New insights into childhood autoimmune hemolytic anemia: a French national observational study of 265 children

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Acknowledgments: the authors wish to thank all patients and families and all CEREVANCE physicians for their participation (cited in appendix).

Funding: this work was initiated and financially supported in part by grants from the Association Française pour le Syndrome d'Evans (AFSE). The clinical research and methodology staff were supported by the GIS - Institut des Maladies Rares - INSERM, and the French Health Ministry (Programme Hospitalier de Recherche Clinique 2005, Rare Diseases Plan 2007).

Manuscript received on 28 October 2010. Revised version arrived on December 16, 2010. Manuscript accepted on January 3, 2011.

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background
Autoimmune hemolytic anemia is a rare condition in children. Little is known about its initial presentation and the subsequent progression of the disease.

Design and Methods
Since 2004, a national observational study has been aiming to thoroughly describe cases and identify prognostic factors. Patients from all French hematologic pediatric units have been included if they had a hemoglobin concentration less than 11 g/dL, a positive direct antiglobulin test and hemolysis. Evans' syndrome was defined by the association of autoimmune hemolytic anemia and immunological thrombocytopenic purpura. Data from patients' medical records were registered from birth to last follow-up. Autoimmune hemolytic anemia was classified as primary or secondary. Remission criteria, qualifying the status of anemia at last follow-up, were used with the aim of identifying a subgroup with a favorable prognosis in continuous complete remission.

Results
The first 265 patients had a median age of 3.8 years at diagnosis. In 74% of cases the direct antiglobulin test was IgG/IgG+C3d. Consanguinity was reported in 8% of cases and first degree familial immunological diseases in 15% of cases. Evans' syndrome was diagnosed in 37% of cases. Autoimmune hemolytic anemia was post-infectious in 10%, immunological in 53% and primary in 37% of cases. After a median follow-up of 3 years, 4% of children had died, 28% were still treatment-dependent and 39% were in continuous complete remission. In multivariate analysis, IgG and IgG+C3d direct antiglobulin tests were associated with a lower rate of survival with continuous complete remission (adjusted hazard ratio, 0.43; 95% confidence interval, 0.21-0.86).

Conclusions
This nationwide French cohort is the largest reported study of childhood autoimmune hemolytic anemia. The rarity of this condition is confirmed. Subgroups with genetic predisposition and underlying immune disorders were identified.

Key words: autoimmune hemolytic anemia, children, prognostic factors, Evans' syndrome, rare diseases.

Citation: Aladjidi N, Leverger G, Leblanc T, Picat MQ, Michel G, Bertrand Y, Bader-Meunier B, Robert A, Nelken B, Gandemer V, Savel H, Stephan JL, Fouyssac F, Jeanpetit J, Thomas C, Rohrlich P, Baruchel A, Fischer A, Chêne G, and Perel Y, for the Centre de Référence National des Cytopénies Auto-immunes de l'Enfant (CEREVANCE). New insights into childhood autoimmune hemolytic anemia: a French national observational study of 265 children. Haematologica 2011;96(5):655-663. doi:10.3324/haematol.2010.036053

Introduction

Autoimmune hemolytic anemia (AIHA) is characterized by the presence of autoantibodies that bind to the erythrocyte surface membrane and lead to premature red cell destruction. AIHA is the main cause of acquired extracorpuscular hemolysis in children.[1] The first descriptions available of this rare disease were provided 40 years ago and were drawn from retrospective series.[2-6] Recent literature contains information from small series, mainly based on laboratory tests, and involving very few centers.[7-10] The prevalence of AIHA in childhood is still unknown, but likely increases with age, as for most autoimmune disorders. Evans' syndrome (ES) was first described as hemolytic anemia with a positive direct antiglobulin test (DAT) and immune thrombocytopenia occurring simultaneously or in succession, in the absence of any known etiology. It is currently defined as autoimmune destruction of at least two hematologic cell types after exclusion of other diagnoses.[11-16]

For some authors, infectious causes of AIHA predominate in children.[2,5] For others, most cases of AIHA are primary.[5,6] The underlying pathogenic mechanisms are poorly individualized.[1] Acute post-infection, self-limited illness, recovering in a few weeks, is commonly differentiated from chronic illnesses often lasting several years, characterized by constant or intermittent hemolysis, with sudden acute relapses, the definitive cure of which is uncertain. In acute phases, hemolysis may be life-threatening. In chronic phases, immunosuppressive treatments are not consistently effective and may have major, life-threatening, secondary effects.[1-4,6]

In 2001, the rarity and severity of this disease, the therapeutic challenges and the absence of any identified national research program led the French Society of Hematology and Immunology (SHIP), in close partnership with families, to create a clinical and laboratory network on AIHA and ES in children. This national pediatric CEREVANCE group was labeled in 2007 as a "Reference Center" under the French Health Ministry's Rare Diseases Plan. A pilot retrospective study had been conducted during the period from 1990 to 2002 in all pediatric hematology units on 36 children with ES.[17] In January 2004, a national observational study of children with AIHA was started. The aim of this first multicenter, non-selective data collection was to describe the presentation and outcome of AIHA, with reference to published statements, and to analyze potential prognostic factors.

