European Organization for Research on Treatment of Cancer Gnotobiotic Project Group Writing Committee: M. Dietrich, W. Gaus, J. Vossen, D. van der Waaij, F. Wendt

Protective Isolation and Antimicrobial Decontamination in Patients with High Susceptibility to Infection

A Prospective Cooperative Study of Gnotobiotic Care in Acute Leukemia Patients. I: Clinical Results

Summary: The efficiency of protective isolation and protective isolation plus gastrointestinal decontamination on the control of infectious complications in patients with decreased defence capacity was investigated prospectively in a cooperative trial with the participation of clinical centers in several European countries (European Organization for Research on Treatment of Cancer, Gnotobiotic Project Group). The study was performed in patients with acute leukemia under remission-induction therapy on the basis of the frequency of such patients in the participating centers. Over a period of five years, 137 cases of acute leukemia were randomly allocated to three different treatment groups as follows: Group A: strict protective isolation in plastic isolation systems or laminar air flow isolators and prophylactic antimicrobial decontamination by non-absorbable antibiotics, Group B: strict isolation alone in one or the other type of isolator, and Group C: routine hospital ward. The results demonstrated that the incidence of contamination and colonization with new bacteria could be decreased significantly by strict protective isolation alone and even more markedly by strict protective isolation and antimicrobial decontamination. They also demonstrated that in Group A there were less frequent episodes of severe infection. More specifically, the incidence of pulmonary infection was reduced significantly in both Groups A and B in comparison with Group C. The remission rate of acute leukemia was higher in patients of Groups A and B versus Group C (69%, 61%, 49%), although this result was not significant. However, the microbiological investigations and the outcome of the study suggest that the techniques of protective isolation and antimicrobial decontamination have to be improved to decrease further the incidence of infection in the compromised host.

Zusammenfassung: Protektive Isolierung und antimikrobielle Dekontamination bei Patienten mit hoher Infektionsgefährdung. Eine prospektive kooperative Studie mit Hilfe der gnotobiotischen Betreuung von Patienten mit akuter Leukämie. I: Klinische Ergebnisse. Die Wirksamkeit der protektiven Isolierung und Isolierung mit zusätzlicher gastrointestinaler Dekontamination zur Beherrschung von infektiösen Komplikationen bei Patienten mit verminderter Abwehrfähigkeit wurde prospektiv in einer Gemeinschaftsstudie unter Beteiligung von einigen klinischen Zentren in einigen europäischen Ländern durchgeführt (European Organisation for Research on Treatment of Cancer, Gnotobiotic Project Group). Die Untersuchung wurde bei Patienten mit akuter Leukämie durchgeführt bei einer zur Remission führenden Therapie. Die Patientenzahl hing von dem in den verschiedenen Zentren zur Verfügung stehenden Krankengut ab. Über einen Zeitraum von fünf Jahren wurden 137 Fälle von drei verschiedenen Behandlungsgruppen randomisiert zugeteilt: Gruppe A (strenge protektive Isolierungssysteme mit Plastikzelten oder Laminar-Airflow-Isoliereinheiten und prophylaktische antimikrobielle Dekontamination durch Antibiotika, die nicht absorbiert werden), Gruppe B (strenge Isolierung als Einzelmaßnahme in einem der betreffenden Isoliersysteme) oder Gruppe C (Routinemäßige Hospitalpflege). Die Ergebnisse zeigen, daß die Inzidenz von Kontamination bzw. Kolonisierung durch neuerworbene Bakterienspezies bei strenger Isolierung allein und noch deutlicher bei einer strengen Isolierung und antibakterieller Dekontamination signifikant herabgesetzt werden konnten. Auch zeigte sich, daß in der Gruppe A weniger häufig schwere Infektionen auftraten. Noch höher signifikant schien das Auftreten von Lungeninfektionen in den beiden Gruppen A und B in Vergleich zu Gruppe C reduziert zu sein. Die Remissionsrate von Patienten mit akuter Leukämie war im Vergleich mit den Gruppen A und B zur Gruppe C höher (69%, 61%, 49%) obwohl dieses Ergebnis statistisch nicht signifikant war. Jedoch lassen die mikrobiologischen Untersuchungen und das Ergebnis der Studie vermuten, daß sowohl die Technik der protektiven Isolierung als auch die der antimikrobiellen Dekontamination verbessert werden müssen, um ein weiteres Absinken der Infektionsinzidenz bei gefährdeten Patienten zu erreichen.

Introduction

The Gnotobiotic Project Group published a "Protocol for an Evaluative Study of the Protective Effect of Isolation Systems and Decontamination in Patients with High Susceptibility to Infection" in 1972 (6). The study lasted four years, randomization of patients being performed from February 1st, 1971 until December 31st, 1974. The data were divided into two parts: the first part consists of the clinical results which are presented in this paper, the second part presenting the detailed microbiological results is in preparation.

Received: 29 December 1976.

