Severe Aplastic Anemia in Children (<19 yrs of age)

Guideline – Diagnosis and treatment of Severe Aplastic anemia (SAA) in children.

Princess Máxima Center for pediatric oncology

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This guideline will be updated every 2 years or earlier if appropriate

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What's new? / Take home messages

SAA and hypoplastic myelodysplasia have been notoriously difficult to distinguish. Moreover, it becomes ever clearer that there is a continuum between low grade myelodysplasia, proceeding to myelodysplasia with increased blast count to AML. Treatment should be weighed involving experienced diagnostic experts, and doctors experienced in both chemotherapy/immunosuppressive therapy and stem cell transplantation.

In SAA, well matched unrelated donors, willing to donate marrow are equivalent to sibling donors. Such transplant scenarios are preferred over immune suppressive therapy (IST). IST has a high inherent risk of late failure.

Conditioning regimens for matched family/sibling and unrelated transplants are identical: a combination of fludarabine, cyclophosphamide and ATG.

Other than in adults, addition of eltrombopag to IST has no clear beneficial effect in children with SAA.

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1. General and regulatory aspects

a. Status of this guideline

The previous version of the treatment guideline for pediatric SAA was exempted from METC approval, since it was judged a best available treatment guideline.
As there is currently no active study on pediatric SAA in Europe, this document is considered a treatment guideline with registration of patients in the European EWOG-SAA database (based in Freiburg) as well as bio banking of samples (after informed consent). Patients of our own centre will be evaluated every 2 years, including data on protocol violation/non-adherence.

b. National and international collaboration

At a national level, the M4C group for marrow failure collaborates with the section of benign hematology that is part of the Dutch Association of Pediatrics (NVK). Twice a year, interesting cases of bone marrow failure are digitally discussed with representatives of all pediatric hematology centers and the M4C group.

Both Dutch pediatric transplant units (Leiden and Utrecht) have regular meetings where they discuss patients, studies and other issues. Marrow failure patients (except for Fanconi anemia and high risk MDS patients, transplanted in Utrecht) are treated in both centers. Their treatment plan is shared in this so-called Platform meeting.

AYA patients (roughly >16 years up to 19 years of age) will be discussed in the monthly meeting with the adult hematology department of the UMC Utrecht. New insights eg regarding treatment with eltrombopag or alternative transplant approaches will thus be taken into consideration.

In Europe, most pediatric hemato-oncology centers (Germany, Nordic Countries, The Netherlands, Belgium, Switzerland, Austria, Czech Republic, Hungary, Poland) cooperate in the Freiburg-based European Working Group (EWOG) – MDS (Myelodysplastic syndrome) in children. EWOG-MDS/SAA | Universitätsklinikum Freiburg (ewog-mds-saa.org) Parallel to this cooperative group a similar cooperation for pediatric acquired aplastic anemia has emerged (EWOG-SAA).

Since pediatric bone marrow failure syndromes, including SAA and MDS are very rare, international cooperation is mandatory to achieve improvement in knowledge and management of these diseases. The cooperation within the EWOG, has contributed greatly to the knowledge on biology of pediatric marrow failure syndromes with – in general – low blast counts as well as for JMML (juvenile myelomonocytic leukemia). Examples are the current knowledge on SAMD9, GATA2, RUNX and JMML biology and its impact on disease.

Unfortunately, clinical studies aiming at improved therapeutics are rarely performed in this consortium. This is caused by a lack of interest of pharmaceutical companies for these rare diseases as well as the different regulations in different European countries hampering cooperative, investigator initiated studies. Current practice is to update consensus guidelines during EWOG meetings, especially for transplant indications and procedures. These guidelines are available through the EWOG website.
The most recent EWOG members meeting is in Frankfurt, May 2022.

EWOG teleconferences, where cases are also discussed, are organized several times a year.
EWOG conferences are biannual, the most recent one in hybrid form due to the pandemic, in Athens, October 2021.

The DCOG has partnered in the EWOG network for decades. M4C Marrow Failure has a strong commitment to continue the EWOG collaboration.

Level of evidence for this guideline
This guideline is based on the EWOG SAA European guideline. Adaptations are limited, to bring procedures in line with local procedures. The authors performed additionally a review of the literature in preparation for this guideline.

c. Data accrual and informed consent

Data have been entered by all national groups in the EWOG databases for decades. In recent years, increased privacy regulations have prompted an update of agreements underlying data sharing. Recently a contract regarding this issue has been signed between the Máxima Center and EWOG, available at the Trial and data centre.

All patients with bone marrow failure receive a patient information form (PIF), explaining data accrual, national and international data registration as well as bio banking. After having obtained informed consent, all data, including transplant data if applicable, are also shared with the EBMT and its working parties (e.g. SAA and Pediatric diseases).

Appropriate informed consent texts are available on the document management system from the Princess Máxima Center, iMáxima.

Material and/or data will only be shared after discussion in the Biobank & Data Access Committee (BDAC) of the Princess Máxima Center.

d. Evaluation

Data on patients treated according to this guideline will be reviewed every 2 years. Number of patients treated, relevant violations of the guideline and their motivation will be taken into account, as well as treatment results. This will be reviewed in a written evaluation.
Transplant results are evaluated in the yearly transplant outcome review (JACIE format).

2. Introduction

a. Definitions

Aplastic anemia (AA) is defined as pancytopenia in combination with a hypo cellular bone marrow. The bone marrow is often characterized by replacement of healthy marrow cells by fat tissue, as well as lymphocytosis. Pancytopenia is the presentation with multiple lineage cytopenia, which is the result of the inability of the bone marrow to form blood cells. The pathophysiology can be very different.

To diagnose AA, at least 2 of the following criteria must be present (1):
- hemoglobin < 6 mmol /L.
- platelets < $50x10^9$ /L.
- neutrophils < 1500 x 10^6/L.

AA can be classified into moderate (MAA), severe (SAA) and very severe (VSAA), based on the peripheral blood values, as originally described by Camitta (2) (Table 1).

Children are included up to and including 18 years of age.

Table 1: *Classification of AA*

	Data accrual and informed consent	Data accrual and informed consent	Data accrual and informed consent
BM cellularity	Hypo cellular marrow	<25%	<25%
Absolute neutrophil count	>500x10^6/L <1500x10^6/L	<500x10^6/L	<200x10^6/L
Platelets	<20x10^9/L >100x10^9/L	<20x10^9/L	<20x10^9/L
Reticulocytes (normal = 25-120x10^9 /l)	<40x10^9/L	<20x10^9/L	<20x10^9/L

b. Causes of AA

Hypo plastic bone marrow can be caused by inherited bone marrow failure syndromes (IBMFS) versus acquired causes such as viral infections, nutritional deficiencies, immune mediated or metabolic disorders and marrow failure secondary to toxic damage. Other hematologic causes are myelodysplasia, hematologic malignancies and/or myeloproliferative diseases.

