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Mechanism of Actions, Efficacy, and Long-term Use of Steroids in Autoimmune Hemolytic Anemia (AIHA)

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Autoimmune hemolytic anemia (AIHA) is a rare condition in which autoantibodies cause the loss of red blood cells. Steroids have been used to treat several illnesses, including AIHA. For now, steroids remain as the first line of treatment for AIHA. In AIHA, especially warm AIHA (wAIHA), steroids suppress autoantibody production and downregulate Fcγ receptors' expression on monocytes to prevent hemolysis. The type of steroids chosen for first-line therapy for wAIHA in pediatrics and adults are Prednisone (Prednisolone) and Methylprednisolone. At the same time, Dexamethasone is used as an alternative treatment in AIHA. Steroids show better therapeutic outcomes in the first 2-3 weeks of administration, but the proportion of patients who remain in remission after steroid discontinuation are still quite low. Long-term administration of steroids may affect bone, blood glucose metabolism, and hypothalamic-pituitary-adrenal axis (HPAA). However, steroids which have a linear pharmacokinetic profile, intermediate-acting glucocorticoids such as Prednisone (Prednisolone) or Methylprednisolone, and also tapering dose of steroids after 2-4 weeks administration will be safe for long term use as AIHA treatment.

Keywords*: steroids, glucocorticoid, corticosteroid, autoimmune hemolytic anemia, AIHA, mechanism of action, efficacy*

Introduction

The annual incidence of autoimmune hemolytic anemia (AIHA) is estimated to be 1-3 per 100,000 individuals, affecting both adults and children, but it rises with age, especially beyond 40 years old.[1,2] In 50-60% of documented cases of warm-type AIHA are primary cases; while secondary cases, which are uncommon, are linked to immunological disorders, infections, solid tumors, and lymphoproliferative disorders.[3] Sixty six percent of patients with warm AIHA (wAIHA) in 27 trials, totaling 4311 individuals, were females[4], compared to a higher prevalence of primary AIHA and Evans syndrome (immune thrombocytopenic purpura (ITP) is associated with AIHA) in women and children[5]. According to a Korean study, 369 infants had hereditary hemolytic anemia between 2007 and 2016, and of those, 71.3% had red blood cells (RBC) embryopathy, 16.04% had hemoglobinopathy, 6.5% had an unclear cause, and 6.2%

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had RBC enzymopathy.[6] Acute renal failure, thrombotic events (pulmonary embolism, stroke, cardiac infarction), co-occurring infections, AIHA with thrombocytopenia (Evans syndrome) were factors which worsen the prognostic in AIHA patients.[5]

The type of autoantibodies, clinical symptoms, severity, coexisting or underlying illnesses, and patient age affect how AIHA is treated. The most frequent form of AIHA, namely wAIHA, has a high chance of recurring in children and adults. Despite the absence of comprehensive research, corticosteroids are widely acknowledged as the best "first-line" treatment for those with wAIHA.[7] Steroids act by altering immunological response, which slow down the destruction of RBC.[8] This review aimed to share detailed information about steroids in AIHA by exploring the selection of steroids used for AIHA in pediatrics and adults, their mechanism of action, efficacy, and long-term administration.

Overview of AIHA

Diagnosis

AIHA is characterized by hemolysis directed toward the RBCs and is mediated by autoregulation. AIHA is also associated with inflammatory disorders affecting the liver and gut, as well as antibodies that perceive RBCs as antigens to be attacked, leading to hemolysis.[9] AIHA is classified into three categories: wAIHA, cold AIHA (cAIHA), and mixed AIHA.[3,9-11]

AIHA is identified when one or more indirect hemolysis-related parameters are present, such as falling down of idiopathic hemoglobin level <11 g/dL (<6 mmol/L) or hematocrit <30%, reticulocytosis, elevated bilirubin or lactate dehydrogenase, or decreased haptoglobin. There is evidence of an underlying autoimmune mechanism, such as the presence of cold agglutinins or immunoglobulin G or C3 DAT positivity.[12] RBC morphology is crucial for an accurate diagnosis. Diagnosing a specific kind of hemolytic anemia may be possible using a standard blood smear showing hemolysis appearance with finding of schistocytes, spherocytes, and cells in the shape of a bite.[13-15] One of the hemolysis markers called ferritin will increase in AIHA. Ferritin is an acute-phase protein that shows an inflammatory disease. Recently, one of newer diagnostic tests to identify the markers of hemolysis is reticulocyte hemoglobin equivalent (Ret-He). Ret-He is used to measure the iron incorporation in hemoglobin and reticulocytes.[16-18]

Clinical Manifestations

The symptoms manifest in wAIHA are usually associated with anemia. Patients may present with fever, abdominal pain, pallor, icterus/jaundice of the skin and whites of the eyes, dark urine, gallstones, and abdominal fullness from an enlarged spleen (splenomegaly or hepatosplenomegaly), as well as hyperpnea, tachycardia, angina, or heart failure in very severe cases, including those with acute onset. Three percent of severe anemia may result in unconsciousness, collapse, or acute renal failure.[19-21]

