Pulmonary complications in patients receiving granulocyte transfusions and amphotericin B

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To evaluate the possibility that in febrile granulocytopenic patients amphotericin B given along with granulocyte transfusions could increase the incidence of pulmonary complications, we studied 43 severely granulocytopenic patients during 46 episodes of fever. Granulocytes were administered as part of the clinical protocol to all 19 patients who had clinically or microbiologically documented infection; the other 24 patients were randomly allocated to treatment with granulocytes (13 patients) or without granulocytes (11 patients). In all, 32 patients received granulocyte transfusions during 35 episodes of fever. Pulmonary complications developed in six patients in each of the two randomized groups. The incidence of pulmonary complications was not influenced by the number of granulocyte transfusions or by the number of granulocytes per transfusion. Pulmonary complications were significantly more likely to occur in patients with fungal infections. Amphotericin B was given according to clinical indications; 21 patients in all received it. Survival was significantly poorer in patients with pulmonary complications, but the administration of amphotericin B was not related either to survival or to the incidence of pulmonary complications. We conclude that pulmonary complications and poor prognosis are related to underlying pulmonary fungal infection and not to any interaction between amphotericin B and granulocyte transfusions.

Afin d'évaluer si, chez le patient fébrile granulocytopénique, l'administration concomitante d'amphotéricine B et de transfusions de granulocytes peut augmenter l'incidence des complications pulmonaires, on a étudié 43 patients en granulocytopénie profonde au cours de 46 épisodes de fièvre. À l'intérieur d'un protocole clinique des granulocytes ont été transfusés chez 19 patients souffrant d'une infection vérifiée par des examens cliniques ou microbiologiques; les 24 autres patients ont été assignés au hasard à un traitement par transfusion de granulocytes (13 patients) ou sans transfusion de granulocytes (11 patients). En tout, 32 patients ont reçu des transfusions de granulocytes au cours de 35 épisodes de fièvre. Des complications pulmonaires sont apparues chez six patients de chacun des deux groupes randomisés. L'incidence des complications pulmonaires n'a pas été affectée par le nombre de transfusions granulocytaires ou par le nombre de granulocytes par transfusion. Les complications pulmonaires étaient significativement plus susceptibles de se produire chez les patients souffrant d'infection fongique. L'amphotéricine B fut administrée selon indication clinique; 21 patients au total en ont reçu. La survie a été significativement moins bonne chez les patients souffrant de complications pulmonaires; toutefois, l'administration d'amphotéricine B n'a été associée ni à la survie ni à l'incidence des complications pulmonaires. Nous concluons que les complications pulmonaires et le mauvais pronostic sont reliés aux infections fongiques pulmonaires sous-jacentes et non à une interaction entre l'amphotéricine B et les transfusions de granulocytes.

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Granulocyte transfusions have been useful adjunctive therapy for febrile granulocytopenic patients with sepsis that fails to respond to therapy with broad-spectrum antibiotics.[1-5] Similarly, because fungal superinfection is frequent in these patients, amphotericin B has been used empirically in such circumstances.[6] Not surprisingly, a combination of these second-line therapies has been used to treat presumed sepsis in this high-risk population. Recently there has been some concern that this combination may contribute to the development of pulmonary complications and produce excessive mortality.[7] We report our observations on the incidence of pulmonary complications and the short-term survival among febrile granulocytopenic patients receiving granulocyte transfusions with and without the administration of amphotericin B.

Patients and method

Patients

Patients with granulocytopenia (fewer than 10^9 cells/L) were eligible for inclusion in the study and

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were managed according to a prospective investigative protocol. Afebrile patients with granulocytopenia (not reported here unless fever developed) received trimethoprim, trimethoprim–sulfamethoxazole or nalidixic acid as antibacterial prophylaxis, with or without being given nystatin orally as antifungal prophylaxis. Febrile patients were treated empirically with an aminoglycoside and ticarcillin (or cefazolin if the patient was allergic to penicillin).