Design and Methods

Selection of patients, data collection and definitions

From January 2004, all French hematologic units were asked to prospectively include children under 18 years old, living in France, diagnosed with or followed-up for AIHA, whatever the context. The research was approved by the relevant institutional ethics committee, CPPRB-A (Bordeaux). Parents gave written informed consent to the anonymous collection of data concerning their children.

The current analysis includes all cases diagnosed up to December 31, 2007, with a hemoglobin concentration less than 11 g/dL, a positive DAT and at least one of the following three laboratory criteria of hemolysis: reticulocyte count greater than 120×10^9/L, haptoglobin less than 10 mg/dL, and total bilirubin greater than 1 mg/dL. Exclusion criteria were inherited hemolytic anemia or thrombocytopenia. ES was strictly defined by the simultaneous or sequential association of AIHA and peripheral immune thrombocytopenia (ITP) with a platelet count less than 100×10^9/L, on at least two occasions,[18] whatever the context. At the end of the follow-up, AIHA was named AIHA/ES when it turned out to be ES, and isolated AIHA when there was no associated ITP. The diagnosis of a well-defined infection was made on the basis of IgM positive serology, seroconversion, or genomic or culture identification: most of the patients underwent testing for mycoplasma, Epstein-Barr virus, cytomegalovirus, parvovirus, human immunodeficiency virus, and hepatitis viruses, and rotavirus, enterovirus, adenovirus, respiratory syncytial virus and flu viruses only if there were clinical manifestations. Immunological disease was defined as the presence of humoral or cellular primary immune deficiency (PID), systemic or organ-specific autoimmune disease (AID) including inflammatory rheumatic conditions, AIHA/ES, or peripheral neutropenia with a white cell count below 0.5×10^9/L which was not drug- or infection-related.

Detailed data were checked from each patient's medical records from each of the centers. The data collection was retrospective before 2004, then prospective. Patients were only included if complete initial inclusion data had all been checked. Consanguinity, malignancy, PID or AID in parents and siblings was recorded. Relevant medical data from the patient's birth to last follow-up were recorded. For children less than 6 months old, maternal alloimmunization was excluded by appropriate tests. The DAT were performed in 14 French laboratories according to local procedures. Infectious and immunological conditions were looked for in all cases. First-line steroid therapy was the rule, with second-line procedures left to the discretion of the physicians.

Our preliminary study had shown the burden of multiple relapses and long-lasting immunosuppressive therapy.[17] The following CEREVANCE criteria, qualifying the status of AIHA at last follow-up, aimed at identifying a favorable subgroup, with no relapse or treatment for 1 year: no remission: hemoglobin less than 7 g/dL; partial remission: hemoglobin 7 to 11 g/dL and/or reticulocytosis greater than 120×10^9/L; complete remission: hemoglobin greater than or equal to 11 g/dL and reticulocytosis less than or equal to 120×10^9/L, irrespective of the DAT and treatment; continuous complete remission: stable complete remission without any specific treatment for more than 1 year.

The end point for analysis was December 31, 2008, ensuring more than 1 year's follow-up for each patient. By this time, 84 children had been excluded from the analysis: 30 of the earliest patients because validation of the AIHA inclusion criteria was inadequate, 22 patients diagnosed with AIHA in 2008 and so with insufficient follow-up, 3 children with acquired AIHA following immunosuppressive treatment, and 29 children with ITP, positive DAT, mild hemolysis, but no anemia who were not counted as having ES.

Statistical analysis

The distribution of categorical variables was compared among groups, using the χ^2 test or Fisher's exact test. Continuous variables were analyzed using Student's test. The Kaplan-Meier method was applied to estimate survival in continuous complete remission. This analysis was conducted among children whose initial diagnosis was made after January 01, 2000, and who had more than 1 year's follow-up, ensuring homogeneous initial assessment and treatment. Potential prognostic variables for which less than 30% of the data were missing were assessed using a Cox proportional hazards model. The potential prognostic variables under consideration were: gender, age, consanguinity, a history of first degree familial immunological disease, immunological

disease, prematurity, well-defined infection at diagnosis, splenomegaly, hemoglobin level, DAT class, associated thrombocytopenia (platelet count <150×10^9/L), neutropenia (neutrophil count <0.5×10^9/L), or lymphocytopenia (lymphocyte count <1.5×10^9/L), hypogammaglobulinemia below two standard deviations for age, and hypergammaglobulinemia above two standard deviations for age. Their impact was analyzed on status in complete remission at 1 month after initial diagnosis (logistic regression), and on survival in continuous complete remission (Cox model). All analyses were conducted using SASv9.1.3 (SAS Institute, Cary, NC, USA).

Results

Demography

Two hundred and sixty-five children were included from the 26 national pediatric CEREVANCE units. The earliest diagnosis was made in June 1986; 44% of the diagnoses were made between 2004 and 2008. The number of new diagnoses per year in this 5-year period ranged from 15 to 35. Age and gender at diagnosis are described in Figure 1 and Table 1.

