The evaluation of acquired aplastic anemia in children and unexpected frequency of varicella-zoster virus association: a single-center study

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In this study, 32 patients under the age of 17 years with acquired aplastic anemia (AAA) were evaluated. Nine patients developed AAA associated with viral infection in which viral hepatitis and varicella infection were nearly equal. Four of the patients were administered drugs before developing AAA. Patients were treated as follows: combined immunosuppressive therapy (CIST) including anti-thymocyte or anti-lymphocyte globulin plus high-dose methylprednisolone (HDMP) and cyclosporin A and granulocyte colony-stimulating factor (G-CSF) (14 patients); mega-dose (30 mg/kg) methylprednisolone (8 patients); and HDMP combined with cyclosporin A or anapolon or cyclophosphamide (6 patients). Complete remission was seen in 10 patients and partial remission in 2 patients. The response rate was similar in the CIST and MDMP groups. The most striking findings of this study were the frequent association of AAA with varicella infection and the low cure rate, which was due to patient non-compliance with the treatment and inadequate isolation conditions in the hospital.

Key words: acquired aplastic anemia, children, varicella-zoster virus.

Acquired aplastic anemia (AAA) is characterized by pancytopenia and bone marrow hypocellularity. It is a rare hematological disease, often of unknown origin, with a high mortality rate[1]. The annual incidence rate reported in the literature ranges from 1.4 to 14 cases per million[1-3], and it seems to vary geographically. There are several causes in the etiology of AAA[1-11], but many studies have suggested the pathophysiologic role of immunologically mediated bone marrow failure, and in practice, most patients with AAA respond favorably to immunosuppressive therapies (IST), which have been an alternative treatment for patients who do not have a suitable donor[12-14].

The present study evaluated the AAA risk factors, therapeutic approaches, and outcome in children in our clinic.

Material and Methods

We evaluated 32 consecutive patients less than 17 years of age (median age: 12 years; range: 1.5-16 years) with the diagnosis of AAA who received treatment at Hacettepe University İhsan Doğramacı Children's Hospital, Department of Pediatric Hematology between May 1994 and December 2005.

The diagnostic work-up included history of infections, drug use as well as environmental exposures, evaluation of complete blood counts (CBC) with differentiation, reticulocyte count, serum biochemistry, levels of vitamin B_{12} and folic acid, quantitative immunoglobulin levels, viral serology, bone marrow smear examination, and hemoglobin electrophoresis. In addition, Ham test/sucrose hemolysis tests and flow cytometric assay to detect CD55 and CD59 for paroxysmal nocturnal hemoglobinuria, diepoxybutane test for Fanconi's aplastic anemia, and cytogenetic studies to identify clonal abnormalities were performed.

The disease was considered severe (SAA) if at least two of the following were noted: absolute neutrophil count (ANC) $<0.5 \times 10^9$/L; platelet count $<20 \times 10^9$/L; and reticulocyte count $<20 \times 10^9$/L with hypocellular bone marrow[15].

AA was considered very severe (VSAA) if the criteria for severe disease were fulfilled and the neutrophil count was <0.2 x 10^9/L. Moderate disease (MAA) was defined by at least two of the following hematologic values: neutrophil count <1 x 10^9/L; platelet count <50 x 10^9/L; and reticulocyte count <60 x 10^9/L with hypocellular bone marrow.

Therapy schedules were as follows:

1. Combined immunosuppressive therapy (CIST): 14 patients were treated with a CIST regimen that included horse anti-lymphocyte globulin (ALG; n=5) or rabbit anti-thymocyte globulin (ATG; n=9) plus peroral (po) methylprednisolone (MP) and cyclosporin A (CsA) and granulocyte colony-stimulating factor (G-CSF). Among the patients treated with CIST, 9 patients received one course and 5 patients two courses. Horse ALG was administered at a dose of 10 mg/kg/d and rabbit ATG at a dose of 5 mg/kg/d, both intravenously for five consecutive days after a sensitization test and prophylactic MP for prevention of allergic reaction. High-dose methylprednisolone (HDMP) was given according to the following tapering schedule: day 1-9, 5 mg/kg/d; day 10-12, 4 mg/kg/d; day 13-15, 3 mg/kg/d; day 16-18, 2 mg/kg/d; day 19-21, 1 mg/kg/d; and day 22-25, 0.5 mg/kg/d. CsA, 6 mg/kg/d in two divided oral doses, was started on day 1 and continued until day 180. The dose of CsA was adjusted to achieve a whole blood trough level of 100 to 200 ng/ml. Recombinant human G-CSF (rhG-CSF) was administered at a dose of 5-10 μg/kg when the ANC was <0.3x10^9/L[14-16].

