IRAQI SOCIETY OF HEMATOLOGIST
GUIDELINES GROUP
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IRAQI GUIDELINE FOR ACQUIRED APLSTIC ANEMIA

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Guideline Title

Guidelines on the diagnosis and management of Acquired Aplastic Anemia

Disease/Condition

- Acquired aplastic anemia (AAA)

Guideline Category

- Diagnosis and Evaluation
- Management and Treatment

Clinical Specialty

- Hematology

Intended Users

- Clinical hematologists

Guideline Objective

- The objective of this guideline is to provide healthcare professionals with clear guidance on the diagnosis and management of patients with AAA. The guidance may not be appropriate to patients with inherited AA.

Target Population

- Adult patients with confirmed diagnosis of AAA.

Methods Used to Formulate the Recommendations

- Expert Consensus

Description of Methods Used to Formulate the Recommendations

- The guideline group was selected to be representative of Iraq experts in hematology. Recommendations are based on the systematic review of published English language literature and guidelines. The writing group produced a draft guideline, which was reviewed and revised by members of the Iraqi Society of Hematology.

Interventions and Practices Considered

Diagnosis/Evaluation

1. FBC, reticulocyte count and blood film
2. HbF%
3. Bone marrow aspirate and biopsy, including cytogenetics
4. Peripheral blood chromosomal breakage analysis to exclude Fanconi anemia if age < 50 years
5. Flow cytometry for GPI-anchored proteins (or Ham test)
6. Urine hemosiderin if Ham test positive or GPI-anchored protein deficiency
7. Vitamin B12 and folate
8. Liver function tests
9. Viral studies: Hepatitis A, B, C, EBV, CMV and HIV
10. Anti-nuclear antibody and anti-dsDNA
11. Chest X-ray
12. Abdominal US scan and ECHO
13. Peripheral blood gene mutation analysis for dyskeratosis congenita if clinical features or lack response to immunosuppressive therapy

Treatment/Management

I. Supportive care
   1. Transfusion support
   2. Hematopoietic growth factors
   3. Prevention of infection
   4. Treatment of infection
   5. Iron chelation therapy
   6. Vaccination

II. Specific treatment
   1. Allogenic bone marrow transplant (BMT)
   2. Immunosuppressive therapy (IST)

Introduction and Epidemiology

- Aplastic anemia is defined as
    - **pancytopenia** (at least two of the following (i) hemoglobin < 100 g/l (ii) platelet count < 50 x 10^9/l (iii) neutrophil count < 1.5 x 10^9/l) and
    - **hypocellular marrow** in the absence of an abnormal infiltration and no increase of reticulin fibrosis.

- A summary review of the incidence of AA in Europe and North America is around 2 per million population per year. The incidence is 2–3 times higher in East Asia reaching to 7.5 per million among Chinese population.
  There is a biphasic age distribution with peaks from 10 to 25 years and >60 years. In almost all studies, there is no significant difference in incidence between males and females, which is unusual for immune-mediated diseases.

Diagnosis and Evaluation

- Clinical presentation: anemia, haemorrhage and infection.
- Careful history and clinical examination is important to help exclude rarer inherited forms.
- A detailed drug and occupational exposure history should always be taken.

Laboratory Assessment

1. ***Full blood count, reticulocyte count, blood film and % HbF***
    - Early stages → isolated cytopenia, particularly thrombocytopenia.
    - Later, pancytopenia (all blood counts are uniformly depressed) although the lymphocyte count is preserved.
    - Anemia accompanied by reticulocytopenia, and macrocytosis is commonly noted.
    - It is essential to exclude the presence of dysplastic neutrophils and abnormal platelets, blasts and other abnormal cells, such as hairy cells (as seen in HCL).
    - The monocyte count may be depressed but the absence of monocytes should alert the clinician to a possible diagnosis of HCL.
    - Anisopoikilocytosis is common and neutrophils may show toxic granulation.
    - Platelets are reduced in number and mostly of small size.
    - HbF should be measured pre-transfusion in children as this is an important prognostic factor in pediatric myelodysplastic syndrome (MDS).

2. ***Bone Marrow examination***

- Both aspirate (assess morphology) and trephine biopsy (assess severity, residual hemopoiesis and to exclude an abnormal infiltrate) are required.
- Bone marrow (BM) aspiration and biopsy may be performed in patients with severe thrombocytopenia without platelet support, providing that adequate surface pressure is applied.
- Difficulty obtaining fragments should raise the suspicion of a diagnosis other than AA.
- The fragments and trails are **hypocellular** with prominent fat spaces and variable amounts of residual hemopoietic cells.
- Erythropoiesis is reduced or absent, **dyserythropoiesis** is very common and often marked (this alone should not be used to make a diagnosis of MDS).
- Megakaryocytes and granulocytic cells are reduced or absent; dysplastic megakaryocytes and granulocytic cells are not seen in AA.
- Lymphocytes, macrophages, plasma cells and mast cells appear prominent.
- In the early stages of the disease, hemophagocytosis may be prominent, as well as background eosinophilic staining representing interstitial oedema.
- The **trephine** is hypocellular throughout but sometimes it is patchy, with hypocellular and cellular areas "hot spot". Thus, a good quality trephine of at least **2 cm is essential**.
- Care should be taken to avoid tangential biopsies as subcortical marrow is normally 'hypocellular'.
- Sometimes lymphoid aggregates occur in AA; in acute phase of the disease or in association with autoimmune diseases.
- The reticulin is not increased and no abnormal cells are seen.
- Increased blasts indicates a hypocellular MDS or evolution to leukaemia.