Familial history

First- to third-degree consanguinity was reported in 8% (18/229) of children. Parents of 3% (8/243) of the children suffered from malignancies. First-degree relatives of 12% (29/243) of the children had AID or PID (Table 2). In addition, in second-degree relatives, ES was reported in two maternal uncles and isolated AIHA with Castelman's disease in one paternal uncle.

Presentation of autoimmune hemolytic anemia

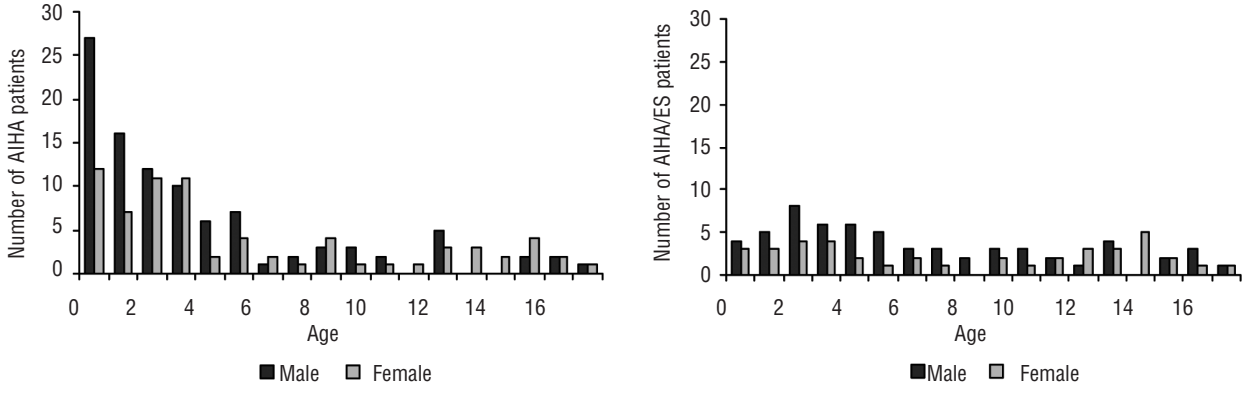
Sixty percent of the cases had a febrile, non-specific clinical illness in the month preceding AIHA. In 3% of cases, collapse, coma or acute renal insufficiency testified to the suddenness of the profound anemia. Jaundice was not constant, while dark urine was reported in 80%, splenomegaly in 31%, and hepatomegaly in 19% of cases. The median hemoglobin level was 6.4 mg/dL (range, 2 to 10.9 mg/dL). In 39% of cases, the rise in the reticulocyte count above 120×10^9/L occurred after reticulocytopenia which lasted a median of 6 days (range, 1 to 70 days) (Table 1). In 22% (49/219) of microbiologically explored cases, the initial diagnosis of AIHA was concomitant with a well-defined infection: Epstein-Barr virus (n=11), mycoplasma (n=9), cytomegalovirus (n=6), parvovirus (n=5), rotavirus (n=4), varicella (n=3), human herpes virus 6 (n=3), *E. coli* (n=3), pneumococcus (n=2), adenovirus (n=1), human herpes virus 1 (n=1), and enterovirus (n=1). The DAT class in 35% of these cases was C3d. A well-defined infection was identified in 27% of cases of C3d AIHA.

Associated diseases from birth to last follow-up

In 9% (16/170) of cases, children were born prematurely before 37 weeks' gestation. In 8% (20/245) of cases, AIHA occurred in association with an abnormal karyotype (n=3) (inv (17), 47, XXY, chromosome 22q11.2 deletion syndrome), neurological (n=6), cardiac (n=3) or digestive (n=1) disorders, cataract (n=2), perception deafness (n=2), dysmorphy (n=2) or onycho-dystrophy (n=1). A single case of B nodular lymphocyte-predominant Hodgkin's lymphoma occurred in a 19-year-old girl, 5.7 years after AIHA. In 15% (41/265) of cases, immunological disease preceded AIHA. In 38% (100/265) of the children, AIHA was the first sign of a previously unknown immunological disease (Table 3). AIHA was isolated in 166 children, and AIHA/ES was diagnosed in 99 children (37%). The characteristics and outcome of the patients with AIHA and AIHA/ES differed significantly (Figure 1, Table 1).

Subgroups of autoimmune hemolytic anemia

At the last follow-up, AIHA was secondary in 63% and primary in 37% of the cases. Secondary forms were purely post-infectious in 10% (26/265) of cases and immunological in 53% (141/265), including AIHA/ES. After a median follow-up of 2 years (range, 0.1 to 11.1 years), 47% (23/49) of children with well-defined infections turned out to have immunological conditions (Table 3). The frequency of immunological AIHA was not significantly different



[1]*Of the whole group of 265 children, 21% (57/265) were less than 1 year old, and 14% (37/265) were over 13 years old. Females predominated in children older than 13 compared with children younger than 13 (65%* versus *40%, P=0.002). For details, see Table 2.*

Figure 1. Age and gender distribution at diagnosis of AIHA in 166 cases of isolated AIHA (left) and 99 cases of AIHA/ES (right) enrolled in the CEREVANCE cohort.

between boys and girls, for all ages. AIHA was immunological in 62% of IgG/IgG+C3d cases and in 38% of the others (P<0.0001).