Dr. *M. Dietrich,* Clinical Department, Bernhard-Nocht-Institute for Tropical and Nautical Diseases, Hamburg, Germany;

Dr. *W. Gaus,* Department of Documentation and Statistics, University of Ulm, Schloss Wiblingen, Ulm, Germany;

Dr. *J. Vossen,* Department of Pediatrics, University Hospital of Leiden, Rijnsburgenweg 10, Leiden, The Netherlands;

Dr. *D. van der Waaij,* Department of Microbiology, University Hospital of Groningen, Oostersingel 59, Groningen, The Netherlands;

Dr. *F. Wendt,* Department of Medicine, Evangelisches Krankenhaus, Essen-Werden, Pattbergstraße 1–3, D-4300 Essen-Werden, Germany.

This prospective randomised study was undertaken to investigate the efficiency of preventive measures in controlling infection in the compromised host. These measures involved:

1. the use of antimicrobial decontamination in patients treated in protective isolation;
2. the use of protective isolation only;
3. ordinary hospital care.

Antibiotic decontamination under open ward conditions was purposely not included in this study since animal experiments indicate a risk of rapid colonization of the gastrointestinal tract with resistant bacteria from environmental sources in such individuals (15), and subsequently an unfavourable effect on morbidity and mortality. The efficiency of the measures aimed at controlling infection was judged by:

1. the incidence of contamination and colonization of the gastrointestinal tract with either exogenous or endogenous microorganisms;
2. the incidence of infections;
3. the incidence of death from infection.

It was decided to perform the study in patients with acute leukemia because infectious complications are the major cause of morbidity and mortality in such patients (7) and because of the relatively frequent occurence of acute leukemia patients in the units participating in the study.
The reduced resistance to microbial invasion is mainly associated with the degree and duration of granulocytopenia and to a lesser extent with the degree and duration of lymphocytopenia (1). Both conditions are due either to the disease itself or to the cytotoxic chemotherapy. The causative agents of infections originate either from the patient's own (endogenous) microflora, especially from the gut flora, or from the environmental (exogenous) microflora (5).
Prospective randomized studies on protective isolation and antimicrobial decontamination in patients with acute leukemia have been performed previously in the United States of America and produced equivocal results (8, 11, 17). Several earlier studies suggested the possible usefulness of this type of supportive care in patients with acute leukemia (2, 3, 4, 10, 14). However, clinical experience was limited by the small numbers of patients investigated by these groups, so that valid confirmation of the advantage of gnotobiotic care was felt necessary, particularly in view of the additional stress for patients and the high costs involved.

Patients and Methods

Minimal requirements had to be observed by the different participating units, located in four European countries, in order to obtain meaningful comparative data on the efficiency of strict protective isolation either with or without the use of gastrointestinal decontamination.

Selection and Randomization of Patients

All patients between 2 and 60 years of age suffering from either acute myelocytic leukemia, acute lymphocytic leukemia or acute undifferentiated leukemia, who were admitted to the participating clinical departments for remission-induction therapy, were reported to the statistical center. Stratas were formed on the basis of age groups (2–15, 16–40, 41–60 years), presence or absence of infection at admission, and number of remission-induction treatments. The patients were allocated at random to one or other of the following three groups:

Group A: treatment with antimicrobial decontamination in strict protective isolation;

Group B: treatment in strict protective isolation only;

Group C: treatment in an open ward under standard hospital conditions.

Some patients were admitted more than once due to relapse or re-treatment. Each cytotoxic treatment period in a patient was considered to be equivalent to a separate case.
Patients were classified as "not eligible" if they were not between 2 and 60 years (unsuitable for recruitment to the study), if they did not agree to enter randomisation or to be confined to strict protective isolation, or if there were psychological and/or psychiatric contraindications. Some patients were not eligible because their kidney function was impaired as demonstrated by a glomerular filtration rate of less than 60 ml/min/1.73 m^2 body surface. Patients were classified as "rejected" when after allocation to Group A or B no isolator was available. Patients were classified as "omitted" after randomisation when treatment was discontinued within 14 days for reasons other than death or imminent death. Several clinical units other than the above-mentioned intended to participate in this study but did not follow the protocol of the study correctly or were unable to achieve the required minimal number of eight patients. This minimal number was set because units admitting fewer cases were believed to be less experienced in gnotobiotic care, thus increasing the risk of variability. These units were therefore excluded from the study.
Ten patients included in the study who died within 14 days after randomisation were classified as "not evaluable". Fourteen patients were admitted twice, and four patients three times to the study; 115 individual patients gave a total of 137 evaluable cases (Table 1). The contribution of the different participating units is given in Table 2.