Inherited bone marrow failure syndromes
Inherited bone marrow failure syndromes (IBMFS) are hematological disorders characterized not only by ineffective hematopoiesis, but also by a predisposition to cancer and often by congenital malformations (3). IBMFS may present with single cell cytopenia, e.g. Diamond Blackfan anemia or severe congenital neutropenia (SCN) or evolve towards or present with pancytopenia. The underlying cause of marrow failure is dependent of the constitutional mutation, such as an impaired DNA repair system in Fanconi anemia (4)– separate Máxima guideline –, defects in maintaining adequate telomere length in Dyskeratosis Congenita (5) or ribosomal biosynthesis (e.g. in Diamond Blackfan anemia).

In addition to the classical IBMFS, indirect damage to the stem cells caused by an impaired immune system or the stem cell niche, due to constitutional gene defects such as CTLA4 (6) or DADA2 (7) mutations, can also cause AA. The proportion of congenital syndromes accountable for AA has been reported quite variable ranging from 5% up to 50%.
Firstly, this can be explained by bias of the investigated population. In children with AA and classical characteristic physical anomalies, the incidence of underlying IBMFS or immune deficiencies is clearly higher as compared to patients without physical anomalies (7)

Secondly, the chance to find an underlying genetic defect depends on the diagnostic approach. With increasing application of full genomic sequencing methods, more genetic defects will be found related to marrow failure. Conversely, this implies that underlying congenital mutations causing marrow failure are currently undetected in a part of the patients diagnosed as AA (8, 9a). This suggests that also in adult patients underlying causes are currently underdiagnosed and should be considered, especially in familial cases, or if malignancies are diagnosed at an unusual young age and/or if treatment with chemo- and/or radiotherapy has unusual severe toxicities such as extremely severe mucositis.

Hypo cellular bone marrow and malignant predisposition
Cytopenia in pediatric patients can be a result of IBMFS associated with hematological and non-hematological malignant propensity (9b, 10), such as in Fanconi anemia. Other germline mutations, such as mutations in GATA2, ETV6 and SRP72 genes are associated with an increased risk of progression to leukemia and are referred to as predisposition syndromes (11 -14). In addition, in childhood myelodysplastic syndrome (MDS) specific somatic chromosomal alterations and mutations such as in RAS, RUNX1, SETBP1 and ASXL1 oncogenes are related to an increased frequency of malignant progression.

Immune mediated aplastic anemia
The group of AA patients without an identifiable cause is often referred to as acquired AA or -more appropriately- idiopathic AA. This type of AA is hypothesized to be often
driven by an immunologic etiology. The strongest evidence for an immune mechanism in these patients is the effect of immunosuppressive therapy (IST) restoring blood counts in a considerable part of AA patients (15, 16). In addition, the role of immune dysregulation is supported by the identification of oligo clonal expanded T-cell populations in experimental settings (17). Extensive experimental investigations have provided data supportive for an immune-mediated pathophysiology. Several related mechanisms have been suggested, including CD8+CD57+ oligo clonal T-cells with direct cytotoxic activity (18), secretion of different inflammatory cytokines such as interferon-gamma - (19), immune disarrangement by increased T-helper type 17 cells (20) or reduced regulatory T cells (Tregs) (21) and associative correlations with certain HLA types (22).
However, who will or will not respond to IST is as yet unknown as determinants to predict therapy response are lacking (16, 22).

Myelodysplastic syndrome with refractory cytopenia of childhood
Myelodysplastic syndrome (MDS) often presents with cytopenia affecting different hematopoietic lineages and accounts for less than 5% of childhood hematological malignancies (12). The most prevalent MDS subtype in pediatric patients is Myelodysplastic syndrome with refractory cytopenia of childhood (MDS-RCC), which is challenging to differentiate from AA (23, 24). Somatic cytogenetic abnormalities are present in approximately 30% of MDS-RCC cases, most frequently monosomy 7 (11). Identification of blood and marrow morphological features of MDS-RCC is important for the differential diagnosis with cytopenia in IBMFS or SAA and is required for the application of distinctive therapeutic decisions (25, 26).

However, morphological distinction between the two entities can be challenging and combined investigation of bone marrow aspirate and trephine biopsy together with cytogenetic analysis and molecular mutational analysis is essential for a comprehensive assessment (27, 28).

Paroxysmal nocturnal hematuria
Paroxysmal nocturnal hematuria (PNH) is clinically associated with intravascular hemolysis, nocturnal hemoglobinuria, thrombosis and marrow failure. Although frequently found in adult AA patients (29), PNH is rarely associated with bone marrow failure in pediatric AA. PNH is caused by a mutation in the phosphatidylinositol glycan anchor biosynthesis, class A (PIG-A) gene in the hematopoietic stem cell, rendering cells susceptible to complement-mediated hemolysis. Pediatric patients with AA often have small PNH-clones, but do not display the characteristic clinical phenotype of PNH. Clone size is an important predictor of clinical symptoms, especially considering the risk for thrombosis. Patients with small PNH-clones should be followed-up at regular intervals for potential progression/evolution of these clones, and/or development of the clinical phenotype of PNH. Treatment of PNH was revolutionized with the advent of complement binding antibodies such as eculizumab.
Due to the rareness of PNH, the treatment of children is centralized at UMC Utrecht and Radboud UMC.

Hepatitis associated aplastic anemia

Hepatitis associated aplastic anemia (HAAA) is a potentially lethal complication of acute hepatitis where pancytopenia develops concordantly or within a few weeks to months after the acute hepatitis episode. As with SAA, the pathogenic mechanism is unclear (30). The (hypothetical) immune attack might be a presentation of auto-immunity since in most reported cases no identifiable viral or otherwise infectious cause for the hepatitis was found. However also characteristic laboratory findings of auto-immune disease lack in most patients. So the etiology of HAAA regarding the hepatitis as well as the following aplastic anemia remains unclear. Pediatric patients with HAAA who recover from hepatitis spontaneously or after treatment with (transitory) IST show excellent outcomes when treated by HSCT for their AA (31).

The management of aplastic anemia in these patients, regarding the supportive care, conditioning regimens, donor selection and follow-up does not differ from other SAA patients. However, prior to the development of bone marrow failure, hepatitis may progress to fulminant hepatic failure. SAA after liver transplantation is more challenging and is associated with high mortality (32, 33). The complexity of HAAA hampers general recommendations within this guideline, therefore, a dedicated multidisciplinary team of hepatologists and hematologists within the HSCT center is needed to tailor diagnostic and treatment strategies. Current registry investigations of the European Society for Blood and Marrow Transplantation (EBMT) should be helpful to improve management of this challenging patient group.

