

TITLE

Clinical features, diagnostic work-up, and treatment outcomes in Cold Agglutinin Disease: results from a monocentric retrospective study

AUTHORS

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Introduction: Cold agglutinin disease (CAD) is a rare autoimmune hemolytic anemia (AHIA) caused by cold agglutinins by activation of the classic complement pathway¹, driven by a clonal B-cell lymphoproliferative disorder (LPD) not meeting lymphoma criteria¹. CAD represents 15% of AIHA cases², with incidence of 1/million/year³; clinical manifestations consistently impact patients' quality of life. Treatment targets the two key pathogenic mechanisms, suppressing autoantibody-producing B-cells and blocking complement activation⁴.

Aim: due to its rarity, CAD population-based studies are lacking. We aimed to describe epidemiology, clinical/pathological features, course, and therapy of CAD in a monocentric retrospective cohort.

Methods: we retrospectively reviewed patients diagnosed with CAD at IRCCS Policlinico San Matteo (1986-2025), per standard criteria (hemolysis, direct antiglobulin test positive for C3d, CA titer $\geq 1:64$, absence of CA syndrome). Clinical, laboratory, histological, and treatment data were collected from diagnosis to death/last follow-up. *MYD88*^{L265P} mutation was assessed using digital droplet PCR. Overall survival (OS) and progression-free survival (PFS) were estimated by the Kaplan-Meier method.

Results: with a median follow-up of 36.5 months, twenty-four patients diagnosed with CAD were included; 14 were female (58.3%). Median age at diagnosis was 68.5 years (range: 36-83). Two patients died (one CAD-related); median OS was not reached. At diagnosis, mean hemoglobin was 9.5 g/dL (range: 5.5–14.7), bilirubin 2.78 mg/dL (range: 0.7–6), and LDH 440 U/L (range: 116–1210). LDH was elevated in 75% of patients, and haptoglobin was reduced in 90% of cases (n=20, [2]). Monoclonal protein was detected in 89%, predominantly IgM-kappa. C4 was reduced in nearly all patients. CA titers ranged from 1:64 to 1:4096. No hepatosplenomegaly was observed. Bone marrow (BM) evaluation (n=22) identified a B-cell LPD clone in 6 cases by histology and 13 by flow cytometry; 6 were detected only by flow cytometry. Immunophenotyping of the clonal population (peripheral blood (PB), n=8; BM, n=13) showed a CD19⁺ CD20⁺ CD79⁺ CD200⁺ CD10[–] CD43[–] profile with kappa light-chain restriction in all evaluated cases. Notably, CD5 was positive in a substantial proportion of clones (75% in PB, 77% in BM), diverging from the classically described CD5-negative CAD-associated B-cell clone. *MYD88*^{L265P} mutation was assessed in 20 patients and found in 3 (15%). Circulatory symptoms occurred in 8.3%, while symptomatic anemia and fatigue were reported in 66.7% and 50%, respectively. Thromboembolic events occurred in 25% (n=6, 4 venous, 2 arterial), with no baseline clinical or laboratory variable predicting risk. Eighteen patients (75%) received 1–4 treatment lines (median: 2). Rituximab-based regimens achieved more durable responses than corticosteroids, immunosuppressants, or chemotherapy. Among the 16 patients treated with rituximab and/or chemoimmunotherapy, median PFS was 84.1 months, with 1- and 2-year PFS of 92%. Hemolytic exacerbations occurred in 8 rituximab-treated patients, mostly infection-triggered, with 3 requiring retreatments.

Conclusions: CAD has a good prognosis, but remains diagnostically and therapeutically challenging. Extensive diagnostic work-up is warranted; the unexpectedly frequent CD5 positivity of LPL-clone requires further immunophenotypic characterization. Our data confirm that B-cell-directed therapies achieve durable responses; however, treatment remains non-curative, with frequent, often infection-triggered re-exacerbations, underscoring the need for novel complement-targeted strategies.

