

Second-line therapy in paediatric warm autoimmune haemolytic anaemia. Guidelines from the *Associazione Italiana Onco-Ematologia Pediatrica* (AIEOP)

Saverio Ladogana¹, Matteo Maruzzi¹, Piera Samperi², Annalisa Condorelli², Maddalena Casale³, Paola Giordano⁴, Lucia D. Notarangelo⁵, Piero Farruggia⁶, Fiorina Giona⁷, Agostino Nocerino⁸, Silvia Fasoli⁹, Maria L. Casciana⁹, Maurizio Miano¹⁰, Fabio Tucci¹¹, Tommaso Casini¹¹, Paola Saracco¹², Wilma Barcellini¹³, Alberto Zanella¹³, Silverio Perrotta³, Giovanna Russo², on behalf of the AIHA Committee* of the *Associazione Italiana di Ematologia ed Oncologia Pediatrica*

¹Paediatric Onco-haematology Unit "Casa Sollievo della Sofferenza" Hospital, IRCCS, San Giovanni Rotondo;

²Paediatric Onco-haematology Unit, Azienda "Policlinico Vittorio Emanuele", Department of Clinical and Experimental Medicine, University of Catania, Catania; ³Department of Women, Children and General and Specialised Surgery, "Luigi Vanvitelli", University of Campania, Naples; ⁴Department of Paediatrics "B. Trambusti", Policlinico Hospital "Giovanni XXIII", Bari; ⁵Paediatric Onco-haematology Unit, Children's Hospital "Spedali Civili", Brescia; ⁶Paediatric Onco-haematology Unit, "Civico" Hospital, Palermo; ⁷Haematology, Department of Cellular Biotechnologies and Haematology, "Sapienza" University, Rome; ⁸Department of Paediatrics, "S. Maria della Misericordia" University Hospital, University of Udine, Udine; ⁹Paediatric Unit, "Carlo Poma" Hospital, Mantua; ¹⁰Clinical and Experimental Haematology Unit, "G. Gaslini" Children's Hospital, Genoa; ¹¹Paediatric Haematology-Oncology Unit, Meyer Paediatric Hospital, Florence; ¹²Paediatric and Adolescent Science Department, University of Turin, Turin; ¹³Onco-haematology, Physiopathology of Anaemia Unit, IRCCS "Ca' Granda Foundation", Ospedale Maggiore Policlinico, Milan, Italy

*Co-Authors are listed in the Appendix 1.

Introduction

Autoimmune haemolytic anaemia (AIHA) is a rare condition in children caused by the presence of auto-antibodies directed against red blood cell surface antigens, leading to premature red cell destruction^{1,2}. It is classified as warm, cold, including cold haemagglutinin disease and paroxysmal cold haemoglobinuria, or mixed, according to the thermal range of the auto-antibody. AIHA can be idiopathic or secondary. The overall annual incidence is reported to be 1-3 cases/100,000 people and approximately 0.2 cases/1,000,000 individuals less than 20 years of age³⁻⁷. AIHA constitutes an important issue for many paediatricians who often have to deal with the diagnosis and treatment of critical patients in the absence of consistent support from the literature. This is particularly true for the second-line therapy. Indeed, while there is an established common practice about management of the acute phase of the disease, including diagnostic work-up and first-line therapy^{8,9}, second-line therapy is more debated. On the basis of these considerations, the Red Cell Working Group of the AIEOP (*Associazione Italiana di Ematologia ed Oncologia Pediatrica*) developed this consensus paper with the objective of providing shared recommendations to paediatricians.

Design and methods

The design and methodology were similar to those adopted for the AIEOP Consensus Guidelines on

sickle cell disease, childhood aplastic anaemia, and immune thrombocytopenias¹⁰⁻¹², using a procedure validated by the AIEOP board. In detail, 20 AIEOP centres and ten other non-AIEOP centres participated in the AIHA Committee. Issues to be addressed in the Recommendations were identified by the whole Committee; every topic was developed by a subgroup in a single document. Each document included a brief description of the state-of-the-art knowledge on the topic, followed by specific recommendations.

For the pre-guideline documents, the literature search was conducted in the MEDLINE database (from January 1, 1991 to December 31, 2015, and then updated in December 2017 during the compilation of the final draft). Search terms included: autoimmune haemolytic anaemia, warm autoimmune haemolytic anaemia, warm antibodies, cold autoimmune haemolytic anaemia, DAT, IAT, therapy, diagnosis, paediatric, and children. The MEDLINE search yielded 326 articles that were examined, of which 59 were selected as they offered data related to the topics of the present paper: The other 267 articles either dealt with aspects other than AIHA treatment or were not robust enough. The search was also extended to older papers, specifically retrieved following cited references, and to haematology textbooks. Every piece of evidence collected was attributed a strength that was scored using the level of evidence criteria reported in Table I.

