ANEMIE EMOLITICHE AUTOIMMUNI: INQUADRAMENTO DELL'ARGOMENTO E DIAGNOSI

A. Zanella

Ematologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano

Rimini, 26 maggio 2018

AIHA - OUTLINE

- **EPIDEMIOLOGICAL & CLINICAL ASPECTS**
- SEROLOGICAL & DIAGNOSTIC ASPECTS

Rimini, 26 maggio 2018

AUTOIMMUNE HEMOLYTIC ANEMIAS (AIHA)

- ✓ ***Incidence*** **0.8-3 per 10^5/year.** ***Prevalence*** **17:100,000.** ***Mortality*** **rate 11%** **[Petz & Garratty 2004; Packman 2008]**
- ✓ ***IDIOPATHIC*** **(50%) or** ***SECONDARY*** **to lymphoproliferative syndromes (20%), autoimmune diseases (20%), infections and tumors (Valent & Lechner 2008)**
- ✓ **Very rare in children (0.2 per 10^5/year); secondary forms associated with immune disorders in 53% of cases (Aladjidi et al, 2011)**
- ✓ ***"WARM"*** **(60-70%),** ***"COLD"*** **(CAD 20-25%, and PCH 1-5%) and** ***"MIXED"*** **(5-10%).** ***"ATYPICAL"*** **cases (DAT negative AIHA; warm IgM AIHA) reported with increasing frequency (Arndt et al, 2009; Kamesaky et al, 2013)**
- ✓ **Variable degree of anemia at onset, from compensated to fulminant**

Factors influencing the autoantibody pathogenicity and the extent of anemia

SEROLOGIC: **Ig class, subclass, thermal amplitude, C activating efficiency, specificity, affinity, ability to react with macrophages, quantity and type of cell-bound C/igG, characteristic of target antigen**

Donath-Landsteiner biphasic hemolysin (PCH)

- **IgG antibody of anti-P specificity, C fixing, which reacts in the cold and hemolyses at 37 °C**
- **hemoglobinuria and acute anemia (usually self-limited process)**
- **direct antiglobulin test negative or weakly positive with IgG**

	IgG (Warm AIHA)	IgM (Cold AIHA)
	37°C (0 - 40°C)	4°C (4 - 34°C)
	rare	common
	extravascular spleen ***Extravascular H.*** ***17.5 mL RBCs/hr***	intra and extra liver (spleen) ***Intravascular H.*** ***200 mL RBCs/hr***
	anti-Rh positive IgG (+C3d)	anti-I/i positive C3d

OTHERS: **- *A*ctivity of the reticuloendothelial system**
- Efficacy of the erythroblastic response

Reticulocytopenia in autoimmune hemolytic anemia

Crosby & Rappaport, Blood 1956

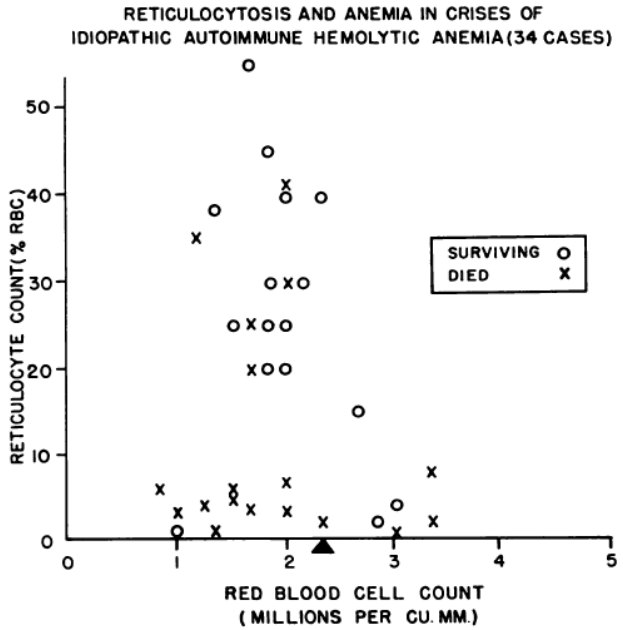


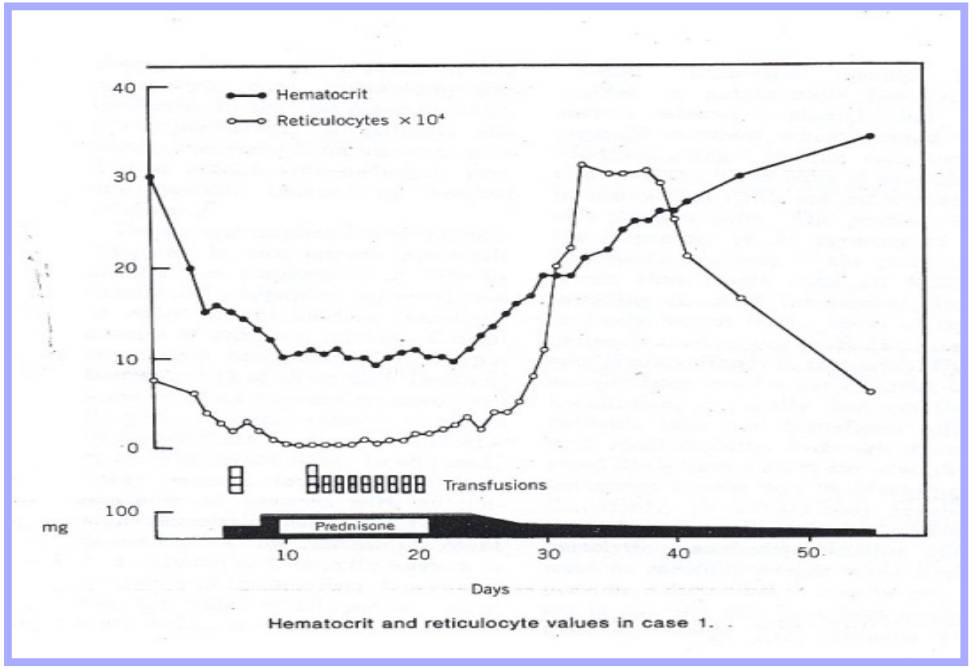
FIG. 1.—Reticulocyte counts versus red cell counts in 34 cases of idiopathic autoimmune hemolytic anemia. A caret on the abscissa is at point representing a hemoglobin concentration of 7 Gm. per 100 ml.

In a survey of cases of AIHA from the files of Armed Forces Institute of Pathology it was noted that a number of patients exibited, at one time or another, a definite reticulocytopenia in the presence of severe hemolytic anemia with a bone marrow that presented a picture of intense erythroid proliferation. The phenomenon appears to have a sinister significance.

Rimini, 26 maggio 2018

AIHA with reticulocytopenia. A Medical emergency

Conley CL et al, JAMA, 1980;244:1688



Hematocrit and reticulocyte values in case 1.