Table 1: *Selection of patients*

Cases notified to the Statistical Centre: n = 361	
"not eligible" because	
• patient too young	1
• patient too old	10
• patient not willing to enter the study or psychological/psychiatric contraindications	73
• creatinine clearance too low	9
• other reasons (stated by the treating clinician)	19
Total	112
"rejected" because no isolator free	66
Total cases not admitted	178
Cases randomized: n = 183	
"omitted" because	
• record not available	9
• unit did not follow protocol or had less than eight cases	20
• patient was not eligible but was randomized by mistake	2
• treatment was discontinued within the first 14 days (other reasons than imminent death)	5
Total	36

Cases on study: n = 147

"not available" because

- deceased within 14 days after randomization 10

Evaluable cases: n = 137

Table 2: *Distribution of evaluable cases according to participating units*

	Clinical units							
	Essen Internal Medicine	Essen Oncology	Essen Werden	Leiden	Pesaro	Ulm	Wien	Total
Group A	3	3	7	1	5	17	6	42
Group B	2	3	6	5	6	21	1	44
Group C	7	4	7	5	5	22	1	51
Total	12	10	20	11	16	60	8	137

Treatment of Patients

Gnotobiotic care: Only systems for strict protective isolation were used for patients in Group A and B, i.e. laminar air flow isolators and plastic isolation systems (5, 14, 16). The systems were sterilized by spraying with a 2% peracetic acid solution before admission of the patient. All supplies were sterilized either by dry heat, autoclaving or ethylene oxide. Disposable utensils sterilized by gamma-irradiation were also used. All handling and nursing of patients was done either through long neoprene gloves fitted in the PVC wall or using aseptic techniques while standing downstream in the laminar air flow. Only steam sterilized diets and beverages were used for the patients. The selection of combinations of poorly absorbable antimicrobial drugs for gastrointestinal decontamination in Group A patients, and of topical antibiotics and disinfectants was left to the attending physician whose aim was to eliminate microorganisms. A standard combination of non-absorbable antibiotics was not chosen because of expected variations in the sensitivity of the microflora in the environment of the different participating units and in the individual patients. Previously described combinations, such as gentamicin and vancomycin, were not available in Europe at the time of the study. Patients treated in the open ward (Group C) received routine hospital care.

Cytotoxic treatment: Cytotoxic treatment was not standardized and was given to achieve complete remission. The treatment was administered to the limit of toxicity, accepting the risk of severe myelosuppression.

Investigation of Patients

The results of the clinical, radiological and laboratory investigations to be performed according to the protocol of the study (6) were recorded daily on special forms designed for coding and key punching. On admission of the patient two fecal samples and two oral washings were taken and stored in liquid nitrogen. These samples were cultured, and the Enterobacteriaceae species and Pseudomonas aeruginosa and Staphylococcus aureus strains isolated were typed by the Central Laboratory to obtain a bacteriological inventory of the patients. Further twice-weekly samples of feces and oral washings were collected for bacteriological evaluation by the Central Laboratory (detailed results will be published in Part II).

Evaluation of the Study

Because the antileukemic, the antimicrobial and the supportive transfusion treatments were not standardized, daily information on their administration was given on the forms. The following data were evaluated: the number of contaminating and colonizing microorganisms found in oral washings and feces, the occurence of fever above 38° and 39° C, the incidence of severe infectious episodes and of death from infection.

"Contamination" of a patient was defined as the finding of a new biotype of an Enterobacteriaceae species not found at the microbiological inventory in one sample only; the same applied for typed strains of P. aeruginosa or S. aureus. "Colonization" was defined as the finding of new bacterial types in two or more consecutive samples. Only severe infectious episodes involving bacteremia and organ invasion (lower respiratory tract infection, urinary tract infection, abcesses or miscellanous infections, such as peritonitis, meningitis, otitis, sinusitis), were considered for evaluation. The episodes were identified by definite clinical signs and symptoms of infection, by positive radiological findings and by positive microbiological cultures. In the latter case, the infectious episodes were defined as "*microbiologically* documented;" the others were defined as "clinically documented." The severe infections were listed according to sites of infection. Infectious episodes were separated from each other by the following: a period of two days or more with a body temperature below 38° C, where possible combined with either negative culture results or negative X-ray findings.

Termination of the Study

The study was terminated either when the patient went into remission and his blood granulocyte count was above 1500/μl; when the patient was transferred from the isolator to the open ward for social or psychological reasons; when death was imminent, or following death.

Results

Comparability of Treatment Groups

The characteristics of the patients allocated to Groups A, B, and C and the mean treatment duration per patient in the three groups were comparable (Table 3). Slightly lower numbers of patients were allocated to Groups A and B than to Group C, since some patients had to be rejected

Table 3: *Patient characteristics*

	Groups			
	A	B	C	Total
Total cases	42	44	51	137
Total days of study	2339	1974	2018	6931
Mean treatment duration/patient-days	55.7	44.9	51.3	
Males/females	24/18	27/17	23/21	81/56
Age range (years)	3–60	2–58	2–59	
Median age (years)	28	24.5	25	
Acute myloid leukemia cases	21	15	29	65
Acute lymphoid leukemia cases	9	9	9	27
Acute undifferentiated leukemia cases	12	20	13	45

when no isolator was available after randomization. The predominance of males over females in all three groups is in accordance with the male-female ratio seen in leukemia. Due to the stratification, the distribution of cases in each group was similar with regard to age, infection status at randomization and the number of cytotoxic induction treatments (Table 4).