Etiology

Hemolytic anemia can occure in acute and chronic illnesses, immunological or non-immune induced conditions, intravascular or extravascular conditions, hereditary or acquired conditions, and intracorpuscular or extracorpuscular conditions.[22-26] The causes of intracorpuscular conditions are abnormalities in the RBCs themselves. RBCs can be damaged internally when there are changes in hemoglobin solubility (hemoglobinopathies), altered membrane or cytoskeleton structure (membranopathies), or decreased metabolic ability (enzymopathies).[22] In contrast, the causes of extracorpuscular are the defect influenced by external factors, including mechanical, immune-mediated, or infectious.[13]

Pathophysiology

According to the immunopathogenesis of AIHA, immunoglobulin G (IgG) antibodies will battle against Rh, Band 3, protein 4.1, or RBC antigens by binding to Fc receptors on macrophages, particularly in the lymph. The antibodies will destroy the red blood cell membrane. In addition, autoantibodies produced by B lymphocytes are characterized by autoreactive T cells (T cell dysregulation), decreased T-regulatory (Treg) cells and increased pro-inflammatory Th 17 cells, and increased reactive oxygen species (ROS), which causes a decrease in activation-induced cell death (AICD) and increases auto-antibody production on RBCs.[3,27,28]

In AIHA, there is also an interaction of T lymphocyte subsets and cytokines. Elevated cytokines were interleukin (IL)-2 and IL-12, which induce differentiation of cluster of differentiation (CD)4^+ naïve T cells into the Th1 subset and IL-4, which triggers Th2 turnover. In addition, elevated levels of transforming growth factor (TGF)-β favoured Th17 subset differentiation, which amplifies pro-inflammatory and autoimmune responses. And there was a decrease in

interferon (IFN)-γ and Treg levels and their decreasing level leads to inhibition of the Th2 response, which amplifies autoantibody-mediated autoimmune diseases and can lead to reduced down-regulation of inflammatory and autoimmune pathways.[29-31]

In wAIHA, IgG is predominantly involved in attacking erythrocyte membrane antigens at 37°C. It also binds to complement and triggers the removal of erythrocyte membrane antigens by lymph and other parts of the reticuloendothelial system.[28,31] Complement-mediated interference is only partial, depending on the subtype of wAIHA and the duration of complement activation.[31,32] The activity of complement C3b that binds to phagocyte receptors on the surface of RBCs and finally produces the C5b-C9 complex later will cause cell opsonization and induce RBC lysis in AIHA.[31,32]

AIHA in Adults and Pediatrics

AIHA is a prevalent disease condition in adults but uncommon in children. However, there is no difference in the type of steroids used to treat AIHA in adult or pediatric patients. The dose of steroids given is adjusted according to body weight. The most commonly given steroids include Prednisone (Prednisolone), Methylprednisolone, and Dexamethason.[33-36] Table 1 showed the comparison of steroid agents for AIHA treatments in the pediatric and adults population. Some studies reported some types of AIHA, based on the impairment conditions. Meanwhile, Table 2 showed the current landscape of steroids clinical pharmacology in special cases of AIHA, such as mixed type AIHA and AIHA in hepatic and renal impairment conditions.

Steroids agents in AIHA treatments

Prednisone (Prednisolone)

Prednisone (Prednisolone) is a type of steroid with an intermediate duration of action and has the ability to act on the hypothalamic-pituitary-adrenal axis (HPAA) for 12-36 hours. The pharmacokinetic characteristic of Prednisone in the body is non-linear protein binding, and based on its pharmacodynamic attributes in the treatment of AIHA, Prednisone has an excellent potential in suppressing the Fc receptor and T-helper. The general therapeutic indications of Prednisone are its beneficial for long-term treatment and as an anti-inflammatory/immunosuppressant due to its high glucocorticoid (GC) activity.[37-39]

Methylprednisolone

Methylprednisolone is also an intermediate-acting steroid with the same HPAA potency as Prednisone. However, based on its pharmacokinetic characteristics, Methylprednisolone has linear protein binding at a steady state, and pharmacodynamically in the treatment of AIHA, Methylprednisolone has the same potency as Prednisone in suppressing Fc receptor and T-helper. Methylprednisolone's general therapeutic indications are anti-inflammatory and immunosuppressant.[37-39]

Dexamethasone

Dexamethasone is a long-acting steroid, potentially suppressing HPAA for 36-72 hours. In addition, Dexamethasone has linear protein binding like Methylprednisolone and has the same potential as Prednisone in suppressing Fc receptors and T-helper in treating AIHA. In general, Dexamethasone is indicated as an anti-inflammatory/immunosuppressant, particularly when retention is not desired due to its low mineralocorticoid action.[37-39]

