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U. Creutzig J. Ritter G. Schellong (Eds.)

Acute Myelogenous Leukemia in Childhood

Implications of Therapy Studies for Future Risk-Adapted Treatment Strategies

With 53 Figures and 68 Tables

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Preface

The results of treatment for childhood acute myelogenous leukemia (AML) have improved considerably during the last ten years. This progress was demonstrated by the two consecutive multicenter studies, AML-BFM-78 and -83, in which almost identical extended multidrug regimes of chemotherapy were administered for eight weeks and followed up by two years maintenance. The main difference in the second study was the addition of an eight-day intensive induction course. Due to this new element, the relapse rate was reduced significantly. Another result of the BFM-83 study was the definition of two risk groups on the basis of standardized treatment, which has lead to a risk-adapted treatment strategy in the third ongoing trial, AML-BFM-87.

This improvement was only possible thanks to the cooperation of pediatricians, physicians, radiotherapists, statisticians, and especially the staff at the hospitals and reference laboratories. Thus, we would like to thank everyone who has been involved in these studies and hope that they will be further encouraged to improve treatment strategies for AML in children.

The coordination, enforcement, and analyses of the studies would not have been possible without the financial support of the Federal Ministry for Research and Technology of the FRG. We are grateful for the generous contributions supporting this book from Lederle and Farmitalia.

Münster, April 1990

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Abbreviations

Forms of leukemia

ALL	acute lymphoblastic leukemia
AML	acute myelogenous leukemia
FAB M1	acute myelogenous leukemia without maturation
FAB M2	acute myelogenous leukemia with maturation
FAB M3	acute promyelocytic leukemia
FAB M4	acute myelomonocytic leukemia
FAB M5	acute monocytic leukemia
FAB M6	acute erythroleukemia
FAB M7	acute megakaryocytic leukemia

Drugs

ADR	adriamycin
ARA-C	cytosine arabinoside
HD-ARA-C	high-dose cytosine arabinoside
CP	cyclophosphamide
DNR	daunorubicin
HAM	HD-ARA-C and mitoxantrone
P	prednisone
6-TG	6-thioguanine
VP-16	VP-16-213 (etoposide)
ADE	cytosine arabinoside, daunorubicin, and etoposide

Other

ANAE	α-naphtyl-acetate-esterase
BFM	Berlin-Frankfurt-Münster
BM	bone marrow
BMT	bone marrow transplantation

CCR	continuous complete remission
CR	complete remission
CSF	cerebrospinal fluid
DC	dose compliance
del	deletion
DNA	deoxyribonucleic acid
ED	early death
EFI	event-free interval
EFS	event-free survival
Eo	eosinophils
FAB	French-American-British Cooperative Group
inv	inversion
ith.	intrathecal
lfu	lost to follow-up
n.a.	not applicable
n.p.	not performed
NR	nonresponder
n.s.	not significant
POX	peroxidase
PR	partial remission
SD	standard deviation
t	translocation
TdT	terminal deoxynucleotidyl transferase
/	last patient of the group (in life table analyses)
WBC	white blood cell count
...; ... (age)	years; months

1 Introduction

1.1 Development of AML Therapy in Children

Clinical research in the field of pediatric oncology has been carried out in the Federal Republic of Germany in the form of multicenter cooperative therapy studies since the early 1970s [101]. One of the first studies, which investigated the treatment of acute lymphoblastic leukemia (ALL), was started by Lampert in 1971 for the Deutsche Arbeitsgemeinschaft für Leukämieforschung und -behandlung im Kindesalter (German Study Group for Research and Treatment of Leukemia in Childhood). For the first time, a high degree of uniformity in treatment was thus achieved in this country [72]. At the same time, Riehm developed a remarkably effective therapy concept for ALL. It consisted of an eight-week combined induction/consolidation phase with eight different cytotoxic agents and irradiation of the central nervous system as well as subsequent maintenance therapy [139, 140]. The same concept was adopted by the first cooperative acute myelogenous leukemia (AML) study BFM-78.

While there are approximately 400 new cases of ALL per year involving children under the age of 15 in the Federal Republic of Germany, there are only about 70 new cases of AML [117]. The ratio between the two diseases is 5–6:1. As the acute nonlymphoblastic leukemias are extremely rare in children, evaluable scientific results can only be obtained by multicenter cooperation.

The AML is characterized by malignant proliferation involving myelogenous precursor cells. Compared with ALL, the disease is more heterogeneous morphologically and in its course. Consequently, implementation of therapy is more difficult than in any other malignant diseases affecting children. This is mainly due to the considerable toxic effect of the treatment on normal hematopoiesis, which nevertheless has to be accepted in an attempt to destroy malignant myelogenous cells originating from the same pool of stem cells. Sequelae of therapy are extended severe bone marrow aplasia with infections and thrombopenic hemorrhage. For these reasons, analysis of

therapy implementation and complications is especially important in AML.

1.1.1 Historical Development

As a disease, leukemia has been known since 1845 [11], when it was described by Bennett in Scotland and Virchow in Berlin. In his monograph on leukemia published in 1856, Virchow already differentiated between splenogenic (current terminology: myelogenous) and lymphatic leukemia. Except for a few cases of somewhat longer duration, the course of acute leukemia in children usually led to death within half a year after diagnosis [87].

In 1930, the prognosis of leukemia was, for the first time, favorably influenced by the use of blood transfusions [165]. Later on, survival periods could be prolonged by exchange transfusions [16] and antibiotic therapy, which had become available by then.

During 1946 and 1947, chemotherapy was developed, starting with urethane [125] and the folic acid antagonists [54]. These were followed by the glucocorticoids [55, 127], the antimetabolite 6-mercaptopurine [30], the alkylating agent cyclophosphamide (CP, [4]), and the Vinca alkaloid derivative vincristine [89]. In the 1960s, cytosine arabinoside (ARA-C) and the anthracyclines were added to the group of antileukemic agents (Table 1). Monotherapy with the above substances succeeded in achieving remissions, although short ones, especially in children with ALL.

Table 1. Historical data on the therapy of acute leukemias

Year	Therapy/agents	Reference
1930	Blood transfusion	Ullrich 1932 [165]
1945	Exchange transfusion	Bernard and Bessis 1948 [16]
1946	Urethane	Paterson et al. 1946 [125]
1947	Follic-acid antagonists	Farber et al. 1948 [54]
1949	Corticosteroids	Pearson et al. 1949 [127]; Farber et al. 1950 [55]
1953	6-mercaptopurine	Burchenal et al. 1953 [30]
1958	Cyclophosphamide	Hoogstraten et al. 1960 [81]
1960	Vincristine	Johnson et al. 1963 [89]
1966	Cytosine arabinoside	Howard et al. 1968 [82]
1966	Anthracyclines	Bernard et al. 1967 [17]

After it was learned that survival time depends on whether or not complete remission (CR) is achieved and that the course after relapse compares with the course in untreated children [60], attempts were made to improve remission quality. During the 1950s and 1960s, 10%–20% of AML patients (adults and children) achieved CR with 6-mercaptopurine monotherapy [21, 59]. The results obtained with monotherapies of ARA-C or daunorubicin (DNR) were already much improved, with CR rates of 20%–50% [18, 156, 157, 178]. However, the duration of CR after monotherapy was short (median eight months) [21]. Only after combination chemotherapy had been introduced did the rates and the duration of CR increase, reflecting an additive effect of the different cytotoxic drugs [ARA-C and 6-thioguanine (6-TG): 44%–60% in CR; median duration of CR: 21 months] [108, 120]. In the 1970s, drug combinations containing anthracyclines were introduced which further improved the rate and duration of remission (Table 2).

Since the late 1970s, treatment strategies prescribing consolidation and maintenance therapy, or intensification, in addition to the conventional induction regimes, have produced increasingly better results (Boston studies [172, 173], study AML-BFM-78 [39, 148]). Close to 80% of the children today will achieve CR. With adequate therapy, about one-half of them will remain in long-term remission.

Table 2. Response rates using mono- and combination chemotherapy in children (C) and adults (A) with AML

Year	Therapy	Patients	CR rate	References
Monotherapy				
1952–60	6-MP	70 (A)	12%	Boggs et al. 1969 [21]
1961–68	6-MP	76 (A)	22%	Boggs et al. 1969 [21]
1957–60	6-MP	11 (C)	9%	Frei et al. 1961 [59]
1967–69	ARA-C	247 (A)	20–38%	SOG 1974 [156]
		34 (C)	41%	Steuber et al. 1978 [157]
1973–75	Daunorubicin	144 (A+C)	42%	Wiernik et al. 1979 [178]
		18 (C)	50%	Wiernik et al. 1979 [178]
Combinations without anthracyclines				
1973–75	ARA-C + 6-TG	162 (A+C)	44%	Wiernik et al. 1979 [178]
	ARA-C + 6-TG	27 (C)	52%	Wiernik et al. 1979 [178]
Combinations including anthracyclines				
1973–75	DAT	154 (A+C)	50%	Wiernik et al. 1979 [178]
	DAT	21 (C)	86%	Wiernik et al. 1979 [178]

CR: complete remission; 6-MP: 6-mercaptopurine; ARA-C: cytosine arabinoside; 6-TG: 6-thioguanine; DAT: daunorubicin + ARA-C + 6-TG

In recent years, particularly good results have been reported after allogeneic bone marrow transplantation (BMT) during first remission, especially in young AML patients [124]. This form of therapy, however, is available for only a small percentage of patients since CR and histocompatibility of a donor are required. Moreover, while relapse rates after BMT are lower than under conventional chemotherapy, treatment-induced lethality is approximately 15% [124]. For these reasons, the development of new strategies in the treatment of AML requires that the potential value of BMT within the therapy concept will be carefully investigated. Pertinent analyses, including pretherapeutic parameters and parameters reflecting the course of the disease, are necessary to evaluate this problem. Consequently, any future, risk-adapted treatment strategy has to meet the requirement of offering neither under- nor overtreatment of the individual patient.

1.2 Prognostic Parameters in AML

In contrast to ALL, uniform factors allowing estimations of prognosis have not yet been identified for AML. Several studies, most of them carried out in the field of internal medicine, could only show that the number of leukemic cells at diagnosis, represented by the number of leukocytes in the peripheral blood, constitutes one risk factor [85, 151, 176]. There are also suggestions that the risk of early death is higher in monocytic leukemias [45, 107]. In adults suffering from AML, advanced age at the time of diagnosis is considered to be prognostically unfavorable [134], whereas in children the youngest age group tends to have the least favorable outcome [174]. Several other variables have been determined by univariate analysis and in small numbers of patients, limiting the value of the conclusions. The possibility of numerous potent cross relationships between potential prognostic factors must also be taken into consideration. Therefore, the influence of one specific factor may well be overestimated with univariate analysis if this factor is closely related to another one. In other instances, division of AML cases into various subgroups will show that one specific factor is of prognostic significance for only a specific group of patients. Comparison of the prognostic factors is even more difficult as they are also decisively influenced by the therapy chosen.

1.3 Objectives of the Analysis

The aim of the present investigation is to develop criteria for estimating at the time of diagnosis or soon thereafter the individual risk to AML patients of early death, nonresponse, and relapse, in order to gain a basis for the use of risk-adapted treatment in later studies. For this purpose, the value of prognostic factors has to be studied by univariate and multivariate analysis.

In view of the heterogeneity of AML, the relationship between various parameters has to be taken into consideration. Distinctive features characterizing the malignant cell, such as morphologic, cytochemical, immunologic, and cytogenetic properties, are often closely related and characterize specific subgroups. Factors such as fast or slow response to therapy may be of greater importance for all patients.

The therapy used in the AML study BFM-83 mainly differs from that in BFM-78 by the addition of the intensive induction phase with ARA-C, DNR, and etoposide (ADE). The effect of this intensification of therapy on prognosis and on the significance of various pretherapeutic factors may indicate the importance of this therapeutic element. An analysis of conducted therapy may allow one to recognize problems of therapy and to improve future therapy schedules.

The objectives of this investigation can be summarized as follows:

1. Determination of pretherapeutic factors providing information on individual prognosis with respect to:
   a) Risk of early death due to hemorrhage and/or leukostasis
   b) Response to therapy
   c) Risk of relapse
2. Evaluation of response-kinetic parameters with regard to prognosis
3. Determination of the value of treatment intensification in study AML-BFM-83 with regard to prognosis
4. Analysis of therapy implementation and subsequent complications in AML-BFM-83 and evaluation with regard to future treatment strategies

Based on the results of this analysis, the development of a risk-adapted treatment strategy can be attempted.

2 Patients and Therapy

2.1 Patient Eligibility

The investigations are based on the pre- and post-therapeutic data from 333 study patients. The following *criteria of patient eligibility* were applied:

1. Primary diagnosis of AML between December 1, 1978, and September 30, 1982, in study AML-BFM-78 and between December 1, 1982, and September 30, 1986, in study AML-BFM-83
2. Age between 0 and 17 years
3. Prior cytotoxic treatment not exceeding two weeks

The diagnosis of AML was confirmed by the diagnostics commission of the respective study, a total of 151 patients being enrolled in AML-BFM-78 and 182 patients in AML-BFM-83. All parents were advised of the procedures and risks involved in the therapy and gave oral or written consent for their child to enter the study. The participating hospitals and the respective numbers of patients are listed in Table 3.

The *criteria for exclusion* were:

1. No AML according to FAB criteria [12]
2. Manifestation of AML as a secondary malignancy
3. Presence of a previous disease prohibiting a cytotoxic therapy according to protocol (Down's syndrome, Fanconi's anemia, severe mental disorders, malformations, cardiac disorders or hepatic and renal failure independent of the leukemia)
4. Lack of consent of the parent/guardian (and the patient) to the therapy according to protocol

Patients who met the criteria of eligibility were enrolled in the study even if they died before the onset of therapy and are called *study patients*. Of these, only those patients who started treatment according to

protocol are referred too as *protocol patients*. Patients fulfilling one or more of the criteria for exclusion were recorded as ineligible and are not included in the present analysis (Table 4).

Table 3. Participating hospitals and number of patients enrolled in studies AML-BFM-78 and AML-BFM-83

	AML-BFM-78	AML-BFM-83
Augsburg (1. Kinderklinik des Krankenhauszweckverbandes)	2	3
Berlin (Universitäts-Kinderklinik)	11	10
Bremen (Prof.-Hess-Kinderklinik)	2	5
Datteln (Vestische Kinderklinik)	1	2
Düsseldorf (Universitäts-Kinderklinik)	9	11
Erlangen (Universitäts-Kinderklinik)	–	7
Essen (Universitäts-Kinderklinik)	9	8
Frankfurt (Universitäts-Kinderklinik)	4	10
Freiburg (Universitäts-Kinderklinik)	9	10
Gießen (Universitäts-Kinderklinik)	7	12
Göttingen (Universitäts-Kinderklinik)	6	5
Hamburg (Universitäts-Kinderklinik)	7	12
Hannover (Universitäts-Kinderklinik)	2	10
Heidelberg (Universitäts-Kinderklinik)	4	4
Homburg/Saar (Universitäts-Kinderklinik)	6	3
Karlsruhe (Städtische Kinderklinik)	2	4
Kassel (Städtische Kinderklinik)	1	1
Kiel (Universitäts-Kinderklinik)	7	3
Köln (Universitäts-Kinderklinik)	5	1
Köln (Städtisches Kinderkrankenhaus)	1	5
Mannheim (Universitäts-Kinderklinik)	1	0
Mainz (Universitäts-Kinderklinik)	3	1
Marburg (Universitäts-Kinderklinik)	–	4
München-Harlaching (Kinderabteilung des Städtischen Krankenhauses)	1	2
München-Schwabing (Universitäts-Kinderklinik)	4	8
München-Poliklinik (Universitäts-Kinderklinik)	3	1
München-von Haunersches Kinderspital (Universitätsklinik)	4	–
Münster (Universitäts-Kinderklinik)	24	22
Nürnberg (Cnopf'sche Kinderklinik)	4	7
Stuttgart (Olgahospital)	4	1
Tübingen (Universitäts-Kinderklinik)	7	6
Ulm (Universitäts-Kinderklinik)	0	4
Worms (Städtische Kinderklinik)	1	0
Total number of patients	151	182

Table 4. Patients recorded as ineligible between Dec. 1, 1978 and Sept. 30, 1986

Different therapy	
pretreatment exceeding 14 days	8
Diagnosis	
diagnosis uncertain and/or insufficient material	16
biphenotypical leukemia	25
Secondary malignancy	8
Preexistent disease	
Down's syndrome	14
statomotor and cerebral retardation	1
Total number of patients	72

2.2 Therapy Schedule for Study AML-BFM-78

The therapy schedule of AML-BFM-78 consisted of up to 14 days of prephase therapy for selected patients, eight weeks of initial therapy divided into two four-week phases of induction/consolidation therapy, and a subsequent two-year period of maintenance therapy.

Prephase therapy was given in cases of high leukocyte counts (>30000/mm^3) and/or severe enlargement of liver and spleen. This involved daily administration of 6-TG, (30 mg/m^2 p.o.) and ARA-C (30 mg/m^2 i.v.) for a maximum of 14 days preceding initial therapy, with the aim of cautiously reducing the leukemic cell mass.

Initial therapy consisted of two four-week treatment phases (phases 1 and 2) with up to 14 days of recovery in between. Details regarding dosage and sequence of drugs and 'prophylactic' CNS treatment are shown in Fig. 1. Prednisone was not given during first days of initial therapy if there was an increased risk of hemorrhage resulting from coagulation defects. During therapy phase 2 treatment was interrupted when severe bone marrow aplasia occurred, but was continued later on.

Maintenance therapy, which started two weeks after completion of phase 2, involved the following drug regimen: 6-TG (40 mg/m^2 p.o. daily), ARA-C (40 mg/m^2 s.c. every 4 weeks for 4 days), and adriamycin (ADR) (25 mg/m^2 i.v. every 8 weeks for the first year up to a maximum cumulative dose of 300 mg/m^2). In patients in continuous first remission, maintenance therapy was discontinued after two years.

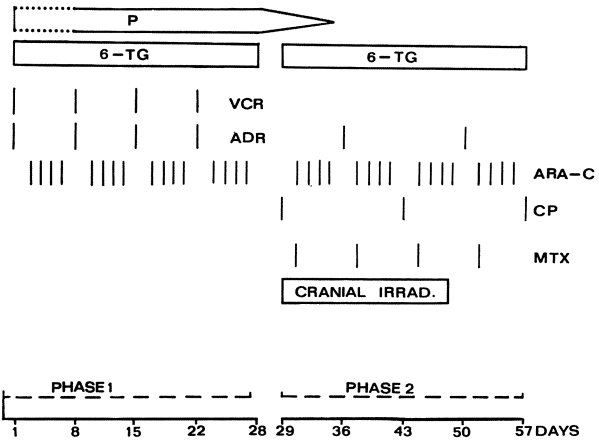


Fig. 1. Scheme of the initial therapy of study AML-BFM-78. *P,* Prednisone 60 mg/m^2 p.o. for 28 days at 3 doses per day, gradual reduction in three 3-day steps with application of 1/2, 1/4, and 1/8 of the initial dose; *TG,* thioguanine 60 mg/m^2 p.o., 2 × 28 days; *VCR,* vincristine 1.5 mg/m^2 i.v. × 4, maximum individual VCR dose 2 mg; *ADR,* Adriamycin 25 mg/m^2 i.v. × 6; *ARA-C,* cytosine arabinoside 75 mg/m^2 i.v. × 32; *CP,* cyclophosphamide 500 mg/m^2 × 3; methotrexate 12.5 mg ith × 4; cranial irradiation (focal dose) 12 Gy in children under 1 year, 15 Gy in children under 2, and 18 Gy in children aged 2 years and older.

2.3 Therapy Schedule for Study AML-BFM-83

In AML-BFM-83, the initial therapy consisted of an eight-day intensive induction block that preceded the two phase therapy schedule used in AML-BFM-78 (Fig. 2). Continuous infusion of ARA-C (100 mg/m^2/day) on days 1 and 2 was followed by ARA-C (100 mg/m^2/12 h; 30 min infusions) on days 3–8, DNR (60 mg/m^2/day; 30 min infusions) on days 3–5, and etoposide (VP-16) (150 mg/m^2 per day; 60 min infusions) on days 6–8.

The response to induction therapy was evaluated by bone marrow (BM) puncture on day 15. When $<5\%$ of blasts remained, hematological recovery of the peripheral blood (rise in neutrophils and thrombocytes) was awaited, and consolidation therapy was usually begun between days 25 and 30. When $\geq 5\%$ of blasts remained, consolidation therapy was started without delay, clinical condition permitting. The induction/consolidation therapy described in the protocol of AML-BFM-78, except for minor alterations, was then followed. Alterations included in phase 1, the dosage of prednisone (40 mg/m^2 p.o., daily)

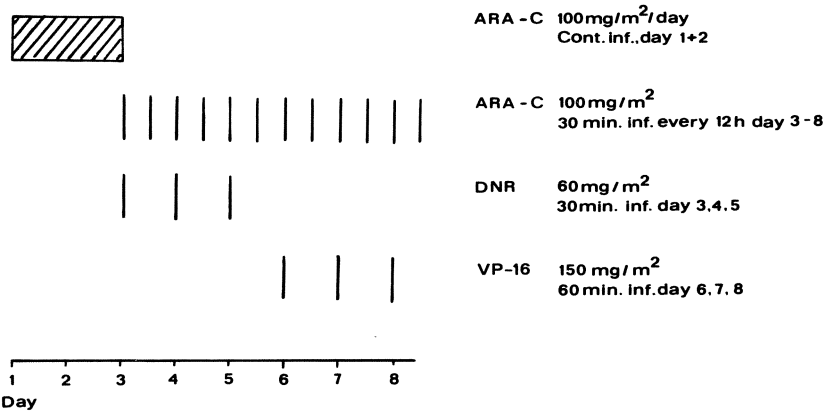


Fig. 2. Induction (ADE) therapy protocol in AML-BFM-83

and ADR (30 mg/m^2 i.v. on days 1, 8, 15, 22), and, in phase 2, the elimination of two ADR doses and of the second CP application. The dosage of intrathecal prophylactic ARA-C CNS treatment and of cranial irradiation was adjusted to age (Table 5).

Maintenance therapy was administered for a total duration (including initial therapy) of two years. ADR (25 mg/m^2 i.v.) was given at eight-week intervals, but four times only, i.e., up to a total dose of 400 mg/m^2 ADR and DNR.

Antibacterial prophylaxis was administered throughout both studies using daily administrations of co-trimoxazole. Polymyxin E and amphotericin B p.o. were given from the first day of induction therapy until the end of neutropenia (>1500/mm^3 of neutrophils), and during consolidation therapy.

BMT during first CR was not included in the treatment schedule of either of the two protocols.

Table 5. Dosages for cranial irradiation and intrathecal ARA-C administration in 'prophylactic' CNS treatment (study AML-BFM-83)

Age	Cranial irradiation	ARA-C i th.
<1 year	12 Gy	20 mg
≥ 1–2 years	15 Gy	26 mg
≥ 2–3 years	18 Gy	34 mg
≥ 3 years	18 Gy	40 mg

3 Definitions and Methods

3.1 Definitions

Early death (ED) refers to deaths in study patients before onset of therapy or in protocol patients within the first six weeks of therapy.

Complete remission (CR) is assumed when less than 5% blasts are detected in the BM and there is no evidence of disease at other sites. The Cancer and Leukemia Group B (CALGB) criteria of complete remission [132] additionally require normocellular BM with regeneration of hematopoiesis (peripheral blood thrombocyte count $\geq 100000/mm^3$ and neutrophil count $\geq 1500/mm^3$). While regeneration of hematopoiesis was awaited after induction therapy in AML-BFM-83, this could not be done in AML-BFM-78 since the induction/consolidation therapy was continued without major interruptions for a duration of 8–10 weeks.

Patients with partial remission ($\geq 5\%$ but $< 25\%$ blasts in the bone marrow) are regarded as *nonresponders* (NR), as are patients with a remission duration of less than four weeks or with renewed blast expansion after a transient phase of aplasia without regeneration of hematopoiesis.

