Approaches in Hematology

Hematology Made Easy

British Society of Hematology BSH guidelines for Classification & approach of AIHA

Background

- AIHA is an acquired hemolysis caused by the host's immune system acting against its own red cell antigens.
- The incidence is approximately 1 per 100 000/year.
- It can occur at any age, but incidence rises with increasing age.
- Serologically, cases are divided into warm type (65%), cold type (29% cold haemagglutinin disease [CHAD], 1% paroxysmal cold hemoglobinuria) or mixed AIHA (5%). Approximately half are primary (idiopathic) AIHA, and half are secondary to associated disorders.

Classification of autoimmune hemolytic anemia:

A. Warm AIHA could be:
   - ❖ Primary (idiopathic)
     Or
   - ❖ Secondary to
     - ➢ Neoplasia (CLL, Lymphoma, Solid organ)
     - ➢ Infection (e.g., Hepatitis C, HIV, CMV, VZV, Pneumococcal infection, Leishmaniasis, Tuberculosis)
     - ➢ Immune dysregulation
     - ➢ Connective tissue disorders (e.g., SLE, Sjögren syndrome, Scleroderma)
     - ➢ Ulcerative colitis, PBC, Sarcoidosis
     - ➢ Post transplantation
     - ➢ Immune deficiency syndromes (e.g., CVID)

B. Cold AIHA includes:
   - Cold Haemagglutinin Disease CHAD might be:
     - Primary (idiopathic)
       Or
     - Secondary to
       - ➢ Malignancy (e.g., CLL, NHL, Solid organ)
       - ➢ Infection (e.g., Mycoplasma, Viral infections, including IM)
       - ➢ Autoimmune disease
       - ➢ Post-allogeneic HSCT
   - Paroxysmal Cold Hemoglobinuria PCH could be:
     - Primary
       Or

- Secondary to Infection (e.g., Adenovirus, Influenza A, Syphilis, CMV, IM, VZV, Measles, Mumps, Mycoplasma pneumoniae, Hemophilus influenzae, E. coli)

C. Mixed type AIHA
   - Primary
     Or
   - Secondary to lymphoma, SLE, Infection

Presentation of AIHA:

- Patients with AIHA may present with symptoms of anemia.
  - Weakness 88%
  - Dizziness 50%
  - Dyspnea 9%
  - Hemolysis (jaundice 21%, dark urine 3%)

- Symptoms of an underlying disorder.
- Without underlying disease, examination may be unremarkable or reveal:
  - Mild pallor
  - Splenomegaly.
  - Less often, severe hemolysis leads to hepatosplenomegaly, hemoglobinuria, and signs of heart failure.

- Cold haemagglutinin disease (CHAD) can present as a primary chronic clonal disorder, usually occurring in middle age or in the elderly.
  - Cold-induced acrocyanosis (dusky blue appearance of toes, fingers, nose tip or ears).
  - Raynaud phenomenon occur in 40–90% of patients.
  - Secondary CHAD can be self-limiting, for example following childhood infection, with its different natural history, secondary CHAD has also been termed cold agglutinin syndrome.

- ✨Paroxysmal cold hemoglobinuria (PCH) is typically transient, presenting 1–2 weeks after an upper respiratory tract infection or other childhood illness with 🔄
  - Acute fever
  - Abdominal, back or leg pain
  - Hemoglobinuria
  - Hemolysis can be severe and intravascular but usually settles over several weeks.

Diagnostic approach to suspected AIHA:

Three questions should be considered. Is there hemolysis, is the hemolysis autoimmune, and what is the type of AIHA?

1) Is there hemolysis?

- Typical laboratory findings in patients with hemolysis:
  - Bilirubin (unconjugated): increased.
  - Reticulocyte count: increased.
  - Lactate dehydrogenase (LDH): may be normal or increased.
  - Haptoglobin: reduced.
  - Blood film: spherocytes, agglutination or polychromasia
  - Urinalysis/dipstick test: positive for blood, but urine microscopy negative for red cells. if hemolysis is intravascular, leading to hemoglobinuria.
  - Urinary hemosiderin - can be detected approximately 1 week after onset of intravascular hemolysis.

