MAJOR ARTICLE

Decreased Infection-Related Mortality and Improved Survival in Severe Aplastic Anemia in the Past Two Decades

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Background. Persistent neutropenia associated with severe aplastic anemia (SAA) is an important risk factor for development of life-threatening infections. Earlier studies underscored the high mortality associated with invasive fungal infections (IFIs) in SAA. However, little is known about the current patterns of infections and the impact of advances in anti-infective therapy on survival in SAA.

Methods. We reviewed the records of 174 patients with SAA admitted to the Hematology Branch at NHLBI from 1989 to 2008 who were unresponsive to initial immunosuppressive therapy (IST) at 6 months. Three patient groups determined by IST protocol and time interval were compared: group 1 (43 patients; December 1989–October 1996), group 2 (51 patients; November 1996–October 2002), and group 3 (80 patients; November 2002–April 2008). Outcome variables included infections, patterns of resistance, survival, and infection-related mortality.

Results. During the past 2 decades, infection-related mortality decreased from 37% in group 1 to 11% in group 3 ($P<.001$), and the frequency of IFIs decreased from 49% in group 1 to 8% in group 3 ($P<.001$). Overall 5-year survival for all patients ($n = 420$) increased from 64% in group 1 to 79% in group 3 ($P<.001$). Among non-responders ($n = 174$), it increased from 23% in group 1 to 57% in group 3 ($P<.001$). In multivariate analysis, younger age, absolute neutrophil count >200 cells/μL before IST, absence of IFIs, and use of voriconazole were independently predictive of survival.

Conclusion. During the past 2 decades, there has been a significant decrease in IFIs, infection-related mortality, and overall mortality in patients with SAA unresponsive to initial IST.

A half century ago, severe aplastic anemia (SAA) was an almost universally fatal disease with treatment options limited to androgens, transfusion support, and antibiotics. Hematologic improvement was infrequent, and infectious complications usually led to death. The introduction of hematopoietic stem cell transplantation (HSCT) in the 1970s [1, 2] and immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine (CsA) in the 1980s [3, 4] led to striking improvement in survival because of hematologic recovery. Despite advances in IST for SAA, infections remain the main cause of death, with invasive fungal infections (IFIs) being the most lethal [5].

There has been a strong link between response to IST and survival across many SAA protocols [6–8]. In general, responders have long-term survival of 80%–90%, whereas for non-responders, survival has been 20% to 30% [9]. In addition, robust improvement in blood counts correlates with better long-term survival rates [9]. Unfortunately, since the introduction of ATG + CsA, hematologic response rate has remained steady at 60%–70% despite efforts to improve on the ATG + CsA platform. The addition of a third drug to the ATG + CsA regimen has not been beneficial [7,10–12], and

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more-potent lymphocytotoxic agents have generated conflicting data [13, 14]. Thus, there remains a sizeable population of patients with SAA who remain pancytopenic after IST who depend upon supportive care measures for management of life-threatening infections and other complications of pancytopenia. Hematological salvage treatments in these unresponsive cases include a repeat course of IST or HSCT from a histocompatible sibling or alternative donor. Therefore, the ability to support IST-unresponsive patients through extended periods of pancytopenia to receive these salvage hematological interventions is critical to their outcome.

Despite the lack of progress in achieving higher response rates in the last 2 decades, we observed marked improvement in survival during the same time period. Here, we report the pattern of infections, the causes related to death, and the impact of newer antifungal agents on survival among non-responders to IST at our institution.

PATIENTS AND METHODS

Patient Demographic Characteristics

Consecutive patients who fulfilled diagnostic criteria for SAA were enrolled in sequential treatment protocols from November 1989 through April 2008 at the Warren Grant Magnuson Clinical Center and Mark O. Hatfield Clinical Research Center at the National Institutes of Health in Bethesda, Maryland. All patients (or their legal guardians) signed informed consent according to the Institutional Review Board of the National, Heart, Lung, and Blood Institute. For protocol entry purposes, SAA was defined as bone marrow cellularity of <30% and severe pancytopenia satisfying at least 2 of the following peripheral blood count criteria: (1) absolute neutrophil count (ANC) <500 cells/μL; (2) absolute reticulocyte count (ARC) <60,000 cells/μL; and (3) platelet count <20,000 platelets/μL [4]. Response was defined as no longer meeting criteria for SAA and was determined at 3 and 6 months after ATG [4].

Study Design and SAA Treatment Regimens

We retrospectively reviewed the records of 174 patients with SAA treated at the Hematology Branch who were unresponsive to initial IST from 1989 through 2008. Three patient cohorts were defined by date of implementation of the different immunosuppression protocols of similar duration at our institution: 43 patients treated from December 1989 through October 1996 (group 1), 51 patients treated from November 1996 through October 2002 (group 2), and 80 patients treated from November 2002 through April 2008 (group 3). Four ATG-based regimens were used, as previously reported: horse ATG (h-ATG)/CsA [4], h-ATG/CsA/mycophenolate mofetil (MMF) [12], h-ATG/CsA/sirolimus [11], and rabbit ATG (r-ATG)/CsA [15].

