ORIGINAL ARTICLE

Aplastic anemia and concomitant autoimmune diseases

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Abstract The association of aplastic anemia (AA) with
other autoimmune diseases (AID) has been described but
so far not systematically evaluated. We assessed the
incidence and the outcome of concomitant AID in a
retrospective, single-center study of 243 patients with
severe AA treated between 1974 and 2006 with either
immunosuppression (186) or hematopoietic stem cell
transplantation (57) and a median follow-up time of
9.3 years (0–33). Clinically manifest AID were observed
in 24 out of 243 (10±3.7%) patients. Age at diagnosis of
AA was significantly younger in patients without AID
compared to patients with AID (median, 20 versus
52 years; $P<0.001$). In 12 patients where the diagnosis
of AID was done before AA therapy, response to
antithymocyte globulin was good for AA (ten out of 12)
but not for AID (2 out of 12). In 13 patients in which AID
occurred after first-line therapy, the median time to the
AID was 7 years (range 3 months–27.5 years).

Keywords Aplastic anemia · Autoimmune diseases ·
Antithymocyte globulin

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Introduction

Aplastic anemia (AA) is defined as a pancytopenia with
unexplained bone marrow hypocellularity. Acquired AA
can be considered in most cases as a T cell-mediated
autoimmune disorder, targeted against the hematopoietic
progenitors, leading to the failure of the bone marrow [1].
Viral infections, drugs, chemical exposure, pregnancy, or
unknown agents seem to trigger the autoimmune dysregu-
lation in patients with predisposition. Associations of AA
and other autoimmune diseases (AID) have been shown in
single case reports [2, 3]. However, so far, there are no
published data on a systematic review of concomitant AID
in AA patients. Moreover, data on the impact of immuno-
suppressive strategies to treat AA, e.g., antithymocyte
globulin (ATG) and cyclosporine A (CSA) on the outcome
of AID, are scarce [4].

We sought to determine the incidence and characteristics
of concomitant AID diagnosed before or during the course
of AA and to compare AA patients with and without AID.

Materials and methods

This single-center, retrospective cohort study included all
243 patients with the diagnosis of AA, treated at the
University Hospital Basel, between 1974 and 2006. The
severity of AA was defined according to the widely
accepted criteria described by Camitta [5]. The general
treatment strategy was uniform with only minor changes
throughout the observation period. Patients younger than
40 years with a matched sibling donor received hemato-
poietic stem cell transplantation (HSCT) as first-line
therapy. Older patients and without an eligible sibling
donor were treated with intensive immunosuppression

containing ATG [6] with or without CSA. Since 2001, patients allocated to immunosuppression were included in the prospective European study and randomized to receive ATG and CSA with or without granulocyte colony-stimulating factor [7]. In order to circumvent serum sickness of ATG, most patients received steroids during the early phase of the treatment. Patients with relapse or nonresponse (NR) at 3 months usually were splenectomized [8] and, if necessary, retreated with a second course of ATG [9]. Response of AA to therapy was defined according to the Camitta criteria [10]: Splenectomy was performed in nonresponder patients before second-line therapy.

Concomitant AID were defined according to international diagnostic criteria [11–17]. We retained in this study only clinically evident AID. Isolated positive antibody titers without further clinical signs were not included. Response criteria for the concomitant AID were those used for patients with autoimmune disorders treated with HSCT, e.g., absence of all clinical signs without additional therapy and normalization of laboratory values [18]. Patients were controlled yearly; clinical outcome data were collected prospectively and stored in our local database. To identify AID, medical records were systematically reviewed. This study was approved by local Institutional Review Boards.

Statistical analysis

Left-truncated Cox models were used to assess the impact of patients' characteristics (gender, presence of HLA-DR2, severity of AA, type of AA treatment [ATG, HSCT], splenectomy) on the probability of developing an AID. Severity of AA, AA treatment, and splenectomy were coded as time-dependent covariates. Similarly, the impact of development of AID on survival was assessed by coding AID as a time-dependent risk factor in a Cox model. P values <0.05 were considered significant.