- **Reticulocytopenia in 20% (23/109) of adults with AIHA in the presence of erythroblastic bone marrow hyperplasia**
  (Liesveld et al, 1987)

- **Reticulocytopenia in 39% (81/208) of children with severe AIHA**
  (Aladjidi et al, 2011)

Case	Age/Sex	Hct	Retics (%)	Duration (d)	RBC units (n)	Outcome
1	52/F	10	0.5	10	19	CR
2	78/F	9	0.5	14	12	CR
3	53/F	10	2.0	90	53	PR
4	35/M	9	0.2	5	5	PR
5	49/F	8	3.0	160	84	PR

Zanella A, personal observation

SEVERE AND LETHAL AIHA CAUSED BY "WARM" IgM AUTOANTIBODIES

•"...AIHAs associated with IgM warm auto Abs are often severe with more fatalities than other types of AIHA…."
(Garratty et al, Vox Sang. 1997,72:124-30).

- Shirey RS et al. Fatal immune hemolytic anemia and hepatic failure associated with a warm-reacting IgM autoantibody. Vox Sang 1987; 52:210-22.
- McCann EL et al. IgM autoagglutinins in warm AIHA: a poor prognostic feature . Acta Hematol 1992;82:120-5.
- Garratty G et al. Severe AIHA associated with IgM warm autoantibodies directed against determinants associated with glycophorin A. Vox Sang 1997;72:124-30.
. Friedman AM et al. Fatal AIHA in a child due to warm-reactive immunoglobulin M antibody. J Pediatr Hematol Oncol 1998;20:502-5.
- Nowak-Wegrzyn A et al. Fatal warm AIHA resulting from IgM autoagglutinins. J Pediatr Hematol Oncol 2001;23:250-2.
- Arndt PA, Leger RM, Garratty G. Serologic findings in autoimmune hemolytic anemias associated with immunoglobulin M warm autoantibodies. Transfusion 2009;49: 235-42.
- Barnstetter CN et al. Severe autoimmune hemolytic anemia in an infant caused by warm- reactive IgM and IgA autoantibodies. J Pediatr Hematol Oncol 2015; 37::468-71.
- Takahashi H et al. Fatal warm autoimmune hemolytic anemia in a child due to IgM -type autoantibodies. Pediatr Int 2016; 58:744-46.

Rimini, 26 maggio 2018

AIHA associated with Warm IgM autoantibodies

Arndt PA, Leger RM, Garratty G, Transfusion, 2009;49:235

TABLE 1. Age and sex distribution of IgM warm AIHA patients and fatalities

Age range (years)	Number of patients (female/male)	Number of fatalities (female/male)
0-10	1 (1/0)	0
11-20	5 (1/4)	1 (0/1)
21-30	3 (2/1)	1 (1/0)
31-40	7 (3/4)	1 (1/0)
41-50	2 (2/0)	0
51-60	2 (1/1)	0
61-70	8 (4/4)	3 (1/2)
71-80	15 (13/2)	5 (5/0)
81-90	4 (2/2)	0
Unknown	2 (1/1)	0
Total	49 (30/19)	11 (8/3)

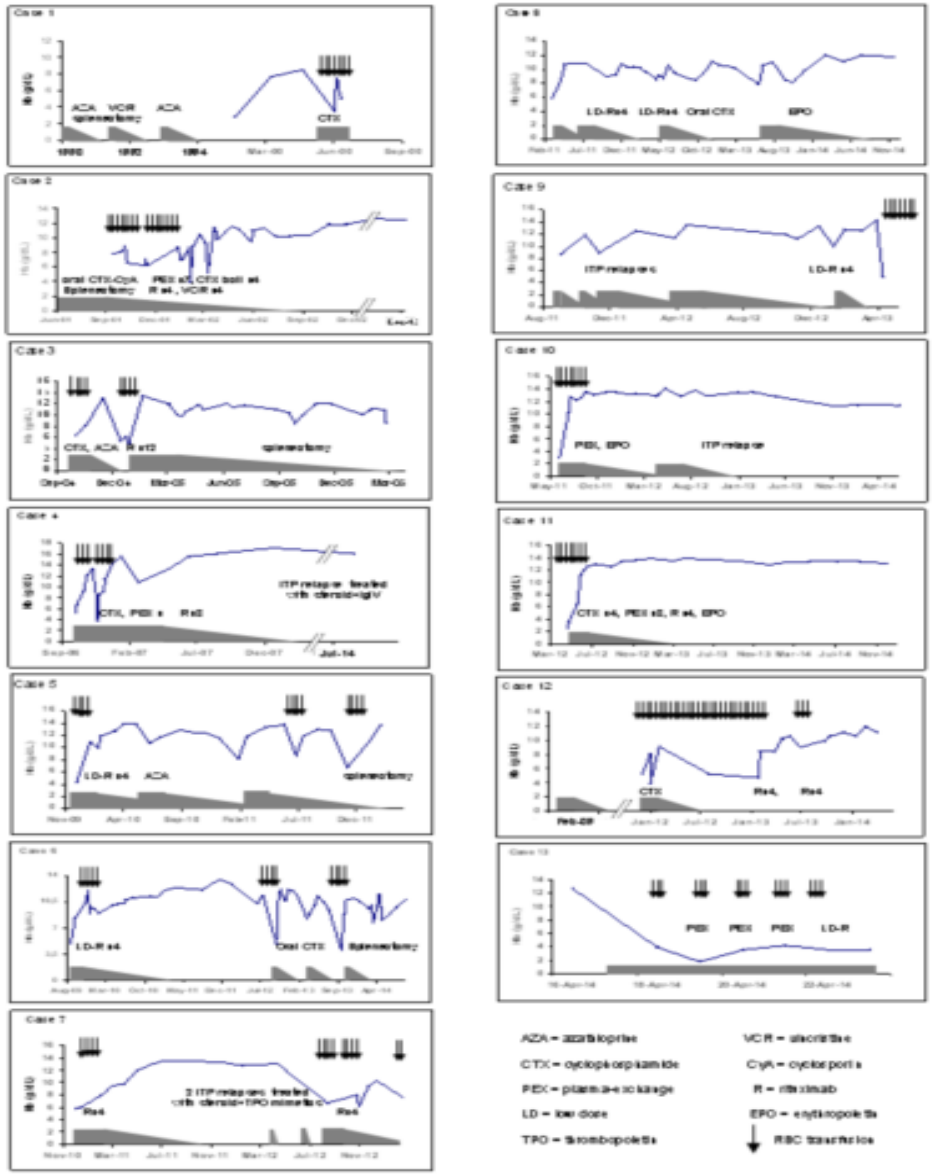
22.5% fatalities in patients with "warm" IgM AIHA

- **Spontaneous in vivo RBCs agglutination due to crosslinking of IgM autoabs at 37 ^{0}C, resulting in tissue ischemia and devasting clinical effects**
- **Can appear to have only C3d on RBCs (more rarely IgG+C3d) or even be DAT- Negative by routine DAT**
- **Difficult and delayed diagnosis**

Lessons from very severe, refractory and fatal primary autoimmune hemolytic anemias Fattizzo B, Zaninoni A, Nesa F, Sciumbata VM, Zanella A, Barcellini W. Am J Hematol 2015; 90:E149-51