Treatment and outcome

A median of 2 (range, 1-17) transfusions was required for 65% of children in the first month. First-line steroid therapy was initiated in 92% of patients (total duration, 1 to 240 months), and complete remission was obtained at the end of the first month in 58% of cases. Prolonged multimodal therapies (median, 2; range, 1 to 7) were necessary for 45% of patients (Table 4). More than simple steroid therapy was required for 35% of children with well-documented infection. For the entire cohort of 265 children, the median evaluable period was 3 years (range, 0.0 to 21.2) and was longer than 1 year for 198 children. At the last follow-up, 4% (10/265) of children had died, 6% (17/265) had no response or were in partial remission, 90% (238/265) were in complete remission while on or off therapy, and 104 (39% of the total cohort) were in continuous complete remission. Twenty-eight percent of the live children (70/255) were still on therapy.

Prognostic factors

The probability of survival in continuous complete remission at 2 and 5 years after diagnosis was 35% and 64%, respectively. In the logistic regression analysis, an IgG/IgG+C3d DAT was associated with the lack of complete remission achievement after 1 month of steroid therapy (hazard ratio 0.5, 95% CI 0.25 to 0.97, P=0.04).

Table 1. Characteristics of the 265 children enrolled in the CEREVANCE cohort, and in the two main subgroups: isolated AIHA and AIHA/ES.[1]

	Total patients n = 265	Isolated AIHA n = 166	AIHA/ES n = 99	P
Clinical characteristics (n=265)				
Mean age (years) (min - max)	3.8 (0.1-17.4)	3.1 (0.1-17.4)	5.7 (0.2-17)	0.0003
Male / female	151 / 114	92 / 74	59 / 40	0.5
Consanguinity (%)	8	5	12	0.04
Familial immunological diseases (%)	14	9	22	0.004
Prematurity < 37 weeks (%)	9	14	3	0.01
Personal immunological manifestations (%)	17	8	41	< 0.0001
Well-defined infection (%)	22	29	10	0.0008
Hematology (n=265)[1]				
Hemoglobin g/dL	6.4 (2-10.9)	5.9 (2-10.6)	7.2 (1.8-10.9)	< 0.0001
Reticulocytes $\times 10^9$/L	208 (2 - 897)	210 (3-805)	203 (2-897)	0.80
Haptoglobin mg/dL	0.31 (0.01-20.2)	0.4 (0.01-20.2)	0.2 (0.01-1.3)	0.34
Bilirubin mg/dL	60 (2-260)	66 (2-260)	45 (5-178)	0.001
Lactate dehydrogenase IU/L	2048 (150-16343)	2483 (150-16343)	1381 (150-6610)	0.0001
Platelets $\times 10^9$/L	289 (1-1000)	374 (31-1000)	96 (1-598)	< 0.0001
Neutrophils $\times 10^9$/L	6 (0-29)	8 (0.1-27)	3 (0-29)	< 0.0001
< 500×10^9/L (%)	10	2	25	
Lymphocytes $\times 10^9$/L	4 (0.5-22)	5 (0.5-22)	3 (0.5-15)	0.0001
< 1500×10^9/L (%)	17	13	24	
Immunology				
DAT (n=265) (%)				< 0.001
Ig G	42	29 (2 CA[2])	65	
Ig G + C3d	32	33 (7 CA)	30	
C3d	24	35 (17 CA)	5 (3 CA)	
Ig A	1	2	0	
Ig G g/dL (n=114)[3] (%)				0.50
< 2 standard deviations	16	20	10	
> 2 standard deviations	20	17	25	
Ig A g/dL (n=113) (%)				0.05
< 2 standard deviations	14	17	11	
> 2 standard deviations	25	17	36	
Ig M g/dL (n=112) (%)				0.76
< 2 standard deviations	12	11	15	
> 2 standard deviations	27	26	28	
Antinuclear antigen-positive (n=123) (%)				0.43
< 1/400	24	23	25	
> 1/400	9	6	12	

[1]Percentages are given according to the data available, and analyses are based on averages, [2]CA: cold agglutinin [3]Before any substitution.

In the univariate survival analysis, age, family or personal history of immunological disease, thrombocytopenia, lymphocytopenia and the DAT type were associated with poor survival in continuous complete remission, while prematurity and the existence of documented infection at initial diagnosis were associated with a higher rate of survival in continuous complete remission. Sex, consanguinity, hemoglobin level and dysgammaglobulinemia did not have any impact on survival in continuous complete remission.

In the multivariate survival analysis, only the IgG/IgG+C3d DAT was significantly associated with a lower rate of continuous complete remission (18% *versus* 71%; hazard ratio 0.43; 95% CI 0.21 to 0.86; P=0.01) (*Online Supplementary Figure S1*).

