CORRESPONDENCE

Clinical characteristics and outcome of patients with autoimmune hemolytic anemia uniformly defined as primary by a diagnostic work-up

To the Editor: Primary autoimmune hemolytic anemia (P-AIHA) is a relatively uncommon and hetereogeneous disease characterized by the destruction of red blood cells due to anti-erythrocyte autoantibodies (AeAbs) in the absence of an associated disease [1–3]. Secondary AHIA is frequently associated with lymphoproliferative diseases (LD) in particular, chronic lymphocytic leukemia, aggressive or indolent lymphomas, autoimmune disorders, malignancies other than lymphoid, and infections [1,2,4].

On the hypothetical assumption that in a significant proportion of cases defined as P-AIHA the clinical heterogeneity could be due to an ignored associated disease, we retrospectively analyzed the clinical characteristics and outcome of patients with a diagnosis of P-AIHA based on a diagnostic work-up aimed at excluding or identifying an associated disease.

From March 1982 to March 2014, 200 adult patients diagnosed as having a P-AIHA on a clinical basis were managed at our institution. AIHA diagnosis was made in the presence of anemia, signs of hemolysis, the positivity of the direct antiglobulin test, and/or the detection of an AeAb in the serum of patients (Supporting Information Material and Methods). For the purpose of this study, two main groups of patients were analyzed, the group with a "G" serological profile including cases with an IgG AeAb ±C3d or, in rare cases, combined with an IgA AeAb, and the group characterized by an "M" profile including cases sustained by a cold IgM AeAb and mixed cases with an IgG AeAb associated with a pathogenic IgM AeAb.

Patients did not report any previous history of drugs, recent infections, or diseases known to be associated with AIHA. Furthermore, none of them showed on examination any overt signs of a concomitant disease related to AIHA. The diagnostic work-up subsequently performed by each patient was retrospectively reviewed. The minimum panel of tests that we considered as sufficient to exclude, or identify, an associated disease included: (1) bone marrow (BM) biopsy; (2) autoantibody profile incorporating at least the following autoantibodies: anti-nuclear; anti-cardiolipin IgG/IgM; lupus anticoagulant, anti-β2glycoprotein1 IgG/IgM, anti-thyroid peroxidase antibody, and anti-thyroglobulin antibody; (3) B and C hepatitis serology (HBsAg; HBeAg; HBsAb; HBcAb; HBeAb, HCVAb); (4) total body CT scan (or at least, an abdomen ultrasonography combined with a chest X-ray). A front-line treatment with steroids [2] was given to all patients, with the exception of two with mild signs of cold AIHA who received only folic acid support. Patients with severe and symptomatic anemia received also intravenous immunoglobulins and/or RBCs transfusions. In patients who achieved a response, steroids were slowly tapered to reach the lowest effective dose (no more than 5–10 mg of prednisone daily or on alternate days) to maintain the Hb value $\geq$10 g/dL. Steroids were discontinued in the presence of a stable Hb value $\geq$12 g/dL, no signs of hemolysis and no longer detectable AeAb at two consecutive examinations. In patients who, after the screening, were re-classified as having a secondary AIHA, treatment was addressed to the underlying disease.

The response to treatment was defined according to the Hb value, the presence of signs of hemolysis and the detection of the AeAb (Supporting Information Material and Method).

We identified 110 patients with an initial diagnosis of P-AHIA who performed the minimum panel of tests that we considered as sufficient to exclude, or identify, an associated disease (Supporting Information Table 1s). The diagnostic work-up did not reveal an associated disease in 72/110 (65.45%) patients whereas in 38 (34.54%) an underlying disease was identified. An ignored malignancy was detected by the CT scan in six patients (5.45%) and an active HCV-RNA positive hepatitis in one patient. In 13 patients (11.81%), the significant positivity of some autoantibodies directed further serological and clinical evaluations allowing the identification of an autoimmune disorder. The proportion of cases with a serological "M" profile was significantly higher in patients with an LD ($P < 0.0001$). The LD was revealed by the CT scan in two cases (enlarged spleen with nodules, 1; enlarged abdominal lymph-nodes, 1). In 16/18 cases, the LD was detected by the BM biopsy in the absence of other clinical manifestations. As previously described [5], the BM involvement by clonal B lymphocytes was usually limited and these patients, defined as having a primary cold agglutinin disease [5] showed a high response rate, 85.71% with a rituximab-based-chemoimmunotherapy. This observation confirms the benefit of chemoimmunotherapy in these cases [6].

The 72 patients with no evidence of an associated disease after the screening, and uniformly diagnosed with P-AIHA, were relatively younger ($P = 0.0056$) and more frequently characterized by a "G" than an "M" profile (79.16% vs. 18.05%) (Supporting Information Table 2s).