3. Diagnostics

a. Diagnostic work-up

Based on the different causes of AA, diagnosis requires a multidisciplinary approach in which clinical features, histopathologic presentation and genetic aberrations have to be considered.
In a stepwise approach from clinical presentation and routine lab diagnostics, to histopathological evaluation of peripheral blood and bone marrow, to extensive genetic and molecular analysis, it is possible to unravel the different causes of cytopenia. (Figure 1)

i. General diagnostic procedures

In initial workup, clinical presentation, clinical and family history, in combination with general laboratory diagnostics may help to identify reversible causes of marrow failure (e.g. viral/immunologic/metabolic/toxic causes) and clear-cut diagnosis of hematologic malignancies such as acute leukemia.

Clinical history should focus on clinical presentation with special attention for infections, intake and deficiencies, toxins and travel history. A detailed family history is needed regarding early death, hematologic diseases, cancer, lung- or liver transplant (fibrosis can be indicative of dyskeratosis congenita) and mental retardation syndromes.

In physical examination, special attention for growth, skin, nail and/or skeletal abnormalities, and dysmorphia is needed, beside regular physical examination and detection of lymphadenopathy and organomegaly.

General lab is needed to have a general insight in the hematologic problem and potential organ failure, to exclude infectious causes (by performing serology) and detect deficiencies. Specific examinations such as trombopoetin, HbF and basic immunologic analysis can be included.

When reversible causes are excluded, diagnostics should be extended to specialized diagnostic procedures and genetic/molecular diagnostics.

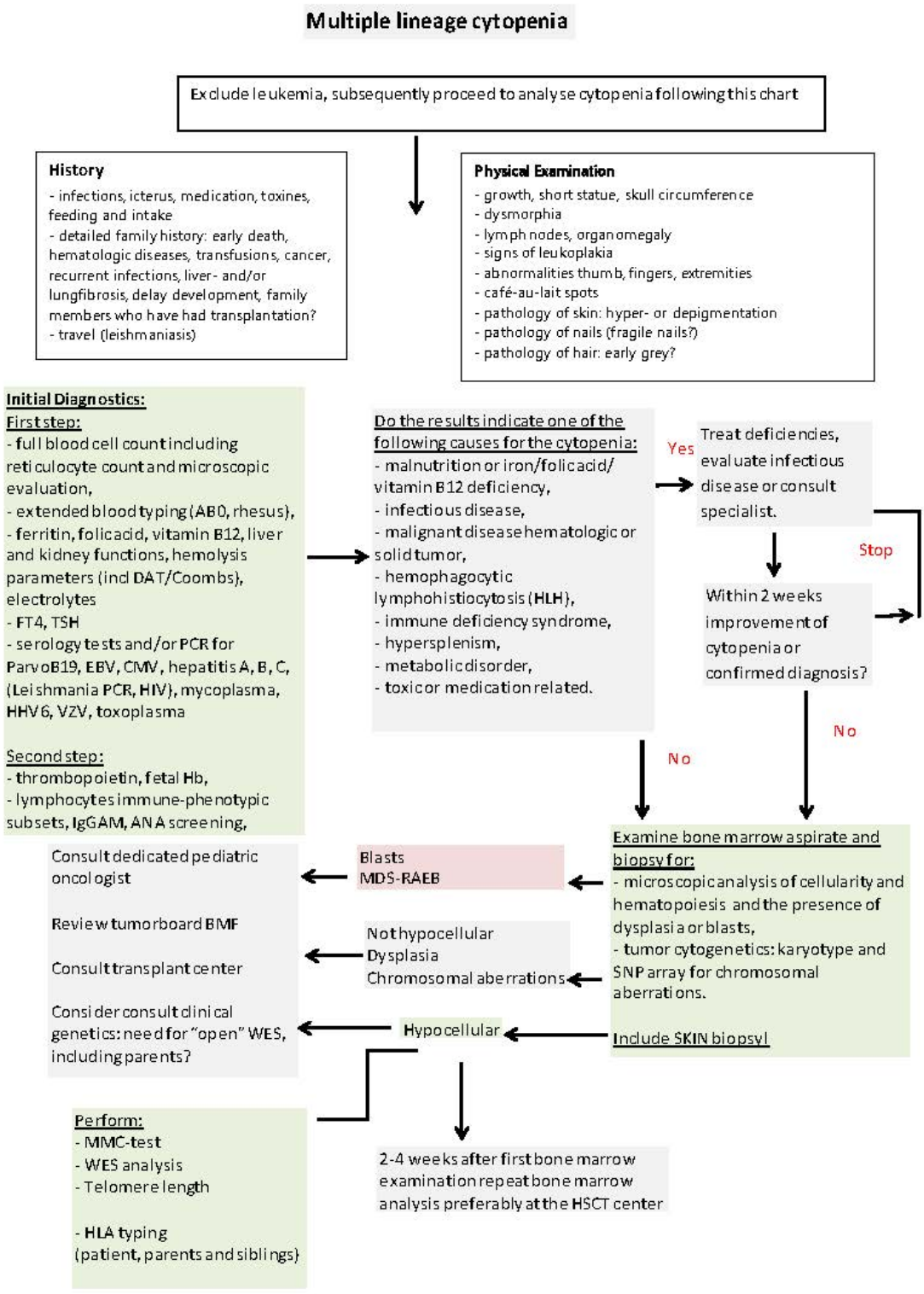

Figure 1: *Diagnostic work-up of multiple lineage cytopenia*

ii. Specialized hematologic diagnostic procedures

If general diagnostic procedures do not reveal a cause (see figure 1); or the pathologic features remain despite adequate treatment, analysis of the bone marrow should be performed. It is advised to repeat bilateral trephine crista biopsies in 2-4 weeks before proceeding to treatment in all patients. Repeated bone marrow assessments may also be necessary to rule out spontaneous recovery of hematopoiesis after a viral infection.

Cytomorphology and histology of bone marrow aspirate and trephine biopsy give insight in the structure of the bone marrow, cellularity, maturation and/or dysplasia of the cells, and abnormal clonal cells.