Results

Of the 243 patients, there were 115 (47%) females. The median age at diagnosis was 20 years (range 1–80 years), the median follow-up time was 9.3 years (0–33). Very severe AA was diagnosed in 128 (53%) patients and severe AA in 115 (47%). Splenectomy was performed in 89 (37%) patients. First-line therapy was ATG in 186 (77%) patients and HSCT in 57 (23%) (Table 1). In 24 out of 243 (10%) AA patients, a concomitant AID was diagnosed. Thirteen of the patients (54%) had an AID before diagnosis of AA, and 11 (46%) after therapy for AA. Four out of these 24 patients had more than one AID. In two of them, the first AID was diagnosed before AA appearance and a second AID after first-line therapy. We identified 16 different types of AID (Table 2). The most frequent AID were autoimmune gastritis (six patients) and autoimmune thyroiditis (six patients). The median age at diagnosis of AA was significant lower for patients without AID than patients with a concomitant AID (20 versus 52 years; $P<0.001$) (Fig. 1a, b). In our cohort, the cumulative incidence according to the age of the patients at diagnosis of AA increases mainly during the first three decades of age. In

Table 1 Pretreatment patients' characteristics comparing patients with and without concomitant AID

	All patients	Without AID	With AID	P value
Number of patients, n (%)	243	219 (90)	24 (10)	
Median age at diagnosis, years (range)	20 (1–80)	20 (1–80)	51.5 (9–75)	<0.001[a]
Median follow-up, years (range)	9.3 (0–33)	9.3 (0–33)	11.5 (0–32)	0.219[a]
Sex, n (%)				
Female	115 (47)	106 (46)	9 (38)	0.310[b]
Male	128 (53)	113 (54)	15 (62)	
Severity, n (%)				
Severe AA	115(47)	101 (46)	14 (58)	0.255[b]
Very severe AA	128 (53)	118 (54)	10 (42)	
Splenectomy, n (%)	89 (37)	81 (37)	8 (33)	0.723[b]
HLA DRB1*15 present, n (%)	32 (49) (n=65)	21 (47) (n=45)	11 (55) (n=20)	0.598[b]
First-line treatment, n (%)				
ATG	186 (77)	164 (75)	22 (92)	0.019[b]
HSCT	57 (23)	55 (25)	2 (8)	
Need for a second-line treatment, n (%)	68 (28)	63 (29)	5 (21)	0.441[b]

[a] Mann–Whitney U test

[b] Chi-square according to Pearson

Table 2 Characteristics of the 24 patients with concomitant AID and SAA

UPN	Sex	Age at diagnosis of SAA (years)	Autoimmune disorder	Severity of AA	HLA-DR15	Time interval diagnosis AID–SAA	Splenectomy	Therapy for AID	Remission of SAA	Outcome AID after ATG
First-line therapy: ATG, AID before AA										
292	M	22	Diabetes type 1	vSAA	No	232 months before	Yes	Insulin	NR	No change
352	F	71	Eosinophilic fasciitis	SAA	Yes	3 months before	No	No therapy	CR	Remission
353	M	9	Chron. juv. polyarthritis	vSAA	No	29 months before	Yes	Not known	CR	Remission
499	M	63	Autoimmune gastritis	SAA	Yes	51 months before	No	Vit. B_{12} substitution	PR	No change
580	M	62	Derm. herp. Duhring	vSAA	Yes	26 months before	No	Danazol, steroids, dapsone	CR	No change
			Celiac disease			27 months after		Diet		NA
			Hashimoto thyroiditis			128 months after		Not known		NA
747	M	49	Autoimmune gastritis	SAA	Yes	At same time	No	Vit B_{12} substitution	PR	No change
748	M	72	Psoriasis	SAA	No	Before, not specified	No	Steroids PUVA	NR	No change
778	M	57	Sjogren syndrome	SAA	Yes	259 months before	No	Prednisone	PR	No change
786	M	31	Autoimmune gastritis	SAA	No	1 months before	No	No periodic vit. B_{12} substitution	CR	No change
883	F	71	ITP	vSAA	Yes	181 months before	Yes	Danatrol, steroids	CR	No change
919	M	75	Microscopic polyangitis	SAA	No	5 months before	No	Cyclophosphamide, prednisone, mesna	PR	No change
1052	M	55	Colitis ulcerosa	SAA	Yes	139 months before	No	Not known	PR	No change
1248	F	65	Guillain–Barré syndrome	SAA	Unknown	61 months before	No	IvIg	NR	Remission before ATG
			Small vessel vasculitis			1 month after		Cortisone injections		NA
First-line therapy: ATG, AID after AA										
19	M	31	Systemic sclerosis	SAA	Yes	330 months after	Yes	Not known	CR	NA
39	M	10	Psoriasis	vSAA	Unknown	Not known, after	Yes	Not known	CR	NA
126	M	26	Autoimmune thyroiditis	vSAA	No	276 months after	Yes	Hormone replacement	CR	NA
286	F	15	Graves' disease	SAA	Unknown	232 months after	No	Thyreostatic treatment	CR	NA
291	F	55	Autoimmune gastritis	SAA	No	238 months after	Yes	Vit. B_{12} surveillance	CR	NA
501	F	22	Hashimoto thyroiditis	vSAA	Yes	123 months after	No	Hormone replacement	CR	NA
			Antiphospholipid syndrome			108 months after		No		NA
507	F	62	SLE with vasculitic neuropathy	SAA	No	87 months after	No	Cyclophosphamide, steroids, azathioprine	CR	NA
			Hashimoto thyroiditis			86 months after		No		NA
			Autoimmune gastritis			61 months after		No		NA
513	F	10	Guillain–Barré syndrome	vSAA	Yes	7 months after	No	IvIg, steroids	CR	NA
646	M	56	Autoimmune gastritis	SAA	Yes	12 months after	No	Vit. B_{12} substitution	CR	NA