- However, there may be confounding factors as these laboratory tests are not highly specific. Some parameters may be normal, especially with mild compensated hemolysis.

- The differential diagnosis of hemolytic anemia is shown below:

  - Hereditary
    - Membrane disorders (e.g., HS, HE)
    - Enzyme disorders (e.g., G6PD, PK deficiency)
    - Hemoglobinopathies (e.g., SCD, Unstable hemoglobins)
  - Acquired
    - Immune
      - Autoimmune (e.g., Warm or cold AIHA)
      - Alloimmune (e.g., HTR, post-allogeneic HSCT) HTR = hemolytic transfusion reaction
      - Drug induced.
    - Non-immune
      - Infection (e.g. Malaria, Clostridium perfringens)
      - Mechanical (e.g. Prosthetic heart valve)
      - PNHTMA (e.g. TTP, HUS)
      - Hypersplenism
      - Oxidant substances (e.g. Dapsone, Arsine gas, Amyl nitrite)
      - DIC

2) Is the hemolysis immune?

- A positive direct antiglobulin test (DAT) indicates the presence of immunoglobulin (Ig)G, IgM, IgA or complement (usually C3d) bound to the red cell membrane.

- In the presence of hemolysis, this suggests an immune etiology, but clinical assessment is required before a diagnosis of AIHA can be made. Typically, monospecific anti-IgG and

anti-C3d antibodies are used in the initial screening and these help to determine the type of AIHA.

- A positive DAT is not specific and is also associated with a wide range of non-hemolytic disease states, possibly through passive deposition of immunoglobulins or immune complexes; examples include liver disease, chronic infection, malignancy, systemic lupus erythematosus (SLE), renal disorders and drugs such as intravenous immunoglobulin (IVIg) or anti-thymocyte globulin.

- The DAT: Recommendation (direct coomb's test)

- At a minimum, the DAT should include monospecific anti-IgG and anti-C3d.
- DAT positive → evidence of hemolysis

- Before diagnosing AIHA, ask the following 4 questions:
  1) Is there a history of blood transfusion in the last 3 months?
     - Consider a delayed hemolytic transfusion reaction (HTR)
  2) Has the patient received a solid organ or allogeneic hematopoietic stem cell transplant (HSCT)?
     - Consider alloimmune hemolysis caused by major ABO mismatch (HSCT) or passenger lymphocyte syndrome (PLS) (solid organ or HSCT).
  3) Has the patient received any relevant drugs?
     - Consider drug-induced immune hemolytic anemia (DIIHA).
  4) Is there another known cause of hemolysis?
     - Given the high prevalence of an incidental positive DAT within the hospital population, consider whether there is an alternative cause of hemolysis or abnormal laboratory values.

- DAT-negative AIHA (around 5 % of cases):
  Rarely, AIHA patients test negative with a tube test DAT, for example due to a low affinity antibody, low levels of red cell bound antibody, or an immunoglobulin not tested for (e.g. IgA-only AIHA).

- A gel column agglutination method is a more sensitive method that is less prone to error than a conventional tube test.

- AIHA can be diagnosed in 3% of patients testing negative with a gel card method by using a red cell elution technique.

- The Donath-Landsteiner test may be considered in children with hematuria and is discussed under investigations. Patients with DAT-negative AIHA generally have a milder anemia and are steroid responsive.

Recommended investigation of DAT-negative hemolysis:

- In patients with unexplained hemolysis and a negative screening DAT, retest with a column agglutination DAT method that includes monospecific anti-IgG, anti-IgA and anti-C3d. If also negative, consider preparing and investigating a red cell eluate.