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Figure 1

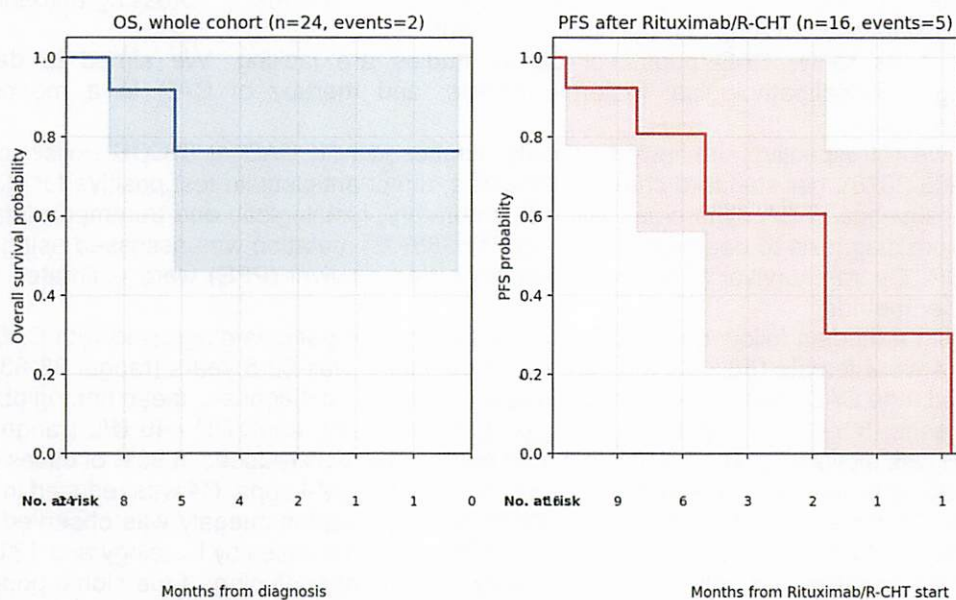


Figure 1: Kaplan-Meier estimates of overall survival (OS, n=24; blue curve, on the left) and progression-free survival after rituximab and/or chemoimmunotherapy (PFS, n=16; red curve, on the right). Shaded areas indicate 95% CI; numbers below each curve show patients at risk.

Figure 2

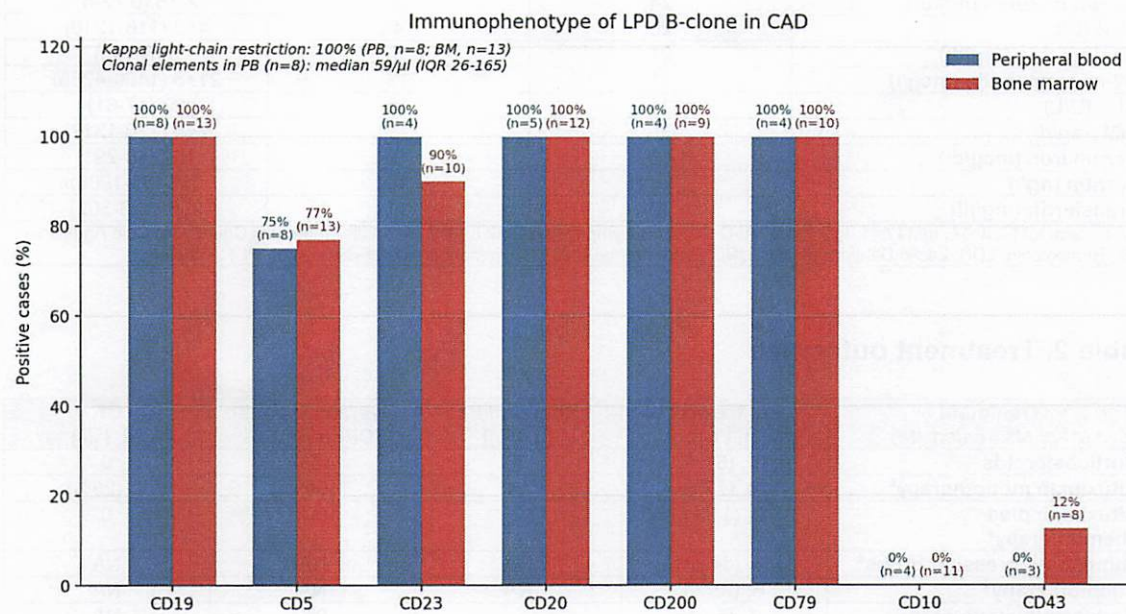


Figure 2: Immunophenotype of the B-cell lymphoproliferative disorder (LPD) Clone in CAD patients: bone marrow and peripheral blood data.

