Management of severe aplastic anaemia

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SUMMARY

Aplastic anaemia is a rare condition characterised by pancytopenia and bone marrow hypocellularity and caused by the immune-mediated destruction of the haematopoietic precursors. The early complications are related to cytopaenias with infections being the major cause of morbi-mortality. The main long-term issue is clonal evolution to myelodysplastic syndrome or acute leukaemia. The diagnosis relies on exclusion of other causes of pancytopenia and characteristic pathologic findings. Severity is stratified according to peripheral blood counts. Nowadays, the survival of treated patients reaches 80-90%. The treatment of the severe form of aplastic anaemia consists on haematopoietic stem cell transplantation in eligible patients and immunosuppressive therapy in non-transplant candidates. Supportive therapy is an option in frail and/or elderly patients. Here, we define and briefly review the pathogenesis of aplastic anaemia. We propose a diagnostic and therapeutic strategy based on existing literature and experts' recommendations. We finally report three cases illustrating particular clinical associations with pregnancy, hepatitis and paroxysmal nocturnal haemoglobinuria.

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INTRODUCTION

Aplastic anaemia (AA) is defined as pancytopenia with bone marrow hypocellularity in the absence of marrow infiltrate or fibrosis. It is characterised by the absence of or by a diminished number of haematopoietic precursors, usually due to injury to the pluripotent stem cell. The disease is estimated to occur in two to four individuals per million per year in Europe, at the median age of 60 years.[1-3]

The designation 'aplastic anaemia' is actually a misnomer because the disorder is defined as pancytopenia rather than anaemia.[1] AA should be distinguished from pure red cell aplasia where only the erythroid precursors are affected and for which the therapeutic strategy is different and from acquired amegakaryocytic thrombocytopenia.[4,5]

Although several etiologic triggers have been proposed in AA, the majority of cases are idiopathic, with a small proportion occurring after an episode of seronegative hepatitis.[6] AA results from the immune destruction of CD34-positive haematopoietic cells, mediated by oligoclonally expanded effector Th1 cells secreting interferon-γ (IFNγ). Specific cytotoxic T cell oligoclones recognising autologous myeloid cells have been identified in AA.[7] By contrast, regulatory T cells, which control and suppress auto-reactive T cells, are decreased at presentation in almost all patients with AA.[8]

The reduced number and function of the marrow is secondary to cell destruction but also to increased apoptosis and impaired regulation of the few remaining elements, as suggested by gene expression profile studies.[6]

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TABLE 1. Severity criteria for AA.

	Severe AA (SAA)
Marrow cellularity	<25% or 25–50% with <30% residual haematopoietic cells
Neutrophil count	$<0.5*10^9$/L
Platelet count	$<20*10^9$/L
Reticulocyte count	$<20*10^9$/L
sAA is defined as marrow cellularity <25% plus at least two lineages deeply affected according to indicated thresholds. Very severe AA (vsAA) is a sAA with neutrophils $<0.2*10^9$/L and moderate AA or non severe AA is AA not fulfilling the criteria for sAA or vsAA.	

TABLE 2. Peripheral blood tests to perform when suspecting AA.

Vitamin B12 and folate	To exclude carential aetiology
Liver function tests	To detect previous or on-going hepatitis
Viral serology	HIV, hepatitis A, B, C and E, EBV, CMV and parvovirus B19
Anti-nuclear antibody and anti-double stranded DNA	To exclude associated autoimmune disease such as lupus erythematosus
Peripheral blood chromosomal breakage analysis: diepoxybutane test (DEB Test)	To diagnose Fanconi anaemia
Flow cytometry for GPI-anchored proteins	To detect associated PNH clone
Cytogenetics and next generation sequencing	To exclude late-onset inherited bone marrow failure syndromes (IBMFS)
Telomere length	Useful for screening of telomere gene mutations in some IBMFS. Short telomeres may also occur in acquired AA

A minority of AA cases may share pathogenic features with inherited bone marrow failure syndromes (IBMFS). Leukocytes in AA typically present short telomeres. Although initially blamed on excessive stem cell turnover, telomere shortening in some cases of acquired AA is due to mutations in components of the telomerase complex, causing low telomerase activity, progressive telomere erosion, and a deficient proliferative capacity of haematopoietic stem cells.[6,9]

AA may finally co-exist with or evolve to clonal disorders, as paroxysmal nocturnal haemoglobinuria (PNH), myelodysplastic syndrome (MDS), or acute myeloid leukaemia (AML) through poorly elucidated mechanisms.[6] The identification and monitoring of these clonal disorders in AA patients may help to better classify the disease and potentially have prognostic significance.[10]