A response to steroids was recorded in 52/68 (76.47%) evaluated patients, with a complete response (CR) in 49 (72.05%). The disappearance of the AeAb was recorded in 12 (17.65%) cases, in 11/56 (19.64%) cases with a "G" profile and in 1/12 (8.33%) with an "M" profile ($P = 0.67$).

Twenty-one patients have died. Refractory hemolysis associated with pneumonia (2 patients), a cerebrovascular event (1 patient), a cardiovascular event (1 patient) were the causes of death in 4 (5.5%) older patients (median age 81 years). In the remaining 17 patients, the most frequent causes of death were cerebrovascular and cardiovascular events. The median overall survival (OS) was 21 years and the median disease-free survival (DFS), 13.21 years (Supporting Information Fig. 1s). Remarkably, the severity of anemia and the serological characteristics of the AeAb did not influence significantly the response to steroids, the DFS and the OS (Supporting Information Fig. 1s) as previously observed by other authors [3]. Even though the small sample size did not allow to recognize statistical differences, patients who achieved a response to front-line steroids showed a better OS ($P = 0.28$) and DFS ($P = 0.17$) than those who did not respond (Fig. 1). In particular, the higher survival probability, 81% at 9 years, was displayed by the subset of patients (20%) who obtained a CR with no more detectable AeAbs (Fig. 1).

Taken together, our results demonstrate that probably true cases of P-AIHA are less frequent than commonly thought. Furthermore, our study shows that patients with AIHA uniformly defined as primary by a stringent diagnostic work-up aimed at excluding an associated disease show a favorable outcome after a steroid treatment.

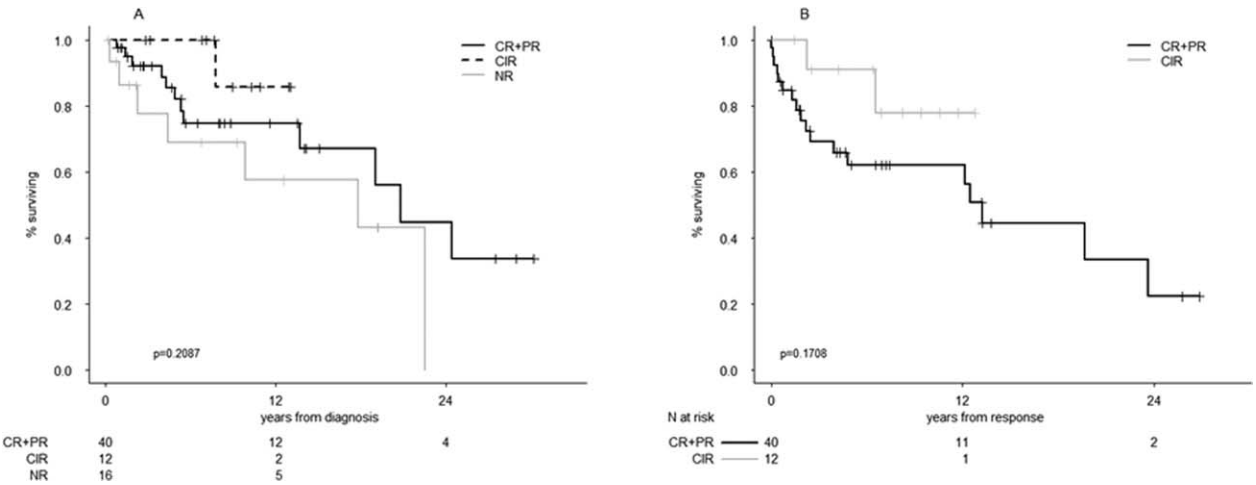

Figure 1. Overall survival and disease-free survival of patients with P-AIHA according to response to frontline steroid treatment. OS, overall survival; DFS, disease-free survival. A: OS at 9 years, patients with immunohematologic response (CIR). CR+PR vs. NR, 80.8% vs. 74.5% vs. 69.2%; $P = 0.21$. B: DSF at 9 years, patients with a CIR vs. patients with a complete or partial response (CR, PR), partial response, 77.9% vs. 62%; $P = 0.17$; CIR, median PFS: not reached; CR+PR, median DFS: 13.2 years.

Author Contributions

FRM managed patients, designed the research, collected, analyzed, and interpreted the data and wrote the paper; SC, GG, performed immunohematologic analyses, collected and interpreted data, reviewed the manuscript; FP performed statistical analysis and reviewed the manuscript; FT, DA, AF,GC, MM,GM, RC, managed patients and collected data; MSP, AG, performed PB flow-cytometric analysis; GG and RF analyzed, interpreted data and reviewed the manuscript.

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Additional Supporting Information may be found in the online version of this article.

Conflict of interest: Nothing to report.

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