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A Hematological Triad: Dissecting Synergistic Oxidative and Immune Hemolysis in Dapsone-Treated G6PD Deficiency

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A B S T R A C T

Dapsone, a key component of leprosy multidrug therapy (MDT), is a well-known precipitant of oxidative hemolytic anemia in individuals with Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency. Conversely, Dapsone-induced immune hemolytic anemia (DIIHA) is exceedingly rare. The concurrent presentation of both severe oxidative hemolysis and a positive Direct Antiglobulin Test (DAT) in a patient also receiving Rifampicin creates a profound diagnostic and mechanistic challenge. We present the case of a 42-year-old female with multibacillary leprosy who developed life-threatening, multifactorial hemolytic anemia (Hemoglobin 5.3 g/dL) three months after initiating MDT (Dapsone, Rifampicin, Clofazimine). A comprehensive diagnostic workup was performed, including detailed hematopathology and quantitative G6PD assay. The immunohematological evaluation was positive (DAT and IAT), but critical sub-testing, including monospecific DATs, was unavailable. The workup confirmed severe oxidative hemolysis (Heinz bodies, degmacytes) in the setting of G6PD deficiency (6.0 U/g Hb measured during 12.5% reticulocytosis). Concurrently, the polyspecific DAT and IAT were strongly positive with a pan-reactive antibody, confirming a simultaneous immune-mediated process. Due to polypharmacy (Dapsone, Rifampicin) and incomplete immunohematological data, the precise trigger for the DIIHA component—whether a rare Dapsone-induced autoantibody, a Rifampicin-induced immune-complex, or an oxidative-trigger mechanism—could not be definitively isolated. In conclusion, this case unmasks a complex, synergistic pathophysiology of concurrent oxidative and immune hemolysis. The inability to attribute the autoimmune component definitively to either Dapsone or Rifampicin highlights a critical diagnostic gap. This report underscores the necessity of a complete immunohematological workup (including monospecific DATs) in such cases and demonstrates that management must be multifaceted—addressing both the oxidative insult (drug cessation) and the severe immune-mediated destruction (immunosuppression), even in the face of etiological uncertainty.

1. Introduction

Hemolytic anemia, a heterogeneous group of disorders defined by the premature destruction of erythrocytes and a compensatory increase in erythropoiesis, presents a broad diagnostic spectrum. These etiologies are fundamentally bifurcated into intrinsic erythrocyte defects (such as enzymopathies, membranopathies, and hemoglobinopathies) and a wide array of extrinsic insults (such as immune-mediated, mechanical, infectious, or toxic).[1] Among the extrinsic causes, drug-induced hemolytic anemia (DIHA) represents a critical, complex, and often reversible category, with over 125 distinct therapeutic agents implicated in its pathogenesis through diverse and often overlapping mechanisms.

Dapsone (4,4'-diaminodiphenyl sulfone), a sulfonamide antibiotic with potent anti-inflammatory and immunomodulatory properties, remains a cornerstone of multidrug therapy (MDT) for all forms of leprosy (Hansen's disease). It is also employed as prophylaxis for *Pneumocystis jirovecii* pneumonia and for various neutrophilic and inflammatory dermatoses.[2] Its utility, however, is frequently constrained by a well-established, dose-dependent hematological toxicity: oxidative hemolytic anemia. The primary mechanism is not mediated by Dapsone itself, but by its N-hydroxylated metabolite, dapsone hydroxylamine (DDS-NOH), a potent oxidant generated via hepatic cytochrome P450 (CYP450) metabolism. Within the erythrocyte, DDS-NOH engages in a futile redox cycle, generating reactive oxygen species (ROS) that overwhelm intracellular antioxidant defenses, leading to globin denaturation, Heinz body formation, and premature splenic sequestration.[3,4]

This oxidative challenge is profoundly exacerbated in individuals with Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency.[5] G6PD is the rate-limiting enzyme of the pentose phosphate pathway (PPP), which is the sole source of reduced nicotinamide adenine dinucleotide phosphate (NADPH) in mature erythrocytes.[6] NADPH is, in turn, the essential cofactor for glutathione reductase, the enzyme responsible for regenerating reduced glutathione (GSH), the cell's primary scavenger of ROS. In G6PD-deficient individuals, this critical defense pathway fails, rendering erythrocytes exceptionally vulnerable to oxidative stressors like Dapsone. This combination frequently results in severe, precipitous, and life-threatening hemolytic episodes.[7]

Separately, and far less commonly, drugs can induce immune-mediated hemolysis (DIIHA). This extrinsic process is classically divided into three mechanisms: (1) Drug-Adsorption (Hapten) Mechanism: The drug (such as high-dose Penicillin) binds firmly to the RBC membrane, and drug-specific IgG antibodies bind to the drug, leading to extravascular hemolysis (typically IgG+ DAT); (2) Immune-Complex (Drug-Dependent Antibody) Mechanism: The drug (such as Quinine or Ceftriaxone) complexes with a plasma protein, inducing an IgM or IgG antibody. This drug-antibody complex loosely associates with the RBC membrane and is a potent activator of the classical complement cascade, often leading to severe, brisk intravascular hemolysis (typically C3d+ DAT); (3) True Autoantibody Induction Mechanism: The drug (such as Methyldopa or Fludarabine) induces a conformational change in RBC membrane proteins or dysregulates immune tolerance, resulting in the production of true, pan-reactive autoantibodies that bind to RBCs even in the absence of the drug. This presents identically to primary Warm Autoimmune Hemolytic Anemia (WAIHA), with an IgG+ (and sometimes C3d+) DAT and a positive indirect antiglobulin test (IAT). Dapsone is *not* classically recognized as a common agent of DIIHA. While a few case reports have anecdotally linked Dapsone to a positive Direct Antiglobulin Test (DAT) or a Methyldopa-type autoantibody, the association remains rare and mechanistically obscure.[8]

