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Evaluation of Refractory Autoimmune Hemolytic Anemia Patients After Splenectomy

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Abstract

Objective: In this study, we aimed to retrospectively evaluate the results of patients with autoimmune hemolytic anemia (AIHA) who had undergone splenectomy.

Methods: The records of 25 patients with AIHA refractory to steroids and who underwent splenectomy between January 1, 2000, and October 31, 2017, and were followed up at Ondokuz Mayıs University were retrospectively reviewed. Patient demographics, AIHA type and secondary causes, direct Coombs subtypes, hemoglobin and lactate dehydrogenase levels at diagnosis and at postsplenectomy, bone marrow biopsy and splenectomy pathologies, liver and spleen size, splenectomy complications, treatments given presplenectomy, status after splenectomy, and relapse treatments were examined.

Results: Twelve of 25 patients (48%) had primary AIHA and 13 (52%) had secondary AIHA. All patients received methylprednisolone before splenectomy. After splenectomy, 14 (56%) of the cases were in remission, while 11 (44%) relapsed. Of the patients who relapsed, steroids were used in 54.54%, rituximab in 18.18%, cyclosporine in 36.36%, intravenous immunoglobulin in 9.09%, and mycophenolate mofetil in 9.09%. One patient underwent plasmapheresis. At the end of all treatments, 6 (54.54%) patients who relapsed after splenectomy were in remission, while 3 (27.27%) relapsed and 2 (18.18%) were refractory. Four patients died. As a complication of splenectomy, sepsis developed in 4 patients (16%) and thrombotic events occured in 6 patients (24%).

Conclusion: Some of the patients with AIHA did not respond to splenectomy. We think that the number of patients who need to undergo splenectomy will decrease with randomized trials using new generation drugs.

Keywords: Autoimmune hemolytic anemia, splenectomy, anemia, recurrence

Introduction

Autoimmune hemolytic anemia (AIHA) is an uncommon disease with an incidence rate of 1-3 cases per 100 000 and a mortality rate of about 11%.[1,2]

The thermal characteristics of autoantibodies and whether there is an underlying illness are used to classify AIHA. It is referred to as primary (idiopathic) AIHA if it is not caused by another illness (pAIHA). Secondary AIHA (sAIHA) occurs when there are underlying disorders (such as collagen vascular diseases or lymphoproliferative diseases).[3] Warm autoantibodies are more active about 37°C, whereas cold autoantibodies bind to erythrocytes more strongly around 0°C-4°C.[4] In warm type AIHA (wAIHA), the direct Coombs test's typically positive for immunoglobulin G (IgG) or IgG+C, whereas in cold type AIHA, autoantibodies are of the IgM type and the direct Coombs tests positive for C3d. There is also a heterogeneous group of atypical AIHAs including negative direct Coombs test, IgA-mediated, and warm IgM types.[5-7] Signs of hemolysis such as low haptoglobin level, elevated lactate dehydrogenase (LDH) and indirect bilirubin levels, and reticulocytosis may also be seen.

Corticosteroids are the main and the first-choice treatment in wAIHA.[1,8] Prednisone (or prednisolone) 1-2 mg/kg is daily used as a corticosteroid regimen.[8] The effectiveness of intravenous immunoglobulin (IVIG) therapy is low in wAIHA.[9] It is given as a 5-day treatment at a dose of 0.5-1 g/kg/day.[10]

Rituximab is usually given at a dose of 375 mg/m^2/week for 4 weeks. It is effective in both warm type and cold type AIHA with complete response (CR) rate of approximately 54%-60%.[11] Splenectomy has long been the primary and preferred second-line therapy in pAIHA. The sustained response after splenectomy is approximately 60%-70%.[12,13]

Treatment options and response rates decrease in cases resistant to both rituximab and splenectomy. Immunosuppressive agents (azathioprine, cyclophosphamide, mycophenolate mofetil, cyclosporine) can be used in third-line therapy. Hematopoietic stem cell transplantation is limited to some refractory cases.[6]

The number of studies evaluating relapse after splenectomy in patients with AIHA is quite limited.