13/157 cases studied from 1996 to 2014
Hb<6 , refractory to at least 2 therapy lines
7 fatal (54%)

- **7 DAT positive IgG+C , 4 IgG, 1 C, 1 DAT neg**
- **All marked intravascular haemolysis**
- **8 reticulocytopenia**
- **6 ITP**
- **8 thrombotic events**
  - **4 pulmonary embolism**
  - **3 DIC**
- **3 acute renal failure**
- **All received transfusions and steroids +/- IvIg**
- **11 rituximab,**
- **9 immunosuppressants**
- **5 plasma-exchange**
- **3 erythropoietin**
- **5 splenectomy**



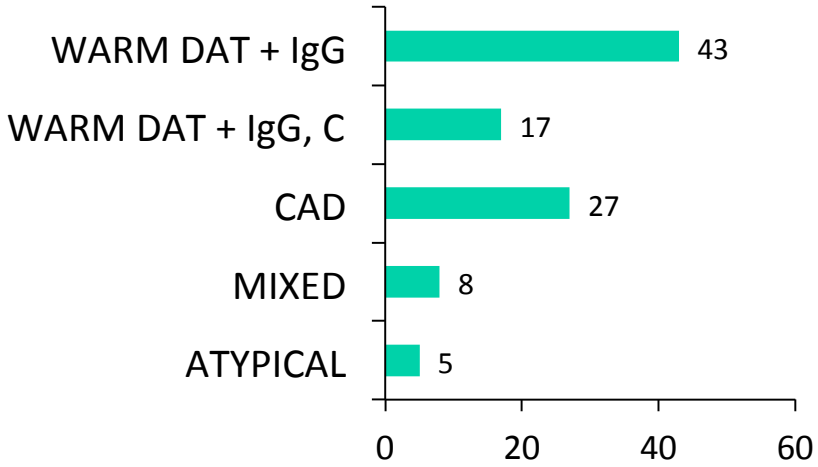
blood®
2014;124:2930-2936

Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients

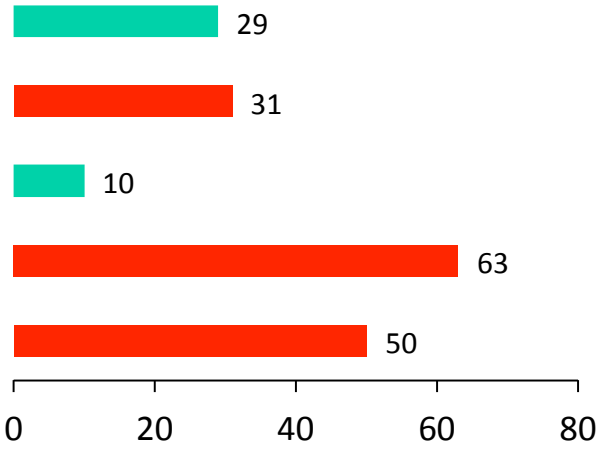
Wilma Barcellini,[1] Bruno Fattizzo,[1] Anna Zaninoni,[1] Tommaso Radice,[1] Ilaria Nichele,[2] Eros Di Bona,[2] Monia Lunghi,[3] Cristina Tassinari,[4] Fiorella Alfinito,[5] Antonella Ferrari,[6] Anna Paola Leporace,[6] Pasquale Niscola,[7] Monica Carpenedo,[8] Carla Boschetti,[1] Nicoletta Revelli,[9] Maria Antonietta Villa,[9] Dario Consonni,[10] Laura Scaramucci,[7] Paolo De Fabritiis,[7] Giuseppe Tagariello,[4] Gianluca Gaidano,[3] Francesco Rodeghiero,[2] Agostino Cortelezzi,[1,11] and Alberto Zanella[1]

Median age at the onset 58 yrs (1-95) Median Follow-up 33 mo. (12-372)

AIHA serological type (% patients)



Patients (27%)with severe onset (Hb<6 g/dL)



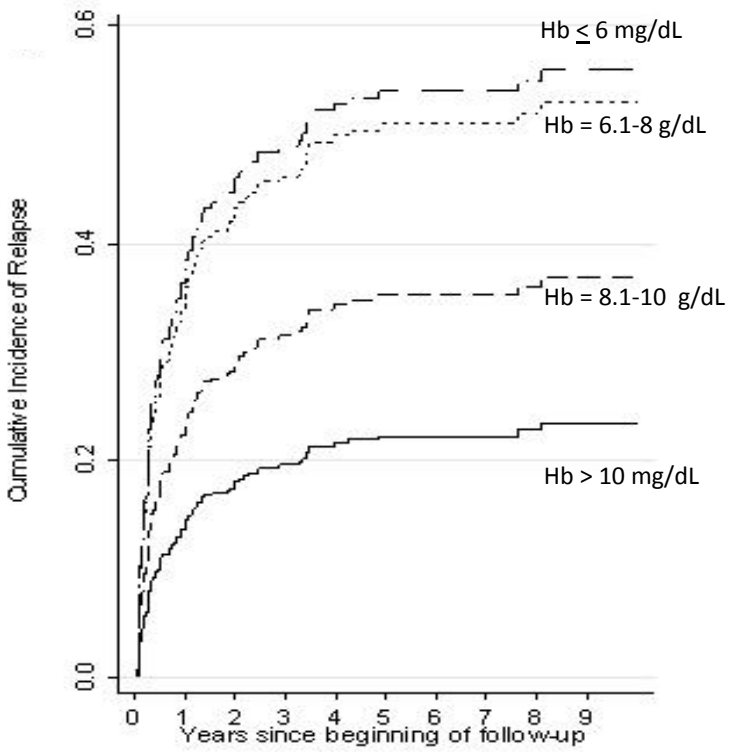
- **The majority of cases (60%) were WAIHA, followed by CAD (27%) and a small fraction (13%) of mixed and atypical** (1 DAT+ for IgA only, 6 MS-DAT pos. only, and 9 DAT-Neg)
- **Severe onset (<6 g/dL) in 27% of pts, more frequently associated with mixed and atypical forms, and with younger age**
- **Inadequate retics (<100x10^9/L) in 18% of cases, mostly severe**