Table I - Levels of evidence for studies evaluating diagnosis and therapy of AIHA in children.

Level of evidence	Study design
I (strongest)	Prospective randomised trial with high statistical value
II	Prospective randomised trial with lower statistical value
III	Non-randomised study with concurrent control group
IV	Non-randomised study with historical control group
V	Case report(s) with no control group

Where possible, an "A" was added for studies conducted on adults, and a "P" for those conducted on paediatric patients.

AIHA: autoimmune haemolytic anaemia.

Each draft was reviewed by the entire Committee and modified accordingly after exhaustive discussion. The Committee prepared statements that were then subjected to validation during the Consensus Conference, in which 44 participants scored the final items.

The strength of this consensus was quantified on a 1-9 scale where 1 represents no consensus and 9 represents a 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 3.1 to 6.9 a practice of uncertain appropriateness; mean scores from 7 to 9 an appropriate/necessary practice. The level of agreement among participants, indicating the rate of consensus, was also scored by evaluating the distribution of the standard deviations (SD) within each statement and then dividing the level of agreement into four categories:

- A:** strong agreement (variation more than 1 SD below the average of the variances, in logarithmic scale).
- B:** moderate agreement (variation less than 1 SD below the average of the variances).
- C:** moderate disagreement (variation less than 1 SD above the average of the variances).
- D:** strong disagreement (variation more than 1 SD above the average of the variances).

Response and relapse criteria

Preliminary to the discussion of therapy, the response criteria were defined. The consensus score is given in brackets. Responses could be complete, partial or none or relapse.

Complete response was defined as the achievement of haemoglobin \geq the lower normal limit for age, with no signs of haemolysis, i.e. normal reticulocyte, bilirubin, haptoglobin and lactate dehydrogenase levels (**8.9-A**).

Partial response was defined as an increase of haemoglobin concentration ≥ 2 g/dL, but not reaching normal values for age (**8.5-B**).

A < 2 g/dL increase of haemoglobin concentration and/or dependence on transfusions was considered to indicate no response (**8.4-B**).

A relapse was defined as recurrence of anaemia, along with haemolysis (i.e. reticulocyte count $> 120,000/\text{mm}^3$; haptoglobin < 10 mg/dL, indirect bilirubin > 1 mg/dL, lactate dehydrogenase twice normal limits), after having reached a complete response (**8.2-B**).

Second-line treatment of warm autoimmune haemolytic anaemia

Corticosteroids are used as first-line therapy of warm autoimmune haemolytic anaemia and 70-80% of patients show improvement after 3 weeks of treatment. In responders the dose can be tapered gradually over a 6-month period, as long as clinical improvement is maintained⁸.

Unfortunately, there are patients who require further treatments after first-line therapy. The largest study of childhood autoimmune haemolytic anaemia reported that, out of 265 cases, 92% of patients started steroid therapy. Among them, 45% of patients required a second-line treatment¹³. In another study 28 patients with warm AIHA were enrolled and, among those treated with steroids (85.7%), only 20.8% needed other treatments¹⁴.

Relapsed patients can usually be managed with their previous treatment (**7.6-C**), while there is an indication to change a patient to a different second-line therapy in the case of no response to the first-line treatment (**9-A**) or steroid dependence, with prednisone dosage ≥ 0.1 - 0.2 mg/kg/day, the Consensus being uncertain on whether to fix the dosage threshold at 0.1 (**5.3-D**) or 0.2 (**6.3-D**) mg/kg/day.

Possible second-line therapy strategies include achieving complete remission with an alternative treatment and/or establishing a maintenance regimen in order to control the disease with acceptable side effects.

Remission induction

Second-line treatments include rituximab, immunosuppressive drugs, splenectomy, alemtuzumab and haematopoietic stem cell transplantation (Table II; Figure 1).

Rituximab is a chimeric monoclonal antibody that targets CD20 antigen expressed by B lymphocytes and is licensed for the treatment of non-Hodgkin's lymphomas, chronic lymphocytic leukaemia and severe rheumatoid arthritis. Rituximab has been used "off label" for the treatment of AIHA and, even though its role has been evaluated in only a small number of prospective and retrospective clinical studies or case series, the results are encouraging: efficacy is reported to be 76% in paediatric patients when the drug is administered at a

Table II - Second-line treatment options for paediatric patients with warm AIHA.