Table 4: *Allocation of cases according to stratification*

	Groups			
	A	B	C	Total
Age range (years): 2–15	7	8	9	24
16–40	26	27	28	81
41–60	9	9	14	32
Infection at randomization:				
present	19	16	22	57
not present	23	28	29	80
Induction therapy:				
first	28	26	31	85
second	8	12	13	33
third and subsequent	6	6	7	19

Tests of serum creatinine and creatinine clearance showed no significant differences between the treatment groups. There was also no difference observed in the results of these tests at the start of the study, during treatment, and at the end of the study. This indicates that glomerular function did not change after possible partial absorption of the large doses of oral antibiotics in patients of Group A. Hematological findings did not differ significantly between the three groups at the commencement of and during treatment. Tests included determination of hemaglobin concentration and counts of erythrocytes, platelets, granulocytes and lymphocytes. The susceptibility to infection in all groups (Tables 5a, 5b, and 5c) was documented

Table 5a: *Percentage of days during treatment with a granulocyte count below 1000/*l of blood*

	Group			
Granulocyte count	A	B	C	Total
< 100	25%	29%	19%	24%
100–500	30%	29%	29%	29%
500–10000	16%	21%	20%	19%
> 1000	29%	25%	32%	29%

Table 5b: *Mean and standard deviation of granulocyte count during treatment*

	Group			
	A	B	C	Total
Mean	769	671	1409	983
Standard Deviation	1100	1197	4751	3075

by the fact that on 4,957 out of 6,931 observation patient-days (72%) a granulocyte count below 1,000/μl was observed, on 1,299 (19%) patient-days the count was between 500 and 1,000/μl, and on 1,641 (24%) patient-days below 100/μl. One patient in Group B and three patients in Group C received a granulocyte transfusion on a total of six occasions. There was no difference within the treatment groups in the administration of blood products, such as washed red blood cells, packed red blood cells, whole blood or platelets.

Table 5c: *Mean number of days between two blood cell countings*

	Group		
	A	B	C
Days	3.48	3.19	3.37

Although not standardized, the various forms of anti-leukemic cytotoxic therapy were very similar. The drug combination and also the mean dose per application did not differ in the three treatment groups (Table 6). It was approved that cytotoxic drugs be given in adequate doses as aggressive chemotherapy.

Table 6: *Anti-leukemic chemotherapy**

Cytotoxic drug	Route of administration	Number of patients treated			
		A	B	C	Total
Daunorubicin	i. v.	30	31	32	93
Vincristin	i. v.	32	33	31	96
Ara-C	i. v.	20	24	35	79
Thioguanin	oral	16	14	20	50
Cyclophosphamide	i. v.	9	8	16	33
6-Mercaptopurine	oral	4	4	12	20
Methotrexate	i. v.	3	4	9	16
L-asparaginase	i. v.	4	4	8	16
Prednison	oral	30	31	37	98
	i. v.	12	16	25	53
Mean dose (mg) per application**					
Daunorubicin	i. v.	100	144	102	
Ara-C	i. v.	168	159	154	
Vincristin	i. v.	2.61	2.38	2.67	
Thioguanin	oral	160	170	146	
Prednison	oral	67	56	61	
	i. v.	91	105	131	

* If the patient did not respond to the chemotherapy, another combination was given to follow up the therapy.

** Only for the most frequently used cytotoxic drugs.

Many patients received systemic antimicrobial therapy for treatment of infections at the time of admission or for an infection acquired during the observation period. The antimicrobial therapy was given to approximately the same number of patients in Groups A, B, and C; the mean dose per application did not differ (Table 7).

Table 7: *Systemic antimicrobial therapy*

Antimicrobial drugs	Route of administration	Number of patients treated			
		A	B	C	Total
Ampicillin	oral	8	9	12	29
	i. v.	8	9	11	28
Carbenicillin	i. v.	15	15	19	49
Cephalotin	i. v.	18	17	21	56
Gentamicin	i. v.	28	33	30	91
Isoniazide	oral	3	6	19	28
Ethambutol	oral	1	1	7	9
Cloxacillin	oral	5	5	5	15
	i. v.	8	9	13	30
Tetracyclin	oral	5	4	9	18
Trimethoprim-Sulfamethoxazole	oral	14	13	8	35
Mean dose (9) per application*					
Ampicillin	oral	4.70	3.73	2.87	
	i. v.	6.90	5.84	10.67	
Carbenicillin	i. v.	23	30	25	
Cephalotin	i. v.	7	7	9	
Gentamicin	i. v.	0.172	0.185	0.172	
Cloxacillin	oral	2.80	2.80	2.60	
	i. v.	24.70	4.90	11.00	
Trimethoprim-Sulfamethoxazole	oral	0.324	0.268	0.154 *r*	

* Only for the most frequently used antimicrobial drugs.

Non-absorbable antibiotics for decontamination were given to patients in Group A only (Table 8) with the exception of antifungal drugs such as nystatin and amphotericin B. The latter drugs were also given in equal doses to approximately the same number of patients in Group B or C. This was permissible since only the culture and typing results of bacteria which are not sensitive to these drugs were used for evalution of the quality of protective isolation. From the list of non-absorbable drugs administered, the combination used in 90% of the cases was polymyxin, bacitracin, neomycin and nystatin (in varying doses).