3. ***Cytogenetic Investigations***

- Abnormal cytogenetic clones occur in up to 12% of patients with AA.
- FISH analysis in particularly for chromosomes 5 and 7.
- The presence of abnormal cytogenetics at presentation in children, especially monosomy 7, should alert to the likelihood of MDS.
- Abnormal cytogenetic clones may arise during the course of the disease.

4. ***Tests to detect a PNH clone***
    - Peripheral blood flow cytometry for GPI-anchored proteins CD55 and CD59 (sensitive and quantitative for PNH clones detection)
    - Small PNH clones, in the absence of hemolysis, occur in up to 50% of patients with AA.
    - The Ham test may be negative if the patient had a recent transfusion, whereas GPI-deficient red cells may still be detected by flow cytometry.
    - Urine should be examined for hemosiderin to exclude intravascular hemolysis which is a constant feature of hemolytic PNH.

5. ***Liver function tests and viral studies***
    - Liver function tests LFTs should be performed to detect antecedent hepatitis, but in post-hepatitic AA the serology is most often negative for all the known hepatitis viruses.
    - The onset of AA occurs 2–3 months after an acute episode of hepatitis and is more common in young males.
    - Blood should be tested for hepatitis A antibody, HBsAg, hepatitis C antibody and EBV.
    - CMV and other viral serology should be assessed if BMT is being considered.

6. ***Vitamin B12 and folate levels***
    - Vitamin B12 and folate (pancytopenia in severe megaloblastic anemia)
    - If B12 or folate deficiency, it should be corrected before diagnosis of AA.
    - BM aplasia due to vitamin deficiency is exceedingly rare.

7. ***Autoantibody screen***
    - Blood should be tested for anti-nuclear antibody (ANA) and anti-DNA antibody in all patients presenting with aplastic anemia.
    - Pancytopenia in SLE may be an autoimmune in nature with cellular marrow or associated with myelofibrosis or rarely hypocellular bone marrow.

8. ***Screen for inherited disorders***
    - Peripheral blood lymphocytes should be tested for chromosomal breakage to identify or exclude Fanconi anaemia.
    - This should be performed in all patients who are BMT candidates.
    - Siblings of Fanconi anaemia patients should also be screened.

9. Radiological investigations

- A chest X-ray is useful at presentation to exclude infection and for comparison with subsequent films.
- Abdominal ultrasound: an enlarged spleen and/or enlarged lymph nodes raise the possibility of a malignant hematologic disorder as the cause.
- In younger patients, abnormal or anatomically displaced kidneys are features of Fanconi anaemia.

Differential diagnosis

1. Hypocellular MDS/acute myeloid leukaemia (AML)
2. Hypocellular acute lymphoblastic leukaemia (ALL)
3. Hairy cell leukaemia
4. Hodgkin's and Non-Hodgkin's lymphomas
5. Mycobacterial infections
6. Anorexia nervosa
7. ITP

Severity Assessment

The severity of the disease is graded according to the blood count parameters and bone marrow findings

Severe AA (Camitta et al, 1975)

- BM cellularity < 25%, or 25-50% with <30% residual hematopoietic cells.
  AND
- 2 out of the following 3 factors:
  - Absolute neutrophil count < 0.5 X 109 /L
  - Platelet count <20 X 109 /L
- Reticulocyte count <20 X 109 /L

Very severe AA (Bacigalupo et al, 1988)

- As for severe AA but neutrophils < 0.2 X 109 /L

Non-severe AA

- Patients not fulfilling the criteria for severe or very severe AA.

Treatment and Management

When to treat?

- SAA and VSAA almost always need immediate and definitive treatment

Whom to treat?

- NOT elderly, NOT feeble, NO serious comorbidity

What definitive treatment is?

- Allo BMT or IST

What is the immediate measure?

- Supportive care

Response Assessment

Responses should be confirmed by two or more blood counts at least 4 weeks apart, and should ideally be measured in patients who are not receiving hemopoietic growth factors.

Response criteria for SAA

None ----------Still severe

Partial ----------Transfusion independent
No longer meeting criteria for severe disease

Complete ----------Hemoglobin normal for age
Neutrophil count >1.5 x 10^9/l
Platelet count >150 x 10^9/l

Response criteria for NSAA

None ----------Worse or not meeting criteria below

Partial ----------Transfusion independence (if previously dependent)
or doubling or normalisation of at least one cell line
or increase of baseline haemoglobin of >30 g/l (if initially <6)
or increase of baseline neutrophils of >0.5 x 10^9/l (if initially < 0.5)
or increase of baseline platelets of >20 x 10^9/l (if initially <20)

Complete ---------- Same criteria as for severe disease

Refractory SAA: blood counts still fulfilling criteria for severe pancytopenia 6 months after initiation of IST.