2. Eight patients were treated only with po mega-dose MP (MDMP) according to the Özsoylu protocol; MP was given with gradually tapering doses beginning with 30 mg/kg/d[17].

3. Three patients were given po HDMP (5 mg/kg) plus CsA.

4. Two patients received po HDMP plus anapolon.

5. One patient was given po HDMP plus cyclophosphamide.

Among the four patients who did not receive therapy, two could not receive AAA-specific therapy due to infection; one patient, who developed AAA following a suicide attempt with an overdose of colchicine, improved without therapy; and the last patient had been prepared for hematopoietic stem cell transplantation (HSCT).

Bone marrow transplantation was performed in patients having an identical donor if the patient's parents consented.

Complete blood counts were performed every two days, or every three days, and then weekly after CBC values stabilized. Patients were assessed for hematologic response according to the following criteria: complete remission (CR) was defined as neutrophils >1.5x10^9/L, platelets >100x10^9/L, and hemoglobin >11g/dl, while partial remission (PR) was defined as neutrophils >0.5x10^9/L, platelets >30x10^9/L, and hemoglobin >8 g/dl[16].

All patients received care at the university hospital for between two and three months, but some were discharged before they attained remission because of inadequate isolation conditions in the hospital, parental refusal or personal circumstances.

Red blood cells and platelets were transfused when indicated by clinical and laboratory findings. Patients who had temperatures exceeding 38.0°C were treated with broad-spectrum antibiotics, or according to microbiological findings.

Results

Etiology and presentation:

Among the 32 patients, 21 had VSAA, 5 had SAA, and 6 had MAA. Clinical and laboratory findings of the patients are presented in Tables I and II. Peripheral blood findings and bone marrow examinations were compatible with AA morphology in all patients. Relative increased bone marrow normoblasts, associated with AA, were also detected in 3 patients.

Viral agents were considered as the cause of AAA in 9 patients; 3 had hepatitis B infection, 1 hepatitis A infection, 3 varicella-zoster infection, 1 parvovirus B19 (PV-B19) infection, and 1 patient was infected with the Epstein-Barr virus. Drug usage prior to development of AAA was detected in 4 patients. Of these, 1 patient used trimethoprim–sulfamethoxazole, 1 chloramphenicol, 1 ibuprofen and amoxicillin for upper respiratory tract infections, and 1 patient used novalgine and ampicillin for a urinary tract infection. Toxic substance

Table I. Clinical Findings and Etiology of Acquired Aplastic Anemia

Clinical findings	
Patient number	32
Age	Median: 12 years, range: 1.5-16 years
Sex M/F	19/13
Etiology	
Viral agents	9
HBV	3
HAV	1
Varicella	3
Parvovirus	1
EBV	1
Drug usage	4
TMP-SMX for URTI	1
Chloramphenicol for URTI	1
Ibuprofen, amoxicillin for URTI	1
Novalgine, ampicillin for UTI	1
Toxic substance exposure	2
DDT	1
Colchicine	1
Unknown etiology	17

HBV: Hepatitis B virus. HAV: Hepatitis A virus. EBV: Epstein-Barr virus. TMP-SMX: Trimethoprim-sulfamethoxazole. URTI: Upper respiratory tract infection. UTI: Urinary tract infection.