Investigations:

The most relevant tests to investigate for an underlying cause for AIHA are shown below:

i. Primary evaluation: Hemolytic screen → FBC, blood film, LDH, haptoglobin, bilirubin, DAT, reticulocyte count ± urine for hemosiderin or urine dipstick and microscopy

ii. Detection of underlying disorders

- Investigation of AIHA
  - Serum Igs and electrophoresis with immunofixation
  - HIV, HBV, HCV
  - Anti-dsDNA, ANA
  - CT chest, abdomen, and pelvis

- Additional investigation in selected patients with AIHA
  - Bone marrow examination:
    CHAD, age ≥60, features in history, examination, FBC or film suggesting possible marrow infiltration.
  - U&E, LFT, clotting, BP, urine dipstick:
    If pregnant or thrombocytopenic, to exclude DIC or pregnancy-associated TMA.
  - Infection screening: Dependent on symptoms, travel history and age.
  - Parvovirus hematinic screening: If reticulocytopenia occurs

- Additional serological investigation in selected patients with AIHA
  - If DAT positive for C3 ± IgG → Do direct agglutination test (DAggT).
  - If DAggT positive → Do cold antibody titer.
  - If DAT-negative AIHA suspected → Do monospecific DAT for IgM, G, A, C3.
  - If (monospecific) DAT- negative AIHA suspected → Do red cell eluate.
  - If DAT is positive for C3 ± IgG and (DAggT negative or insignificant CAs) and (age <18 years or hemoglobinuria or cold associated symptoms or atypical serology) → Do Donath-Landsteiner

- Cold auto-agglutinin thermal amplitude If clinical significance of cold auto-agglutinin unclear

Important Notes:

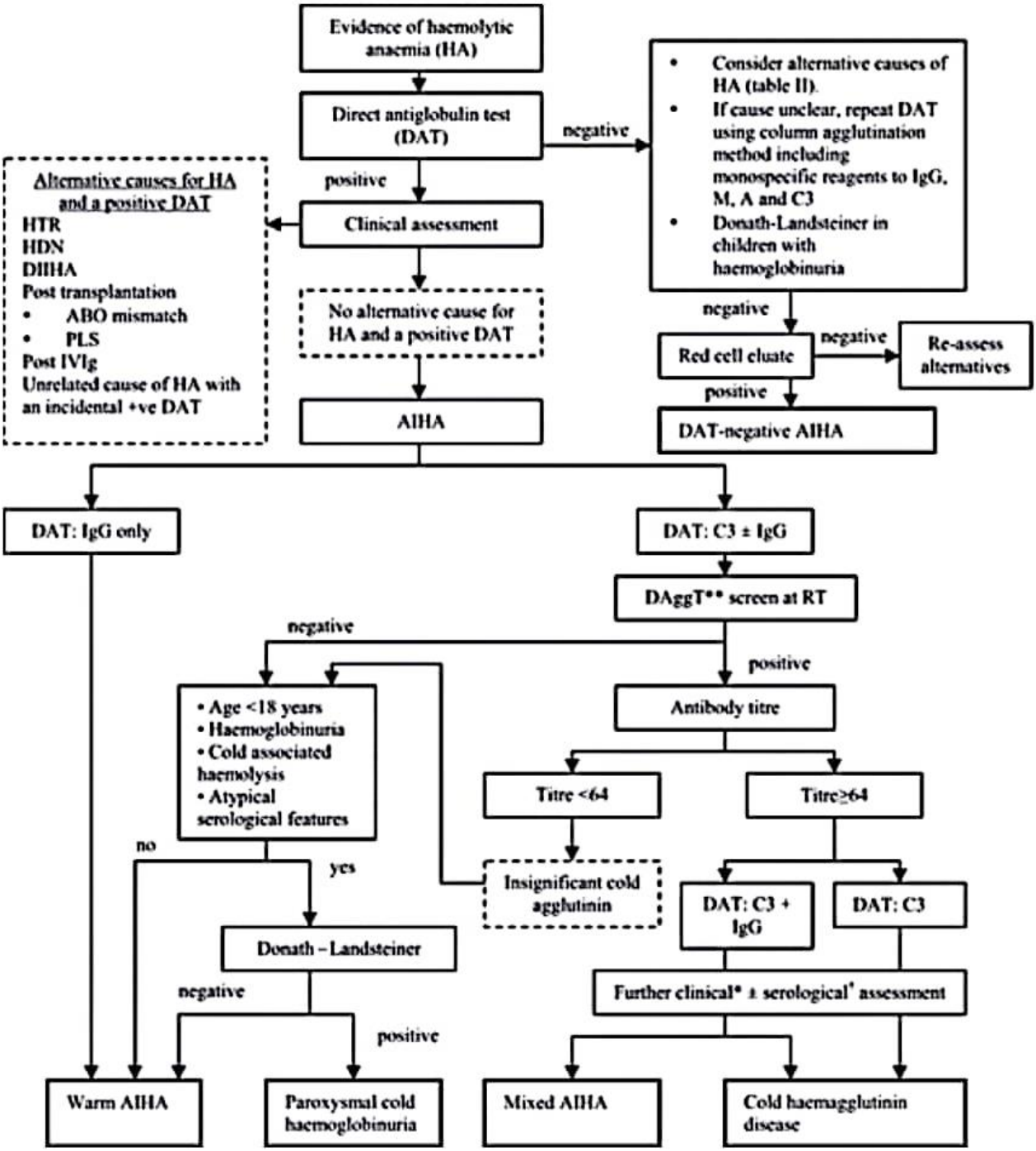
- Although reticulocytopenia can occur in the acute phase of AIHA; hematinic deficiency, marrow infiltration, aplastic anemia and parvovirus B19 infection should be considered if it is present.