The 3 h-ATG regimens have yielded virtually identical outcomes [4, 11, 12]. In addition, 2 non–ATG-containing regimens were used, as previously described: cyclophosphamide/CsA [13] and alemtuzumab [16]. The majority of patients received an ATG-based regimen ($n = 154$), whereas only 20 received a non–ATG-containing regimen. G-CSF and prophylactic antifungal or antibacterial agents were not administered in the IST protocols.

Statistical Analysis

Summary statistics including mean values, proportions, and their corresponding standard errors were used to describe patients' age, sex, and other baseline characteristics. To evaluate differences in baseline characteristics, response rates, and survival over time, patients were divided into 3 groups based on the years when they received their initial IST. *P* values based on multiple-sample tests for proportions, analysis of variance F-tests and log-rank tests in survival analysis were used to compare patients' baseline characteristics and survival probabilities across the 3 time periods. Because all patients had been followed up for at least 12 months, the effects of covariates on the probability of mortality within 12 months were analyzed using multivariate logistic regression models, as well as Cox proportional hazard models. Probabilities of long-term survival for patients with different time periods for receipt of the first IST and other baseline risk factors were evaluated using Kaplan-Meier estimates and Cox proportional hazard models. Subgroup analyses were performed separately for IST responders, non-responders, and patients who received antifungal agents. Differences in proportions of causes of bloodstream infections between groups were performed with Fisher's exact test. Numerical results were computed by S-PLUS statistical package (Insightful).

RESULTS

Among 420 patients who received IST for SAA from 1989 through 2008 according to different treatment protocols, 174 were non-responders; their characteristics are shown in Table 1.

Bacterial Infections

Results of blood cultures were compared among groups 1–3; organisms that were isolated over time are shown in Figure 1. To avoid overrepresentation of isolates from individuals with multiple cultures of the same organism, blood cultures that were repeatedly positive for the same species in the same patient were counted only once. There was no apparent change in overall distribution of bacterial infections or resistance patterns that would account for improved survival in SAA.

During the past 2 decades, the following patterns of bloodstream infections occurred: decreased prevalence of infections due to coagulase-negative *Staphylococcus* species (from 53% to 25%; relative risk = 2.14; 95% CI, 1.34–3.42; $P = .003$),

Table 1. Patient Characteristics of Nonresponders to Immunosuppressive Therapy

Variable	All patients		Group 1 (1989–1996)		Group 2 (1996–2002)		Group 3 (2002–2008)		*P*[a]
	No. (%) of patients	Mean value ± SE	No. (%) of patients	Mean value ± SE	No. (%) of patients	Mean value ± SE	No. (%) of patients	Mean value ± SE	
Total	174		43		51		80		
Age, years		38.9 ± 1.5		46.0 ± 2.9		37.7 ± 2.7		35.4 ± 2.4	.019
Sex									
Male	103 (59)		22 (51)		32 (63)		49 (61)		.732
Female	71 (41)		21 (49)		19 (37)		31 (39)		
Baseline (/uL)									
ANC		290 ± 23		253 ± 44		279 ± 42		320 ± 36	.019
ANC <200	82 (47)		23 (53)		19 (37)		40 (50)		.705
ALC		1143 ± 43		1072 ± 99		1120 ± 81		1199 ± 57	.138
ARC		14,304 ± 1148		12,505 ± 1.789		14,012 ± 1,983		15,554 ± 1,979	.500
Platelet count		10,094 ± 650		10,283 ± 1003		12,040 ± 1589		8662 ± 791	.052
Salvage therapy									
Repeat IST	72 (41)		1 (2)		22 (42)		49 (61)		
HSCT	43 (25)		3 (7)		14 (26)		28 (35)		

NOTE. Baseline values are given. ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ARC, absolute reticulocyte count; IST, immunosuppressive therapy; HSCT, hematopoietic stem cell transplantation; SE, standard error.

[a] Log (X + 1) transformations for X=ANC, ALC, ARC, platelet count were used for calculating the *P* values.

increased prevalence of infections due to gram-positive bacilli (from 2.3% to 15%; relative risk = 0.15; 95% CI, 0.03–1.34; P= .03), and no significant change in the prevalence of infections due to gram-negative bacilli (from 46% to 41%; relative risk = 1.13; 95% CI, 0.74–1.70; P = .70). The distribution of bacteria and *Candida* species at the species level as causes of bloodstream infections is summarized in Table 2.

Antibiotic susceptibility profiles of bacterial isolates recovered from patients in groups 1–3 over the 20-year period are presented in Table 3. An increase in the pattern of resistance developed over time in *Pseudomonas* species and *Klebsiella* species against ciprofloxacin, ceftazidime, imipenem, and tobramycin. Resistance to ciprofloxacin occurred similarly in *Escherichia coli*. No carbapenemase-producing *E. coli* or *Klebsiella* species were identified in this cohort. Among the gram-positive bacteria, *Enterococcus faecium* emerged as vancomycin resistant in group 2.