Recurrence of $> 5\%$ of myelogenous blasts in the BM after CR with unequivocal expansion of the blast clone or evidence of leukemic infiltration at other sites is defined as *relapse.*

CNS involvement is assumed when there is unequivocal evidence of leukemic cells in the CSF, specifically a cell count of $> 10/mm^3$, or when cerebral leukemic infiltration is apparent, e.g., by biopsy or operation.

Extramedullary organ involvement, as used here, is defined as leukemic infiltration in sites other than liver, spleen, or CNS. The AML-BFM-83 study also records orbital, renal, and skeletal infiltration in addition to the tumorous manifestations documented in AML-BFM-78 (Table 7).

Hyperleukocytosis signifies a leukocyte count in the peripheral blood of $\geq 100000/mm^3$. *Leukostasis* is defined as sludging of blasts in the blood vessels with subsequent occlusion and can only be confirmed by autopsy.

Complications include minor and severe hemorrhage and infections.

	Hemorrhage	*Infections*
Minor	Petechiae, hematomas	Local infections predominantly of the upper respiratory tract
Severe	Cerebral, gastric, or intestinal hemorrhage or hematuria	Disseminated bacterial, viral, or fungal infections, fever of unknown origin, pneumonia, abscesses

For the analysis of therapy implementation the following definitions are used:

dose compliance (DC) = actual dose/prescribed dose
time compliance = actual time/prescribed time

Calculation of the prescribed dose, which was done at the study center, was based on the patient's body surface area. A DC of $\geq 80\%$ is defined as fulfilling the requirements of the protocol (other studies set limits between 75% and 85% [23, 66]).

The individual phases of therapy – induction, consolidation, and maintenance – were evaluated separately. Regarding the induction treatment, only DC, not time compliance, was taken into consideration since deviations from the required eight day duration did not occur, except for three ED. These deaths were not included in the evaluation of this therapy phase. In addition, all children under the age of one year were excluded from the analysis because, in these patients, the prescribed dose was calculated with reference to body weight, not to body surface area as in the older children, and would have resulted in a distortion in the calculation of the prescribed dose.

The cut-off date for the evaluation was March 1, 1989.

3.2 Diagnostic Methods

In both studies, AML was diagnosed from bone marrow (BM) and peripheral blood smears on the basis of cytomorphologic and cytochemical criteria. All smears were forwarded to the study center at the University Children's Hospital in Münster by the hospitals in charge of treatment, stained, inspected, and checked by the diagnostics commission.

The morphologic subgroups of AML were classified according to the FAB criteria [12]. In addition to the Pappenheim stain the following cytochemical stainings were performed: PAS (periodic acid-Schiff), acid phosphatase, α-naphthyl-acetate-esterase (ANAE), and peroxidase (POX). These tests were also able to exclude ALL.

Auer rods and eosinophils were retrospectively counted by use of the original Pappenheim stained BM smears. In some cases (Tables 10 and 11), however, this test could not be done due to the poor technical quality of the smears (bleached preparations) or missing BM smears. For each smear, 1000 (in a few cases only 500) leukemic blasts were examined for their Auer rod status. 200 cells were counted for determination of eosinophilia. For a considerable proportion of the patients in AML-BFM-83 (Tables 11 and 12), the following tests were also performed:

a) Central immunophenotyping of the blasts using a panel of monoclonal antibodies and determination of terminal deoxynucleotidyl transferase (TdT, for method see [112])
b) DNA measurement by flow cytophotometry (for method see [76])
c) Cytogenetic analyses (for methods see [100])

3.3 Statistical Methods

The calculation of the probabilities of survival and remission was based on the life table method [93]. The following definitions apply:

- *Event-free survival* (EFS): Absence of any events leading to remission failure (early death, nonresponse) or termination of first remission (relapse, death during remission) in the total group of protocol patients.
- *Event-free interval* (EFI): The period of time until first relapse or death during remission in the patients who entered CR (remission group).

Patients who dropped out due to BMT in first remission, or because they were lost to follow-up were censored at the time these events happened.

This investigation tried to describe the considerable variation in the duration of survival and remission on the basis of predominantly pretherapeutic variables. Both studies provided data with respect to the following variables: age, sex, WBC, thrombocyte count, hemoglobin, liver and spleen enlargement, extramedullary organ and CNS involvement, Auer rods and eosinophils in the BM. Additionally, in AML-BFM-83, the results of cytochemical, immunological, flow cytometric and cytogenetic tests were recorded. The proportion of blasts in the BM on day 15 and the time of CR were analyzed in both studies as measurements of response kinetics.

The life table method and statistical analysis employing the log rank test [130] offer first indications on potential prognostic factors. In addition, the multiple stepwise regression analysis developed by Cox [37] allows one to determine the relationship between a target variable (EFS, duration of continuous CR) and potential prognostic factors. Using this form of analysis, several variables can be evaluated simultaneously and interactions and high correlations can be taken into consideration regardless of whether these variables are continuous (e.g., leukocytes) or categorical (e.g., sex).

The multiple stepwise regression model requires complete sets of data for all variables in each patient ([19] Program L2). Furthermore, each variable must first be tested individually; the variable with the highest significance is then entered into the model for multivariate analysis. Next, the remaining variables are tested, and again, the variable with the highest significance is determined. These steps are repeated until there is no remaining variable reaching a significance level above 10% (arbitrary value).

For each variable tested in the Cox model, the relative risk can be defined as exp(Cox coefficient $\times$ variable). This equation allows determination of the individual relative risk of failure for each patient. This means an increase or decrease of the risk for this patient with a variation in one variable compared with a different patient who has the same prognostic factors in all other respects.

Yule's constant of association and the χ^2 test were used to determine the relationship between initial variables [26].

4 Results

4.1 Analysis of Pretherapeutic Patient Data

4.1.1 Total Group

An overview of the initial study patient data is given in Table 6.

For the 180 patients in the AML-BFM-83 study with known case histories, the median duration of symptoms was four weeks (range: two days - 51 weeks). Quantitative information on more uncharacteristic symptoms, such as fatigue and exhaustion, fever, bleeding tendency, and weight loss, was not available.

Table 6. Pretherapeutic data on study patients AML-BFM-78 and AML-BFM-83

	AML-BFM-78		AML-BFM-83	
	(*n*)	(%)	(*n*)	(%)
No. of patients	151	100	182	100
Age, median (years;months)	9;11		8;2	
Sex				
Boys	81	54	99	54
Girls	70	46	83	46
Initial CNS involvement	13	9	10	6
Extramedullary organ involvement	27	18	52	32
Hepatomegaly				
≥5 cm below costal margin	38	25	39	21
Splenomegaly				
≥5 cm below costal margin	39	26	33	18
Total blood counts:				
WBC, median ($\times 10^3/mm^3$)	23.6		24.9	
Platelets, median ($\times 10^3/mm^3$)	39.0		49.0	
Hemoglobin, median (g/dl)	8.4		8.1	
No data submitted:				
Hepatomegaly	1		-	
Splenomegaly	1		4	
Initial CNS involvement (doubtful)	4		6 (7)	
Extramedullary organ involvement	2		18	

The median age of the patients was 9 years 11 months in AML-BFM-78 and 8 years 2 months in AML-BFM-83. Figures 3a and 4a show the age distribution of the patients. A moderate peak was seen for children under the age of two, and there is a gradual increase in the number of patients aged nine years and older. These data are representative only until the age of 15, since adolescents are frequently treated in institutions other than pediatric hospitals. The cumulative frequency (Fig. 4a) shows a steeper slope in children under the age of two years, and again, in patients between nine and 15. The differences in age distribution between the two studies are discussed in detail in Sect. 4.1.2. The sex distribution was the same, with a slight predominance of boys (54%) (Table 6).

With respect to initial symptoms, quantitative information is available on fever and bleeding in AML-BFM-83. Of 178 patients, 63 (35%) had primary bleeding symptoms, such as petechiae, hematomas, epistaxis, or severe gastrointestinal and cerebral bleeding. Fever of known or unknown origin was present in 111/177 patients (63%).

Clinical Findings. A total of 13/147 patients (9%) in AML-BFM-78 and 11/175 (6%) in study AML-BFM-83 showed initial CNS involvement (>10 cells/mm^3 of CSF). Liver and spleen enlargement of ≥ 5 cm below the costal margin were found in 25% and 26% respectively of the patients in AML-BFM-78, and in 21% and 18% respectively of the children in AML-BFM-83.

Unusual extramedullary organ involvement (Table 7), that is, in sites other than liver, spleen, and CNS, was found in skin, tonsils, salivary and lacrimal glands, testes, dental laminae, cervical lymph nodes (unusual tumorous swellings), intestines, lungs, pericardium, and muscles, in 18% of the patients in each study. In addition, 13% of the children in study AML-BFM-83 showed infiltration of organs not documented in AML-BFM-78 (kidneys, skeleton, and orbita). This observation must be attributed to the more frequent documentation of extramedullary organ involvement in AML-BFM-83.

Hematological Findings. Data obtained from peripheral blood analysis were similar in the two studies. The median WBC in both studies was 24000/mm^3. In AML-BFM-78, the median platelet count was 39000/mm^3 and the median hemoglobin was 8.4 g/dl. In AML-BFM-83, the median platelet count was 49000/mm^3 and the median hemoglobin was 8.1 g/dl.

The WBC distribution in the patients of both studies, either combined or separately (Fig. 5), resembled a normal distribution, with only minor differences between the two groups. The proportion of children with a low WBC (<10000/mm^3) was slightly higher in AML-BFM-78

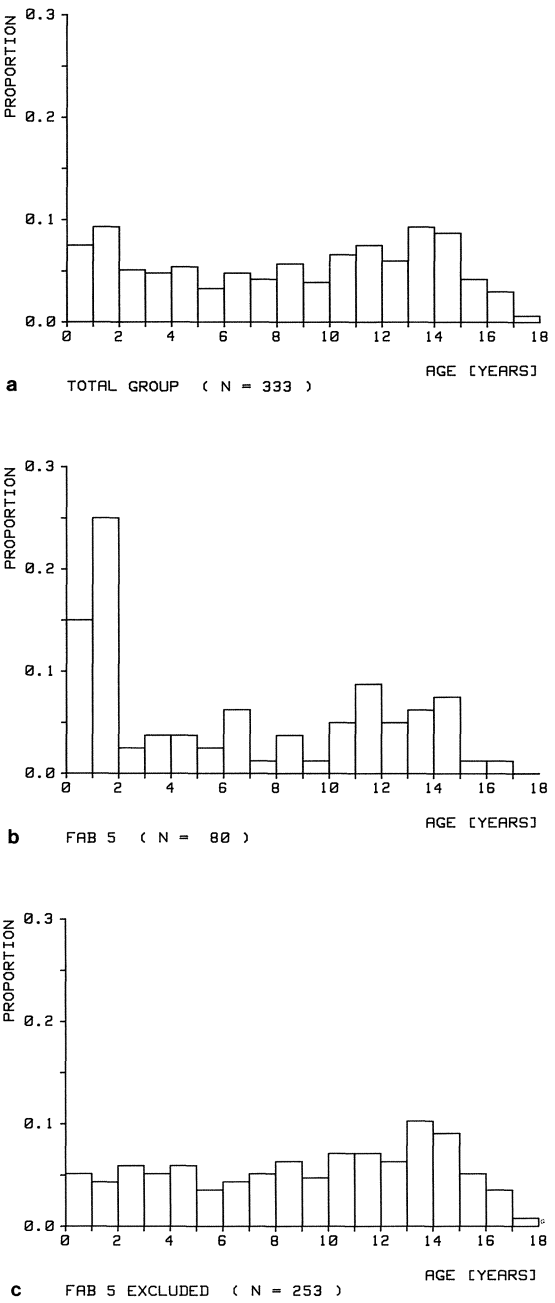


Fig. 3 a–c. Age distribution of AML patients in both studies combined (total group $n = 333$). **a** All FAB types; **b** FAB M5 and **c** all except FAB M5

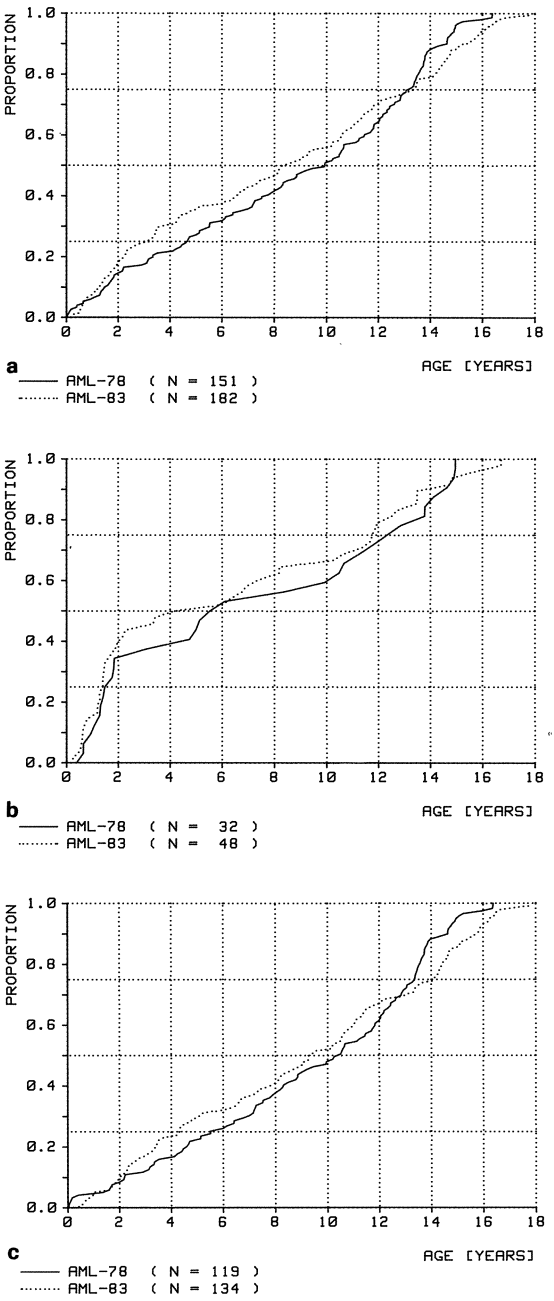


Fig. 4 a–c. Comparison of age distribution in the two studies as cumulative frequencies; compare with **3a–c**

than in the subsequent study (see the graphs of cumulative frequencies, Fig. 5b).

All other morphologic, immunologic, cytogenetic, and flow cytophotometric findings are discussed in detail in the following section.

Table 7. Extramedullary organ involvement in studies AML-BFM-78 and AML-BFM-83

	AML-BFM-78		AML-BFM-83	
	Total	with FAB M5	Total	with FAB M5
No. of patients	27/149 (18%)	9/30 (30%)	52/164 (32%)	21/42 (50%)
Skin	8	4	13	2
Tonsils	4	1	7	2
Salivary glands	3	–	4	2
Lacrimal glands	1	–	–	–
Dental lamina/gingiva	3	1	2	1
Lymphoma	2	1	1	–
Intestines	1	–	–	–
Muscular system	–	–	1	1
Pericard	1	1	1	–
Lungs	1	–	–	–
Testes	2	1	–	–
Testes and skin	–	–	1	1
All organs	1	–	–	–
Kidney	not documented		10	5
Bone			8 (13%)	5 (29%)
Orbita			4	2

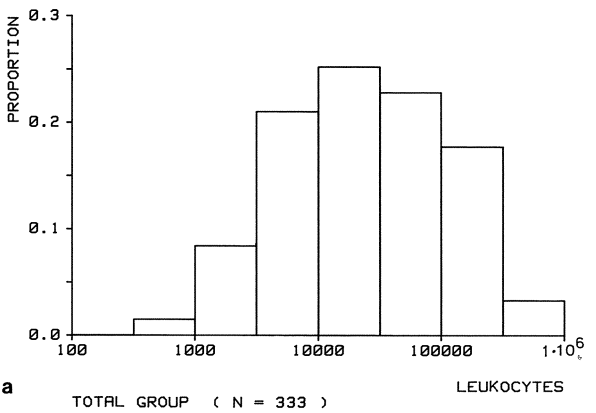
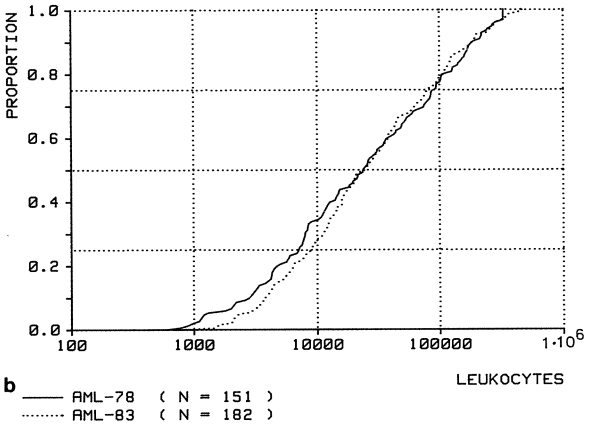
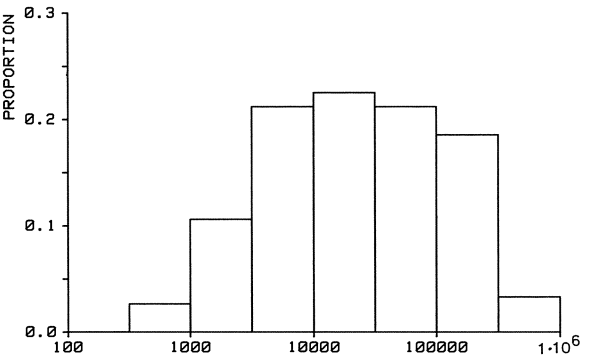


Fig. 5 a–d. Distribution of leukocyte counts in AML-BFM-78 and AML-BFM-83. **a,** **b** Both studies together, **c** study AML-BFM-78, **d** study AML-BFM-83



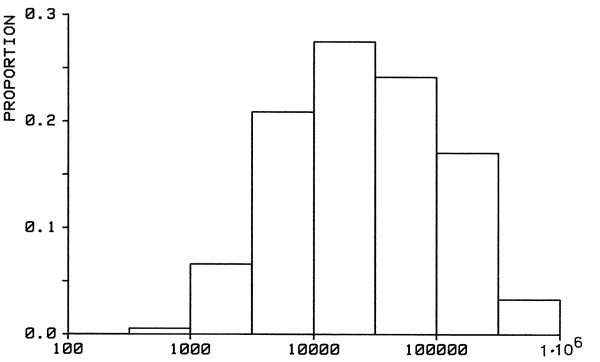
b

—— AML-78 (N = 151)
····· AML-83 (N = 182)



c

TOTAL GROUP (N = 151)



d

TOTAL GROUP (N = 182)

4.1.2 FAB Subtypes

Table 8 shows a comparison of FAB subtyping in the two studies. The distribution of subtypes among the study patients differed only in the slightly higher number of patients in AML-BFM-83 with monocytic leukemias (FAB M5) (26% vs. 21%), and the slightly lower number of patients with FAB M1/M2 (40% vs. 47%). The initial clinical and hematological data by FAB subtypes for both studies are described below and listed in Table 9.

FAB M1 and M2 (acute myeloblastic leukemia without and with maturation). The initial data showed no relevant differences from those of the total group of AML patients.

FAB M3 (acute promyelocytic leukemia). In 11 patients overall, notable findings were a low median WBC (2000/mm^3 in AML-BFM-78, and 10000/mm^3 in AML-BFM-83) and marked thrombocytopenia (median 25000/mm^3 and 20000/mm^3). Organ enlargements were not seen, except in one patient with hepatomegaly.

FAB M4 (acute myelomonocytic leukemia). The median leukocyte count was high (64000/mm^3 in AML-BFM-78, and 37000/mm^3 in AML-BFM-83); extramedullary organ involvement and hepatosplenomegaly, as well as primary CNS involvement were frequent.

FAB M5 (acute monocytic leukemia). Characteristic features were low median leukocyte counts (12000/mm^3 and 20000/mm^3) as well as hy-

Table 8. Morphological subtypes according to the FAB classification in AML-BFM-78 and AML-BFM-83

	AML-BFM-78		AML-BFM-83	
	(*n*)	(%)	(*n*)	(%)
No. of patients	151	100	182	100
FAB M1	36	24	37	20
FAB M2	34	23	37	20
FAB M3	6	4	5	3
FAB M4	40	26	45	25
FAB M5	32	21	48	26
FAB M6	3	2	7	4
FAB M7	–	–	3	2

Table 9. Initial clinical and hematological data by FAB subtypes

FAB subtype	M1		M2		M3		M4		M5		M6		M7		Total	
Study BFM	78	83	78	83	78	83	78	83	78	83	78	83	78	83	78	83
No. of patients	36	37	34	37	6	5	40	45	32	48	3	7	–	3	151	182
Percentage of patients in study	24	20	23	20	4	3	26	25	21	26	2	4	–	2	100	100
Age, median (years;months)	9;11	10;6	10;5	11;1	13;7	2;4	9;4	9;3	5;4	3;8	7;11	3;3		3;5	9;11	8;2
Sex																
Boys (%)	47	51	68	62	67	40	53	62	44	48	67	43		33	54	54
Girls (%)	53	49	32	38	33	60	47	38	56	52	33	57		67	46	46
Clinical data:																
CNS involvement (%)	3	14	3	0	0	0	26	15	3	2	0	0		0	9	6
Extramedullary organ involvement[a] (%)	6	19	15	24	0	0	28	36	27	50	0	29		0	18	32
Hepatomegaly ≥ 5 cm below costal margin (%)	20	19	6	3	17	0	40	22	39	38	0	29		33	25	21
Splenomegaly ≥ 5 cm below costal margin (%)	31	24	6	5	0	0	40	22	31	19	0	29		33	26	18
Total blood counts:																
WBC median ($\times 10^3/mm^3$)	26	26	15	32	2	10	64	37	12	20	4	9		4	24	25
Thrombocytes median ($\times 10^3/mm^3$)	36	56	41	40	25	20	37	45	34	73	12	20		101	39	49
Hemoglobin median (g/dl)	8.4	8.5	8.7	8.6	7.7	8.3	8.7	8.3	8.2	7.7	5.0	7.7		7.6	8.4	8.1

[a] Distribution, see text

perleukocytosis (Fig. 6). Fig. 6b shows a shift towards higher leukocyte counts in AML-BFM-83 as compared to the first study.

Extramedullary organ involvement, including hepatosplenomegaly, was predominantly found in leukemias with either partially or purely monocytic differentiation (Tables 7 and 9). Overall, 55/150 children with FAB M4 and M5 showed extramedullary organ involvement, as compared to 24/163 children with FAB types without monocytic differentiation ($p = 0.0001$, χ^2 test). In each of the two studies, only one child with FAB M5 presented with initial CNS involvement.