Flow cytometry has to be performed to exclude a clonal hematologic malignancy and to identify PNH-clones (in peripheral blood) by detecting glycophosphoinositol-linked cell surface membrane proteins (CD55/CD59). Patients with small PNH-clones should be followed-up at regular intervals for potential progression/evolution of these clones, and/or development of the clinical phenotype of PNH.
According to Shah et al (34) finding a PNH clone highly supports the diagnosis of aplastic anemia as opposed to iBMF syndromes and MDS/RCC (positive predictive value 100%, negative predictive value 48,5%, specificity 100% and sensitivity 46%).

iii. Genetic and molecular diagnostics, functional assays

Cytogenetic and molecular diagnostics need to be performed on the bone marrow sample to identify MDS-related genetic abnormalities. Standard cytogenetics comprise karyotyping and FISH to reveal chromosomal abnormalities, i.e. monosomy 5 or 7 and trisomy 8.
Newer techniques, such as SNP array and RNA sequencing are more sensitive to detect smaller cytogenetic and molecular aberrations.
The finding of 6p CN-LOH would support the diagnosis of aplastic anemia (34).
Also whole exome sequencing (WES), focusing on the detection of known bone marrow failure related mutations, is routinely performed in our center. WES data are analyzed by a gene defined filter, now identifying 126 genes. (see Appendix) .The gene filter is yearly evaluated in collaboration with the genetic department and collaborating laboratories, with newly identified gene mutations.

Not all genes have been identified, justifying the need to continue applying functional assays such as mitomycin C induced chromosomal breakage testing and telomere length assays.
A negative mitomycin C test (or DEB test) is needed in all cases to rule out Fanconi Anemia, even in case of absence of other clinical features indicative of Fanconi Anemia (note: in case of strong suspicion of Fanconi anemia a negative mitomycin C test may not be sufficient, and a mitomycin C test on fibroblasts may be required, as mosaicism may obscure a correct diagnosis).

Clonal abnormalities may or may not be present at initial diagnosis both in AA as well as in MDS, although a large clone of monosomy 7 or 5q- is probably more suggestive of MDS than of AA. It remains questionable whether reported cases of AA with clonal abnormalities should not be classified as hypoplastic MDS. In such cases one could consider repeating the trephine biopsies as mentioned above. In one study, 4% of typical AA patients (n=176) diagnosed with trephine biopsies were reported to have clonal cytogenetic abnormalities at diagnosis. These abnormalities may disappear spontaneously or may disappear after immunosuppressive treatment (IST), without compromising survival. However, newly developing clonal aberrations after IST may be associated with progression to MDS/AML.

b. Differentiating from inherited bone marrow syndromes

A positive family history, findings of specific abnormalities on physical examination can indicate in the direction of an inherited bone marrow syndrome. Please be referred to e.g. the SKION Guideline on Fanconi anemia or specific literature on e.g. dyskeratosis congenita.
Specific diagnostic procedures include gene panel assays as well as functional assays:
Mitomycin C (for increased chromosomal breakage in Fanconi anemia) and telomere length (for dyskeratosis congenital). The relevance of these diagnoses lies in concomitant comorbidities and thus adapted therapies as well as in genetic counseling.

c. Differentiation of SAA from refractory cytopenia (MDS-RCC)

Inherited BMF syndromes, refractory cytopenia of childhood (RCC) and acquired severe aplastic anemia (SAA) are the most important diagnoses with different causes that can have an overlap in clinical presentation and are difficult to distinguish. Differential diagnosis of SAA and iBMF is described above.

Differentiation of myelodysplastic syndrome – refractory cytopenia of childhood (MDS-RCC)
MDS-RCC is the most common subtype of myelodysplastic syndrome in children and can present similar to SAA. Although distinguishing MDS-RCC from SAA can be quite challenging in the absence of chromosomal abnormalities, the diagnosis of MDS-RCC is much clearer when next to cytopenia and dysplastic features, chromosomal (-7/7q-; -5/5q-) or molecular aberrations are present (35). Besides traditionally mutated genes in MDS (i.e. NRAS, TP53, RUNX1 and ATRX); a number of additional mutational targets including NPM1, TET2, CBL, EZH2, FLT3, KRAS, MPL, ASXL1, PTPN11 and KIT have been identified thanks to further progress on advanced sequencing technologies (36). These developments in molecular technologies have also improved our understanding of the pathogenesis of pediatric MDS by identifying germline mutations, particularly those in GATA2, SAMD9, and SAMD9L (14, 37).

A clear histopathological differentiation between SAA and RCC is needed, because treatment protocols are not entirely identical (e.g. conditioning regimen in transplantation).

The morphological criteria for the distinction between SAA and RCC have been published and the most recent World Health Organization (WHO) classification included RCC as a provisional entity (38).
It may also be necessary to repeat the trephine biopsy or to perform bilateral biopsies to increase the reliability of a correct diagnosis. Especially small trephine biopsies may not show patchy areas with islands of erythropoiesis, hence obscuring a correct diagnosis of MDS.

Decisive pattern for differentiation of RCC and SAA:
The pattern of SAA shows complete aplasia or –as stated above- severe hypoplasia of hematopoiesis with scarcely distributed marrow cells, without patchy areas, especially of erythroid precursors and no evidence of megakaryocytes with dysplasia. Also, in SAA the bone marrow is often characterized by fat tissue replacing healthy bone marrow and lymphocytic infiltration (>95% fatty marrow) (Table 2)(26).
The histopathological pattern of RCC consists of islands of immature (left-shifted) erythroid precursors accompanied by sparsely distributed myeloid cells. In RCC megakaryocytes are significantly decreased or absent and few dysplastic micro megakaryocytes are detected on immunohistochemistry (CD61 staining). These features (especially patchy erythroid islands with a marked left shift) are mandatory for the diagnosis of RCC and are used to separate RCC from SAA.

Table 2: *Histopathological criteria of RCC and SAA*

	Refractory cytopenia	Severe aplastic Anemia
Erythropoiesis	Patchy distribution Left shift Increased mitoses	Lacking or single small focus with less than 10 cells with maturation
Granulopoiesis	Marked decrease Left shift	Lacking or marked decrease Very few small foci with maturation
Megakaryopoiesis	Marked decrease Dysplastic changes Micro megakaryocytes	Lacking or very few megakaryocytes No dysplastic megakaryocytes
Lymphocytes	May be increased Focally or dispersed	May be increased Focally or dispersed
CD34+ cells	No increase	No increase

Timing of diagnostics
Work-up for diagnosis can be divided into three time-periods, spanning a 2 month period (see Figure 2):

1) First presentation with general work-up for pancytopenia. Analysis of family history, clinical history, physical examination and other routine diagnostics with focus on infectious, metabolic and immunologic causes. In this first phase a hematologic malignancy (acute leukemia) needs to be excluded.
   Therefore peripheral blood, as well as bone marrow aspirate and trephine biopsy needs to be examined.