Patients became eligible for granulocyte transfusions if severe granulocytopenia (fewer than 0.1×10^9 cells/L) and fever (temperature above 38°C) developed and if they had been unresponsive to broad-spectrum antibiotic therapy given for at least 72 hours. Granulocytes were given if an infection was documented either microbiologically or clinically. If there was no focus of infection the patient was randomly allocated to either receive granulocytes or be a control subject. All gave informed consent.

Procurement of granulocytes

Granulocytes were harvested from healthy, unrelated volunteers by discontinuous flow centrifugation using the Haemonetics model 30 cell separator. The sedimenting agent was hydroxyethyl starch (6%). Each donor was ABO-compatible with a particular patient and was selected, if at all possible, to have at least two of the HLA (histocompatibility) antigens found in that patient. If this was not feasible, donors with antigens cross-reactive to those of the patients were chosen. The results of lymphocytotoxic and erythrocyte cross-matches were negative in all cases. Dexamethasone (6 mg) was given orally 6 to 12 hours before leukapheresis.

Evaluation

On a patient's entry into the study infections were classified as microbiologically documented if a pathogen was identified either in culture or in a histopathological preparation from an infected focus, as clinically documented if no pathogen was recovered, and as a possible infection if, in the absence of other causes of fever (such as underlying disease, drugs or transfusion with blood products), the febrile illness was compatible with infection but had no identifiable focus. Some possible infections were eventually documented in the course of the study.

Patients were followed up for 21 days after becoming eligible for granulocyte transfusions to assess their survival of the episode, the type of infection, the response to treatment and any complications of therapy. Pulmonary complications were defined as either the appearance of new pulmonary infiltrates or the clear progression of pre-existing pulmonary infiltrates on serial chest x-ray films after the patient had become eligible for granulocyte transfusions. In each case follow-up chest roentgenography was done in response to a change in the patient's clinical status. Patients were considered as having survived the episode of infection if they lived at least 21 days after becoming eligible for granulocyte transfusions.

We assessed differences in proportions by chi-square analysis (using Yates's correction for continuity when appropriate) and Fisher's exact test. The *t*-test was used to compare the difference between means at $p < 0.05$.

Study population

Forty-three patients were eligible for evaluation during 46 episodes of prolonged, life-threatening granulocytopenia. Infections were clinically or microbiologically documented in 19 patients (22 episodes). All 19 received granulocyte transfusions during each episode. A second group of 24 patients (24 episodes) had possible infections; 13 were randomly allocated to receive granulocyte transfusions and 11 to be controls. Accordingly, granulocyte transfusions were administered to a total of 32 patients during 35 granulocytopenic episodes.

Three with acute nonlymphocytic leukemia had a second episode of fever with documented infection and received granulocytes. Only the second episodes in these patients were considered in the analysis of survival.

Overall, the patients had received broad-spectrum antibiotics for a mean (and standard deviation [SD]) of 7.1 ± 3.7 days before entering the study. The patients with documented infections had received slightly longer courses of empiric antibiotic therapy (9.2 ± 5.4 days) than had the patients with possible infections (6.0 ± 2.0 days). This difference was not statistically significant and reflected a skew created by the extended courses of antibiotic therapy given a number of patients with documented infections because a temporary response had been followed by recrudescence of the signs and symptoms of infection. A majority of the patients eligible for granulocyte transfusions, therefore, had received antibiotics for longer than the minimum suggested by the protocol as an indication of failure of empiric antibiotic therapy.

The patients received a mean of 6.4 ± 3.8 granulocyte transfusions, with a mean of $0.87 \pm 0.35 \times 10^{10}$ granulocytes and $6.38 \pm 2.91 \times 10^{11}$ platelets per transfusion. There were no significant differences in age, sex or underlying illness between the groups with documented and possible infections. Similarly, there were no significant differences in the number of granulocyte transfusions or the number of granulocytes per transfusion between the group with documented infections and the group with possible infections who received granulocytes.