Treatment (dose)	Treatment objective	Efficacy	Indication	Pitfalls
Rituximab (375 mg/m ² /week for 4 weeks)	Remission	84-100%	Main option in children with severe warm AIHA resistant to steroids	Grade 4 neutropenia (2%); infections (7%); contraindication in ALPS
Cyclophosphamide 10 mg/kg/day i.v. for 10 days or 50 mg/kg/day i.v. for 4 consecutive days	Remission	Only data from small series and case reports	Third-line treatment in severe, selected cases. It is preferred over splenectomy	Common side effects: nausea, vomiting, transient alopecia, and symptomatic neutropenia
Splenectomy	Remission	No reports in paediatric patients	Children who failed rituximab and cyclophosphamide	Short term surgical complications; long term risk of post-splenectomy sepsis; contraindicated in children <5 years old
HSCT	Remission	Single case reports	Patients with severe, transfusion-dependent warm AIHA who failed 3 lines of therapy, including splenectomy	Rejection, GvHD; toxicity; infections
Alemtuzumab	Remission	Small series reports	Patients with severe, transfusion-dependent warm AIHA who failed 3 lines of therapy, including splenectomy	Significant toxicity; viral reactivation (CMV); bacterial infections
Mycophenolate mofetil	Maintenance	62-82%	Steroid-sparing drug in steroid-dependent patients	Several weeks may be required to generate a response, therefore it should be initially associated with a more aggressive therapy, such as high-dose steroids.
Azathioprine	Maintenance	60%	Steroid-sparing drug in steroid-dependent patients	Myelotoxicity, gastrointestinal toxicity

AIHA: autoimmune haemolytic anaemia; ALPS: autoimmune lymphoproliferative syndrome; i.v.: intravenous; HSCT: haematopoietic stem cell transplantation; GvHD: graft-versus-host disease; CMV: cytomegalovirus.

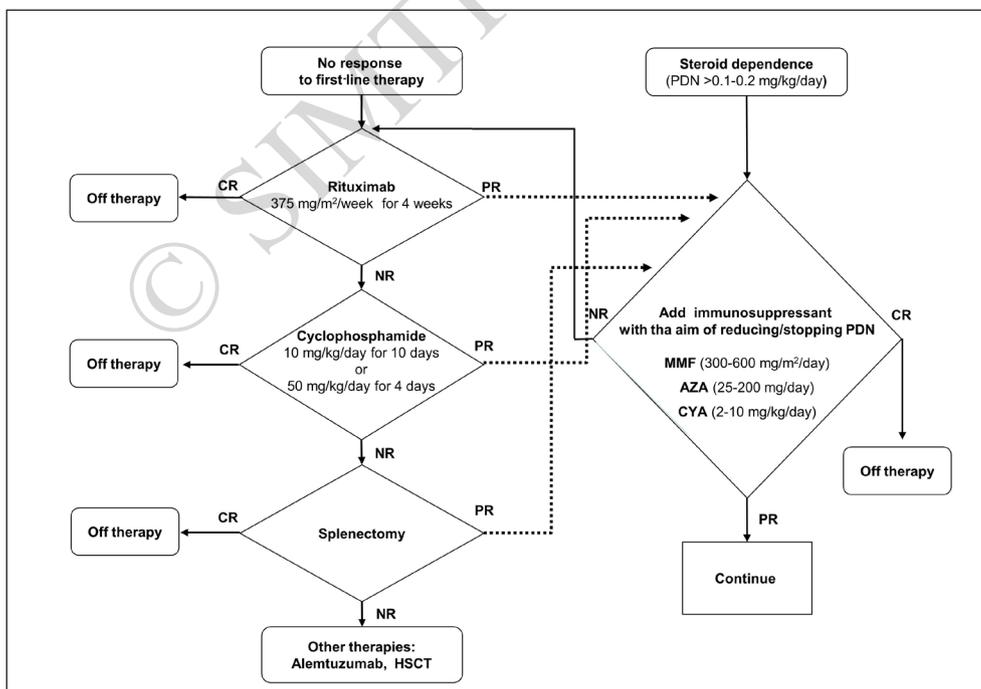


Figure 1 - Second-line therapy of warm antibody AIHA.

AIHA: autoimmune haemolytic anaemia; AZA: azathioprine; CR: complete response; CYA: cyclosporine A; HSCT: haematopoietic stem cell transplantation; MMF: mycophenolate mofetil; NR: no response; PDN: prednisone; PR: partial response.

dose of 375 mg/m² weekly for a total of three to four doses¹⁵. Low doses of rituximab (4 weekly infusions of 100 mg) have been proved to be as effective in a prospective study of 23 adult patients¹⁶.