Table II. Laboratory Findings of Patients at Diagnosis

Hemoglobin*	6.3±2.1
Mean corpuscular volume	91.8±10.3
Reticulocyte	0.47±0.47
White blood cell count	$2.6x10^9$/L±2.6 $x10^9$/L
Absolute neutrophil count	$0.43x10^9$/L±0.55 $x10^9$/L
Platelet	10.1±9.7 $x10^9$/L
Hemoglobin F ↑	4/32
Paroxysmal nocturnal hemoglobinuria tests	
CD55↓	4/12
CD59↓	4/12
Acid Ham (+)	1/21
Sucrose lysis test	1/21
Cytogenetic (trisomy 22)	1
Bone marrow	Aplasia Relative normoblastic hyperactivity (n=3)

*mean±SD.

exposure was found in 2 patients: dichloro diphenyltrichloroethane (DDT) in 1 patient and colchicine overdose In an attempted suicide, in the other. Paroxysmal Nocturnal Hemoglobinuria (PNH) panel tests were conducted in 12 patients and the results were abnormal in 4. Acid Ham and sucrose lysis tests were given to 21 patients, and all but 2 were normal. Cytogenetic examinations were normal in all but 1 patient who had trisomy 22. The levels of serum vitamin B_{12}, folic acid, and immunoglobulin were normal in all 32 patients. Increased hemoglobin F level was detected in 4 of 32 patients.

Therapy response and outcome:

The results of each therapy schedule are shown in Table III. Among the 9 patients who received 1 course of CIST, 3 patients developed CR, 4 patients died, 1 patient was not followed-up, and 1 patient underwent HSCT. Of the 5 patients who received 2

Table III. Therapy Schedule and Outcome of Patients

Therapy schedule	n	Disease severity n	Response n	Outcome
				2 alive (60 mo, 30 mo)
Combined IST			CR 2	4 exitus
One course		VSAA 7	NR 5	1 alive (60 mo after HSCT)
				1 alive (40 mo)
			CR 1	1 lost to follow-up (5 mo)
	9	MAA 2	NR 1	
		VSAA 1	NR 1	
				1 exitus
		SAA 3	NR 2	
				1 (alive 18 mo after HSCT)
			PR 1	1 exitus
				1 alive (14 mo)
		MAA 1	CR 1	
Two courses	5			1 alive (50 mo)
		VSAA 5		
			CR 1	Alive (60 mo)
			NR 4	2 exitus
				1 lost to follow-up (1 mo)
				1 (alive 48 mo after HSCT)
		SAA 2		
			NR 2	1 exitus
				1 lost to follow-up (1.5 mo)
		MAA 1		
MDMP (Özsoylu protocol)	8		CR 1	1 alive (132 mo)
				2 exitus
		VSAA 2	NR 2	
				1 alive (8 mo)
HDMP + CsA	3	MAA 1	PR 1	
				1 exitus
		VSAA 1	NR 1	
				1 exitus
HDMP + anapolon	2	MAA 1	NR 1	
				1 exitus
HDMP+ cyclophosphamide	1	VSAA 1	NR 1	
				2 exitus
				1 alive (2 mo, spontaneous remission)
No therapy	4	VSAA 4		1 preparing for HSCT (2 mo)

IST: Immunosuppressive therapy. MDMP: Mega-dose methylprednisolone (30 mg/kg). HDMP (5 mg/kg): High-dose methylprednisolone. VSAA: Very severe aplastic anemia. SAA: Severe aplastic anemia. MAA: Moderate aplastic anemia. CsA: Cyclosporin A. CR: Complete remission. PR: Partial remission. NR: No response. HSCT: Hematopoietic stem cell transplantation.

courses of CIST, 1 patient (with trisomy 22) developed CR, 1 patient developed PR, 1 patient underwent HSCT, and 2 patients died. Among the 8 patients treated with MDMP, 2 patients showed CR, 3 patients died, 2 patients were lost to follow-up, and 1 patient underwent HSCT. All 3 patients who underwent HSCT showed CR. Among the 3 patients treated with HDMP and CsA, 1 showed PR and 2 died. Two patients treated with HDMP and anapolon and 1 patient treated with HDMP and cyclophosphamide died. The types of AA and the different treatment protocols of each patient are shown in Table II. There was no response superiority according to severity of AAA. Of the 6 patients revealing positivity for PNH tests, 3 of them responded to therapy, and at the writing of this report were still alive.