Discussion

Epidemiology and initial presentation

Through the active collaboration of all French units, this first nationwide study has provided unique epidemiological data from non-selected children with AIHA, confirming the rarity of this condition. Recruitment biases are obvious, AIHA occurring during the course of PID, systemic lupus erythrematosus or hematopoietic stem cell transplantation not being systematically included. Some cases of AIHA might have been missed, as a negative DAT can occur in authentic AIHA in less than 5% of cases,[1] and such cases were excluded from our study. The annual incidence had been previously estimated to be around 0.2 per million individuals under 20 years old.[2-10] Given a minimum of 15 new cases per year over the last 5 years, and around 15 million people under 19 years old in France (http://www.insee.fr, 2008), the incidence in children and adolescents might be 10 to 20 times higher than previous estimates in other countries. The registration of children is improving over the years with the growing participation of primary-care pediatric hospitals. The potential bias towards more severe and prolonged cases is, therefore, being reduced. Ongoing studies, with registry-based methodology crossing various data sources, will help to improve this input.

The well-known characteristics of AIHA in children are accurately described in this large study. It is a disease of young children, and for the first time a high 21% prevalence in infants was found. Teenagers, occasionally referred to adult care units, may still be underrepresented. In this age-group, females predominated significantly, as in other autoimmune diseases.[1] The cardiovascular adaptation of children at diagnosis is remarkable, while deaths related to anemic cardiovascular ischemia have been reported in adult patients.[19] There was an initial transient reticulocytopenia in up to 39% of the children. Concomitant parvovirus infection was documented in only three cases, so other hypotheses to explain this finding include transient sideration of erythropoiesis or

Table 2. Disease in first-degree relatives for 37/243 children with AIHA enrolled in the CEREVANCE cohort.

Relevant diseases	Number of children	Members affected
Neoplastic diseases	**8**	
Breast	2	2 mothers
Lymphoma	2	1 mother, 1 father
Acute myeloid leukemia	2	1 father[1]
Thyroid	1	1 mother
Colon	1	1 mother
Autoimmune diseases	**27**	
Thyroid	9	7 mothers[2], 1 father, 1 brother[2]
Systemic lupus erythematosus	5	4 mothers, 1 brother
Rheumatic disease	3	1 father, 1 brother, 1 sister
Evans' syndrome	3	1 mother, 2 brothers and sister[1]
AIHA	2	2 brothers[3]
ITP	2	1 mother, 1 father
Type 1 diabetes	1	1 mother
Immune neutropenia	1	1 mother
Myasthenia	1	1 brother
Primary immunodeficiencies	**2**	
Combined immunodeficiency	1	1 sister
Autoimmune lymphoproliferative syndrome	1	1 mother

[1]Brother and sister suffering from ES, whose father had acute myeloid leukemia. [2]Girl suffering from AIHA and autoimmune thyroiditis, whose mother and brother have autoimmune thyroiditis. [3]Twin brothers suffering from AIHA and encephalopathy. The two brothers were equally affected by AIHA in 1/3 monozygous twin pairs.

Table 3. Secondary immunological AIHA in 265 children of the CEREVANCE cohort.

Immunological diseases[4]	Number of children	
	Before AIHA diagnosis	After AIHA diagnosis
Autoimmune disease	**35**	**83**
ITP (ie AIHA/ES)[2]	28	71
Auto-immune hepatitis	1	4
Thyroiditis	2	1
Systemic lupus erythematosus		3
Immune neutropenia	1	
Rheumatic disease	1	
Type 1 diabetes	1	
Type 1 diabetes and hepatitis	1	
Graves' disease		1
Vitiligo		1
Giant cell hepatitis		1
Giant cell hepatitis and Crohn's disease		1
Primary immunodeficiencies	**6**	**17**
Humoral deficiency (adult "CVID")	4	6
Cellular non identified PID		5
Combined immunodeficiency	1	2
Autoimmune lymphoproliferative syndrome	1	2
Adenosine deaminase deficiency		1
HLA class 2 deficiency		1

CVID: combined variable immuno-deficiency. [1]Immunological disease in 141/265 children concerned 99 AIHA/ES, 19 AID, and 23 PID, including 23 children with initially well-documented infection. [2]AIHA/ES began by ITP first in 28% of cases (28/99), by AIHA in 23% of cases (23/99), and by simultaneous ITP and AIHA in 49% of cases (48/99). For dissociated AIHA/ES, the median period between the first and second cytopenia was 2.9 years (range, 0.1 to 11.3 years).

autoimmune attack of bone marrow progenitors (autoimmune erythroblastopenia).[1] In 75% of these non-selected cases, the DAT class was IgG/IgG+C3d, whereas it was previously thought that in the pediatric age-group, the C3d post-infectious type predominated.[2,5]