IFIs

A marked decrease in microbiologically documented fungal pneumonia and sinusitis occurred after 2000 (Table 4). Fungal pneumonias decreased from 30% to 3% (P<.001), whereas fungal sinusitis decreased from 16% to 5% (P = .05). *Aspergillus* species were the most common fungal pathogens recovered in this patient population.

Overall Survival

Figure 2 presents survival by Kaplan-Meier estimates for all patients with SAA (n = 420) who were responders (n = 246) or non-responders (n =174) to IST. The overall 5-year survival among all patients (n = 420) with SAA increased from 64% in group 1 to 79% in group 3 (P<.001; Figure 2). There was no difference in survival among responders to IST (n =246) between groups (Figure 2). However, there was a significant increase in survival observed among non-responders to IST (n =174), with 5-year survival increasing from 23% in group 1 to 57% in group 3 (P<.001; Figure 2).

When 6-month survival during the neutropenic phase was analyzed separately, a similar improvement was observed, with survival increasing from 61% in group 1 to 82% in group 2 and 89% in group 3 (P<.001; Figure 2). Usage of antifungal agents was distinct in the protocol-specified time period groups: in group 1, antifungal therapy was primarily with deoxycholate amphotericin B (DAmB); in group 2, therapy was primarily with lipid formulations of amphotericin B in the early phase and voriconazole in the later phase; and in group 3, therapy was predominantly with voriconazole. Therefore, the improved 6-month survival observed in groups 2 and 3 corresponds to the introduction of liposomal formulations of amphotericin B and voriconazole in these groups, respectively, during the period of persistent pancytopenia. IFI was commonly diagnosed within the first 6

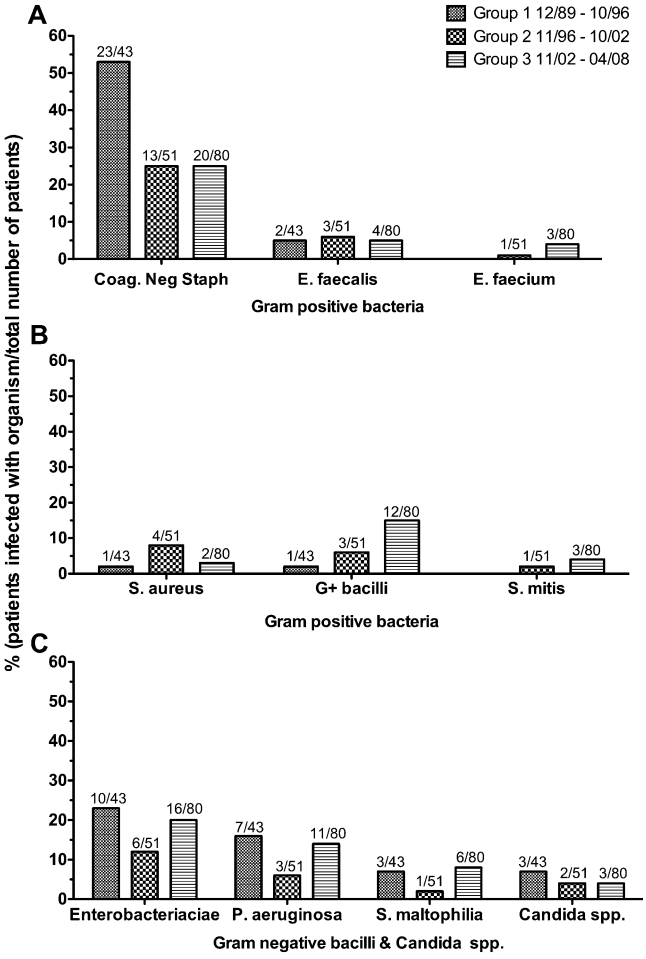

Figure 1. Organisms identified in blood cultures from specimens obtained from patients with severe aplastic anemia in the 3 different study groups. Cultures from the same patient that were repeatedly positive for the same organism were counted only once to avoid overrepresentation. The absolute number of occurrences of each organism is shown as the numerator above each bar, with the total number of patients for each time period shown as the denominator.

months after immunosuppressive therapy. No substantial change occurred in this pattern across the study cohorts.

Among non-responders to IST, infection-related mortality decreased from 37% (16 of 43 patients) in group 1 to 11% (9 of 80 patients) in group 3 ($P<.001$). In a logistic regression model, factors that were independently associated with 1-year mortality in multivariate analysis of non-responders to IST were older age, pretreatment ANC <200/μL, and IFIs (Table 5). To understand the effect of specific antifungal agents, multivariate analysis using DAmB compared with voriconazole was performed in patients who received a diagnosis of IFIs. The use of voriconazole (primarily in group 3) was independently associated with better 1-year survival, compared with survival among those who received DAmB, which was administered primarily to those in group 1 (Table 5). Thus, infectious diseases supportive care with newer antifungal agents contributed to the superior 1-year survival rate in recent years among patients with SAA who were unresponsive to initial IST.