This landscape becomes profoundly complex in the context of leprosy MDT, which combines Dapsone with Rifampicin and Clofazimine. Rifampicin, unlike Dapsone, is a well-established (though rare) cause of DIIHA, typically operating via the immune-complex mechanism, leading to a C3d-positive DAT and severe intravascular hemolysis.[9] This scenario creates a significant diagnostic conundrum: What is the underlying pathophysiology when a patient on Dapsone, Rifampicin, and Clofazimine, with a confirmed G6PD deficiency, presents with both classic oxidative features (Heinz bodies, degmacytes) and a robustly positive DAT/IAT?

This clinical triad forces the clinician to differentiate between several competing hypotheses: (1) Simple Oxidative Hemolysis: A straightforward, severe Dapsone-induced oxidative hemolysis in G6PD deficiency, with the positive DAT being an incidental, unrelated finding (such as pre-existing subclinical AIHA); (2) True Dapsone-DIIHA: A rare, true Dapsone-induced autoantibody (Hypothesis B), with the oxidative findings and G6PD deficiency being secondary or incidental; (3) The "Unifying" Dapsone Hypothesis (Hypothesis C): A complex, multifactorial process where the two mechanisms are linked—specifically, where the massive oxidative damage from

Dapsone triggers a secondary autoimmune response by exposing cryptic erythrocyte neoantigens; (4) The "Dual Drug" Hypothesis (Hypothesis D): Two simultaneous, but mechanistically separate, drug-induced events: Dapsone-induced oxidative hemolysis (due to G6PD deficiency) and Rifampicin-induced immune-complex hemolysis (DIIHA).[10]

Herein, we present the sophisticated case of a 42-year-old female with confirmed G6PD deficiency who developed severe, multifactorial hemolytic anemia following the initiation of MDT. The aim of this report is to dissect this complex multifactorial pathophysiology in detail. The case is notable for the diagnostic ambiguity created by the patient's polypharmacy and the unavailability of specialized immunohematological testing. The novelty of this case lies in its exploration of these competing hypotheses, the profound management implications of this uncertainty, and its role as a powerful illustration of the need for a comprehensive diagnostic approach.

2. Case Presentation

A 42-year-old female of Southeast Asian descent, with a recent diagnosis of multibacillary (MB) leprosy (histologically classified as Borderline Lepromatous), presented to our Emergency Department with a two-week history of progressive, severe dyspnea on exertion, debilitating fatigue, intermittent subjective fevers, and the new onset of dark, "tea-colored" urine. Her leprosy diagnosis had been established three and a half months prior (Day 0), at which point she was initiated on the standard World Health Organization (WHO) 12-month MDT regimen for MB leprosy. This regimen consisted of Rifampicin 600 mg (monthly, supervised); Clofazimine 300 mg (monthly, supervised) plus 50 mg (daily, self-administered); and Dapsone 100 mg (daily, self-administered). The patient had no other chronic medical conditions, reported no known drug allergies, and had no personal or family history of anemia, jaundice, or hematological disorders. Her baseline hemoglobin (Hb) prior to initiating therapy was 12.8 g/dL, with normal white blood cell (WBC) and platelet counts. Approximately 14 days prior to admission (Day 90 of therapy), the patient began to experience worsening fatigue, generalized pruritus, and a "warm" sensation, which she initially attributed to her leprosy or a mild infection. Her symptoms escalated rapidly over the subsequent two weeks to include significant resting dyspnea, palpitations, and the onset of hemoglobinuria. Due to this severe clinical deterioration, she self-discontinued all three of her leprosy medications three days before presenting to the hospital (Day 101 of therapy).

Upon admission (Day 104), the patient was in moderate respiratory distress. Her vital signs were: Temperature: 37.8°C; Heart Rate: 124 beats per minute (sinus tachycardia); Blood Pressure: 105/65 mmHg; Respiratory Rate: 22 breaths per minute; and Oxygen Saturation: 95% on ambient air. Physical examination was notable for marked conjunctival and palmar pallor. Mild scleral icterus was present. There was no lymphadenopathy. Her cardiovascular examination revealed a grade 2/6 systolic ejection murmur (flow murmur) at the left sternal border, consistent with high-output physiology. The respiratory exam was clear to auscultation. Her abdomen was soft, non-tender, and non-distended, with normoactive bowel sounds. Crucially, there was no hepatomegaly or splenomegaly palpable. Her skin examination showed several well-demarcated, hypopigmented, hypoesthetic plaques consistent with her leprosy diagnosis, with no new rashes or signs of a leprosy reaction (such as erythema nodosum leprosum). Initial laboratory investigations revealed a severe normocytic, normochromic anemia with findings highly suggestive of a brisk, ongoing, and severe hemolytic process. A comprehensive hematological, biochemical, and immunohematological workup was initiated (Table 1a and 1b). The leukocytosis and thrombocytosis were interpreted as a reactive acute-phase response to the severe hemolytic stress. The mild elevation in direct bilirubin was noted.