Table 8: *Non-absorbable antimicrobial drugs for decontamination**

Antimicrobial drug	Number of cases	Mean dose per application	Mean total dose per patient treated	
Polymyxin E	18	4986	123278	10/U
Polymyxin B	20	213	7471	mg
Bacitracin	38	1636	68808	10/U
Gentamicin	17	546	13651	mg
Neomycin	39	3.56	154.49	g
Cephaloridin	3	3051	11216	mg
Paromomycin	2	1161	22750	mg
Nystatin**	37	5049	118123	10/U
Amphotericin B***	7	626	34645	mg

* Group A patients only

** Nystatin was also given to 20 patients of Group B and 24 patients of Group C as a prophylactic fungistatic.

*** Amphotericin B was also administered to 4 patients of Group B and 6 patients of Group C as a prophylactic fungistatic.

Efficiency of Gnotobiotic Care

The microbiological findings (Table 9) show a significantly lower rate of contamination per patient per week ($p<0.01$) and also a significantly lower rate of colonization in Group A versus Group B and C, and in Group B versus Group C

Table 9: *Microbiological data*

Contamination/colonization infectious episodes	Group A	Group B	Group C	Statistical analysis
Mean number of bacterial contamination/patient/week	0.83	1.59	2.35	significant
Mean number of bacterial colonization/patient/week	0.1	0.41	0.6	significant
Episodes of severe infection/total number of patients	35	45	54	—
Number of clinically* documentated lower respiratory tract infections:				
a start of the study	7/70 (17%)	3/44 (7%)	9/50 (18%)	not significant
during the study	9/45 (6%)	8/122 (7%)	39/163 (24%)	significant
Number of severe infections at time of death during treatment	4	5	8	—
Number of severe infections at time of death/total number of patients	0.09	0.11	0.16	—

* Documentated by signs and symptoms of infection and by positive radiological findings.

($p<0.01$). Episodes of severe infection, expected in Group C, also occured in Groups A and B despite the use of preventive measures. However, cases in Group A tended to have fewer episodes of severe infections, and severe infection occured less often at death in the observation phase. Whereas at the start of the study the rate of positive

Table 10: *Causes of treatment termination*

	Groups A	B	C	Total
No longer increased susceptibility to infection and going into remission	29 (69%)	27 (61%)	25 (49%)	81 (59%)
Chemotherapy interruped, no remission	4 (10%)	6 (14%)	12 (14%)	22 (16%)
Psychological reasons	0	0	2 (4%)	2 (1%)
Death	5 (12%)	8 (18%)	11 (22%)	24 (18%)
Other reasons	4 (10%)	3 (7%)	1 (6%)	8 (6%)
Total number of cases	42	44	51	137

chest X-rays did not differ significantly, it was significantly lower during the observation phase in Groups A and B as compared with Group C ($p < 0.01$). The number of days where the peak temperature was above 38 °C per number of patient-days did not differ in Group A (478 / 2,339 = 20%), Group B (478 / 1,974 = 24%) or Group C (368 / 2,618 = 21%). This was also the case for days where the temperature was above 39 °C (Group A: 218 / 2,339 = 8%; Group B: 203 / 1,974 = 10%; Group C: 235 / 2,618 = 9%).

Cases in Groups A and B tended to have higher remission rates than cases in Group C (Table 9). While 29/42 (69%) cases in Group A no longer had an increased susceptibility to infection and went into remission at the end of their treatment period, the respective number in Group B was 27/44 (61%) and in Group C 25/51 (49%) (figure 1).

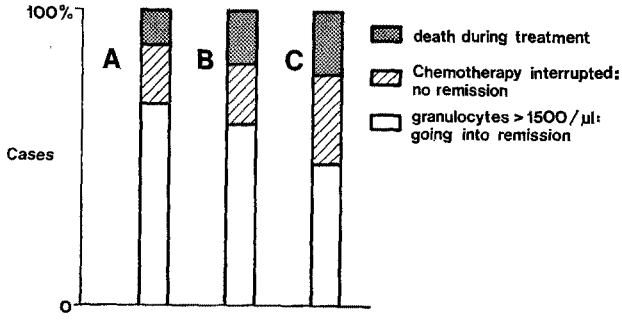

Figure 1: *Outcome of patients in Groups A, B and C at termination of observation period.*

Chemotherapy was interrupted without remission in more patients in Group C than in Group A or B. In Group C death was more often the reason for termination of treatment than in Group B or A. However, survival until Day 30 after termination of treatment, which was chosen prospectively as a clearly defined reference date, did not differ significantly in Group A: 33/42 (79%), Group B: 35/44 (79%) or Group C: 38/51 (75%). Further analysis of the survival ratio 30 days after termination of treatment (figure 2) shows that the improvement in the results when