2) After transient causes have been excluded, molecular and genetic analysis is needed to reveal iBMF and MDS with somatic or germline mutation. Histopathological work-up of bone marrow aspirate and trephine biopsy needs to be done and if non-conclusive or difficult to interpret, repeated and/or reviewed.
   Review is organized via the Máxima diagnostic laboratory (Valérie de Haas). European review is from there through the Freiburg EWOG laboratory services.
   At this stage workup for allogeneic transplant (including HLA typing and a global donor search) should be considered. This is done through the Máxima transplant team and procedures (SCT coordination team).

3) Subsequently, a bone marrow aspirate and trephine biopsy needs to be repeated within 2-4 weeks in case of differential diagnosis SAA versus RCC (without molecular/genetic substrate) and to follow-up progression of hypoplasia and dysplasia. In this period, additional tests like mitomcyin C breakage test and telomere assay need to be done.

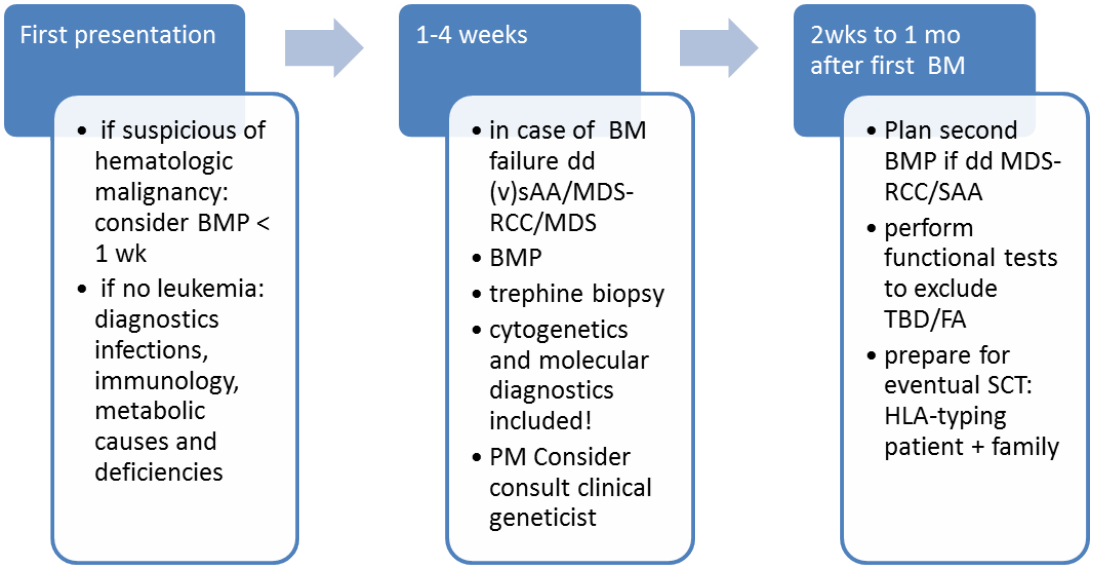

Figure 2: *Timing of diagnostics*

4. Therapy

a. Choosing between HSCT or IST as first line treatment

Allogeneic hematopoietic stem cell transplantation (HSCT) can be life-saving in patients with (acquired) bone marrow failure. It can be applied upfront or in case of failure of alternative therapies, mainly immunosuppressive therapy (IST). IST ultimately fails in up to 50% of cases, despite improvement – mainly in adults- with the addition of eltrombopag. Initial response to IST may be good in a majority of patients, but late relapse, development of clonal disease or unacceptable toxicity of prolonged use of cyclosporine at high levels impacts on the IST success.

b. HSCT

In case a HLA identical sibling is available, HSCT has been the frontline treatment of choice for decades, especially in younger patients (39-41). A more contemporary paper by Dufour et al (42) examined the outcome of SAA in 563 children aged 0-12 years, reported to the EBMT (thus biased towards transplant as a treatment).
OS after IST and matched sibling transplant were 87 and 91 % respectively. However, EFS after IST was limited to 33%. This should be interpreted with some caution, again as there is bias since transplanted patients are overrepresented in this cohort. Matched family transplant results are excellent. Chronic GvHD was limited to 6 %. Conditioning regimens contained cyclophosphamide in >90% of cases, often combined with fludarabine. GvHD prophylaxis was cyclosporine based in general, in 54% of cases combined with a short course of methotrexate.

The role of unrelated or alternative donor (such as haploidentical donors or cord blood donors) has been more controversial and evolved over time:

Unrelated, matched transplantation for SAA
The EBMT registry contained data on 74 European patients receiving an unrelated graft upfront between 2010 and 2018. Petit et al (43) report their outcome: median age was 20 years. 60 patients had a matched unrelated donor. Graft failure was limited to 8%. Acute GvHD II-IV was also limited, 13%. 2 year overall survival was 89%. GvHD/relapse free survival (GRFS) was 86% at 2 yrs. Time from diagnosis to transplant was rather prolonged with 7 months.

Although the use of peripheral blood as stem cell source is well known to lead to increased chronic GvHD compared to marrow, this was not obvious in this particular study.

All French SAA patients treated between 2000 and 2012 with an unrelated donor as first transplant were analyzed to build a predictive score using age over 30, time from diagnosis to transplant (over 12 months) and the use of a 9/10 matched unrelated donor (versus a 10/10 matched donor). This score was then confirmed in an independent cohort from the EBMT of 296 patients resulting in the prediction that survival was shorter in the presence of at least 2 of the identified risk factors. Children were included in both cohorts. OS with 0-1 risk factor was 74-76% at 4 yrs in both cohorts respectively (44).
In the UK, Samarasinghe et al (45) published results of children with SAA transplanted with an unrelated donor with promising results: in 44 children receiving a (10/10) MUD graft 5 yrs failure-free survival was 95%. There are some remarks: 40 children had failed previous IST treatment and the conditioning regimen contained alemtuzumab (CAMPATH) as serotherapy. Campath is not readily available in many countries (for this indication).

In a next study, Dufour et al (46) explored upfront unrelated donor transplantation: this was compared to historical data with IST and HLA identical sibling transplantation. 29 children were transplanted with an unrelated donor between 2005 and 2014. 2 year survival was excellent, using the same approach with alemtuzumab and a chemo-based conditioning (fludarabine and cyclophosphamide): 96%. Matched sibling transplantation and IST survival were 91 and 94 %. Thus, Samarasinghe et al (47) proposed a UK pediatric SAA treatment guideline in 2012 where IST and MUD HSCT were equivalent treatment options in the absence of a matched sibling donor.

Haplo identical transplantation for SAA
15 studies on haplo identical HSCT for SAA were analyzed by El Gohary et al (48). Jointly, 577 patients were included. Successful engraftment was achieved in 97% of cases. TRM was 6.7% / year. These retrospective studies thus showed encouraging results.