Unlike in the group of patients with other forms of the disease, girls outnumbered boys among the children with monocytic leukemias in both studies (56% and 52% of girls, boys/girls n.s., χ^2 test). Furthermore, the median age was very low (5;5 years and 3;8 years). The distribution of the age groups shows a high proportion of children under two with FAB M5 (32/80, 40%), as compared to the other subtypes (24/232, 9.5%) (Figs. 3b, c and 4b, c). The differences in age distribution between the total groups of the two studies may be attributed to the higher proportion of patients with FAB M5 in study AML-BFM-83 (26% vs. 21%, see Table 8). When these patients are excluded, the difference in median age between the two studies is reduced (AML-BFM-78: 10;3 years; AML-BFM-83: 9;3 years). The cumulative frequency curve of the age distribution shows a steady slope after exclusion of the FAB M5 patients (Fig. 4c), since children under two are no longer overrepre-

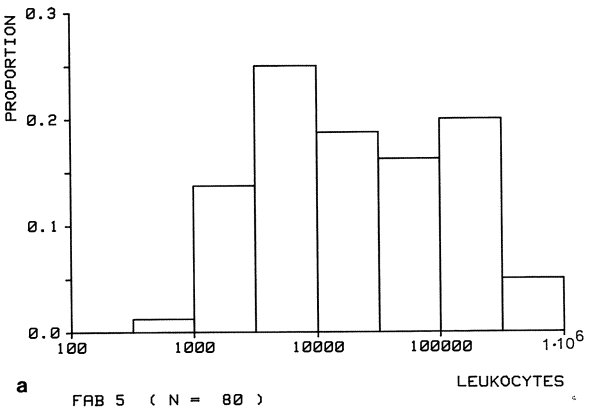
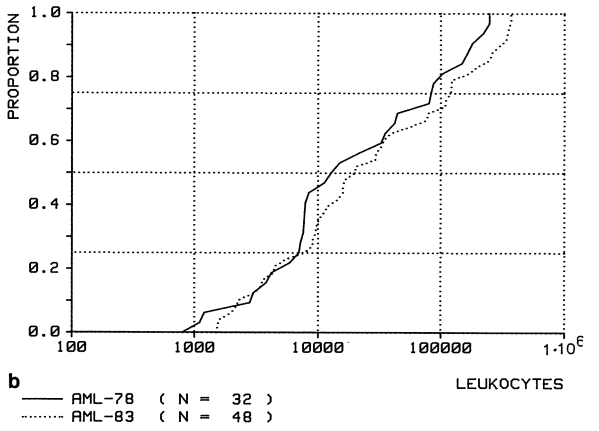
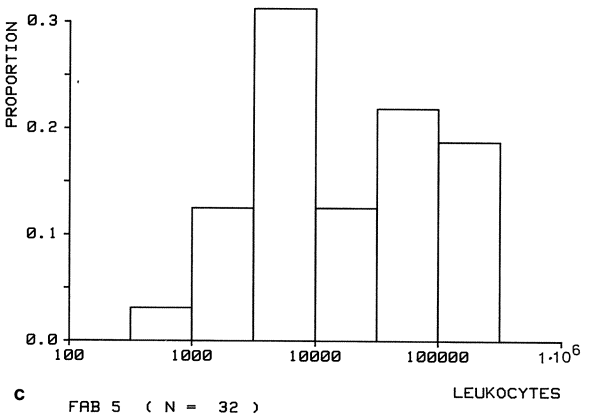


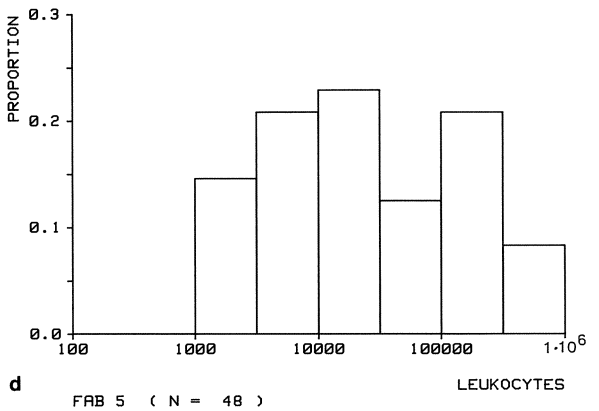
Fig. 6 a–d. Distribution of leukocyte counts in AML-BFM-78 and AML-BFM-83 for patients with FAB M5. **a, b** Both studies together, **c** AML-BFM-78, **d** AML-BFM-83


PROPORTION
1.0
0.8
0.6
0.4
0.2
0.0
100
1000
10000
100000
1·10^6
LEUKOCYTES
AML-78 (N = 32)
AML-83 (N = 48)

b


PROPORTION
0.3
0.2
0.1
0.0
100
1000
10000
100000
1·10^6
LEUKOCYTES
FAB 5 (N = 32)

c


PROPORTION
0.3
0.2
0.1
0.0
100
1000
10000
100000
1·10^6
LEUKOCYTES
FAB 5 (N = 48)

d

sented. However, Fig. 4b clearly shows a steep slope in the curve for children under two with FAB M5.

FAB M6 (acute erythroblastic leukemia). This morphological type, seen in only 10 out of 333 patients (3%), is rare. Except for two children who initially showed increased WBC (32000/mm^3 and 40000/mm^3), initial pancytopenia was observed.

FAB M7 (acute megakaryoblastic leukemia). Morphologic findings of undifferentiated, nongranular blasts with cytoplasmic blebs strongly supported the diagnosis of this subtype in three patients of study AML-BFM-83. In two patients, the monoclonal antibody J15 against the platelet glycoprotein IIb/IIIa was positive (>20%).

4.1.3 Morphologic and Laboratory Findings

Tables 10 and 11 present further morphologic and laboratory findings by FAB types for studies AML-BFM-78 and AML-BFM-83, respectively.

Auer Rods. Quantitative results on Auer rods are available for 85% of the patients in each of the two studies. In both studies, the FAB types M1-M3, characterized by granulocytic differentiation, included a predominance of Auer rod positive patients (73%, AML-BFM-78 and 69%, AML-BFM-83), whereas Auer rods were seen in only 28% (AML-BFM-78) and 25% (AML-BFM-83) of the FAB types M4 and M5, characterized by partially or purely monocytic differentiation, ($p<0.0001$, χ^2 test). The distribution of Auer rod positive and negative patients among the FAB types was not appreciably different between the two studies (n.s., χ^2 test).

Eosinophils. Quantitative information is available on 74% of the patients in AML-BFM-78 and 86% of the patients in AML-BFM-83. All the following data refer to these patients.

In 73 out of 269 patients (27%) in both studies the initial BM smear showed eosinophilia, defined as ≥3% immature or mature eosinophils. This condition was found most frequently in children with FAB types M2 (28/64 = 44%) and M4 (29/70 = 41%). Atypical eosinophils were only detected with ≥3% of these cells, predominantly in FAB type M4 (20/29 = 71%). The dysplastic eosinophils showed a wide range of maturation stages (Fig. 7). They often contained a mixture of eosinophilic and basophilic granules in the cytoplasm. In FAB type M4, there was a

Table 10. Distribution of initial morphological data by FAB types in study AML-BFM-78

FAB type	M1	M2	M3	M4	M5	M6	Total
No. of patients	36	34	6	40	32	3	151
Auer rods							
negative	11	6	–	21	23	–	61
positive	17	26	5	16	1	3	68
no data submitted	8	2	1	3	8	–	22
Eosinophils in BM							
<3%	18	17	2	20	17	2	76
≥3%	6 (1)	14 (2)	1	12 (8)	2	1	36 (11)
no data submitted	12	3	3	8	13	–	39

Parentheses, number of patients with atypical eosinophils

Table 11. Distribution of initial morphological, cytochemical, and immunological data by FAB types in study AML-BFM-83

FAB type	M1	M2	M3	M4	M5	M6	M7	Total
No. of patients	37	37	5	45	48	7	3	182
Auer rods								
negative	11	10	–	22	38	2	2	85
positive	18	23	5	18	2	3	–	69
no data submitted	8	4	–	5	8	2	1	28
Eosinophils in BM								
<3%	29	19	5	21	38	5	3	120
≥3%	4 (3)	14 (2)	–	17 (12)	1	1	–	37 (17)
no data submitted	4	4	–	7	9	1	–	25
POX								
positive	35	33	5	43	24	4	1	145
negative	–	1	–	–	21	2	1	25
no data submitted	2	3	–	2	3	1	1	12
ANAE								
positive	17	21	5	43	42	5	2	135
negative	17	13	–	–	3	–	–	33
no data submitted	3	3	–	2	3	2	1	14
TdT								
<10%	23	19	4	25	31	4	3	109
≥10%	6	4	–	3	1	–	–	14
no data submitted	8	14	1	17	16	3	–	59

Parentheses, number of patients with atypical eosinophils

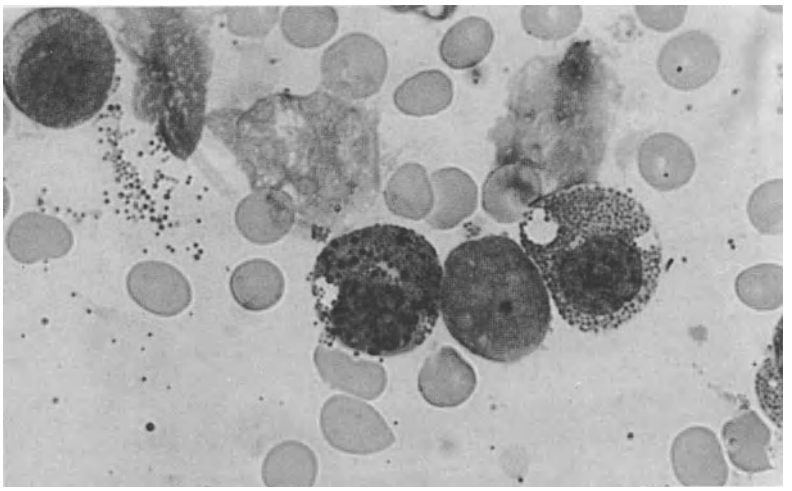


Fig. 7. Abnormal, dysplastic eosinophils with eosinophilic and basophilic granules in a patient with FAB M4 (Pappenheim staining, × 1000)

large variation in the percentage of eosinophils in the bone marrow (Fig. 8a); an abnormal morphology was found predominantly in patients with ≥ 5% eosinophils. In the other FAB types, the percentage of children with ≥ 5% eosinophils was low and abnormal eosinophilic cells were rarely seen (Fig. 8b).

The distribution of patients with and without eosinophilia in the BM in the two studies was not significantly different in those FAB types with representative numbers of patients (M1, M2, M4, M5) (n.s., χ^2 test).

Cytochemistry. Quantitative information on the cytochemical reactions POX and ANAE is available only for AML-BFM-83 (Table 11). In those FAB types with predominantly granulocytic differentiation (M1–M4), the POX reaction, with one exception, was always positive (116/117 patients). In FAB M5, a slightly positive POX reaction was found in 24/45 patients (53%).

The ANAE reaction was positive in 135/168 patients (80%). In those FAB types with partially or purely monocytic differentiation (M4 and M5), a negative reaction was rare (3/85 patients, 3.5%). By contrast, a negative reaction occurred much more frequently in those FAB types with granulocytic differentiation, (M1 and M2, 30/68 patients, 44%).

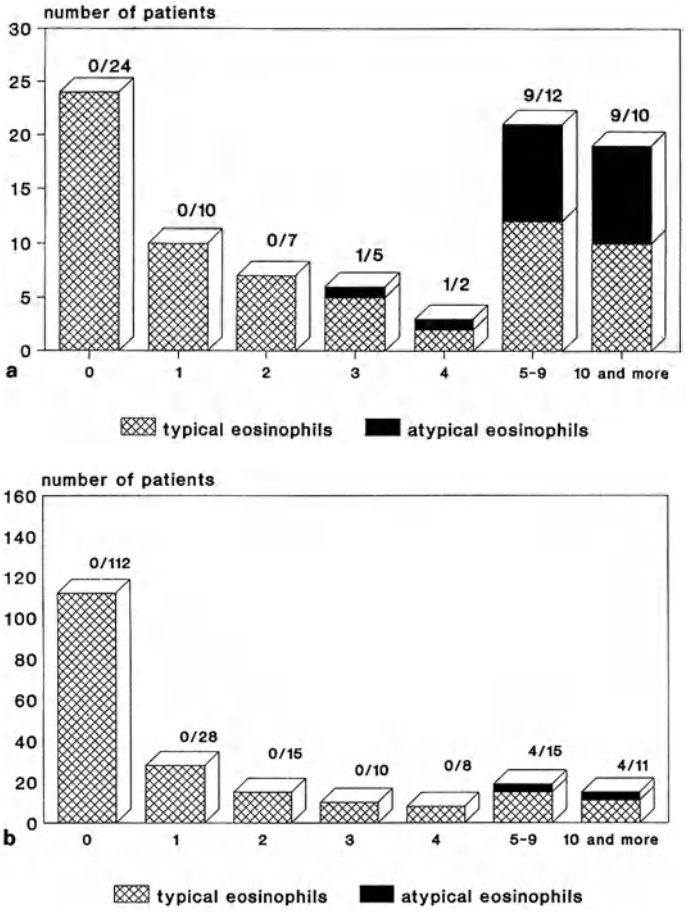


Fig. 8 a, b. Distribution of the proportion of eosinophils in the bone marrow in patients, **a** with FAB M4 and **b** with morphologic types other than M4

Figure 9 shows that the POX and ANAE reactions were characteristic of a large proportion of the FAB types. Only two patients with FAB M5, as determined by the morphologic findings, showed negative results with both reactions.

Immunology. Data are available for 123 patients in AML-BFM-83, although they are sometimes incomplete for all of the markers. For TdT, 14/123 patients (11%) had clearly positive results ($\geq 10\%$ of cells, Table 11). These were mainly patients with FAB types M1 and M2 (10/52,

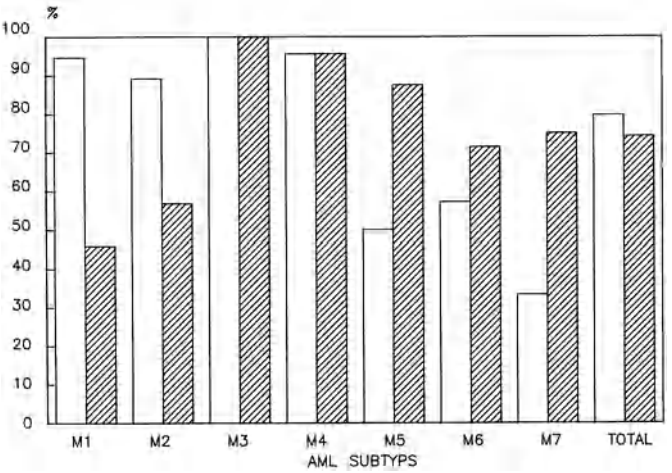


Fig. 9. Proportion of POX (open columns) and ANAE (hatched columns) positive patients by FAB types in AML-BFM-83

19%). The results of surface marker analysis using monoclonal antibodies (My7, My9, VIM-D5, OKM1, glycophorin A and platelet glycoprotein) in 81 patients of AML-BFM-83 have been published elsewhere [112].

Cytogenetics. Findings are available for 59/182 patients in AML-BFM-83 (32%; Table 12). Of 59 patients, 21 (36%) showed no aberrations. The translocation (t)8; 21 was found in FAB M1 and M2 (8 patients), t(15; 17) in three patients with FAB type M3; t(9; 11) in four children with FAB M5; deletion 11q23 in five patients of FAB types M4 and M5, and in one child with FAB M2. Inversion 16 was detected in one child with FAB M4. The remaining aberrations were distributed among all morphologic subtypes.

Flow cytometry. Data are known for 69/182 patients (37%). DNA aneuploidy (DNA index > 1) was found in 20/69 patients (29%). The distribution of aneuploidy among the morphologic subtypes (Table 12) shows only that aneuploidy was more frequent in FAB M5 (8/21 patients) than in the other FAB types which had larger patient numbers. Both patients with FAB M7 who were tested showed an increased DNA index. These data should be evaluated with caution since many data are missing. Nonetheless, in most instances, the cytogenetic data were consistent with the data obtained by flow cytometry. Out of nine

Table 12. Distribution of initial cytogenetic and flow cytometry data by FAB types in study AML-BFM-83

FAB type (n)	M1	M2	M3	M4	M5	M6	M7	Total
Cytogenetic:								
No data submitted	22	20	2	19	27	4	2	96
Test failure	3	6	–	10	7	1	–	27
No aberration	8	1	–	8	4	–	–	21
t(8; 21)	3	5	–	–	–	–	–	8
t(9; 11)	–	–	–	–	4	–	–	4
t(15; 17)	–	–	3	–	–	–	–	3
11q23-aberration	–	1	–	2	3	–	–	6
inv(16)	–	–	–	1	–	–	–	1
+8	–	1	–	1	2	–	–	4
−7	–	–	–	–	–	1	–	1
Other	1	3	–	4	1	1	1	11
ICP: no data submitted	22	26	4	28	27	5	1	113
DNA index = 1	12	9	1	13	13	1	0	49
</> 1	3	2	–	4	8	1	2	20

ICP, flow cytometry

patients with aneuploidy and cytogenetic data, eight had an abnormal karyotype and only one was found to be normal. Out of 18 children with diploidy, 10 had abnormal and eight had normal cytogenetic findings.

4.2 Correlation of Initial Parameters Including Kinetics of Blast Cell Reduction

The contingency table (Table 13) for calculating Yule's association coefficient describes the relationship between initial parameters in protocol patients of AML-BFM-83. EDs have been excluded and will be discussed separately (Sect. 4.6). If the coefficient is >0.50, it signifies a relationship between parameters; values ≤ 0.50 indicate an independent variable.

Significance levels on which the parameters given in the contingency table are associated are shown in Table 14. Hyperleukocytosis correlates with both hepatomegaly and compromised blast reduction, as measured on day 15. There is also a weak association with initial CNS involvement. The presence of Auer rods correlates with little or no enlargement of the liver and with $\geq 80\%$ POX positive blasts in the BM;

Table 13. Association of initial parameters in patients of AML-BFM-83 (early deaths excluded). Data indicate number of patients

	WBC ($\times 10^3$/mm^3)		CNS		Organ		Liver (cm)		Age (years)		% Blasts (day 15)		% POX		% EOS	
	<100	≥100	+	−	+	−	≤5	>5	<10	≥10	<10	≥10	<80	≥80	<3	≥3
Auer																
+	57	7	4	56	13	49	**61**	* **3**	30	34	43	13	**20**	* **36**	41	20
−	62	13	4	67	23	43	**60**	* **14**	46	29	53	13	**34**	* **16**	53	12
WBC ($\times 10^3$/mm^3)																
<100			**6**	* **127**	36	93	**124**	* **14**	80	59	**98**	* **13**	54	49	42	28
≥100			**3**	* **17**	7	11	**15**	* **7**	12	10	**14**	* **6**	9	6	12	6
CNS																
+					4	4	9	0	4	5	5	2	5	3	8	0
−					39	93	123	20	83	61	102	25	55	48	96	31
Organ																
+							35	7	30	13	29	6	14	12	29	9
−							92	12	55	49	74	21	46	39	72	21
Liver (cm)																
≤5									77	62	94	26	**54**	* **53**	95	31
>5									15	6	17	3	**9**	* **2**	12	3
Age (years)																
<10											66	15	27	37	61	48
≥10											46	14	28	26	18	16
% Blasts (day 15)																
<10													44	38	**68**	** **28**
≥10													12	9	**25**	**1**
% POX																
<80															**40**	* **8**
≥80															**29**	* **19**

Yules' association coefficient: ≤|0.5| no association; >|0.5|; * positive or negative association, >|0.8|; ** strong positive or negative association

Table 14. Calculation of significance values for association between parameters listed in Table 13

Parameter A	Parameter B	χ^2	*p* value
WBC	Liver ≥5 cm	7.82	.01
	Blasts day 15	4.57	.04
	CNS involvement	3.45	.1
Auer positive	POX ≥80%	11.02	.001
	Liver <5 cm	6.44	.02
	Eo ≥3%	3.41	.07
	Organ negative	3.05	.11
Liver ≥5 cm	POX <80%	3.94	.06
Blasts day 15	Eo <3%	7.24	.008
POX ≥80%	Eo ≥3%	6.24	.02

there is a weak association with eosinophilia and with an absence of extramedullary organ involvement. With hepatomegaly, the proportion of POX positive blasts required for association tends to be lower, whereas a high proportion of POX positive blasts is associated with eosinophilia in the BM. An increased proportion of blasts on day 15 correlates negatively with eosinophilia, and positively with hyperleukocytosis.

There is no association between age and any other parameters. Extramedullary organ involvement is only weakly associated with liver enlargement. Initial CNS involvement could not be adequately evaluated due to the small number of patients. There is no relationship between initial hyperleukocytosis and the presence of Auer rods.

4.3 Outcome

The proportion of children who died before onset of therapy was higher in AML-BFM-83 (9/182, 5%) than in AML-BFM-78 (2/151, 1%), whereas the number of EDs after onset of therapy was lower in study AML-BFM-83 (12/173, 7%, compared with 17/149, 11%, Table 15). By contrast, at 22/173 (13%), the number of nonresponders (including partial responders) was slightly higher in AML-BFM-83 than in AML-BFM-78 (13/149, 9%). Overall, in both studies, 80% of the protocol patients achieved CR.

The proportion of children who entered CR by six weeks was slightly higher in AML-BFM-83 (82/139, 59%) than in AML-BFM-78 (63/119, 53%, n.s.). The median time until CR was 41 days (AML-BFM-78) and 38 days (AML-BFM-83).

Table 15. Outcome of patients in the AML-BFM-78 and AML-BFM-83 studies

	AML-BFM-78	AML-BFM-83
No. of patients	151	182
Death prior to therapy	2	9
Patients treated	149	173
Early deaths		
during induction therapy		
due to hemorrhage/leukostasis	12	9
other complications	5	3
Nonresponders	13	22
CR achieved	119 (80%)	139 (80%)
Death in CCR	6	4
Withdrawals[a]	6 (2)	7 (7)
Relapses[b]	55 (9)	46 (7)
In CCR	52	82
Alive	63	97
Follow-up (months)	77–122	30–75

[a] Number of patients receiving BMT in parenthesis
[b] Number of patients with CNS involvement in parenthesis

Out of the 10 patients in the two studies combined who died in CCR, nine had severe infections (mycosis, pneumonia, tüberculosis). In seven of these patients the infections were concurrent with bone marrow aplasia which occurred during or after consolidation therapy (for further details see Sect. 4.8).

Out of nine children in both studies (2 and 7 patients) who underwent BMT during first CR, three died from therapy-related complications within the first two months after transplantation. The remaining six patients have been in CCR for periods of 2;11 to 6;0 years (Fig. 53).

In AML-BFM-78, four children were lost to evaluation. In two of these, protocol-appropriate therapy was either discontinued after the induction phase or continued with different cytostatic agents (both children have remained in CCR); the other two were lost to follow-up.

As of the cut-off-date for the evaluation, 55 (AML-BFM-78) and 46 (AML-BFM-83) relapses, predominantly affecting the bone marrow, had occurred (Table 16). In 9/55 (16%) and 7/46 (15%) children, the relapse involved the CNS. The four cutaneous relapses occurred in patients with FAB M4 and M5. Out of 10 patients with isolated CNS, testicular, or skin relapses, one remained in continuous second remission for 22 months and one for 5;6 years.

Table 16. Sites of relapses in the AML-BFM-78 and AML-BFM-83 studies

Site of relapse	AML-BFM-78	AML-BFM-83
BM	40	37
CNS	1	4
Testes	1	–
Skin	2	2
BM/CNS	6	2
BM/testes	2	–
BM/intestinal tumor	1	–
BM/CNS/testes	1	1
BM/CNS/pleura	1	–
Total no. of patients	55	46

In AML-BFM-78, 52 children were in CCR for a median observation period of 8;5 years and 63 patients survived. The probability of a 10-year EFS is 0.37 (SD 0.04) for the whole group, and 0.46 (SD 0.05) for the children who achieved remission (EFI) (Fig. 10). In AML-BFM-83, 82 children remained in CCR, and 97 were alive after a median follow-up period of 4.5 years. The probability of a six-year EFS is 0.49 (SD 0.04), a six-year EFI has a probability of 0.61 (SD 0.04) (Fig. 10). The difference between the EFI curves of the two studies as determined by the log rank test is significant ($p = 0.03$).

Documentation on the achievement of second remissions is incomplete. The median times of survival after relapse were 5,2 and 5,4 months (Fig. 11a).

Out of 21 patients in both studies who underwent BMT for nonresponse to therapy (6 patients) or after relapse (15 patients), six died from complications following BMT (4 of these were nonresponders). Seven children suffered a hematological relapse after 3–15 months and eight patients remained in CR for periods from 1;4 to 8;6 years (Table 17). Even if the smaller number of patients is taken into consideration, the probability of survival after relapse is markedly higher in patients with BMT than in those treated differently ($p < 0.001$, see Fig. 11b).