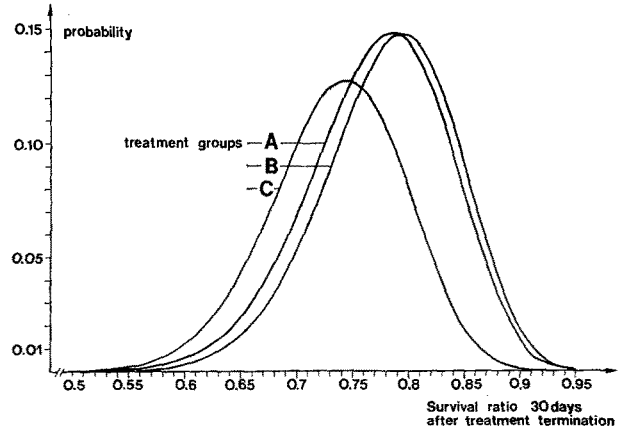

Figure 2: *Survival ratios for the three groups 30 days after terminating treatment at different levels of probability.*

treating leukemia by supportive care, as in Group A or B, can be expected to lie between 0% and 30%. This means that with $p > 0.95$ the beneficial effect of isolation, or isolation and decontamination, did not exceed a 30% improvement in the survival ratio. According to the survival curves* of patients in Groups A, B, and C, patients in Group A tended to have a better survival rate than those in Group C (figure 3).

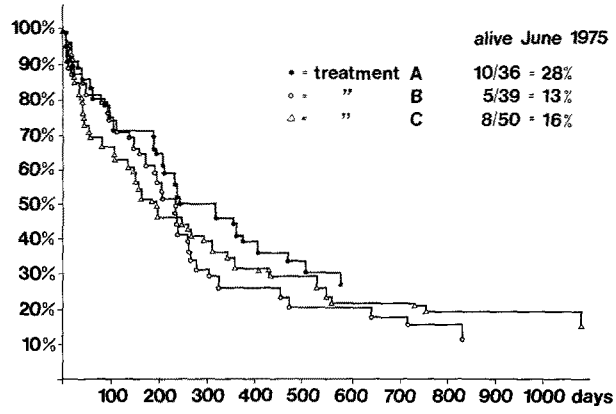

Figure 3: *Survival rate of the three groups related to treatment.*

Prognostic Parameters

Morphological diagnoses: Patients with acute lymphocytic leukemia survived longer than patients with acute myelocytic leukemia, and patients with acute myelocytic leukemia fared better than patients with acute undifferentiated leukemia (figure 4). Equal distribution of patients with

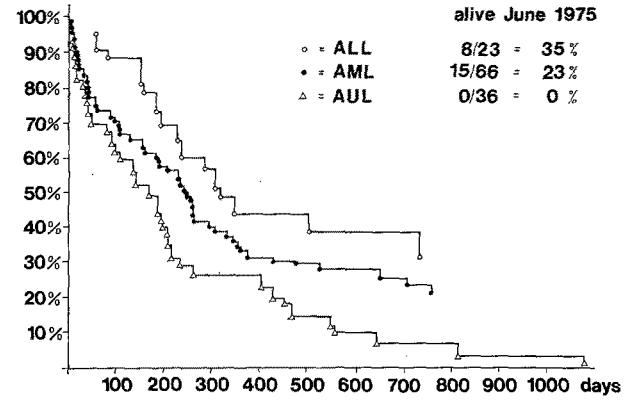

Figure 4: *Survival rate related to morphological classification (ALL = acute lymphocytic leukemia; AML = acute myloid leukemia; AUL = undifferentiated leukemia).*

* All survival curves are based on patients, not on "cases": one observation period was selected randomly for those patients who were studied more than once for use in the life table analyses. The ten cases who died within 14 days after randomization (Table 1) were included in the survival curves. At the end of the period of data collection (June 1975) some patients were still alive. This should be noted for the interpretation of life curves because these patients may actually have shorter survival rate than indicated by the end-point of the curves.

the diagnosis acute myelocytic leukemia, acute lymphocytic leukemia or acute undifferentiated leukemia thus proved to be important.

Infection at admission: The survival curve* shows that patients with neither fever nor other signs of infection at randomization had a better survival rate than patients with signs of infection (figure 5). This indicates that this factor must be taken into account when stratifying during the randomization procedure.

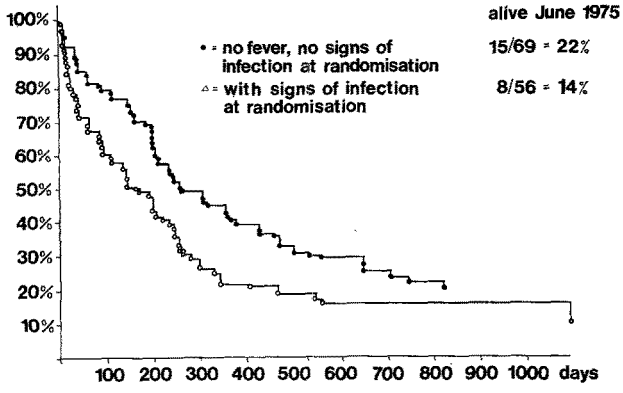

Figure 5: *Survival rate related to status of infection at randomization.*

Induction therapy: When considering survival rate* in relation to the first, second, third or subsequent therapies, the curves of the first and second induction therapy are found to cross, and the curve for third and subsequent induction therapies shows a shorter survival period (figure 6). However, the duration of remission was found to be longest with the first induction therapy, as expected.