In this study, we investigated the demographic and laboratory characteristics, AIHA types and etiologies, complications of splenectomy, and treatments for postsplenectomy relapse in patients with AIHA who had received splenectomy.

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Methods

The records of 25 patients with AIHA who were refractory to steroids and had undergone splenectomy between January 1, 2000, and October 31, 2017, and followed up at Ondokuz Mayıs University were retrospectively reviewed. There were 123 AIHA patients followed up and treated at Ondokuz Mayıs University between these dates. Approval for this study was granted by the Ethics Committee of the Faculty of Medicine of Ondokuz Mayıs University (Approval no: 2019/247, Date: March 14, 2019). Since the subject analyzed in the present study does not contain any confidential information and personal or patient data, informed consent was not obtained.

The inclusion criteria were patients who were 18 years of age or older when included in the study, steroid-resistant cases who underwent splenectomy, and had a positive direct antiglobulin test before splenectomy.

Sex, age, date of AIHA diagnosis, comorbidities, hemoglobin, white blood cell count, platelets, LDH, haptoglobin, corrected reticulocyte percentage and absolute reticulocyte count, direct–indirect Coombs test results at the time of diagnosis and after splenectomy, liver spleen size, bone marrow pathologies, date of splenectomy and spleen pathology, complications after splenectomy, final status after splenectomy if relapse occurred; the date of relapse and doses of treatments received were studied.

All patients were vaccinated with pneumococcal, meningococcal and Haemophilus influenzae (Hib) vaccines before splenectomy.

Those with a spleen size ≥13 cm were classified as splenomegaly, and those with a liver size ≥ 16 cm as hepatomegaly.

Complete response was defined as an increase in hemoglobin to a normal level and the absence of active hemolysis. Partial response (PR) was defined as an increase in hemoglobin to >10 g/dL and at least 2 g/dL increase from baseline in the absence of recent transfusion. Cases that did not comply with the concepts of complete response and PR were assessed as no response (NR).[14]

Rituximab was not covered by health insurance and it required off-label approval. Splenectomy was performed because some patients did not respond to rituximab and required transfusions.

Statistical Analysis

In this study, statistical parametric analyses were carried out using Microsoft Excel and the Statistical Package for Social Sciences version 25.0 software (IBM Corp.; Armonk, NY, USA). Mean, standard deviation, median, lowest, highest, frequency, and ratio values were used.

Results

Twenty-five patients were included in the study, 9 (36%) were female, 16 (64%) were male. The median age was 60 years and the mean age was 56.56 ± 16.39 years (11-77 years). The mean follow-up period was 47.80 months and the median was 37.0 months (2-228 months). While 12 (48%) of the patients were diagnosed with pAIHA, 13 (52%) had sAIHA. Among the patients with sAIHA, 4 developed secondary to non-Hodgkin lymphoma, 3 to chronic lymphocytic leukemia (CLL), 2 to Hodgkin lymphoma (HL), 2 to hairy cell leukemia (HCL), 1 to autoimmune lymphoproliferative syndrome, and 1 to sarcoidosis (Table 1). The mean hemoglobin value of the 25 patients at the time of diagnosis was 8.03 ± 1.69 g/dL (5.0-12.6 g/dL). The mean LDH values at the time of diagnosis were 739.16 ± 471 U/L (190-2088 U/L). All 25 patients had a positive direct Coombs test.

No pathology was found in the bone marrow biopsy of 8 (32%) patients who underwent a bone marrow biopsy before splenectomy. The pathology of 9 (36%) patients was interpreted as lymphoproliferative disease infiltration. The pathology of 3 patients was interpreted as CLL, 1 with HCL, 3 with B-cell lymphoma, 1 with T-cell lymphoma, 1 with HL infiltration, 2 (8%) with hypoactive hypocellular bone marrow. In 2 (8%) bone marrow biopsies, sufficient material could not be obtained. Bone marrow aspiration biopsy was not performed in 4 patients.