Results

Pulmonary complications were observed in 18 (51.4%) of the 35 febrile episodes treated with granulocyte transfusions. The groups with and without such complications were comparable with respect to age, sex and underlying illness (Table I). Similarly, there were no significant differences between these groups in the number of transfusions or the number of granulocytes per transfusion. Prolonged life-threatening granulocytopenia due to acute leukemia was associated with 77% of the episodes.

Table II shows the differences in the types of infections documented in the two groups of patients. Documented fungal infections were significantly more frequent ($p = 0.008$) in the group with pulmonary complications than in the group without

such complications, occurring in 10 of 18 as compared with 2 of 17 febrile episodes. Of the former 10 episodes 6 were due to *Aspergillus* pneumonia (1 involved both *Aspergillus* and *Candida albicans*), 2 were due to *C. albicans* pneumonia associated with multisystem involvement detected at postmortem examination, and 2 were due to *C. albicans* fungemia associated with pulmonary infiltrates. Of the remaining eight episodes in the group with pulmonary complications six were due to pneumonia for which no pathogen was found, one was in a patient with clinically documented cellulitis, and one was in a patient with disseminated herpes zoster.

Documented bacterial infections were significantly more frequent ($p = 0.008$) in the group without pulmonary complications than in the group with such complications, occurring in 6 of 17 as compared with none of 18 episodes. Five of these six infections were bacteremias (two due to *Staphylococcus epidermidis*, one to a viridans group *Streptococcus*, one to *Escherichia coli* and one to *Klebsiella pneumoniae*) and one was a perirectal abscess due to *Pseudomonas aeruginosa*. One of the two fungal infections in the group without pulmonary complications was *C. albicans* pneumonia documented at postmortem examination; the other was disseminated *C. tropicalis* infection in which the organism was recovered from the blood and urine and through biopsies of several erythematous maculopapular skin lesions. Of the remaining nine febrile episodes in this group five were due to clinically documented pneumonia, one was due to a clinically documented perirectal abscess, and the other three were classified as presumed, for no focus or pathogen was identified.

Table II further shows that of the 17 infection-related deaths 9 (53%) were due to documented fungal infection. A higher proportion of patients died of fungal infection in the group with pulmonary complications than in the group without such complications (8 of 12 v. 1 of 5), but the difference was not statistically significant.

There were pulmonary complications in 12 (57%) of the 21 episodes treated with a combination of amphotericin B and granulocytes, and in 6 (43%) of the 14 episodes treated with granulocytes alone. The difference was not significant.

Among the patients treated with amphotericin B and granulocytes no significant differences were observed between the groups with and without pulmonary complications in the total dose of amphotericin B, the number of transfusions, the number of granulocytes per transfusion, or the sequence of drug and granulocyte administration (Table III). Similarly, among the patients who did not receive amphotericin B no differences were observed between the groups with and without pulmonary complications in the number of transfusions or the number of granulocytes per transfusion.

The impact of pulmonary complications and the use of amphotericin B on the short-term survival of the febrile episode in the 32 transfused patients who had a single granulocy-

Table I—Clinical characteristics of 32 patients and their 35 episodes of sepsis treated with granulocyte transfusions

Characteristic	Mean ± standard deviation (SD) or no. of patients	
	With pulmonary complications (n = 18)	Without pulmonary complications (n = 17)
Age (yr)	52.6 ± 12.4	45.1 ± 20.0
Sex		
Male	9	12
Female	9	5
Underlying illness		
Leukemia		
Acute nonlymphocytic	11	11
Acute lymphocytic	3	2
Chronic lymphocytic	1	1
Non-Hodgkin's lymphoma	1	1
Carcinoma	1	1
No. of granulocyte transfusions	6.2 ± 4.0	6.4 ± 3.4
No. of granulocytes ($\times 10^{10}$) per transfusion	0.86 ± 0.21	0.91 ± 0.14

Table II—Classification of infections in 35 febrile episodes treated with granulocyte transfusions