MANAGEMENT OF APLASTIC ANAEMIA

DIAGNOSIS

Patients are usually referred upon complications of cytopaenias such as bleeding, infections or symptomatic anaemia. Infection is the main cause of death in AA patients. The diagnose of AA requires at least two of the following: haemoglobin concentration (Hb) <10 g/dL together with reticulocytopenia ($<60*10^9$/L with automated counting), platelet count $<50*10^9$/L and neutrophil count $<1.5*10^9$/L (*Table 1*).[2] The neutrophil count determines severity according to the modified Camitta criteria. Severe aplastic anaemia (sAA) is defined as marrow cellularity <25% plus at least two lineages deeply affected according to indicated thresholds. Very severe AA (vsAA) is a sAA with neutrophils <0.2*109/L and moderate AA or non severe AA is AA not fulfilling the

TABLE 3. Bone marrow examination in AA.

Cytology	• Hypocellularity. • Erythropoiesis is reduced or absent; dyserythropoiesis is very common and does not distinguish from myelodysplastic syndromes (MDS). • Megakaryocytes and granulocytic cells are markedly reduced or absent. Dysplastic megakaryocytes and granulocytic cells are not seen in AA, which can help distinguish with hypocelluar variant of MDS. • Lymphocytes, macrophages, plasma cells and mast cells often appear prominent.
Flow cytometry	• To exclude the presence of leukemic blasts, large granular lymphocyte leukaemia and associated PNH clone.
Cytogenetics	• Karyotyping may be unsuccessful in hypocellular marrow with insufficient metaphases. FISH analysis for chromosomes 5, 7, 8 and 13 should be performed. • Identification of some typical clonal abnormalities can suggest diagnosis of MDS but cannot firmly exclude AA. • A baseline molecular analysis is mandatory. The appearance of a new abnormality provides evidence of clonal evolution.
Trephine biopsy	• To assess overall cellularity and morphology of residual haematopoietic cells. • To exclude myelofibrosis. • To exclude marrow infiltration and haemophagocytic lymphohistiocytosis (HLH).

criteria for sAA or vsAA (*Table 1*).[6,7] Before making a firm diagnosis of AA, inherited and other acquired causes of pancytopenia with hypocellular bone marrow should be excluded, following a work-up recently recommended by European AA experts (*Tables 2 & 3*).[2,13]

Peripheral blood examination

Beside pancytopenia, frequent microscopic findings include macrocytosis and anisopoikilocytosis. Neutrophils may show toxic granulation. Platelets are mainly small in size. Cytological examination should exclude the presence of dysplastic neutrophils, abnormal platelets, blasts and other abnormal cells, such as 'hairy' cells particularly in presence of monocytopenia.[2] Other useful blood tests are listed in *Table 2*.[2]

Bone marrow examination

Examination of bone marrow and trephine biopsy are mandatory and will provide information gathered in *Table 3*.[2]

Radiology

It is recommended to perform a standard chest X-ray or a CT thorax to exclude a thymoma, since this condition can be associated with AA, and an abdominal ultrasound as screening for underlying organomegaly, adenopathies or underlying neoplasia.[14]

PROGNOSIS

Over the past few decades, the overall outcome of patients with sAA has improved drastically due to the increased availability of haematopoietic stem cell transplantation (HCT), more effective immunosuppressive therapy (IS) and significantly improved supportive care. To date, survival rates are as high as 80-90%. Of note, the one-year mortality rate of untreated sAA is as high as 70%. The major factors that affect prognosis are the severity of pancytopenia, response to initial therapy, and patient age.[3,13]

THERAPEUTIC STRATEGY

In case an underlying cause has been identified, the offending agent should be withdrawn and/or treated appropriately. However, delaying (>2-3 months) adequate therapy while awaiting marrow recovery is not advised because it increases the risk of serious complications.[13]

Herein, we describe the recommended therapy for sAA. For patients with non-severe AA, the optimal therapy is not well established yet. A more individualised approach, based on the patient's age, clinical status, and degree of cytopaenia, may be considered.

The following treatment algorithm for sAA was recently proposed. (*Figure 1*).[13] As a rule of thumb: donor search should be started as soon as the diagnosis of sAA has been made.