Various therapeutic protocols were used in our patients because of the difficulty in obtaining some drugs, and the lack of both patient health insurance and patient treatment compliance

(Table III). Three patients were lost to follow-up during therapy, at 1, 1.5 and 5 months after the diagnosis. A total of 13 of 32 patients (40.6%) were alive. Ten (31.2%) were in CR, 2 (6.3%) were in PR, and 1 (3.1%) had been preparing for HSCT without any AAA-specific treatment. The patient with colchicine intoxication spontaneously developed remission within three weeks after the diagnosis of AA. The median follow-up duration for patients who were alive was 40 months (range: 2-132 months). Thirteen patients who did not respond to therapy and 2 patients who could not receive AAA-specific therapy due to infection died (median time after the diagnosis: 2 months; range: 0.5-24 months) (Table III).

Causes of death are provided in Table IV. Two patients died due to intracranial hemorrhage and 14 patients died with infection. Among the 8 patients who died due to fungal infection, 4 of them died due to pulmonary fungal infection, 2 died of sinus aspergillosis, and 2 due to sinus mucormycosis. Pulmonary infection with unknown etiology was the cause of death in 4 patients (typhilitis secondary to pulmonary infection in 1 patient) and sepsis in 2 patients. Of the 16 patients who died, 13 had VSAA, and 11 of them died of infection.

Discussion

In this retrospective single-center study, we aimed to evaluate the etiology of AAA and its response to therapy, as well as treatment outcome and cause of death in patients with AAA. Almost half of the study patients came from families of low-level socioeconomic status. One of these patients had a history of toxic substance (DDT) exposure. It is known that DDT is used in agriculture in Turkey. For that reason, although we did not obtain a clear history about exposure to DDT, we think that more patients may have a higher rate of DDT exposure than we detected. Viral infection associated with AAA was detected by history and clinical and serological findings in 9 (28.1%) patients. In the present study, 4 patients with hepatitis progressed to AAA, which was comparable with results found in the literature[4]. One of the patients had fulminant hepatitis, a condition in which AAA occurs[18]. The most interesting finding of our study was the high ratio of varicella-associated AAA (3/32, 9.4%). To the best of our knowledge, progression of varicella infection to AAA is very rare in the literature[9]. The etiological role of varicella infection in AAA may also be associated with the use of drugs such

Table IV. Causes of Death of the Study Patients with Aplastic Anemia

Patient no.	Disease severity	Therapy schedule	Therapy response	Cause of death	Follow-up time (months)
1	SAA	MDMP	NR	Pulmonary infection	1.5
2	VSAA	HDMP+Cyclophosphamide	NR	Pulmonary infection	1.5
3	VSAA	HDMP+CsA	NR	Pulmonary infection, typhilitis	2.5
4	VSAA	MDMP	NR	Pulmonary fungal infection	1.5
5	VSAA	One course CIST	NR	Intracranial hemorrhage	2.0
6	VSAA	HDMP+anapolon	NR	Sinus aspergillus	2.5
7	MAA	HDMP+anapolon	NR	Sinus mucormycosis	0.5
8	VSAA	One course CIST	NR	Pulmonary infection	2.0
9	VSAA	Two courses CIST	NR	Sinus aspergillus	3.5
10	VSAA	One course CIST	NR	Sinus mucormycosis	3.0
11	SAA	Two courses CIST	NR	Sepsis	24.0
12	VSAA	No therapy		Sepsis	1.0
13	VSAA	No therapy		Pulmonary fungal infection	4.0
14	VSAA	MDMP	NR	Intracranial hemorrhage	2.0
15	VSAA	HDMP+CsA	NR	Pulmonary fungal infection	5.0
16	VSAA	CIST	NR	Pulmonary fungal infection	18.0