باعتبارها واحده من اسباب ال hemolytic معلومه هاامه ،، وهذا استثناء وليس القاعده الطبيعى ان ال AIHA بتعلى مع ال retics %

- Further serological investigation is required to determine the type of AIHA (e.g. warm, CHAD, PCH) as the approach to treatment differs.
- Finally, if the patient requires blood, investigations are needed to exclude underlying alloantibodies and identify units suitable for transfusion. In adults, two 7 ml EDTA samples

are usually sufficient for initial serological investigation. A clotted sample is also required for investigation of suspected PCH or DIIHA.

- CHAD can be diagnosed in patients with AIHA and a DAT positive to C3 ± IgG, with a consistent clinical picture and a high titre cold reactive antibody. The thermal amplitude may be considered as a supportive serological investigation where diagnostic uncertainty exists.



Treatment strategy of AIHA (BSH guidelines)

Primary Warm AIHA

- First line treatment:

ℛ Prednisolone 1 mg/kg/day.

- 80% of patients respond to steroids at a dose equivalent to prednisolone 60–100 mg/day.
- Nearly two-thirds of the patients achieve complete remission (CR).
- The initial response may take several weeks but absence of response by 21 days should be considered a steroid failure.
- In responding patients, an incremental taper can begin, for example once Hb >10 g/dl or after a maximum of 3 weeks, reducing to 20–30 mg over 4–6 weeks, and then by 5 mg every month.
- Relapse was more common if steroids were tapered to ≤10 mg in less than 2 months and if stopped in less than 6 months.
- Approximately 20% of patients remain in remission after steroids are discontinued.
- Although a further 40% can maintain an acceptable Hb on maintenance prednisolone <15–20 mg, due to the long-term side effects of steroids, second line therapy should be considered.
- Dexamethasone data are limited but do not suggest that dexamethasone is superior to prednisolone.

- Second line treatment

ℛ Rituximab

ℛ Splenectomy.

- The best-studied and most efficacious treatments used.
- Second line therapy should be considered if:
  1) No response to 1 mg/kg/day after 3 weeks.
  2) Relapse during or after steroid reduction
- Approximately 70% of cases respond to splenectomy but even higher response rates are reported with rituximab.
- Following splenectomy, refractory or relapsing patients often require immunosuppression, and the rate of serious infection appears higher post-splenectomy.
- Given the significance of infection and chronic course of AIHA, most patients will benefit from an effective steroid-sparing agent prior to consideration of splenectomy.

Rituximab

- Response rates of 100% have been reported following rituximab for primary warm AIHA.
- Prior splenectomy does not adversely affect outcome, although better outcome is associated with shorter duration of AIHA.
- Median time to response is approximately 3–6 weeks (range 2–16 weeks). The long-term remission rate is unknown, but relapse occurs in 14–25% after a median of 15–21 months.