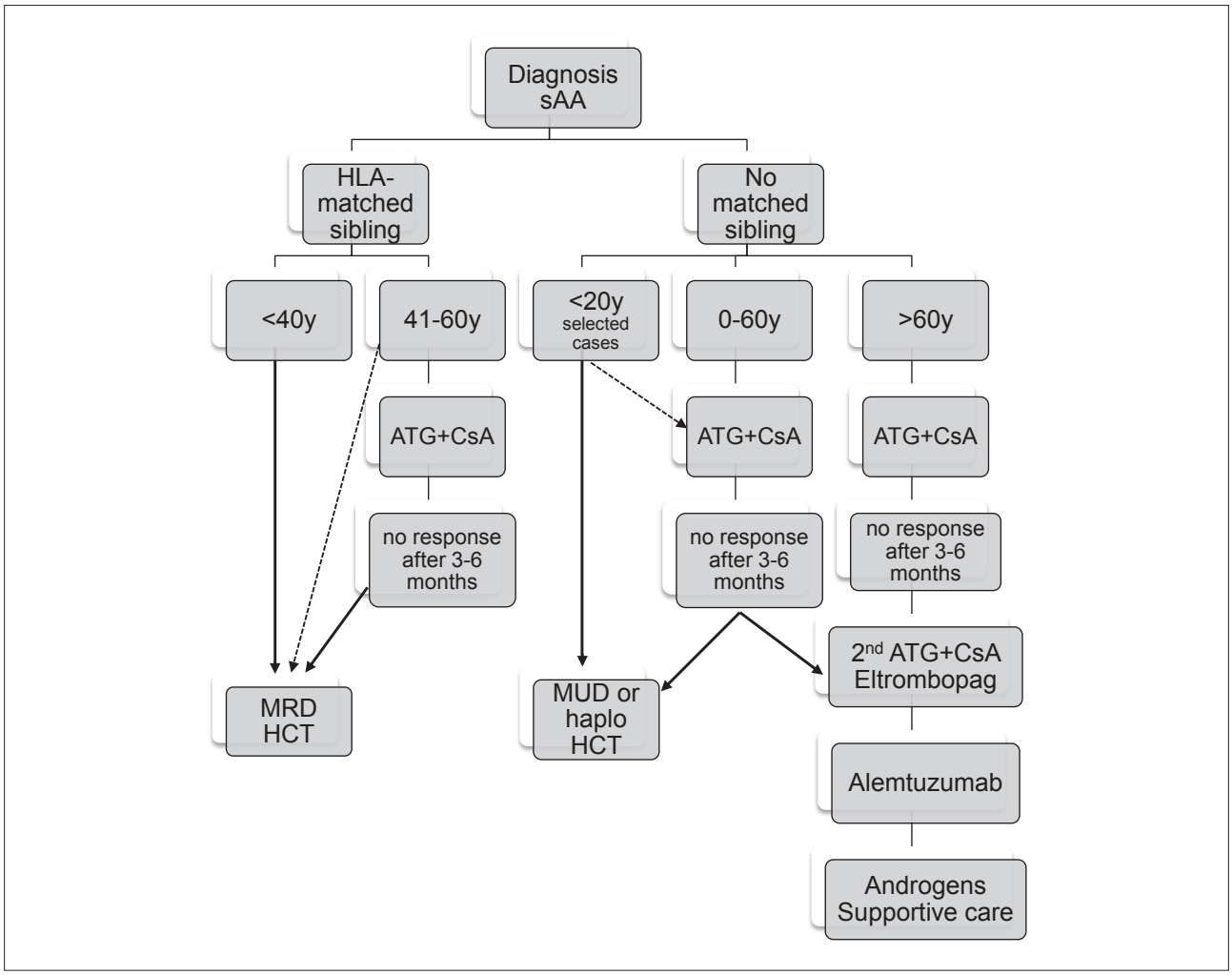

FIGURE 1. Treatment strategy in sAA according to donor availability and patient age.
MRD, matched related donor; HCT, haematopoietic stem cell transplantation; ATG, anti-thymocyte globuline; CsA, cyclosporine A; MUD, matched unrelated donor; haplo, haploidentical.[2,13]

Bone marrow transplantation

The outcome after HCT strongly correlates with the recipient age.[13] For young individuals with a compatible sibling donor, HCT can (and should) be initiated within six to eight weeks after diagnosis. Patients who would benefit from a HCT but without a timely suitable donor are best treated with immunosuppressive (IS) therapy as described below. However, search for a compatible donor should continue, especially for younger patients with severe disease. Recent progress in molecular diagnosis might help better define candidates for upfront transplantation. For instance, patients with mutations in *ASXL1* or *DNMT3A* have a poorer response to IS and a greater propensity for clonal evolution.[15] Available data also suggest a poorer outcome in case of monosomy 7 and these patients should be transplanted upfront.[16,17]