SAA: Severe aplastic anemia. VSAA: Very severe aplastic anemia. MAA: Moderate aplastic anemia. MDMP: Mega-dose methylprednisolone. HDMP: High-dose methylprednisolone. CsA: Cyclosporin A. CIST: Combined immunosuppressive therapy. NR: Non-response.

as antibiotics or antihistaminics during the incubation or overt infection periods. The second explanation may be the sub-clinical undetected immunocompromised condition of the patients, although they had normal immunoglobulin levels and unpredictable histories for immuno deficiency. Turkey's neonatal vaccination program does not include varicella-zoster vaccination. Although immunocompromised patients may be more likely to progress to bone marrow failure due to viral infection, our patient with PV-B19 infection had normal immunity. Patients with AAA associated with PV-B19 infection, which was reported previously, were also immunocompetent[5-6]. Although PV-B19 is known to have predilection to red cell progenitors, it has also recently been implicated in cases of AAA. In a very recent report, Ku80 autoantigen, which functions as a novel receptor for PV-B19 and is found on human bone marrow erythroid cells, CD20 positive B-cells, and CD3 positive T cells, was demonstrated[19].

Four patients in the present study used several drugs, including antibiotics and analgesics: for upper respiratory tract infection (URTI) in 3 patients and for urinary tract infection (UTI) in 1 before the development of AAA. Although it was considered that drugs were responsible for the development of AAA in these cases, it was not possible to differentiate the contribution of infection, especially viral infection, or drugs as etiologic factors. A total of 10 patients developed CR. One of them, in whom AA developed after colchicine intoxication, recovered spontaneously. Lee et al.[20] showed that 13% of patients with AAA had spontaneous remission, and that most cases with spontaneous remission might be associated with external factors such as drug use or infection. In the present study, 2 patients were in PR and 1 was waiting for HSCT. All 3 patients who underwent HSCT were alive with CR between 18 and 60 months. The effect of MDMP in AAA has been demonstrated by Özsoylu in our clinic[17]. The rate was 25.0% (2/8) in the patients treated with MDMP and 28.6% (4/14) in the patients treated with 1 or 2 courses of CIST. One patient who was responsive to MDMP had novalgine-related AAA. This retrospective study revealed that the therapy response rate in patients treated with MDMP seems to be similar to that of patients treated with CIST. Therefore, MDMP can be used in patients who do not have a suitable donor and who develop an allergic reaction to ATG or ALG.

Sixteen of 32 patients (50%) died due to infection, principally fungal, with sinus or pulmonary localization (Table III). This finding highlights the low cure rate related with early death due to the extremely delicate conditions of AAA patients, and can be attributed to insufficient patient compliance, insufficient duration of hospitalization and inadequate isolation conditions in the hospital. Three patients were lost to follow-up and did not continue therapy at our hospital because of the lack of both treatment compliance and health insurance. The rate of CR in this study may not precisely show the actual CR rate because among the patients who died, most died due to infection before the time required for therapeutic response had elapsed.

In conclusion, the results did not reflect those found in the literature with respect to etiological factors and therapeutic response according to therapy type and disease severity. The cure rate was very low in comparison to what was expected[16]. Patients with AAA should be adequately followed-up in-hospital in special isolation conditions for the time required for a therapeutic response. The socioeconomic level of patients must also be taken into account when considering compliance to therapy. Unfortunately, our study group was lacking in both conditions and poor outcomes were observed. A recent report has shown that patients with VSAA were more responsive to immunotherapy than patients with less severe disease[22]. In our group, most of the VSAA patients died of infection; therefore, we could not evaluate the response of AAA to the therapy according to AAA severity. Varicella virus-associated AAA seems to be of similar importance to hepatitis virus-associated AAA in our pediatric population. Patients with relative normoblast activity in the bone marrow died and did not appear to be more responsive than patients with no normoblast activity in bone marrow. The MDMP therapy cure rate was almost equal to the CIST rate. Furthermore, therapy with MDMP is less expensive than CIST. These findings, in total, may be considered a new contribution to the AAA perspective.

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