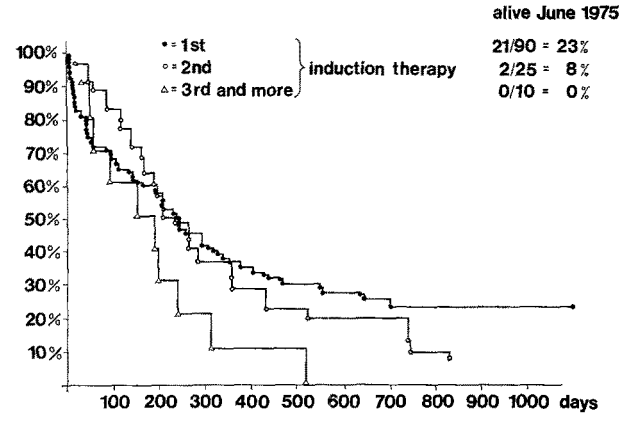

Figure 6: *Survival rate related to number of induction chemotherapy.*

Age at diagnosis: Retrospectively, as far as the age groupings 16–40 and 41–60 years were concerned, this factor was not important in the stratification. Both groups had a similar survival rate*. The number of cases in age group 2–15 years was too small to draw any conclusion in this regard.

Discussion

The results of the clinical part of this study show that protective isolation and antimicrobial decontamination under conditions of protective isolation did not prevent infectious complications in patients with acute leukemia during cytotoxic induction therapy.

The conclusions of this study with regard to a possible beneficial effect of protective isolation and antimicrobial decontamination in patients with impaired defence were valid, provided patients allocated to the three treatment groups were equally susceptible to microbial contamination, colonization and infection. That this was presumably the case with regard to susceptibility to infection could be concluded from the hematological follow-up, and from the similarity of the type and dosage of cytotoxic therapy administered to the patients in the three groups. Equal susceptibility to contamination and colonization could be concluded from the retrospective observation that systemic antimicrobial therapy and basic supportive care were similar with regard to duration, dosage and type in all three groups during treatment.

In our study the reduction in the number of infections in Groups A and B was not as striking as that described in three other randomized studies (8, 11, 17). This could, at least in part, have been due to the use of different antibiotics. In our study most patients were decontamined with a combination of neomycin and bacitracin (in some cases supplemented with polymyxin B), while in the other investigations a combination of gentamicin and vancomycin was used. It is well known that resistance to the antibiotics employed in our study is much more common than is the case with the gentamicin-vancomycin combination. The Gnotobiotic Project Group is therefore now investigating methods of improving the efficiency of antibiotic decontamination. The results of treatment with a standard antibiotic regimen are being compared with the results of treatment with a combination of antibiotics selected on the basis of a sensitivity test on the patient's microflora.

Data obtained by typing species of S. aureus and P. aeruginosa, and biotyping Enterobacteriaceae species demonstrated clearly that protective isolation alone (Group B) reduced the incidence of bacterial contamination and colonization. However, a considerable number of contaminations still occurred. The additional use of non-absorbable antibiotics for decontamination in Group A further reduced the incidence of contamination and colonization markedly. Another interesting finding was that there was a significantly smaller number of radiologically confirmed lower respiratory tract infections in Groups A and B compared with Group C, which confirms previous publications (8, 11, 17). This supports the hypothesis that a certain number of these infections are airborne in hospitals and that airborne transmission can be reduced markedly by strict protective isolation.

There was a higher rate of patients going into remission in Group A, and to some extent also in Group B, compared

with Group C. Although providing evidence of the beneficial effect of gnotobiotic care, these differences were not statistically significant.

Levine et al. (9) recently reviewed the effects of "protective environments" and "protective environment and prophylactic antibiotics" on infection and remission in acute leukemia in seven clinical studies, each conducted on more than 20 patients. In three studies it was suggested that a "protective environment" alone lowers the incidence of infection, while in all seven studies the combination of "protective environment" and "prophylactic antibiotics" apparently reduced infection. In one study it was suggested that the remission rate was increased by a "protective environment," in three studies the same was ascribed to "prophylactic antibiotics" and a "protective environment," while in three studies the remission rate was not changed by "prophylactic antibiotics" in combination with "protective environment." In one study it was suggested that there was no change in the remission rate due to a "protective environment" only. Three of the studies were prospective clinical trials; in the remaining studies the patients were matched retrospectively. All of these studies involved small numbers of patients.

Schimpff et al. (12) regard the results of different studies on "reverse isolation and microbial suppression" as inconclusive or equivocal.

In our opinion such studies cannot even be compared unless the quality of isolation and decontamination is monitored at the same time. The treatment of a patient in an isolator does not guarantee that the patient is really bacteriologically isolated, and the oral administration of non-absorbable antibiotics in sufficient doses does not ensure successful decontamination. Studies of this kind are consequently of minimal value unless adequate quality control is available, both for isolation and decontamination. Gnotobiotic care needs further development and careful evaluation before being recommended as standard supportive therapy in the treatment of high-risk patients, such as those suffering from acute leukemia.

Acknowledgments

The authors gratefully acknowledge the assistance of Dr. *S. Selwyn* (Westminster Medical School, London) in the preparation of the manuscript.