Matched related donor (MRD) HCT remains the first line treatment of sAA. Fortunately, outcome of matched unrelated donor (MUD) HCT has improved over time, with comparable survival rates as MRD, although with increased risk for graft versus host disease (GVHD). In the absence of a matched donor, haploidentical HCT has become a valid option.[18-22] Recent literature favours bone marrow rather than peripheral blood as optimal stem cell source.[23] However, nowadays, many centres prefer peripheral blood stem cells for practical reasons. At present, the conditioning regimen usually includes immunosuppressive drugs such as fludarabine and cyclophosphamide, without total body irradiation. The pre-transplant work-up follows local standards and should include fertility counselling when appropriate.

Immunosuppressive agents

Although HCT is the only curative treatment of sAA, IS can lead to haematological recovery in 50-70% of cases and excellent long-term survival among responders.[24] The stan-

dard regimen for first-line IS remains anti-thymocyte globulin (ATG) and cyclosporine A (CsA). This therapy is recommended for all patients without a matched sibling donor but also for patients with a matched sibling who are older than 40 years (*Figure 1*).[2]

Only rabbit ATG is available in Belgium and is commonly administered at the dose of 3.5 mg/kg daily for five days. There are controversial data in the literature on whether to use horse or rabbit ATG. One randomised trial on 120 patients clearly favours horse.[25] A more recent retrospective study shows similar efficacy of the two products but with longer time to reach recovery with rabbit ATG, which was partly due to more profound lymphocyte depletion.[26] Beside immunosuppression, the main side effect of ATG is serum sickness, to be treated with methylprednisolone at 1 mg/kg daily, and continuing for two weeks, followed by a rapid taper and discontinuation.

CsA is started upon tapering of steroids and given at an initial dose of 5 mg/kg daily, administered orally in two equally divided doses. Subsequent dosing is titrated based on trough levels to a target of approximately 200-400 ng/ml. Many patients develop hypertension which will be preferably treated with amlodipine because of minimal overlap with CsA toxicities.[16] CsA is generally continued for approximately six months. Data to guide the duration are limited. Some authors reduce the dose after the first month to a trough level of 200-250 ng/ml. Once recovery of blood counts is reached, CsA should be tapered very slowly, over a period of six to twelve months.[13,16]

Response to IS treatment should be evaluated after three and six months and is defined as any improvement in blood counts sufficient to no longer satisfy criteria for sAA and transfusion independency.[24] A complete response can be defined as an absolute neutrophil count of at least $1*10^9$/L, a haemoglobin level of at least 10 g/dL, and a platelet count of at least $100*10^9$/L.[27] After failing a first course of IS, patients with a MUD should undergo a transplant if clinically fit or receive a second course of IS if clinically unfit or in the absence of suitable donor (*Figure 1*).[13] Initial IS does not affect the prognosis of later HCT, provided a donor is available and the patient remains in adequate health to tolerate the procedure.

Growth factors

Levels of haematopoietic cytokines such as granulocyte colony-stimulating factor (G-CSF), thrombopoietin (TPO) receptor agonists and erythropoietin (EPO) are generally quite high in individuals with AA at baseline. Therefore, and because of concern for clonal evolution, these growth factors generally are not used as part of the routine management of sAA.[28] Exceptions include the possible use of granulocyte colony-stimulating factor (G-CSF) in individuals with frequent or severe infections, and the use of thrombopoietin (TPO) receptor agonists.

Combining ATG+CsA with G-CSF does not improve survival.[29] However it allows rapid neutrophil recovery and identifies non responders who could benefit from urgent HCT.[13]

Recent data demonstrate the efficacy of the TPO receptor agonist eltrombopag as first line therapy in combination with standard IS. The dose is 150 mg orally once a day for Caucasian adults of non-Asian ancestry, starting on day one and continued for six months. Individuals of East or Southeast Asian ancestry are given half the normal dose. The dose is reduced and/or temporarily held for high platelet counts (>200-400$*10^9$/L) or transaminase elevation.[27] This combination regimen lead to a complete response rate at six months of 94% with marked increase in peripheral counts of the three lineages, bone marrow cellularity, CD34+ cell count and frequency of early haematopoietic progenitors, possibly through evasion of the detrimental effects of IFNγ on TPO signalling.[24,27,30] In Belgium, reimbursement criteria currently limit access to this product to adults with refractory sAA after one therapy-line and who are not eligible for HCT.