- Rituximab is largely well tolerated although severe neutropenia, transient infusion-related reactions or infections have been reported.
- Reactivation of hepatitis B virus (HBV) is a potentially fatal complication and pre-administration screening with serology for HBV surface antigen and HBV core antibody is recommended.
- Progressive multifocal leukoencephalopathy is a rare complication.
- The standard regimen is 375 mg/m2 weekly for four consecutive weeks

- Third line treatment

- The treatment options are listed alphabetically so as to show no preference for a particular therapy.
  - ℛ Azathioprine
  - ℛ Ciclosporin
  - ℛ Danazol
  - ℛ Mycophenolate mofetil
  - ℛ Splenectomy

Azathioprine

- Approximately 60% of AIHA patients respond to azathioprine 100–200 mg/day 2–2·5 mg/day with prednisolone.

- However, the number achieving steroid independence and the duration of response is unclear. Thiopurine methyltransferase (TPMT) deficiency prevents azathioprine metabolism and should be excluded prior to commencing therapy.

Ciclosporin

- Case reports and small series suggest some efficacy in AIHA. Where specified, the ciclosporin dose was typically 5 mg/kg/day.

Danazol

- Six out of 7 patients with primary warm AIHA (3 treated first line) responded to danazol 200 mg 3–4 times/day, added to prednisolone while, 3/3 patients with secondary AIHA responded to danazol 200 mg three times daily.

Mycophenolate (MMF)

- Small series and case reports suggest some efficacy in primary and secondary AIHA.
- Most patients had received multiple previous therapies and were treated with MMF 500mg twice daily, titrated up to 1 g twice daily. Responses typically took 3–4 months.

Splenectomy

- Larger series of unselected patients with AIHA suggest 50–85% of patients respond (improved Hb or increased sensitivity to steroids). Response rates appear higher in primary vs. secondary AIHA.
- Most responses occur within the first few months of surgery but slower responses (5–6 months) have been reported. Approximately a third of patients relapse after splenectomy.

- Radioisotope studies to determine the main site of red cell destruction are not currently recommended when considering splenectomy.
- Patients should be counselled on infection risk and be vaccinated at least 2 weeks before splenectomy.
- There should be a low threshold for investigating patients with post-operative fever, abdominal pain or ileus with Doppler ultrasound to exclude portal or splenic vein thrombosis.
- Patients without a contra-indication should receive thromboprophylaxis with low molecular weight heparin (LMWH) following splenectomy.
- Extended prophylaxis following discharge may be considered in patients considered high risk.
- After splenectomy, patients should be discharged on prophylactic antibiotics, provided with a course of antibiotics for emergency use and given advice on risk factors for infection. Long-term follow-up should be organized for revaccination in primary or secondary care.

- Treatment options for patients failing third line therapies.

℟ **Alemtuzumab**
  - Case reports suggest some efficacy in AIHA although dosing regimens have varied.

℟ **Cyclophosphamide**
  - Although some success has been reported with low dose oral cyclophosphamide (e.g. 50–100 mg daily) with or without prednisolone.
  - Higher intravenous doses also appear effective, for example 50 mg/kg/day for 4 days or 1 g monthly for 4 months.

℟ **Hematopoietic stem cell transplantation (HSCT)**
  - Given that toxicity and treatment-related mortality is significant, HSCT should be restricted to carefully selected patients with refractory life-threatening disease following multidisciplinary review.

Warm AIHA caused by IgA antibodies.

- Warm AIHA caused by isolated IgA occurs in 0·1–2·7% of cases and usually responds to conventional treatment including steroids and splenectomy.

Mixed AIHA

- Mixed AIHA is usually described as causing severe hemolysis.
- Approximately 50% are primary while secondary cases are often associated with SLE.
- Mixed AIHA is steroid responsive but most often leads to chronic hemolysis. Splenectomy was unsuccessful.

- Occasional success has been reported with IVIg and plasma exchange for acute hemolysis, with chemotherapy for underlying lymphoma and with cyclophosphamide.

℞ First line therapy for mixed AIHA is prednisolone 1 mg/kg/day.
℞ If AIHA is secondary, optimize treatment of the underlying disorder.
℞ If AIHA is primary, consider immunosuppression as second line therapy similar to primary warm AIHA.