Childs et al describe 29 patients (19 SAA, 10 MDS), aged 4-48 years in a phase 2 trial combining single cord with haplo CD34 selected grafts, to speed up engraftment. 97% had neutrophil engraftment at median 10 days, in the early phase from the haplo graft. Hematopoiesis was eventually taken over by the cord graft. With a median FU of 7.5 years, Graft-versus-host free, relapse free survival (GRFS) was 69 % (OS 83%) (48). Dezern et al using an approach of haplo identical transplantation with post-transplant cyclophosphamide treated 20 relapsed/refractory and 17 treatment-naïve (TN) SAA patients, including patients with inherited marrow failure (non-Fanconi). 3/17 patients in the TN group had primary graft-failure after 200 cGy TBI, thus this dose was increased to 400 cGy. Although a high OS at 2 years of 94% was achieved, the use of radiotherapy makes this approach less valuable in children (50). In Europe, between 2011 and 2017, 33 patients with SAA were transplanted with an haplo identical transplant combined with cyclophosphamide post-transplant and reported to the EBMT. Prata and colleagues report on their outcome: neutrophil engraftment was achieved in 67% at day 28. 2 year OS was 78% and 2 yr GRFS was 63%. 39 % of reported patients were children in this retrospective study (51).

Cord blood transplantation in SAA
A French national study evaluated 26 SAA patients, median age 16, transplanted with 1 or 2 cord blood units (to achieve a minimum cell dose of 4.10*7 nucleated cells/kg) after a fludarabine, cyclophosphamide, ATG and 2 Gy TBI conditioning regimen. This last resort treatment lead to 88.5 overall survival after 1 year. Incidences of grade II-IV aGvHD and chronic GvHD were relatively high with percentages of 46 and 36 %, respectively (52). This compares favorably to historical data on survival of cord blood transplantation in SAA with 2 year OS of 41 % (Japan, Yoshimi et al)(53) and 3 year OS of 38% (Europe, Peffault de Latour)(54).

Conclusion: the role of upfront HSCT in acquired aplastic anemia in children.

HSCT Indication and position versus immunosuppressive therapy
As first line treatment both an HLA identical sibling donor as well as a readily available (1-2 months after diagnosis) 10/10 matched unrelated donor, willing to donate marrow (to reduce the risk of chronic GvHD) are first choice. It is pivotal that a donor search is started as soon as possible after the (suspected) diagnosis has been made: in real life, delay in starting the treatment is a clear risk. Treatment with IST should not be postponed for prolonged time periods, awaiting donor issues. It should be kept in mind that treatment-start should be ultimately 2-3 months after diagnosis. Infectious complications as well as potential bleeding issues may develop in prolonged waiting time. IST takes time to evaluate for efficacy, and so it may take easily up to 6 months in case of IST failure to proceed to transplant, a long period to be aplastic. In case of IST failure best available donor (including haplo) should be considered.

The role of cord blood or alternative approaches such as haplo (using in vivo or ex vivo T cell depletion) versus IST are less clear at this point in time.

HSCT Conditioning regimen
The recommendation for conditioning in SAA, both with identical sibling donors and with 10/10 matched unrelated donors is:

ATG (Genzyme, dose according to individualized dosing, local SOP)
Fludarabine 4x30 mg/m2 Day -6, -5, -4, -3,
Cyclophosphamide 4x 25 mg/kg Dag -6, -5, -4, -3

Supportive care according to local SOPs.

GvHD prophylaxis: cyclosporine for 6 months, short course methotrexate (10 mg/m2 on days 1,3,6)

In case of haplo-transplant with Cy(clophosphamide) post-transplant:
Cy Post Tx: 50 mg/kg on day 3,4 (cum 100 mg/kg) add MMF 30 days, cyclosporine 6 months

Stem cell source
Marrow as a stem cell source leads to a lower incidence of chronic graft-versus-host disease compared to peripheral blood stem cells. For this reason there is a strong recommendation to use marrow.
Cord blood may be used, according to local guidelines on matching and stem cell dose (local SOP).
In a haplo setting with T cell depletion, a high stem cell dose is necessary. Consult local SOP.

Side effects of stem cell transplantation
In general, allogeneic transplant is a complex procedure with several short and long term side effects. Mucositis, due to chemotherapy, infectious complications are the major short term side effects. The graft may fail, leading to persistent pancytopenia. This may require a second transplantation. Graft-versus-host disease is a complication due to donor immunity, directed against recipient tissues. This may be a major complication, with impact on quality of life.

Before every transplant, patients and parents are counseled and informed on their personal treatment plan, including age-adapated information texts.

Long term effects
There are some reports on late secondary malignancies in patients that had been transplanted for SAA. Especially irradiation (as part of the conditioning regimen) and (chronic) skin GvHD seem risk factors for a clearly elevated incidence of secondary malignancies in well studied transplanted populations. Socie et al provide a recent overview (55).

Fertility may be damaged due to drugs used in the preparative chemotherapy. This is taken into consideration in the pre-transplant work up.

c. Immunosuppressive therapy for severe aplastic anemia

Patients with SAA have been treated with immunosuppressive therapy (IST) for decades, (currently a combination of h(orse)ATG with Cyclosporine (CsA), prednisolone and sometimes G-CSF, (when neutrophils are < 0,2x10.9/l)). IST was generally chosen historically when a matched sibling donor for HSCT was not available. Responses are often slow, rendering the patients at risk for severe infections, bleeding and transfusion dependency for some time. Many patients do not reach complete response, though many do show partial responses. A high percentage of relapses and clonal evolution is maybe the biggest concern.
In 2018 Rogers et al (56) published the American results; 314 children treated with IST for SAA from 2002-2014. Of this group 264 were treated with hATG and Cyclosporine. Since this is the largest group of pediatric SAA patients published recently it will be discussed in more detail.

Responses were seen in 71,2% of patients (complete response (defined in this study: platelets>100x10.9/l) in 59,8% and at least good partial response (platelets> 50x10.9/l) in 68,2%). No response was noted in 25%. Five year OS was 93%. However EFS without further treatment was only 64%. Clonal evolution occurred in 7% of patients and almost 2% developed MDS/leukemia.
Median time to first response was 6 months. Responses 6 months after initiation of hATG/CsA were CR 21,6%, VGPR 19,7%, PR 8% and non-response 47%. The estimated probability of sustained response was 94% at 24 months and 84% at 60 months. However, there was no plateau for loss of response observed over time, even 5 years post treatment.

Of 171 patients in whom follow-up BM was obtained, clonal evolution during follow-up was seen in 12 (7%), after a median of 25 months from initiation of IST. Four of those developed MDS.