Erythropoietin is not used in sAA, because there are insufficient erythroid precursors and endogenous EPO levels are already high.[16,31]

Follow-up

Follow-up post treatment is based on peripheral blood counts. However, bone marrow aspirate remains useful to detect clonal evolution, especially in individuals with decreasing counts. We propose, according to expert recommendations, to follow all patients with serial bone marrow examination six and twelve months after the start of IS treatment and then yearly as monitoring.[16,24]

Supportive care

Transfusions should be administered with classical thresholds. Phenotype (Rh and Kell) matched blood should be considered to reduce the risk of allo-immunisation. Transfusion-induced iron overload should be treated with iron chelators according to reimbursement criteria or venesection following successful HCT.[2] Intriguingly, deferoxamine and deferasirox have been suggested to improve erythroid response of AA patients in a multicentre retrospective study but reimbursement criteria limit their use for this indication in Belgium.[32]

There are no specific guidelines regarding prophylactic antimicrobial therapy in patients with sAA.[2] EBMT guidelines recommend the use of per oral fluconazole 400 mg daily

and of levofloxacine 500 mg daily as long as neutrophil count stays below 0.5*10^9/L. Acyclovir 400 mg twice daily as anti-herpes simplex prophylaxis and monthly pentamidine aerosol therapy when the CD4 count decreases under 0.2*10^9/L are also recommended. One should also monitor regularly aspergillus antigen in peripheral blood in order to promptly diagnose invasive aspergillosis in patients with long-term neutropenia or under IS treatment.[33]

Disease relapse

Relapses occur in approximately 10% of individuals whose initial disease responded to IS, once the treatment is tapered or stopped. The likelihood of relapse is not predicted by age or disease severity, and relapse in turn does not appear to be predictive of increased mortality.[34] Importantly, many patients whose disease responded to ATG and CsA initially (with or without eltrombopag) can be retreated with the same regimen.[35,36]

Refractory disease

Approximately 25% of sAA patients fail to IS, because of persistent immune attack on haematopoietic cells or persistent stem cell deficiency. Potential approaches are directed at either (or both) of these problems and include salvage HCT, eltrombopag, alemtuzumab and androgens.[2,13,24]

All non-responding fit patients should be considered for second line HCT, with matched unrelated, haploidentical or cord blood stem cell donor (*Figure 1*).[2,13]

A repeat course of IS is an effective option when tolerable for the patient. Rabbit ATG was shown to be capable of rescuing blood counts in two-thirds of relapsing patients and one-third of patients refractory to an initial course of horse ATG/CsA. However, a second course of rabbit ATG in patients who did not respond to a first course of rabbit ATG showed disappointing results.[24,36,37]

Eltrombopag is efficient in the treatment of refractory disease and should be proposed to all non-responders, with 40% response, including bi-lineage and tri-lineage responses.[38] Further experience with eltrombopag in the refractory disease setting is awaited. Unanswered questions include the duration of response, selection of patients for drug discontinuation, and safety of continuing (or initiating) therapy in individuals with clonal cytogenetic abnormalities or dysplasia.[39]

Alemtuzumab (Campath) is a third line option for treating refractory disease that does not respond to eltrombopag plus IS, or for patients unable to tolerate CsA. Prospective response rate and three year overall survival were of 37% and 85%, respectively in refractory sAA patients.[24,40]

Androgens such as danazol, oxymetholone or testosterone can be used in non- transplant candidates who fail to achieve haematopoietic recovery after standard IS. One potential mechanism of action could be up-regulation of telomerase function.[24] A proposed strategy is to use testosterone 40 mg per day, three to five days a week.[13] Danazol can be used at the dose of 300-600 mg/day, especially in patients with inherited or acquired shortened telomeres.[24,41,42]

High dose cyclophosphamide has also been proposed but barely used because of unacceptable toxicity.[24] Similarly, there is no place for mycophenolate-mophetil or sirolimus in the treatment of refractory sAA.[2,16]

Treatment strategy for elderly

Elderly patients with sAA should be individually assessed for frailty and their specific wishes respected. First-line HCT is rarely offered to patients older than 60 years because of high transplant-related mortality. Patients between 60 and 70 years of age should be first treated with conventional ATG+CsA, optionally with G-CSF to achieve faster neutrophil recovery. Fit patients above 70 years can be treated with standard IS whereas frail elderly are preferably treated with CsA plus androgens.[2,13] Patients unfit for, who decline or who are intolerant to IS should be offered best supportive care.[2]

Eltrombopag could be valuable in elderly patients unsuitable for HCT or standard IS [24,27] but Belgian reimbursement criteria only permit its use after failing of first line IS.