blood®
2014;124:2930-2936

Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients

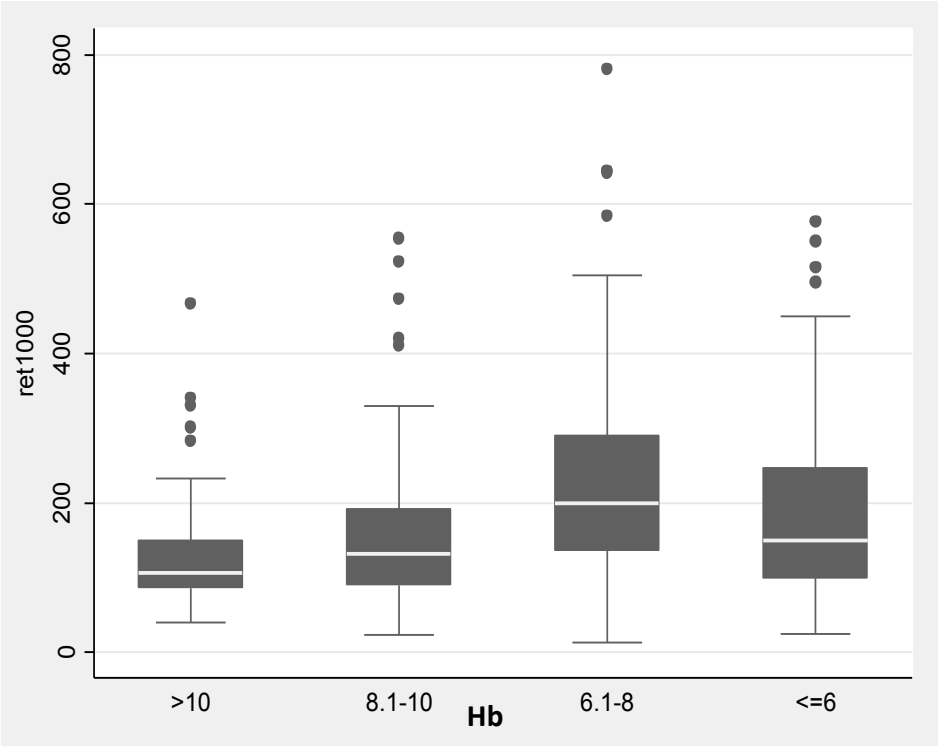
Wilma Barcellini,[1] Bruno Fattizzo,[1] Anna Zaninoni,[1] Tommaso Radice,[1] Ilaria Nichele,[2] Eros Di Bona,[2] Monia Lunghi,[3] Cristina Tassinari,[4] Fiorella Alfinito,[5] Antonella Ferrari,[6] Anna Paola Leporace,[6] Pasquale Niscola,[7] Monica Carpenedo,[8] Carla Boschetti,[1] Nicoletta Revelli,[9] Maria Antonietta Villa,[9] Dario Consonni,[10] Laura Scaramucci,[7] Paolo De Fabritiis,[7] Giuseppe Tagariello,[4] Gianluca Gaidano,[3] Francesco Rodeghiero,[2] Agostino Cortelezzi,[1,11] and Alberto Zanella[1]

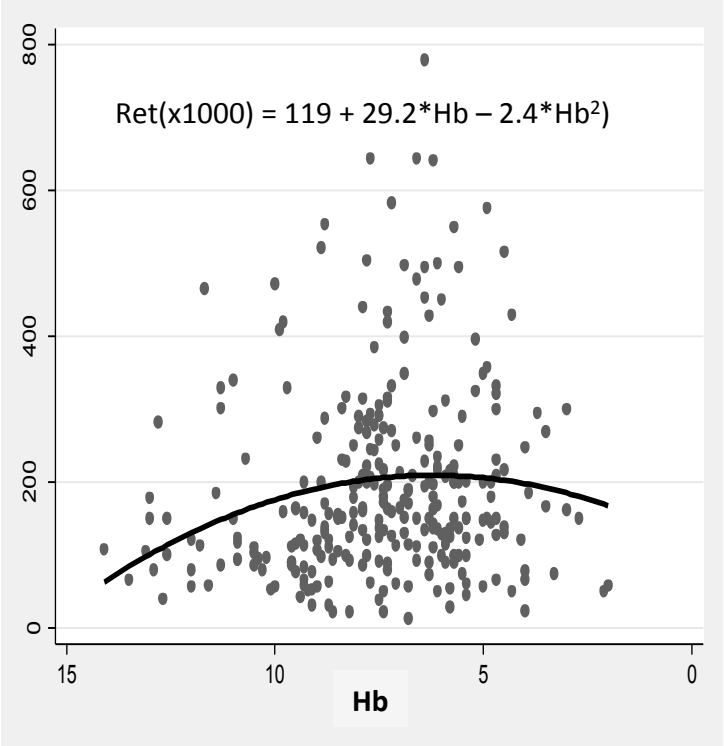
- **47% of cases (mainly IgG warm-AIHA) required a single line of therapy**
- **26% require at least two lines of therapy, 13% three, and 4% four or more lines**
- **Risk of relapse higher in pts with severe anemia at onset**



Clinical follow-up of 378 patients with autoimmune hemolytic anemia from 9 italian centers: prognostic impact of Hb levels, autoAb class, and reticulocytopenia at onset on the relapse risk and outcome. Barcellini W, Zaninonii A, Fattizzo B, Giannotta JA, Lunghi M, Ferrari A, Leporace AP, Maschio N, Scaramucci L, Cantoni S, Chiurazzi F, Consonni D, Rossi G, De Fabritiis P, Gaidano G, Zanella A, Cortelezzi A. Am J Hemat submitted

Absolute reticulocyte counts as a function of Hb at onset

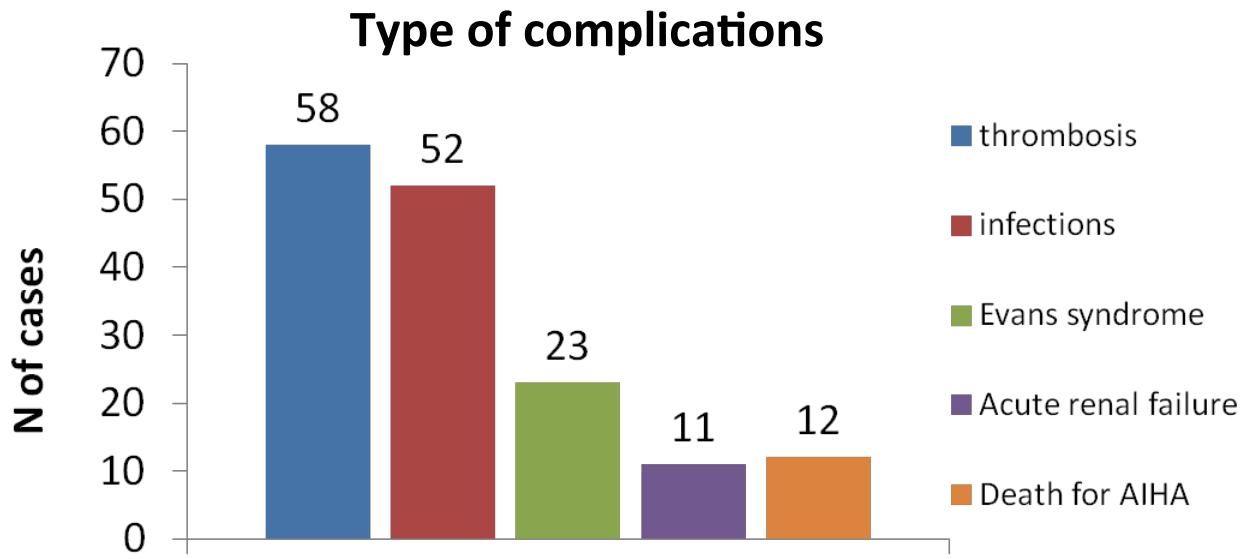




- **absolute reticulocytes were mostly reduced in CAD and mixed forms**
- **the reticulocyte index was lower in cases with Hb<6 g/dL** (65 vs 98, p<0.001)**, along with more frequent inadequate reticulocytosis** (87 vs 70%, p=0.01)