That patients with unresponsive SAA were supported more effectively in groups 2 and 3 also enabled them to survive to receive hematologic salvage therapies. Although 6-month survival among those with unresponsive SAA was significantly improved in both groups 2 and 3 (Figure 2), 5-year survival (Figure 2) was significantly better in those unresponsive patients in group 3. We therefore sought to understand the effect of hematological salvage therapies in groups 2 and 3. Hematologic salvage therapies consisted of a second cycle of IST or allogeneic HSCT, which were implemented more frequently in groups 2 and 3 because of the development of salvage protocols in these time periods for those who experienced failure of an initial course of IST. In group 2, ~70% (36 of 51) of the patients who experienced failure of initial IST underwent hematological salvage therapy, and in group 3, 96% (77 of 80) of the patients underwent salvage therapies (Table 1). In contrast, only 9% (4 of 43) of the patients underwent salvage therapies in group 1. In group 2, 13 patients (36%) had successful hematological salvage (ie, responded to repeat IST or are alive after HSCT), and in group 3, 42 patients (55%) had successful salvage therapy.

DISCUSSION

This study of a large cohort of patients with SAA demonstrates that, among those who are unresponsive to initial IST, survival has markedly improved during the past 20 years. This improvement in overall survival has occurred in conjunction with decreased infection-related mortality and decreased frequency of IFIs. Multivariate analysis identified younger age, ANC >200/μL before IST, absence of IFI, and use of voriconazole as independent variables associated with survival. Occurrence of bacteremia without IFI was not predictive of mortality.

In assessing potential risk factors for the development of infection across the 3 groups, a significant difference is observed in Table 1 for ANC, from a mean of 253 cells/μL to a mean of 320 cells/μL. However, this difference is unlikely to be biologically significant. Instead, the proportion of patients with an ANC <200 cells/μL, which is a powerful predictor of infections in neutropenic hosts [17], did not differ among the groups over time.

The severe morbidity and mortality associated with infectious complications in patients with SAA before 1990 were well described, with the greatest mortality caused by invasive aspergillosis [5]. The current study demonstrates an approximately 5-fold reduction in the frequency of IFIs in SAA, from 49% in group 1 to 8% in group 3. This reduction is predominantly

Table 2. Bloodstream Isolates in Patients with Severe Aplastic Anemia

Microbiological organism	Total (*n*=174)	Group 1 (*n*=43)	Group 2 (*n*=51)	Group 3 (*n*=80)
Coagulase-negative Staphylococcus species	56	23	13	20
Unspecified coagulase-negative *Staphylococcus* species	11	5	2	4
Staphylococcus epidermis	31	14	8	9
Staphylococcus haemolyticus	6	3	0	3
Other gram-positive cocci[a]	8	1	3	4
Staphylococcus aureus	7	1	4	2
Enterococcus faecalis	9	2	3	4
Enterococcus faecium	4	0	1	3
Enterococcus species	2	0	1	1
Gram-positive bacilli	16	1	3	12
Unspecified gram-positive bacilli species	4	1	0	3
Corynebacterium species	6	0	1	5
Clostridium ramosum	1	0	0	1
Clostridium speticum	2	0	0	2
Other gram-positive bacilli[b]	3	0	2	1
Enterobacteriaceae	32	10	6	16
Escherichia coli	9	3	1	5
Enterobacter cloacae	5	0	2	3
Enterobacter aerogenes	2	2	0	0
Proteus mirabilis	1	0	0	1
Klebsiella pneumonia	11	4	2	5
Klebsiella oxytoca	3	1	0	2
Fermenter species	1	0	1	0
Stenotrophomonas (Xanthomonas) maltophilia	10	3	1	6
Pseudomonas aeruginosa and non–lactose fermenters	21	7	3	11
Pseudomonas aeruginosa	13	3	2	8
Other *Pseudomonas* species	4	3	0	1
Sphingomonas paucimobilis	1	1	0	0
Capnocytophaga sputigena	1	0	0	1
Acinetobacter species	2	0	1	1
Candida species and other yeasts	8	3	2	3
Candida albicans	3	1	1	1
Candida tropicalis	1	1	0	0
Candida krusei	1	1	0	0
Candida glabrata	2	0	1	1
Malassezia furfur	1	0	0	1
Filamentous fungi- *Alternaria* species	1	0	0	1
Anaerobic bacteria	12	3	4	5
Bacteroides species	3	2	1	0
Leptotrichia species	1	0	0	1
Bifidobacterium species	1	1	0	0
Propionibacterium acnes	8	0	3	5
Atypical mycobacteria[c]	3	0	0	3

[a] *Staphylococcus captitis ureolyticus, Staphylococcus hominis, Micrococcus, Leuconostoc mesenteroides, Stomatococcus mucilaginosus,* and *Gemella morbillorum.*

[b] *Lactobacillus* species, *Brevibacter casei,* and *Rothia mucilaninosus.*

[c] *Mycobacterium chelonae, Mycobacterium porcinum,* and *Mycobacterium mycogenicum.*