PARTICULAR SITUATIONS

Pregnancy

Case #1

A 27 year old and 31 weeks pregnant woman consulted the emergency department because of syncopal symptoms and nausea. She had no relevant medical history except a normal previous pregnancy. Since one month she presented with progressive fatigue and spontaneous gum bleeding. The complaints had worsened the last week. Clinical examination showed ecchymoses on the limbs. Peripheral blood counts showed deep anaemia (haemoglobin 4,5 g/dL) and thrombocytopenia (24*10^9/L). Reticulocyte count was of 9*10^9/L. There were no signs of hemolysis. Blood analyses including auto-immunity and virologic tests were further normal. Detailed interrogation of the patient indicated no infection or exposition to toxics. Peripheral blood and bone marrow cytologic examination as well as trephine biopsy showed hypocellularity affecting the erythroid and megakaryocyte lineages (*Figure 2*). The patient met the criteria of non-severe aplastic anaemia. She received steroids for foetal lung maturation and was treated supportively with transfusions until at term normal vaginal delivery. The postpartum period was

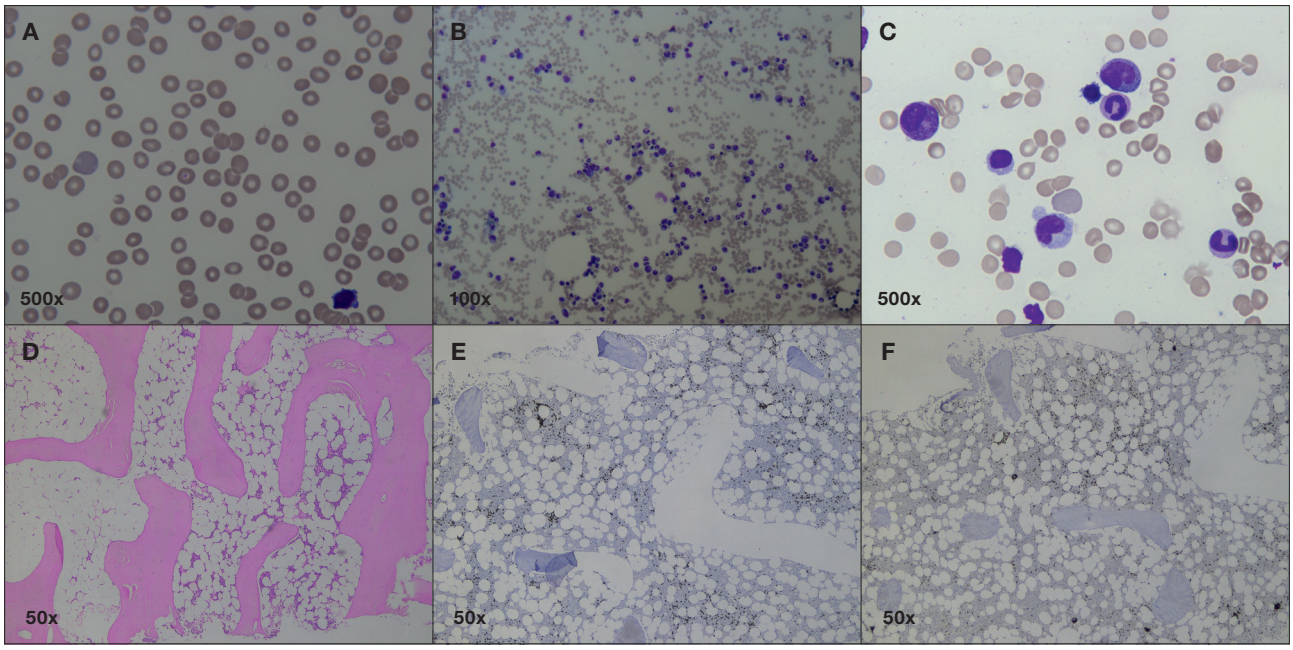

FIGURE 2. Non-severe aplastic anaemia in pregnancy. Panel **A** shows the peripheral blood cytology, characterised by normocytair normochrome anaemia, anisopoikilocytosis and deep real trombopenia. The myeloid lineage was normal (not shown). Panels **B** and **C** show the bone marrow cytology with important hypoplasia of the erythroid and megakaryocytic lineages. Lower panels show the histological findings from the corresponding trephine biopsy. We notice hypocellularity for the age (**D**, hematoxylin-eosin staining) as well as very weak or absent erythroid (**E**, glycophorin staining) and megakaryocytic lineages (**F**, linker for activation of T cells (LAT) staining).