Table 2 (continued)

UPN	Sex	Age at diagnosis of SAA (years)	Autoimmune disorder	Severity of AA	HLA-DR15	Time interval diagnosis AID–SAA	Splenectomy	Therapy for AID	Remission of SAA	Outcome AID after ATG
First-line therapy: HSCT, AID after AA										
2	F	30	Graves' disease	SAA	Unknown	21 months after	No	Radiojod-therapy	CR, AR	NA
521	M	45	ITP	vSAA	No	9 months after	Yes	Splenectomy	CR	NA

The patients are grouped according to their first-line therapy (ATG versus HACS) and the time of appearance of the AID (before SAA, after first-line therapy). Some of the patients presented more than one AID

SAA severe aplastic anemia, *NR* no response, *vSAA* very severe aplastic anemia, *CR* complete remission, *AR* autologous reconstitution, *PR* partial remission, *NA* not applicable

contrast, the cumulative incidence of AA patients with concomitant AID increases predominantly after the fifth decade of life (Fig. 1c).

In multivariate analysis comparing patients with and without AID, there were no differences with respect to gender (hazard ratio [HR]=0.540; 95%CI=0.232–1.260; P=0.154), severity of the disease (HR=1.343; 95%CI= 0.503–3.588; P=0.556), presence of HLA-DRB1*15 (HR= 0.731; 95%CI=0.297–1.796; P=0.494), type of treatment (HR=0.994; 95%CI=0.406–2.434; P=0.990), and splenectomy (HR=0.987; 95%CI=0.214–4.547; P=0.987).

We evaluated the influence of the AA treatment on the outcome of the concomitant AID in the 12 patients where the AID appeared before the diagnosis of AA (one patient was not included because Guillain–Barré syndrome resolved before ATG therapy). Five patients obtained complete response (CR=41.5%) of the AA, five obtained partial response (PR=41.5%), and two were nonresponders (NR=17%). Complete response of both diseases (AA and AID) was observed only in two out of 12 (17%) patients. One patient presented an eosinophilic fasciitis and the other a chronic juvenile polyarthritis. In the other ten patients, the course of the AID was not changed by the treatment of AA.

In 13 patients, the AID occurred after first-line therapy for AA (11 patients, ATG; two patients, HSCT); we evaluated the influence of this treatment on the development of the concomitant AID. Two out of 13 patients had had a first AID before diagnosis and therapy for AA. The median time from ATG treatment to diagnosis of the first AID was 7 years (range 3 months–27.5 years). At onset of the AID, the AA was in CR in seven patients and in PR in three patients. In two patients, there was NR and in one patient, the type of response was unknown. After allogeneic HSCT, two patients developed an AID. One patient with graft rejection and subsequent autologous reconstitution developed Graves' disease 2 years later. He is now in CR of the AA since more than three decades. A second patient developed an immune thrombocytopenia controlled by splenectomy. None of their donors had had a documented AID.

