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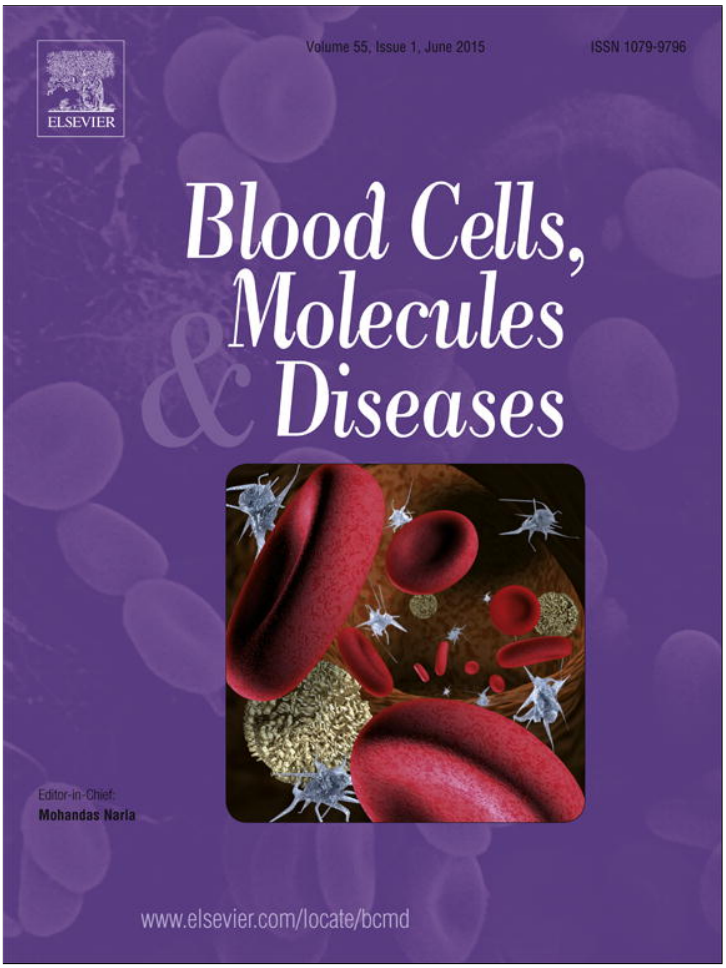

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Diagnosis and management of acquired aplastic anemia in childhood. Guidelines from the Marrow Failure Study Group of the Pediatric Haemato-Oncology Italian Association (AIEOP)

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ABSTRACT

Acquired aplastic anemia (AA) is a rare heterogeneous disease characterized by pancytopenia and hypoplastic bone marrow. The incidence is 2–3/million inhabitants/year, in Europe, but higher in East Asia. Survival in severe aplastic anemia (SAA) has markedly improved in the past 2 decades because of advances in hematopoietic stem cell transplantation, immunosuppressive and biologic drugs, and supportive care. In SAA hematopoietic stem cell transplant (HSCT) from a matched sibling donor (MSD) is the treatment of choice. If a MSD is not available, the options include immunosuppressive therapy (IST) or unrelated donor HSCT. The objective of this guideline is to provide healthcare professionals with clear guidance on the diagnosis and management of pediatric patients with AA. A preliminary, evidence-based document issued by a group of pediatric hematologists was discussed, modified and approved during a series of "Consensus Conferences" according to procedures previously validated by the AIEOP Board. The guidelines highlight the importance of referring pediatric patients with AA to pediatric centers with long experience in diagnosis, differential diagnosis, management, supportive care and follow-up of AA.

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1. Introduction

Aplastic anemia (AA) is a rare disorder of the bone marrow (BM) in which hematopoietic stem cells get destroyed by an autoimmune attack. The disease has an estimated incidence of 2–3/million inhabitants/year in Europe and America but up to three-fold higher in the Far East. This figure is lower in childhood and adolescence and this limits the possibility to run large controlled studies in these age groups. In spite of this, the outcome of the disease thanks to hematopoietic stem cell transplantation (HSTC) and immunosuppressive treatment has remarkably improved over last decades. In order to maintain and further improve these achievements there is need to optimize diagnosis and treatment tools. Moreover in pediatric age this task is complicated by the confounding effect of constitutional marrow failures that sometimes may present without classical somatic abnormalities thus fully mimicking an acquired aplastic anemia; hence, the need to have comprehensive guidelines for diagnosis and treatment of AA in the age between 0 and 18 years. With this aim the Marrow Failure Syndrome Group (MFSG) of the AIEOP (Associzione Italiana Emato-Oncologia Pediatrica) elaborated the present document.