The mechanisms of action of steroids (GC) in AIHA

Since GCs are naturally lipophilic hormones, they can migrate freely through cell membranes. They exert their effects at the gene expression level by attaching to the GC receptor (GR), a transcription factor that controls a number of genes either positively or negatively.[40]

One possible mechanism of steroids in helping to stabilize anemia in patients is the entry of oxygenated cholesterol derivatives into the RBC membrane, causing membrane expansion. In an isotonic environment, there is no effect of hemolysis, but in a hypotonic environment, the ratio of cells to surface increases toward volume, stabilizing the RBCs and helping reduce hemolysis. Another study mentioned that interactions between steroids and RBC membranes naturally occur. Steroids have been postulated to interact with a class of phospholipids in the erythrocyte membrane, specifically dimyristoyl phosphatidylcholine (DMPC). Steroids cause gradual hydration of DMPC, accumulating water in the RBC membrane and cell will dilate, which can further prevent hemolysis. The interaction of steroids with phospholipids further alters the permeability of RBC membranes. Therefore, steroids may help ameliorate hemolysis by increasing oxygenated cholesterol in the RBC membrane or interacting with

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Table 1. Comparison of the use of steroid agents for AIHA treatments in pediatrics vs adults population.

Steroids Use in AIHA	The dose of steroids in AIHA Treatment		Duration of Action (hours)	Explanations	Reference
	Pediatrics	Adults			
Prednisone	- First line therapy - Dose: 1-2 mg/kg/day, maximal 4-6 mg/kg/day orally for 3-6 weeks, then tapering slowly until 4-6 months	- First line therapy - Dose: 1-2 mg/kg/day, max 30-80mg/day orally for 3-4 weeks, then tapering slowly until 3-4 months	12-36	- Intermediate-acting glucocorticoid; - Prednisone has a higher immunosuppressant potency than methylprednisolone and Dexamethasone; - Prednisone is used for long-term therapy because it has high glucocorticoid activity	38,58,70,71
Prednisolone	- First line therapy - Dose: 1-2 mg/kg/day for 3-6 weeks	- First line therapy - Dose: 1-6 mg/kg/day for 3-6 weeks, then tapering slowly until 2-3 years	12-36	- Similar with prednisone	38,58,72,73
Methylprednisolone	- First-line therapy in severe condition - Dose: 1-2 mg/kg/day for 1-3 days	- First-line therapy in severe condition - Dose: a. Megadose 250-1000 mg iv in first 72 hours (1-3 days) b. 100-200 mg/day 7-14 days	12-36	- Intermediate-acting glucocorticoid; - Methylprednisolone has better immunosuppressant ability than Dexamethasone	38,58,70,73
Dexamethasone		- Alternative regimen therapy - Dose: 40 mg/day for 4 days or 2-6 times every 2-4 weeks	36-72	- Long-acting glucocorticoid; - Dexamethasone is used briefly in severe and acute conditions; - Dexamethasone was shown to be approximately 18 times more potent than prednisolone in suppressing the growth rate	36,38,58, 74–76

Table 2. The current landscape of steroids clinical pharmacology in special cases of AIHA.

Cases of AIHA	Therapeutic Uses of Steroids	Reference
Mixed-type AIHA	Prompt steroid therapy is typically effective in mixed-type AIHA (associated with low titer cold agglutinins) and requires few or no transfusions. However, therapy in combination with immunosuppressive medications, such as monoclonal anti-CD 20 (Rituximab), may be beneficial in some circumstances, such as CAD and refractory wAIHA.	77,78
AIHA with hepatic impairment condition	Steroids raise the risk of electrolyte imbalances and fluid retention-related cirrhosis decompensation. In this situation, steroids may raise the death rate from severe infections. Additionally, liver failure affects the pharmacokinetics of Prednisone and Prednisolone. Therefore the dose should be adjusted according to the concentration of albumin serum.	9,79,80
AIHA with renal impairment condition	Steroid therapy increases the risk of steroid-associated adverse events (SAAE) in patients with specific conditions of renal impairment, such as immunoglobulin A (IgA) nephropathy and primary proteinuric kidney disease. The SAAE includes hypertension, diabetes, weight gain, short stature, fractures, and infections.	81,82