Table 1. Baseline characteristics of CAD patients (N=24)

Variables	N	Missing	%
Female sex	14/24	0	58.3%
LDH>UNL	18/24	0	75%
Haptoglobin reduced: yes	20/22	2	90%
M-protein*	16/18	6	89%
C4 reduced: yes	7/8	16	88%
HBV-positivity	5/23	1	22%
HCV-positivity	0/23	1	0%
HIV-positivity	0/23	1	0%
Symptomatic anemia	16/24	0	68%
Circulatory symptoms	2/24	0	8.3%
Thromboembolic event	6/24	0	25%
Fatigue	12/24	0	50%
Transfusion need	7/17	0	30%
Hepatomegaly	0/24	0	0%
Splenomegaly	0/24	0	0%
Cholecystectomy	7/17	0	29%
Bone marrow evaluation	22/24	2	92%
LPD clone by flow cytometry	13/16	6	81%
LPD clone by histology	6/20	2	30%
MYD88 ^{L265P} mutation	3/20	4	15%
Non hematological malignancies	5/24	0	21%
Autoimmune disease	3/24	0	12.5%
Variables	N	Missing	Median, (range)
Age at diagnosis (yrs)	24	9	68.5 (36.8- 83.3)
CA title	24	0	1:64 (1:64-1:4096)
Hb (g/dl)	24	0	9.5 (5.5-14.7)
WBC (/mmc)	24	0	7455 (4400-16480)
ANC (/mmc)	21	3	4750 (2150-13870)

ALC (/mmc)	19	5	2190 (850-21400)
PLT (/mmc)	23	1	295000 (37000-436000)
Serum bilirubin (mg/dl)	24	0	2.78 (0.72-6)
LDH (U/L)	20	4	440 (116-1210)
Haptoglobin (mg/dl)	21	3	6 (6-143)
B2-microglobulin (mcg/l)	11	13	2173 (1480-4265)
ALT (U/L)	11	13	29 (17-61)
IgM (mg/dl)	6	18	248 (130-1312)
Serum iron (mcg/dl)	15	9	151 (48-291)
Ferritin (ng/l)	14	10	356 (33-1966)
Transferrin (mg/dl)	13	11	256 (163-302)

*M-protein: IgM-k n=14, IgM-l n=1, IgG-k n=1. ALC: Absolute Lymphocyte Count. ANC: Absolute Neutrophil Count. CA: Cold Agglutinin. Hb: Hemoglobin. LDH: Lactic Dehydrogenase. UNL: upper normal limit. LPD: lymphoproliferative disorder. PLT: Platelets.

Table 2. Treatment outcomes

Treatment (n° of pts treated: 18)	Pts treated, n, (%)	Responders [n, (%)]	Overall response (CR or PR), %	CR [n, (%)]
Corticosteroids	11, (61%)	5	45%	0
Rituximab monotherapy [‡]	14, (77%)	14	100%	4, (29%)
Rituximab plus chemotherapy*	2, (11%)	2	100%	0
Immunosuppressant drugs [#]	8, (44%)	NA	NA	NA
Chemotherapy [§]	6, (33%)	NA	NA	NA
Bortezomib alone or in combination	1, (5%)	0	0%	NA
Erythropoietin	2, (11%)	NA	NA	NA

[‡]Two patients received 2 course of rituximab monotherapy. *Rituximab plus fludarabine (n=1), rituximab plus cyclophosphamide and prednisone (n=1). [#]Azathioprine (n=8), cyclosporin (n=2, in combination with azathioprine). [§]Cyclophosphamide (n=6), chlorambucil (n=2, in combination with cyclophosphamide).

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