Rimini, 26 maggio 2018

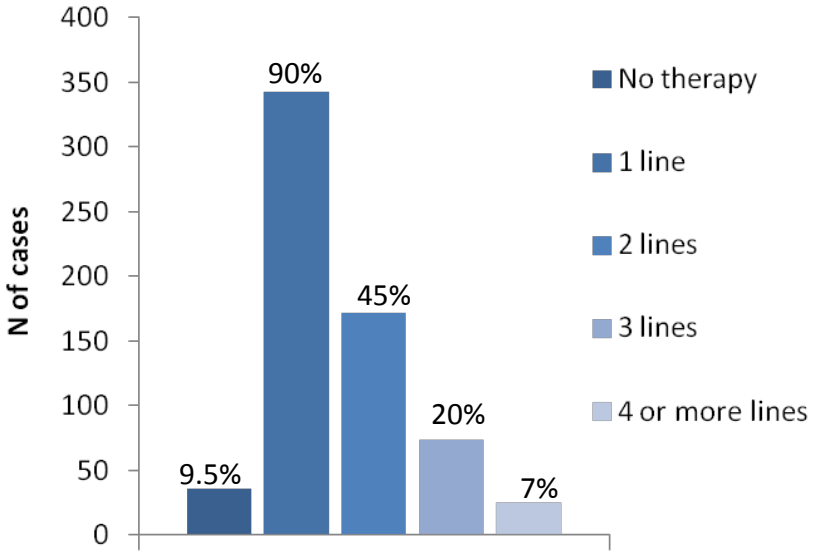
Clinical follow-up of 378 patients with autoimmune hemolytic anemia from 9 italian centers: prognostic impact of Hb levels, autoAb class, and reticulocytopenia at onset on the relapse risk and outcome. Barcellini W, Zaninonii A, Fattizzo B, Giannotta JA, Lunghi M, Ferrari A, Leporace AP, Maschio N, Scaramucci L, Cantoni S, Chiurazzi F, Consonni D, Rossi G, De Fabritiis P, Gaidano G, Zanella A, Cortelezzi A. Am J Hemat in press

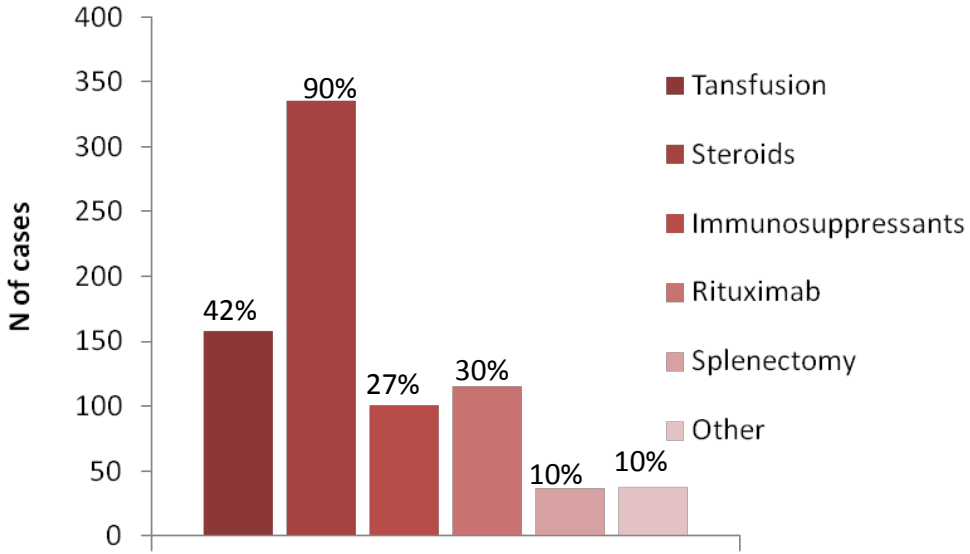


- **Thrombosis (15%), mostly in WIgG+C and atypical AIHA (severe cases with high LDH)**
- **Infections (14%), mostly in mixed and multi-treated AIHA**
- **Evans' syndrome (6%), more frequent in mixed or atypical cases**
- **Acute renal failure (3%), no relationship with AIHA type/Hb values**
- **12 (3.1%) died because of AIHA-related acute complications. Higher mortality was observed for Evans' syndrome** (HR 8.3, 95% CI)**, acute renal failure** (HR 7.6, 95% CI) **and infections** (HR 5.8, 95% CI)

Clinical follow-up of 378 patients with autoimmune hemolytic anemia from 9 italian centers: prognostic impact of Hb levels, autoAb class, and reticulocytopenia at onset on the relapse risk and outcome. Barcellini W, Zaninonii A, Fattizzo B, Giannotta JA, Lunghi M, Ferrari A, Leporace AP, Maschio N, Scaramucci L, Cantoni S, Chiurazzi F, Consonni D, Rossi G, De Fabritiis P, Gaidano G, Zanella A, Cortelezzi A. Am J Hemat in press

Number and type of therapy lines





- I[st] line therapy (steroids) was administered in almost all cases but 32 (mostly CAD)
- 2[nd] line therapy (rituximab, immunosuppressants, splenectomy) was mostly required in IgG+C wAIHA, and mixed forms (p=0.005).
- The ultra-refractory cases requiring 4 or more lines of therapy were mainly mixed and atypical
- Transfusions were required in 42% of cases

Hb<8 g/dL at onset predictive of relapse after I[st] line, and refractoriness to further lines

Rimini, 26 maggio 2018

AIHA - OUTLINE

- EPIDEMIOLOGICAL & CLINICAL ASPECTS
- **SEROLOGICAL & DIAGNOSTIC ASPECTS**

Rimini, 26 maggio 2018

AIHA - DIAGNOSTIC ASPECTS

- **Positive DAT not specific for AIHA**

  Direct antiglobulin test (DAT)
  Antibody screening & identification (serum & eluate)
  Complete erythrocyte phenotyping
  Additional serological investigations (DL abs, drug-induced abs, others)

DAT +	AIHA		Allo-IHA	DRUG-induced
	Auto	Auto+Allo		
496	385	69	37	5

Zanella A., personal observation

- **Atypical cases (Warm IgM AIHA / DAT-Neg AIHA)**

A dual antiglobulin test for the detection of weak or nonagglutinating immunoglobulin M warm autoantibodies

T. Bartolmas and A. Salama Transfusion. 2010 May;50(5):1131-4.

TABLE 2. DDAT for the detection of IgM on patient's RBCs*

Antiglobulin test	Anti-IgG	Anti-IgM	Anti-IgA	Anti-C3d
DAT	–	–	–	3+
DDAT	NT	2+	NT	NT

* The data in the first line of the table represent the result of a standard DAT test. Data in the second line represent the result of the DDAT after incubation with primary rabbit IgG antibodies anti-human IgM and secondary goat anti-rabbit IgG.

NT = not tested.