marked by serious bleeding events for which obstetrical intervention and transfusions were needed. We then started cyclosporine monotherapy with target blood levels of 300 ng/ml. Currently, five months after delivery, the patient is still under this therapy and her blood counts progressively improve.

Pregnancy-associated AA is frequently self-limited, spontaneously resolving after delivery in 25-30% of patients.[43] It is uncertain whether or not pregnancy is causally related to the development of aplasia in these patients.[44] Supportive care is the mainstay of treatment of AA in pregnancy, aiming to maintain the platelet count above $20*10^9$/L with platelet transfusions. CsA is safe in pregnancy if needed.[45] ATG, allogeneic HCT or androgens for AA during pregnancy are not recommended[2] and data are lacking for the use of eltrombopag in pregnant women with AA.[27] Close monitoring of blood counts and collaboration between obstetricians and haematologists are crucial for pregnancy-associated AA. The mode of delivery should be determined on obstetric grounds.[2]

Relapse occurs during 19% of pregnancies in AA patients who have previously responded to IS, especially those with partial response. Normal blood counts before conception did not guarantee freedom from relapse of AA during pregnancy.[46] Pregnancy does not trigger relapse of the disease in patients who had undergone successful HCT.[2]

Paroxysmal nocturnal haematuria (PNH)

Case #2

A 36 year old man without medical history was referred because of pancytopenia. A first bone marrow examination and trephine showed hypocellularity and no clear dysplasia nor fibrosis. Because he did not match the criteria of severe aplastic anaemia and was transfusion independent, he was only observed. Three years later he developed Coombs-negative haemolytic anaemia in the absence of any GPI-negative clone by flow cytometry. More than six years later he developed a full blown PNH (40% GPI-negative granulocytes). After two more years' follow-up, the symptoms related to anaemia worsened leading to indication of transfusions and eculizumab therapy. A control of the bone marrow examination and trephine were then compatible with a MDS with multilineage dysplasia (MLD) with a significant component fibrosis (Tefferi 2/3). He was then proposed for MUD allogeneic HCT. He underwent the transplantation after a classical conditioning regimen (revised Slavin) and received an ATG/cyclosporine/mycophenolate based GVHD prophylaxis. Post transplantation we observed complete haemato-