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phospholipids to cause water accumulation in the RBC membrane which helps stabilize and prevent hemolysis or by directly stimulating erythropoiesis or having an effect on spleen remodelling.[41-43] Fc receptor plays a significant part in preventing autologous erythrocyte opsonization in hemolytic anemia. Monocyte and macrophage receptors for the Fc portion of IgG (Fcγ receptor (FcR)) are involved in many physiological and pathophysiological responses). They play an important role in recognizing specific opsonization by macrophages, then removed by the reticuloendothelial system of immune complexes, pathogenic bacteria, and autoimmune diseases.[8,44,45] GCs interfere with FcR function, and GC reduce the number of FcR on promyelocytic cells in *in vitro* studies. GC inhibit FcR function in cell phagocytosis, making them effective for treating autoimmune diseases. GCs directly reduce the amount of FcR in macrophage cells by inhibiting the production of FRAF (FcR augmenting factor).[44,46] Another study states that Fc receptors are specific granulocyte and lymphocyte subtypes that bind Fc immunoglobulin of cell membrane receptors on macrophages. FcR play an important role in initiating phagocytosis through antibody binding to the surface of particles coated by phagocytic cells. They are involved in immune system processes which mediate cytotoxicity activity. GCs inhibit the role of FcR in phagocytosis by reducing the number of FcR on phagocytic cells.[47,48]

In treating hemolytic anemia, GCs also affect the production of T cell growth factor. T cell growth factor is produced by mitogens or antigens that stimulate lymph cells, which are required for the proliferation of T lymphocytes and are responsible for the expansion of T cell responses to antigens. In peripheral monocellular, 95% of GCs were found to inhibit T cell growth factor. The inhibitory action by GCs on T cell blastogenesis induced by antigen or mitogen is related to the inhibition of T cell growth factor production.[47]

Recent studies have also mentioned almost the same thing about the mechanism of GCs in the treatment of AIHA, which include: causing downregulation of FcR on phagocytes in lymph cells and reducing IL-2 production, suppressing the sequestration of RBCs that are opsonized by splenic macrophages, reducing the binding affinity of autoantibodies from B cells for RBCs, and decreasing extravascular erythrocyte damage (hemolysis).[28,49-52] This inhibitory mechanism produced by GCs may be responsible for the delay in the primary immune response.[47] By interfering with the production of soluble growth factors necessary for the expansion of the lymphocyte response to antigens, GCs will delay the development of the immune system as fewer cells are responsive to antigen.[47,53] Based on the potential ability of GC to suppress the HPAA, hydrocortisone, and cortisone acetate have the lowest suppression potential; Prednisone, Prednisolone, Methylprednisolone, and Triamcinolone have moderate suppression potential, while Dexamethasone has the most potential of HPAA suppression compared to others. Suppressing the HPAA will cause an adrenal crisis which can interfere with the body's response to acute stress, such as infection or surgery.[54]

By lowering the quantity of FcR on phagocytic cells, GC contributes to suppressing the function of FcR in the phagocytosis process. The quantity of FcR significantly dropped after 24 hours in the administration of 100 nM Dexamethasone, whereas, after 48-72 hours, FcR decreased by 65% of the initial concentration.[47]

It is clear that GCs affect the phagocytosis and destruction of antibodies lining autologous tissues, a feature of many autoimmune diseases. This study also observed that patients with autoimmune diseases such as hemolytic anemia and thrombocytopenia often show obvious improvement after 24-48 hours of treatment with GC, which can be explained by decreased FcR after GC administration.[47]

Prednisolone has the largest T-helper cell suppression effect compared to other steroids. An RCT study comparing the ability to suppress T-helper cells and cortisol among Hydrocortisone, Prednisolone, Methylprednisolone, and Dexamethasone showed that Dexamethasone has almost two-fold higher activity than Hydrocortisone in suppressing T-helper cells.[55]

However, further analysis with the post-hoc test using the Bonferroni method showed no significant difference; Prednisolone and Methylprednisolone had almost the same T-helper cell suppression potential as Dexamethasone. Likewise, Prednisolone and Dexamethasone showed a larger effect than Hydrocortisone and Methylprednisolone in cortisol suppression. However, after further analysis with the post-hoc test using the Bonferroni method, it also did not show significant difference.[55,56] A retrospective study in renal transplant patients suggested that Methylprednisolone was superior in maintaining immunosuppressant effects compared to prednisolone.[55,57,58] Steroids (GC) are lipophilic and are usually given in prodrug form for intravenous administration. GC is well absorbed after oral administration

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with a 60-100% bioavailability. The protein binding of steroids is biologically relevant because it depends on the free drug that can reach the biophase (site of action) and interact with the receptor.[55,59] When Prednisolone-protein binding achieves a stable state, it falls non-linearly from 95% to 60-70% while its concentration rises from 200 g/L to 800 g/L. Being dose-dependent will therefore result in a rise in the volume of distribution (VD) and a fall in Prednisolone clearance (CL). CL of Prednisolone will decrease in high-dose administration (dose >20 mg) due to the saturation of its elimination mechanism.[55,60]

The Methylprednisolone (6α-methylprednisolone) and Dexamethasone (9α-fluoro-16α-methylprednisolone) have no affinity for transcortin and bind only to albumin. Therefore, Methylprednisolone's pharmacokinetics is linear, not dependent on the doses. A comparison of the pharmacokinetic profile between Dexamethasone and Methylprednisolone includes the T½ of Dexamethasone sodium phosphate that was longer than Methylprednisolone (4.6±1.2 hours *vs.* 3.0±1.7 hours), the VD at steady state (Vss) of Dexamethasone was greater than that of Methylprednisolone injection (81.6±16.6 L *vs.* 71.5±13.9 L). Still, the CL of Dexamethasone was lower than Methylprednisolone (12±4 L/h *vs.* 24±8 L/h).[55]