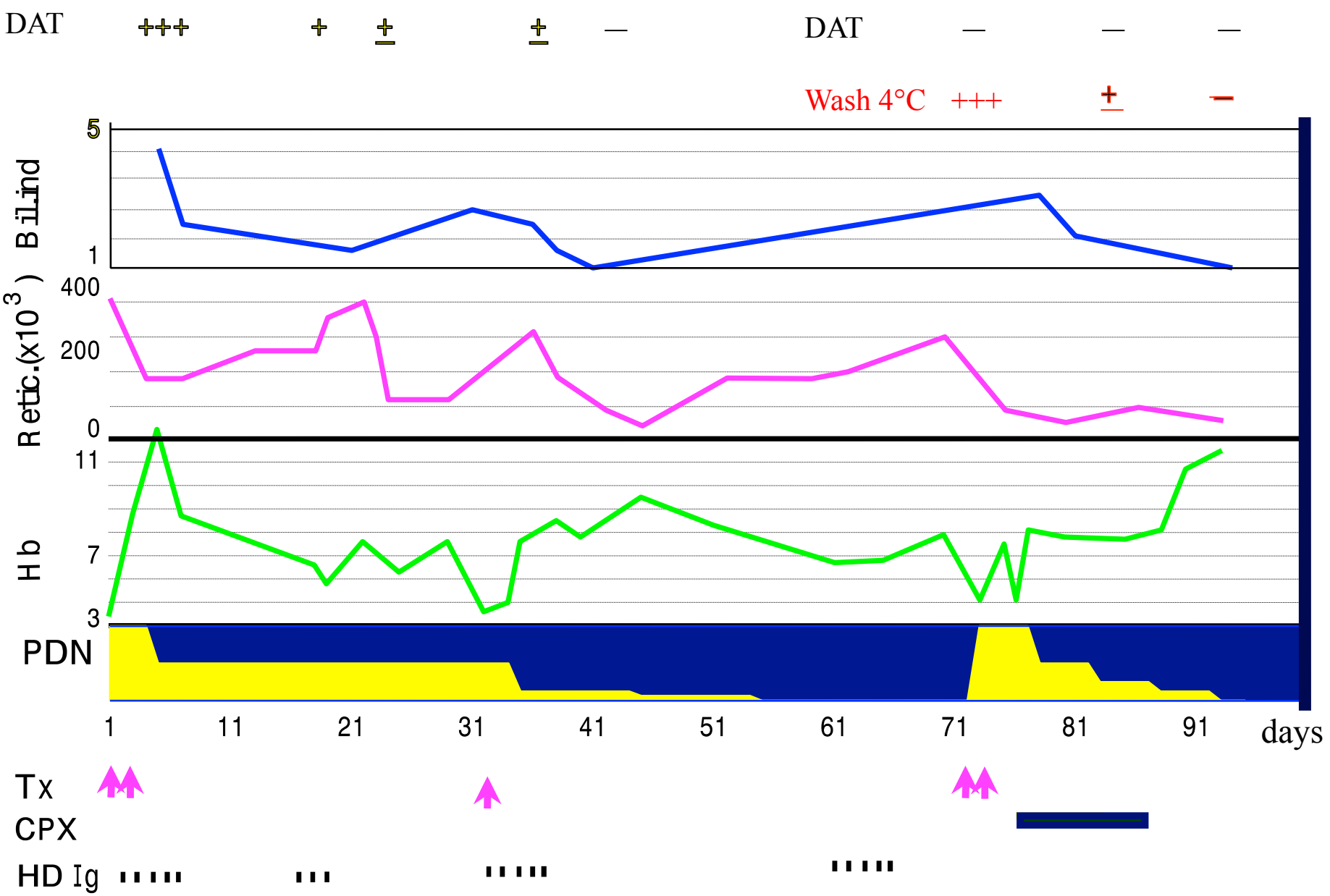
The hemolysis was refractory to treatment with prednisolone alone and plus azathioprine, but gradually improved after treatment with prednisolone plus cyclophosphamide.
CONCLUSION: Weak or nonagglutinating RBC-bound IgM warm antibodies can be identified by DDAT

Rimini, 26 maggio 2018

DAT negative AIHA

- **Represent 11% of AIHA according to Petz e Garratty (Immune hemolytic anemias. New York: Churchill Livingstone, 2004)**

Causes of false neg results	Possible solutions
IgA auto Abs	Use on monospecyfic anti-IgA antisera
Low-affinity IgG auto Abs	Wash with saline at 4^0C Wash with low-ionic solutions (LISS) Wash with polyethylene glycol (PEG)



Rimini, 26 maggio 2018

DAT negative AIHA

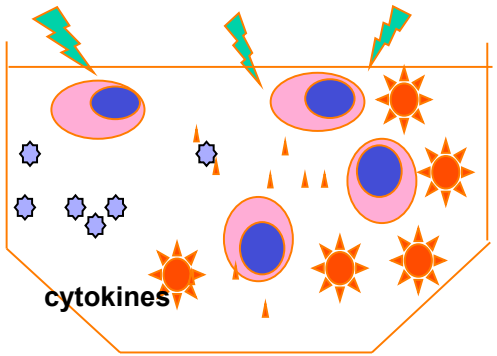
- **Represent 11% of AIHA according to Petz e Garratty (Immune hemolytic anemias. New York: Churchill Livingstone, 2004)**

Causes of false neg results	Possible solutions
IgA auto Abs	**Use on monospecyfic anti-IgA antisera**
Low-affinity IgG Abs	**Wash with saline at 4^0C** **Wash with low-ionic solutions (LISS)** **Wash with polyethylene glycol (PEG)**
Low test's sensitivity	**More sensitive techniques** ➢ ***Microcolumn agglutination (CAT)*** ➢ ***Solid phase agglutination*** (Yamada, 2008) ➢ ***Immunoenzimatic assay*** (Bencomo et al,2003) ➢ ***Flow cytometry*** (Lin et al, 2009) ➢ ***Mitogen-stimulated (MS)-DAT*** (Barcellini et al. 2010)

Comparison of traditional methods and mitogen stimulated direct antiglobulin test for detection of anti-red blood cell autoimmunity. Barcellini W, Revelli N, Imperiali FG, Villa MA, Manera MC, Paccapelo C, Zaninoni A, Zanella A, Int J Hematol 2010;91:762-9)

Mitogen-stimulated DAT (MS-DAT)