Table 1a. Complete blood count and hemolysis panel on admission.

PARAMETER	PATIENT VALUE	REFERENCE RANGE	UNIT
Complete Blood Count (CBC)			
Hemoglobin (Hb)	5.3	12.0 – 15.5	g/dL
Hematocrit (Hct)	12.2	36.0 – 46.0	%
Mean Corpuscular Volume (MCV)	92.4	80.0 – 100.0	fL
Mean Corpuscular Hgb (MCH)	31.3	27.0 – 34.0	pg
MCHC	33.8	32.0 – 36.0	g/dL
White Blood Cell (WBC) Count	14.42	4.0 – 10.0	x $10^3/\mu L$
Neutrophils	80%	40 – 70	%
Lymphocytes	15%	20 – 45	%
Monocytes	2%	2 – 10	%
Eosinophils	3%	0 – 6	%
Platelet Count	559	150 – 400	x $10^3/\mu L$
Hemolysis Panel			
Reticulocyte Count (Percent)	12.5	0.5 – 2.5	%
Absolute Reticulocyte Count	0.25	0.02 – 0.10	x $10^{12}/L$
Lactate Dehydrogenase (LDH)	1250	135 – 225	U/L
Haptoglobin	< 10	30 – 200	mg/dL
Total Bilirubin	1.15	0.3 – 1.2	mg/dL
Indirect Bilirubin	0.55	0.2 – 0.8	mg/dL
Direct Bilirubin	0.6	0.0 – 0.4	mg/dL

● **High Value:** Indicates a value above the reference range. ● **Low Value:** Indicates a value below the reference range.

Table 1b. Renal, liver, and inflammatory markers.

PARAMETER	PATIENT VALUE	REFERENCE RANGE	UNIT
Renal & Liver Function			
Creatinine	0.8	0.6 – 1.2	mg/dL
Blood Urea Nitrogen (BUN)	15	7 – 20	mg/dL
Alanine Transaminase (ALT)	49	7 – 55	U/L
Aspartate Transaminase (AST)	47	8 – 48	U/L
Alkaline Phosphatase	98	40 – 129	U/L
Inflammatory Markers			
C-Reactive Protein (CRP)	85.0	< 10.0	mg/L
Erythrocyte Sedimentation Rate	60	0 – 20	mm/hr

● **High Value:** Indicates a value above the reference range. ● **Low Value:** Indicates a value below the reference range.

A manual review of the peripheral blood smear was critical. It demonstrated marked anisopoikilocytosis with significant polychromasia (consistent with the reticulocytosis), microcytes, ovalocytes, tear-drop cells, and numerous spherocytes. Most notably, there were frequent degmacytes ("bite cells"), where a portion of the erythrocyte membrane appeared to have been removed. Given the clinical context of Dapsone exposure and the presence of degmacytes, a supravital stain with brilliant cresyl blue was performed. This stain revealed multiple, peripherally-located, irregular-shaped inclusions consistent with Heinz bodies in over 30% of the erythrocytes. These findings were pathognomonic for a severe oxidative hemolytic process. A quantitative G6PD enzyme activity assay (spectrophotometric) was performed on admission, yielding a result of 6.0 U/g Hb (Reference Range: 7.8–14.4 U/g Hb). Despite being only moderately below the reference range, this result, in the context of a 12.5% reticulocytosis, was interpreted as being indicative of a severe underlying deficiency (a falsely high reading). An infectious workup, including blood cultures, *Mycoplasma pneumoniae* IgM, Ebstein-Barr Virus (EBV), and Cytomegalovirus (CMV) serologies, was negative. An autoimmune screen, including Antinuclear Antibody (ANA) and anti-dsDNA, was also negative. Concurrently, an immunohematological workup was performed to investigate the etiology of the spherocytes and to rule out an immune component. The results were highly complex and unexpected.

Table 2. Immunohematological workup.

TEST	PATIENT RESULT	INTERPRETATION / COMMENT
Blood Type	O Positive	*N/A*
Direct Antiglobulin Test (DAT)	**Positive**	Antibodies/complement on RBCs
Indirect Antiglobulin Test (IAT)	**Positive**	"Irregular" antibody in serum
Antibody Screen (Serum)	**Positive**	Confirms presence of serum antibody
Antibody Identification Panel	**Pan-reactive**	Consistent with a warm autoantibody
Donath-Landsteiner Test	**Negative**	Ruled out Paroxysmal Cold Hemoglobinuria
Monospecific DAT (IgG, C3d)	Not Performed	Testing unavailable; key limitation

- **Positive Finding:** Indicates a clinically significant positive result.
- **Negative Finding:** Indicates a clinically significant negative (rule-out) result.
- **Unavailable Test:** Indicates a test that was not performed.