2. Design and methods

Design and methodology reflected those adopted for the "Congenital and acquired neutropenias consensus guidelines on therapy and follow-up in childhood" [1] of the AIEOP. In brief the Marrow Failure Syndrome Group (MFSG) of the AIEOP elaborated in 2006 a document on recommendations for management of children with aplastic anemia. In 2012, it was decided to review and update this document and assigned this task to a group of experts who wrote two pre-guidelines documents.

2.1. Literature review and assessment of evidence

2.1.1. Data source

For the pre-guideline documents, experts extracted evidence from literature searching in the Medline database from 1971 to 31.12.2013 and then updated in March 2014 during the compilation of the final draft. Search terms included: adolescents, aplastic anemia, idiopathic, acquired, congenital, granulocyte-colony-stimulating factor, bone marrow transplantation (BMT), myelodysplasia, G-CSF receptor, children, paroxysmal nocturnal hemoglobinuria (PNH), immunosuppressive treatment, anti-thymocyte globulin, horse, rabbit, cyclosporine A, antibiotic treatment, anti-fungal treatment, vaccinations, transfusion, and chelation. The Medline search sorted out a total of 224 articles that were examined and from which 54 were included in the present paper. The search was also extended to hematology textbooks and proceedings of international hematology meetings. Every collected evidence was attributed a strength that was scored using level of evidence criteria reported in Supplementary Table I.

2.2. Consensus conference

When controlled and non-controlled studies and case-report series (representing the ground for level of evidence from I to V) were not available, issues were regarded as experts opinion (EO) both in case they were contained within published literature or represented the opinion of the panel of experts. The strength of this consensus was quantified on a 1–9 scale where 1 represented no consensus and 9 full consensus regarding the appropriateness and necessity of the practice. For each statement a mean score was calculated. Mean scores from 1 to 3 indicated an inappropriate practice; mean scores from 3.01 to 7 a practice of uncertain appropriateness; mean scores from 7.01 to 9 an appropriate/necessary practice. The level of unanimity of the opinions, indicating the level of consensus was evaluated as in Supplementary Table II.

Based on this system in the text after each statement, the following symbols will be found in brackets: level of evidence in Roman numbers from I to V or EO if expert opinion; strength of consensus in Arabic numbers from 1 to 9; level of consensus in capital letters from A to D.

3. Definition and classification

The term aplastic anemia (AA) indicates a disease characterized by tri-lineage peripheral blood (PB) cytopenia due to reduced or absent production of hematopoietic cells by the BM without extrinsic or intrinsic cellular infiltration [2]. Incidence of AA in the Western world is 2/million inhabitants/year. This figure is 2–3-fold higher in the Far East [3,4]. The majority of cases (70–80%) is considered idiopathic (i.e., with a non-demonstrable cause). In a minority of cases, a drug, a chemical or an infectious agent might be identified.

AA is a multifactorial disease in which different mechanisms are involved often concomitantly. One of the prevalent is autoimmunity, according to which following an usually unknown antigenic stimulus, cytotoxic clones of T cells are activated and release effectors harmful to the BM cells like TNF-alpha, interferon-gamma, that in the end cause destruction of marrow hematopoietic cells [5,6]. Cases were reported in whom chemicals or drugs were associated to the occurrence of AA but an association between the risk of AA and deficiency of detoxifying enzymes was not shown [7]. Table 1 reports the main substances whose use is potentially harmful to the BM. It is usually extremely difficult to prove their etiological role in AA. However if a drug is suspected for having caused AA, cost benefit ratio should be weighted regarding the possibility of withdrawing the substance/drug.

Based on the degree of the reduction of cells in PB, AA can be classified in three forms: moderate or non-severe (NSAA), severe (SAA) and very severe VSAA [8,9] (Table 2).

4. Diagnosis

Diagnostic work up should be extensive and accurate in order to confirm the diagnosis, to define the severity of the disease and to exclude other possible causes of pancytopenia with hypocellular marrow

Table 1
Agents reported to be associated with the occurrence of AA.