Discussion

In this study, we show that about one in ten AA patients will develop a concomitant AID during their lifetime, which can appear at any time before and/or after the onset of the AA. The frequency of a concomitant AID is higher in older AA patients. Hence, more than 25% of AA patients diagnosed after 50 years of age presented a concomitant AID. The main type of concomitant AID appeared to be either gastritis or thyroiditis. AA response to ATG was

Fig. 1 Age repartition at diagnosis of AA without AID (**a**) and with AID (**b**). The data are presented in percentage of the whole group. Patients are divided into three age groups: <20, 20–50, and >50 years ($P<0.001$). AA patients without AID are significantly younger than AA patients with a concomitant AID. **c** Cumulative incidence of AA and the concomitant AID according to the age at diagnosis of each disease. The slope of the curve is different for both diseases. In AA, the cumulative incidence increases mainly during the first three decades of age, whereas in concomitant AID, the cumulative incidence increases predominantly after the fifth decade of life
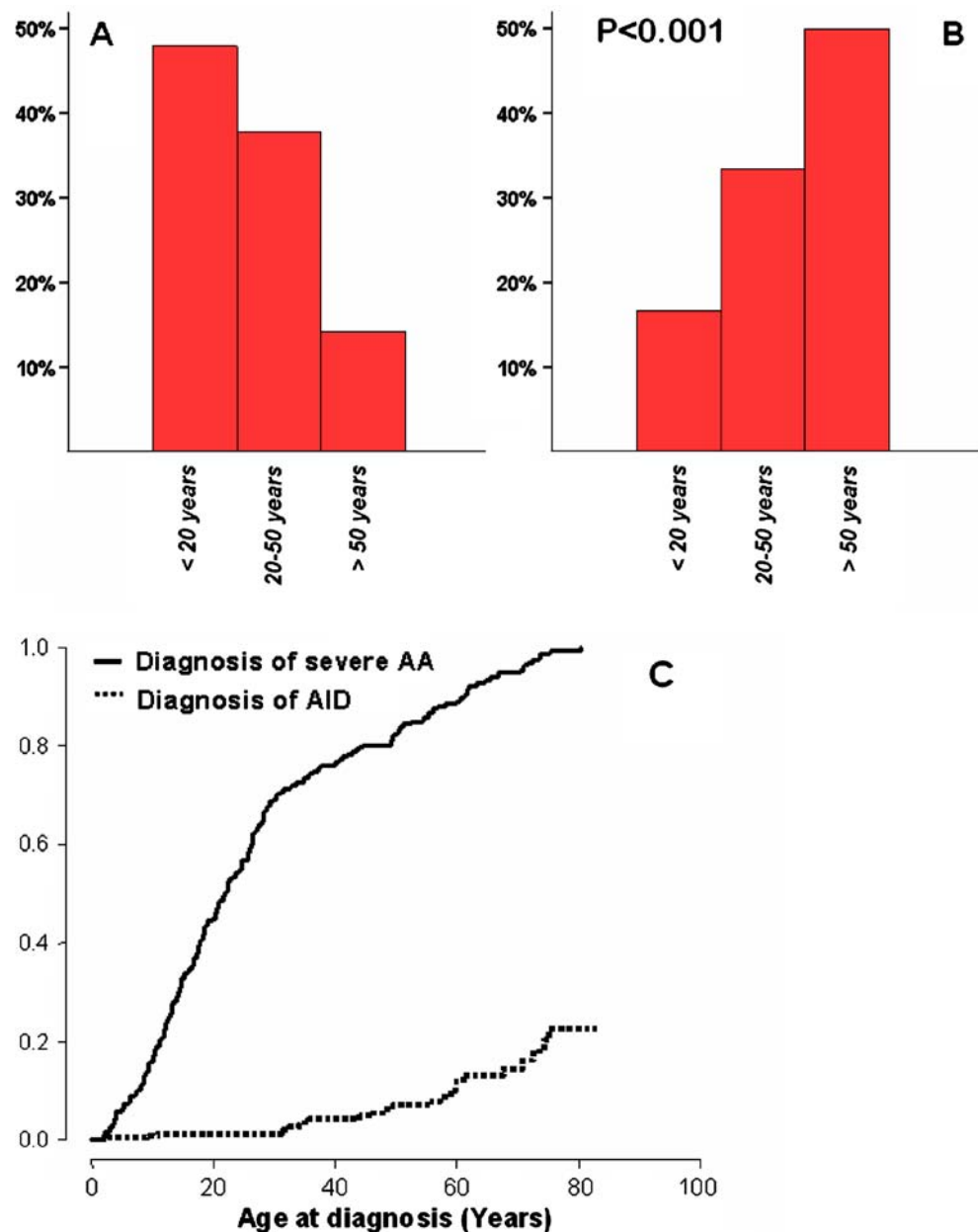
similar in patients with or without AID, but AID response to ATG was poor.