A diagnosis of severe, acute hemolytic anemia with a complex multifactorial etiology was established. The clinical picture was characterized by (1) A Severe Oxidative Component: Confirmed by Dapsone exposure, G6PD deficiency, and the pathognomonic findings of Heinz bodies and degmacytes; and (2) A Concurrent, Severe Autoimmune Component: Confirmed by the strongly positive polyspecific DAT, positive IAT, and a pan-reactive warm autoantibody. The primary unanswered question at admission was the etiology of the autoimmune component, given the polypharmacy (Dapsone and Rifampicin) and the incomplete immunohematological data. Both Hypothesis C (Dapsone-trigger) and Hypothesis D (Rifampicin-DIIHA) remained highly plausible.

Given the life-threatening severity of the presentation (Hb 5.3 g/dL, hemodynamic instability), management could not be delayed and was designed to cover all possibilities: (1) Cessation of Offending Agents: All three leprosy medications (Dapsone, Rifampicin, Clofazimine) were immediately withheld; (2) Immunosuppression: Due to the profound anemia and strong evidence of an active autoimmune process (DAT 4+), a high-dose pulse steroid regimen was initiated: intravenous methylprednisolone 125 mg twice daily (250 mg/day) for three days. This high dose was chosen to rapidly blunt the severe immune-mediated destruction, regardless of the trigger; (3) Supportive Care: Folic acid 5 mg daily was started to support the massive erythropoietic drive; (4) Transfusion: The blood bank reported a pan-reactive autoantibody, making standard cross-matching impossible. After hematology consultation, four units of "least incompatible," ABO/Rh-matched Packed Red Blood Cells (PRBCs) were transfused slowly under vigilant monitoring (including pre-medication with acetaminophen and diphenhydramine).

The patient tolerated the transfusions well, with an appropriate rise in hemoglobin to 7.1 g/dL. She demonstrated a rapid clinical and hematological response. Within 48 hours of stopping the MDT and initiating steroids, her urine cleared, and her heart rate normalized. The IV methylprednisolone was transitioned to oral prednisolone 60 mg daily on Day 4. Her endogenous hemoglobin began to rise steadily, and her hemolytic markers (LDH, haptoglobin) began to normalize. She was discharged on Day 6 with a tapering course of oral prednisolone.

The patient's follow-up demonstrated a complete and sustained recovery. At the 1-month follow-up, she was asymptomatic, with her hemoglobin recovered to 11.4 g/dL and hemolytic markers normalized (LDH 210 U/L, Haptoglobin 55 mg/dL, Reticulocyte Count 1.8%). By the 3-month follow-up, the prednisolone had been fully tapered, and her hemoglobin was stable at 12.6 g/dL; notably, a repeat polyspecific DAT was now negative. A repeat quantitative G6PD assay, intended to be performed in a non-hemolytic, non-reticulocytic state, was planned for the 4-month follow-up, but the patient was lost to hematology follow-up at this stage. According to leprosy clinic records, her leprosy treatment was successfully restarted using a modified, Dapsone-free regimen. She reportedly tolerated this new regimen, though specific details on whether Rifampicin was included were not available for this report.

3. Discussion

This case report details not merely a drug reaction, but a life-threatening hematological episode that unmasked a complex, synergistic, and ultimately ambiguous pathophysiology. The clinical presentation of a patient on multidrug therapy (MDT) for leprosy—specifically Dapsone and Rifampicin—developing simultaneous profound oxidative hemolysis and a robust autoimmune hemolytic process, represents a diagnostic vortex. This challenge was significantly compounded by the unavailability of specialized, second-line immunohematological testing, forcing a reliance on clinical reasoning and syndromic management.[11]

The primary and most evident mechanism at play was a catastrophic acute oxidative hemolytic event. This process represents the "expected," or at least, the most easily identifiable pathology. The patient's clinical profile provided a textbook triad for this diagnosis: a susceptible host, a potent oxidizing agent, and the classic morphological evidence of the resultant damage. The patient's severe Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency provided the necessary substrate, representing the inherent "Achilles' heel" of her erythrocytes. The G6PD enzyme is the gatekeeper for the pentose phosphate pathway (PPP), a metabolic route that, in the mature, anucleated erythrocyte, stands as the *sole* source of reduced nicotinamide adenine dinucleotide phosphate (NADPH). This molecule, NADPH, is the indispensable cofactor for the enzyme glutathione reductase, which is tasked with regenerating reduced glutathione (GSH) from its oxidized state (GSSG). Reduced glutathione, in turn, is the primary intracellular scavenger of reactive oxygen species (ROS).[12]

Table 3. Treatment, follow-up, and outcome of the patient.