Table 3. Susceptibility Profiles of Selected Organisms

Organism and antibiotics	No. of resistant isolates/total isolates (%)		
	Group 1 (*n*=43)	Group 2 (*n*=51)	Group 3 (*n*=80)
Staphylococcus aureus			
Oxacillin	0/1 (0)	0/7 (0)	0/1 (0)
Enterococcus faecium			
Vancomycin	0/0 (0)	13/14 (93)	5/5 (100)
Enterococcus faecalis			
Vancomycin	0/2 (0)	0/2 (0)	0/5 (0)
Pseudomonas species			
Ciprofloxacin	0/5 (0)	3/15 (20)	5/11 (45)
Ceftazidime	0/5 (0)	9/16 (56)	4/11 (36)
Cefepime	NT	0/0 (0)	2/10 (20)
Imipenem	0/6 (0)	11/17 (68)	4/11 (36)
Meropenem	NT	1/2 (50)	4/11 (36)
Tobramycin	0/6 (0)	0/17 (0)	5/11 (45)
Eschercheria coli			
Ciprofloxacin	0/3 (0)	0/1 (0)	4/8 (50)
Ceftazidime	0/3 (0)	1/1 (100)	0/8 (0)
Cefepime	NT	0/0 (0)	0/8 (0)
Imipenem	0/3 (0)	0/1 (0)	0/8 (0)
Meropenem	NT	0/0 (0)	0/8 (0)
Tobramycin	1/3 (33)	1/1 (100)	0/8 (0)
Klebsiella species			
Ciprofloxacin	0/5 (0)	0/2 (0)	2/7 (29)
Ceftazidime	0/5 (0)	2/2 (100)	2/7 (29)
Cefepime	NT	0/0 (0)	2/7 (29)
Imipenem	0/5 (0)	0/2 (0)	0/7 (0)
Meropenem	NT	0/0 (0)	0/7 (0)
Tobramycin	0/5 (0)	2/2 (100)	2/7 (29)
Enterobacter cloacae			
Ciprofloxacin	0/0 (0)	6/10 (60)	0/3 (0)
Ceftazidime	0/0 (0)	10/10 (100)	0/3 (0)
Cefepime	NT	7/8 (88)	0/3 (0)
Imipenem	0/0 (0)	0/10 (0)	0/3 (0)
Meropenem	NT	0/8 (0)	0/3 (0)
Tobramycin	0/0 (0)	8/10 (80)	0/3 (0)

NOTE. NT, not tested.

related to a marked diminution in the frequency of invasive pulmonary aspergillosis. This decrease occurred in the setting of structured management of potential infections, which included empirical antifungal therapy throughout the past 20 years. Antifungal prophylaxis was not used for antimicrobial supportive care in these patients.

IFIs in persistently neutropenic patients are difficult to diagnose. At the same time, early initiation of antifungal therapy improves outcome. Pizzo et al [18] described the rationale for empirical antifungal therapy as providing early treatment for patients with clinically suspected IFIs and prevention of mycoses in persistently neutropenic hosts. Since the use of empirical antifungal therapy with DAmB for persistently febrile neutropenic patients was introduced in group 1 [18, 19], there has been considerable improvement in the safety and tolerability of the newer antifungal agents used in groups 2 and 3. Liposomal amphotericin B was introduced in the early period of group 2 [20], and voriconazole [21] was used in the latter portion of group 2 and throughout group 3, both during the investigational period and after approval. The improved safety profile of these drugs permitted their use throughout extended periods of neutropenia in patients, including those with pulmonary infiltrates. Our findings are consistent with those of Cordonnier et al[22], which demonstrated the preventive effects of empirical antifungal therapy in persistently neutropenic patients.

The beneficial effects of this strategy of empirical antifungal therapy also occurred in the context of early and improved diagnostic procedures. Diagnostic CT imaging was commonly used in febrile patients. Patients with SAA and pulmonary infiltrates routinely underwent bronchoalveolar lavage, and those with sinus abnormalities were evaluated by direct endoscopic visualization. Although serum galactomannan and (1→3)-β-D-glucan were studied as investigational biomarkers, they were not used in routine patient care in this SAA population.

Because several variables may have contributed to the improved outcome of IST unresponsive patients with SAA, we conducted a logistic regression analysis of the probability of death within the first year after IST. The logistic regression model demonstrated that older age, ANC <200/μL, and the presence of IFIs were positive predictive covariates for 1-year mortality. Although age and neutropenia are well-known determinants of mortality in SAA [23, 24], to our knowledge, this is the first report describing the independent impact of IFIs in a logistic regression model. We then further explored whether newer antifungal therapy was an independent predictor of improved outcome among the patients receiving those compounds. In comparison with patients with IFIs who received DAmB (mainly those ingroup 1), those who received voriconazole (mainly those in group 3) had a significantly improved outcome, with approximately 12-fold odds ratio as an independent variable. Also notable in this higher risk group receiving antifungal therapy is the importance of neutropenia, which had a 13-fold odds ratio for mortality.

