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ORIGINAL ARTICLE

Epidemiology of infections in children with acquired aplastic anaemia: a retrospective multicenter study in Italy

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Abstract

Infection is a significant cause of death in patients with aplastic anaemia (AA). However, few studies have examined the characteristics of infections in patients with AA, especially in children. The aim of this retrospective study was to evaluate the incidence and types of infections in a large cohort of paediatric patients with AA referred to eight AIEOP (Italian Association of Paediatric Oncology and Haematology) centres in Italy. The study included 78 patients, 45 boys and 33 girls, median age 9.29 yrs (1st–3rd quartile 3.59–13.09) diagnosed with AA. During the study period, 111 infectious episodes were observed in 42 (54%) patients. Fifty-one (46%) episodes were fever of unknown origin and 60 (54%) were documented infections (DI). In this group, microbiologically documented infection (MDI) with bacteremia accounted for 23 (38%) episodes, MDI without bacteremia for 7 (12%), clinically documented infection for 25 (42%) and invasive fungal diseases for 5 (8%). The rate (episodes/1000 d at risk) was similar in severe aplastic anemia and very severe aplastic anemia both before and after day 120. During the first 120 d from diagnosis, the cumulative risk of a DI was 21% (95% CI 12–29) with the last episode at day 117, but the 50% of episodes were observed in the first 24 d. After day 120, the cumulative risk of DI was again 21% (95% CI 12–29), with the last episode at day 445 of follow-up, with 50% of episodes observed in the first 120 d of observation (240 d from the diagnosis of AA). We found a statistically significant association between the grade of aplasia at diagnosis and the incidence of IEs ($P = 0.0002$). No association was found between gender, age at diagnosis, response at day +120 and at day +180, use of G-CSF and occurrence of IEs. The actuarial overall survival at 5 yrs was 90% ± 3.6. The mortality rate attributable to infection complication was 9%. This is a large paediatric cohort study reporting the epidemiology of infectious complications in children with AA and that allow us to compare the epidemiological data in this diseases with that of the most recent studies in neutropenic children with cancer. Our findings confirm that infections represent the main cause of death in patients with AA and they are important for the design of management strategies of febrile neutropenia in these patients.

Key words aplastic anaemia; infection; children

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Acquired aplastic anaemia (AA) is a bone marrow failure syndrome characterised by the reduction in haematopoietic stem and progenitor cells and pancytopenia. The optimum treatment for children with AA is haematopoietic stem cell transplantation (HSCT) from a human leucocyte antigen (HLA)-identical sibling donor. This is associated with a survival rate of up to 90%. Combined immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine A (CyA) is the alternative gold standard first-line treatment for children lacking a family matched donor (1–6).

Addition of granulocyte colony-stimulating factor (G-CSF) to the combination increases neutrophil counts and decreases the rate of infections and the days of hospitalisation but has no impact on overall survival, event-free survival, remission and death rates. Prolonged use of G-CSF has to be weighed against possibly higher risks of myelodysplastic syndrome/acute myelogenous leukaemia (MDS/AML) (7).

Patients with AA have primarily a decrease in the blood counts resulting in severe and prolonged neutropenia and increased susceptibility to recurrent bacterial sepsis or invasive fungal infection; treatment modalities may as well increase the risk of infections (8). Profound persistent neutropenia is the dominant risk factor for infections that are the leading cause of death in AA. Only few studies have examined the characteristics of infections in patients with AA, especially in children (8–10).

The aim of our study was to describe the incidence and types of infections in a large cohort of paediatric patients with AA referred to eight AIEOP (Italian Association of Paediatric Oncology and Haematology) centres in Italy.

Patients and methods

Study design

All the centres of the Italian Association of Paediatric Haematology and Oncology (AIEOP) were invited to retrospectively report all patients aged 0–18 yrs diagnosed with AA between March 1996 and February 2007 and treated with IST.