PHASE	INTERVENTION / FINDING	DETAILS
Initial Management (Admission)	Cessation of Agents	All MDT drugs (Dapsone, Rifampicin, Clofazimine) immediately withheld.
	Immunosuppression	IV Methylprednisolone 125 mg twice daily (250 mg/day) for 3 days.
	Supportive Care	Folic Acid 5 mg daily.
	Transfusion	4 units "least incompatible" PRBCs due to pan-reactive autoantibody.
Hospital Outcome	Clinical Response	Rapid improvement within 48 hours; urine cleared, heart rate normalized.
	Medication Taper	Transitioned to oral Prednisolone 60 mg daily on Day 4.
	Discharge	Discharged on Day 6 with a tapering course of oral Prednisolone.
Long-Term Follow-up	1-Month Follow-up	Asymptomatic. Hb recovered to 11.4 g/dL. Hemolytic markers normalized.
	3-Month Follow-up	Prednisolone tapered off. Hb stable at 12.6 g/dL. Repeat DAT: **Negative**.
	4-Month Follow-up	Patient lost to follow-up for planned repeat G6PD assay.
	Final Outcome	Leprosy treatment successfully restarted on a **Dapsone-free regimen**.
	Key Unresolved Data	Details on whether the new regimen included Rifampicin were unavailable.

○ **Initial Management:** Interventions at admission. ○ **Hospital Outcome:** Patient response before discharge.
Long-Term Follow-up: Post-discharge status.

Into this metabolically vulnerable environment, the patient was administered Dapsone, the "oxidant fuel." While Dapsone itself is relatively inert, its hepatic metabolite, dapsone hydroxylamine (DDS-NOH), is a powerful oxidant.[13] This metabolite enters the erythrocyte and initiates a futile, high-velocity redox cycle. It directly oxidizes GSH to GSSG, creating a massive, sudden demand for GSH regeneration. In a healthy individual, the G6PD/PPP pathway would ramp up NADPH production to meet this demand, and the oxidative threat would be neutralized. In this G6PD-deficient patient, this pathway failed. The result was the immediate and complete depletion of the cell's entire antioxidant capacity. With no GSH to scavenge them, the ROS generated by DDS-NOH—superoxide, hydrogen peroxide, and hydroxyl radicals—were free to attack intracellular components. Their primary target was the cysteine residue at the 93rd position of the hemoglobin beta-globin chain. This oxidation denatured the globin chains, causing them to dissociate from the heme iron (forming hemichromes) and subsequently cross-link and precipitate. These intracellular inclusions of denatured globin are the pathognomonic Heinz bodies.[14]

These Heinz bodies are not inert. They are toxic aggregates that bind to the N-terminus of the inner-leaflet membrane protein, Band 3. This binding induces cross-linking and clustering of Band 3, damaging the membrane's structural integrity, reducing its deformability, and signaling for removal. As these rigid, damaged cells attempted to navigate the microcirculatory maze of the splenic cords, they were intercepted by splenic macrophages of the reticuloendothelial system (RES). These macrophages, recognizing the damaged membrane complexes, "pitted" the Heinz bodies from the cells. This "pitting" process is not subtle; it removes a portion of the cell membrane, creating the "bite cell" or degmacytemorphology seen on the patient's smear. The resulting cell, now possessing less membrane for the same internal volume, reseals as a microspherocyte. This spherocytic, rigid cell is then unable to pass through the splenic fenestrations on its next circuit and is phagocytosed. This entire cascade—oxidative stress, Heinz body formation, splenic pitting, and extravascular sequestration—is the classic pathophysiology of Dapsone-induced oxidative hemolysis.[15]

A key interpretive point, crucial for understanding the severity of this event, is the quantitative G6PD level. The initial admission value of 6.0 U/g Hb was measured during a state of massive, compensatory reticulocytosis (12.5%). Reticulocytes, being metabolically vigorous young cells, possess three to five times more G6PD activity than mature erythrocytes. The patient's measured enzyme level is an average of this small, surviving, enzyme-replete reticulocyte population and the profoundly deficient mature cells, the vast majority of which had already been destroyed. The fact that this average (6.0 U/g Hb) was still below the laboratory's normal range, even with the massive compensatory influx of high-activity reticulocytes, strongly implies that the patient's true baseline G6PD activity in her mature cells was near-zero.[16] This indicates a profoundly severe (likely WHO Class II) deficiency, which fully explains the catastrophic, life-threatening reaction to a standard 100 mg dose of Dapsone. This massive oxidative insult represents the "first hit" in her multifactorial process, an event that, by itself, would be sufficient to cause severe anemia. A follow-up G6PD assay at a hematological baseline (3-6 months post-event) was unfortunately not obtained, representing a limitation in confirming the precise severity, but the admission data are clinically conclusive.[17]

The central conflict of this case, and its most compelling diagnostic feature, was the positive immunohematological workup. The oxidative hemolysis, while severe, was an expected complication (Figure 1). The discovery of a 4+ Polyspecific Direct Antiglobulin Test (DAT), a positive Indirect Antiglobulin Test (IAT), and a pan-reactive warm autoantibody in the serum and eluate was not. This confirmed a severe, concurrent immune-mediated hemolytic process, fundamentally altering the diagnostic and therapeutic landscape. The etiological trigger for this immune process, however, remained unresolved due to two key, insurmountable factors at the time of admission: (1) the patient's polypharmacy, involving two drugs (Dapsone and Rifampicin) both implicated, albeit with vastly different frequencies, in DIIHA; and (2) the lack of monospecific DAT and drug-specific antibody testing. A polyspecific DAT is merely a screening tool; it confirms that something (either IgG antibody or C3d complement) is coating the erythrocytes. It cannot differentiate the mechanism. This differentiation is the critical step, and its absence forces us to rely on clinical reasoning to evaluate the competing hypotheses.