The role of improved antifungal supportive care is more clearly defined in the first 6 months following IST among nonresponders, because alternative modalities that may confer better survival, such as repeat IST and/or HSCT, were not routinely employed. The improvement in survival beyond 6 months also benefited from repeat IST and/or HSCT introduced primarily in groups 2 and 3 (Table 1). Improved infectious disease supportive care during the 6-month period

Table 4. Infections and Causes of Death in Patients with Severe Aplastic Anemia

Variable	Total(n=174)	Group 1(n=43)	Group 2(n=51)	Group 3(n=80)
Infection				
Pneumonia				
Bacterial[a]	6 (3)	3 (7)	2 (4)	1 (1)
Fungal[b]	18 (10)	13 (30)	3 (6)	2 (3)
Viral	0 (0)	0 (0)	0 (0)	0 (0)
Sinusitis				
Bacterial[c]	4 (2)	0 (0)	0 (0)	4 (5)
Fungal[d]	12 (7)	7 (16)	1 (2)	4 (5)
Mucocutaneous HSV	20 (11)	0 (0)	13 (25)	7 (9)
Viral respiratory infections[e]	6 (3)	0 (0)	3 (6)	3 (4)
GI parasitic infections[f]	18 (10)	6 (14)	8 (16)	4 (5)
Pulmonary tuberculosis	2 (1)	2 (5)	0 (0)	0 (0)
Ecthyma				
Bacterial[g]	1 (1)	1 (2)	0 (0)	0 (0)
Fungal[h]	1 (1)	1 (2)	0 (0)	0 (0)
Meningitis[i]	2 (1)	0 (0)	0 (0)	2 (3)
Total deaths	84 (48)	37 (86)	25 (49)	22 (28)
Infection-related deaths[j]	39 (22)	16 (37)	14 (27)	9 (11)
Fungal[j]	17 (10)	8 (19)	7 (14)	2 (3)
Sepsis	16 (9)	4 (9)	6 (12)	6 (8)
Pneumonia	6 (3)	4 (9)	1 (2)	1 (1)
Hemorrhage	6 (3)	1 (2)	2 (4)	3 (4)
MDS/leukemia	6 (3)	1 (2)	2 (4)	3 (4)
HSCT	16 (9)	5 (12)	4 (8)	7 (9)

NOTE. Causes of death were attributed by the patient's primary care physician. Causes of death not confirmed to be infectious, secondary to other co-morbidities, surgeries, accidents or of unknown causes are not depicted.GI, gastrointestinal tract; HSCT, hematopoietic stem cell transplantation;HSV, herpes simplex virus; MDS, myelodysplasia.

[a] *Stenotrophomonas maltophilia* (2), *Pseudomonas aeruginosa* (3), and *Mycobacteria avium* (1).

[b] *Aspergillus* species (4), *Aspergillus fumigatus* (1), *Aspergillus flavus* (1), *Aspergillus versicolor* (1), *Aspergillus terreus* (2), *Acremonium* species (1), *Zygomycetes* (3), *Fusarium* (3), *Cryptococcus neoformans* (1), and *Mucor* species (1).

[c] *Stenotrophomonas maltophilia* (3) and *Achromobacter xylosxidans* (1).

[d] *Aspergillus* specis (3), *A. flavus* (4), *A. terreus* (1), *Fusarium* species (3), and *Alternaria* species (1).

[e] Respiratory syncytial virus (1), influenza type A (2), and adenovirus (1).

[f] *Ascaris lumbroicoides* (3), Hookworm (1), *Trichuris trichuiuria* (2), *Giardia lamblia* (4), *Blastocystis hominins* (6), and *Entamoeba coli* (2).

[g] *Stenotrophomonas maltophilia* (1).

[h] *A. fumigatus* (1).

[i] *Eschercheria coli.*

[j] $P< 0.01$

of severe neutropenia allowed IST-unresponsive patients with SAA to survive to receive these salvage hematological therapies. Therefore, the ability to maintain patients alive with persistent pancytopenia after IST and the higher success of hematological salvage therapies in recent years resulted, among patients with unresponsive SAA, in the best survival for those in group 3.

Bacterial infections also pose a serious threat to patients with SAA. Despite the emergence of resistant bacteria, our limited antimicrobial armamentarium has allowed successful treatment. Gram-positive cocci represented the preponderance of bacterial isolates in our cohort. Although we considered that *Corynebacterium* species and coagulase-negative staphylococci maybe contaminants, we considered all bloodstream bacterial isolates as true pathogens in these persistently neutropenic hosts. The frequency of gram-negative bacillary bacteremias in patients with SAA has been relatively constant. However, the trends in antibacterial resistance reflect those that have occurred nationwide [25, 26]. Among the cases of *Pseudomonas* infection in group 1, virtually all were initially susceptible to third-generationcephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. Since 1996, that secular trend shows more resistance among *P. aeruginosa* isolates.