Relapsed defined when reintroduction of IST is required for decreasing blood counts, usually but not always, accompanying reinstitution of transfusions. (oscillating numbers can occur normally in the setting of infection)

Supportive Care

1. ***Transfusional support to maintain a safe blood count***
   - Platelet transfusion
     - Prophylactic platelet transfusions when the platelet count is **<10 x 10^9/l** (or **<20 x 10^9/l** in the presence of fever).
     - For invasive and surgical procedures, platelet transfusion(s) must be given to achieve appropriate levels as recommended by BCSH guidelines.
     - Alloimmunization to leucocytes (HLA and non HLA antibodies) can result in platelet refractoriness and increased risk of graft rejection after allogeneic BMT ➔ pre-storage leucocyte depletion of all units of red cells and platelets.
     - HLA-matched platelets should be used for patients with alloimmunization.
     - Other caused of platelets refractoriness are infections and drugs
   - Red cell and platelet transfusions should be given to maintain a safe **Hb level (>80** g/l, although this will depend on co-morbidities) and safe platelet count and not be withheld for fear of sensitising the patient.
   - Good dental hygiene, the use of oral tranexamic acid and control of menorrhagia with norethisterone are measures help to prevent bleeding
   - CMV-negative blood products until the patient's CMV status is known
   - *The irradiated blood components empirically used for patients receiving IST and continue until the lymphocyte count recovers to > 1.0 x 10^9/l.*
   - Granulocyte transfusions can be used as supportive therapy in patients with life-threatening neutropenia.

2. ***Hematopoietic growth factors***
   - There are currently **no effective and safe hemopoietic growth factors** to support red cell and platelet counts in patients with AA.
   - The routine use of rHuEpo in AA is not recommended.
   - There have been no clinical studies of rHu-TPO in AA
   - There have been no controlled studies evaluating the use of G-CSF in the treatment of severe infection in patients with AA.
   - However, a short course of G-CSF at a dose of 5 micg/kg per day may be considered for severe systemic infections that are not responding to intravenous antibiotics and antifungals. If there is no response by 1 week, it is then reasonable to discontinue the drug.

3. Prevention of infection

- Patients with AA are at risk of bacterial and fungal infections including Aspergillus. Aspergillus infections have a very high mortality in SAA patinets.
- Patients who are severely neutropenic (<0.5 x 10 9/l) should ideally be nursed in isolation when in hospital and should receive prophylactic antibiotics and antifungals, regular mouth care including an antiseptic mouthwash, such as chlorhexidine, and food of low bacterial content.
- Laminar air-flow facilities are not essential but should be used when available.
- Prophylactic antibiotics are given to help prevent Gram-negative sepsis, either (neomycin and colistin), or (a quinolone antibiotic, such as ciprofloxacin).
- For children, it is not standard practice to use prophylactic antibiotics; ciprofloxacin is not licenced, and non-absorbable antibiotics are very unpalatable.
- Fluconazole provides no cover against Aspergillus species. The drugs of choice is itraconazole.
- There is no indication for routine prophylactic measures against Pneumocystis jirovecii, or anti-viral prophylaxis in untreated patients with AA.
- Antiviral prophylaxis with acyclovir is essential for all transplanted patients and is commonly given during and for the first 3–4 weeks after BMT with ATG.
- Prophylaxis against PCP is essential post BMT for all patients regardless of diagnosis.

4. Treatment of infection

- Fever may require immediate hospitalisation and treatment before the results of bacterial investigations are available.
- The most frequently initial synergistic combination of antibiotics is an aminoglycoside and a b-lactam penicillin.
- If fever persist, systemic antifungal therapy is introduced into the febrile neutropenia regimen.
- Pulmonary infiltrates and sinus infection should be taken as indicators of likely fungal infection in patients with SAA.

5. Iron chelation therapy

- S/c desferrioxamine should commence when the serum ferritin is >1000 mg/l.
- Oral iron chelators, Deferiprone and Deferasirox, is not routinely used.
- For iron over-loaded patients following response to ATG or successful BMT, venesection is the standard way to remove iron.

6. *Vaccinations*

- There have been anecdotal reports of vaccination producing bone marrow failure or triggering relapse of AA, so vaccinations, including influenza vaccination, should only be given when absolutely necessary.
- All live vaccines should be avoided after BMT and ATG, indefinitely.
- After BMT, AA patients should be routinely vaccinated as recommended for all allogeneic BMT recipients.