This work was supported by grants from the following institutions: Landesamt für Forschung des Landes Nordrhein-Westfalen; Dutch Ministry of Public Health; Deutsche Forschungsgemeinschaft im SFB 112 „Zellsystemphysiology".

Participating Units and Representatives

Department of Internal Medicine, University of Essen, Essen, Germany, *(G. Brittinger, G. Linzenmeier);* Department of Oncology, University of Essen, Essen, Germany, *(J.H.Beyer);* Evangelisches Krankenhaus Essen-Werden, Essen, Germany, *(F. Wendt);* Department of Pediatrics, University of Leiden, The Netherlands, *(J. Vossen);* Ospedale San Salvatore, Pesaro, Italy, *(C. Lucarelli);* Division of Hematology, Department of Internal Medicine, University of Ulm, Ulm, Germany, *(M. Dietrich);* Innere Medizinische Klinik, University of Wien, Vienna, Austria, *(N. Honetz).*

Central Services

Microbiology: REPGO Institutes TNO, Rijswijk, The Netherlands, *(D. van der Waaij);* St. Mary's Hospital, London, Great Britain, *(H. Gaya);* Statistics: Department of Documentation and Statistics, University of Ulm, Ulm, Germany, *(W. Gaus).*

Literature

1. *Bodey, G., Buckley, M., Sathe, Y. S., Freireich, E. J.:* Quantitative relationships between circulating leukocytes and infection with acute leukemia. Ann. intern. Med. 64 (1966) 328–340.

2. *Bodey, G. P., Hart, J., Freireich, E. J., Frei, E.:* Studies of a patient isolator unit and prophylactic antibiotics in cancer chemotherapy. Cancer 22 (1968) 1018–1026.

3. *Bodey, G. P., Rodriguez, V., Freireich, E. J., Frei, E.:* III, Protected environment, prophylactic antibiotics and cancer chemotherapy. In: Recent results in cancer research (Ed.: *G. Mathé*), vol. 29, p. 16. Springer Verlag, Berlin 1970.

4. *Dietrich, M., Fliedner, T. M., Heimpel, H.:* Isolierbettsystem zur Infektionsprophylaxe bei verminderter Resistenz. Dtsch. med. Wschr. 94 (1969) 1003–1009.

5. *Dietrich, M., Abt, C., Pflieger, H.:* Experiences with a new isolated bed system in the treatment of acute leukemia. Med. Progr. Technol. 3 (1975) 85–89.

6. *EORTC Gnotobiotic Project Group:* Protocol for an evaluative study of the protective effect of isolation systems and decontamination in patients with high susceptibility to infection. Europ. J. Cancer 8 (1972) 367–371.

7. *Hersh, E. M., Bodey, G., Nies, B. A., Freireich, E. J.:* Causes of death in acute leukemia. J. Amer. med. Ass. 193 (1965) 105–109.

8. *Levine, A. S., Siegel, S. E., Schreiber, A. D., Hauser, J., Preisler, H., Goldstein, I. M., Seidler, F., Simon, R., Perry, S., Bennett, J. E., Henderson, E. S.:* Protected environments and prophylactic antibiotics. A prospective controlled study of their utility in the therapy of acute leukemia. New Engl. J. Med. 288 (1973) 477–483.

9. *Levine, A. S., Robinson, R. A., Hauser, J. M.:* Analysis of studies on protected environments and prophylactic antibiotics in adult acute leukemia. Europ. J. Cancer II (1975) Suppl., p. 57–63.

10. *Levitan, A. A., Perry, S.:* The use of an isolator system in cancer chemotherapy. Amer. J. Med. (1968) 234–242.

11. *Schimpff, S. C., Greene, W. H., Young, V. M., Fortner, C. L., Jepsen, L., Cusack, N., Block, J. B., Wiernik, P. H.:* Infection prevention in acute nonlymphocytic leukemia. Ann. intern. Med. 82 (1975) 351–358.

12. *Schimpff, S. C.:* Laminar air flow room reverse isolation and microbial supression to prevent infection in patients with cancer. Cancer Chemother. Rep. 59 (1975) 1055–1060.

13. *Schneider, M.:* Les infections bactériennes et fungiques au cours des leucémies aigue. Sem. Hôp. Paris 43 (1967) 438–444.

14. *Schwartz, S. A., Perry, S.:* Patient protection in cancer chemotherapy. J. Amer. med. Ass. 197 (1966) 623–627.

15. *Waaij, D. van der, Vries, J. M. de, Lekkerkerk, J. E. C.:* Colonisation resistance of the digestive tract and the occurrence of spread of the bacteria to lymphatic organs in mice. J. Hyg. (Lond.) 70 (1972) 55–63.

16. *Waaij, D. van der, Vossen, J. M., Korthals Altes, C.:* Patient isolators designed in the Netherlands. In: Germfree research, biological effect of gnotobiotic environments (Ed.: *J. B. Heneghan*), p. 31. Academic Press, New York 1975.

17. *Yates, J., Holland, J. F.:* A controlled study of isolation and endogenous microbial suppression in acute myelocytic leukemia patients. Cancer 32 (1973) 1490–1498.