With structured antimicrobial therapy and careful antibiotic stewardship, our initial regimen for new onset of fever in patients with SAA has remained ceftazidime for >20 years. A back-

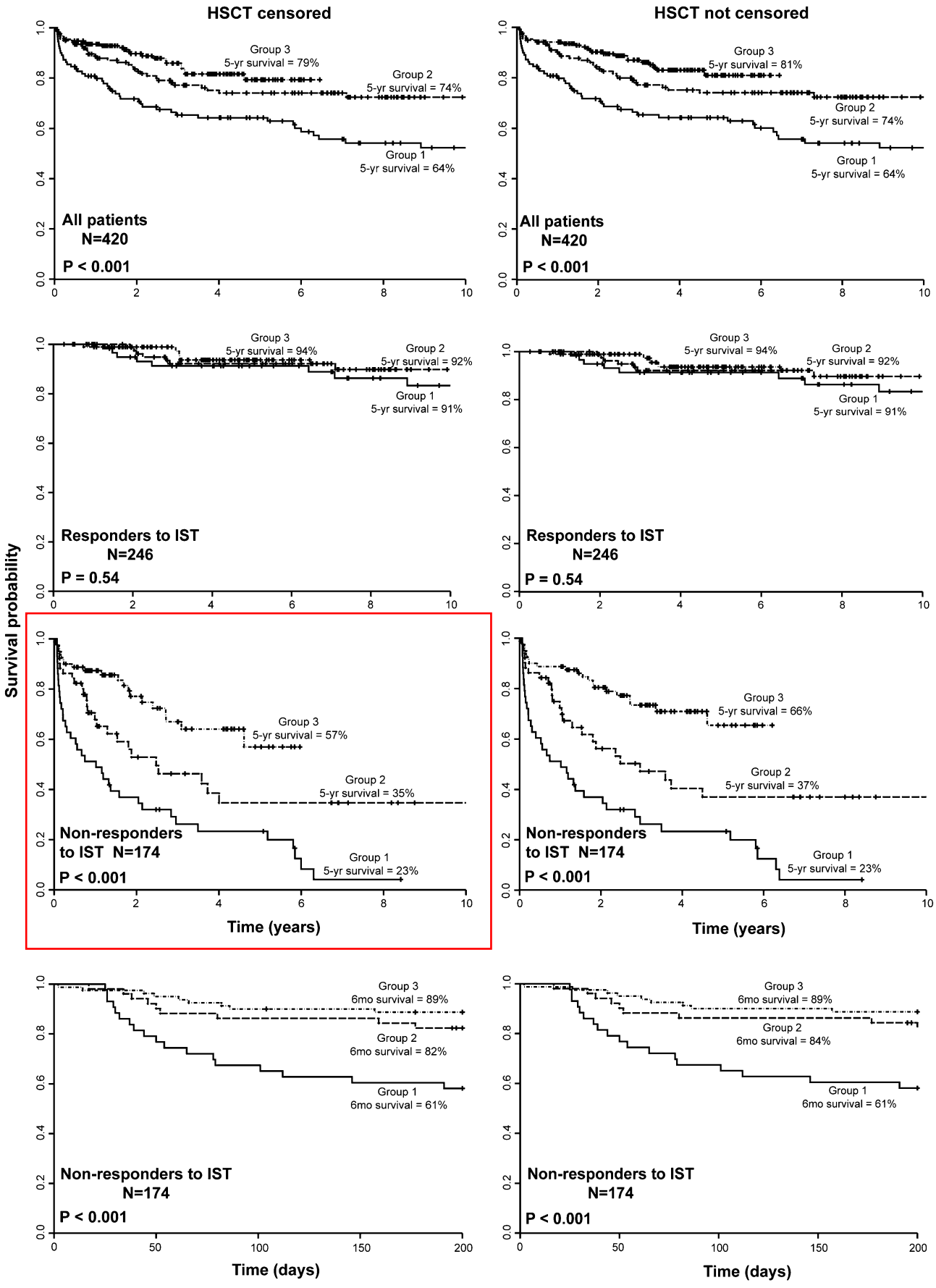
HSCT censored
HSCT not censored
Survival probability
Group 3
5-yr survival = 79%
Group 2
5-yr survival = 74%
Group 1
5-yr survival = 64%
All patients
N=420
P < 0.001
Group 3
5-yr survival = 81%
Group 2
5-yr survival = 74%
Group 1
5-yr survival = 64%
All patients
N=420
P < 0.001
Group 3
5-yr survival = 94%
Group 2
5-yr survival = 92%
Group 1
5-yr survival = 91%
Responders to IST
N=246
P = 0.54
Group 3
5-yr survival = 94%
Group 2
5-yr survival = 92%
Group 1
5-yr survival = 91%
Responders to IST
N=246
P = 0.54
Group 3
5-yr survival = 57%
Group 2
5-yr survival = 35%
Group 1
5-yr survival = 23%
Non-responders
to IST N=174
P < 0.001
Time (years)
Group 3
5-yr survival = 66%
Group 2
5-yr survival = 37%
Group 1
5-yr survival = 23%
Non-responders
to IST N=174
P < 0.001
Time (years)
Group 3
6mo survival = 89%
Group 2
6mo survival = 82%
Group 1
6mo survival = 61%
Non-responders to IST
N=174
P < 0.001
Time (days)
Group 3
6mo survival = 89%
Group 2
6mo survival = 84%
Group 1
6mo survival = 61%
Non-responders to IST
N=174
P < 0.001
Time (days)