Splenomegaly was present in 75% of pAIHA and 84.6% of sAIHA. Hepatomegaly was present in 75% of pAIHA and 69.2% of sAIHA.

Splenectomy pathology of 17 (68%) patients was reported as extramedullary hematopoiesis, 7 (28%) were lymphoproliferative disease (2 HCL, 2 CLL, 2 HL, 1 marginal zone lymphoma (MZL)), 1 (4%) was reported as granulomatous inflammation. No involvement was observed in the splenectomy material of a patient whose bone marrow biopsy was compatible with CLL. Bone marrow biopsy of the patient whose splenectomy pathology resulted as HCL was reported as hypoactive hypocellular bone marrow. No pathology was found in the bone marrow biopsy of the patient whose splenectomy material was interpreted as HL involvement. Bone marrow biopsy of the patient whose splenectomy pathology resulted as MZL was reported as B-cell lymphoma infiltration. No findings in favor of lymphoproliferative disease were found in the splenectomy material of 2 of the patients with B-cell lymphoma infiltration in their bone marrow biopsy and of the patient with T-cell lymphoma infiltration.

Complications of splenectomy occurred in 9 patients, of whom 4 had sepsis, 4 had portal vein thrombosis, 1 had deep vein thrombosis, 1 had myocardial infarction, and 1 had pulmonary thromboembolism (1 had portal vein thrombosis and then sepsis, and 1 had portal vein thrombosis and then pulmonary thromboembolism) (Table 2).

All cases received methylprednisolone before splenectomy. While 21 (84%) cases were treated with a single agent, i.e., methylprednisolone, 2 (8%) were treated with methylprednisolone + IVIG, and 1 (4%) was treated with methylprednisolone, fludarabine, cyclophosphamide, rituximab, vincristine, and chlorambucil for the treatment of the underlying disease. The other patient received methylprednisolone, IVIG, fludarabine, cyclophosphamide, rituximab treatment for both AIHA and the underlying disease (Table 1).

While 14 (56%) of the cases were in remission after splenectomy, relapse occurred in 11 (44%) cases. The mean hemoglobin value after splenectomy was 11.51 ± 2.83 (4.0-17) g/dL. Of the patients with relapse, 2 (18.18%) died before treatment response could be observed. Four of the patients were given 1 mg/kg methylprednisolone after splenectomy. Partial response was observed in 1 of them, CR was observed in 1, and NR was obtained in 2 of them. One patient was given cyclosporine + methylprednisolone treatment, and NR was obtained. One patient received 4 cycles of rituximab at a weekly dose of 375 mg/m^2 (weekly RTx), and a CR was achieved. Three patients were given treatment for the underlying disease. One of these patients had AIHA secondary to CLL, fludarabine + cyclophosphamide + rituximab regimen was given because bendamustine was not available in the hospital pharmacy at that time, and PR was achieved. The other patient had AIHA secondary to splenic MZL, rituximab + bendamustine regimen was given, and CR was achieved.

Patient 8 (Table 3), while being followed up with the diagnosis of pAIHA, relapsed 24 months after splenectomy, and de novo AML M1. He was given 7 + 3 and high-dose cytarabine (ARA-C) protocol chemotherapy, PR was achieved.

Table 1. Characteristics of the Patients and Treatments Given Before Splenectomy.