Efficacy of steroids in AIHA

Table 3 showed about various studies reported that the most widely used steroids for treating AIHA, especially wAIHA are Prednisone (Prednisolone), Methylprednisolone and Dexamethasone. There is no difference between adult and pediatric doses. The recommended dose of Prednisone (Prednisolone) starts from 1-2 mg/kg/day, given as an initial dose for 1-4 weeks, then tapering off the dose until 4-6 months of administration. Methylprednisolone is given with a mega dose of 250-1000 mg/day for 1-3 days or 100-200 mg/day for 7-14 days or 30 mg/kg/day for 72 hours and then tapering off the dose. Meanwhile, Dexamethasone is given as an alternative therapy at 40 mg/day for 1-4 days. The efficacy (response rate) of AIHA patients to steroid administration reached 70-85% after the first 2-3 weeks of steroid administration, but only 20-30% of patients remained in remission after discontinuing steroids.

Effects of long-term steroid administration

Corticosteroids induce diabetes in 20% of patients, worsen pre-existing diabetes (10%), osteoporosis with fractures (10%), and osteonecrosis of the femur (4%).[11,61] Particular types of diabetes can be induced by external factors, such as drug- or chemical-induced diabetes, exocrine pancreatic disorders, and monogenic diabetic syndrome, and steroids are one of the causes.[62] Besides that, the most frequent cause of non-traumatic femoral head osteonecrosis is the use of GCs.[63,64] Apoptosis, vascular endothelial injury, oxidative stress, abnormal fat metabolism, and osteoporosis are some theories that have been put forth.[63,64] And another undesirable effect of GC treatment is the suppression of the HPAA, which can lead to adrenal insufficiency. Table 4 showed the mechanisms of the long-term effects of steroid use.

There are ways to mitigate the adverse side effects of long-term steroid use, including using GC rationally by single morning dose or alternate day dose, ensuring adequate calcium and vitamin D intakes, preventing malnutrition, encouraging early light exercise (walking), avoiding strenuous activity, avoiding to get up suddenly from supine position for prevention of spinal compression, adding therapy of bisphosphonates (Pamidronate, Alendronate, Zoledronate) to reduce fracture risk, and combining therapy with growth hormone (rhGH) agents to prevent growth suppression in children.[65,66] Since there is limited data about the duration and adverse effects of bisphosphonate therapy in children, it should only be used sparingly.[65,67] To prevent fractures in adults and children, there are some recommendations for vitamin D and calcium supplementation. Vitamin D 600-800 units/day combined with calcium 1000-2000 mg/day should be given to adults.[66,68] Then, vitamin D 400-1000 units/day, a maximum of 2000 units/day, is still safe for children. There are some variations of calcium doses for children such as 500 mg/day in children 1-3 years of age, 800 mg/day in children 4-8 years of age, 1000 mg/day in children up to 9 years of age.[69]

Conclusion

Based on the literature studies, Prednisone (Prednisolone) and Methylprednisolone are preferred recommendations as first-line therapy for adults and children in wAIHA over Dexamethasone. Even though Methylprednisolone and Dexamethasone both have almost the same potential in suppressing T cell growth factor and FcR, judging from its pharmacokinetic properties Dexamethasone has a long duration of action (long-acting) compared to Prednisolone and Methylprednisolone. It indicates that Dexamethasone will last longer in the body than Prednisone

Table 3. The updated studies of the use and efficacy of steroids in wAIHA.