Table 5. Multivariate Logistic Regression Analysis of the Probability of Death within 1 Year for Patients Unresponsive to Initial Immunosuppressive Therapy

Risk factor	Effect of covariate and risk of death[a]				Effect of antifungal therapy[b]			
Baseline risk	Coefficient (β)	SD	OR	*P*	Coefficient (β)	SD	OR	*P*
Age	0.033	0.012	1.034	.004	0.064	0.024	1.066	.007
ANC <200 cells/μL	2.056	0.633	7.818	.001	2.623	1.234	13.777	.034
ARC	0.114	0.234	1.120	.627	…	…	…	…
ALC	-.297	0.399	0.743	.457	…	…	…	…
Platelet count	-.096	0.267	0.908	.719	…	…	…	…
Bacteremia only	0.919	0.542	2.508	.090	…	…	…	…
Fungal infection	2.751	0.665	15.666	<.001	…	…	…	…
Voriconazole	…	…	…	…	-2.550	0.967	0.078	.008

NOTE. The bacteremia-only group did not include patients with a concomitant fungal infection. Fungal groups were those who had a fungal isolate identified in blood, secretions, and/or tissue (seeTable 4). The amphotericin B group does not include liposomal formulations. Voriconazole refers to any voriconazole-containing regimen. Natural log-transformed counts log(ANC+1), log(ARC+1), log(ALC+1), and log(platelet+1) were used to reduce the skewness of these variables. ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ARC, absolute reticulocyte count; OR, odds ratio; SD, standard deviation.

[a] Includes age, ANC, ARC, ALC, Platelet count, bacteremia-only infection, and fungal infection.

[b] After fungal infection was found to be an independent variable predicting probability of death, a separate analysis was performed to assess the effect of voriconazole vs amphotericin B as a predictor of death (includes age, ANC, and antifungal therapy defined by 0 if amphotericin B was used and 1 if a voriconazole-containing regimen was used). There were insufficient numbers of patients receiving echinocandins to be included in this analysis. Covariates not statistically significant in the first analysis were not included in the antifungal analysis.

up regimen consisting of carbapenem, aminoglycoside, and vancomycin is used for suspected resistant bacteria. *Enterobacter cloacae* expressing class C or type 1 stably derepressed β-lactamase emerged as an important problem in group 2 (1996–2003), but subsequent isolates have been uniformly susceptible to ceftazidime. Increasing resistance is observed, however, among-*Klebsiella* species isolates, with the appearance of extended-spectrum β-lactamase (ESBL) beginning in 1996 in group 2 and continuing into the present. Carbapenemase-producing *Klebsiella pneumoniae* has not been observed in our population, and thus, carbapenems remain the mainstay for treating refractory infections or infections due to ESBL strains. Consistent standards of infectious diseases supportive care and careful antibiotic stewardship are continually maintained to avoid the emergence of highly drug-resistant bacteria.

Our analysis has the limitation of being a retrospective study of a prospectively observed patient population. The study analyzes clinical outcomes for patients treated over a 20-year period in different treatment protocols for SAA. Cultural, socioeconomic, and referral patterns may have varied during the same period. This study provides an assessment of both overall mortality and attributable mortality over time. Attributable mortality was determined by the treating physician at the time of death. Attribution of mortality by the bedside physician carries advantages in being prospective and determined by the provider most knowledgeable of the various factors contributing to death. Although a uniform definition of attributable mortality prospectively used over a 20-year period would be desirable, the causes of death in IST-refractory SAA usually results from complications of pancytopenia (hemorrhage or infection). However, despite these limitations, we believe that our observations are valid in a uniformly defined protocol-based patient population in which the overall strategies of infectious diseases supportive care have been consistently applied.

In summary, this study demonstrates that patients with IST-unresponsive SAA attained a marked improvement in survival in association with a significant decrease in infectious diseases–related mortality and frequency of IFIs, particularly invasive aspergillosis. These improved outcomes have paralleled advances in infectious diseases supportive care, especially antifungal therapy with voriconazole. The impact of infectious diseases supportive care in IST-unresponsive SAA serves as

Figure 2. Improvement in survival over time. Five-year survival is depicted for all patients (*first row*), responders to immunosuppressive therapy (IST) (*second row*), and non-responders to IST (*third row*). Six-month survival, as a surrogate for neutropenia, in non-responders to IST is presented in the fourth row. The first column depicts hematopoietic stem cell transplantation (HSCT)–censored Kaplan-Meier survival curves and the second column HSCT non-censored curves. Overall survival for all patients was improved in group 3 (*first row*) compared with groups 1 and 2. Among responders to immunosuppressive therapy, no difference in survival was noted over time (*second row*). Among non-responders, a marked improvement in survival occurred in group 3, compared with survival in groups 1 and 2 (*third row*). The 6-month survival after IST among non-responders was also markedly higher in group 1 than in group 3 (*fourth row*).

a potential model for the benefits of such strategies in other neutropenic patient populations.

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