Classification and type of infection; organisms	No. of patients*	
	With pulmonary complications (n = 18)	Without pulmonary complications (n = 17)
Microbiologically documented	11 (9)	8 (3)
Blood infection		
Gram-negative cocci	0	3
Gram-negative bacilli	0	2 (1)
Candida sp.	2 (1)	0
Pneumonia		
Candida sp.	2 (2)	1 (1)
Aspergillus sp.	6 (5)	0
Skin infection		
Herpes zoster virus	1 (1)	0
Candida sp.	0	1
Perirectal infection		
Pseudomonas aeruginosa	0	1 (1)
Clinically documented	7 (3)	6 (2)
Pneumonia	6 (3)	5 (1)
Skin infection	1	1
Perirectal infection	0	1 (1)
Possible	0	3

*Numbers in parentheses are numbers of deaths caused by infection.

topenic episode is shown in Table IV. A larger proportion of patients in the group without pulmonary complications survived at least 21 days ($p < 0.036$). There was a similar trend in the patients with pulmonary complications who received amphotericin, but this difference was not statistically significant. No such trend was observed in the group without pulmonary complications who received amphotericin.

The 24 patients with possible infection were also evaluated separately. There were no significant differences between the groups receiving and not receiving granulocyte transfusions in the number with pulmonary complications (6 of 13 [46%] and 6 of 11 [55%] respectively), the 21-day survival rate (8/13 [62%] and 7/11 [64%] respectively), the mean time until the abatement of fever (9.1 ± 3.0 days and 12.9 ± 7.0 days respectively) and the number of patients receiving amphotericin B (10 of 13 [77%] and 5 of 11 [43%] respectively). Infections were documented by the end of the 21-day study period in 10 (77%) of the patients who received granulocytes — 3 had fungal pneumonia (due to *Aspergillus* sp. in 2 and *C. albicans* in 1), 2 had clinically documented cellulitis, 2 had clinically documented pneumonia, 2 had bacteremia (due to *S. epidermidis* in 1 and viridans group *Streptococcus* in the other), and 1 had fungemia due to *C. albicans* — and in 6 (55%) of the 11 in the control group — 5 had clinically documented pneumonia, and 1 had pulmonary aspergillosis.

Discussion

The frequency with which adverse reactions occur during granulocyte transfusion therapy ranges from less than 10% to over 75%, depending on the method of granulocyte procurement.[1,4,8-10] Granulocytes harvested by discontinuous flow centrifugation have been associated with adverse reactions in 68% to 72% of patients.[9,10] The most important reactions are pulmonary; these have been reported in over half the patients receiving granulocytes.[9,10] The mechanisms involved have recently been reviewed.[8]

Many severely granulocytopenic patients who fail to respond to broad-spectrum antibiotic therapy are candidates not only for granulocyte transfusion therapy but also for the empiric use of amphotericin B.[6] It has recently been postulated that the combined use of these two forms of treatment is a cause of potentially fatal pulmonary reactions.[7,9] Amphotericin B has been shown to damage granulocyte membranes,[11] interfere with chemotaxis and inhibit chemoluminescence in vitro[12] and, in animal models, to promote the aggregation of leukocytes and their accumulation in the lungs.[13] Wright and colleagues[7] reported that among 22 patients receiving both granulocyte transfusions and amphotericin B, pulmonary complications developed in 14 (64%), while this happened in only 2 (6%) of 33 patients who received granulocyte transfusions alone. These observations have major implications for the management of severely ill patients with granulocytopenia. However, the association of an excessive incidence of pulmonary toxic effects with the combined use of granulocyte transfusions and amphotericin B has not been observed by other investigators.[14]

We did not find any relations between the use of amphotericin B and either the appearance of pulmonary complications or the short-term survival of febrile episodes among our patients receiving granulocytes. Our overall 51% incidence of pulmonary complications was similar to the 57% reported by Winston and associates[9] and the 64% reported by Strauss and coworkers,[10] all of us having used discontinuous flow centrifugation to harvest the granulocytes. Among our patients receiving both amphotericin B and granulocytes, pulmonary complications de-

Table IV—Short-term survival of febrile granulocytopenic patients

	Proportion of patients surviving at least 21 days after onset of febrile episode		
Amphotericin B	With pulmonary complications	Without pulmonary complications	Total
Administered	3/10	6/8	9/18
Not administered	1/6	4/8	5/14
Total	4/16*	10/16*	14/32

*The difference between the groups was significant at $p < 0.05$.