AA was diagnosed by means of morphology (bone marrow biopsy), blood counts and negative diepoxybutane (DEB) chromosome fragility studies. Severe AA (SAA) was defined by bone marrow cellularity $<25\%$, or 25–50% with $<30\%$ residual haematopoietic cells and two of the following: neutrophil count $<0.5\times10^9$/L, platelet count $<20\times10^9$/L, reticulocyte count $<20\times10^9$/L. Very severe AA (VSAA) was defined as severe but with a neutrophil count $<0.2\times10^9$/L. Other forms of AA were classified as non-severe AA (NSAA) according to the international criteria (11).

The standard IST included: horse ATG (IMTIX, Lyon, France) (15 mg/kg/d on days 1–5), methylprednisolone (2 mg/kg/d for 5 d followed by a taper until discontinuation on day +30) and CyA (5 mg/kg/d orally) from day +1 for at least 6 months, adjusted to blood levels (therapeutic range from 150 to 300 ng/mL) and then tapered according to the response. In all the centres, CyA blood levels (during CyA treatment before tapering) were measured with radio-immune monoclonal assay at basal, predose, timing. Also, patients treated with CyA (5 mg/kg/d orally) alone were included in this study. The patients could also receive G-CSF (5 μg/kg subcutaneously) from day +1 to day +90 (2, 12, 13). Non-responders could be retreated with a second course of rabbit ATG (Thymoglobulin, IMTIX).

In patients receiving IST, the response was evaluated at +120 and +180 d from IST. Complete responses were defined as transfusion independence associated with haemoglobin (Hb) >11 g/dL, neutrophil count $>1.5\times10^9$/L and platelet count $>100\times10^9$/L. We defined partial responses as transfusion independence associated with Hb >8 g/dL, neutrophil count $>0.5\times10^9$/L and platelet count $>30\times10^9$/L. Transfusion dependence was taken as evidence of no response (2).

Because the aim of the study was to describe the infectious complications of IST, patients with an HLA identical sibling donor, and thus eligible for HSCT as first-line therapy, were not included.

Before including patients in the database, informed consent was obtained from all parents. Information on patient age, clinical and haematological features at diagnosis, gender and therapy were collected. For each infectious episode (IE), date of diagnosis, localisation and aetiology were retrospectively recorded. An episode was defined as diagnosed concomitantly to the diagnosis of AA whether occurring ±7 d from the diagnosis of AA. All IEs were recorded from diagnosis until death, HSCT as second-line therapy or censored at 31 December 2007.

Febrile episodes were classified as documented infections (DI), and in more detail as microbiologically documented infection (MDI) with bacteraemia, MDI without bacteraemia, clinically documented infection (CDI) and fever of unknown origin (FUO) (14). Invasive fungal diseases (IFD) were classified separately according to the European Organisation for Research and Treatment of Cancer/Mycoses Study Group criteria (15). Bacteraemia or fungemia or skin and soft tissue (exit site and tunnel) infections, related with the presence of an indwelling central venous catheter (CVC), were diagnosed according to previous definitions (16). However, as data regarding the presence of CVC and data of insertion/removal were not available for all patients, no specific analysis was performed on the epidemiology of CVC-related infections in children with AA. Death was considered as

infection-related if occurring within 14 d from a diagnosis of bacteraemia or 90 d from a diagnosis of IFD.

No specific standard of prophylaxis, with the exception of *P. jiroveci* pneumonia (PCP) prophylaxis, or therapy of the infectious complications was recommended. So management strategies were defined by any single centre on the basis of local considerations and experience.

Statistical analysis

Descriptive statistics were reported in terms of absolute frequencies and percentages. Distribution of quantitative data was described as median values and interquartile range owing to the non-normal (Gaussian) distribution of most of them. Continuous variables were compared with the Student's *t*-test, and percentages were compared with the chi-square test or the Fisher exact test.