Responses to rituximab were observed in patients with either primary or secondary AIHA regardless of prior therapy¹⁷. Retreatment with rituximab has been shown to be effective¹⁸⁻²². Rituximab is a well-tolerated therapy. Indeed, most patients with AIHA receiving rituximab experienced mostly mild adverse events (including hypotension, fever and chills, upper airway oedema), while only few serious adverse events have been reported (2% grade 4 neutropenia, 7% infections)¹⁷.

Given all the above-mentioned evidence, rituximab should be considered the main option in children with severe warm AIHA resistant to first-line therapy (**8.6-B**).

The use of high-dose cyclophosphamide has been described, mostly in adult patients, and is considered as a third-line therapy in severe, selected cases (**8.3-B**). It was used by Moyo *et al.* to treat nine patients with AIHA who had failed a median of three other treatments²³. Six patients achieved a complete remission and three patients a partial remission. There is also a case report of a 5-month old infant with life-threatening AIHA refractory to conventional therapy who responded to high-dose cyclophosphamide²⁴. Although paediatric data are scarce and safety in children is still a concern, high-dose cyclophosphamide is preferred over splenectomy for the treatment of patients who are not responsive to rituximab (**7.3-C**).

Splenectomy reduces the sequestration of red blood cells and the production of antibody by the spleen. In fact, the warm-type auto-antibodies of AIHA are usually IgG, which do not fix complement and cause red blood cell destruction predominantly in the spleen as the result of the interaction between antibody-coated cells and phagocytes²⁵.

Splenectomy has a proven high short-term efficacy in adults with warm AIHA, while no high quality data are available regarding long-term remissions. Of 162 adults with warm AIHA treated with splenectomy and described in the literature, 71 (45%) achieved a complete remission and 35 (22%) had a partial remission²⁶⁻³². The percentages of complete and partial short-term remissions range from 38 to 82%³³ depending on the number of secondary cases, which seem to be less responsive than the idiopathic form³⁴. Although many patients require therapy with steroids after splenectomy, dosages are usually lower than those before surgery³⁵, with a clinical improvement, as observed also in patients with immune thrombocytopenia³⁶. Given the lack of data in the literature about paediatric patients with AIHA treated with splenectomy, the actual effectiveness of this treatment in children is not clearly defined. The limits

of this approach are the absence of reliable predictors of the outcome (disease duration, response to steroids and the extent of splenic sequestration are not predictive)³⁷, along with the risks of surgical complications and overwhelming post-splenectomy sepsis. Splenectomy has not, therefore, been considered as second-line therapy (**3.6-D**), but its indication has been placed as third-line treatment in children with warm AIHA not responsive to rituximab (**7.2-C**). Splenectomy should be avoided in children younger than 5 year-old because of the higher risk of severe infection³⁸ (**7.9-D**). Furthermore, the Consensus failed to express a preference of splenectomy over cyclophosphamide, even in children over 5 years (**4.6-D**).

Alemtuzumab is a humanised anti-CD52 monoclonal antibody approved for the treatment of chronic lymphocytic leukaemia. Alemtuzumab was responsible for the complete remission of 13 of 16 patients with idiopathic AIHA resistant to conventional treatment³⁹⁻⁴¹ and 11 of 12 patients with AIHA secondary to chronic lymphatic leukaemia⁴²⁻⁴⁵. In addition, alemtuzumab induced complete remission in three paediatric cases with AIHA secondary to giant cell hepatitis, stem cell transplantation and intestinal transplantation^{46,47}. Side effects include viral reactivation (in particular cytomegalovirus infection) and bacterial infections⁴¹; therefore, the paucity of treated cases and the consistent toxicity prevent specific recommendations being made regarding the use of alemtuzumab in children, other than as a "last resort" option for individual patients with AIHA refractory to all the other available therapies³³.

Another option is haematopoietic stem cell transplantation, although available data are limited^{48,49}. Transplantation is indicated for those patients who suffer from severe, transfusion-dependent AIHA and have failed three lines of therapy, including splenectomy (**8.3-C**). Recommended procedures are allogeneic transplantation, from a family HLA-matched donor, harvesting stem cells from explanted bone marrow. Descriptions of condition regimens and graft-versus-host disease prevention are beyond the scope of the present paper, since candidates for haematopoietic stem cell transplantation must be referred to Transplant Units that have specific clinical experience in treating children with autoimmune disorders.