Complications after initiation of IST were significant bleeding (23,5%), severe infection (53,4%) and renal failure requiring dialysis (1,9%).
Second-line therapy for relapsed or refractory disease was given in 92 (35%) patients (38 HSCT and 54 2nd course IST) and 3rd and 4th line treatment in 13 and 1% respectively. Of all 92 patients receiving 2nd line treatment OS was almost 84% while EFS was 53% with a median time to failure of 64 months.

Due to longer FU after 2nd IST as compared to HSCT, data were censored at 36 months. This analysis showed significantly better EFS after HSCT as 2nd line treatment compared to IST (56).

In a somewhat older study Kosaka Y et al (Blood 2008) (57) already showed that estimated failure free survival (defined as survival with response) was much better after SCT with alternative donor as 2nd line treatment after failed IST, compared to 2nd IST; 83,9% and 9,5% respectively.

For patients with a diagnosis of (very) severe aplastic anemia IST is the treatment of choice for patients who do not have a matched sibling donor or a 10/10 matched unrelated donor who is willing to donate bone marrow within a timeframe of 1-2 months.

Immunosuppressive therapy (IST): treatment protocol

The standard IST treatment (see also Figure 3 below) schedule consists of:

- **Horse-ATG (ATGAM®)** is administered at a dose of 40 mg/kg/day for 4 consecutive days, in 4 hrs. iv. Infusion-related side-effects should be prevented with anti-histamines and methylprednisolone (see below). Note; the dose of ATGAM we recommend is based on the NEJM publication (Scheinberg 2011)(58) showing higher efficacy of ATGAM over Thymoglobulin (rabbit), and differs from the label text, where lower dosages per day are given (10-20 mg/kg) for a higher number of days (8-14 days). Note: “artsenverklaring” and ordering-form are needed for ATGAM, please contact the hospital pharmacy.
- **CsA** 5 mg/kg/day in 2 doses, orally, for at least 6 months, after which it should be slowly tapered (see below) in patients who have achieved a CR, or continued in patients with a GPR. Trough CsA levels need to be checked regularly, and should be in the 0,15-0,25 mg/l range. Tapering of CsA treatment is discussed below. Beware of interaction with azoles (see below), macrolide antibiotics and several anti-epileptic drugs.
- Methylprednisolone 1 mg/kg/day IV for 5 days, as a 30 min. infusion shortly before the first and subsequent ATG infusions. Patients with infusion-related side-effects may need additional dosages of methylprednisolone (1-2 mg/kg IV) during the ATG infusion. Methylprednisolone is also given to reduce the likelihood of serum-sickness.
- From day 6-14 use oral prednisolone 1 mg/kg/day divided in 3 dosages. At day 14 start tapering the dose by 50% every 5 days, until it stops at day 29.
- GCSF 5 µg/kg s.c. or i.v. once daily, will be given to patients with granulocyte counts below $500x10^6$/l at day 1 of IST . If there is a granulocyte response (ANC $>500x10^6$/l) at day 28 or at any later time point, the dose may be tapered by lengthening the injection interval from 24 to 48 hrs., and subsequently to 72 hrs., after which it may be stopped. If the WBC decreases again to $0.5x10^9$/l, G-CSF is restarted. GCSF therapy should not last longer than 60 days in total. Patients with an ANC $>0.5.10^9$ / l at day 1 who subsequently develop severe neutropenia should not necessarily receive G-CSF.

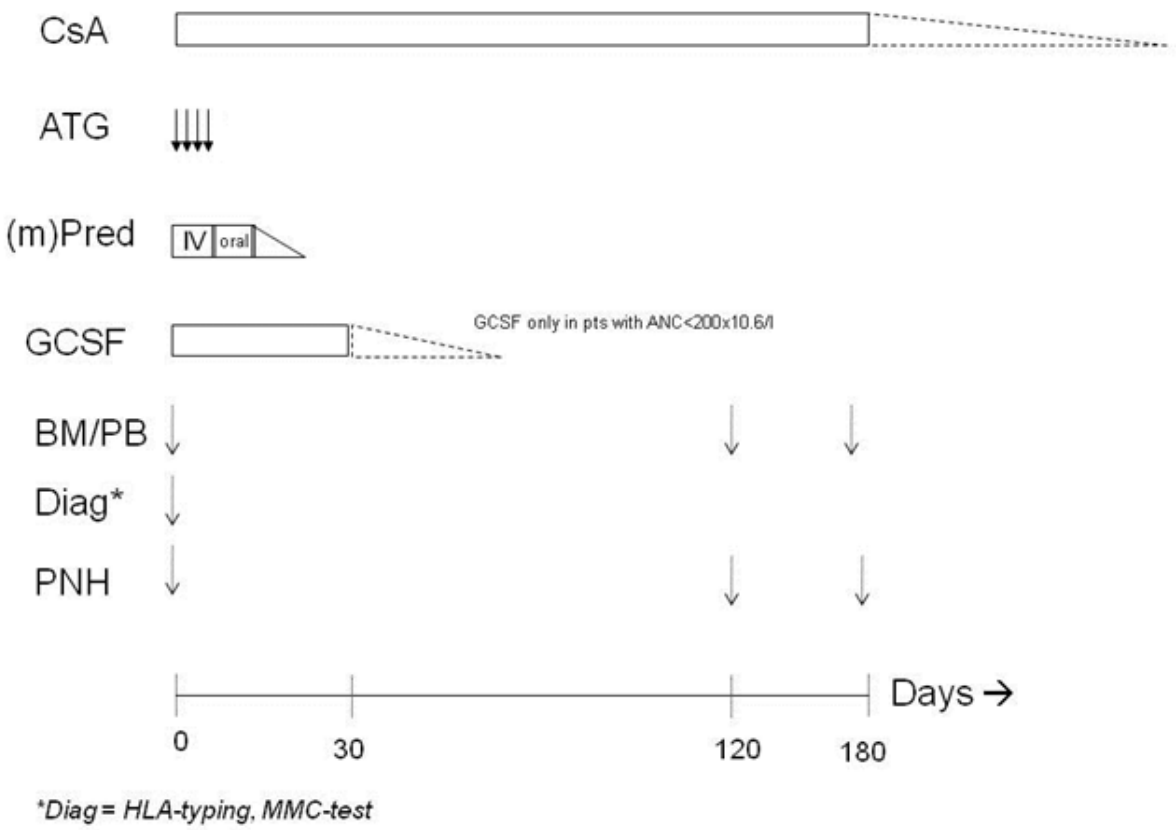

Figure 3: *Standard IST treatment*

Supportive care/infection-prophylaxis during IST :

Blood- and platelet transfusions:

- Hb <4,3 mmol/l (may be somewhat higher if anemia is symptomatic)
- Platelets: Local guideline of the Prinses Máxima Centrum will be followed. Currently (2021) this means that platelets only will be transfused in case of clinical bleeding tendency. Local guidelines will be followed for invasive procedures as well.