Genetic predisposition to childhood autoimmune hemolytic anemia

Until recently, it was said that "there is no familial hereditary component" in AIHA,[20] and familial cases of AIHA or ES were very rare.[3,21,22] Due to the large size of this study and the systematic collection of family data, we are now able to refute this point. A genetic predisposition to AIHA, of autosomal recessive or dominant transmission, can be suspected for a small subgroup of patients, since consanguinity (8%) was observed in specific ethnic groups and a history of immunological diseases in first-degree relatives (14%) in all ethnic groups. In AIHA/ES, a family history of immunological diseases was observed in 22% of cases, with five cases of ES in first- or second-degree relatives.[21,22] However, incomplete concordance in monozygotic twins highlights the importance of multigenic systems and/or environmental factors. The slightly high 9% rate of prematurity draws attention to potential antenatal events. Associated malformations or chromosomal abnormalities in 8% of children might be a simple coincidence. Significant inroads into the understanding of the genetic basis of autoimmune disorders come from the comprehension of rare defects in immune homeostasis, such as impaired apoptosis of auto-reactive lymphocytes in autoimmune lymphoproliferative syndrome,[23] disruption of thymic negative selection in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome,[24] and absence of regulatory T cells in immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome.[25] None of these PID was found in the registered patients, so new avenues need to be explored.

Secondary childhood autoimmune hemolytic anemia

With a median follow-up of 3 years, secondary AIHA was identified in 63% of cases. Immune disorders or malignancies may still occur decades after the diagnosis. Lessons have been learnt over the years from this collaborative experience and an algorithm for a standardized workup, performed before starting treatment and repeated annually according to the clinical context, has been designed (Table 5). Making an etiological diagnosis has a clear impact on treatment, since in PID, adequate immune reconstitution via transplantation may be preferred to an escalation of immunosuppression. Specificities of childhood AIHA compared to the adult type justify a distinct workup.[19] Firstly, childhood AIHA should not be considered a paraneoplastic condition. Cold agglutinin AIHA was identified in 11% of pediatric patients, mainly in C3d DAT transient cases, unlike the chronic "cold agglutinin disease" of adults, related to B low-grade lymphoma in 10-20% of cases.[27] Secondly, the rare association of AIHA with giant cell hepatitis, a specific disease of infants, has never been described in adults: one of the two children enrolled died, in line with the known dismal prognosis of this combination of diseases. The exact post-infectious or immune etiology of the disease is still unclear.[28,29]

Evans' syndrome

The main dichotomy in childhood AIHA is between isolated AIHA and AIHA/ES. In the long-term context of our survey, ES accounted for 37% of all cases of AIHA; previous estimates ranged from 13% to 73%.[12,14,16] The definition of ES was restricted here to red blood cell and platelet

Table 4. Treatment and outcome in children enrolled in the CEREVANCE cohort for AIHA, according to underlying context: isolated AIHA *versus* AIHA/ES for all 265 patients with AIHA, and immunological *versus* non-immunological cases for the 166 patients with isolated AIHA.

	Total n = 265	Isolated AIHA n = 166	AIHA/ES n = 99	Isolated AIHA Immunological n = 42	Isolated AIHA primary and post infectious n = 124
Treatments					
Observation (%)	7	10	4	9	10
Steroid alone (%)	48	57	31	31	66
Steroid + others[1] (%)	45	37	65	60	24
Follow-up					
Follow-up (years) : mean (min-max)	4 (0.1-21.1)	3.1 (0.1-21.1)	5.6 (0.1-18.9)	4.5 (0.2-21.2)	2.4 (0-10.5)
Treatment duration[2] (years) : mean (min-max)	3.6 (0-21.1)	1.6 (0.4-21.1)	4.3 (0-16.8)	3.7 (0 - 21.2)	0.8 (0-5.8)
Treatment duration > 6 months (%)	72	62	88	86	53
Status at last follow-up					
Continuous complete remission (%)	39	42	35	33	44
Completed remission (%)	51	50	51	50	50
Partial response/no response (%)	6	7	5	12	6
Died[3] (%)	4	1	8	5	0
Off therapy (live patients) (%)	**70**	**80**	**59**	**60**	**86**

[1]*Other treatments used, with heterogeneous indications and modalities, and a median number of 2 (range, 1 to 7) per patient, were rituximab (n=69), azathioprine (n=41), cyclosporine (n=40), splenectomy (n= 37), mycophenolate mofetil (n=14) and hematopoietic stem cell transplantation [geno-identical donor (n=2) for AIHA/ES and for HLA class 2 deficiency, and unrelated donor (n=1) for AIHA/ES].* [2]*Mean total treatment duration was 1.1 years (range, 0.0 to 21.2 years) for C3d DAT* versus *3.1 years (range, 0.0 to 16.1 years) for IgG /IgG+C3d DAT (P=0.0002). Treatment lasted more than 6 months in 36% of cases of C3d DAT* versus *83% of cases of IgG /IgG+C3d DAT (P<0.0001).* [3]*Of the children who died, two had isolated AIHA (1 of giant cell hepatitis and 1 of cytomegalovirus infection after geno-identical marrow transplantation for PID), and eight had AIHA/ES (4 of cerebral hemorrhage, 3 of infection, including one* Pseudomonas aeruginosa *neutropenic septic shock 3 months after rituximab in refractory AIHA/ES, 1 of stroke).*

Table 5. Recommended CEREVANCE/SHIP workup at initial diagnosis for newly diagnosed AIHA in children.