For each patient, the number of days at risk was recorded, calculated from the date of diagnosis of AA to the day +120 of treatment and from the day +121 of treatment to day 1096 (3 yrs of follow-up), bone marrow transplant, or death, or censored at 31 December 2007, whichever occurred first. The stratification before and after day 120 from diagnosis of AA was based on previously reported response criteria (2). Cumulative risk estimates and infection rates (IRs) were used as measures of the burden of infectious complications. The cumulative risk estimates considered only the first infectious episode, if any, occurring in any given patient and were calculated according to the Kaplan–Meier method. The IR was calculated as the number of events divided by the person-days at risk (pdr) and expressed as episodes/1000 pdr with 95% confidence intervals (95% CI). Differences in IR values among different AA diagnostic groups were evaluated by the log-rank test. Cumulative survival was calculated by means of the Kaplan–Meier method. All statistical tests were two-sided and significant for a P value < 0.05.

Results

Study population

The study included 78 patients, 45 boys and 33 girls, median age 9.29 yrs (1st–3rd quartile 3.59–13.09) diagnosed with AA. In particular, 6 were classified as NSAA, 34 as SAA and 38 as VSAA, with a total of 65038 pdr. The main features at diagnosis and the details on IST of these patients are summarised in Table 1.

Infectious episodes

During the study period, 111 IE were observed in 42 (54%) patients. No IE was reported in patients with NSAA, while 74 (67%) were observed in the 38 patients with VSAA and 37 (33%) in the 34 patients with SAA. Diagnosis of IE was FUO in 51 (46%) episodes while 60 (54%) were DI. Table 2 summarises the aetiologies and localisations of DI. In this group, MDI with bacteremia accounted for 23 (38%) episodes, MDI without bacteremia for 7 (12%), CDI for 25 (42%) and IFD for 5 (8%). The 30 MDI (50% of all DI) were owing to Gram-positives in 18 (60%) episodes (16, 69%, of the 23 bacteremias) and to Gram-negatives in 12 (40%) (7, 31%, of the 23 bacteremias). *S. aureus* was the most frequently isolated pathogen followed by coagulase negative staphylococci , streptococci, KES (*Klebsiella-Enterobacter-Serratia group*) and *E.Coli*. *Pseudomonadaceae* were isolated only in two episodes (one *P. aeruginosa* and one *B. cepacia)*. As regards the site of DI, bacteremia was the most frequent localisation (23/60, 38%), followed by pneumonia (13/60, 22%), severe stomatitis (10/60, 15%), and skin and soft tissues (8/60, 13%), including CVC exit site/tunnel. CVC-related infections accounted for a total of nine episodes (15%), six bacteremias (26% of bacteremic episodes) and three exit site/tunnel infections (two MDI, *Streptococcus* sp and *S. cromogenes*, and 1 CDI). IFD accounted for 8% of episodes: three proven/probable and two possible IFD, all localised at the respiratory tract (three pneumonias and one sinusitis). Another episode of pneumonia was because of the reactivation of latent tuberculosis. In 8 (10%) patients, the diagnosis of AA was concomitant with an infectious episode: 6 FUO, 1 CDI (stomatitis) and 1 MDI with bacteremia (*Streptococcus* viridians).

Fifteen IEs (three of which were bacterial infections) were diagnosed in patient receiving antibacterial prophylaxis with ciprofloxacin (7/15), amoxicillin-clavulanate (6/15) and ceftriaxone or cefixime, one episode each. Four fungal infections occurred during antifungal prophylaxis, three with fluconazole and one during Amphotericin B administration. Prophylaxis against PCP was administered to all 42 patients with oral trimethoprim-sulfamethoxazole (21 patients) or aerosolic pentamidine (23 patients), and no episode of PCP was observed. Fifty-six out of 111 IEs (50%) occurred during G-CSF therapy: 50% (28/56) of them within the first 3 months from diagnosis while 34% (19/56) occurred within 12 months from diagnosis, and all but two occurred in non-responders patients. The remaining 16% (9/56) of IEs were documented after the first year from diagnosis, during treatment with G-CSF; six out of nine of these patients were no responders while three were partial responders. During the study period, the actuarial overall survival at 5 yrs was 90% ± 3.6. (Fig. 1). The mortality attributable to IE was 9% (7/78).