Maintenance therapy

Immunosuppressive drugs, other than cyclophosphamide, are a valid option for second-line treatment, since they can be used as steroid-sparing drugs in steroid-dependent patients. Given that these drugs need rather a long time to generate a response, ideally, they should be overlapped early with more

aggressive therapy, such as steroids (Table II; Figure 1). Mycophenolate mofetil, a potent inhibitor of the enzyme inosine-5'-monophosphate dehydrogenase, which mainly affects lymphocytes, is the most indicated in steroid-dependent patients in order to reduce the dosage of steroids (7.3-B). Its efficacy both in AIHA and Evans syndrome is becoming ever more evident and it has few or no side effects⁵⁰⁻⁵³. Long-term remission after its discontinuation has also been reported⁵³.

Azathioprine can also be used as a steroid-sparing drug^{54,55}, but since published data rely on early literature and side effects are quite a concern (Table II), azathioprine is considered a second choice after mycophenolate mofetil (7.6-C).

Cyclosporine is an immunosuppressive drug that can be used only in selected patients with AIHA resistant to steroid therapy (7.3-C). It was effective in four patients with AIHA refractory to steroids^{56,57}. Cyclosporine was also used in association with prednisone to improve the clinical course of a 6-year old child with Evans syndrome⁵⁸ and to treat a case of AIHA of perinatal onset⁵⁹.

A comprehensive therapeutic algorithm, summarising the proposed choice priorities, is available in Figure 1.

Conclusions

The present report represents a valuable effort to support paediatricians with a guide to the management of second-line therapy of childhood AIHA.

In summary, a second-line treatment is indicated in children with AIHA who do not respond to the first-line therapy or become steroid dependent. Rituximab is the first choice as second-line treatment; in patients resistant to rituximab, cyclophosphamide can be used, before splenectomy. In patients who develop steroid dependence, an immunosuppressive drug - preferably mycophenolate mofetil, but also azathioprine or cyclosporine - is indicated in order to reduce the dose of the steroid and eventually suspend it. Alemtuzumab and haematopoietic stem cell transplantation are last choices for refractory, severely symptomatic cases.

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Correspondence: Giovanna Russo
 Paediatric Onco-haematology Unit
 Azienda Policlinico Vittorio Emanuele
 Department of Clinical and Experimental Medicine, University of Catania
 Via Santa Sofia 78
 95123 Catania, Italy
 e-mail: diberuss@unicit.it

Appendix 1

List of co-Authors (in alphabetical order)

- Carlo BARONCI
Department of Paediatric Haematology and Oncology, "Bambino Gesù" Paediatric Hospital, Rome
- Anna Maria CASADEI
Department of Paediatrics, "La Sapienza" University of Rome, Rome
- Giovanni CAZZANIGA
Paediatric Clinic, University of Milan-Bicocca, "M. Tettamanti" Research Centre, Monza
- Andrea CILIBERTI
Paediatric Onco-haematology Unit "Casa Sollievo della Sofferenza" Hospital, IRCCS, San Giovanni Rotondo
- Serelina COLUZZI
Immunohaematology and Transfusion Medicine Unit, Policlinico Umberto I, "La Sapienza" University of Rome, Rome
- Paola CORTI
Paediatric Department, University of Milano-Bicocca, "San Gerardo" Hospital, Monza
- Giovanni C. DEL VECCHIO
"F. Vecchio" Paediatric Unit, University Hospital, Policlinico, Bari
- Elena FACCHINI
Department of Paediatrics, "Lalla Seràgnoli" Haematology-Oncology Unit, University of Bologna
- Gabriella GIRELLI
Immunohaematology and Transfusion Medicine Unit, Policlinico "Umberto I", "La Sapienza" University of Rome, Rome
- Ilaria LAZZARESCHI
Division of Paediatric Oncology, Catholic University of Rome, Rome
- Nicoletta MASERA
Paediatric Department, University of Milano-Bicocca, "San Gerardo" Hospital, Monza
- Paolo PERSEGHIN
Transfusion Medicine Service, Apheresis Unit, "San Gerardo" Hospital, Monza
- Daniela PERUCCIO
Immunohematology, OIRM "Sant'Anna", Turin
- Angelamaria PETRONE
Paediatrics, APSS Trento, Rovereto
- Antonella SAU
Paediatric Onco-haematology Unit, Department of Haematology, P.O. "Spirito Santo", Pescara
- Raffaella SCHIRÒ
Paediatric Haematology, "Buzzi" Hospital, ICP, Milan
- Manuela TUMINO
Paediatric Onco-haematology Clinic, University of Padua, Padua
- Isabella VASTA
Paediatric Onco-haematology Unit, "Vito Fazzi" Hospital, Lecce
- Federico VERZEGNASSI
Paediatric Onco-haematology Unit, "Burlo Garofolo" Hospital, Trieste