Drugs
Antibiotics:
chloramphenicol (no evidence for eye drops and tablets), sulphonamide, cotrimoxazole, linezolid
Antirheumatics:
gold salts, penicillamine.
Anti-inflammatory:
indomethacin, phenylbutazone, naproxen, diclofenac, piroxicam, sulfasalazine
Anticonvulsants:
phenytoin, carbamazepine
Thyroid drugs:
carbimazole (neutropenia), thiouracil
Antidepressants:
phenothiazine, dothiepin
Hypoglycemic drugs:
chlorpropamide, tolbutamide
Antimalarials:
chloroquine
Others:
mebendazole, allopurinol, thiazide diuretics
Chemicals
Benzene and other dissolvents
Pesticidals:
organochlorine and organophosphate, pentachlorophenol, DDT and carbamate
Oils and other lubricant agents
Narcotic drugs:
ecstasy, methylene dioxy-methamphetamine (MDMA)
Others:
Exposure to non-drinkable water, to non-sterile needles, farmers in contact with fowls

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especially constitutional forms [10]. Table 3 shows an extensive list of investigations necessary for an appropriate diagnostic work-up (*Level of evidence EO*; *Strength of consensus 8.6*; *level of consensus B*).

Some tests are considered as mandatory whereas other as ancillary. Personal and family history, exposure to toxics and/or infectious agents are to be carefully evaluated. Physical examination should be focused on malformations and other somatic abnormalities characterizing constitutional marrow failure syndromes, like cafè au lait spots, nail dystrophy, microcephaly, hypogonadism, and mucosal abnormalities such as erythro/leukoplakia [11].

Full blood count usually shows normochromic (normal MCH and MCH) normocytic (normal MCV) or macrocytic (increased MCV) anemia along with neutropenia and thrombocytopenia. In early stages mono- or bi-lineage cytopenia, usually thrombocytopenia, may be observed. Reticulocytes are always reduced.

PB film should be examined for the search of dysplastic and blast cells that may address diagnosis toward myelodysplastic syndrome (MDS) or leukemia.

BM aspiration does not allow a correct quantification of the hematopoietic cellularity, since a hypocellular marrow may depend on technical problems and, although rarely, it may happen to observe a normal cellularity if the aspiration fell in an area in which hematopoiesis is still preserved. BM aspiration is useful for the detection of dysplastic and blast cells thus supporting differential diagnosis with MSD and hypocellular leukemias.

BM trephine biopsy is the key test for diagnosis of AA. The hallmark is the reduction of the hematopoietic cellularity below 30% with fat cells replacing hematopoietic cells. This test is also very helpful in differentiating AA from hypo-cellular leukemias, where blast cells are seen, and from MDS where maturation and morphological alterations (of one ore more lineages), often not detectable on marrow aspiration, are found [12].

The differential diagnosis should always take in account congenital forms of AA. In 15-20% of cases an AA which appears as acquired, indeed unveils an inherited or constitutional form, like Fanconi anemia (FA), dyskeratosis congenita, Shwachman Diamond syndrome, and congenital amegakaryocytic thrombocytopenia.

Some patients that harbor mutations of TERC or TERT genes may have silent family history and be asymptomatic or only display subtle changes of blood cells like macrocytosis (increased MCV). These subjects tend to have reduced levels of hematopoiesis and their identification is critical to avoid to select them as family marrow donors.

Chromosomal fragility test is also mandatory in AA patients undergoing HSCT to prevent misdiagnosis of FA and consequent selection of wrong conditioning regimen.

Table 3
Diagnostic work-up of aplastic anemia.