Table 1 Individual patient characteristics

PT	Age at diagnosis (months)	Grade of AA	Aetiology	First IST	G-CSF	Response at +120 d	Response at +180 d	Following treatment	Last follow-up
1 (M)	95	VSAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	HSCT
2 (M)	245	SAA	Idiopathic	ATG + CYA	Yes	PR	PR	2nd ATG	HSCT
3 (M)	45	VSAA	Idiopathic	ATG + CYA	Yes	PR	PR	2nd ATG	HSCT
4 (F)	148	VSAA	Idiopathic	ATG + CYA	Yes	NR	Exitus (2*)	–	–
5 (M)	112	SAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	HSCT
6 (M)	159	VSAA	Idiopathic	ATG + CYA	Yes	NR	PR	–	REM
7 (M)	41	VSAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	REM
8 (F)	24	SAA	Idiopathic	ATG + CYA	Yes	NR	NR	–	HSCT
9 (F)	42	SAA	Idiopathic	ATG + CYA	Yes	NR	NR	–	HSCT
10 (M)	99	VSAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	REM
11 (F)	33	VSAA	Idiopathic	ATG + CYA	No	PR	CR	–	REM
12 (M)	33	VSAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	REM
13 (F)	114	SAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	REM
14 (M)	186	VSAA	Idiopathic	ATG + CYA	No	PR	CR	–	REM
15 (F)	219	VSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
16 (M)	89	VSAA	Hepatitis	ATG + CYA	Yes	PR	PR	2nd ATG	REM
17 (M)	136	VSAA	Idiopathic	ATG + CYA	Yes	CR	CR	–	REM
18 (F)	42	VSAA	Idiopathic	ATG + CYA	Yes	CR	CR	–	REM
19 (F)	182	VSAA	Idiopathic	ATG + CYA	Yes	NR	PR	2nd ATG	REM
20 (F)	39	VSAA	Idiopathic	ATG + CYA	Yes	CR	CR	–	REM
21 (M)	24	SAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	HSCT
22 (M)	24	SAA	Idiopathic	ATG + CYA	Yes	CR	CR	–	REM
23 (F)	186	VSAA	Idiopathic	ATG + CYA	Yes	PR	PR	–	HSCT
24 (F)	23	SAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	Exitus
25 (F)	120	SAA	Hepatitis	ATG + CYA	Yes	NR	Exitus (5*)	–	–
26 (M)	230	VSAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	Exitus
27 (M)	19	VSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
28 (M)	198	VSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
29 (M)	83	SAA	Idiopathic	ATG + CYA	No	PR	PR	2nd ATG	HSCT
30 (M)	32	VSAA	Idiopathic	ATG + CYA	No	CR	CR	–	REM
31 (F)	59	SAA	Adenovirus	ATG + CYA	No	PR	PR	–	REM
32 (M)	73	SAA	Hepatitis	ATG + CYA	Yes	PR	PR	2nd ATG	REM
33 (M)	135	VSAA	Idiopathic	ATG + CYA	No	PR	PR	2nd ATG	HSCT
34 (M)	170	SAA	EBV	ATG + CYA	No	CR	PR	2nd ATG	HSCT
35 (M)	134	VSAA	Hepatitis	ATG + CYA	Yes	CR	CR	–	REM
36 (F)	167	VSAA	Hepatitis	ATG + CYA	No	NR	NR	2nd ATG	REM
37 (F)	83	VSAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	HSCT
38 (M)	161	SAA	Idiopathic	ATG + CYA	Yes	NR	NR	–	Exitus
39 (M)	10	VSAA	Idiopathic	ATG + CYA	Yes	CR	PR	–	REM
40 (F)	161	VSAA	Idiopathic	ATG + CYA	Yes	PR	PR	2nd ATG	REM
41 (F)	91	VSAA	Idiopathic	ATG + CYA	Yes	NR	Exitus (4*)	–	–
42 (M)		VSAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	Exitus
43 (M)	194	VSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
44 (M)	202	NSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
45 (M)	161	VSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
46 (M)	116	NSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
47 (F)	169	SAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
48 (M)	60	SAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
49 (F)	157	SAA	Idiopathic	ATG + CYA	Yes	PR	PR	–	REM
50 (M)	106	VSAA	EBV	ATG + CYA	Yes	CR	PR	–	CyA d
51 (M)	78	SAA	Idiopathic	ATG + CYA	Yes	PR	PR	–	CyA d
52 (F)	198	NSAA	Idiopathic	CYA alone	No	PR	CR	–	CyA d
53 (M)	158	NSAA	Hepatitis	ATG + CYA	Yes	NR	NR	2nd ATG	HSCT
54 (M)	156	SAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	REM
55 (F)	9	VSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
56 (M)	111	VSAA	Idiopathic	ATG + CYA	Yes	PR	PR	2nd ATG	HSCT