Types and Dosage of Steroids	Duration of Administration	Description	Efficacy	References
- Prednisone 1-1.5 mg/kg/day - Methylprednisolone IV 250-1000 mg/day	1-3 weeks until hemoglobin reaches more than 10 g/dL 1-3 days	- If there is a slight improvement in the 2nd or 3rd week, the therapy is considered ineffective; - Treatment for rapid or severe hemolysis; - After stabilization of hemoglobin, prednisone is tapered off 10-15 mg each week with 20-30 mg daily, then 5 mg every 1-2 weeks up to 15 mg, and 2.5 mg every two weeks until the drug is discontinued; - AIHA should be treated for at least 3-4 months with a low dose of prednisone of ≤10 mg/day	- 70-85% of warm AIHA patients; - 14-35% of cold AIHA patients	83
- Prednisone 1-1.5 mg/kg/day - Methylprednisolone 100-200 mg/day - Methylprednisolone 250-1000 mg/day	3-4 weeks 10-14 days 1-3 days	- Being able to enhance hemoglobin and control hemolysis until 70-85%, especially for patients with rapid hemolysis and very severe anemia; - Taper at regular intervals and discontinue after 4-6 months	- 70-85%	30
Predniso(lo)ne 1 mg/kg/day	2-3 weeks	- Prednisolone doses ≤10 mg daily with or without steroid-sparing immunosuppression control AIHA effectively and can be an appropriate long-term therapy for wAIHA	- 1.80% of patients respond to a daily dose equivalent to Prednisone (lone) 60-100 mg; - Although alternative glucocorticoids such as Dexamethasone have therapeutic activity, unlike in ITP, data in wAIHA are sparse; - Early reports concluded that Prednisone (Prednisolone) at doses higher than 60 mg or 1-1.5 mg/kg did not achieve higher response rates. Therefore, most adult patients starting treatment should receive oral Prednisone (Prednisolone) at 1 mg/kg daily	11
- In the first 72 hours, the dose varies widely from 1 to 2 mg/kg/dose of Prednisone every 8-12 hours to high-dose steroids, *e.g.*, Methylprednisolone 250-1000 mg/day - After the first 72 hours, the dose is reduced to 1-2 mg/kg/day in children and 30-80 mg/day in adults	1-3 weeks 1-3 weeks	- The disease is considered responsive to steroids if stabilization of hemoglobin increases >10 g/dL within 1-3 weeks; - After partial remission, steroid doses should be continued for ≥ 6 months with prolonged weaning; - Long-term steroid therapy reduces the risk of recurrence	- About 80% of wAIHA patients respond favorably to steroids within 1 to 3 weeks	70
Adult dosage : - Initial dose of Prednisone 1 mg/kg/day orally or Methylprednisolone IV - If the target is reached, the Prednisone dose is lowered to 20-30 mg/day within one week. After that, it is slowly reduced to 2.5-5 mg/day/month while hemoglobin and reticulocyte levels are monitored - If the patient is still in remission after 3 to 4 months at a dose of 5 mg, steroids can be discontinued	 3 weeks 3 weeks 3 weeks	- The initial dose is given until the hematocrit value is >30% or the hemoglobin level is >10 g/dL; - If the target is not achieved within three weeks, 2nd line therapy is given	- It is not known precisely how many patients remain; - In remission after steroid withdrawal and possibly recover, it is estimated that this occurs in less than 20% of patients	84
Pediatric dose: - Prednisolone 1-6 mg/kg/day. In children, the response is seen at a dose of 1-2 mg/kg - The dose can be increased in the first 72 hours to 4-6 mg/kg/day	 3 weeks - 6 months 3 weeks - 6 months	- The total dose can be given for two weeks in patients who show a partial response after the first three weeks. Then, It is slowly reduced in less than six months. If it does not show any response, switch to 2nd line treatment	- Remission occurs in 80-85% of pediatric patients who respond to Prednisolone administration at 1-2 mg/kg/day	73

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Table 3. The updated studies of the use and efficacy of steroids in wAIHA (cont.).

Types and Dosage of Steroids	Duration of Administration	Description	Efficacy	References
First-line therapy: corticosteroids, *i.e.* , Prednisolone 60-100 mg/day	Several weeks	- Failure of steroid therapy is assessed after 21 days of steroid administration; - If a response is characterized by Hb >100 g/l or after three weeks of steroid administration, the steroid dose needs to be decreased gradually to 20-30 mg for 4-6 weeks and then by 5 mg monthly; - High-dose intravenous methylprednisolone may have a role in fulminant cases, but the risk of severe infection may also be increased; - Data on the use of Dexamethasone are limited but do not suggest that Dexamethasone is superior to Prednisolone	- About 80% of patients respond to corticosteroids, and two-thirds achieve complete remission; - About 20% of patients remain in remission after steroids are discontinued; - 40% can maintain Hb with maintenance Prednisolone doses <15-20 mg	34
Prednisolone 1 mg/kg/day	2 weeks	- Once hemoglobin stabilization is achieved, lower the prednisolone dose to 20 mg/day for two weeks. If hemoglobin remains stable, the dose is further reduced to 10 mg/day every month. After that, steroids may be discontinued after two weeks	- Steroids induce partial remission in 60-70% of patients, and complete remission is achieved in 10-15% of patients	51
Prednisolone 1.5 mg/kg/day	3 weeks, then tapered and discontinued after 2-3 years of treatment		- Response rate achieves 90% (62% complete response and 28% partial response)	72
Dosage in pediatrics: Prednisolone 2 mg/kg/day		- To overcome anemia quickly; - Then maintenance therapy by giving azathioprine		85
The initial dose of oral Prednisone is 1 mg/kg/day or methylprednisolone IV. Tapering Dose: 20-30 mg/day within one week. It was then tapered again slowly to 2.5-5 mg/day every month while monitoring hemoglobin levels and reticulocyte counts. If the patient is in remission after 3-4 months, the dose is reduced to 5 mg/day; then the steroid can be stopped	3 weeks, continued 3-4 months	- The initial dose is given until the hematocrit value is > 30% or the hemoglobin level is > 10 g/dL; - If therapeutic goals are achieved, tapering off Prednisone; - An alternate regimen is given to reduce the adverse effects of steroids		86
Pediatric dose: Prednisone 2-6 mg/kg/day every 8-12 hours or use an initial dose of methylprednisolone above 30 mg/kg/day IV		- After hemoglobin becomes normal, steroids are tapered slowly for about six months; - Tapering too quickly will lead to relapse	- Steroid response can reach above 80% and is seen after 24-72 hours of an initiating dose	87
First-line therapy: - Predniso(lone) 1-2 mg/kg/day for 3-4 weeks - Methylprednisolone 100-200 mg/day for 7-10 days or 250-1000 mg/day for 1-3 days - Dexamethasone 40 mg/day for four days, 2-6 cycles every 2-4 weeks	3-4 weeks, continued 4-6 months 1-3 days 4 days, 2-6 cycles every 2-4 weeks	- Response time: 7-25 days; - Gradual tapering after 4-6 months; - Bolus steroids are given for severe acute conditions (Methylprednisolone IV); - Dexamethasone as first line for CLL in secondary wAIHA	- Response rate: 80-90%	36
- First-line therapy: Prednisone 1 mg/kg per day - Alternative regimen: high dose Dexamethasone (40 mg/day), which is considered equivalent to prednisone standard in primary AIHA	3-4 weeks, then slowly tapered over the following 1-2 months. 4 days	- Dexamethasone is an alternative therapy for treating CLL (chronic lymphocytic leukemia) in secondary AIHA or complications of AIHA but requires further investigation regarding efficacy and safety.		74