Definitive Treatment

A. *Allogenic BMT*

1. Newly diagnosed SAA and VSAA (without serious comorbidities)
2. Age of 40 years or less and have an HLA identical sibling
3. Children having NSAA in whom treatment is indicated
4. Age less than 50 years (or 50 – 60 years with good performance status) who failed to ATG + CSA (MUD if no HLA identical sibling)

B. *ATG and CSA*

1. SAA and VSAA (no HLA identical sibling)
2. SAA and VSAA with age more than 40 years to 60 years
3. NSAA with transfusion dependent (blood or platelets)
4. NSAA (not transfusion-dependent) but have significant neutropenia

ATG antithymocyte globulin, CSA cyclosporine A, MUD matched unrelated donor

Treatment for Refractory AA

1. High dose cyclophosphamide without stem cell support …. Is not recommended.
2. Mycophenolate mofetil …. Is not effective.
3. Alemtuzumab (Campath-1H)
4. Oxymethalone
5. Long term use of G CSF and other growth factors

Clinical Algorithm(s)

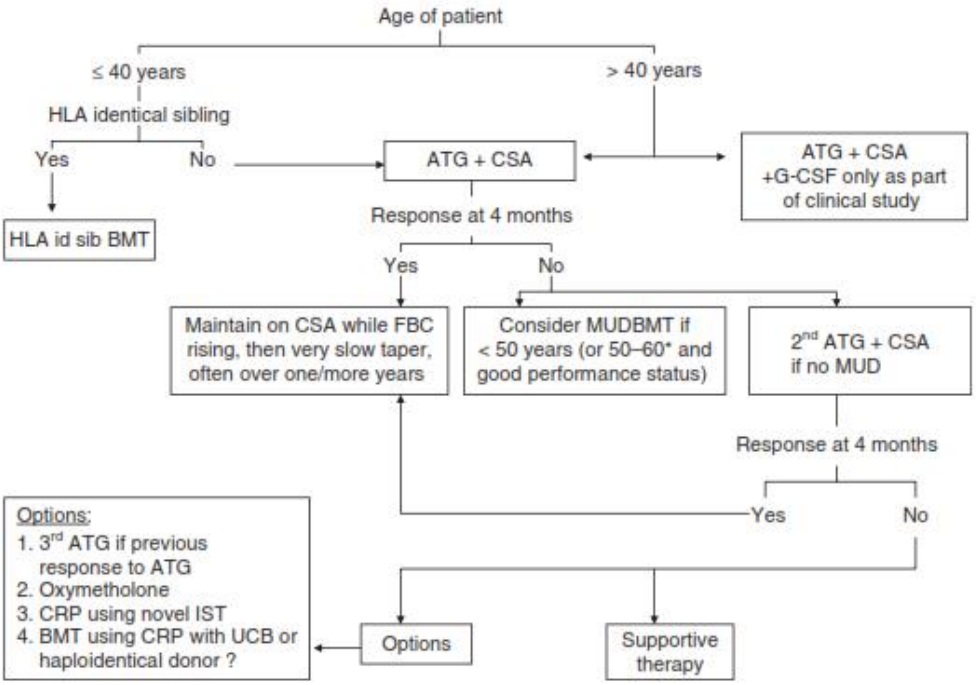

Fig 1. Treatment of acquired severe aplastic anaemia. FBC, full blood count; CRP, clinical research protocol; IST, immunosuppressive therapy; UCB, umbilical cord blood; MUD, matched unrelated donor; ATG, antithymocyte globulin; CSA, ciclosporin; G-CSF, granulocyte colony-stimulating factor; BMT, bone marrow transplantation; HLA id sib, human leucocyte antigen-identical sibling. *For patients older than 60 years, there is currently insufficient data on the role of HSCT in severe AA although data for MDS suggests that this may be a future option (see text).