Table 1 (Continued)

PT	Age at diagnosis (months)	Grade of AA	Aetiology	First IST	G-CSF	Response at +120 d	Response at +180 d	Following treatment	Last follow-up
57 (F)	108	VSAA	Idiopathic	ATG + CYA	Yes	PR	PR	–	HSCT
58 (F)	105	VSAA	Idiopathic	ATG + CYA	Yes	PR	CR	–	REM
59 (M)	14	NSAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	HSCT
60 (M)	43	VSAA	Hepatitis	ATG + CYA	Yes	CR	CR	–	REM
61 (M)	131	SAA	Idiopathic	ATG + CYA	Yes	PR	PR	–	CyA d
62 (F)	139	SAA	Idiopathic	ATG + CYA	Yes	NR	NR	2nd ATG	HSCT
63 (F)	26	SAA	Idiopathic	ATG + CYA	Yes	PR	PR	–	REM
64 (F)	6	NSAA	Idiopathic	CYA alone	No	PR	PR	–	REM
65 (M)	44	SAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	HSCT
66 (F)	191	SAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	NR
67 (M)	193	SAA	Idiopathic	ATG + CYA	No	PR	PR	2nd ATG	REM
68 (M)	78	SAA	Hepatitis	ATG + CYA	No	NR	NR	2nd ATG	HSCT
69 (F)	58	SAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	REM
70 (M)	153	SAA	Idiopathic	ATG + CYA	No	CR	CR	–	REM
71 (M)	210	SAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	REM
72 (F)	106	SAA	Idiopathic	ATG + CYA	No	NR	NR	–	HSCT
73 (F)	126	SAA	Idiopathic	ATG + CYA	No	NR	NR	–	HSCT
74 (M)	30	SAA	Idiopathic	ATG + CYA	No	NR	NR	2nd ATG	HSCT
75 (F)	155	SAA	Idiopathic	ATG + CYA	Yes	CR	CR	–	REM
76 (F)	126	VSAA	Idiopathic	ATG + CYA	Yes	PR	PR	–	REM
77 (M)	127	VSAA	Hepatitis	ATG + CYA	Yes	NR	NR	2nd ATG	REM
78 (F)	130	SAA	Idiopathic	ATG + CYA	Yes	PR	PR	2nd ATG	HSCT

PT, patient; F, female; M, male; VSAA, very severe aplastic anaemia; SAA, severe aplastic anaemia; NSAA, non-severe aplastic anaemia; IST, immunosuppressive therapy; CR, complete response; PR, partial response; NR, no response; *, months from diagnosis; HSCT, hematopoietic stem cell transplantation; REM, remission; CyA d, Cyclosporine dependence; ATG, antithymocyte globulin.