Mandatory tests for the diagnosis of AA
✓ Full blood count with differential count
✓ Reticulocyte count
✓ Peripheral blood film
✓ Liver function tests
✓ Liver virus tests (antibodies and DNA/RNA)
✓ Bone marrow aspirate for morphology, cytogenetics, immunophenotype, Pearl's staining (for intra-cytoplasmic iron)
✓ Bone marrow trephine biopsy with immunostaining for CD34 and CD117
✓ Flow cytometry for PNH clones
✓ Autoantibody screening (anti-nucleus and anti-DNA for SLE detection)
✓ Vitamin B12 and folate serum levels
✓ Fibrinogen and serum ferritin (detection of HLH)
✓ Stool pancreatic elastase, serum pancreatic lipase (for identification of Shwachman Syndrome)
✓ Serum bilirubin and LDH
✓ Chest X-ray
✓ Abdomen US scan and echocardiography (for liver, spleen, lymph node enlargement and malformations)
Mandatory tests for differential diagnosis with constitutional marrow failure syndromes
✓ Chromosomal fragility tests (MMC or DEB). Gold standard for the diagnosis of Fanconi Anemia
✓ TERC mutation analysis (detection of hidden forms of DKC)
✓ TERT mutation analysis (for those who do not respond to IST)
Ancillary tests for the diagnosis of AA
✓ Search for mycobacteria infection (atypical mycobacteria more frequently than TB mycobacteria)
✓ Marrow progenitor assay (not available in all centers)
✓ MRI of vertebral column
Ancillary tests for differential diagnosis with constitutional marrow failure syndromes
✓ TNF2, NHP2, NOP10, DKC1 and cMPL mutation analysis
✓ Shwachman-Diamond Syndrome mutation analysis, telomere length measurement

5. HLA typing

HLA typing, including DRB1*15, that may predict response to immunosuppressive treatment (IST), of the patient and relatives is recommended at diagnosis both in the patient and in relatives (*Level of evidence EO*; *Strength of consensus 8.7*; *level of consensus B*).

If a matched family donor (MFD) is not found, the search of a matched unrelated donor (MUD) has to start immediately. This is to rapidly provide those patients who, on day +120 since diagnosis, will not respond to IST (or have an early recurrence after initial response) a MUD HSCT as back-up treatment option [13].

6. Specific treatment

It is highly recommended that patients be taken in charge during the diagnosis, treatment and follow-up itinerary in centers with experience in the management of children and adolescents affected with AA (*Level of evidence EO*; *Strength of consensus 8.8*; *level of consensus B*).

Aims of the treatment are to restore hematopoiesis by either HSCT or IST with the combination of anti-thymocyte globulin (ATG) and cyclosporine A (CsA). Timing and type of treatment depend on the severity of AA and on the availability of an HLA matched family donor (MFD) (see Fig. 1).

NSAA patients are transfusion independent in about 1/3 of cases and may experience spontaneous remission with no specific treatment [14]. The remaining 2/3 may either remain stabile for months to years, or progress to SAA. IST is potentially toxic and there is no clear evidence that an early start be beneficial in transfusion independent NSAA.

Based on this, in stable non-transfusion dependent NSAA it looks reasonable to propose an initial observation with only supportive treatment.

Table 2
Classification of AA based on the severity.

Moderate or non-severe (NSAA)	Severe	Very severe
> Hematopoietic marrow cellularity < 30% > Neutrophils > 0.5×10^9/l but <1.0×10^9/l or: lack of criteria for severe and very severe	> Hematopoietic marrow cellularity < 30% > At least two of the following conditions: – Neutrophils < 0.5×10^9/l – Platelets < 20×10^9/l – Reticulocytes < 20×10^9/l[a]	Like severe but with neutrophils <0.2×10^9/l

[a] If reticulocytes are measured manually. Values should be <0.6×10^9/l if reticulocytes are measured with automatic coulter since the instrument may over-estimate lower values.