Infection-prophylaxis:

- PJP prophylaxis only in case of persistent lymphopenia (CD3<300, very rare in these patients). Anti-fungal prophylaxis with Itraconazole 5 mg/kg/d (max 200 mg/d) as long as neutrophil count is below 0,5x10.9/l. Levels should be monitored. Beware of interaction between Itraconazole and Cyclosporine. On stopping Itraconazole, Cyclosporine dose should be increased with at least 50% and levels closely monitored.
  In case of persistent deep neutropenia Ciproxin prophylaxis could be considered (30 mg/kg/d in 2 doses, max 2 dd 500 mg).

d. Response evaluation (EWOG criteria), IST tapering in responders

All responses must be stable over at least a 4 week period.

Complete response:

- No transfusion dependance and
- Hb normal according to age and gender and
- Neutrophils>1,5x10.9/l and
- Platelets> 150x10.9/l

Good partial response:

- No transfusion dependance and
- Hb> 3,8 mmol/l and
- Neutrophils 1,0-1,5x10.9/l and
- Platelets 50-100x10.9/l

Poor partial response:

- No transfusion dependance and
- Hb> 3,8 mmol/l and
- Neutrophils 0,5-1,0x10.9/l and
- Platelets 20-50x10.9/l

Note: In the US different response criteria are used; Complete response Hb>6, neutrophils>1, platelets>100; VGPR Hb >5, neutrophils > 0,5, platelets >50 and partial response Hb>5, neutrophils>0,5, platelets>20.

Follow-up

Response evaluation:

- A complete blood count (CBC) should be performed monthly after the start of IST, hence at day +30, 60, 90, 120, 180, 240 and 360, 18 months, 2 years and later yearly after start of IST.
- A bone marrow aspirate and trephine biopsy should be performed at day +120 after the start of IST and in case of relapse. Genetic analysis (karyotyping and SNP array) should be performed too, for early detection of clonal evolution.

Response is formally assessed at day +120 following the start of IST.

CAUTION: EWOG data show that patients with persistent severe neutropenia (<0.2.10^9 / l) at day 90 have a high risk of mortality. SCT should be seriously considered.

After evaluation at day +120, all patients should be discussed in the tumor board hemato-oncology/bone marrow failure. Four different response categories can be distinguished, which determine how to proceed:

- Non-responders / poor partial responders: these patients should be discussed with the SCT team. Most likely the best available donor, including haplo-identical transplant donors should be considered. In the unlikely case no donor can be identified: consider a second IST course.
  It is advised to perform the SCT not earlier than the day +180 time-point, given that some late responses may occur, unless the neutrophil count at day 90 is $<0.2.10^9$ / l.

Tapering IST in responders

- Good partial responders: continue CsA at least until day 180 and re-asses response at day +180. At day 180: decide on management as given below. In case of relapse at day +180 (defined as a loss of GPR), consider a second IST course, especially in cases with a poorly matched donor (less than 9/10 match). In case of a 9/10 matched unrelated donor a SCT may also be considered, depending on the clinical situation of the patient. A 5/6 or 6/6 matched cord-blood may also be considered as well as haplo identical transplantation.
- Complete responders: start tapering CsA. Tapering of CsA always has to be done slowly, to prevent relapse. As a rule of thumb, the CsA dose may be tapered 10% every month, provided the blood counts are stable.[20]

After evaluation at day +180 (in patients with PPR and/or GPR at day +120):

- Complete responders: taper CsA as mentioned above
- GPR: continue CsA at the same dose for another 4 months and re-determine response at that time-point.
- Since many patients do not reach platelet counts >150x10.9/l but otherwise fulfill criteria for complete response, tapering CsA could also be considered when platelet count remains above 100x10.9/l for several months, while closely monitoring the blood count.

Day 360 after start IST:
CsA should be tapered slowly (10% / month) regardless of response status.

e. Adding eltrombopag to IST: is there a role in treating children with SAA ?

Townsley et al described the efficacy of adding eltrombopag (rTPO) to standard IST in adults with SAA (59). 3 administration regimens were compared with historical controls. Especially early rTPO start after diagnosis led to a remarkably better response rate (94 vs 66% at 6 months treatment) . This improved response did not lead to higher relapse or clonal evolution rates. In the limited experience so far this has not been observed in children with SAA (60). Some reports from China, combining eltrombopag with unusual IST regimens (e.g. porcine ATG) did show some benefit (61). At this stage it is too early to advocate the use of rTPO in the upfront treatment of pediatric SAA.

5. Cost effectiveness

This guideline does not lead to additional cost, all care described is currently state of the art and cost-effective, given the very high cure rate for this life-threatening condition.

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Appendix I – Overview gene panel BMF

Overview gene panel BMF (122 genes) – clinical genetics UMCU:

ABCB7, ABCD4, ABCG5, ABCG8, ACBD5, ACD, ACKR1, AK2, AMN, ANKRD26, AP3B1, ATR, BRCA2, BRIP1,
CD40LG, CECR1, CLCN7, CLPB, CSF3R, CTC1, CTLA4, CUBN, CXCR4, CYCS,
DDX41, DHFR, DKC1, DNAJC21,
EFL1, EIF2AK3, ELANE, ERCC4, ERCC6L2, ETV6,
FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF , FANCG , FANCI, FANCL, FYB1,
G6PC3, GATA1, GATA2, GBA, GFI1, GP1BA , GP1BB, GP9, GRHL2,
HAX1, HOXA11,
IVD,
JAGN1,
KLF1, LIG4,
LYST,
MASTL, MECOM, MPIG6B, MPL, MTR, MTRR, MYH9, MYSM1,
NBEAL2, NHEJ1, NHP2, NOP10,
OSTM1,
PALB2, PARN, PLEKHM1, PRF1,
RAB27A, RAC2, RBM8A, RMRP, RPL11, RPL35A, RPL5, RPS10, RPS19, RPS24, RPS26, RPS29, RPS7, RTEL1 , RUNX1,
SAMD9, SAMD9L, SBDS, SH2D1A, SLC19A2, SLC25A38, SLC46A1, SLX4, SRC, SRP72, STIM1, STK4, STN1,
TAZ, TBXAS1, TCIRG1, TCN2, TERC, TERT, THPO, TINF2, TNFRSF11A, TNFSF11, TUBB1,
UBE2T, USB1,
VPS13B, VPS45,
WAS, WRAP53