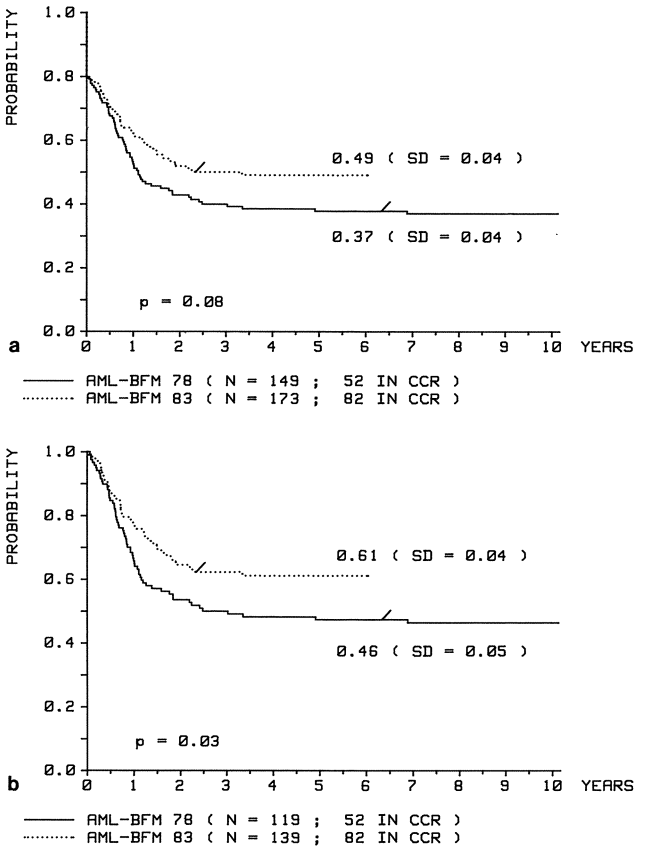


Fig. 10. a Probability of EFS duration, study AML-BFM-78 vs. AML-BFM-83. **b** Probability of EFI duration, study AML-BFM-78 vs. AML-BFM-83

Table 17. Results of BMT in primary nonresponders (NR) or relapse patients

	AML-BFM-78		AML-BFM-83		Total	
	NR	Relapse	NR	Relapse	NR	Relapse
Death due to complications following BMT	2	2	2	-	4	2
Recurrent relapse	-	2	1	3	1	6
In CCR	-	2	1	6	1	7
Total no. of patients	2	6	4	9	6	15

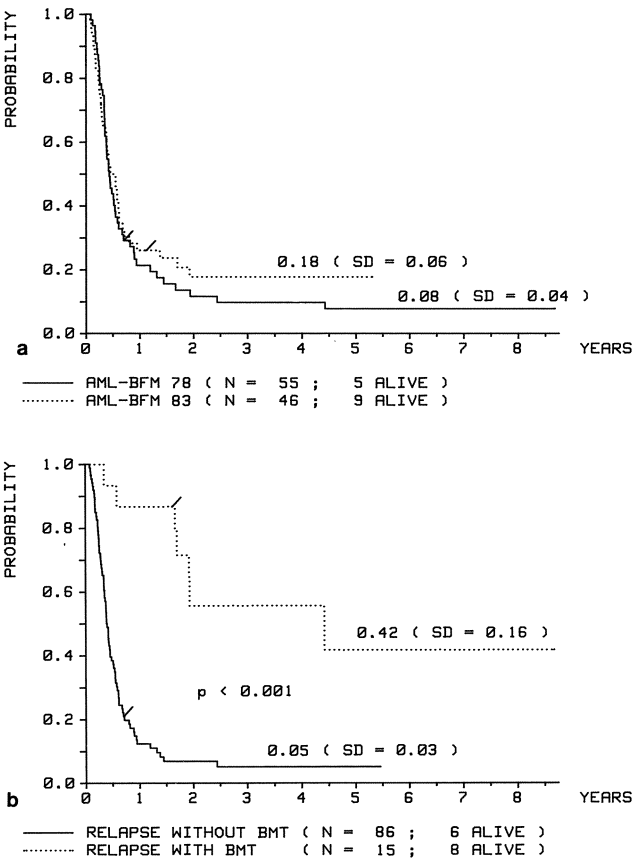


Fig. 11. a Probability of survival after relapse in AML-BFM-78 vs. AML-BFM-83. **b** Probability of survival after relapse without BMT and with BMT in both studies combined.

4.4 Results by Morphologic Subtypes

The results of the two studies by FAB types are listed in Table 18. In both studies, EDs due to hemorrhage and/or leukostasis, before or after onset of therapy, were mainly seen in monocytic leukemia (19/32, 59%) (compare Sect. 4.6.). Nonresponders occurred in all of the more frequent FAB types. In study AML-BFM-78, relapses were most often seen in the FAB types M2 and M4, unlike study AML-BFM-83, where they were most frequent in M5.

Table 18. Results by morphological subtypes of AML in the AML-BFM-78 and AML-BFM-83 studies

FAB	M1		M2		M3		M4		M5		M6		M7		Total	
BFM study	78	83	78	83	78	83	78	83	78	83	78	83	78	83	78	83
No. of patients	36	37	34	37	6	5	40	45	32	48	3	7	–	3	151	182
Death prior to therapy	–	–	–	–	–	–	–	2	2	7	–	–	–	–	2	9
Death during induction therapy	3	1	–	3	3	1	4	2	7	5	–	–	–	–	17	12
NR	4	5	1	4	–	–	5	6	3	5	–	1	–	1	13	22
CR achieved	29	31	33	30	3	4	31	35	20	31	3	6	–	2	119	139
(percentage)	(81)	(84)	(97)	(81)			(78)	(81)	(67)	(76)					(80)	(80)
Death in CCR	1	–	–	1	1	1	3	1	1	1	–	–	–	–	6	4
Withdrawals	3	–	–	–	–	–	2	4	1	2	–	1	–	–	6	7
Relapses	10	8	19	9	–	1	16	10	7	16	3	1	–	1	55	46
EFS*	46	62	41	54			28	52	42	30					38	49
EFI*	57	74	42	67			36	63	63	40					47	61

* Kaplan-Meier method of 6 years in both studies (standard deviations according to patient numbers: 7%–11%)

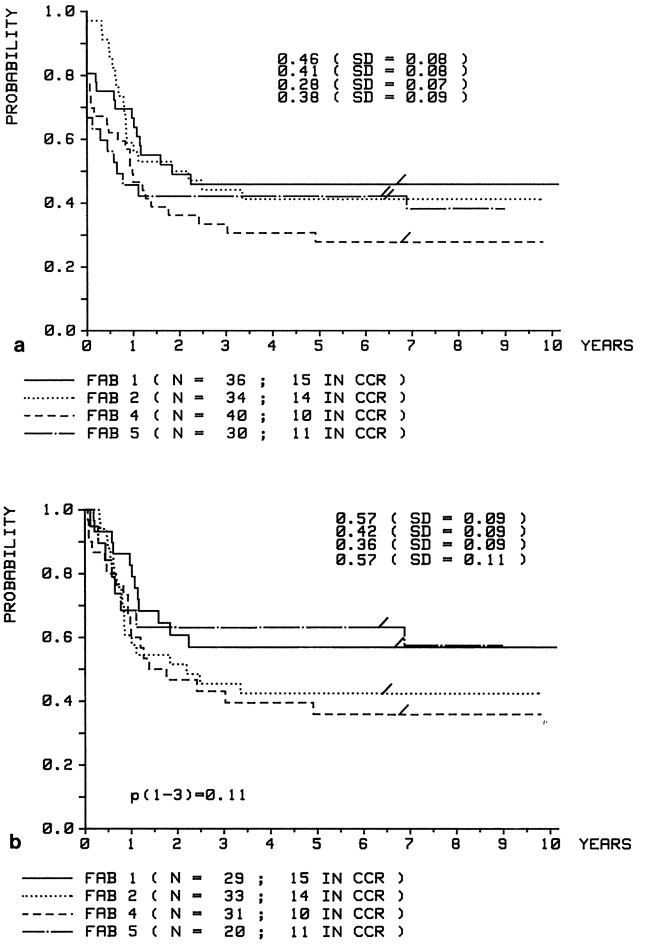


Fig. 12. a Probability of EFS duration in the FAB types M1, M2, M4, and M5 (AML-BFM-78). **b** Same for EFI duration

The life table analyses for the most frequent FAB types M1, M2, M4, and M5 in studies AML-BFM-78 and AML-BFM-83 are shown in Figs. 12 and 13.

FAB M1. In both studies, the results for an EFS (Fig. 14a) were slightly better in FAB M1 than in the other frequent FAB types. At 0.74 (SD 0.08), the probability of a six-year EFI in study AML-BFM-83 is not

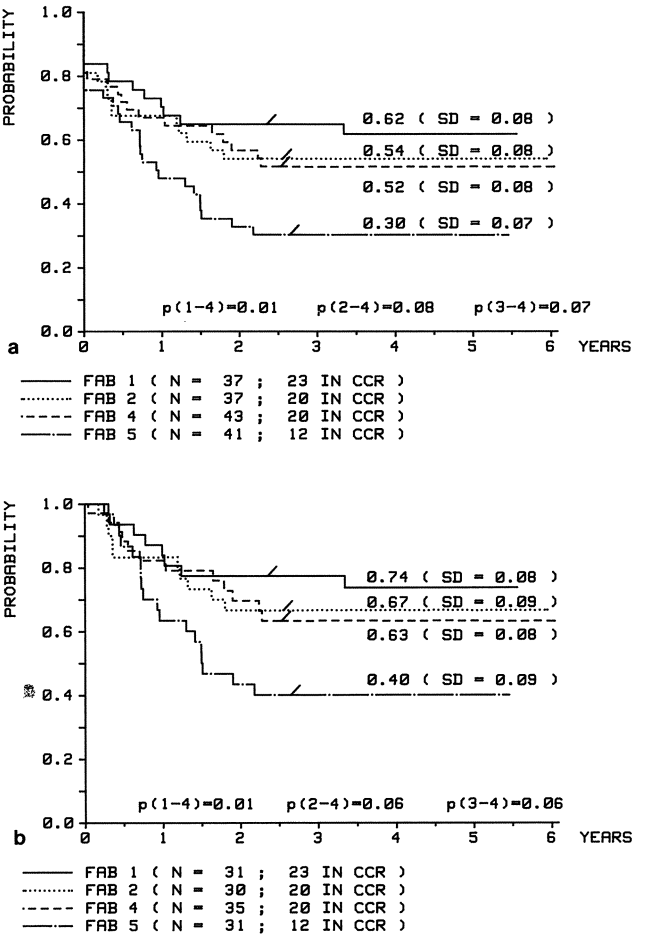


Fig. 13. a Probability of EFS duration in the FAB types M1, M2, M4, and M5 (AML-BFM-83). **b** Same for EFI duration

significantly higher than in study AML-BFM-78 (0.57, SD 0.09), (Fig. 14b, $p = 0.19$).

FAB M2. At 30/37 children (81%), the proportion of M2 patients who entered remission was lower in AML-BFM-83 than in AML-BFM-78 (33/34 patients, 97%) (Fig. 15a); however, few relapses occurred in AML-BFM-83. These differences were not significant ($p = 0.08$, Fig. 15b).

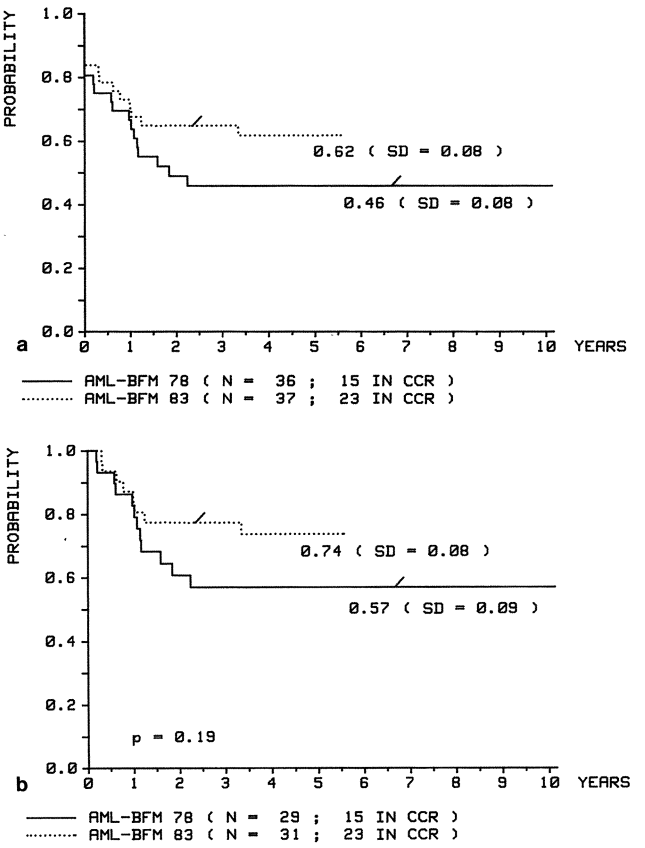


Fig. 14. **a** Probability of EFS duration in patients with FAB M1 in AML-BFM-78 vs. AML-BFM-83. **b** Same for EFI duration

FAB M3. Out of a total of 11 patients with acute promyelocytic leukemia, two suffered ED from intracranial hemorrhage, one from ulcer bleeding and one from sepsis. Of the two children who died in CCR, one died after six weeks from the sequelae of anthracycline-induced cardiotoxicity, the other from undetected tuberculosis after eight months. There was only one relapse after six months. Four patients remained in CCR, for 39, 54, 92, and 116 months respectively (Table 19).

FAB M4. The number of EDs, nonresponders, and relapses in FAB M4 was high in both studies (Table 17, Fig. 16a). Prolongation of the EFI in AML-BFM-83 ($p = 0.04$) is shown in Fig. 16b.

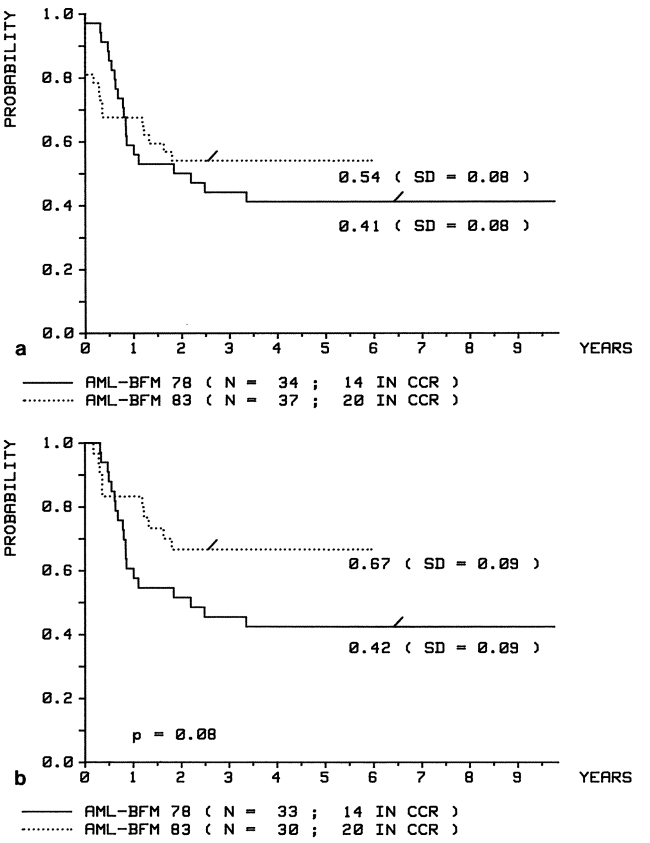


Fig. 15. **a** Probability of EFS duration in patients with FAB M2, AML-BFM-78 vs. AML-BFM-83. **b** Same for EFI duration

Table 19. Treatment results in patients with FAB M3

	AML-BFM-78	AML-BFM-83
No. of patients	6	5
Death during induction therapy		
hemorrhage	2	1
other complications	1	–
CR achieved	3	4
Death in remission	1	1
Relapse	–	1
In CCR	2	2

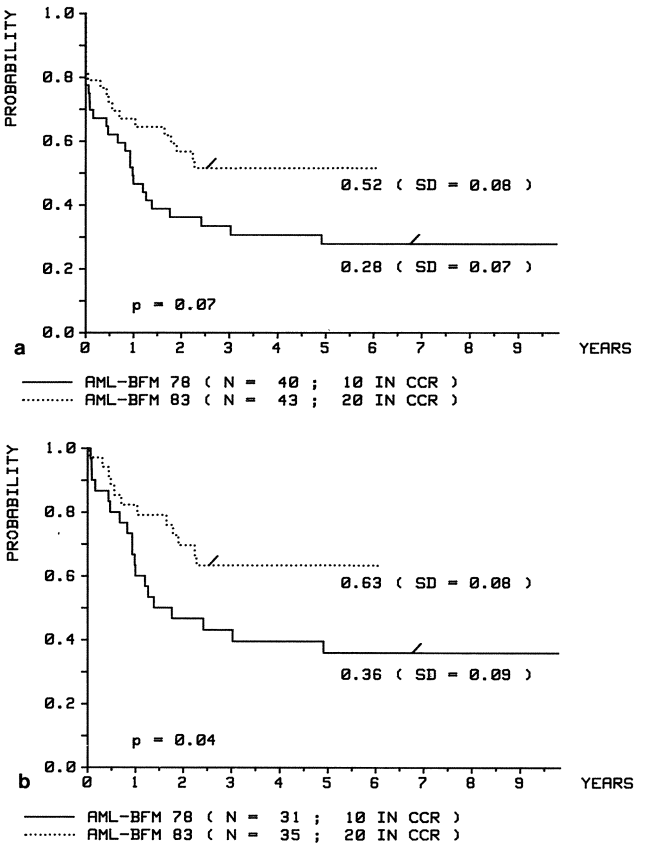


Fig. 16. **a** Probability of EFS duration in patients with FAB M4, AML-BFM-78 vs. AML-BFM-83. **b** Same for EFI duration

FAB M5. The percentage of EDs and nonresponders was comparable in the two studies (Table 18, Fig. 17a). Relapses in patients in AML-BFM-78 were mainly seen within the first 14 months after achievement of CR; there was only one late relapse, which occurred after seven years. The difference in the EFI duration between the studies was not significant ($p = 0.26$, Fig. 17b).

FAB M6. Out of 10 children with acute erythroleukemia, nine achieved CR. All three children in AML-BFM-78 relapsed. Out of six CR patients in AML-BFM-83, five were in continuous first remission for 31 months to six years, one of them after BMT (Fig. 18).

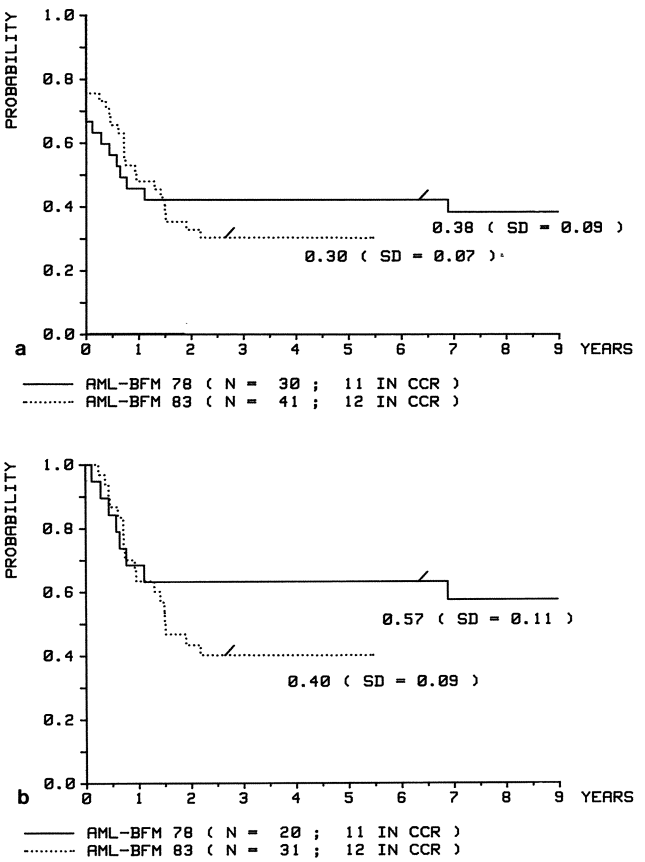


Fig. 17. a Probability of EFS duration in patients with FAB M5, AML-BFM-78 vs. AML-BFM-83. **b** Same for EFI duration

FAB M7. Out of three patients with acute megakaryoblastic leukemia in AML-BFM-83, one was in CCR for 28 months; the other two died within five months after achieving partial remission or suffering early relapse.

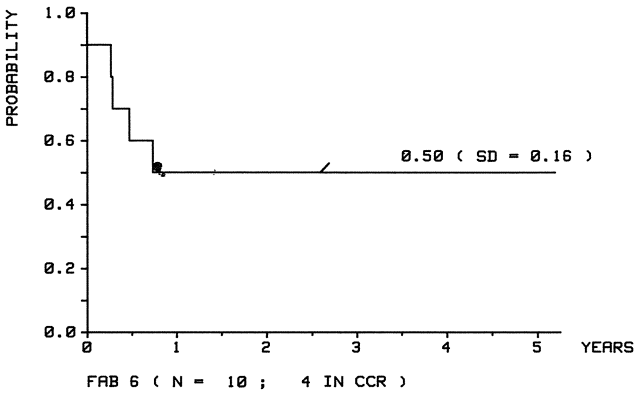


Fig. 18. Probability of EFS duration in patients with FAB M6. Combined analysis of AML-BFM-78 and AML-BFM-83

4.5 Risk Factor Analysis of Pretherapeutic and Response-Kinetic Parameters

The following analysis refers to protocol patients. Risk factors predicting failure to achieve remission as well as relapse are analyzed first; analysis of the risk factors predicting ED is carried out separately (Sect. 4.6).

The significance of therapy intensification in AML-BFM-83, compared to the first study, was evaluated by investigating the influence of the individual variables for each study separately; a combined evaluation was performed only in exceptional cases. Tables A–C of the appendix present the treatment results according to initial clinical and hematological findings, as well as selected response kinetic parameters.

4.5.1 Sex

Sex had no influence on prognosis in either of the two studies. Figure 19 shows the same results for an EFS in boys as in girls. Only the CR rate was lower in girls (76%) than in boys (84%, n.s.).This was attributable to the higher frequency of early deaths resulting from hemorrhage and/or leukostasis, and not to treatment failure. Evaluation of the two studies separately showed similar results (Table 20).

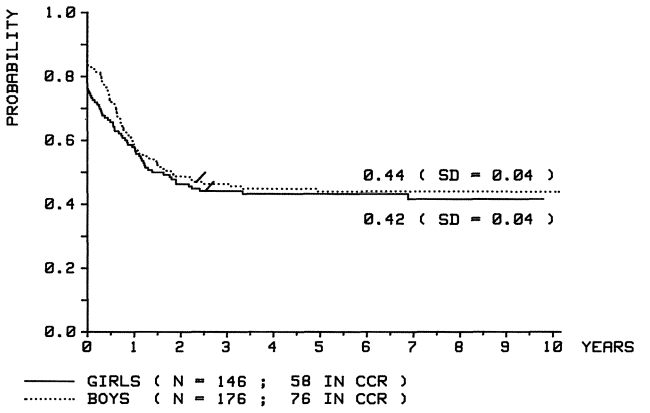


Fig. 19. Probability of EFS duration, girls vs. boys. Combined analysis of AML-BFM-78 and AML-BFM-83

4.5.1.1 Influence of Sex in the FAB Types

Table 20 shows the results by FAB types. Only 70% of girls with FAB M1 achieved CR, as compared to 94% of boys ($p = 0.01$). The difference in EFS (Fig. 20) was not significant ($p = 0.13$).

In the FAB types M2 and M5, the EFS and EFI curves show no differences between girls and boys. Although there was a higher relapse rate in boys with FAB M4 (Fig. 21), the difference was not significant ($p = 0.18$).