- Complete blood count (including reticulocytes)
- Blood smear (exclusion of inherited disease and schistocytes)
- Coagulation tests (lupus anticoagulant and antiphospholipid antibodies)
- Extended phenotyped blood grouping
- Bone marrow aspiration, if cytopenia and reticulocytopenia
- Urea, creatinine, AST, ALT, bilirubin, GGT, haptoglobin, LDH
- Urine examination (hemoglobinuria, hematuria, proteinuria)
- Direct and indirect DAT (contact with Blood Center hemobiologist)
- IgG, IgA, IgM dosage (IgG subclasses if > 2 years old) (before initiation of intravenous immunoglobulins)
- Lymphocyte immunophenotyping (before steroids or immunosuppressive treatments): o $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+$, $CD56^+$. If hypogammaglobulinemia, naïve ($CD19^+IgD^+CD27^-$) and memory B ($CD19^+CD27^+$) o Double-negative T cells: $CD3^+$ $CD4^-$ $CD8^-$, TCR α/β^+ o If splenomegaly, hypergammaglobulinemia and elevated double-negative T cells: IL10, circulating FASL, Fas-mediated apoptosis functional tests. If abnormal: further sequencing of *FAS*, *FASL*, *CASP10*.[46]
- Antinuclear antibody (before starting intravenous immunoglobulins). If elevated titer, anti-dsDNA antibodies, other autoantibodies.
- C3, C4, CH50 Microbial serology, genomic or culture identification, and freezing of serum (wide and systematic)
- Chest X-ray and abdominal sonography (spleen size, tumoral syndrome)

destruction, as in the first reports on ES. Numerous cases of ITP with neutropenia and/or compensated hemolysis and positive DAT are registered separately in our database, whereas such cases were considered as ES according to recent definitions in the literature.[19,30,31] As in celiac disease and autoimmune thyroiditis, the predictive value of isolated autoantibodies needs to be assessed. As in adults, even after an apparent cure, the second cytopenia may appear up to 10 years after the first one.[19] An overlap of ES and autoimmune lymphoproliferative syndrome has been suggested in nearly half of cases of childhood ES.[30,31] In our country, for these selected patients, in cases of splenomegaly, hypergammaglobulinemia or elevated double-negative T cells, an active search for autoimmune lymphoproliferative syndrome, respecting revised international diagnostic criteria was the rule, and only revealed an authentic autoimmune lymphoproliferative syndrome in 3/265 children.[32]

Infections

Little was known about the exact implication of infections in childhood AIHA.[2,5,6] Well-defined infection was identified in 22% of our cases but half the cases turned out to be immunological and chronic (Table 4). The main causative agents were Epstein-Barr virus, mycoplasma, parvovirus and cytomegalovirus. The DAT was of C3d type in only 35% of cases. Infectious initiating agents which are not yet included in a systematic workup may be involved in the majority of cases of C3d type transient AIHA and primary AIHA. Whatever the exact role of initial infection may be,[1] it seems in most cases to be an initiating rather than a causal factor. We suggest that complete immunological investigations should be done, even if a well-defined infection is diagnosed, (Table 5) and that treatment monitoring and long-term follow-up are essential.

Primary immunodeficiency

A specific PID was revealed through the initial workup of isolated AIHA in 13% of children, and may still occur later in adult life. The frequency of the main classical diagnoses is emphasized here.[33,34] Common variable immunodeficiency was reported as associated with AIHA or ITP in 5% to 10% of cases.[35,36] However, in children less than 5 years old, symptomatic humoral deficiencies have to be separated into molecular entities. Other PID such Wiskott-Aldrich syndrome, autoimmune lymphoproliferative syndrome, hyper-IgM syndrome, nucleoside phosphorylase deficiency or newly described entities, were not found in these children.[37-41] Wider studies of AIHA occurring in the context of these PID is possible through the French participation in the European Society for Immunodeficiencies (ESID) registry.[37]

Autoimmune disease

Authentic AID accompanied 11% of the non-selected cases of isolated AIHA; the main types of AID were complete or incomplete systemic lupus erythematosus, hepatitis and thyroiditis including Hashimoto's thyroiditis. Some of these conditions are known to be more frequent in women, as in our series (Table 2). Pediatric systemic lupus erythematosus is said to be associated with AIHA in 10% of cases and with ITP in 26% to 74% of cases, with the hematologic forms having a worse prognosis.[42]

Prognostic factors and outcome of childhood autoimmune hemolytic anemia

With 18% of patients in continuous complete remission 2 years after diagnosis, the pejorative value of an IgG/IgG+C3d DAT, suspected in earlier studies,[2-6] was here demonstrated in the multivariate analysis as an independent factor. It has an impact on both initial status and long-term status off-therapy. In contrast, the good prognosis of C3d isolated AIHA, even with a shorter follow-up, was demonstrated, since the 2-year continuous complete remission rate was 71%. In contrast to the findings of earlier retrospective studies,[3,4] sex, age, and triggering infection were not associated with survival in continuous complete remission in the context of current therapies. The dismal prognosis of patients with AIHA/ES was shown to be related to the IgG/IgG+C3d DAT, which might explain the high level of steroid resistance, longer treatment dura-

tion and worse status at last information collection. New avenues are to be opened to study potential factors modulating hemolysis.[43]