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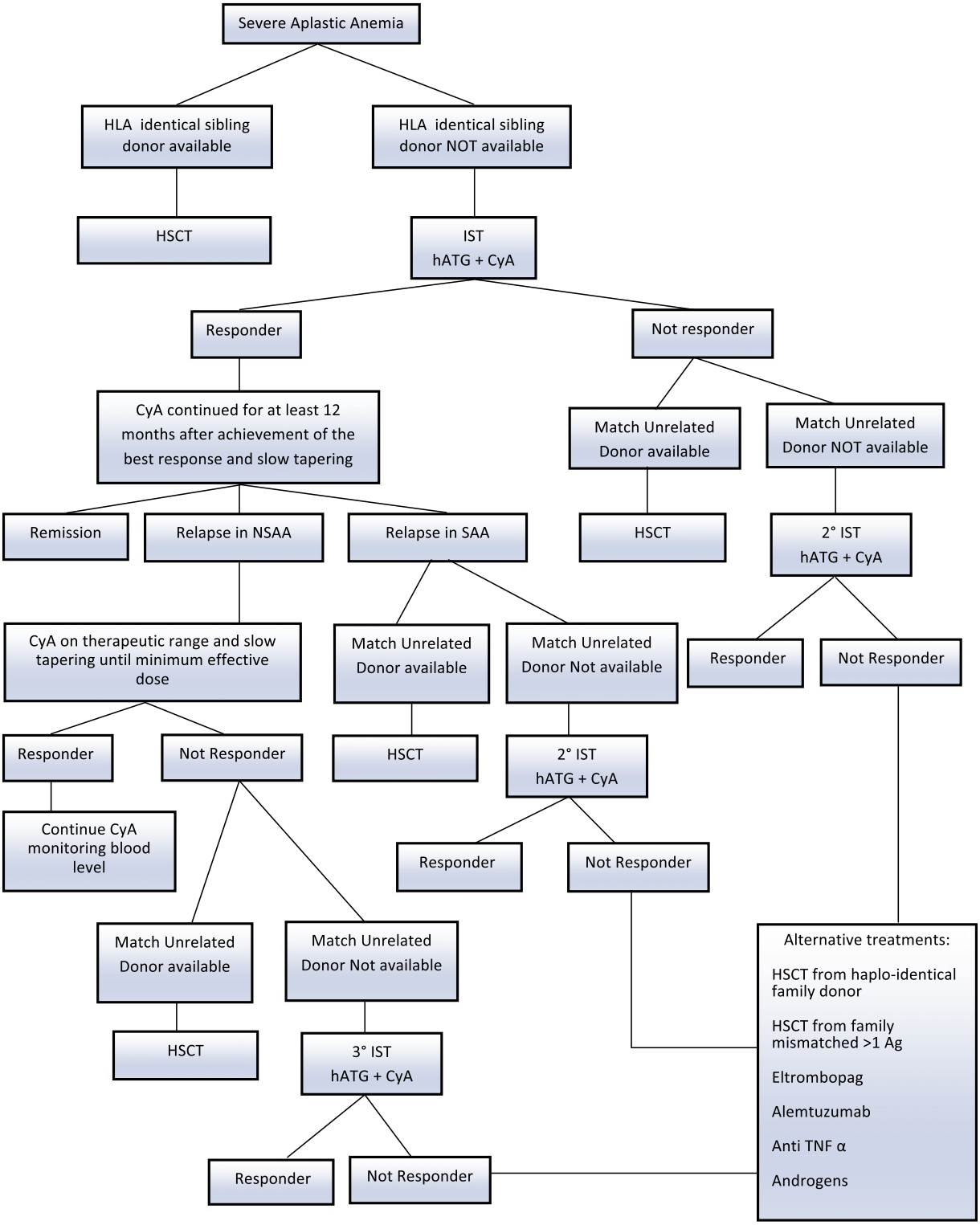

Fig. 1. Therapeutic algorithm in children with severe aplastic anemia. HLA: human leucocyte antigen. HSCT: hematopoietic stem cell transplant. IST: immunosuppressive therapy. hATG: horse anti-thymocyte globulin. CyA: cyclosporine. NSAA: non-severe aplastic anemia. SAA: severe aplastic anemia. TNF: tumor necrosis factor.

Specific treatment (IST or HSCT from MFD) can be started in case of progression to SAA (*Level of evidence II*; *Strength of consensus 8.3*; *level of consensus B*).

On the contrary, patients with transfusion-dependent NSAA, with SAA and with VSAA, very rarely achieve spontaneous remission. In addition, an interval diagnosis–treatment longer than 2–3 months is known to worsen prognosis [13,15]. Therefore after diagnostic work-up, specific therapy must be started [16,17].

6.1. Matched family donor HSCT

If a MFD is found, then HSCT using BM stem cells is the treatment of choice [13,18] in SAA, VSAA and in transfusion-dependent NSAA (*Level of evidence II*; *Strength of consensus 8.5*; *level of consensus B*).

Recommended conditioning regimen in patients undergoing HSCT from HLA compatible, family donor is ATG 2.5 mg/kg on days −4, −3, and −2 plus cyclophosphamide 50 mg/kg on days −5,

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−4, −3, and −2 (*Level of evidence II*; *Strength of consensus 8.4*; *level of consensus B*).