The most significant and plausible confounder was the concurrent administration of Rifampicin. From an internal medicine standpoint, this is the most parsimonious explanation (Dapsone-oxidative + Rifampicin-immune hypothesis). Rifampicin is a known, if infrequent, cause of DIIHA.[18] Critically, it operates via a mechanism that perfectly aligns with the most severe aspects of this patient's presentation. Rifampicin typically induces drug-dependent antibodies, classically via an "immune-complex" mechanism. In this model, an antibody (often IgM, a potent pentamer) is formed not against the red cell itself, but against the drug, or a complex of the drug and a plasma protein. This Rifampicin-antibody immune complex then loosely adsorbs onto the surface of the patient's erythrocytes, making the red cell an "innocent bystander" in the immune assault. The key pathophysiological event follows. The binding of these immune complexes (especially if IgM-mediated) places multiple antibody Fc regions in close proximity, providing an ideal platform for activating the classical complement cascade. This triggers a runaway amplification, leading to the cleavage of C3 and the deposition of millions of C3b molecules on the red cell surface. This massive C3b coating has two consequences. First, it opsonizes the cell for extravascular clearance by RES macrophages (which have C3b receptors). Second, and more catastrophically, it drives the complement cascade to its terminal lytic phase: the formation of the Membrane Attack Complex (MAC), C5b-9. The MAC complex literally punches holes in the erythrocyte membrane, causing immediate, massive, and uncontrolled intravascular hemolysis. This hypothesis is highly compelling as it fits many of the patient's most dramatic findings: exposure to a known culprit (Rifampicin), a clinical picture of fulminant, rapid-onset hemolysis, and strong, classic evidence of intravascular hemolysis-the "tea-colored" urine (hemoglobinuria), the profoundly elevated LDH (1250 U/L), and the absent haptoglobin (completely saturated by free plasma hemoglobin). This intravascular picture is the hallmark of complement-mediated Rifampicin-DIIHA.

The lack of a monospecific DAT is the critical missing piece of this puzzle. A C3d-only DAT result would have been pathognomonic for this mechanism, as the immune complex (IgG or IgM) often dissociates after fixing complement, leaving behind only the C3d "footprint" on the cell.[19] An IgG+C3d result would be more ambiguous, but still possible. The pan-reactive IAT is slightly less consistent, as Rifampicin-dependent antibodies typically only react in vitro when the drug is added to the test system. However, the patient's follow-up, which ambiguously noted a "Dapsone-free regimen" but lacked specific data on the re-introduction of Rifampicin, prevents us from clinically excluding this highly plausible hypothesis. This remains a major, unresolved confounder.

The "unifying" dapsone mechanism is more speculative but scientifically elegant, as it posits that Dapsone is the sole offending agent for both the oxidative and immune phenomena. This is a unifying, sequential pathophysiology for which this case is highly suggestive, but ultimately cannot be proved. In this proposed two-hit process: (1) First Hit (The Oxidative Insult): as detailed before, Dapsone, in the setting of severe G6PD deficiency, initiates a massive, uncontrolled oxidative burst; (2) Membrane Modification & Neoantigen Exposure: This is the immunological turning point. The profound oxidative stress does more than just precipitate globin. It is proposed that ROS directly damages and chemically alters key RBC membrane structural proteins, notably the Band 3 anion exchanger and various glycophorins.[20] This damage can cause protein cross-linking, aggregation, and conformational changes, thereby exposing cryptic antigens (epitopes normally hidden within the protein's tertiary structure) or creating novel "neoantigens" that are not recognized as "self" by the immune system. This includes the premature, en masse clustering of Band 3 to form "senescence antigens," which are normally exposed only on very old (120-day) erythrocytes to signal for their quiet, physiological removal; (3) Second Hit (The Immune Response): The immune system (specifically B-cells and antigen-presenting cells) recognizes these damaged, clustered, or altered proteins as a "danger signal." This breaks immune tolerance, resulting in the production of true, warm-reactive autoantibodies (IgG) that bind to these "neoantigens" on all of the patient's red cells, not just the Dapsone-damaged ones. This "oxidative-trigger" hypothesis is scientifically compelling. It would explain the presence of a pan-reactive warm autoantibody in the serum (IAT positive) and eluate, as this true autoantibody would react with the commercial reagent red cells (which, of course, have normal Band 3) just as it does with the patient's own cells. A monospecific DAT would be expected to be IgG-only (classic WAIHA) or IgG+C3d if the autoantibody was also capable of fixing complement. This hypothesis is supported by a body of literature suggesting a mechanistic link between oxidative stress and the breakdown of self-tolerance in other autoimmune diseases. However, without definitively excluding Rifampicin (Hypothesis D), this remains an unproven, though compelling, speculation.