There are some particular features of the AID occurring in AA patients. In contrast to the general population where AID are more common in females [19], in AA patients, AID were more frequently observed in males (15 out of 24, 62%). Interestingly, we did not observe cases of rheumatoid arthritis and only one case of systemic lupus erythematosus. HLA-DRB1*15 has been shown to be involved in the development and the outcome of AA and other autoimmune disorders [20–23]. It seems unlikely that HLA-DRB1*15 plays a relevant role in the appearance of concomitant AID in AA patients.

Most cases of acquired AA can be considered as autoimmune disorders characterized by T cell-mediated, organ-specific destruction of bone marrow hematopoietic cells. However, the usual trigger of this autoimmune reaction remains unclear. In individual patients, the aberrant immune response can sometimes be linked to a viral infection or to drug or chemical exposure. There is much less evidence for other mechanisms including the association with other AID. In consideration of the high frequency of a concomitant AID in AA patients, it is unlikely that both diseases appear together just by chance. It, therefore, raises the question of related pathophysiologic mechanisms. In posthepatitis AA, which typically occurs in young,

healthy males with self-limited but severe liver inflammation, a common inciting infectious cause could be involved [24]. Indeed, in hepatitis-associated AA, similar skewed T cell repertoires have been detected in the liver and in the peripheral blood lymphocytes, suggesting that a similar antigen-driven pathogenic mechanism is involved for both diseases [25]. This might be different for the concomitant AIDs. Here, there are arguments in favor of distinct mechanisms: the different age repartition of AA patients with and without concomitant AID; the nonresponse of the AID in patients responding to immunosuppressive treatment for the AA and the fact that AA is a T cell-mediated AID, while in many of the concomitant AID, autoantibodies are involved. Common genetic backgrounds, additional immunogenetic, environmental, or hormonal factors may be responsible for the formation of subsets of AID clustering [26]. The AIDs occurring after successful allogeneic HSCT with full donor chimerism do not probably belong to the same category. Late secondary autoimmune-like phenomena have been described after allogeneic HSCT as a possible consequence of skewed immune reconstitution [27].

The older age of our AA patients with concomitant AID suggests that immunosenescence could play a role. Recent studies in healthy octogenarian patients indicate that the immune system, instead of suffering a generalized deterioration, undergoes a remodeling/readjustment of its major functions. Two divergent phenomena may coexist in immunosenescence: a decrease in the capacity of immune response and, simultaneously, autoantibody production [28].

Our study has limitations arising mainly from its retrospective, single-center character and the lack of a control population. There is a relatively small number of patients at risk; however, considering that AA is a rare disease, this is the first and largest study reporting on the frequency of concomitant AID in AA patients followed up systematically over a long period of time. The advantage of a single-center study is the homogeneity of therapeutical approaches and the consistent follow-up. At last control, 80% of the long-term survivors had a follow-up of more than 7 years.

In conclusion, in this study, we show that the development of a concomitant AID is frequent, particularly in older AA patients. The AID may appear at any time before or after the AA, and the outcome of the AA is not impaired by the concomitant AID, but the AID does not usually respond to the immunosuppression applied for the AA. The difference in response to ATG therapy between AA and AID suggests independent underlying immune mechanisms. Alternatively, one of these diseases could be the trigger for a second immune dysregulation.

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