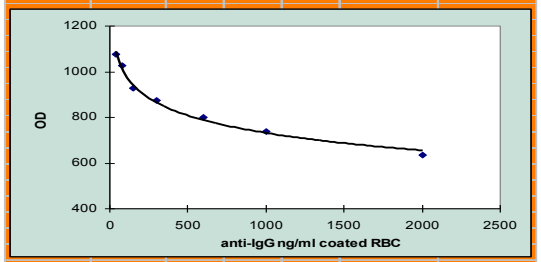
Mitogenic and/or cytokine stimulation

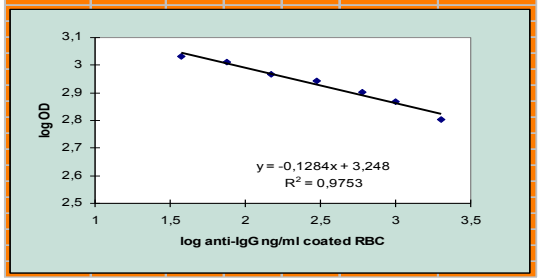


Auto-Ab in culture

Binding of Auto-Ab to autologous RBC

ng/ml	OD mean	OD 1	OD 2	OD 3	ng/ml log	OD log
2000	636	648	632	629	3,301030	2,803685
1000	739	721	746	750	3,000000	2,868644
600	800	795	801	805	2,778151	2,903271
300	876	845	850	933	2,477121	2,942504
150	927	932	948	901	2,176091	2,967080
75	1027	1065	1056	961	1,875061	3,0117114
38	1077	1062	1069	1100	1,574031	3,032216
0	1089	1098	1120	1050		

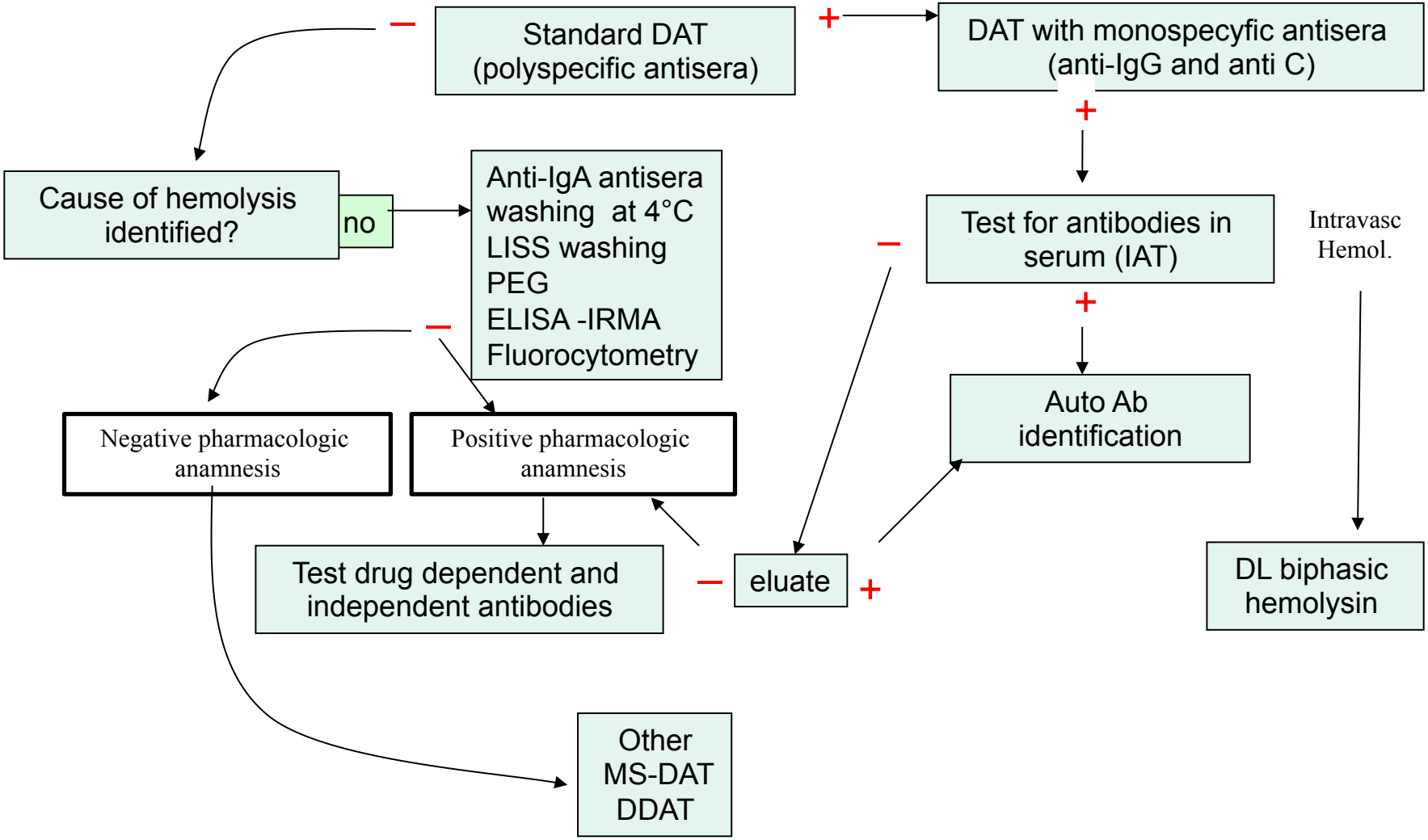




"In the last 10 years we collected 16 cases of DAT-negative AIHA (tube, micro- column and solid phase) in which the diagnosis was made on the basis of a positive MS-DAT. These cases represent roughly 10% of total AIHA observed in the same period".

Rimini, 26 maggio 2018

Diagnosis and management of newly diagnosed childhood autoimmune hemolytic anaemia. Reccomendation from the Red Cell Study Group of the Pediatric Haemato-Oncology Italian Association Ladogana S, Maruzzi M, Samperi P, Perrotta S,De Del Vecchio G,Notarangelo L, Farruggia P, Verzegnassi F, Masera N, Saracco P, Fasoli S, Miano M, Girelli G, Barcellini W, Zanella A, Russo G. Blood Transfusion 2017; 15:259-67



AIEOP - Diagnostic flow-chart for suspected AIHA in children

AIHA - TAKE HOME MESSAGES

✓**AIHAs are very heterogeneous diseases with sometimes a rapid fatal outcome, thus requiring particular attention by clinicians**

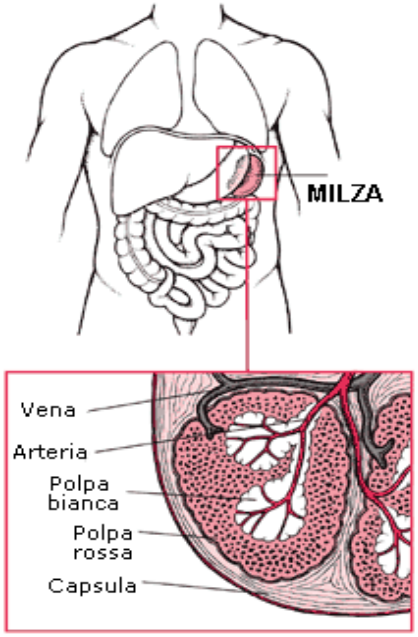
✓**Mixed, atypical and warm IgG+C AIHA (~ 30% of cases) have more frequently a severe onset (Hb<6 g/dL) and require multiple therapy lines.**