Table 2 Aetiologies and localisations of documented infections in children with aplastic anaemia

Diagnosis	Clinical picture	Day 0–120 from IST		Day >120 from IST	
		Aetiology	Number of episodes	Aetiology	Number of episodes
MDI	Bacteremia	*S. aureus*	3	*Coagulase negative staphylococci*	4
		Streptococcus viridans	3	*S. aureus*	3
		K. pneumoniae	1	*Corynebacterium* spp	2
		E. colacae	1	*Alpha-haemolyticus Streptococcus*	1
		E. coli	1	*K. pneumoniae*	1
				E. cloacae	1
				B. cepacia	1
				Proteus spp	1
	Skin and soft tissues[1]	*Streptococcus* spp	1	*Coagulase negative staphylococci*	1
		P. aeruginosa	1		
	Urinary tract	*E. coli*	1	*E. coli*	2
				Morganella sp	1
	Pneumonia	–	–	*Aspergillus* sp	2
	Sinusitis	*Mucor ramosissimus*	1	–	–
CDI	Pneumonia[2]	–	5	–	6
	Skin and soft tissue[1]	–	3	–	2
	Stomatitis/pharyngitis	–	7	–	3
	Varicella	–	–	–	1

MDI, microbiologically documented infection; CDI, clinically documented infection.
[1]Including three cases of CVC exit site/tunnel infections (2 MDI, 1 CDI), [2]two cases evaluated as possible invasive fungal disease, 1 as reactivation of latent tuberculosis (all within day 120).

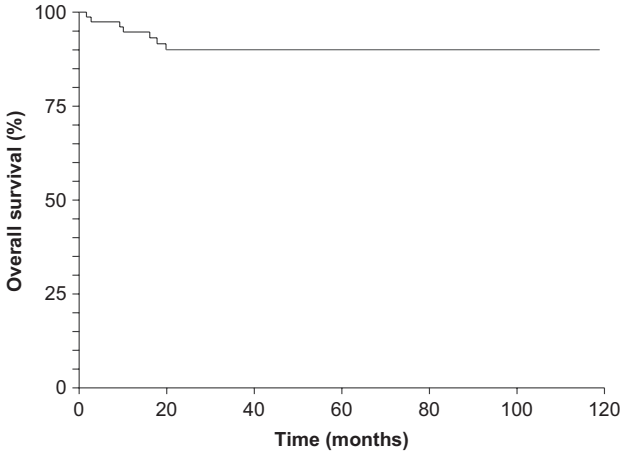

Figure 1 Kaplan–Meier plot of survival time: all patients.

Epidemiology

The epidemiology of IE was analysed stratifying the episodes in two categories: as occurring in the first 120 d after diagnosis or later. Because no infectious episode occurred in patients with NSAA, these subjects were excluded by any further analysis. All patients with SAA ($n = 34$) and VSAA ($n = 38$) were included in the period 0–120 d, while after day 120, the follow-up was restricted to 32 (94%) of those with SAA and 37 (97%) of those with VSAA, because three patients ended the follow-up before day 120 (one died and two received HSCT). After day 121 from the start of treatment, the follow-up was closed in 25 patients because of HSCT or death.

Table 3 reports the rates of different infectious complications observed before and after day 120, stratified by the type of AA. No IE was observed in patients with NSAA, while in the other cases, the highest rates of IE were observed during the first 120 d from the diagnosis of AA, without statistically significant differences between SAA and VSAA. The cumulative risk of infectious complications was calculated only for DI. During the first 120 d from diagnosis, the cumulative risk of a DI was 21% (95% CI 12–29) with the last episode at day 117, but the 50% of episodes were observed in the first 24 d. After day 120, the cumulative risk of DI was again 21% (95% CI 12–29), with the last episode at day 445 of follow-up, with 50% of episodes observed in the first 120 d of observation (240 d from the diagnosis of AA).

Risk factors

The comparison between the 42 patients who experienced one or more IE and the 36 patients never developing IEs showed a significant association between the grade of aplasia at diagnosis and incidence of IEs ($P = 0.0002$). No association was found between gender, age at diagnosis, response at day +120 and at +180 d, use of G-CSF and occurrence of IEs (Table 4).