Table III—Amphotericin B and granulocyte administration in patients with and without pulmonary complications

	Mean ± SD			
	Amphotericin B administered		Amphotericin B not administered	
Variable	With pulmonary complications (n = 12)	Without pulmonary complications (n = 9)	With pulmonary complications (n = 6)	Without pulmonary complications (n = 8)
Total dose of amphotericin B (mg)	476 ± 472	514 ± 834	–	–
No. of granulocyte transfusions	7.1 ± 5.3	7.7 ± 4.2	5.5 ± 2.7	5.0 ± 2.2
No. of granulocytes ($\times 10^{10}$) per transfusion	0.89 ± 0.38	0.88 ± 0.58	0.84 ± 0.37	0.98 ± 0.36

veloped in 12 (57%) of 21, an incidence similar to a previous report of 64%.[7] However, the occurrence of pulmonary complications in 6 (43%) of our 14 patients receiving granulocytes alone stands in contrast to the 6% reported by Wright and colleagues.[7] In common with Strauss and coworkers[10] we could not identify a direct quantitative relation between either the number of granulocyte transfusions or the number of granulocytes per transfusion and the development of pulmonary complications or short-term survival, regardless of whether amphotericin B was administered.

Dana and collaborators[14] noted a relation between the development of pulmonary complications and the frequency with which fungi were recovered in culture among patients receiving both granulocytes and amphotericin B. We, too, documented invasive fungal infections in 56% of our patients with pulmonary complications but in only 12% of those without ($p = 0.007$). This suggests that the development of pulmonary complications is related more to underlying fungal infection of the lungs than to the cytotoxic effects of amphotericin B on transfused granulocytes.

The differences between our results and those of Wright and colleagues[7] remain unexplained. It is possible that the excessive incidence of pulmonary toxic effects attributed to amphotericin B was obscured by the large number of fungal pneumonias we observed. Alternatively, we could speculate that volume overload due to the combined administration of amphotericin B and granulocytes suspended in the sedimenting agent, hydroxyethyl starch,[9] was a factor preventing us from recognizing an excessive incidence of pulmonary toxic effects. These considerations seem unlikely, though, in view of the similar incidence rates of pulmonary complications in all our patient groups.

The problem of pulmonary complications in some patients may instead be related to HLA antibodies.[15] Many investigators do not perform leukoagglutinin or lymphocytotoxicity cross-matching before administering granulocytes.[16] We can further speculate that an excessive incidence of pulmonary toxic effects was not apparent because we used granulocytes from donors cross-matched for lymphocytotoxicity.

The role of granulocyte transfusions in the management of febrile granulocytopenic patients is not clear; there appears to be a lack of therapeutic[16] or prophylactic[9,10] efficacy when granulocytes harvested by discontinuous flow centrifugation are transfused. There are also recent suggestions that the risk of transmitting cytomegalovirus infection[9] may be increased. These considerations could make it difficult to justify further intensive study along the lines of our investigation.

We did not find any relation between the combined administration of amphotericin B and granulocytes and the development of pulmonary complications; instead, we identified an association between new pulmonary complications and systemic fungal infections. Accordingly, we feel that in the absence of a positive cross-match for lymphocytotoxicity, hypersensitivity to amphotericin B or acute pulmonary edema, there appears to be no contraindication to the combined use of amphotericin B and granulocytes in febrile, severely granulocytopenic patients.

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