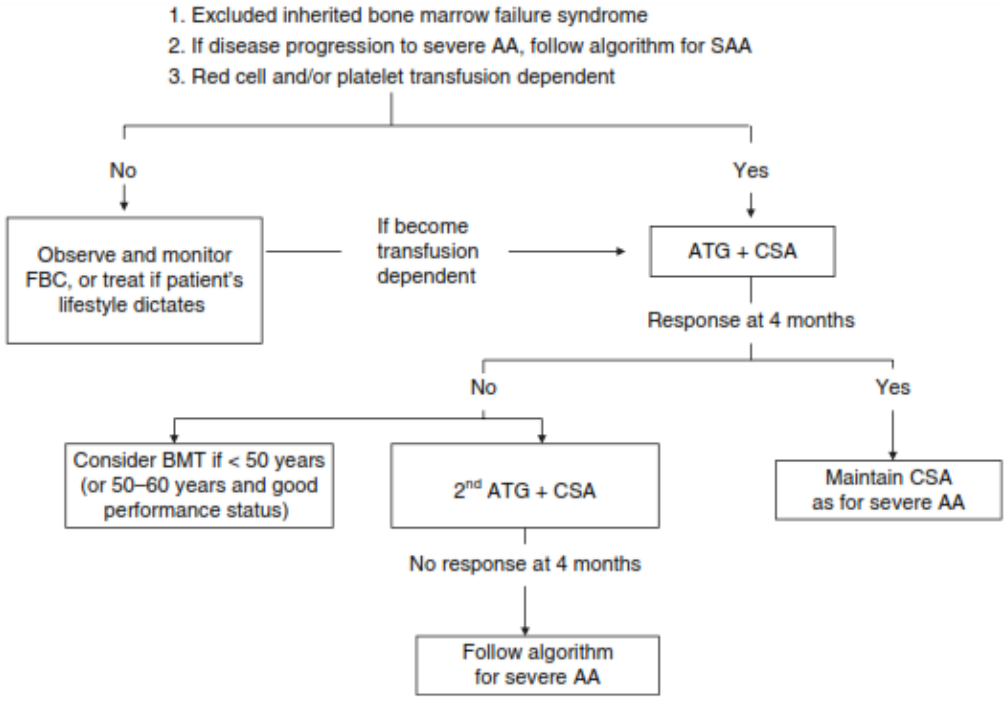

Fig 2. Treatment of non-severe acquired aplastic anaemia in adults. SAA, severe aplastic anaemia; FBC, full blood count; ATG, antithymocyte globulin; CSA, ciclosporin; BMT, bone marrow transplantation.

ANTI-THYMOCYTE GLOBULIN AND CYCLOSPORIN A

Before considering using ATG it is important that hematologists are aware of the following points relating to the administration of this drug:

- ATG is only used by physicians who are familiar with giving ATG and the medical and nursing teams are aware of the side effects and how to treat these promptly and appropriately.
- ATG is highly immunosuppressive. It should only be used in hematology centres. ATG must never be given as an out-patient.
- Patients over the age of 60 years need to be carefully assessed medically to determine whether they are fit enough to tolerate ATG treatment.

Preparations of ATG

1. Lymphoglobuline (Genzyme) = Horse ATG (each vial contains 100 mg of immunoglobulin).
2. Thymoglobuline (Genzyme) = Rabbit ATG (each vial contains 25 mg of immunoglobulin).

Dosage

For both horse and rabbit ATG, the dose is 1.5 vials/10 kg body wt/day for 5 days.

This is equivalent to 15mg/kg/day for horse ATG and 3.75mg/kg/day for rabbit ATG.

Assessment of the patient before starting ATG

1. Investigations to confirm a diagnosis of AA
2. Medical assessment prior to starting ATG
    a. Exclude active infection
    b. Chest X-Ray
    c. ECG (for patients > 60 years, consider ECHO)
3. Assessment of platelet transfusional requirements
    - ensure an adequate platelet increment > 10 – 20 x 10^9/l
    - If refractory to random donor platelets, postpone ATG treatment until further investigated.
    - Obtain informed written consent. A patient information sheet is available.

Prophylactic drug to use with ATG

1. Oral antibiotic prophylaxis: give oral ciprofloxacin 500mg 12 hourly instead.
2. Chlorhexidene mouthwash 10ml 6 hourly.
3. Anti-fungal prophylaxis: itraconazole suspension 2.5mg/kg 12 hourly. If unable to tolerate, use either i.v. itraconazole 200mg daily or low dose amphotericin B i.v. 0.25mg/kg alternate days.
   NB, if using itraconazole, the dose of CSA must be reduced to half normal dose (that is, 2.5mg/kg daily) because itraconazole increases CSA drug levels.
4. Anti-viral prophylaxis: oral acyclovir 200mg 8 hourly
5. Pneumocystis prophylaxis is unnecessary after ATG.
6. Duration of prophylaxis: for all patients, prophylaxis should continue for a minimum of 4 weeks, and longer if VSAA with neutrophil count < 0.2 x 10^9/l.
7. Lansoprazole 15mg daily.
8. Norethisterone 5mg 8 hourly for pre-menopausal females

Administration of ATG

1. Patients must be admitted as an in-patient for ATG treatment. The average length of stay required is 2½-3 weeks providing there are no complications, such as infections, which may necessitate a longer in-patient stay.
2. It is recommended that patients should remain an in-patient for at least the following 2 weeks so they can be monitored closely and treated promptly for serum sickness and other possible complications.
3. **Proposed plan for dates of treatment:**
   (a) Day -1 admit for medical assessment, central line insertion (under platelet transfusion cover), and blood transfusion if needed.
   (b) Day 0 test dose of ATG.
   (c) D1 – 1st full dose of ATG.
   (d) D2-5 – subsequent daily doses of ATG.
4. Always give ATG through a central line. Severe thrombophlebitis may occur if ATG is administered via a peripheral vein.
5. A test dose must always be given before the first full dose. The test dose is 1/10th of a vial diluted in 100 ml of N Saline given intravenously over 1 hour. The test dose must be supervised by a doctor with adrenaline, chlorpheniramine and hydrocortisone drawn up beforehand.
   NB. A severe systemic reaction or anaphylaxis to the test dose is an absolute contraindication to proceeding with ATG treatment.