Discussion

Infections represent a severe complication in patients with AA. Only three, single centre, large series reporting infections in these patients, all involving a majority of adults (8–10, 17), have been published. In the present retrospective multicentre study, we describe the epidemiology of infectious complications in a large cohort of children with AA treated at tertiary care hospitals, providing information on the type of infections as well as outcome. In spite of the limitations present in all retrospective studies, this large paediatric cohort study reporting the epidemiology of infectious complications in children with AA, allows the comparison of epidemiological data in these diseases with that of the most recent studies in neutropenic children with cancer that are generally adopted to design management strategies of febrile neutropenia also in AA. Therefore, these results may be very useful to choose management strategies (18, 19).

IEs were mainly observed in the first 120 d from diagnosis. While there was no infection in children with NSAA, the rates of IE, bacteraemia and IFD resulted similar in SAA and VSAA both before and after day 120 from diagnosis. The cumulative risk of developing at least one DI was 21%, both before and after day 120. This value is lower than that observed in the other studies in patients with AA, which included both children and adults (9, 17). Interestingly, the 50% of the episodes was observed in the first month after diagnosis, while after this day, the episodes were stretched over a longer period (50% within other 90 d). In any case, this observation is consistent with that of Torres and co-workers who reported the majority of episodes within 6 months from diagnosis.

The methodology of this study also allowed the comparison of the epidemiology of infectious complications in children with AA with that observed in those with cancer, the most studied group of immunosuppressed, neutropenic children. In VSAA and SAA patient, the overall frequency of febrile episodes was below that observed not only in children with leukaemia, but also in those with solid tumours not aggressively treated (Table 5). This fact is probably due to the near complete absence of mucositis in AA, which on the other hand represents an important risk factor during chemotherapy (20). However, it is interesting to note that in the first 120 d after diagnosis, rates of bacteraemia were similar to those observed in solid tumour or in a miscellaneous of antineoplastic treatments (19, 21),

Table 3 Epidemiology of infectious episodes in children with aplastic anaemia (episodes/1000 patient-days at risk)

	Day 0–120						After day 120					
	Days of follow-up	DI (*n*)	Rate of DI (95% CI)	Bacteremias (*n*)	Rate of bacteremias (95% CI)	IFD (*n*)	Rate of IFD (95% CI)	Days of follow-up	DI (*n*)	Rate of DI (95% CI)	Bacteremias (*n*)	Rate of bacteremias (95% CI)	IFD (*n*)	Rate of IFD (95% CI)
SAA	3949	11	2.78 (1.54–5.03)	5	1.27 (0.52–3.04)	2	0.51 (0.13–2.02)	22104	12	0.54 (0.31–0.95)	5	0.23 (0.09–0.54)	1	0.04 (0.006–0.32)
VSAA	4506	16	3.55 (2.17–5.80)	4	0.88 (0.33–2.36)		0.22 (0.03–1.57)	30424	19	0.62 (0.40–0.98)	9	0.29 (0.15–0.57)	1	0.03 (0.004–0.23)
NSAA	720	0	0	0	0	0	0	3335	0	0	0	0	0	0
P (log-rank test)	–	–	0.31		0.61		0.68			0.26		0.49		0.92
Total	9175	27	2.94 (2.02–4.29)	9	0.98 (0.51–1.88)	3	0.33 (0.10–1.01)	55863	31	0.55 (0.39–0.79)	14	0.25 (0.15–0.42)	2	0.03 (0.009–0.14)

VSAA, very severe aplastic anaemia; SAA, severe aplastic anaemia; NSAA, non-severe aplastic anaemia; DI, documented infections; IFD, invasive fungal disease; *n*, numbers.
Number in parenthesis is 95% confidence intervals.