Primary Cold Haemagglutinin Disease (CHAD)

Recommendations:

- Patients should be advised to avoid cold exposure where possible.
- For all patients, avoid cold exposure where possible to reduce the risk of severe exacerbations, dressing to protect the head, face, and distal extremities in cold weather.
- Indications for treatment: symptomatic anemia, severe circulatory symptoms or transfusion dependence

℞ First line treatment: rituximab, or if clonality has been demonstrated, the addition of fludarabine may be considered.
The overall response rate to rituximab 375 mg/m2 weekly for 4 weeks was 51%

Rescue (emergency) therapy

- General Strategies for all AIHA:
  - Investigations may reveal a treatable underlying cause, such as infection.
  - If drug induced AIHA is suspected, relevant medication should be stopped.
  - Blood transfusion
  - Full compatibility testing can take 4–6 h or more.
    - If anemia is life threatening in the time required for full compatibility testing, transfuse with ABO, Rh and K matched red cells.
    - Consider the use of a blood warmer for transfusion in patients with cold AIHA (CHAD, mixed AIHA and PCH)

- Warm AIHA in emergency: These are the options available in emergency situations:
  - Consider IVIg or plasma exchange for severe or life-threatening anemia.

℞ Immunoglobulins: Evidence from case series suggests that 40% of patients respond to IVIg 0·4–0·5 g/kg/day for 5 days and most responders maintained their Hb for ≥3 weeks

℞ Plasma exchange: The evidence for plasma exchange is largely limited to case reports and any benefit is temporary. Plasma exchange has been used in patients with severe hemolysis while attempting control with other therapies, such as immunosuppression.

- ℛ Methylprednisolone: The experience of high dose intravenous methylprednisolone is limited to case reports. Methylprednisolone may have a role in fulminant cases, but the risk of serious infections may also increase.

- ℛ Emergency splenectomy and splenic embolization
  - Patients with severe transfusion-dependent hemolysis who have not responded to immunosuppression may require urgent splenectomy.
  - If the patient is not vaccinated 2 weeks prior to splenectomy, this should be deferred until 14 days post-splenectomy as functional antibody responses are improved.
  - Case reports have documented success with partial splenic embolization.

Primary Cold Haemagglutinin Disease (CHAD) in emergency

- Consider plasma exchange or steroids for severe or life-threatening anemia.

- ℛ Steroids in primary CHAD:
  - The overall response of CHAD to steroids can be disappointing with response rates of 14–69% in larger series.
  - Responses are often partial, and cannot be sustained without an unacceptably high steroid dose. However, given limited therapeutic options, a trial of prednisolone 1 mg/kg/day may be considered as a rescue therapy.

- ℛ Plasma exchange
  - Responses are often transient and like warm AIHA, its role may be in stabilizing patients with severe disease in conjunction with alternative therapy.
  - Agglutination can occur within the cell separator and its tubing, especially if the agglutinin is active at 37°C and the room and extracorporeal circuit may need a high temperature setting.
  - Daily or alternative day exchange of 1–1·5 times plasma volume with albumin has been recommended.

Non-emergency management

- ℛ Thromboprophylaxis: Venous thrombo-embolism (VTE) prophylaxis
  - Thromboprophylaxis with low molecular weight heparin is recommended for in-patients with an acute exacerbation of hemolysis and should be considered in ambulatory patients during severe exacerbations (Hb <8.5 g/dl)
- ℛ Patients with AIHA should receive folic acid supplementation.
- ℛ Gastric protection by using additional PPI to steroids.
- ℛ Osteoporosis prevention:

- Osteoporotic (particularly vertebral) fracture occurs in up to 30–50% of adults receiving long term glucocorticoids.
- All patients should receive oral calcium and vitamin D supplements while taking corticosteroids.
- Calcium and vitamin D supplements (typically 1200–1500 mg calcium and 800–1000 units vitamin D) reduce bone loss and are recommended for all patients receiving corticosteroids.
- Postmenopausal women and men age ≥50 years commencing corticosteroids should receive a bisphosphonate when treatment is anticipated to be ≥3 months at a dose of prednisolone ≥7·5 mg/day.