6. Dilute ATG in 250-500 ml N Saline and infuse over 12-18 hours. It should be administered within 18 hours of being made up. Infuse the first full dose over 18 hours. If tolerated, subsequent doses can be given over 12 hours.
7. **Precede each daily dose of ATG with:**
    - **a.** Platelets (one random donor pack or one apheresis pack. If platelet count is > 20 x 10^9/l, withhold platelets on that day. Do not give platelet transfusions during the ATG infusion, because of the anti-platelet activity of ATG.
    - **b.** Methylprednisolone 2mg/kg i.v./30 min infusion, 30 min before ATG.
    - **c.** Chlorpheniramine 10 mg IV.
    - **d.** If possible, avoid giving more than one unit of blood each day of the 5 days of ATG, to
        - help reduce the risk of fluid overload and
        - help ensure that the ATG administration starts in the morning.

Side effects of ATG

A. Immediate side effects (during administration of ATG):

1. Lymphopenia, neutropenia and thrombocytopenia.
2. Fevers and rigors (worse on 1st day and diminish with subsequent doses).
3. Rash, pruritis, urticaria.
4. Fluid retention occurs commonly. Acute pulmonary edema and cardiac failure can develop rapidly if left untreated. Very close monitoring and early treatment with furosemide is recommended. It is usually multi-factorial in origin,N Saline diluent, blood and platelet transfusions, corticosteroids, chronic anaemia.
5. Hypotension or hypertension.
6. Elevation of serum transaminases occur commonly.
7. Cardiac arrhythmias may occur: bradycardia or tachycardias.
8. Chest pain, loin pain, back pain occasionally.
9. Nausea, vomiting, diarrhea may sometimes occur
10. Positive direct antiglobulin test and difficulty with cross matching blood due to the presence of anti-red cell antibodies in ATG
11. Phlebitis can occur when administered through a peripheral vein
12. Anaphylaxis
13. Other rare reported side effects are acute hemolysis, massive pulmonary hemorrhage and adult respiratory distress syndrome, acute renal failure and renal impairment.

B. Late side effects after administration of ATG due to serum sickness

The onset of serum sickness is typically 7-14 days after starting ATG. If a second course of horse ATG is given, serum sickness can occur earlier.

The manifestations of serum sickness are:

1. Fever, rash (maculopapular or urticarial starting on trunk or extremities).
2. Serpiginous palmar-plantar distribution is classical. Rash may become purpuric due to platelet consumption during the time of serum sickness.
3. Arthralgia, myalgia, nausea, vomiting, proteinuria (usually mild), rarely splenomegaly and lymphadenopathy.
4. Increased platelet transfusion requirements due to platelet consumption.
5. Glycosuria and/or hyperglycaemia due to corticosteroids.

C. **Other late side effects of ATG**

1. Rarely, worsening of autoimmune thyroid disorders and fibrosing alveolitis, and precipitation of Guillan Barre syndrome.
2. AA patients treated with ATG are at increased risk of later clonal disorders such as MDS, AML and PNH, and to a lesser degree, solid tumours.

D. Prevention of serum sickness

- Day 1-5: Methylprednisolone (1mg/kg/day with Horse ATG, 2mg/kg/day with Rabbit ATG) i.v./30 min infusion, start 30 min before each dose of ATG.
- Day 6-14: oral prednisolone 1mg/kg/day.
- Day 15-29, taper off prednisolone.

E. Monitoring of patient

Carefully monitor patient clinically for evidence of bleeding, infection, fluid retention and hypo- or hypertension:

- weigh patient twice daily
- keep fluid balance chart daily
- 4 hourly temperature, pulse, BP and respirations
- daily urine test for glucose
- daily FBC, U&Es and LFTs

F. Treatment of immediate side effects

- Immediate allergic side effects usually respond to a dose of hydrocortisone and chlorpheniramine. If persistent, give pethidine 25mg i.v.
- Pyrexia during ATG may also be due to infection, so broad spectrum i.v. antibiotics must be commenced after obtaining blood cultures.
- Treat fluid retention promptly with furosemide and review fluid balance later the same day. If the patient gains more than one kg in weight, or if the amount in is one litre more than the amount out in 24 hours, then give a dose of furosemide. However, assess clinically first, because if febrile, and increased insensible loss, furosemide may not be appropriate.
- If patient is hypertensive, treat any fluid retention if present, and use appropriate anti-hypertensive.
- For anaphylaxis, discontinue ATG immediately and treat appropriately.
- If bleeding occurs during ATG, stop the ATG infusion and give additional platelets. Resume ATG when bleeding has resolved. Also, check the coagulation screen if bleeding persists despite adequate platelet increment.

G. Contraindications

1. Severe systemic reaction to the test dose.
2. ATG may exacerbate viral and parasitic infections, so do not give ATG in the presence of active infection.
3. There is a theoretical risk of acute hemolysis in patients with hemolytic PNH. However, ATG can be given to AA patients with a small PNH clone with no evidence of clinical or laboratory hemolysis.