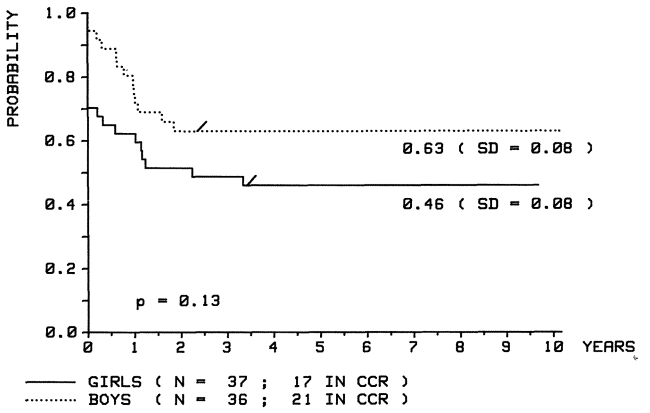


Fig. 20. Probability of EFS duration in FAB M1, girls vs. boys. Combined analysis of AML-BFM-78 and AML-BFM-83

Table 20. Prognostic significance of sex by FAB types in the AML-BFM-78 and AML-BFM-83 studies

	CR rate (%)						EFI (%)					
	AML-BFM-78		AML-BFM-83		AML-BFM-78/83		AML-BFM-78		AML-BFM-83		AML-BFM-78/83	
	M	F	M	F	M	F	M	F	M	F	M	F
FAB M1	94	68(*)	95	72(*)	94	70**	52	62	78	69	67	65
FAB M2	96	100	87	71	91	84	45	36	70	60	57	48
FAB M4	76	79	73	94	75	86	27	46	58	71	41	58
FAB M5	62	71	81	70	74	70	50	64	41	39	44	48
Total	83	77	84	75	84	76	42	52	63	59	53	55

M, male; F, female; * $p \leq .05$; ** $p \leq .01$; (*) $p \leq .10$

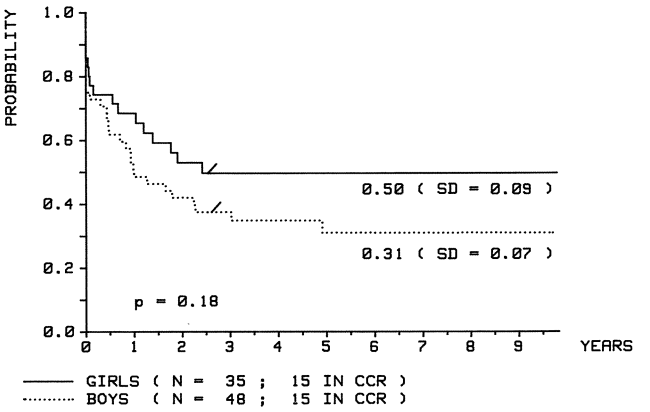


Fig. 21. Probability of EFS duration in FAB M4, girls vs. boys. Combined analysis of AML-BFM-78 and AML-BFM-83

4.5.2 Age at Diagnosis

Grouping of patients by age at: (a) under 2, (b) 2–10, and (c) over 10 years, as is commonly done in studies on childhood ALL, studies AML-BFM-78 and -83 showed no difference in prognosis (Fig. 22). Separate evaluation of the studies showed an extended EFI in children over 10

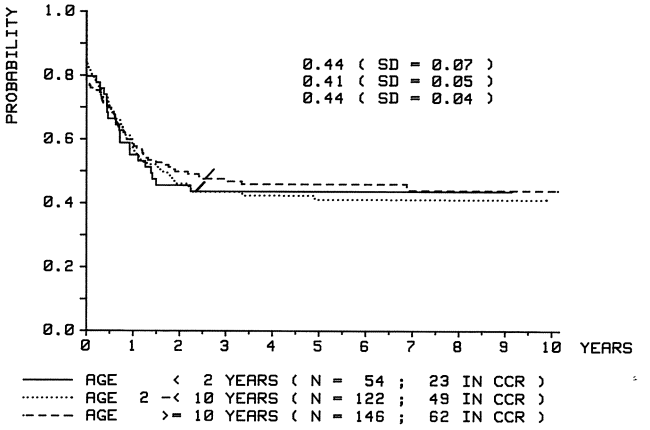


Fig. 22. Probability of EFS duration in three age groups. Combined analysis of AML-BFM-78 and AML-BFM-83

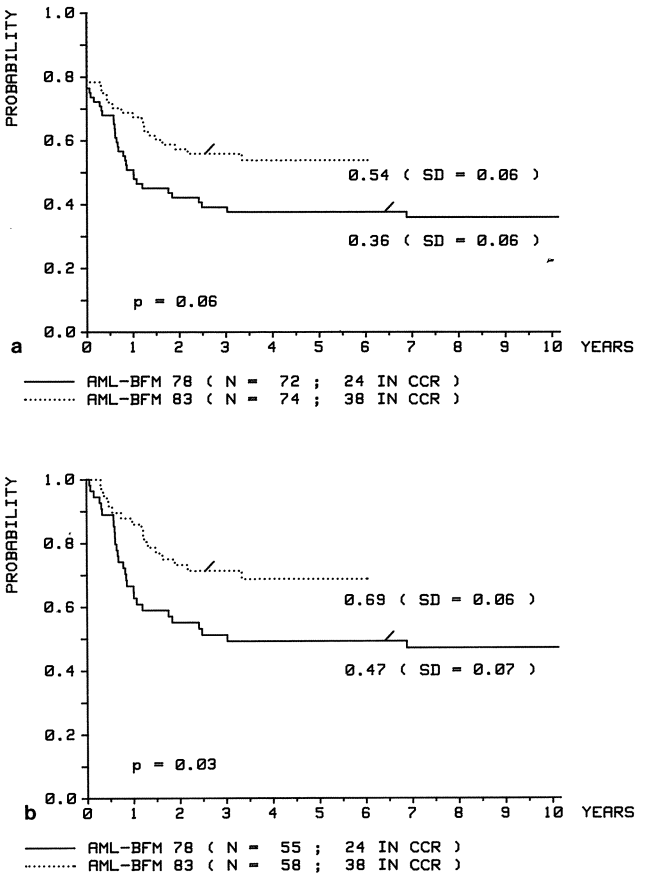


Fig. 23. **a** Probability of EFS duration in patients over 10 years, AML-BFM-78 vs. AML-BFM-83. **b** Same for EFI duration

years in AML-BFM-83 compared to the preceding study (Fig. 23b). Concerning EFS, improvement of prognosis was less obvious ($p = 0.06$; Fig. 23a). In all other age groups, the difference between the two studies was not significant (Tables A and B of the appendix).

4.5.2.1 Influence of Age in the FAB Types

As children with FAB M5 predominate in the age group under two years (57%) (Sect. 4.1.2) it can only be determined that low age did not have a negative effect on prognosis in this FAB type. Out of 30 children under age two with FAB M5 23 (77%) achieved CR; the corresponding proportion in older patients was 68%. In general, the reason that younger children did not achieve remission was early lethal hemorrhage and/or leukostasis (6 out of 7 patients), whereas treatment failure was an additional important cause in older children (7 NRs, 6 EDs).

4.5.3 White Blood Cell and Blast Count

The relevance of the initial WBC was investigated by comparing patient groups with WBCs over and under 20000/mm^3, and with or without hyperleukocytosis ($\geq / < 100000$/mm^3). Tables 21 and 22 present an overview of CR rates and EFI duration in these groups. Patients with hyperleukocytosis always had a higher risk of ED and treatment failure (Fig. 24, Table 22), but CR rates of the patient groups with WBCs over and under 20000/mm^3 differed only in AML-BFM-83 (Table 21).

EFI duration in patients with hyperleukocytosis, as compared to those without, showed no marked reduction when the two studies were evaluated separately (Fig. 25, Table 22). Only the combined analysis of all protocol patients, which was based on a larger number of cases, showed a significant difference (Table 22). Compared to children with a WBC below 20000/mm^3, patients with WBCs exceeding 20000/mm^3 showed a tendency toward reduced EFI duration in study AML-BFM-83, and a significantly different EFI in the combined analysis of both studies (Table 21). When the results of the two studies were compared, it was above all patients with low WBCs (< 20000/mm^3) in AML-BFM-83 who showed a prolongation of EFI ($p = 0.11$, Fig. 26a); the difference between the studies was less distinct in patients with higher WBCs (Fig. 26b).

Table 21. Prognostic significance of initial WBC by FAB types in AML-BFM-78 and -83 (Threshold value: WBC 20000/mm³)

	CR rate (%)						EFI (%)					
Study	AML-BFM-78		AML-BFM-83		AML-BFM-78/83		AML-BFM-78		AML-BFM-83		AML-BFM-78/83	
WBC × 10³/mm³	<20	≥20	<20	≥20	<20	≥20	<20	≥20	<20	≥20	<20	≥20
FAB M1	75	85	86	83	80	84	58	56	81	68	70	63
FAB M2	95	100	100	71	97	82	56	27(*)	100	41**	74	34**
FAB M4	89	74	95	68*	93	72*	33	36	74	47	62	40
FAB M5	72	58	91	56**	83	57*	57	57	40	40	43	47
Total	80	79	90	71**	86	75*	51	42	68	53(*)	59	47*

* $p \leq .05$; ** $p \leq .01$; (*) $p \leq .10$

Table 22. Prognostic significance of initial WBC by FAB types in AML-BFM-78 and -83 (Threshold value: 100000/mm³)

	CR rate (%)						EFI (%)					
Study	AML-BFM-78		AML-BFM-83		AML-BFM-78/83		AML-BFM-78		AML-BFM-83		AML-BFM-78/83	
WBC × 10³/mm³	<100	≥100	<100	≥100	<100	≥100	<100	≥100	<100	≥100	<100	≥100
FAB M1	85	67	87	71	86	69					66	64
FAB M2	97	100	90	50*	93	67*	patient numbers too low				58	25*
FAB M4	88	60*	89	43*	89	55**					56	22(*)
FAB M5	73	25(*)	85	38**	80	33**					–	–
Total	85	63**	87	50**	86	56**	50	30	62	53	56	39*

* $p \leq .05$; ** $p \leq .01$; (*) $p \leq .10$

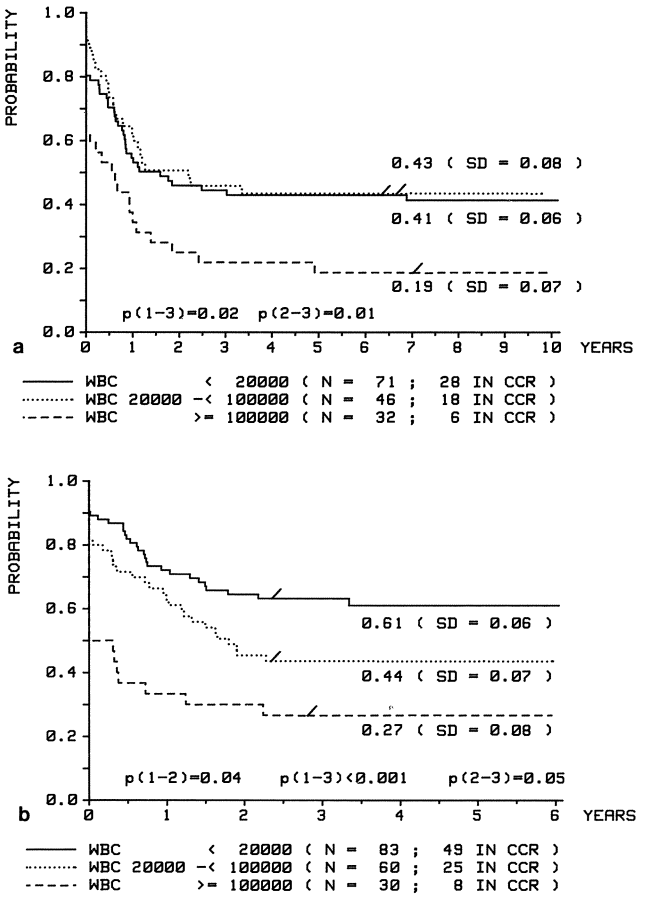


Fig. 24. **a** Probability of EFS duration by WBC in AML-BFM-78. **b** Same in AML-BFM-83

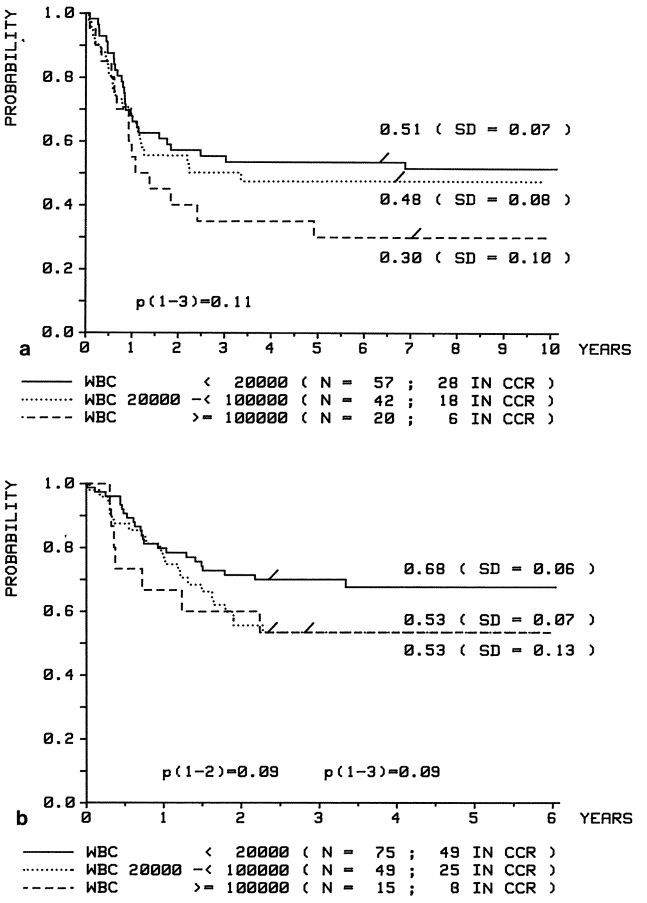


Fig. 25. **a** Probability of EFI duration by WBC in AML-BFM-78. **b** Same in AML-BFM-83

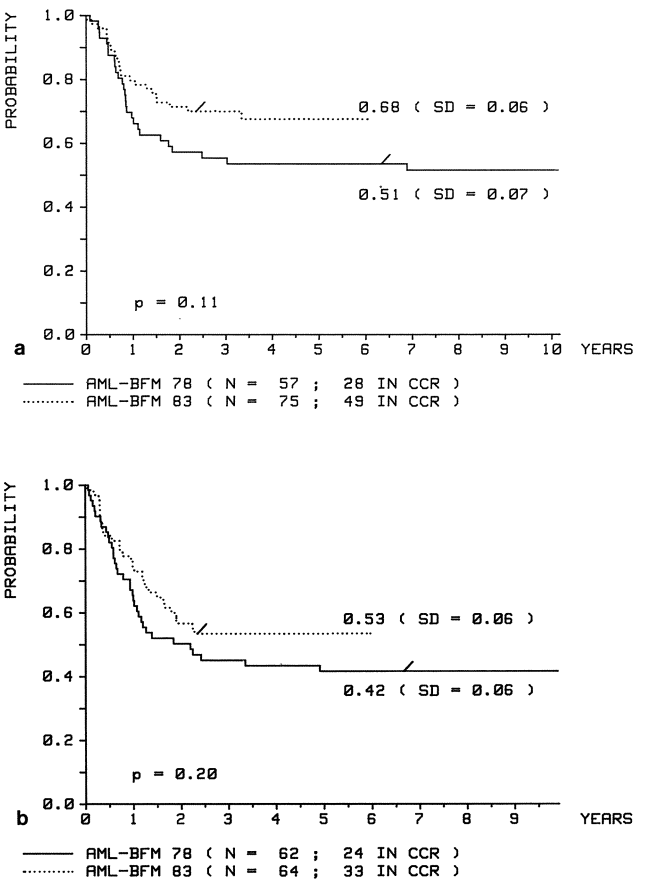


Fig. 26. **a** Probability of EFI duration in patients with initial WBC $<20000/mm^3$, AML-BFM-78 vs. AML-BFM-83. **b** Same in patients with initial WBC $\geq 20000/mm^3$

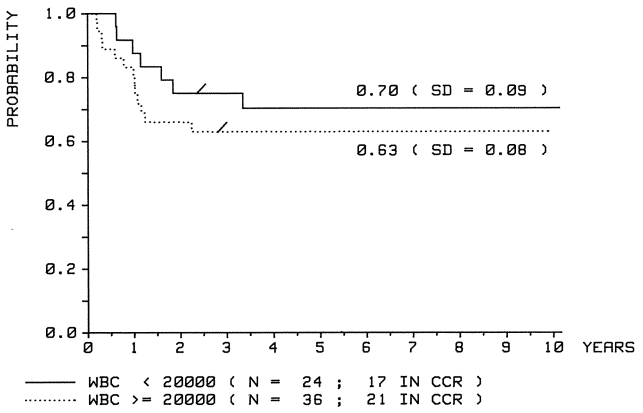


Fig. 27. Probability of EFI duration in patients with FAB M1 and WBC $</\geq 20000/mm^3$. Combined analysis of AML-BFM-78 and AML-BFM-83

4.5.3.1 Influence of WBC on Prognosis in the FAB Types

FAB M1. In neither study were CR rate and initial WBC related (Tables 21, 22). Similarly, there were no significant differences in EFI duration between patients with WBCs over or under $20000/mm^3$ (Table 21, Fig. 27).

FAB M2. The CR rate in study AML-BFM-83 was clearly lower in children with hyperleukocytosis than in patients with WBCs under $100000/mm^3$ (Table 22). Similarly, the risk of relapse and initial WBC were related in the patients in both studies (Fig. 28a and Tables 21, 22). There were no relapses in children with WBCs under $20000/mm^3$ in study AML-BFM-83 (Fig. 28b).

FAB M4. In both studies, hyperleukocytosis had an unfavorable effect on achieving CR (Fig. 29, Table 22). Children with FAB M4 and a WBC under $20000/mm^3$ in AML-BFM-83 showed a longer EFI (Table 21), but the difference to patients with a higher WBC was not significant ($p = 0.15$).

FAB M5. In these children, the untoward effect of hyperleukocytosis on the achievement of CR mainly consisted of early lethal bleeding complications rather than treatment failure (see Sect. 4.6 and Table 18). The risk of relapse was not related to the initial WBC (Fig. 30).

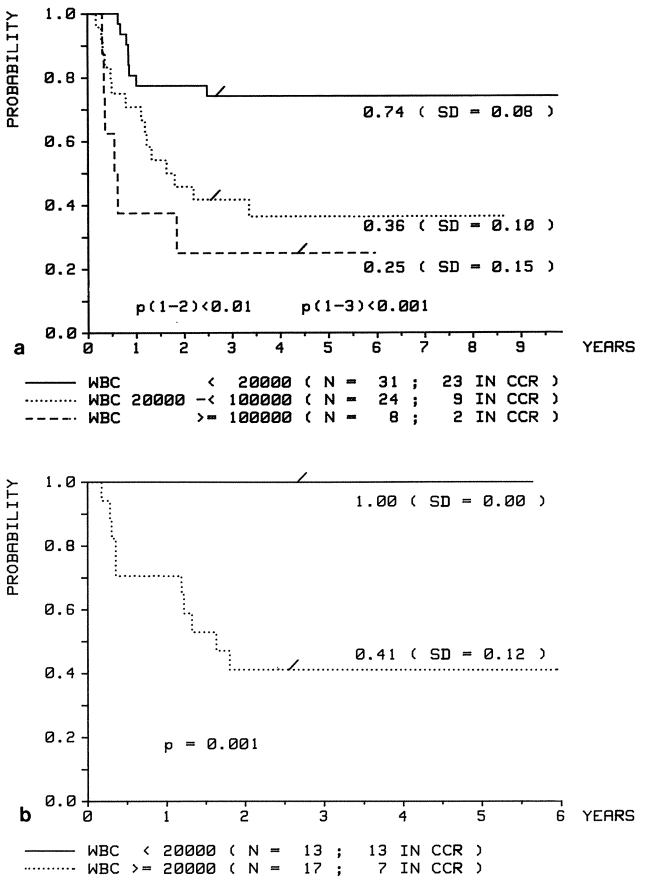


Fig. 28. a Probability of EFI duration in patients with FAB M2 by WBC. Combined analysis of AML-BFM-78 and AML-BFM-83. **b** Probability of EFI duration in patients with FAB M2 by WBC in AML-BFM-83

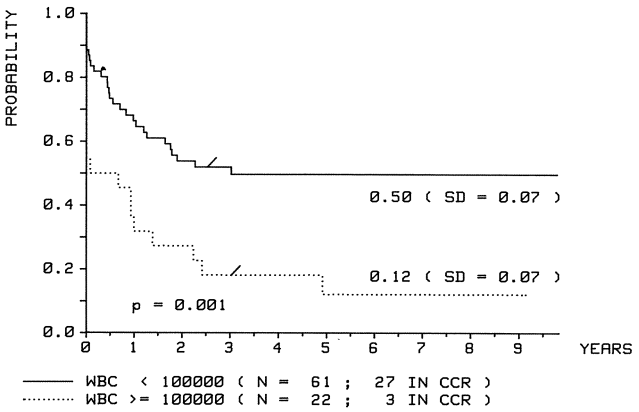


Fig. 29. Probability of EFS duration in patients with FAB M4 and WBC $</\geq 100000/mm^3$. Combined analysis of AML-BFM-78 and AML-BFM-83

In all AML patients, independent of the FAB type hyperleukocytosis was associated with a high risk of failure to achieve CR due to early lethal hemorrhage and/or leukostasis, or treatment failure.

Patients in both studies showed a tendency toward a higher incidence of relapses with a higher initial WBC. In study AML-BFM-78, this was observed in children with WBCs exceeding $100000/mm^3$ (Fig. 25a), whereas a more favorable prognosis in children with low WBC was apparent in study AML-BFM-83 ($<20000/mm^3$, $p=0.09$, Fig. 25b, 26a, and Table 21). The increasing risk of relapse was most obvious in FAB type M2 (Fig. 28).

4.5.3.2 Initial Blast Count

Initial blast and white blood cell counts in the peripheral blood were clearly correlated (Fig. 31). The results obtained in patients with absolute blast counts over and under $20000/mm^3$ differed only slightly from those found with comparable WBCs (see Tables 21, 23). Patients with absolute blast counts exceeding $100000/mm^3$ had an especially low CR rate (8 of 20, 40%); for this reason, patient numbers were too small to be evaluated for EFI.

Blast count in BM was also evaluated in relation to the initial WBC. Patient groups with a WBC under $20000/mm^3$ and either less or more than 80% of blasts in the BM showed no significant differences, nor did patient groups with a WBC above $20000/mm^3$ and either less or more

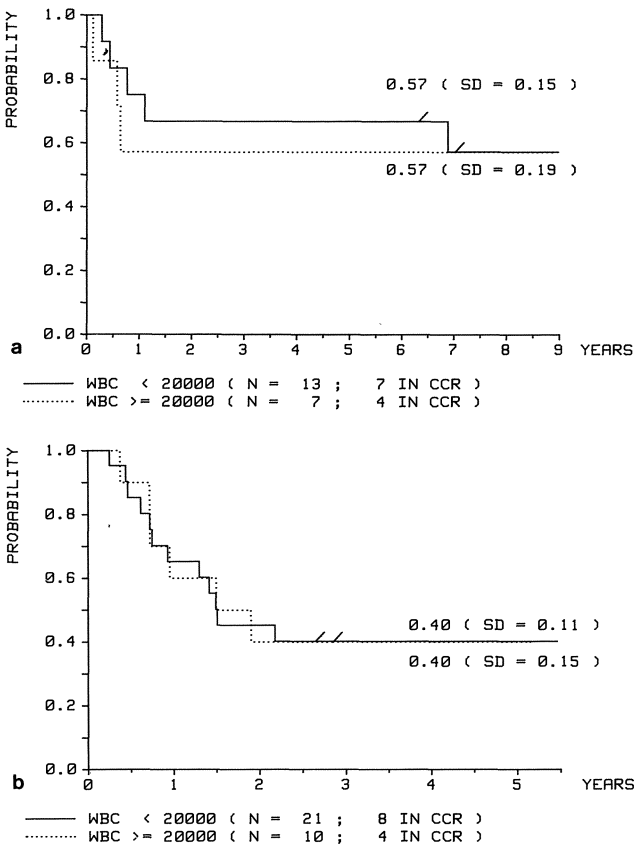


Fig. 30. **a** Probability of EFI duration in patients with FAB M5 and WBC $< / \geq 20000/mm^3$ in AML-BFM-78. **b** Same in AML-BFM-83

Table 23. Prognostic significance of initial peripheral blast count in AML-BFM-83 (Threshold blast count: $20000/mm^3$)

Blast count	$<20000/mm^3$	$\geq 20000/mm^3$
CR rate (%)	88	69**
EFI (%)	66	52*

* $p = .06$; ** $p < .01$

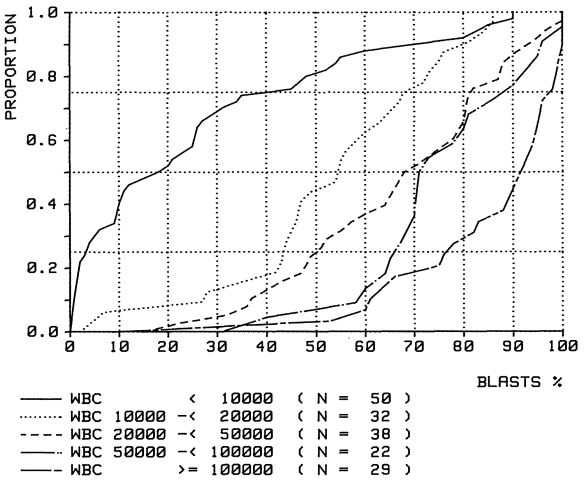


Fig. 31. Cumulative frequencies of peripheral blast counts in patients of AML-BFM-83 grouped by initial leukocyte count.