The true art of internal medicine lies in reconciling data that appears, at first glance, to be contradictory. This case presented two notable clinical findings that were discordant with a typical, simple hemolytic picture and, in fact, served as powerful clues. A key discordant finding was the exceptionally mild hyperbilirubinemia (Total Bilirubin 1.15 mg/dL) relative to the profound, life-threatening degree of hemolysis (Hemoglobin 5.3 g/dL, LDH 1250 U/L, Haptoglobin <10 mg/dL). With this degree of red cell catabolism, one would expect a massive flood of unconjugated bilirubin, overwhelming the liver's conjugating capacity and leading to overt jaundice with a bilirubin level in the 3-8 mg/dL range. The absence of this finding strongly suggests a predominantly intravascular component to the hemolysis. In a typical extravascular hemolysis (like WAIHA or spherocytosis), red cells are phagocytosed by splenic macrophages. Inside the macrophage, heme is converted by heme oxygenase to biliverdin, and then to unconjugated bilirubin, which is released into the blood, causing hyperbilirubinemia.

In contrast, during intravascular hemolysis, red cells lyse directly within the circulation. This releases free hemoglobin directly into the plasma. This free hemoglobin is highly toxic, particularly to the kidneys. The body's first line of defense is the scavenger protein haptoglobin, which binds free hemoglobin and is cleared by the liver. In this patient, the hemolysis was so massive that haptoglobin was instantaneously saturated and depleted (hence, <10 mg/dL). The remaining, unbound free hemoglobin dimers are small enough to be filtered by the glomerulus, leading to hemoglobinuria (the "tea-colored" urine) and renal clearance. This process bypasses the liver's metabolic pathway. The heme is lost in the urine, not converted to bilirubin. Therefore, the low bilirubin is not a sign of mild hemolysis; it is a critical, pathognomonic sign of severe intravascular hemolysis. This finding is highly consistent with both leading hypotheses: severe, fulminant G6PD-related oxidative hemolysis can have a major intravascular component, and complement-driven Rifampicin-DIIHA (Hypothesis D) is classically intravascular.

Another diagnostically significant finding was the lack of palpable splenomegaly on physical exam. This is also unusual. Both classic mechanisms proposed—splenic "biting" of Heinz bodies (oxidative) and splenic sequestration of IgG-coated cells (autoimmune)—are functions of the spleen that, when occurring at this magnitude, typically lead to work hypertrophy and reactive splenomegaly. The absence of this finding may also point to the rapidity and severity of the intravascular component. If a large fraction of the red cells are lysing in the vessels before they even reach the spleen, the spleen is partially bypassed as the primary site of erythrocyte clearance. This would preclude the development of the reactive splenomegaly typically seen in more indolent extravascular processes. This finding, much like the low bilirubin, argues against a pure, classic IgG-mediated WAIHA (which is the prototypical cause of splenomegaly) and favors a mechanism with a dominant intravascular, complement-mediated (Hypothesis D) or fulminant G6PD component.

The unmasking of this dual mechanism, and the profound ambiguity of its precise trigger, carries critical, life-saving clinical implications for both diagnosis and management. This case must serve as a powerful argument that a positive Coombs test in a G6PD-deficient patient on polypharmacy should never be dismissed as incidental. It signals a concurrent and clinically significant immune-mediated process that requires independent, aggressive management. It transforms the case from a "simple" drug-toxicity problem to a complex immunohematological emergency. Furthermore, this case illustrates why a full immunohematological workup is mandatory. The lack of a monospecific DAT (for IgG and C3d) was the central confounding factor. This single test could have provided a powerful clue: a C3d-only result would have pointed strongly toward Rifampicin, while an IgG-only result would have favored a Dapsone-induced autoantibody. Ideally, drug-dependent antibody testing (testing the patient's serum against reagent cells in the presence and absence of Dapsone and Rifampicin) would have been performed. The unavailability of this testing is the reason this case remains a diagnostic puzzle rather than a solved mystery.[17,18]