H. Repeat courses of ATG

If horse ATG is used for the first course, Rabbit ATG is usually given for a second course. It is possible to use horse ATG again for the second course, provided no serious reaction. More than one course of the same ATG preparation can be given but the risks of side effects and anaphylaxis are increased, and the onset of serum sickness occurs earlier than after a first course. Always give a test dose before the second course of either preparation of ATG.

I. Time to response

Response to ATG does not usually begin to occur before 3-4 months, so red cell and platelet transfusions will need to be continued as needed until the peripheral blood counts start to improve. Continue oral prophylactic antibiotics and antifungals while the patient is severely neutropenic.

THE USE OF CICLOSPORIN (CSA) WITH ATG

- The current standard immunosuppressive regimen for the treatment of AA is the combination of ATG and cyclosporine (CSA).

- The dose of CSA is 5mg/kg/day aiming to keep whole blood trough drug level between 150 and 250ųg/l. The dose must be reduced if using itraconazole with CSA.

- CSA is now commenced on the first day of ATG.

A. Dosage

- Initial dose of oral CSA is 2.5 mg/kg twice daily.
- For elderly patients, start with lower dose eg 1.25 mg/kg twice daily if > 60 y old and adjust according to renal function, blood pressure and CSA levels.

B. Clinical formulation

- Oral CSA is available as capsules (10mg, 25 mg, 50mg and 100 mg) or in an oily yellow solution (100 mg/ml).
- Intravenous CSA is given as; i.v. infusion in 100ml N/Saline or 5% Dextrose/ 2 hrs.

C. Side effects

1. Nephrotoxicity:

- Increases in s. creatinine are dose and plasma level related.
- Additive nephrotoxicity occurs particularly with aminoglycosides, vancomycin and amphotericin B (also with ACE inhibitors, NSAID, quinolones and trimethoprim).
- Hyperkemia may occur with long-term use of CSA. Increased risk with renal impairment or drugs such as ACE inhibitors and K+ sparing diuretics e.g. amiloride. Avoid high dietary potassium intake.
- CSA can aggravate hypomagnesemia in the setting of BMT as in renal transplants.

2. Hypertension:

Often associated with fluid retention, and potentiated by methylprednisolone

3. Neurological:

- Grand mal fits, usually occur in patients with fluid retention, uncontrolled hypertension and high CSA blood levels, often during combined CSA and methylprednisolone therapy.
- Tremor - suggests overdose
- Muscle cramps, paraesthesiae of hands and feet.

4. **Gastrointestinal Tract:**
    - Anorexia, nausea, vomiting
    - Gingival hypertrophy
5. **Hepatotoxicity:**
    - Associated with hyperbilirubinaemia.
    - Potentiated by erythromycin, voriconazole, norethisterone, oxymetholone.
    - Monitor LFTs carefully while on itraconazole.
6. **Anaphylaxis:**

    Reactions are due to the drug or emulsifying agent in the i.v. preparation.
7. **Hypertrichosis**

D. Assessment of patient prior to commencing CSA

Check blood pressure (BP), serum electrolytes, urea, creatinine (U&Es), and LFTs. Review current medication patient is taking, and ask about herbal remedies.

E. Monitoring of patient on CSA

- Aim to keep trough whole blood CSA level 150 and 250ųg/l
- CSA levels should be measured from day +2, then twice weekly on inpatients.
- For outpatients, weekly CSA levels until stable. Then be checked every 2-3 weeks. If renal and hepatic function is abnormal ➔ checked more frequently.
- **Sample Required**

  12 hr trough blood levels are measured before the morning dose of CSA.
  Send 3 ml blood in EDTA. Always take sample from a peripheral vein.
  Never take blood for CSA level from the CSA infusion line of the Hickman catheter, even after thorough flushing of the line, otherwise falsely high levels will be obtained.
- **Renal function (RFTs) and other electrolytes**

  The most frequent dose limiting toxicity is renal impairment.
  Monitor RFTs daily and LFT 3 times weekly whilst patient is an in-patient.
  Monitor serum $Ca2^{+}$ and $Mg2^{+}$ weekly.
  Slow rise in creatinine to 120-130µmol/l is common in 1^{st} few weeks of therapy.
  If the creatinine is > 130 µmol/l a dose adjustment should be made.
  If a rapid rise in creatinine occurs, stop CSA for 1-2 doses, monitor RFTs function and CSA level, make appropriate dose adjustment.
- **Blood Pressure**

  Monitor BP regularly.
  Antihypertensive therapy may be necessary (use atenolol or amlodipine)

F. Timing of CSA after ATG

CSA is commenced on the first day of ATG.

G. Duration of treatment

- CSA is given for a minimum of 12 months after complete remission and usually much longer.
- If a response occurs, CSA is continued at full dose until the blood count has stopped rising and has plateaued.
- CSA is tailed off very slowly often over many months or even longer (1-2 years) depending on the FBC.
- Too rapid dose reduction is associated with a high incidence of relapse of aplastic anemia. Some patients, however, are CSA dependent and will need a low dose for a long period of time. In these patients it may be impossible to stop the CSA completely.