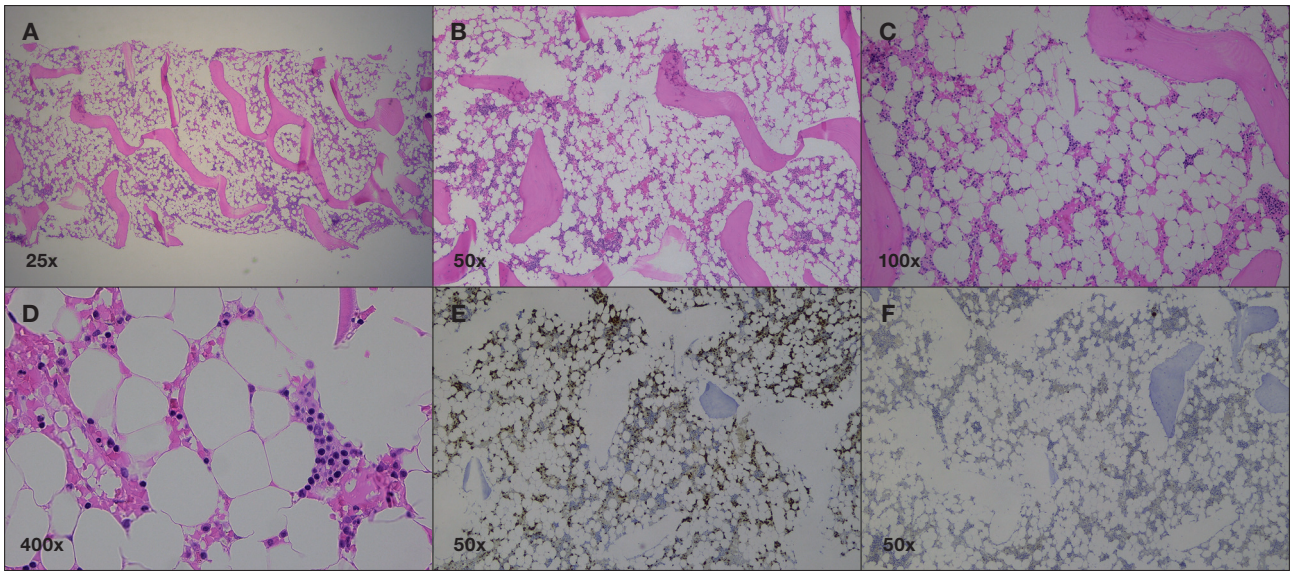

FIGURE 3. Seronegative hepatitis-associated severe aplastic anaemia. Panels **A** to **D** illustrate the major hypocellularity on the trephine biopsy at indicated magnifications after hematoxylin-eosin staining. The myeloid lineage was present at all stages of maturation altough limited in number of precursors (**E**, myeloperoxydase (MPO) staining). The megakaryocytic lineage was weak and the observed megakaryocytes were small in size and disseminated accross the marrow (**F**, LAT staining).

logical response, which is currently maintained after five years follow-up. Unfortunately the patient developed severe chronic GVHD for which he still needs specific treatment. This case illustrates thus a non-severe aplastic anaemia with occurrence of a PNH clone and then evolution to an aggressive MDS.

As mentioned above, patients should be screened for PNH at the diagnosis of AA. A PNH-clone is present in 50% of the AA-patients.[47] If persistently negative, test bi-annually for two years and then move to annual testing unless symptoms/signs develop. If the PNH screen is, or becomes positive, test three monthly for the first two years and only reduce the frequency if the proportion of the PNH cells has remained stable.[2]

In AA patients, a PNH-clone can be small without clear signs of haemolysis: this condition is called subclinical PNH and does not need to be treated. In case of a bigger clone with haemolysis, the condition is called PNH associated with bone marrow failure syndromes. Dependent on the severity of both AA and PNH and their respective clinical manifestations, it will be indicated to first treat AA or PNH. It is now widely accepted that the two conditions behave as two separate entities, fluctuating over time.[2]

Hepatitis-associated AA

Case#3

A 22 year old patient without relevant medical history was referred for pancytopenia two months after an episode of acute severe hepatitis for which no aetiology could be identified. In particular all tested serologies were negative. A bone marrow examination and trephine biopsy showed hypocellularity affecting mostly the myeloid and megakaryocytic precursors (*Figure 3*). His peripheral blood counts at presentation met the criteria of sAA. Because of the young age and the availability of a matched sibling donor, the patient received allogeneic HCT upfront after fertility counselling and cryopreservation of reproductive cells. A reduced intensity conditioning regimen containing fludarabine, cyclophosphamide and ATG was used with CsA as GVHD prophylaxis. After ten years of follow up we didn't observe recurrence of AA nor major post HCT complications.

A preceding (or concurrent) history of hepatitis is seen in a small percentage of individuals with AA (2-5% in some series), almost always in adolescent boys or young men.[48,49] In some cases, a known viral cause is identified, but in many patients serology remains negative. The mechanism may involve T cell activation with release of cytokines or activation of a cytotoxic T cell clone that recognises similar target antigens on both liver and bone marrow cells.[50,51] A typical presentation is development of sAA approximately two to three months following an episode of acute hepatitis. Management of sAA associated with an episode of viral hepatitis follows the same algorithm as sAA (*Figure 1*). In this setting, elevated transaminases do not contra-indicate the use of the standard conditioning regimen or immunosuppression even if they contain hepatotoxic agents.[52]

KEY MESSAGES FOR CLINICAL PRACTICE

1 Aplastic anaemia is a rare entity.

2 Because of the excellent prognosis of treated patients, the management of AA should be aimed at early and accurate diagnosis for urgent initiation of the first line therapy.

3 Stem cell donor search should be started at diagnosis in all patients eligible for stem cell transplantation.

4 Depending on age, comorbidities and donor availability, stem cell transplantation will be performed in first or second line after IS.

5 Classical IS in Belgium consists of rabbit ATG+CsA.

6 Eltrombopag is emerging as a major component of management of refractory disease and possibly also in first line.

7 Supportive care includes transfusion and infection prophylaxis.

8 Evaluation and monitoring of GPI-negative clones as well as other clonal evolution by molecular and cytogenetic analyses are mandatory.

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