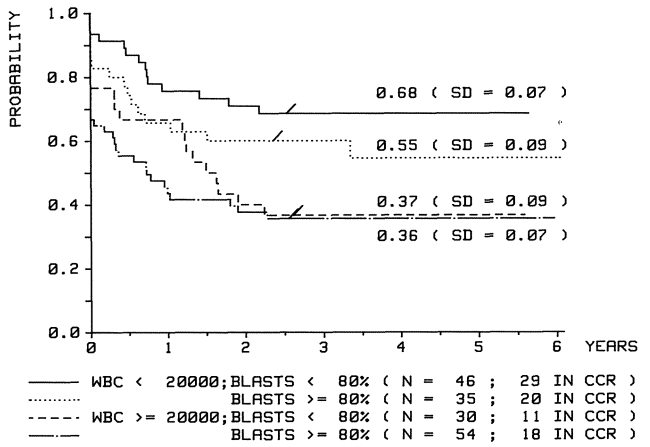


Fig. 32. Probability of EFS in patients of AML-BFM-83 with leukocyte counts $</\geq 20000/mm^3$ and a blast count in BM of $<80\%$ or $\geq 80\%$

than 80% of blasts in BM. Only the already described difference in prognosis for children with a WBC under $20000/mm^3$ and those whose WBC was above $20000/mm^3$ remained significant (Fig. 32).

4.5.4 *Initial CNS Involvement*

There were no differences between patients with or without initial CNS involvement with respect to the probability of achieving CR (Tables A and B of the appendix). An analysis of EFI in the patients of both studies showed a less favorable prognosis in patients with CNS involvement (CNS positive, $p = 0.04$, Fig. 33). Analysis of the two studies separately showed the same trend (Tables A, B, appendix).

Evaluation of the relapse sites (Table 24) showed a higher percentage of relapses that included the CNS in children with initial CNS involvement. Out of 10 relapses in initially CNS positive patients, four were again localized in the CNS, whereas there were only 10 CNS relapses among 91 (11%) CNS negative patients ($p \leq 0.03$).

There were no isolated (CNS only) or combined (CNS and additional sites) CNS relapses in patients with FAB M2 (Table 25). Only one child with this FAB type initially had blasts in the CSF, whereas involvement was evenly distributed in the other FAB types.

Table 24. Isolated and combined CNS relapses in children with and without initial CNS involvement (AML-BFM-78 and -83)

Initial involvement (positive/negative)	(*n*)	Total relapses	Relapse without CNS involvement	Relapse in CNS		CNS relapses as percentage of total relapses
				isolated	combined	
CNS positive	23	10	6	2	2	40*
CNS negative	283	91	81	3	7	11*

Out of 17 patients without data on initial CNS involvement, two relapsed with CNS involvement.
* $p \leq .03$

Table 25. Isolated and combined CNS relapses by FAB types in AML-BFM-78 and -83

	Isolated CNS relapse	Combined CNS relapse	Total
FAB M1	1	4	5
FAB M2	–	–	–
FAB M4	1	3	4
FAB M5	3	4	7
Other	–	–	–
Total	5	11	16

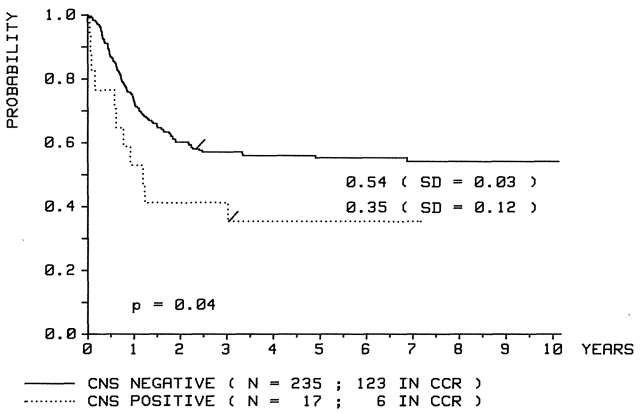


Fig. 33. Probability of EFI duration in patients with and without initial CNS involvement. Combined analysis of AML-BFM-78 and AML-BFM-83

4.5.5 Extramedullary Organ Involvement

Extramedullary organ involvement in sites other than liver, spleen or CNS was especially frequent in the FAB types with monocytic differentiation, M4 and M5 (Table 9). In AML-BFM-78, these patients were the only ones at high risk of failing to achieve CR when the infiltrations described were present (Table 26, Fig. 34a). In AML-BFM-83, there was no difference between FAB M5 patients with or without extramedullary organ involvement (Fig. 34b). When the combined studies were analyzed, the variable "organ involvement" was not associated with any significant differences in EFI in FAB M5 patients (Fig. 34c, Table 27).

Relapses with extramedullary manifestation occurred mainly in those patients who also had initial organ involvement: out of 12 patients with isolated or combined extramedullary relapse (Table 28), seven had initial organ involvement. For one child the relevant information was not available.

4.5.6 Other Pretherapeutic Variables

According to the analysis of both studies the potential risk factors low hemoglobin (Hb) and thrombocytopenia were of no relevance in predicting either achievement of CR or risk of relapse (Tables A and B, appendix). Enlargement of liver and spleen also had no decisive effect

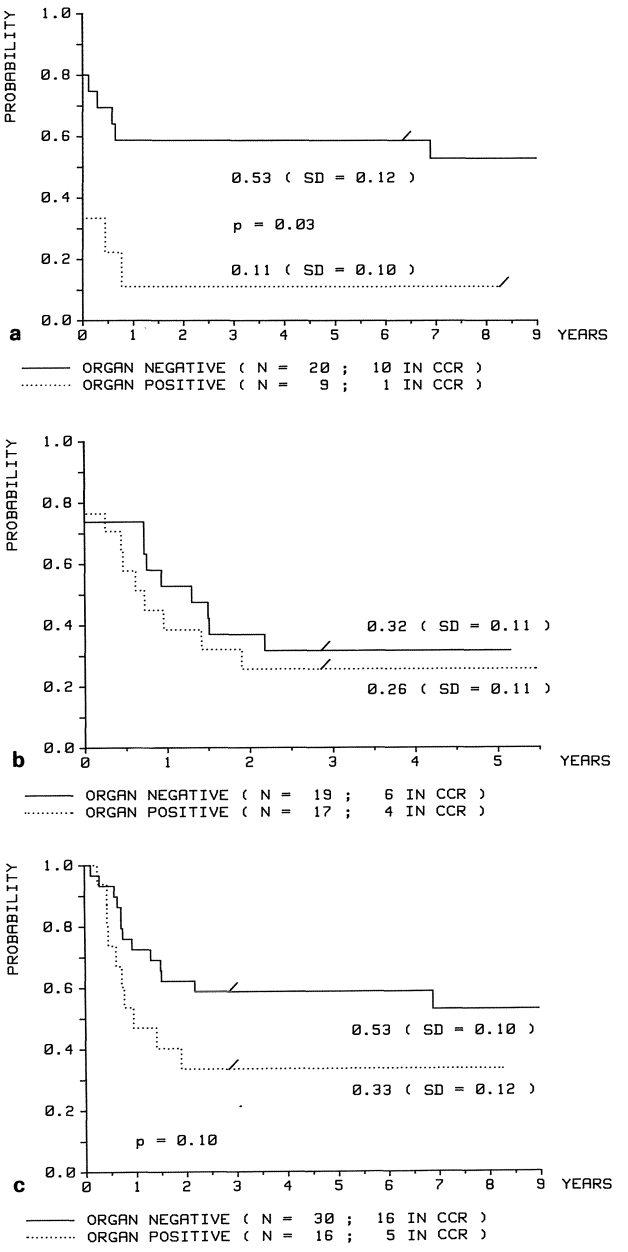


Fig. 34. a, b Probability of EFS duration in FAB M5 patients with or without extramedullary organ involvement. **a** AML-BFM-78; **b** AML-BFM-83. **c** Probability of EFI duration; combined analysis of AML-BFM-78 and AML-BFM-83

Table 26. Prognostic significance of extramedullary organ involvement for CR rate by FAB types in AML-BFM-78 and -83

	CR rate (%)					
Study	AML-BFM-78		AML-BFM-83		AML-BFM-78/83	
Organ involvement	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
FAB M1+M2	89	86	87	67	88	73(*)
FAB M4	86	55(*)	80	85	83	71
FAB M5	80	33*	74	76	77	62
FAB M4+M5	84	45*	77	80	81	66*

* $p \leq .05$; (*) $p \leq .10$

Table 27. Prognostic significance of extramedullary organ involvement for CCR by FAB types in AML-BFM-78 and -83

	EFI (%)					
Study	AML-BFM-78		AML-BFM-83		AML-BFM-78/83	
Organ involvement	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
FAB M1+M2	47	67	68	90	57	81(*)
FAB M4	41	17	67	61	51	44
FAB M5	66	(33)[a]	43	34	53	33(*)
FAB M4+M5	51	22	57	46	51	39

(*) $p \leq .10$

[a] Number of patients too low ($n = 3$)

Table 28. Isolated and combined relapses with extramedullary organ involvement by FAB types in AML-BFM-78 and -83

	Isolated extramed. relapse	Combined relapse	Total
FAB M1+M2	–	3	3
FAB M4	2	2	4
FAB M5	2	3	5
Other	–	–	–
Total	4	8	12

Isolated or combined CNS involvement was disregarded, except in two patients with FAB M4 and M5 and concurrent BM/CNS and extramedullary organ involvement.

on prognosis. While distinctly fewer patients in AML-BFM-78 with hepatomegaly of more than 5 cm achieved CR (19 out of 29, as compared to 99 out of 119 with no or only slight liver enlargement; $p=0.03$, χ^2-test), the difference was not significant in AML-BFM-83 (Table B, appendix).

4.5.6.1 Morphologic Variables: Auer Rods and Eosinophilia

Auer Rods. In both studies, absence of Auer rods was negatively related to achievement of CR (Tables A and C, appendix, Table 29). Absence or presence of Auer rods did not influence the risk of relapse in AML-BFM-78, whereas Auer rod positive patients in the later study had a distinctly better prognosis than Auer rod negative patients (Fig. 35, Table 29).

Evaluation by FAB types showed that the improvement in prognosis of Auer rod positive patients concerned only those with granulocytic leukemia without maturation, FAB M1 (Fig. 36). This difference in EFS, in the fairly small number of M1 patients in study AML-BFM-83, was impressive. The differences between Auer rod negative and positive patients were not significant in the other FAB types which had larger numbers of patients, M2, M4, and M5 (Table 29).

Eosinophilia in the Bone Marrow. For the evaluation of the prognostic significance of eosinophilia in the BM, a threshold value of 3% eosino-

Table 29. Prognostic significance of Auer rods by FAB types in AML-BFM-78 and -83

	CR rate (%)				EFI (%)			
Study	AML-BFM-78		AML-BFM-83		AML-BFM-78		AML-BFM-83	
Auer rods	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
FAB M1	82	94	64	100**	42	60	29	88**
FAB M2	100	96	80	83	50	35	75	68
FAB M4	71	81	86	76	27	54	54	82
FAB M5[a]	64	no data	76	no data	–	–	–	–
Total	73	88*	77	87*	41	48	46	75**

* $p \leq .05$; ** $p \leq .01$

[a] Number of patients with Auer rods too low; in AML-BFM-78, one patient, in AML-BFM-83, two patients; all achieved CR.

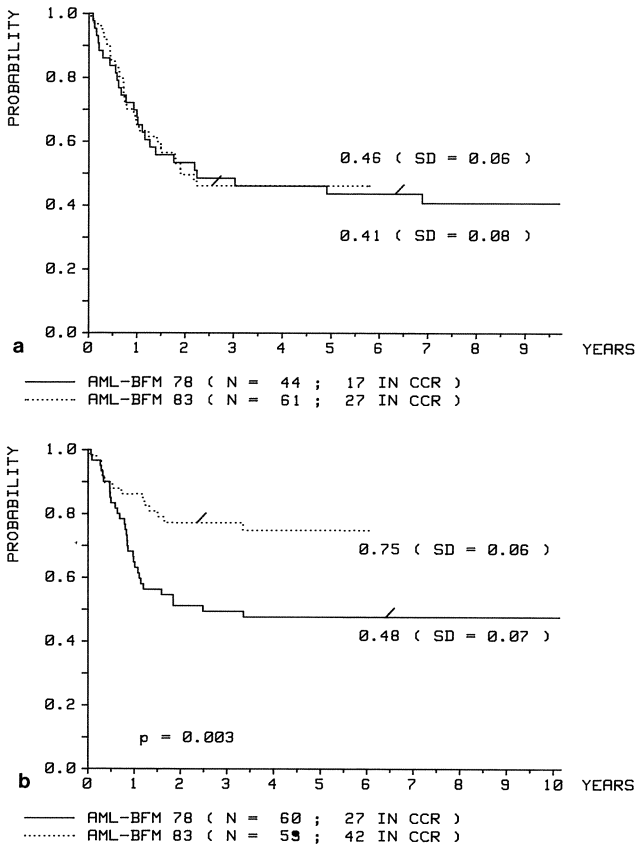


Fig. 35. a Probability of EFI duration in Auer rod negative patients, AML-BFM-78 vs. AML-BFM-83. **b** Same in Auer rod positive patients

phils was established, since atypical forms are found only if 3% or more of these cells are present. The CR rate in AML-BFM-83 was slightly higher with eosinophilia than without (Table 30). Concerning relapses, the reduced incidence in AML-BFM-83 patients with 3% or more eosinophils was impressive when compared to the incidence in patients in the previous study and in those without eosinophilia (Fig. 37).

Evaluation by FAB types showed an improvement in prognosis particularly for patients with the FAB type M4 and eosinophilia (Fig. 38, Table 25).

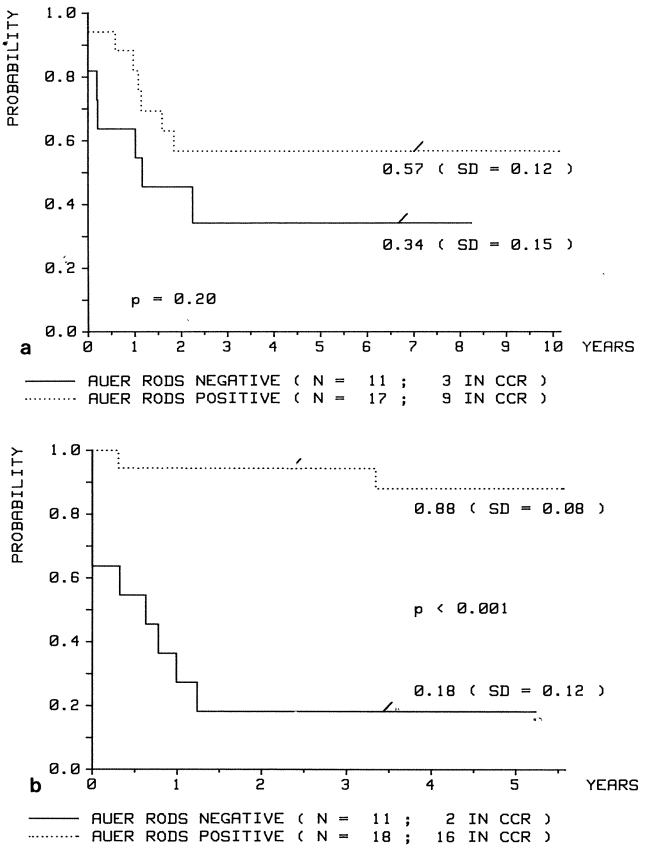


Fig. 36. a Probability of EFS duration in patients with FAB M1, with and without Auer rods, in AML-BFM-78. **b** Same in AML-BFM-83

Abnormal dysplastic eosinophils were found only in children with eosinophilia in the BM and predominantly in FAB type M4 (Tables 10, 11, and Fig. 7). Out of 12 patients in AML-BFM-83 with M4 and atypical eosinophils, 10 survived in CR and two died during the initial phase. Out of the eight patients in study AML-BFM-78 with atypical eosinophils, three suffered a relapse (one with CNS involvement) and one died during the initial phase. In the combined studies, of eight children with FAB types M1 or M2 and atypical eosinophils, seven have achieved CCR. The only relapse occurred in one patient, who showed particularly unusual eosinophils with bubbly granules (Fig. 39).

Table 30. Prognostic significance of eosinophilia (≥3%) in BM by FAB types in AML-BFM-78 and -83

	CR rate (%)				EFI (%)			
Study	AML-BFM-78		AML-BFM-83		AML-BFM-78		AML-BFM-83	
Eosinophils	<3%	≥3%	<3%	≥3%	<3%	≥3%	<3%	≥3%
FAB M2	100	93	79	86	41	36	67	67
FAB M4	70	83	76	88	31	50	37	93**
Other	82	78	80	100	55	57	54	83
Total	83	82	78	89	46	45	54	81*

* $p \leq .05$; ** $p \leq .01$

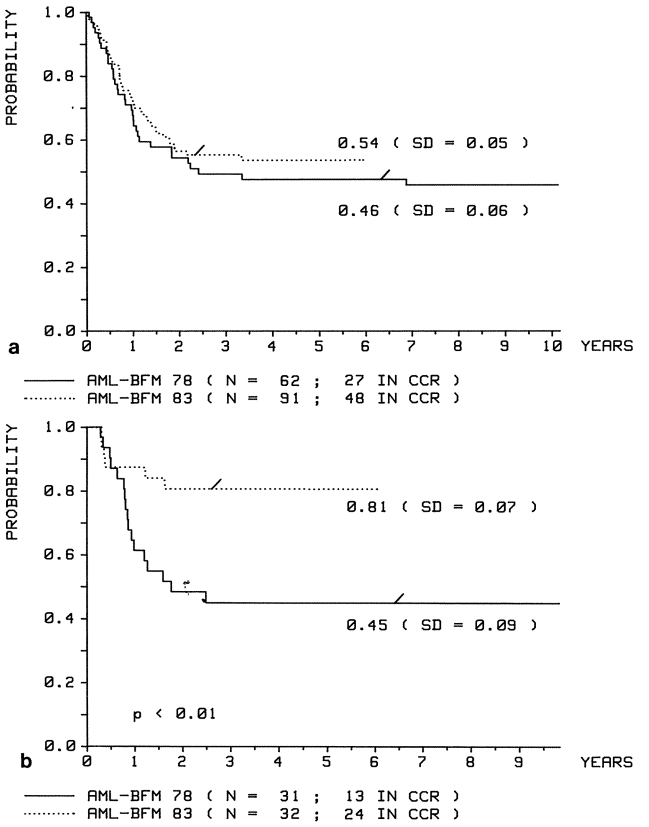


Fig. 37. **a** Probability of EFI duration in patients without eosinophilia (<3%) in the BM, AML-BFM-78 vs. AML-BFM-83. **b** Same in patients with eosinophilia (≥3%) in the BM, AML-BFM-78 vs. AML-BFM-83

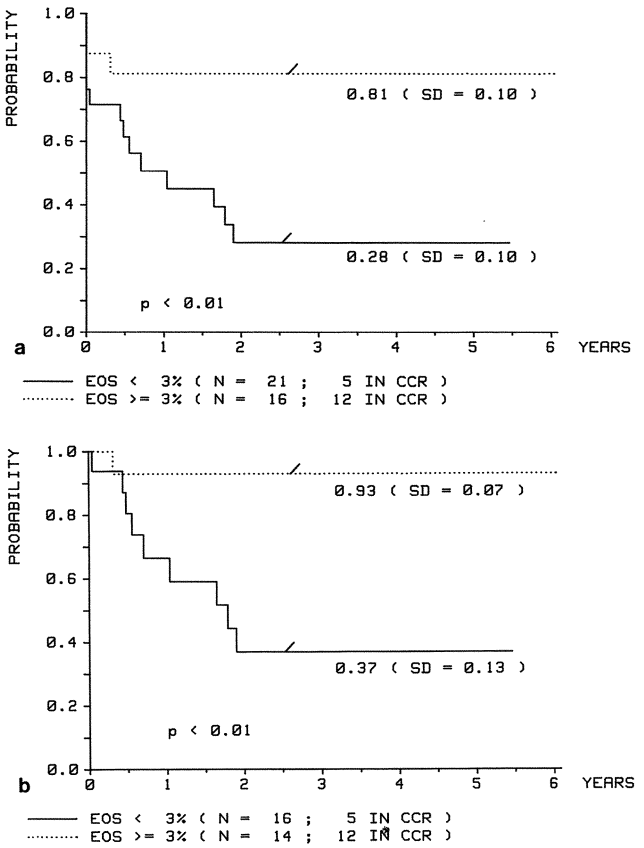


Fig. 38. **a** Probability of EFS duration in FAB M4 patients, with and without eosinophilia in the BM in AML-BFM-83. **b** Same for EFI duration

4.5.6.2 Cytochemical, Immunological, Flow Cytometric, and Cytogenetic Findings

Cytochemical Reactions. In view of the fact that, in the majority of patients, at least some blasts showed a positive POX reaction, the prognostic relevance of a high ($\geq 80\%$) versus a low ($< 80\%$) count of POX positive blasts in BM was investigated. However, this parameter was not predictive of achievement of CR but clearly influenced the risk of relapse (Table C, appendix, and Fig. 40). Nonetheless, the proportion of POX positive blasts was not an independent variable; the specific relationship with other initial parameters has already been demonstrated (see Sect. 4.2).