Recommended GVHD prophylaxis is methotrexate (MTX) plus cyclosporine (CsA). MTX can be either used at the dose of 8 mg/m^2 day on days +1, +3, +6, and +11 or of 15 mg/m^2 on day +1 followed by 10 mg/m^2 on days +3, +6, and +11. Suggested dose of oral CsA is 1.5 mg/kg every 12 h maintaining serum trough level of 150–250 ng/ml until 9–12 months after the transplant. After this time doses should be slowly tailored off in at least three months still in the absence of GVHD. Periodical monitoring of chimerism is also recommended (*Level of evidence III*; *Strength of consensus 8.5*; *level of consensus C*).

6.2. Immunosuppressive treatment

If a MFD is not available, then IST with the combination of ATG + CsA, still represents the first line choice (*Level of evidence II*; *Strength of consensus 8.5*; *level of consensus B*).

Ten year overall survival (OS) in younger populations peaks to about 90% [13,19]. Past studies showed a 10 year cumulative incidence of relapse <15% [20] but recent analyses reported a far higher rate of failure peaking to over 50% [13]. A prospective randomized trial [21] has shown that both hematological response and survival are far superior in patients treated with horse ATG (68% and 96% respectively) vs rabbit ATG (response 37%, survival 76%). These results were confirmed by another controlled study [22]. As for CsA management a slow and gradual tapering was shown to be associated with a lower relapse rate in both children [23] and adults as compared to fast reduction [24].

Therefore horse ATG is the recommended source for the first course of IST. Use of rabbit ATG is considered only if horse ATG is not available. CsA at full dose of 5 mg/kg/day should be continued for at least 12 months after achievement of the best response. After response has been achieved slow tapering (5–10% every month) can be initiated until stop that should not occur before 24 months since best response (*Level of evidence II*; *Strength of consensus 7.8*; *level of consensus C*).

6.3. Matched unrelated donor HSCT

At the moment there is a lot of debate on the role of upfront MUD HSCT for SAA in adult [25] and in children [16] who lack a MSD. With the use of reduced intensity conditioning, in subjects who failed one course of IST, this transplant provided excellent overall survival (OS) and failure free survival (FSS) above 90% [26]. Recently a multicenter national UK study of children with SAA/VSAA who lacked a MFD and were treated with upfront UD HSCT provided excellent OS and EFS [27]. This led both UK Children's Cancer and Leukaemia Group (CCLG) and EBMT SAAWP to recommend that if a MUD can be found quickly, then HSCT may be considered as upfront treatment in children who lack a MFD [28]. However controlled studies comparing front-line MUD with front-line MSD HSCT are awaited to further corroborate this change in the algorithm for treatment of SAA.

IST induces short and long-term side effects and is associated with a significant risk of relapse and of clonal evolution [29,30] up to 21% at 7 years [13]. Usually relapse occurs within 2–4 years since diagnosis [31–33] whereas the risk of clonal evolution tends to increase overtime [13]. Therefore patients treated with IST need to be thoroughly monitored long-term after treatment.

Patients who relapse after initial response or who do never respond to IST, if they have a 10/10 or 9/10 HLA matched unrelated donor, must undergo HSCT. This treatment proved to be far superior to a second course of IST [34] and recently was shown to be the best back-up treatment option post-failed IST with an OS of 78% and an EFS of 71% [13,26] (*Level of evidence II*; *Strength of consensus 8.5*; *level of consensus B*).

Suggested conditioning regimen is fludarabine 120 mg/m^2 plus cyclophosphamide 120 mg/kg and ATG 7.5 mg/kg on days −2 and −3. In patients older than 14 years or multi-transfused (>20 transfusions) the addition of TBI 2 Gy should be considered (*Level of evidence II*; *Strength of consensus 8.4*; *level of consensus B*).

However, given the excellent results achieved in children with the FCC regimen that is fludarabine 30 mg/m^2/day for 5 days, cyclophosphamide 60 mg/m^2/day for 2 days, alemtuzumab 0.3 mg/kg/day for 3 days [26] this combination can also be considered.

GVHD prophylaxis should be done with MTX/CsA. Suggested dose of CsA is 1.5 mg/kg every 12 h, maintaining trough serum level of 150–250 ng/ml until 9–12 months after the transplant. The dose should be slowly tapered off in the next 3 months in the absence of GVHD. Periodical monitoring of chimerism is recommended too. MTX can be used at 10 mg/m^2 on day +1, 8 mg/m^2 on days +1, +3, and +6, or 10 mg/m^2 on days +1, +3, and +6 (*Level of evidence III*; *Strength of consensus 8.5*; *level of consensus C*).