The earliest pediatric AIHA series reported mortality rates ranging from 11% to 32%.[6] The high 10% mortality in the AIHA/ES subset is mainly due to hemorrhagic events, which are more severe than in isolated ITP. The 4% mortality rate we observed could be related to an improvement in supportive care, a greater enrollment of patients with benign forms or to the benefit of recently used drugs. There are no controlled studies or evidence-based data for the best treatment of childhood AIHA.[44,45] The low complete remission rate of 58% at the end of the first month of steroid therapy, together with the toxicity of these drugs, necessitates earlier introduction of second-line therapy. For 61% of children not in continuous complete remission at the last follow-up, establishing continuity of care with the corresponding adult team is a special concern of the French Network for Rare Diseases.

Conclusion

The historical SHIP network and partnership with the families were the key points in this national mobilization for a rare disease. In conclusion, childhood AIHA is a rare and underestimated disease, which differs from the adult form. There is a genetic predisposition to the development of this disease in a subgroup of patients and there is underlying immune deregulation in more than half of cases, warranting long-term homogeneous multidisciplinary follow-up. Finally, we found that the independent prognostic value of the IgG/IgG+C3d DAT is unfavorable. The prospects are now to exploit the bio-bank and establish DAT risk-adjusted therapeutic studies with adequate power.

Appendix

The list of participating physicians from the French Society of Pediatric Hematology and Immunology (SHIP) is as follows: *Dr Brigitte Pautard, Dr Valérie Li Thiao Te (Amiens), Dr Isabelle Pellier, Dr Xavier Rialland (Angers), Pr Pierre Rohrlich, Dr Véronique Laithier, Dr Emmanuel Plouvier (Besançon), Pr Christian Berthou, Dr Philippe Le Moine, Dr Liana Carausu (Brest), Dr Patrick Boutard, Dr Odile Minckes, Dr Gaetane Mousset (Caen), Pr François Demeocq, Dr Catherine Paillard (Clermont-Ferrand), Dr Gérard Couillault (Dijon), Pr Dominique Plantaz, Dr Corinne Armari-Alla, Dr Anne Pagnier (Grenoble), Dr Brigitte Nelken, Dr Françoise Mazingue, Dr Anne Lambilliote, (Lille), Dr Christophe Piguet, Dr Caroline Oudot (Limoges), Pr Yves Bertrand, Dr Corinne Pondarre, Dr Valérie Mialou, Dr Kamila Kebaili, Dr Jean-Marie Andre (Lyon), Pr Michel Gérard, Dr Hervé Chambost, Dr Isabelle Thuret, Dr Claire Galambrun, Dr Catherine Curtillet, Dr Vincent Barlogis (Marseille), Dr Eric Jesiorski, Dr Geneviève Margueritte, Dr Frédéric Bernard (Montpellier), Pr Pierre Bordigoni, Dr Fanny Fouyssac (Nancy), Dr Caroline Thomas, Dr Françoise Mechinaud, Dr Nadège Corradini (Nantes), Dr Fabrice Monpoux, Dr Nicolas Sirvent, Dr Anne Deville (Nice), Pr Alain Fisher, Dr Brigitte Bader-Meunier, Pr Stéphane Blanche, Pr Pierre Quartier, Pr Jean-Louis Casanova (Paris-Necker), Dr Corinne Guitton, Pr Isabelle Kone-Paut (Kremlin Bicêtre), Dr Thierry Leblanc, Pr André Baruchel, Pr Jean-Hugues Dalle, Dr Benoit Brethon, Dr Karima Yacouben, Dr Brigitte Lescoeur (Paris-Robert Debré), Pr Guy Leverger, Dr Judith Landman-Parker, Dr Marie Dominique Tabone, Dr Anne Auvrignon, Dr Catherine Dollfus, Dr Jean Donadieu (Paris-Trousseau), Dr Frédéric Millot, Dr Laurence Blanc (Poitiers), Dr Martine Munzer, Dr Stéphanie Gorde-Grosjean (Reims), Pr Edouard Le Gall, Dr Virginie Gandemer, Dr Sophie Bayart, Dr Sophie Taque (Rennes), Pr Jean-Pierre Vannier, Dr Pascale Schneider, Dr Aude Marie-Cardine (Rouen), Pr Jean-Louis Stephan, Dr Claire Berger (Saint Etienne), Pr Patrick Lutz, Dr Stéphane Ducassou (Strasbourg), Dr Alain Robert, Dr Geneviève Plat, Dr Hervé Rubie, Dr Marlène Pasquet, Dr Ségolène Clayssens (Toulouse), Pr Philippe Colombat, Dr Pascale Blouin, Dr Odile Lejars (Tours).*

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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