Table 3. The updated studies of the use and efficacy of steroids in wAIHA (cont.).

Types and Dosage of Steroids	Duration of Administration	Description	Efficacy	References
- First line: Prednisolone 1 mg/kg/day - Alternative regimen: Dexamethasone 40 mg	3-4 weeks, then slowly tapered 1-2 months 4 days		- The response rate is 84 - 90%; however, patients may become steroid dependent. Therefore, steroids should be tapered slowly over 1-2 months; - Pulsed high-dose Dexamethasone resulted in a 100% response rate (ORR) in 7 refractory cases of secondary AIHA patients with various underlying disorders, with one patient achieving a complete response (CR) and no documentation of adverse events	12
Dosage in paediatrics: Prednisolone 2 mg/kg per day	Until hemoglobin reaches normal or for 6 weeks or earlier	- The dose is reduced/tapered by 10% of the starting dose, and the patient is maintained at this dose for 3-4 weeks before further tapering; - The dose is returned to the starting dose if hemoglobin drops on Prednisolone dose reduction	- Oral Prednisolone showed remission in 81% of patients	49
High-dose Methylprednisolone at 30 mg/kg/day for three days, followed by Prednisone at 2 mg/kg/day	3 days (for high-dose Methylprednisolone)		- Response rate: 70-85%	88
Initial therapy: Prednisone 1 mg/kg/day	3 weeks		- Although about 80% of patients will respond to steroids, only 30% can be completely reduced in steroid dose	89
First-line therapy: Prednisone		- Maintaining hemoglobin levels requires the equivalent of more than 10-15 mg of Prednisone	- Response rate: 70 - 85%. However, only one-third of patients remain in long-term remission after the drug is discontinued, 50% require maintenance doses, and about 20-30% require additional second-line therapy, including splenectomy and other immunosuppressive agents	90
First-line treatment is Glucocorticoids (Prednisone and Methylprednisolone): - Oral Prednisone: 1-2 mg/kg/day - Methylprednisolone intravenous: 500-1000 mg/day		- Start tapering slowly for up to 4-6 months if the hemoglobin level >10 g/dL after 1-3 weeks of treatment.	- It is achieved in 80% of patients over 2-3 weeks. However, only 20-30% of patients remain in remission after Prednisone is discontinued	91
Initial therapy of choice for wAIHA in adults: Prednisone 1-5 mg/kg/day (or 60-100 mg/day)	1-3 weeks	- After a period of stabilization, the steroid dose should be lowered gradually. Sudden dose reduction or rapid progressive reduction may lead to relapse. If relapse occurs, the dose should be increased; - Most physicians consider a daily maintenance dose of prednisone greater than 15 mg to prevent at least 30% of therapeutic failures		92
- Prednisolone/prednisone 1-2 mg/kg for 1-3 weeks - Dexamethasone 4×40 mg/day, 1-4 cycles / 2-4 weeks C4	1-3 weeks Few days		- Early therapeutic response: 70-80%; - Long-term therapeutic response: ≤20%	35
- Prednisone 1-2 mg/kg/day for 28 days - Dexamethasone 40 mg/day for four days	28 days 4 days		- A cohort study of 46 patients showed a response rate of 78%	2
Prednisone 1-1.5 mg/kg/day	3-4 weeks	- Tapering over 4 - 6 months - Tapering too fast has a high risk of relapse	- Response rate: 70-80% - 20-30% of patients have a durable remission after initial therapy, but the rest have chronic relapses - 10-20% of the patient do not respond to corticosteroids or require unacceptably high doses	71

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Table 4. The mechanisms of long-term effects of steroid uses.

