs can influence the immune system, leading to the production of autoantibodies. It is suggested that some drugs (e.g., methyldopa, fludarabine) support autoantibody production through molecular mimicry, drug adsorption that alters erythrocyte antigens, or immune system dysregulation [2]. Serological test results for DIIHA are typically consistent with warm-type autoimmune hemolytic anemia (AIHA).

Among the many drugs known to induce autoantibody production, methyldopa is the most thoroughly studied [3]. It is an antihypertensive drug with a central mechanism of action [13]. The reduction in blood pressure is primarily achieved by decreasing peripheral vascular resistance, with varying effects on cardiac function [14]. It is estimated that approximately 10–20% of patients treated with methyldopa develop autoantibodies, and around 1% of these patients develop autoimmune hemolytic anemia [3]. In such cases, the hemolysis is always extravascular and mediated by IgG antibodies [4]. Methyldopa-induced autoantibodies typically appear between 4 months and 1 year after the initiation of the therapy. In patients who developed methyldopa-induced hemolysis, discontinuation of the drug generally leads to resolution of hemolysis [3]. Methyldopa-induced hemolytic anemia is serologically indistinguishable from warm-type autoimmune hemolytic anemia (AIHA). Laboratory findings include a positive direct antiglobulin test (DAT), a positive indirect antiglobulin test (IAT), and a reactive eluate from the tested red blood cells. Hematological improvement following the discontinuation of methyldopa confirms the diagnosis of drug-induced immune hemolytic anemia (DIIHA). Despite the similarity to warm-type AIHA, the mechanisms underlying methyldopa-induced DIIHA are more complex and heterogeneous [2].

Presently, methyldopa is used primarily for treating hypertension in pregnant women. Diagnosing drug-induced immune hemolytic anemia (DIIHA) during pregnancy is challenging because of the presence of physiological anemia that commonly occurs in pregnancy [4]. Methyldopa--induced DIIHA lacks typical features, and symptoms can range from mild fatigue to shortness of breath, respiratory failure, and eventually death if not treated [2]. Methyldopa-induced immune hemolytic anemia is rare during pregnancy, with only a few cases reported to date.

The likelihood of immune alloantibody production in pregnant women is higher due to the possibility of alloimmunization associated with pregnancy and childbirth. The immune response depends on several factors, including genetic predisposition, red blood cell phenotype, antigen immunogenicity, as well as the dose and structure of the antigen on the erythrocytes. Immune alloantibodies in pregnant women can cause Hemolytic Disease of the Fetus and Newborn (HDFN), which is why screening tests are performed to enable early detection of antibodies [15, 16].

The clinical significance of antibodies against red blood cell antigens relies on:

— the reaction temperature (clinically relevant antibodies are active at 37°C),
— specificity,
— the antibody class and IgG subclasses,
— complement activation capability.

According to current guidelines, a screening test for immune alloantibodies must be performed on every pregnant woman, regardless of whether she is RhD negative or positive. Detailed information on the frequency and scope of immunohematological testing in pregnant women is provided in the Announcement of the Minister of Health dated March 30, 2021, regarding the standards of good practice for blood collection and its components, testing, preparation, storage, issuance, and transportation for public blood service organizations [17].

When antibodies are detected in an indirect antiglobulin test (IAT) during pregnancy, their identification is necessary, especially for alloantibodies active at 37°C. Monitoring the antibody titer

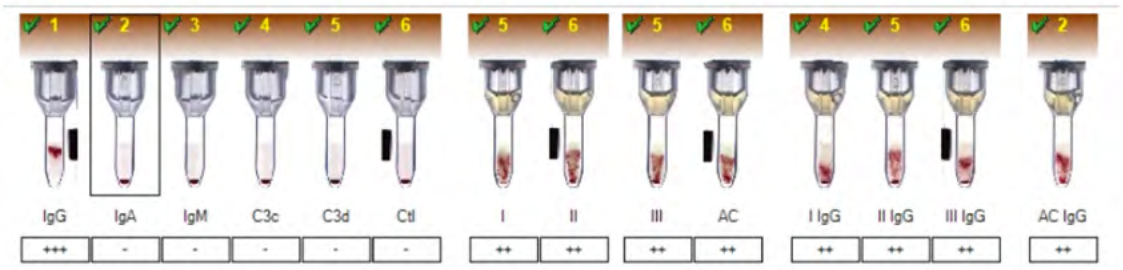

Figure 1. Results of red blood cell testing in the DAT and serum testing in the IAT for the first patient

and its progression is also essential, comparing each new sample with a previously frozen serum sample. If alloantibodies are detected, and the autocontrol is positive, a direct antiglobulin test (DAT) is performed. This test detects antibodies and/or complement components bound *in vivo* to red blood cells. It is used in diagnosing hemolytic disease of the fetus and newborn, hemolytic transfusion reactions, and autoimmune hemolytic anemia [16, 18].

Case reports

This study presents two cases of pregnant women undergoing treatment for hypertension, in whom antibodies were detected.

Case 1

A 31-year-old woman was referred for antibody consultation at the 12th week of her first pregnancy due to a positive indirect antiglobulin test (IAT). Positive reactions were observed with all screening red blood cells, along with a positive autocontrol and a direct antiglobulin test (DAT) with a result of 3+ (Fig. 1). An elution which was performed on the tested red blood cells confirmed the presence of IgG autoantibodies (Fig. 2). The presence of immune alloantibodies was excluded through alloadsorption (Fig. 3). During a follow-up phone consultation, it was revealed that the patient had been taking methyldopa. The patient had no history of anemia or other hematological issues.