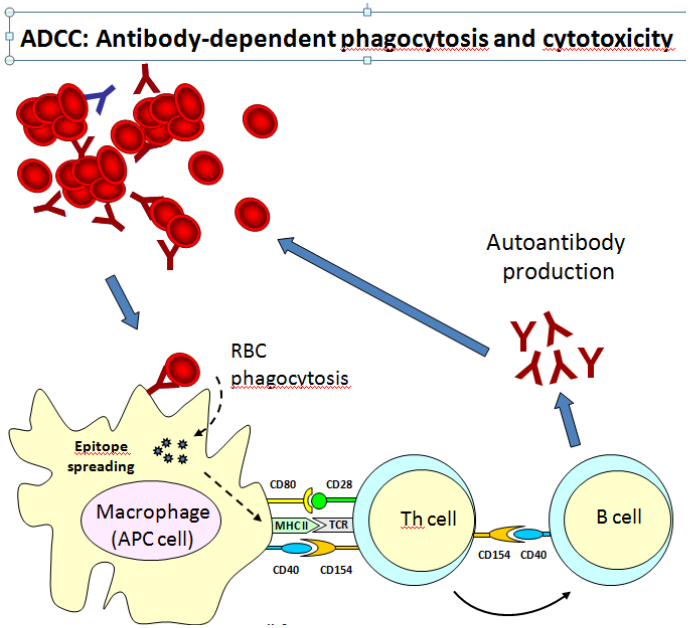
✓**Infections, acute renal failure, Evans' syndrome, and multi-treatment were predictors of fatal outcome. Death was not associated with thrombotic events**

✓ **A very close cooperation between clinical hematologists and immunohematologists in observation of the recommended diagnostic flow-charts is needed for a correct and timely diagnosis and management of the most complex cases**

THE END

Role of the spleen in the pathogenesis of AIHA





- **Major organ for antibody synthesis**
- **Major site of immune destruction of IgG-coated erythrocytes**
- **Splenectomy usually ineffective in CAD since most RBC destruction occurs in liver**
- **Effective in about 70% of warm AIHA**
- Ineffective in about 30% of cases, presumably because there is still an elevated **synthesis of autoantibodies in other lymphoid organs**, and the **liver replaces the spleen's function of RBC destruction**
- The rate of splenectomy is about **10-15%** of all AIHA cases, but is **nowadays decreasing** given the availability of **equally effective second line medical treatments**, primarily rituximab
- No reliable predictors of its effectiveness, such as the extent of **splenic sequestration** assessed by radiolabeled erythrocytes, **disease duration** and the **response to steroids**
- **The emergence of long-lived autoreactive plasma cells in the spleen of primary warm AIHA patients relapsed after rituximab**. Spleens (obtained after splenectomy) showed the presence of non-proliferative memory B-cells and plasma cells, possibly sustained by increased BAFF (new therapeutic options?)

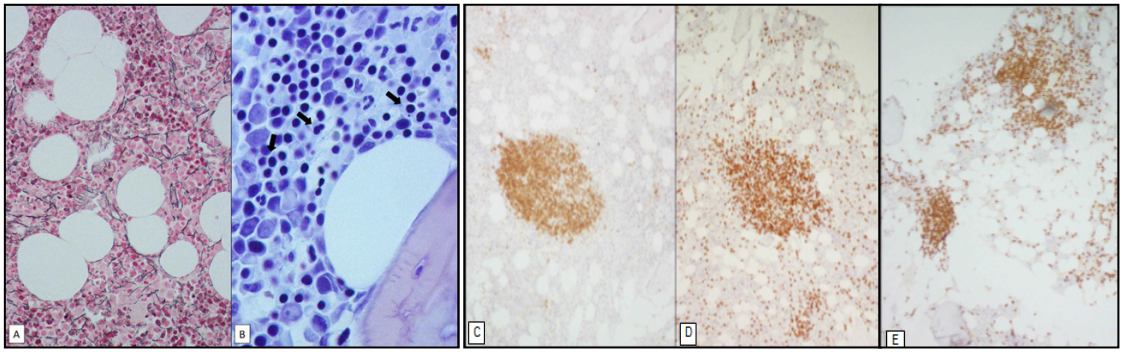
Mahévas M et al, J Autoimmun. 2015 Aug;62:22-30

Am J Hematol. 2017 Dec 23. doi: 10.1002/ajh.25020. [Epub ahead of print]

Prognostic impact of bone marrow fibrosis and dyserythropoiesis in autoimmune hemolytic anemia.

Fattizzo B[1], Zaninoni A[2], Gianelli U[3], Zanella A[2], Cortelezzi A[1], Kulasekararaj AG[4], Barcellini W[2].

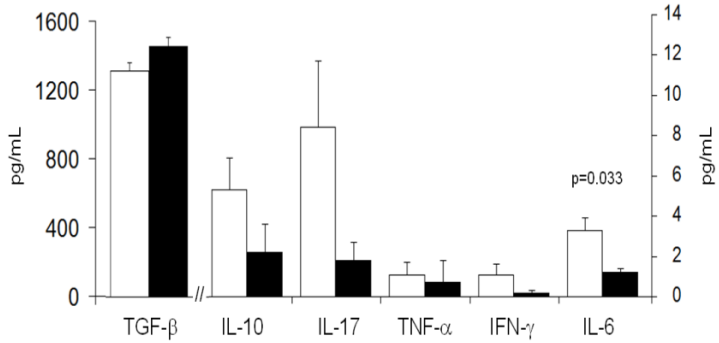
STUDY GROUP	MF0 N=30	MF≥1 N=17
Hb, g/dL,	7.4 (3.5-13.1)	8 (2-11)
LDH x UNL	1.27 (0.4-7)	1 (0-3)
Ret x10^3/mmc	113 (13-275)	157 (58-284)*
Bone marrow characteristics		
Cellularity, %	52.5 (25-90)	65(20-99)**
Normo, N (%)	12 (40)	2 (12)
Hypo, N (%)	2 (7)	1 (6)
Hyper, N (%)	16 (53)	14 (82)*
Diserythro N (%)	16 (53)	14 (82)*
Lymphoid infiltrate		
T-cell, N (%)	8 (26)	2 (12)
B-cell, N (%)	4 (13)	3 (18)
Mixed, N (%)	10 (30)	10 (59)



(A) loose network of reticulin fibers with many intersections (MF-1), (B) The hyperplastic erythropoietic series shows some grade of dyserytropoiesis

(C) centro-lacunar lymphoid aggregate predominantly composed of CD20-positive small B lymphocytes, (D) with scattered small CD3-positive T lymphoid cells. (E) focal and interstitial lymphoid infiltrate composed mainly of CD3+ small T-lymphoytes

Cytokine serum levels in patients with (black) and without (white) bone marrow fibrosis



- **Reticulinic fibrosis (MF1) is present in 36% of AIHA cases and correlates with the presence of a hypercellular dyserythropoietic bone marrow , increased levels of TGF-β , and a higher rate of relapse and treatment requirement**
- **These chronic refractory cases may show clinical/pathologic features similar to low-risk myelodysplastic syndromes and recently described idiopathic cytopenia/dysplasia of unknown significance (ICUS/IDUS)**
- **MF0 AIHA patients seems to present with a more florid hemolytic disease, increased immune activation and cytokine release, more frequent thrombotic events, and a better response to immunesuppression**