Patient	Age at the Time of Splenectomy	Sex	AIHA	Direct Coombs Type	Treatment Given Before Splenectomy
1	68	M	PA	IgG+C	MP
2	73	M	PA	IgG+C	MP
3	60	F	PA	IgG	MP
4	61	F	PA	IgG+C	MP
5	63	M	PA (ES)	IgG	MP
6	47	F	PA	IgG+C	MP
7	59	M	PA	IgG+C	MP
8	48	M	PA	IgG	MP
9	49	F	PA	IgG+C	MP
10	55	M	PA (ES)	IgG+C	MP+IVIG
11	77	F	PA	IgG	MP
12	59	M	PA	IgG	MP
13	11	M	SA (AILPS)	IgG+C	MP
14	50	M	SA (HCL)	IgG+C	MP
15	42	F	SA (NHL)	IgG	MP
16	51	M	SA (HCL)	C	MP
17	60	M	SA (CLL)	IgG	MP+IVIG+TUD
18	41	M	SA (HL)	IgG+C	MP
19	71	M	SA (IndL)	IgG	MP
20	71	M	SA (CLL)	IgG	MP+TUD
21	68	F	SA (CLL)	IgG	MP+IVIG
22	18	M	SA (HL)	IgG+C	MP
23	64	F	SA (SMZL)	IgG+C	MP
24	72	F	SA (Sarc)	IgG	MP
25	76	M	SA (IndL)	IgG+C	MP

AILPS, autoimmune lymphoproliferative syndrome; C, complement; CLL, chronic lymphocytic leukemia; ES, Evans syndrome; F, female; HCL, hairy cell leukemia; HL, Hodgkin lymphoma; IgG, immunoglobulin G; IndL, indolent lymphoma; IVIG, intravenous immunoglobulin; M, male; MP, methylprednisolone; NHL, non-Hodgkin lymphoma; PA, primary autoimmune hemolytic anemia; SA, secondary autoimmune hemolytic anemia; Sarc, sarcoidosis; SMZL, splenic marginal zone lymphoma; TUD, treatment of the underlying disease.

In patient 13 (Table 3), second relapse occurred 32 months after the first relapse following splenectomy and 1 mg/kg methylprednisolone was given, reaching PR. A relapse was seen for the third time after 11 months, and 1 mg/kg methylprednisolone+mycophenolate mofetil+IVIG was administered, reaching CR. The fourth relapse occurred after 19 months, plasmapheresis was applied, IVIG and prednisone were given, reaching PR. The fifth relapse occurred after 13 months, plasmapheresis was applied, 1 mg/kg methylprednisolone+cyclosporine+4 weeks of weekly RTx were given, achieving PR. The sixth relapse occurred after 15 months, and 1 mg/kg methylprednisolone+4 weeks of weekly RTx was given, achieving CR. The seventh relapse occurred after 5 months, methylprednisolone at a dose of 1 mg/kg+4 weeks of weekly RTx+IVIG was given, and CR was reached. In the first recurrence after splenectomy, methylprednisolone was given to patient 15 (Table 3), NR was obtained. Subsequently, cyclosporine was initiated, but she was refractory to cyclosporine and died. In patient 19 (Table 3), the second recurrence occurred 4 months after the first recurrence following splenectomy. Prednisolone+cyclosporine was administered, reaching CR. A third relapse was seen after 31 months, with weekly RTx given for 4 weeks+methylprednisolone at a dose of 1 mg/kg daily and PR was achieved. The fourth relapse occurred after 2 months, and methylprednisolone was administered at a dose of 1 mg/kg, achieving CR.

Discussion

There is a small series of patients with AIHA who have undergone splenectomy. Although splenectomy is performed in patients with AIHA, recurrences are observed. These are difficult to treat. In our study, we analyzed patients with AIHA who underwent splenectomy.

Table 2. Splenectomy Complications

Patient	Splenectomy Complication	Time Between Splenectomy and Complication (Month)
1	MI	36
4	Sps	1
5	PVT, PTE	6, 56
8	DVT	120
12	Sps	4
13	PVT	123
15	PVT, Sps	2, 12
23	PVT	11
25	Sps	1

DVT, deep vein thrombosis; MI, myocardial infarction; PVT, portal vein thrombosis; PTE, pulmonary thromboembolism; Sps, sepsis.

We found that some AIHA patients relapsed despite splenectomy and developed sepsis and thrombosis due to the surgery.