6.4. Patients refractory to a first course of IST and with no MUD available

If such a donor is not available, the following options are to be considered:

- Patients who *relapsed* may benefit of a second course of IST that offers a probability of survival of 75%.
- Patients *refractory* to a first course of IST may undergo a second one if a MUD is not found. Chances of response in this case are 30–60% [33].

6.5. Patients refractory to a second course of IST and with no MUD available

- Patients *refractory* to a second course of IST may have the following options: (i) Third course of IST that proved to be successful in some patients who have previously responded to IST [35], (ii) HSCT from haplo-identical family donor, (iii) HSCT from cord blood (CB) and (iv) alternative non-HSCT treatments. The choice of HSCT from haplo-identical donor vs cord blood vs third course of IST should be balanced on the degree of neutropenia, on the infectious risk, on the refractoriness to transfusions and on the risk of clonal evolution of each single patient. However haplo-identical HSCT and HSCT from CB nowadays are still experimental therapies and should be performed within the framework of clinical trials (*Level of evidence II*; *Strength of consensus 8.5*; *level of consensus B*).

Regarding alternative non-HSCT treatments, limited options are available for patients who relapse after or are refractory to IST or are not eligible to a HSCT.

Alemtuzumab, a monoclonal antibody anti-CD52, originally used in the treatment of lymphoid neoplasms has been tested in AA refractory patients showing a 4 year OS of 67% and a Disease Free Survival of 37% with an acceptable safety profile and limited infectious risk [36].

Eltrombopag is a thrombopoietin receptor (TPOr) agonist [9], the main endogenous regulator of platelet production. The rationale for its use is that TPOr is expressed also on hematopoietic stem cells. In a phase II study on AA patients refractory to at least one course of IST [37], 44% of subjects (11/25) obtained a hematological response after 12–16 weeks of treatment with minimal side effects. A follow up study showed a 20% rate of clonal events in patients receiving this drug long-term and the possibility of drug suspension with maintenance oh hematological response in another 10% [38].

Androgens

Oxymetholone, a synthetic androgens has been used in association with ATG obtaining high OS and response rates (78% and 77% respectively) [39] with low occurrence of clonal events. In spite of this there is some concern in using this androgen in children due to the relevant side effects including virilization, liver adenomas, premature growth arrest, liver toxicity and behavioral changes. Danazole, another synthetic

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androgen, induced response in 30–45% of patients [40,41] with very limited masculinization. Its addition to ATG and CsA increased response rate over ATG and CsA alone (67.9% vs 57.1%) [29]. Due to its lower side effect might be preferred in childhood.

High and moderate dose cyclophosphamide

A cyclophosphamide dose of 200 mg/kg has been used both in naive and resistant/relapsing patients with good response but high rate of fungal infections, particularly in resistant subjects [42]. Recently at the NIH [43] the combination of moderate dose (120 mg/kg) and low dose CsA was prospectively tested. Filamentous fungal infection occurred in about 1/4 of patients, clonal evolution rate was 22% and the survival rate at 2 years of 78%. Due to the high toxicity the Safety Data Safety Monitoring Board recommended termination of accrual of this study. Overall cyclophosphamide, because of the high toxicity seems can be considered as a salvage option in refractory patients.

Daclizumab

Daclizumab is anti-CD 25 monoclonal antibody inhibiting IL-2 dependent lymphocyte activation pathway that in NSAA induced an overall response rate of 42% with trilineage response of 14% [44].

7. Supportive therapy

7.1. Transfusions

Concentrated red cells should be given to patients symptomatic for anemia or in those subjects who are asymptomatic with hemoglobin levels of <8 g/dl [2,45].

Platelet concentrates should be given when the count is <10,000/mmc or <20,000/mmc, in case of fever, sepsis or bleedings.

Single donor concentrates obtained by platelet apheresis offer a better rise of the count with reduced exposure to donors and are preferred over preparation obtained by the buffy coat from multiple donors (*Level of evidence EO*; *Strength of consensus 8.4*; *level of consensus B*).

Granulocyte concentrates should be limited to life-threatening infections during neutropenia as a bridge treatment in the vicinity of neutrophil recovery (*Level of evidence V*; *Strength of consensus 7.5*; *level of consensus D*).