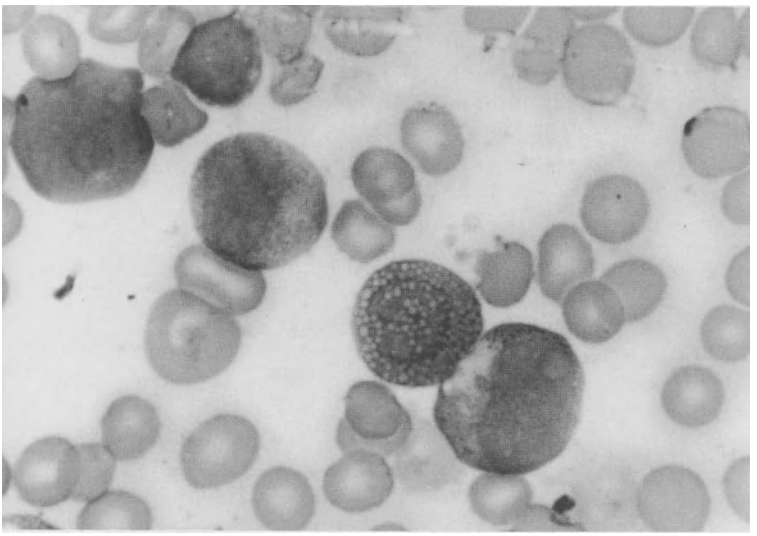


Fig. 39. Patient with FAB M2: atypical eosinophils containing bubbly granules (Pappenheim staining, × 1000)

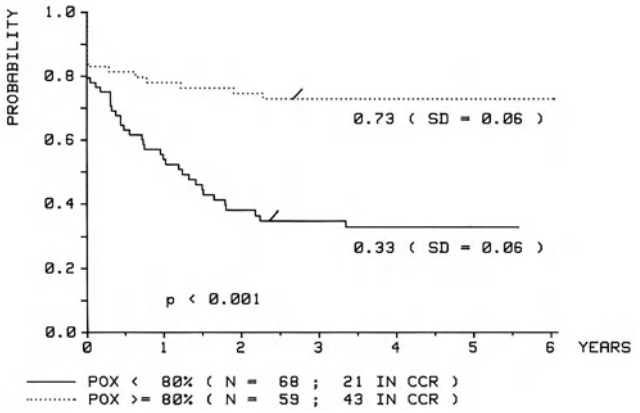


Fig. 40. Probability of EFS duration in patients with $<80\%$ vs. $\geq 80\%$ POX positive blasts in the bone marrow (AML-BFM-83)

Immunologic Markers. Among the immunologic markers only TdT was evaluated, since data on the other markers were incomplete. There was no prognostic significance in the percentage of TdT positive cells. (Table C of the appendix and Fig. 41).

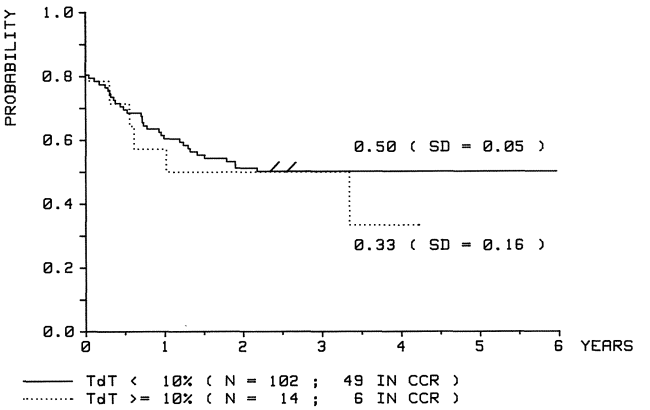


Fig. 41. Probability of EFS duration in patients with $<10\%$ vs. $\geq 10\%$ TdT positive cells (AML-BFM-83)

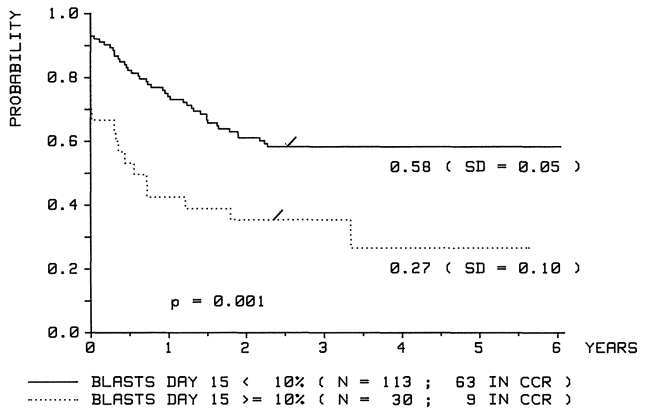


Fig. 42. Probability of EFS duration in patients with $<10\%$ vs. $\geq 10\%$ blasts in the BM on day 15 (AML-BFM-83)

Flow Cytometric Findings. Due to incomplete data, the ability to evaluate the prognostic significance of DNA aneuploidy was limited. The analysis of 67 protocol patients showed no influence of aneuploidy on prognosis (Table C, appendix).

Cytogenetic Findings. Results regarding the relationship between the cytogenetic findings and outcome are listed in Table 31. The data suggested a good prognosis in patients with t(8; 21).

Table 31. Results of cytogenetic findings (AML-BFM-83)

Cytogenetics	Study patients (*n*)	ED prior to therapy (*n*)	ED after therapy (*n*)	NR (*n*)	CR (*n*)	CR (%)	CCR (*n*)	CCR (%)
No data submitted	96	9	8	9	71	82	41	47
Test failure	27	–	4	3	20	73	13	46
No aberration	21	–	–	4	17	81	8	38
t(8; 21)	8	–	–	–	8		7	
t(9; 11)	4	–	–	1	3		1	
t(15; 17)	3	–	–	–	3		2	
11q23 aberration	6	–	–	3	3		1	
inv(16)	1	–	–	–	1		1	
+8	4	–	–	–	4		3	
−7	1	–	–	–	1		1	
Other	11	–	–	3	8	73	5	45

Table 32. Prognostic significance of blasts in the BM on day 15 with respect to treatment success

Proportion of BM blasts	no CR	CR achieved
< 10%	8	105
≥ 10%	9	21

$\chi^2 = 11.89$; $p = .002$

4.5.7 Parameters of Response Kinetics

4.5.7.1. Blast Reduction in the BM

The proportion of blasts in the BM on day 15 was measured only in AML-BFM-83. A proportion of blasts above 10% was associated with a high risk of treatment failure (Table 32, Table B of the appendix, and Fig. 42).

The difference in EFI duration between patients with low and high blast counts on day 15 was not significant ($p = 0.09$, Table B of the appendix).

4.5.7.2 Time Until CR

Late achievement of CR (≥ 10 weeks) was associated with a high risk of relapse in AML-BFM-83 ($p = 0.04$; Fig. 43b). This was not observed in

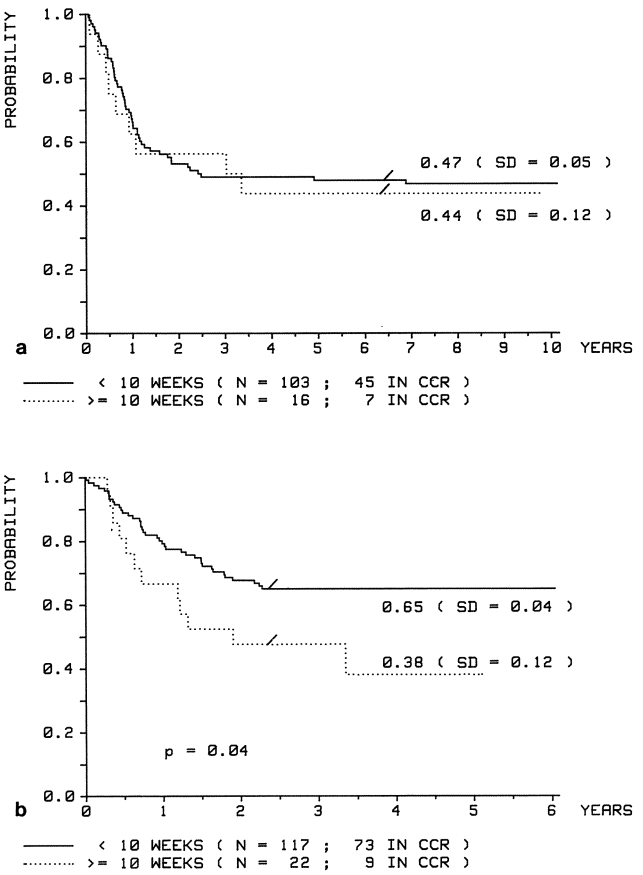


Fig. 43. **a** Probability of EFI duration in patients who achieved CR within the first 10 weeks or later in AML-BFM-78. **b** Same in AML-BFM-83

AML-BFM-78 (Fig. 43). EFI duration in patients who entered CR within six weeks of treatment versus those who achieved CR later was not significantly different.

4.5.8 Prognostic Relevance of Pretherapeutic and Response-Kinetic Parameters: Cox Analyses

The multivariate influence of pretherapeutic and response-kinetic parameters on prognosis was investigated by use of Cox's multiple step-

wise regression analysis (see Sect. 4.2). The following parameters were tested: age; sex; WBC and thrombocyte count; eosinophils and Auer rods in the BM; size of liver and spleen; CNS and extramedullary organ involvement; and, as a parameter of response kinetics, time until CR.

For continuous variables, the following limits were applied:

Age	<10 years	≥ 10 years
WBC	$<20000/mm^3$	$\geq 20000/mm^3$
Eosinophils	$<3\%$	$\geq 3\%$
Thrombocytes	$<50000/mm^3$	$\geq 50000/mm^3$
Liver below costal margin	≤ 5 cm	>5 cm
Spleen below costal margin	≤ 5 cm	>5 cm
CR	<10 weeks	≥ 10 weeks

All events causing failure to achieve CR (ED, nonresponse) or cessation of CCR (relapse, death in CCR) were evaluated in the analysis of protocol patients. For the CR group, only failure in CCR (relapse, death in CR) was recorded as an event. The associated relative risk of failure was calculated for each determined variable using the Cox model [37].

4.5.8.1 Results of AML-BFM-78 Study

Protocol Patients. Complete sets of data were submitted to the study center for 100 of the 149 patients (including 16 NR/ED patients, 42 relapses and 4 deaths in CCR). Overall, both univariate and multivariate analysis showed that only the initial WBC was of predictive relevance for prognosis. This means that, for the group of patients with hyperleukocytosis, the risk of treatment failure was increased by a factor of 2.29 over children with a lower WBC.

4.5.8.2 Results of AML-BFM-83 Study

Protocol Patients. Complete data were available for 111 of 173 patients (including 20 NR/ED patients, 30 relapses and 3 deaths in CCR).

The parameters, WBC, eosinophils, and Auer rods were independent prognostic factors in both univariate and multivariate analysis. The relative risk of failure for patients with a WBC $\geq 20000/mm^3$, as com-

Table 33. Cox analysis of protocol patients in AML-BFM-78

Variable	*p* value		Relative risk
	Univariate	Multivariate	
Age	.97		
WBC	.002	.04	2.29 (≥ 100 vs. < 100)*
Eosinophilia	.92		
Auer rods	.18		
Sex	.68		
Hepatomegaly	.08		
Splenomegaly	.97		
CNS involvement	.19		
Platelet count	.74		
Organ involvement	.09		

* $\times 10^3/mm^3$

Table 34. Cox analysis of protocol patients in AML-BFM-83

Variable	*p* value		Relative risk
	Univariate	Multivariate	
Age	.194		
WBC	.032	.039	1.81 (≥ 20 vs. < 20)*
Eosinophilia	.005	.005	2.56 ($< 3\%$ vs. $\geq 3\%$)
Auer rods	.025	.051	1.72 (neg. vs. pos.)
Sex	.957		
Hepatomegaly	.749		
Splenomegaly	.715		
CNS involvement	.856		
Platelet count	.364		
Organ involvement	.558		

* $\times 10^3/mm^3$

pared to below $20000/mm^3$, is 1.81 when the other two prognostic factors (Auer rods and eosinophilia) are identical.

Patients without eosinophilia have a higher risk than those with eosinophilia (relative risk 2.56), as do Auer rod negative patients compared with Auer rod positive patients (relative risk 1.72).

CR Patients. Out of 139 CR patients with complete sets of data, 91 were analyzed (including 3 deaths in CCR and 30 relapses). The prognostic relevance of the length of time until CR was also evaluated.

Absence of eosinophilia and of Auer rods, age below 10 years, and late CR all predicted unfavorably for CCR. The variable WBC had no significant predictive value (Table 35).

4.5.8.3 Results by FAB Group Allocation of AML-BFM-83 Study

FAB M1–M4. Table 36 shows the results in the FAB subtypes M1–M4 for all evaluable patients (81 of 122 protocol patients).

For the group of CR patients (69 of 100 patients, Table 37), age, Auer rods, and time until CR were additional relevant parameters. As in the total group of protocol patients WBC remains a risk factor.

Table 35. Cox analysis in the CR group of AML-BFM-83

Variable	*p* value		Relative risk
	Univariate	Multivariate	
Age	.004	.004	3.23 (<10 yrs vs. ≥10 yrs)
WBC	.128		
Eosinophilia	.005	.002	5.26 (<3% vs. ≥3%)
Auer rods	.030	.077	2.00 (neg. vs. pos.)
Sex	.342		
Hepatomegaly	.876		
Splenomegaly	.475		
CNS involvement	.605		
Platelet count	.817		
Organ involvement	.978		
Time to achieve CR	.057	.015	3.95 (≥10 wks vs. <10 wks)

Table 36. Cox analysis in protocol patients with FAB M1 to M4 in AML-BFM-83

Variable	*p* value		Relative risk
	Univariate	Multivariate	
Age	.372		
WBC	.008	.008	2.72 (≥20 vs. <20)*
Eosinophilia	.040	.059	2.22 (<3% vs. ≥3%)
Auer rods	.309		
Sex	.705		
Hepatomegaly	.347		
CNS involvement	.469		
Platelet count	.123		
Organ involvement	.964		

* $\times 10^3/mm^3$

Table 37. Cox analysis of CR patients with FAB M1 to M4 in AML-BFM-83

Variable	*p* value		Relative risk
	Univariate	Multivariate	
Age	.034	.022	2.78 (< 10 yrs vs. ≥ 10 yrs)
WBC	.012	.012	2.49 (≥ 20 vs. < 20)*
Eosinophilia	.028	.038	4.55 (< 3% vs. ≥ 3%)
Auer rods	.157	.080	2.38 (neg. vs. pos.)
Sex	.603		
Hepatomegaly	.670		
Splenomegaly	.741		
CNS involvement	.295		
Platelet count	.284		
Organ involvement	.597		
Time to achieve CR	.022	.027	4.33 (≥ 10 wks vs. < 10 wks)

* $\times 10^3/mm^3$

4.5.8.4 Results by Individual FAB Types

The results of the individual analyses of FAB M1, M2, M4, and M5 are shown in Table 38. Remarkably, there was no variable of prognostic significance in M5. In FAB M1, as compared to the other FAB types, Auer rods were the only variable that was significant in both univariate and multivariate analyses. In patients with FAB M2, initial WBC was found to be an important prognostic variable in protocol patients; late

Table 38. Cox analysis of individual FAB types M1, M2, M4 and M5 in AML-BFM-83

	Variable	*p* value		Relative risk
		Uni-variate	Multi-variate	
FAB M1	Auer rods	< .0001	< .0001	25.0 (neg. vs. pos.)
FAB M2	WBC	.0004	< .0001	14.05 (≥ 20 vs. < 20)*
	Time to achive CR	.08	< .04	8.96 (≥ 10 wks vs. < 10 wks)
FAB M4	Eosinophilia	.007	.007	7.14 (< 3% vs. ≥ 3%)
FAB M5	No significant variable			

* $\times 10^3/mm^3$

achievement of CR was an additional risk factor in the CR group. In FAB M4, eosinophilia was the only factor determined by multivariate analysis to be of prognostic significance.

4.5.8.5 Relationship Between the Implementation of Therapy and Pretherapeutic Prognostic Parameters

The results pertaining to the implementation of therapy are presented in detail in Sect. 4.7. Briefly, the negative effect of reduced VP-16 dose compliance, apparent in the univariate analysis ($p < 0.05$), was irrelevant after inclusion of the initial prognostic parameters, WBC and Auer rods. In addition, there was no significant difference between patients who received induction therapy according to protocol (cumulative total dose > 80%) and those showing reduced dose compliance when allocation to prognostic groups, based mainly on pretherapeutic parameters (Table 55), was entered into the evaluation.

4.6 Early Death Due to Hemorrhage and/or Leukostasis and Other Complications

A detailed discussion of EDs due to hemorrhage and/or leukostasis has been published elsewhere [43]. Therefore, the following chapter will present only the most important, updated results.

Out of 333 patients enrolled in the two studies, 32 (10%) died from hemorrhage and/or leukostasis prior to or during the first 12 days of treatment (Table 14). No AML-specific therapy had been started in 11 of these children, but three had received steroids, with or without vincristine for an originally suspected ALL.

Out of 322 protocol patients who had at least started prephase with low-dose ARA-C and 6-TG, 64 (20%) never achieved CR. This group includes 35 (11%) NRs, 21 (7%) EDs due to hemorrhage and/or leukostasis, and eight (2%) deaths due to infection or secondary hemorrhage during the phase of BM depression.

4.6.1 Early Death Due to Hemorrhage and/or Leukostasis

Initial hyperleukocytosis, extramedullary organ involvement, hepatomegaly, and female sex were characteristic for the group of ED due to

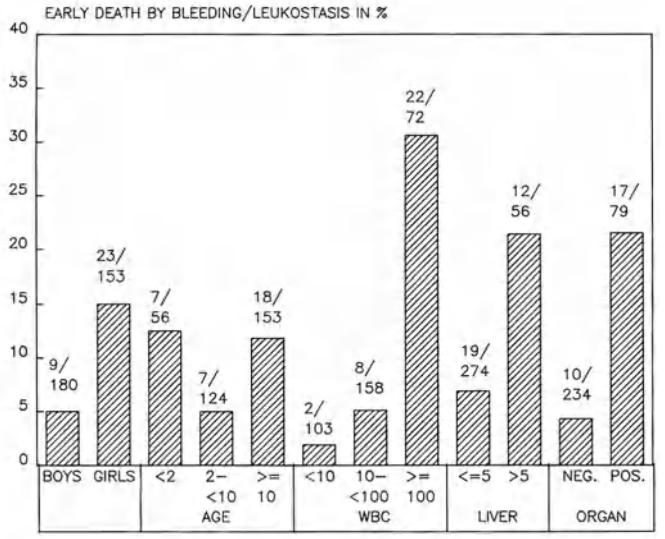


Fig. 44. Relationship between ED due to hemorrhage and/or leukostasis and prognostic factors (AML-BFM-78 and AML-BFM-83). p values (χ^2 test): sex $p < .001$; age n.s.; WBC $\geq 100000/mm^3$ vs. $< 10000/mm^3$ $p < .0001$; hepatomegaly > 5 cm below costal margin vs. ≤ 5 cm below costal margin $p < .01$; extramedullary organ involvement $p < .00001$

hemorrhage and/or leukostasis (Fig. 44). The proportion of patients with initial CNS involvement, splenomegaly, anemia, and thrombocytopenia in this group did not differ from the total group of protocol patients.

Yule's analysis of association (Table 39) showed a relationship between hepatomegaly (> 5 cm below costal margin) and hyperleukocytosis, and also female sex. In the group of ED patients, extramedullary organ involvement correlated with hyperleukocytosis and young age (< 2 years). Age above 10 years may also have been an independent, negatively correlated factor.

The most important risk factor predicting ED from hemorrhage and/or leukostasis was the morphologic type FAB M5 (Table 40). ED was found to occur mainly in FAB M5 patients with accompanying hyperleukocytosis and/or extramedullary organ involvement. Of the patients with monocytic leukemias, 24% (19 of 80) suffered ED. In children with FAB M5 and concurrent organ involvement, the risk of ED increased to 40%, and up to 70% with concurrent hyperleukocytosis.

Aside from the initial parameters discussed, the data on response kinetics such as change in blast count and hemostasis were of considera-

Table 39. Association of prognostic factors for ED from hemorrhage and/or leukostasis

	Extramed. organ involvement	Liver >5 cm	Age 0–2 years	Age ≥10 years	Sex (female)
WBC $>100\times10^3/mm^3$	+	+	−	0	− −
Extramedullary organ involvement	1	0	+	0	− −
Liver >5 cm	0	1	−	− −	+ +
Age 0–2 years	+	−	1	0	0
Age ≥10 years	0	− −	0	1	0
Sex (female)	− −	+ +	0	0	1

Yule's coefficient of association: ≤|50|: 0, no association; |1.00|: 1, complete association; >|50|: + or −, postive or negative association; >|80|: + + or − −, strong positive or negative association

Table 40. ED due to hemorrhage and leukostasis: association with FAB types, hyperleukocytosis, and extramedullary organ involvement

FAB	ED/Total group		WBC $\geq100\times10^3/mm^3$		Extramed. organ involvement	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
M 1/2	6/144	4	4/28	14	1/22	5
M 3	2/11	8	−/−	−	−/−	−
M 4	5/85	6	4/24	17	4/25	16
M 5	19/80*	24	14/20*	70	12/30*	40
Other	−/13	−	−/−	−	−/2	−
Total	32/333	10	22/72*	31	17/79*	22

* χ^2 test $p<.05$

ble relevance in the evaluation of ED. The patients who died from hemorrhage and/or leukostasis before or during the initial phase can be divided into three different groups: *Group A,* ED before onset of therapy ($n=11$); *Group B,* ED from hemorrhage and/or leukostasis after onset of therapy, with concurrent hyperleukocytosis (generally WBC $\geq100000/mm^3$) ($n=14$); and, *Group C,* ED from hemorrhage with WBC $<20000/mm^3$ ($n=7$). A fourth group of patients who suffered

ED as a result of other complications *(Group D)* will be discussed separately.

Group A. Eleven children died before onset of therapy (Table 41). The median WBC of 220000/mm^3 was extremely high, but there was no unusual thrombocytopenia (median of 79000/mm^3). The morphologic diagnosis was FAB M5 in nine of these patients, FAB M4 in the other two. At admission, seven of the 11 children presented with symptoms of hemorrhage, including two with signs of cerebral bleeding. The median time to occurrence of lethal hemorrhage was one day after admission (range 0–6 days). In six patients, cerebral bleeding was the immediate cause of death. Of these, two children also showed intracerebral infiltration. One child died from pulmonary hemorrhage. In another two children, leukostasis or leukemic infiltration resulted in respiratory and myocardial failure. One child with blasts in the CSF developed stupor and died; clinically, central respiratory dysregulation due to infiltration was assumed. Although the data for the last patient were incomplete, a sudden decrease in Hb and blood pressure indicated acute hemorrhage. The three group A patients with leukostasis died without any severe bleeding.

Group B. This group included all those patients who died from hemorrhage and/or leukostasis after onset of therapy and who had increased WBC. The median WBC in these 14 patients was 176000/mm^3 (range 42000–370000/mm^3). Only two patients had an initial WBC under 100000/mm^3. One of them experienced a rise in WBC from 42000 to 125000/mm^3 within the first two days of treatment. The other child had a WBC of 87000/mm^3. Leukostasis and leukemic infiltration should also be considered as possible causes of death in eight patients, four of whom also showed a WBC exceeding 240000/mm^3. All of these children also had bleeding.

In six patients lethal hemorrhage occurred during rapid blast decrease following administration of anthracyclines (three patients), ARA-C infusions (one patient), or after cell separation and exchange transfusion (two patients). Coagulation abnormalities were present in all children, thrombopenia (<20000/mm^3) in four of them.

Group C. In seven deaths from hemorrhage within the first 12 days of therapy occurring in patients with low WBC (<20000/mm^3), the causes of bleeding were heterogeneous. Two children with acute promyelocytic leukemia (FAB M3) died from cerebral hemorrhage with signs of fibrinolysis after a decrease in blast count; in two patients, hemorrhage was associated with pneumonia and sepsis. Another two chil-

Table 41. Clinical data of ED patients

	Group A Death prior therapy	Group B ED from H/LS with hyperleukocytosis	Group C ED from H with WBC $<20 \times 10^3/mm^3$	Group D ED from other complications
	$n=11$	$n=14$	$n=7$	$n=8$
Age, years	12;6	12;2	2;0	8;1
median (range)	(0;7–16;6)	(0;6–15;10)	(0;4–13;8)	(1;10–15;2)
Sex, m:f	4:7	5:9	0:7	6:2
WBC $\times 10^3/mm^3$	220	176	11	43
median (range)	(10–350)	(42–370)	(2–18)	(2–245)
Platelets $\times 10^3/mm^3$	79	38	20	23
median (range)	(16–126)	(10–180)	(3–118)	(8–220)
FAB M 1/2	–	4	2	1
M 3	–	–	2	2
M 4	2	2	1	3
M 5	9	8	2	2
Extramedullary organ involvement	6	9	3	1
Survival, days	1	3	10	37
median (range)	(0–6)	(2–9)	(4–12)	(17–41)
Cause of death				
hemorrhage	6	6	7	(2)
leukostasis	3	–	–	–
hemorrhage/ leukostasis	2	8	–	–
other	–	–	(2)	8

H, hemorrhage; LS, leukostasis, H/LS, hemorrhage and leukostasis; other, see text

dren whose leukemia did not respond to induction therapy, suffered lethal thrombopenic cerebral bleeding 12 days after therapy had been started. At autopsy, leukemic infiltration was found in the visceral organs. One child had thrombocytopenic cerebral bleeding at the time of admission.