In the study by Roumier et al.,[14] bone marrow analysis was performed in 29 patients (19 cases with biopsies, 10 aspirates). The biopsies showed hyperplasia in the erythroid rows. Lymphoid hyperplasia was found in 13 of 19 patients, including 4 with 3 or more atypical lymphoid islets, 3 with well-characterized lymphomas, and 1 with features of Waldenstrom macroglobulinemia.[6] In our study, the pathology of 9 (36%) patients who underwent a bone marrow biopsy was interpreted as infiltration of lymphoproliferative disease. The diagnosis can be made by bone marrow aspiration and biopsy prior to splenectomy. Diagnostic splenectomy has been performed in patients whose lymphoma subtype could not be determined on bone marrow biopsy.

In the study by Prabhu et al.,[15] 14 (42%) of 33 patients with pAIHA were found to have splenomegaly. 7 of them had wAIHA, and 7 had cold agglutinin disease. Splenomegaly was found in 44% of wAIHA patients.[15] In our study, 9 (45%) of the patients with splenomegaly had pAIHA, 11 (55%) had sAIHA. Splenomegaly was present in 75% of patients with pAIHA and in 84.6% of patients with sAIHA. Splenomegaly was found more frequently in our study compared to the literature.

According to the meta-analysis of Kojouri et al.,[16] the prevalence of infectious events after splenectomy was approximately 3.2%. In the study by Yılmaz et al.,[17] postoperative respiratory problems and fever were observed in 6 patients (20%), bleeding in 2 (6.6%) patients, subphrenic abscess in 1 (3.3%), and sepsis in 1 (3.3%) patient. No surgery-related complications were observed in 20 patients (66.6%); perioperative death was observed in 2 patients. The causes of death were pulmonary sepsis and embolism with multi-organ failure in 1 patient, intra-abdominal hemorrhage in the other patient.[17] In the study by Ciftciler et al.,[18] the postsplenectomy infection rate was 14.7%, and thrombosis was observed after splenectomy with a rate of 4.2%. In 1 patient (0.9%), hematoma developed after splenectomy. In our study, sepsis developed in 4 patients (16% of all patients, 44.44% of patients with complications), and thrombotic events in 6 patients (24% of all patients, 66.66% of patients with complications). In our study, more thrombotic and infectious events were observed than in the literature, and thrombotic events were more frequent than infectious events. One patient died of sepsis and was vaccinated. We do not have any data about postoperative care and thromboprophylaxis because we could not access the surgical files of the patients. The reason for the higher rates of sepsis and thrombotic events in our study compared to other studies in the literature may be due to inadequate postoperative care and thromboprophylaxis. Late sepsis can be explained by the failure of management of infections during the use of immunosuppressive agents. The tendency to thrombosis increases in AIHA. The late thrombotic complications after splenectomy in some cases in our study may be related to the lack of thromboprophylaxis in the relapse of AIHA after splenectomy. According to the study by Ho et al.,[19] patients with AIHA who underwent splenectomy are at significantly increased risk of both immediate (≤90 days after splenectomy) and late postoperative (>90 days after splenectomy) venous thromboembolism and long-term risk of sepsis. We found similar results in our study.

In the study by Roumier et al.,[14] splenectomy was performed in 9 patients with AIHA. Of these, 6 had CR and 3 had PR. Relapse was observed in 3 patients.[6] In our study, although 14 (56%) cases were in remission after splenectomy, 11 (44%) cases relapsed. Compared to the studies in the literature, the rate of relapse after splenectomy was higher in our study.

In the study by Moyo et al.,[20] splenectomy was performed in 3 out of 9 patients. All 3 had a relapse after splenectomy. One of the patients who underwent splenectomy was given oral cyclophosphamide, cyclosporine, and then high-dose cyclophosphamide, and PR was reached. The other patient received azathioprine, hydroxychloroquine, ascorbic acid, IVIG followed by high-dose cyclophosphamide, and CR was achieved. Another patient received vincristine, IVIG, danazol, plasmapheresis, ascorbic acid followed by high-dose cyclophosphamide and CR was reached.[20] In the study by Barcellini et al.,[21] 32 patients underwent splenectomy as second-line treatment. Relapse was observed in 8 patients. One patient with wAIHA received bortezomib and eculizumab as 5th and 6thline treatment after steroid + IVIG + cyclophosphamide + rituximab + plasma exchange, achieving a favorable response. Unlike studies in literature, we found that we used steroids more frequently in the treatment of relapses after splenectomy and preferred other immunosuppressive agents less frequently. We think that a better and long-term treatment response can be achieved with the use of other immunosuppressives.