Blood products obtained from relatives are contra-indicated because they may sensitize the recipient to minor HLA or leukocyte antigens of the donor (*Level of evidence EO*; *Strength of consensus 8.7*; *level of consensus B*).

Red cell and platelet concentrates should be leuko-depleted [46–48] and irradiated (c 25 Gy) to avoid that residual lymphocytes contained in the bags may engraft causing post-transfusion graft versus host disease in the recipient [49,50] (*Level of evidence IV*; *Strength of consensus 9*; *level of consensus A*).

7.2. G-CSF

G-CSF in a daily schedule is recommended during the first 30 days of treatment for patients with VSAA and SAA undergoing IST.

The use of G-CSF is also accepted between days 30 and 90 since IST may start in patients with less than 200/mmc neutrophils. This use can either be on a daily schedule or "on demand" during only febrile neutropenia in SAA and VSAA patients (*Level of evidence EO*; *Strength of consensus 7.8*; *level of consensus C*).

7.3. Anti-infection treatment

There are no controlled studies on safety and efficacy of anti-microbial agents in treatment and prophylaxis of pediatric patients with AA. Most data come from meta-analyses and controlled trials conducted in adult oncology patients with febrile neutropenia.

7.3.1. Prophylaxis

Antibiotic prophylaxis may be considered in patients with neutrophils of <200/mmc, between day 30 and day 90 after IST start (*Level of evidence EO*; *Strength of consensus 7.3*; *level of consensus C*). Anti-fungal prophylaxis may be considered in subjects with neutrophil persistently <200/mmc (*Level of evidence EO*; *Strength of consensus 7.1*; *level of consensus C*). Prophylaxis for anti-*Pneumocystis jiroveci* is indicated with oral cotrimoxazole or with pentamidine by aerosol if lymphocyte values are low ($CD4^+ < 400$/mmc or total lymphocytes < 1000/mmc) (*Level of evidence EO*; *Strength of consensus 8.7*; *level of consensus B*). Anti-viral prophylaxis may be taken in account in patients with severe lymphopenia after ATG (*Level of evidence EO*; *Strength of consensus 7.1*; *level of consensus C*).

7.3.2. Empirical treatment of bacterial and fungal infections

Given the lack of controlled studies in children with AA, it looks logical to rely on available sources represented by guidelines for management of febrile neutropenia in patients with tumors or undergoing HSCT and by the indications for empirical treatment in adults with AA [51,52].

Initial antibiotic treatment should be wide spectrum and based on epidemiology of infections of the center where he patient is admitted. Following adjustments should not occur before 72–96 h of initial treatment unless differently oriented by microbiological data (infectious agent isolation). If fever persists beyond the first 96 h or if suggested by clinical signs, laboratory and imaging findings, the start of anti-*Aspergillus* treatment is recommended (*Level of evidence EO*; *Strength of consensus 8.4*; *level of consensus B*).

7.3.3. Vaccinations

No studies on this topic are available in literature. Only anecdotal reports of AA occurring after vaccination are known [53]. In patients still receiving CsA vaccinations are not recommended (*Level of evidence EO*; *Strength of consensus 7.8*; *level of consensus B*).

In general, anti-viral vaccinations are not recommended also in patients who have responded and are off therapy after IST (*Level of evidence EO*; *Strength of consensus 5*; *level of consensus D*).

7.4. Chelating treatment

There is some evidence for recommending the start of iron chelation in patients with ferritin serum level > 1000 ng (*Level of evidence IV*; *Strength of consensus 8*; *level of consensus B*). Deferasirox at the oral dose of 20–30 mg/kg/day is the first choice drug (*Level of evidence EO*; *Strength of consensus 8.1*; *level of consensus B*). This is mainly because Deferasirox is the only chelator tested on large numbers of AA patients [54]. Deferiprone, because of the risk of agranulocytosis is precautionary not suggested in AA patients. Deferoxamine brings about compliance problems related to the way of administration (subcutaneous or intravenous) and to the increased risk of *Yersinia* infection.

Since reduction of iron over-load does not occur quickly, treatment should be started 2–3 months before HSCT. In cured patients who have iron overload, venesection is indicated.

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Disclosure statement

CD provided consultations to Pfizer.

Appendix A. Supplementary data

Supplementary information is available at Blood Cells, Molecules and Diseases' website. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bcmd.2015.03.007.

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