Table 4 Analysis of risk factors for the development of infectious complications in children with aplastic anaemia

	Group 1[1]	Group 2[2]	*P*
No. of patients	42	36	
Gender (male/female)	25/17	20/16	0.82*
Mean age at diagnosis (months)	103	114	0.28**
Type of AA			
VSAA	28	10	0.0002*
SAA	14	20	
NSAA	0	6	
Aetiology			
Idiopathic	35	31	1*
Hepatitis	5	4	
Virus	2	1	
Response at +120 d			
CR	8	4	0.22*
PR	14	19	
NR	20	13	
Response at +180 d			
CR + PR	15	10	0.07*
NR	13	20	
NA	14	6	
Use of G-CSF			
Yes	31	24	0.8*
No	11	12	

VSAA, very severe aplastic anaemia; SAA, severe aplastic anaemia; NSAA, non-severe aplastic anaemia; CR, complete response; PR, partial response; NR, no response; NA, not available.
[1]Patients who experienced one or more infectious episodes; [2]patients who do not experienced infectious episodes.
*Fisher exact probability test; **Single sample t-test.

while after day 120, these rates were by far lower. On the other hand, the rate of IFD, which represents one of the most greatest fears in patients with AA because of its high mortality (8–10, 17), was quite similar to that observed in leukaemia or lymphoma receiving aggressive therapy (18, 19). This probably confirms that the long-lasting neutropenia together with immunosuppression because of the disease and its treatment represents important factors for the development of this complication. However, we clearly showed that the severity of AA at onset was the most important factor associated with the development of an infectious complication. On the contrary, the patient gender, the age at diagnosis, the response to IST and the use of G-CSF seem to not have an impact on the occurrence of IEs.

We recognise that this study has also limitations, mainly because of its retrospective fashion. First of all, specific data about insertion/removal of a CVC during the entire follow-up were not available for all AA patients, not allowing an analysis of the epidemiology of CVC-related infections. Moreover, no data regarding other possibly important factors like, for example, the

Table 5 Rates of infectious complications in children with AA (present study) and in neutropenic children with cancer

Patients' group	Overall	Bacteriemias	IFD	FUO	References (notes)
NSAA	0	0	0	0	Multicenter, present study (No infectious complications)
SAA					
0–120 d from IST	9.71	3.24	1.29	2.59	Multicenter, present study
>120 d from IST	0.99	0.23	0.04	0.41	
VSAA					
0–120 d from IST	13.42	1.22	0.30	8.24	Multicenter, present study
>120 d from IST	0.99	0.29	0.03	0.36	
AL-NHL aggressively treated/alloHSCT/autoHSCT	37.70	6.41	1.77	26.53	Castagnola *et al.* 2007 (Prospective evaluation of
ST aggressively treated	24.71	1.49	0.10	21.03	neutropenic episodes in children with cancer.
AL-NHL/ST not aggressively treated	13.88	0.88	0.63	10.09	Rates calculated as episodes/1000 pdr)
Maintenance AL/NHL	5.02	0	0	5.02	
Children receiving antineoplastic chemotherapy or HSCT	4.80	2.52	0.46	NA	Multicentre, Ammann *et al.* 2008

NSAA, non-severe aplastic anaemia; SAA, severe aplastic anaemia; VSAA, very severe aplastic anaemia; IST, immunosuppressive therapy; AL, acute leukaemia; NHL, non-Hodgkin lymphoma; ST, solid tumour; HSCT, hematopoietic stem cell transplant; IFD, invasive fungal diseases; FUO, fever of unknown origin.

number of blood transfusion or the diagnostic and treatment strategies of the IE were recorded. Therefore, the analysis of risk factors and of the effectiveness of management strategies, especially prophylaxis, is incomplete or still open to questions.

Anyway, in spite of these flaws, these observations give important information regarding the epidemiology of IE in children with AA showing that the severity of underlying disease is the most important factor for developing IE and that these complications are more frequent in the first 120 d from diagnosis. These data should be useful for the design of management strategies and/or clinical trials.

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