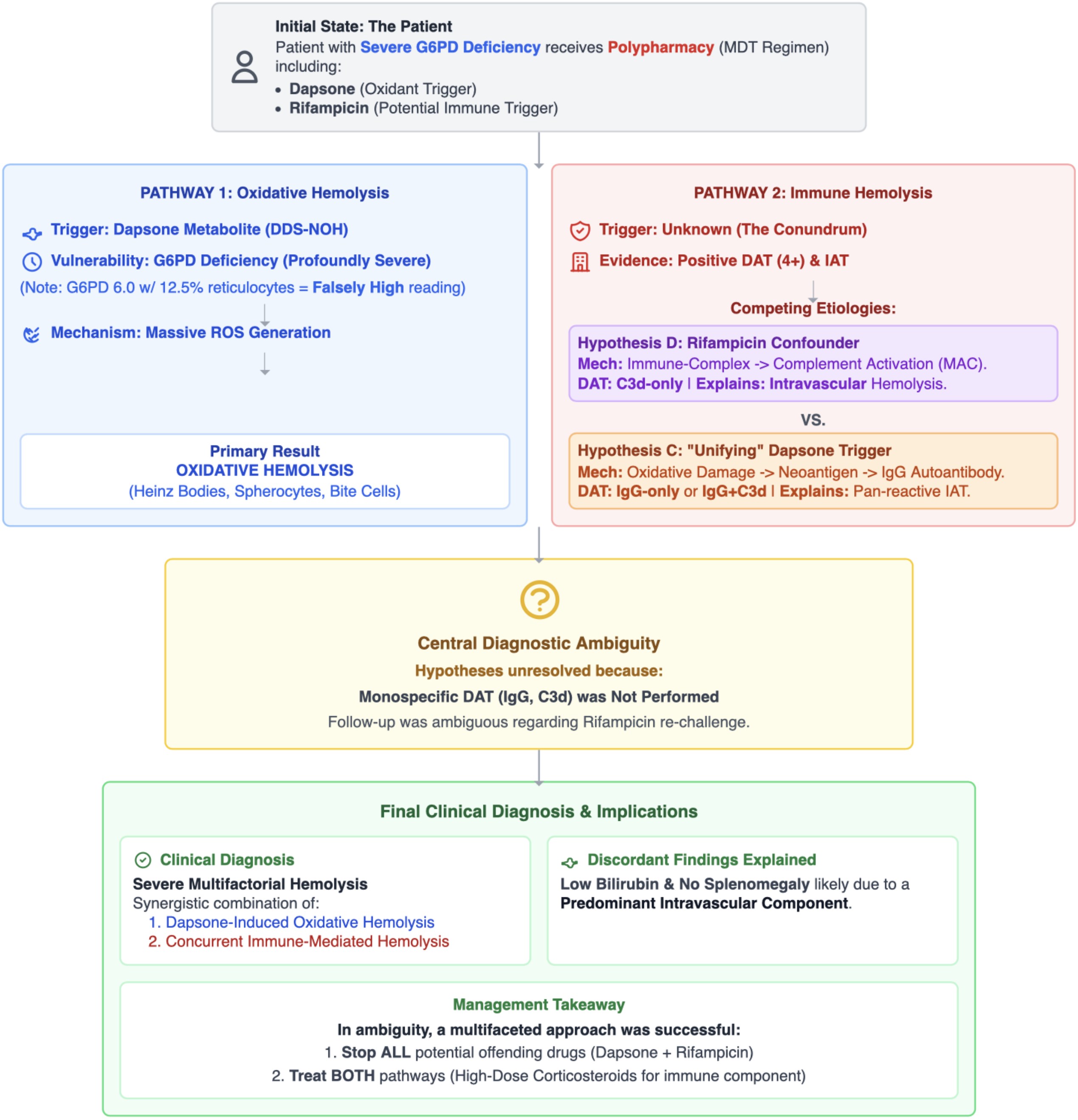

Figure 1. Hypotheses for a severe multifactorial hemolytic anemia.

In the face of such life-threatening ambiguity, management must be multifaceted and aggressive, designed to cover all plausible, worst-case scenarios; (1) Cessation of Agents: Stopping all potentially offending agents (Dapsone and Rifampicin) was the essential first step. It would have been a critical error to "guess" and stop only Dapsone. This removed the "oxidant fuel" (Dapsone) and the potential "immune trigger" (Rifampicin); (2) Aggressive Immunosuppression: This is the key therapeutic lesson. The oxidative component (G6PD crisis) does not respond to corticosteroids. The only reason to initiate steroids was to treat the active, severe immune component. The patient's rapid clinical improvement, which began after the initiation of high-dose corticosteroids (in addition to drug cessation), provides the strongest possible evidence that the dual-mechanism theory was correct. Stopping the drugs alone would likely have been insufficient to control the now-active autoimmune process, which (regardless of its trigger) had become self-sustaining. The use of high-dose "pulse" steroids (IV methylprednisolone 250 mg/day), rather than standard oral prednisone, was fully justified by the patient's hemodynamic instability and profound anemia, as this regimen is designed to rapidly "shut down" autoantibody production and macrophage-mediated clearance. In summary, this case provides a rare window into a hematological "perfect storm," where oxidative and immune pathways converged with devastating, synergistic force. The diagnostic ambiguity, far from being a simple limitation, serves as the central teaching point: it highlights the critical importance of a complete immunohematological workup and demonstrates the necessity of a multifaceted management strategy that simultaneously addresses all active pathophysiological processes.[19,20]

4. Conclusion

We have presented a unique case of severe, multifactorial hemolytic anemia in a G6PD-deficient patient, characterized by the concurrent presentation of massive oxidative hemolysis and a severe, autoantibody-mediated immune hemolysis. The patient's polypharmacy (Dapsone and Rifampicin) and the unavailability of specialized immunohematological testing created a critical and highly plausible diagnostic confounder. We were unable to definitively isolate the trigger for the immune component. Both the "unifying" Dapsone-trigger hypothesis and the "dual-drug" Dapsone/Rifampicin hypothesis remain plausible explanations for the patient's presentation. This case unmasks a complex, synergistic interplay between oxidative and immune pathways and serves as a powerful illustration of the diagnostic challenges posed by polypharmacy in complex hematological crises. It underscores that clinicians must be vigilant for such overlapping etiologies and that recognizing both components of the hemolysis is crucial for tailoring successful management. This requires the immediate withdrawal of all offending oxidant and potential immune-triggering drugs, as well as the concurrent initiation of high-dose immunosuppression to control the life-threatening autoimmune component.

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