In our hospital, some of the splenectomies were performed for diagnostic purposes and some were performed to treat patients with AIHA. We found a discrepancy between the diagnosis of bone marrow biopsy and splenectomy. In some patients, the diagnosis was made by splenectomy. Bone marrow biopsy may be insufficient for diagnosis in some patients.

According to the Health Practice Memorandum, rituximab was used in a small number of patients because it required off-label approval for a period of time and some patients did not receive approval and some patients did not want to use rituximab. Rituximab has been widely used in recent years for benign hematological conditions such as immune thrombocytopenic purpura, cold agglutinin disease, and AIHA, and the response to treatment has been found to be extremely good in trials especially in AIHA.[22] Studies are being conducted with doses of 100 mg and 375 mg/m^2. Due to the high response rate, splenectomy rates decreased in such patients. In recent years, clinical trials of new generation drugs (fostamatinib, isatuximab, ANX005, M281, APL-2, parsaclisib, interleukin 2, acalabrutinib, ibrutinib) have been conducted in relapsed/refractory AIHA.[23-33]

Table 3. Post-splenectomy Status of the Patients and Their Treatments.

Patient	Post-splenectomy Status	Treatment Response	Second Relapse	Treatment Response	Multiple Relapse Treatment Response	Latest Status
1	RL (MI) (Ex)					RL (Ex)
2	RM					RM
3	RM					RM
4	RM					RM
5	RL	MP→NR				RF
6	RM					RM
7	RM					RM
8	RL	TUD→PR				RM
9	RM					RM
10	RM					RM
11	RL (Sps) (Ex)					RL(Ex)
12	RL	Cs+MP→ (Sps)(Ex)				RL(Ex)
13	RL	MP→PR	RL	MP→PR	Third RL-IVIG + MP + MMF→CR Fourth RL-PF + IVIG + PRE→PR Fifth RL-PF + MP + Cs+ 4× Rtx→PR Sixth RL-MP + 4xRtx→CR Seventh RL-MP + 4× Rtx + IVIG→CR	RM
14	RM					RM
15	RL	MP→NR	RF	Cs→NR	RF (Sps) (Ex)	RF(Ex)
16	RM					RM
17	RL	TUD→PR				RM
18	RM					RM
19	RL	4 × Rtx→CR	RL	PR+Cs→CR	Third RL-4×Rtx + MP→PR Fourth RL-MP→CR	RM
20	RM					RM
21	RL	MP→CR				RM
22	RM					RM
23	RL	TUD→CR				RM
24	RM					RM
25	RM					RM

CR, complete response; Cs, cyclosporine; Ex, exitus; 4× Rtx, 4 cycles of rituximab; M, month; MI, myocardial infarction; MMF, mycophenolate mofetil; MP, methylprednisolone; NR, no response; PF, plasmapheresis; PR, partial response; PRE, prednisolone; RF, refractory; RL, relapse; RM, remission; Sps, sepsis; TUD, treatment of the underlying disease; Y, year.

Study Limitations

Our study is a retrospective file review. Since we could not access the files of some patients, we could not include them in the study. Some patients could not be included in the study because some information was missing in their files. A small number of patients were handicapped in this study.

Conclusion

In our study, we analyzed the clinical course of AIHA patients who underwent splenectomy. We found that the majority of patients with AIHA responded to splenectomy, but some did not. According to the results of new studies, we expect that treatment alternatives for AIHA patients will be developed and splenectomy treatment will decrease.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ondokuz Mayıs University (Approval no: 2019/247, Date: March 14, 2019).

Informed Consent: Since this is a retrospective study informed consent cannot be obtained from the subjects.

Peer-review: Externally peer-reviewed.

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