H. CSA drug interactions

1. **Anticonvulsants**
   - Phenytoin, phenobarbital, and carbamazepine: CSA levels may fall dramatically on starting these drugs (and will rise on discontinuation).
     ➔ACTION: Monitor CSA levels. May need to increase dose 2-4 fold (2-3 fold reduction on stopping anticonvulsants.

2. **Antiemetics**
   - **Metoclopramide**: increases CSA absorption.
     ➔ACTION: Monitor levels and reduce CSA dose if necessary.

3. **Antihypertensives**
   - **Nifedipine and amlodipine**: edema may worsen. Can increase risk of gingival hyperplasia.
   - **Potassium sparing diuretics**: may increase risk of hyperkalemia.

4. **Antibacterials**
   - **Aminoglycosides (gentamicin, amikacin), co-trimoxazole, trimethoprim, vancomycin and quinolones (Ciprofloxacin)**: may increase nephrotoxicity of CSA.
     ➔ ACTION: Monitor CSA levels and renal function. Reduce antibiotic dose accordingly.

- **Macrolides (erythromycin and clarithromycin)**: causes a marked increase in CSA levels (4-5 fold) on starting the drug and a similar decrease on stopping.
  ➔ ACTION: Use an alternative e.g. azithromycin if appropriate otherwise reduce CSA dose and monitor levels closely. Increase CSA dose once Erythromycin stopped.
- **Rifampicin**: CSA levels are markedly reduced. Conversely an OVERSHOOT of CSA levels will occur when Rifampicin is stopped.
  ➔ACTION: Increase CSA dose according to levels. May need to increase dose 3-5 fold. Reduce dose when stopping Rifampicin.
- **Imipenen/cilastatin**: isolated reports of increased CSA levels also disorientation, confusion, motor aphasia and tremor.
  ➔ACTION: Monitor renal function.

5. **Antifungals**
   - **Amphotericin B:** increases nephrotoxicity of CSA (not with Ambisone or Abelcet).
     ➔ACTION: Monitor renal function and CSA levels closely.
   - **Itraconazole**: increases CSA levels through inhibition of metabolism (up to 3-4 times).
     ➔ACTION: Monitor CSA levels and adjust dose accordingly when introducing/discontinuing Itraconazole.
   - **Fluconazole**: possible increase in CSA levels of up to 2-3 fold after 2-3 days through inhibition of clearance. Perhaps more likely at higher doses of Fluconazole.
     ➔ACTION: Monitor CSA levels and adjust dose accordingly particularly when Introducing/discontinuing doses of Fluconazole above 100 mg.
   - **Voriconazole:** increased CSA levels.
     ➔ACTION: Dose of CSA should be halved

6. **Antivirals**
   - **Acyclovir:** isolated reports of increases in s. creatinine particularly with IV
   - ACTION: Monitor RFTs and adjust Acyclovir dose if necessary.
   - **Foscarnet:** increased risk of nephrotoxicity.

7. **Anti-ulcer agents**
    - **H2 antagonists (cimetidine, ranitidine):** these do not appear to affect serum CSA levels. Deterioration in renal function may occur.
      ➔ACTION: Monitor renal function.
    - **Omeprazole**: isolated case reports of increased CSA levels.
      ➔ACTION: Monitor CSA levels.

8. **Steroids**
    - **Methylprednisolone**: convulsions, associated with fluid retention and hypertension, have been reported in BMT patients on CSA and Methylprednisolone 5-20 mg/kg/day.
      ➔ACTION: Monitor CSA levels.
    - **Norethisterone**: isolated reports that withdrawal of Norethisterone has reduced CSA levels. In addition, exacerbation of fluid retention and hypertension may occur.
      ➔ACTION: Monitor CSA levels when introducing/discontinuing Norethisterone.

9. **Miscellaneous**
    - **Methotrexate:** previous or concurrent treatment with CSA may increase the risk of liver toxicity.
      ➔ACTION: Monitor LFTs.
    - **Oxymetholone:** risk of hepatoxicity
      ➔ACTION: Avoid the combination of CSA and oxymetholone.
    - **Grapefruit juice:** can increase CSA levels.
      ➔ACTION: Advise patients not to drink grapefruit juice whilst on CSA.
    - **Chinese herbal remedies:** can interfere with CSA levels.
      ➔ACTION: Advise patients not to take whilst on CSA.

Date Released

January 2016

Guideline Status

This is the first release of the guideline.

This guideline needs updates and review according to a scheduled time.

Guideline references

- This guideline is modified from BCSH and APHCON guidelines.
- The epidemiology of acquired aplastic anemia. Haematologica April 2008 93: 489-492.
- How I treat acquired aplastic anemia. Blood, August 2012, volume 120, number 6.
- Optimizing outcome of aplastic anemia in Iraq. Beirut meeting report, April 2015.
- ATG and Cyclosporine; drug information from different references

Guideline Availability

ISOH