Population	Outcome	References
Effects on bones		
Paediatrics and adults	Growth-inhibiting effects by glucocorticoids (GC), as well as inducing osteoporosis, predominantly occur in the first 3-6 months of treatment	38,86,93,94
Paediatrics and adults	- Steroids affecting growth factors in paediatrics have the same mechanism as the mechanism of osteoporosis in geriatrics; - GCs disrupt the homeostatic balance of osteoblast, osteocyte, and osteoclast in bone by affecting RANKL/OPG signaling, WNTs and their inhibitors microRNAs, IL-11, BMP/notch signaling, and apoptotic effectors, leading to suppression of osteoblastogenesis in the bone marrow and promoting osteoblast and osteocyte apoptosis; - GCs also directly affect 11β-HSD expression, thereby increasing resorption activity and promoting osteoclast growth and differentiation, leading to increased osteoclast survival and reduced osteoclast production; - In addition, GC will interfere with calcium absorption in the gastrointestinal tract, resulting in decreased calcium absorption and increased renal calcium loss, resulting in increased bone remodelling and osteoclastic activity	66,95,96
Effects on the metabolism of blood glucose		
Adults	- GC have been shown to impair pancreatic β-cell function after two weeks of exposure to GC; - Administration of high doses of prednisone for 2-3 months will trigger an increased incidence of diabetes, usually preceded by mild hyperglycemia that occurs between the second and fourth weeks of Prednisone administration; - Several studies show several mechanisms by which steroids can alter insulin secretion, increase endogenous glucose production, increase gluconeogenesis and antagonize the metabolic action of insulin, enhance the effects of other counter-regulatory hormones, such as glucagon and epinephrine that increase endogenous glucose synthesis, induce increased expression of nuclear peroxisome proliferator-activated receptor necessary for increased endogenous glucose production, reduce peripheral glucose uptake at the level of muscle and adipose tissue, inhibit insulin production and secretion from pancreatic cells and induce cell failure indirectly by lipotoxicity	62,97–100
Effects on the hypothalamic-pituitary-adrenal axis (HPAA)		
Paediatrics andaAdults	- Factors that influence the risk of GC-induced adrenal insufficiency include the amount of daily dose administered, duration of therapy (daily administration for more than 2-4 weeks), mode of administration, the timing of administration (night time administration), type of GC used (based on short, medium or long duration), as well as other concomitant medications that may interfere GC metabolism and individual susceptibility; - GC entering the systemic circulation will give negative feedback to the HPAA by decreasing the hypothalamic corticotropin-releasing hormone (CRH). This will cause an acute effect on the pituitary by suppressing the synthesis of pro-opiomelanocortin (POMC) which results in a decrease in the amount of adrenocorticotropic hormone (ACTH) and other POMC-derived peptides; and, in the long term, will cause the development of atrophy of corticotroph cells and Crooke cells. Without ACTH, the adrenal cortex will lose the ability to produce cortisol and androgens and may eventually atrophy. When the hormone cortisol is inadequately produced, responses to stressors (*e.g.*, trauma, surgery, inflammation) may be impaired, and defences against infection will be inadequate; - Daily cortisol production is about 10 mg in healthy people and can increase to 400 mg in severe stress conditions. In other studies, the normal production of cortisol is 6-9 mg/m^2 Endogenous cortisol concentrations show a circadian pattern, where high concentrations are reached in the morning between 6-9 AM (approximately 160 µg/L at 8 am in healthy people) and low concentrations at night between 8 PM and 2 AM. Therefore, to reduce the risk of HPAA suppression by GC, GC should be given in the morning when cortisol levels are still high	11,55,65, 101–103

and Methylprednisolone, and Dexamethasone can suppress the hypothalamic-pituitary-adrenal axis (HPPA) most potently compared to other steroids. Hence, it is more at risk of causing adverse effects in long-term use compared to Prednisone and Methylprednisolone. On long term-use, steroids affecting growth factors in pediatrics have the same mechanism as osteoporosis in geriatrics, which both disrupt the homeostatic balance of osteoblast, osteocyte, and osteoclast. Steroids also affect blood glucose metabolism, which can lead to diabetes and affect the HPPA. So, we suggest tapering the dose of steroids gradually and adding supplement therapy such as vitamin D, calcium, and bisphosphonates to protect the bones in adults and children.

Authors Contribution

YT and FNU were involved in concepting the topic of the manuscript. SDY prepared the manuscript draft, and designed the tables. All authors took parts in giving critical revision of the manuscript.

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