Case 2

A 37-year-old woman was referred for consultation due to positive IAT reactions. Antibody screening detected immune antibodies with anti-M specificity from the MNS system and a positive DAT (2+) (Fig. 4). Elution from red blood cells confirmed the presence of IgG autoantibodies. In the test request, the patient included information about the medications she was taking (*inter alia* methyldopa).

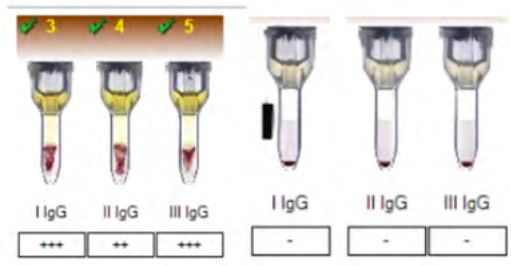

Figure 2. Results of eluate and supernatant testing for the first patient

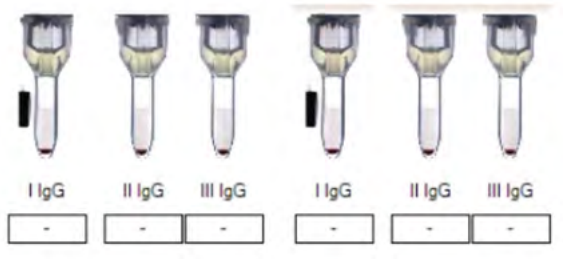

Figure 3. Results of serum testing after alloadsorption for the first patient

Discussion

Charalampos Grigoriadis et al. [4] described a case of autoimmune hemolytic anemia dependent on methyldopa in a woman during her third trimester of pregnancy. The patient was admitted at 37 weeks of pregnancy for a planned caesarean section. Her medical history included hypertension treated with methyldopa at a dosage of 500 mg twice daily since the 24th week of pregnancy. Laboratory tests revealed anemia, a decrease in lymphocyte count, and reticulocytosis. The physical examination of the pregnant woman indicated easy fatigability, tachycardia, and pallor. A decline in hemoglobin with no signs of bleeding from the surgical site was noted postpartum. Over the next three days, a further decrease in hemoglobin was observed. The direct antiglobulin test was strongly

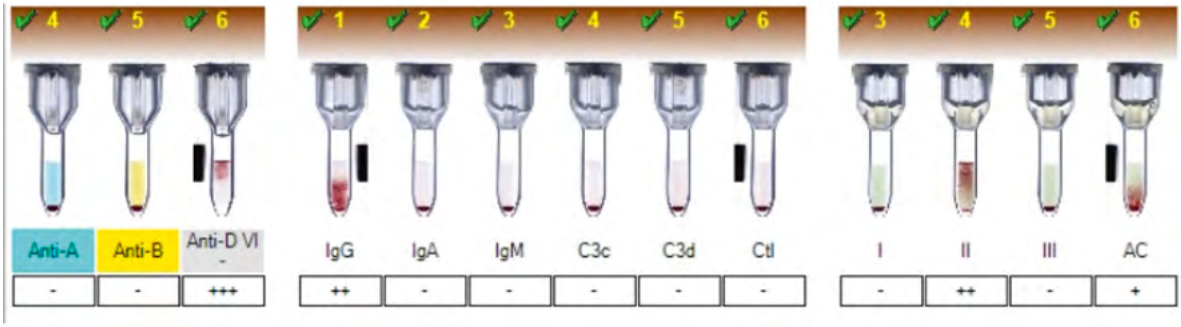

Figure 4. Overview of the test results for the second patient (in sequence): abbreviated blood group, red blood cell count in DAT, and serum analysis in IAT

positive (IgG 3+). Methyldopa was immediately discontinued, and the hemoglobin level returned to normal level one month after, despite the DAT remaining positive. The physical examination of the newborn showed no abnormalities, and no signs of hemolysis were noted throughout the infant's hospital stay.

However, Ozdemir et al. [19] reported a case of a newborn with jaundice and a positive direct antiglobulin test, without signs of hemolysis, whose mother was treated with methyldopa during pregnancy. Blood group incompatibility between the mother and the newborn was also excluded. The mother was taking methyldopa at a dosage of 250 mg twice daily. The physical examination of the newborn was normal, except for the presence of jaundice. Blood tests revealed a positive DAT in the newborn, as well as a positive DAT and a positive serum analysis in the mother. Neither the newborn nor the mother showed signs of hemolysis in the blood smear. By the ninth week postpartum, the newborn's DAT was negative, while the mother's was still positive.

The ongoing argument surrounding why some patients taking methyldopa develop autoantibodies to red blood cells, while most patients with a positive DAT do not exhibit hemolysis, remains unresolved [18]. Pregnant women represent a unique patient population, making accurate diagnosis crucial. Distinguishing warm autoimmune hemolytic anemia (AIHA) from drug-induced immune hemolytic anemia (DIIHA) is both challenging and time-consuming. Typically, a consistent serological investigation combined with a thorough clinical history is sufficient for an accurate diagnosis [2]. However, in obstetric settings, this may not always be feasible due to the urgency of procedures in certain cases, emphasizing the importance of a detailed clinical history [4].

Conclusions

1. Medications can influence the results of immunohematological tests.
2. Methyldopa induces the production of autoantibodies that react with standard red blood cells in serum analysis, presenting a picture similar to autoimmune hemolytic anemia (AIHA).
3. Information regarding medications taken is crucial for the diagnostic process. The absence of a thorough medical history impairs and prolongs the diagnosis.
4. Interferences caused by methyldopa delay the reporting of antibody results and complicate blood selection.

Conflict of interest: none declared.

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