Coagulation disorders were initially recorded for all patients in groups A, B, and C, but follow-up documentation covered only the patients at Münster. It was found that a plasminogen level that was initially low or dropped within days (<60 mg%) predicted for lethal hemorrhage, whereas thrombopenia and the parameters prothrombin time, activated partial thrombin time (aPTT), antithrombin III (AT III), fibrinogen, and fibrinogen degradation products (FDP) did not [160].

The autopsy reports described leukostasis true to definition in only four of the patients in groups A, B, and C, whose vessels were filled with leukemic cells (Fig. 45). One patient manifested clinical signs of leukostasis with priapism. The autopsy reports of another two patients described perivascular leukemic infiltration. Two patients showed leukemic infiltration in the visceral organs or the CNS (autopsy report or computerized tomography) and two others had blasts in the CSF and signs of leukostasis (massive organ infiltration; clinical symptom: stupor). Overall, leukostasis was a potential cause of death in 11 children.

The therapy protocol prescribed initial slow cytoreduction with low doses of ARA-C and 6-TG for children with hyperleukocytosis. Such cytoreduction had not been applied in three patients who suffered ED (group B). These children died from hemorrhage following rapid blast reduction under continuous ARA-C infusion or after the first DNR application. Conversely, four patients experienced an increase in peripheral blast counts under this pretreatment regimen. After protocol-appropriate treatment, lethal hemorrhage occurred following sudden cytoreduction due to therapy with DNR (three patients) or leukapheresis (one patient).

Other prophylactic or therapeutic measures in these high-risk patients were the administration of various coagulants or anticoagulants, and of blood components. In at least one of these cases, whole blood transfusion, in association with the increase in cytocrit, was responsible for the aggravation of leukostasis and subsequent hemorrhage. In one patient, bleeding was temporarily stopped by neurosurgical intervention; leukapheresis and blood exchange were performed once each, but after the onset of cerebral bleeding.

Retrospective analysis of the prophylactic and therapeutic procedures followed in 45 surviving patients with hyperleukocytosis showed the following result: 18 children had severe (11 patients) or mild signs

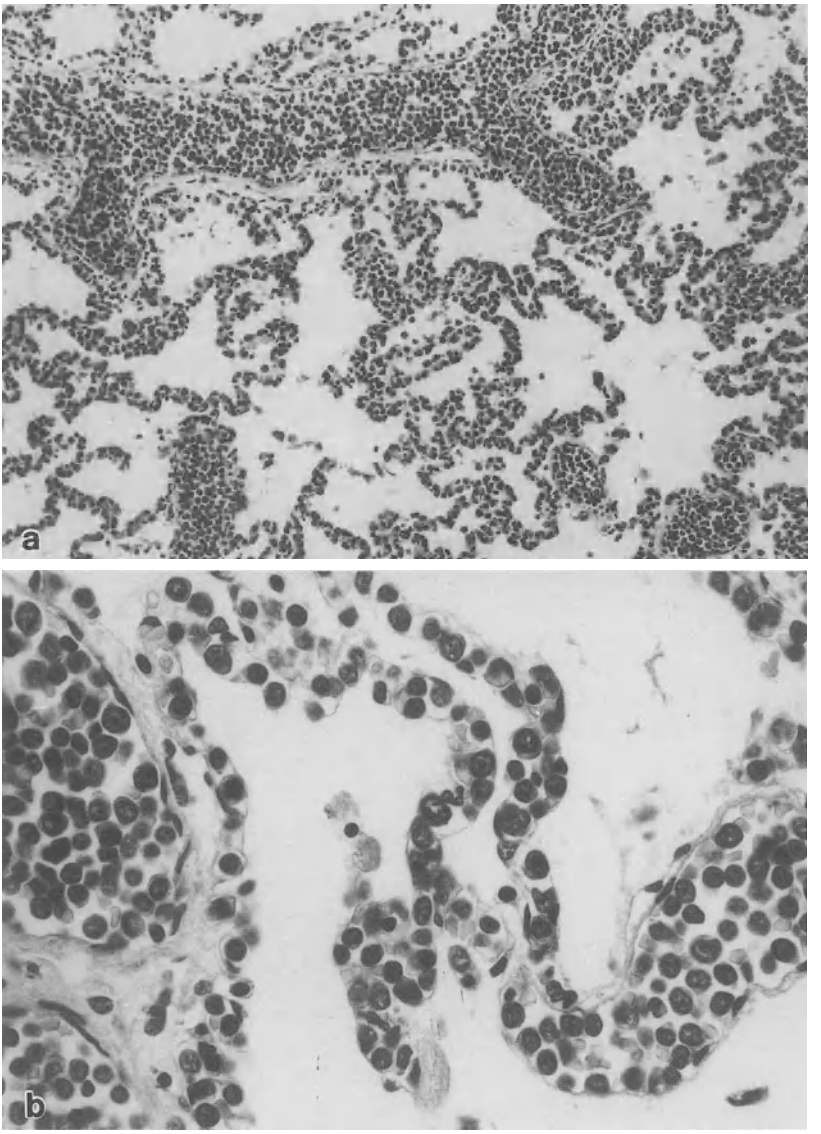


Fig. 45. **a** Leukostasis in pulmonary precapillaries and alveolar capillaries; patient with FAB M5, group A (HE staining, × 130). **b** Section of **a**; HE staining, × 520. We are indebted to Prof. H. J. Stutte, Pathologisches Institut, und Dr. V. Gerein, Universitäts-Kinderklinik, Frankfurt, for these illustrations)

of bleeding and/or signs of pulmonary leukostasis (2 patients) within the first few days; three patients had symptoms of cerebral hemorrhage. Six patients with FAB type M4 and extreme hyperleukocytosis ($>200\,000/mm^3$) were severely affected. Only one patient with FAB type M5 and hyperleukocytosis survived severe hemorrhage. In three patients, a therapeutic effect was obtained by slow cytoreduction with the described prephase or hydroxyurea. Five patients received exchange transfusions. In four children, bleeding was mainly attributable to thrombocytopenia and could therefore be controlled by platelet transfusions.

4.6.2 Early Death Due to Other Complications Within the First Six Weeks After Onset of Therapy

Group D. Eight children died from complications other than those mentioned above for Groups A–C, and death occurred considerably later (17–41 days after onset of therapy; Table 41). One child with the morphologic diagnosis of FAB M4 and initial hyperleukocytosis of $245\,000/mm^3$ died on day 17, in aplasia, from uremia and sepsis after three exchange transfusions. Death was attributed to sepsis and aplasia in three patients, and to lethal thrombopenic hemorrhage during therapy-induced BM depression in two others. Out of these five, two patients had acute promyelocytic leukemia. Another two children probably died from anthracycline-induced cardiomyopathy.

4.7 Analysis of the Implementation of Therapy in AML-BFM-83 Study

Data on the implementation of therapy were submitted for 161 out of 173 protocol patients (93%), but in some instances were not complete for all phases (Table 42).

Cytoreductive prephase therapy, mainly with ARA-C and 6-TG, was performed in 78 out of 161 patients (48%) for 1–14 days, with a median of four days. The relationship between the reduction in peripheral WBC (median about 69%, range 0%–97%) and prognosis was studied ignoring the variation in length of this phase and was found to be non-significant (with cytoreduction by $<69\%$: EFS = 0.49, SD 0.08; with cytoreduction $\geq 69\%$: EFS = 0.56, SD 0.08; both values for six years' duration).

Table 42. Patient data by individual therapy phases analyzed in relation to therapy results (AML-BFM-83)

Therapy results	Protocol patients		Data on therapy course					
			Total	Pre-phase	Induc-tion	Consolidation		Main-tenance
						Phase 1	Phase 2	
	(*n*)	(%)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)
ED	12	7	6	3	6	–	–	–
NR	22	13	20	11	20	14	11	1
Relapses	46	27	40	18	40	39	38	9
in CCR	82	47	84	41	81	81	81	72
Death in CCR	4	2	4	2	4	2	1	–
BMT in first CCR	7	4	7	3	7	7	7	1
Total	173	100	161	78	158	143	138	83

Table 43. Dose compliance in induction ($n = 138$)*

	< 80% PD (*n*)	$\bar{X}$ % PD	SD % PD
ARA-C (days 1 and 2)	8	102.5	29.8
ARA-C (days 3–8)	15	96.2	10.9
DNR	13	96.1	11.1
VP-16	14	95.5	11.8

* ED and patients under 1 year of age excluded
$\bar{X}$, mean; SD, standard deviation; PD, prescribed dose

During induction the average dosage of ARA-C (days 3–8), DNR, and VP-16 actually administered amounted to 96% of the prescribed dose. In 21% of the children older than one year, less than 80% of the prescribed dose of at least one drug was received. Considerable deviations from the treatment protocol occurred in two children given continuous instead of 30 min infusions of ARA-C over eight days, and in a 2½-year-old girl who received no continuous infusion of ARA-C at all, and only 50% of the DNR dose (Table 43).

There was no difference in relapse rate between children with less than 80% DC and those with more than 80% DC ($p > 0.1$ for the difference between EFS values) for the drugs used in induction. The proportion of NR, however, is clearly higher in the groups with reduced DC (Table 44). In Fig. 46 the results of the life table analysis for VP-16 are shown as one example.

Table 44. Relation between dose compliance in induction and therapy result

Drug	DC	NR	Relapses	CCR	*p* value[a]
ARA-C (days 1 and 2)	<80%	2	1	5	–
	≥80%	15	33	71	
ARA-C (days 3–8)	<80%	4	3	7	.008
	≥80%	13	31	69	
DNR	<80%	3	3	6	.02
	≥80%	15	31	70	
VP-16	<80%	4	4	5	.001
	≥80%	14	30	71	

Patients who died in first CCR or had a BMT are not listed.
[a] χ^2 test, NR vs. CCR patients (NR + relapses vs. CCR patients, n.s.)

Of the 27 patients with a DC below 80% for one or more drugs, five (19%) did not achieve CR, compared to 12 out of 99 (12%) with a DC exceeding 80% for all cytostatics (χ^2 test, n.s.).

The median duration of cytopenia (calculated from the first day of induction until the time when the neutrophil count exceeded 1500/mm^3 and thrombocytes rose above 100000/mm^3) was 24 days (0–79 days). The median treatment-free interval between induction and start of consolidation was 21 days (2–57 days). With this range of variation, the duration of cytopenia and treatment-free interval were of no apparent

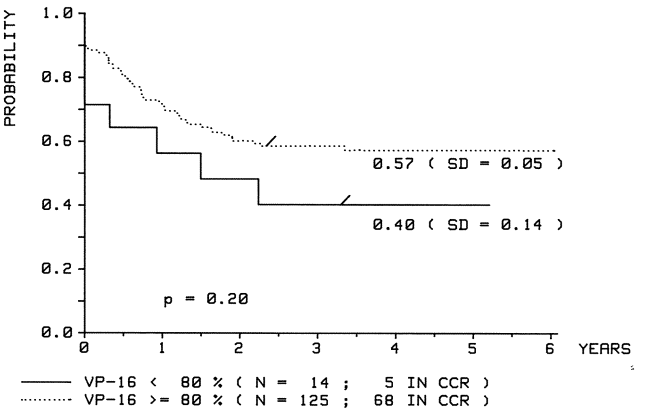


Fig. 46. Probability of EFS duration in children older than 1 year, comparison of AML-BFM-83 patients who received more or less than 80% of the prescribed VP-16 dose during induction

prognostic significance (cytopenia <24 days: EFS=0.59, SD 0.07; cytopenia ≥24 days: EFS=0.51, SD 0.06; treatment-free interval <21 days: EFS=0.53, SD 0.07; treatment-free interval ≥21 days: EFS=0.60, SD 0.06; values for six years duration).

During the consolidation phase the average percentage of the prescribed dosage actually given exceeded 87% for CP and 93% or more for all other drugs. There was, however, a broad range of variation (Table 45), mainly in the group of NRs who received this part of therapy, not as "consolidation", but as a second attempt at inducing remission. The 18 NRs received either no consolidation therapy (four patients), only phase 1 of the consolidation (three patients), or less than 80% of the prescribed dose of one or several drugs (12 patients). In some instances (four patients), the consolidation phase 2 was considerably extended in duration (maximally by six weeks). By comparison, only 37 of the 109 children (34%) who achieved remission had reduced DC for one or more drugs.

Children with reduced DC for ARA-C in phase 1 had a comparatively poorer prognosis (p=0.01). The difference, however, was attributable to the rate of NRs rather than relapses (Fig. 47). The corresponding life table evaluations for the other drugs showed no significant differences related to DC.

The median duration of consolidation phase 1 was 29 days (20–63 days). The consolidation phase 2 had a median length of 35 days (9–87 days). The median treatment-free interval between consolidation phases 1 and 2 was nine days (0–56 days). Comparison of the total lengths of consolidation showed only minor differences in prognosis (duration of

Table 45. Dose compliance during consolidiation therapy (only patients >1 year)

Drug	Patients (*n*)	<80% PD (*n*)	$\bar{X}$ % PD	SD % PD
Phase 1				
Prednisone	129	4	109.5	20.3
6-TG	129	17	97.4	17.6
VCR	131	17	95.2	17.3
ADR	132	12	98.5	18.4
ARA-C	132	17	96.1	11.8
Phase 2				
6-TG	113	23	94.3	27.0
ARA-C	128	24	93.0	19.4
CP	127	31	87.4	26.6
ARA-C ith.	126	14	94.5	15.7

PD, prescribed dose

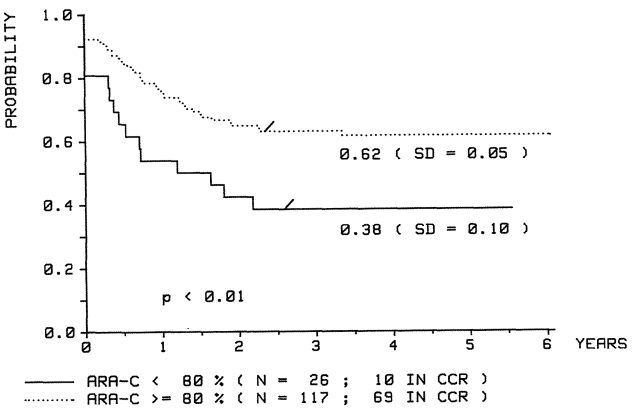


Fig. 47. Probability of EFS duration in children older than 1 year, comparison of AML-BFM-83 patients who received more or less than 80% of the prescribed ARA-C dose in consolidation phase

therapy <64 days: EFS $=0.51$, SD 0.07; duration of therapy ≥ 64 days: EFS $=0.66$, SD 0.06, values for six years' duration, $p=0.07$).

In the NRs, high dose-time correlations during consolidation were found for all drugs except prednisone. In CCR patients the dosage of 6-TG, ARA-C, and prednisone during phase 1 correlated with time of treatment (Table 46).

The range of variation in the therapy compliance of NRs resulted from the fact that consolidation therapy was discontinued in some patients because a response was no longer expected or because of severe complications. In other children, the dosage was considerably reduced

Table 46. Dose-time relationship during consolidation therapy

	NR	Relapses	CCR patients
6-TG	+	n.s.	+[a]
ARA-C	+	n.s.	+[a]
Prednisone	n.s.	+	+
ADR	(+)	n.s.	n.s.
VCR	(+)	(+)	n.s.
CP	(+)		n.s.

Positive correlation: + $p<0.05$; (+) $0.05<p<0.10$

[a] Phase 1 of consolidation only

due to cytopenia. By contrast, the consolidation phase 2 was extended for four NRs in an attempt to finally achieve blast reduction.

Data on maintenance therapy were documented for 83 patients. Of these, 72 were in CCR, nine relapsed, and two underwent BMT, one in first CR and the other in partial remission.

The median duration of maintenance therapy in 70 CCR patients was 85 weeks (53–108 weeks). In two children, treatment was discontinued after 21 and 35 weeks respectively because of hepatotoxicity (see Sect. 4.8). With an intended total treatment period of two years (104 weeks) reduction of this phase was due to extension of the consolidation phase therapy and of the treatment-free intervals by a median of 3–5 weeks over the protocol schedule (the treatment-free interval between consolidation and maintenance therapy varied from 2 to 4 weeks). The prescribed therapy with 6-TG, ARA-C, and ADR was essentially adhered to; however, 6-TG was sometimes omitted for periods of 14 days or longer. Due to persisting thrombocytopenia, four patients received considerably reduced cytostatic doses of ADR for the first year (50% of the dose). Overall, 47% of the children had thrombocytopenias with counts below 50000/mm^3 during the first year, as opposed to only 28% during the second year.

4.8 Toxicity During the Individual Therapy Phases of AML-BFM-83 Study

4.8.1 Complications During Induction

Out of 157 patients, 99 (63%) experienced bleeding during the induction phase. Severe bleeding events, which occurred in every fourth child (26%) were mostly gastrointestinal, but there were also three instances of cerebral bleeding with lethal outcomes. Details were not available on the induction therapy in another six children who suffered lethal hemorrhage during the first 14 days after onset of therapy. The rate of severe bleeding complications in those patients who later turned out to be NRs (5/19) was not markedly higher than in those who achieved remission (31/130).

Out of 157 children, 135 (86%) had infections during induction or during the subsequent BM depression. Fever (of known or unknown origin) was most frequent (54%), followed by enteritis (20%), and pneumonia (9%). Abscess formation and infections of the urinary tract (3% each), and other minor infections (22%) were rare (repeated infections of the same kind in one patient not accounted for). Children with se-

vere infections, such as sepsis, pneumonia, or abscess formation, showed no significant differences in prognosis compared with the other patients ($p = 0.14$, Fig. 48).

4.8.2 Complications During Consolidation

During consolidation therapy, 15 out of 137 children (11%) had severe hemorrhage in phase 1, and eight of 128 (6%) in phase 2, again mostly gastrointestinal. The proportion of patients with infections was 40% in both therapy phases (phase 1: 44%, and phase 2: 38%). However, severe infections (Table 47) were less frequent in the second phase. Children

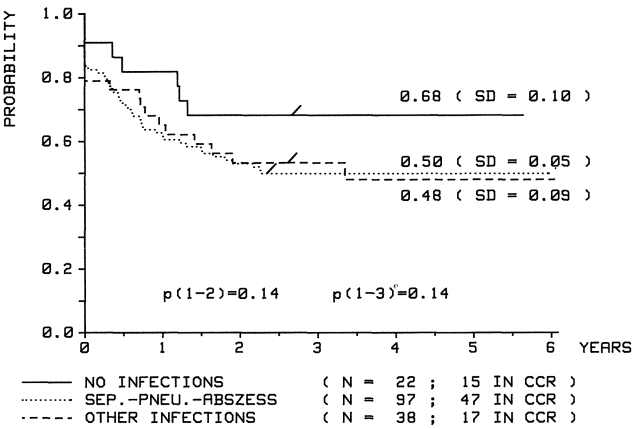


Fig. 48. Probability of EFS duration in AML-BFM-83 patients with no, severe, or minor infections during induction

Table 47. Proportion of severe complications by therapy phases

	Induction		Consolidation				Maintenance[a]	
			Phase 1		Phase 2			
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Hemorrhage	40/157	26	15/137	11	8/128	6	6/76	8
Infection	97/157	62	45/135	33	31/134	23	13/76	17
Other	21/157	13	13/137	9	12/134	9	10/76	13

[a] Allowing for the considerably longer duration of maintenance therapy, severe complications were found to be less frequent in this than in the initial therapy phases

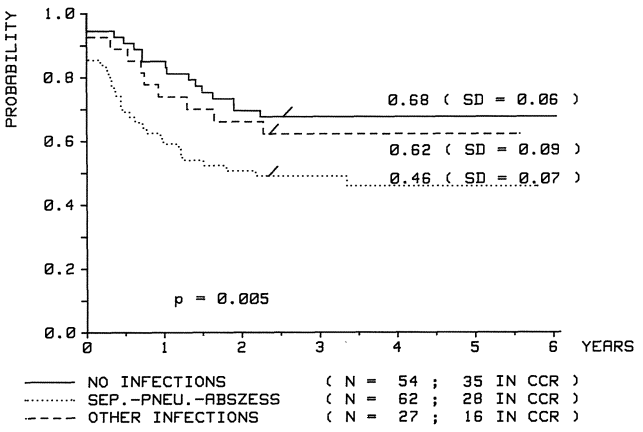


Fig. 49. Probability of EFS duration in AML-BFM-83 patients with no, severe, or minor infections in both phases of consolidation

without infections in either therapy phase had a clearly better prognosis than patients with severe infections such as sepsis, pneumonia, and abscess ($p = 0.005$, Fig. 49). Comparison of patients with or without infections during consolidation therapy showed a much higher number of infections in NRs than in CCR patients (phase 1: 9/13 NRs vs. 35/89 CCR patients, $p = 0.05$; phase 2: n.s.). There was also a tendency toward a higher incidence of infections in patients who relapsed or died with intercurrent complications later on (phase 1: n.s.; phase 2: 15/31 relapse patients vs. 31/93 CCR patients, $p = 0.08$).

Other complications in induction and consolidation therapy are listed in Table 48. Four patients died from the following complications: one NR from progressive renal failure and three children from suspected anthracycline-induced cardiomyopathy after 180 mg/m^2 of DNR given within three days during induction. Allergic or anaphylactic reactions, mainly during induction therapy, were reported in 10 children. There were two incidences of anaphylactic shock, each occurring after application of DNR and platelet transfusion. Urticaria was observed after DNR and VP-16, and during antibiotic treatment. Table 47 shows a decrease in frequency of severe complications from the induction through the first and second phases of consolidation therapy.

Table 48. Complications excluding hemorrhages and infections during induction and consolidation therapy

	Induction	Consolidation	
		Phase 1	Phase 2
	(*n*)	(*n*)	(*n*)
Skin/mucosal ulcer	3	4	3
(Sub)-Ileus	3	1	–
Peptic ulcer	1	–	–
Colitis	–	–	1
Anorexia	–	–	4
Renal failure	2[a]	3[c]	–
Hepatopathy	2[a]	2[a]	2
Anaphylaxis	2	2	–
Exanthema	5	–	1
Cerebral seizure	–	1	–
Cardiomyopathy	3[b]	–	1

[a] Complications in the same patient in different therapy phases
[b] Three patients died with suspected cardiomyopathy.
[c] One NR died from renal failute.

4.8.3 Complications During Maintenance

Evaluation of patients after completion of maintenance therapy (72 CCR patients and 4 late relapses) showed mostly mild thrombopenic bleeding during maintenance therapy in 18 of 76 children (24%). The majority of patients (63%) had infectious complications, with minor infections of the upper respiratory tract predominating (Table 49). Herpes zoster and varicella infections were found in two children and enteritis and sepsis/pneumonia in eight and ten children, respectively. Other, mainly noninfectious complications affected 11 patients. In four children with hepatopathy, infections as well as drug toxicity have to be considered as possible causes. One patient developed hepatic cirrhosis after hepatitis B complicated by bleeding from esophageal varices and a cerebral abscess. Nonetheless, in the majority of children (73 of 76), the general condition at the end of maintenance therapy was not seriously affected.

Table 49. Complications during maintenance therapy ($n = 76$)

Infections	Patients (n)	Other complications	Patients (n)
Infections of the upper respiratory tract[a]	30	Hepatopathies	4
Sepsis/pneumonia	10	Dental complications	2
Phlegmon	1	Venous thrombosis	1
Enteritis	8	Coxitis/femoral head necrosis	2
Infection of the urinary tract	1	Relapsing pleural effusion	1
Varicella	3	Irreversible alopecia	1
Herpes zoster	9		
Epstein Barr infection	2		
Generalized cytomegaly infection	1		
Hepatitis B	1		
Total	48/76 (63%)	Total	11/76 (14%)

Only patients with completed maintenance therapy are listed.
[a] Individual patients had up to 6 infections of the upper respiratory tract.

4.8.4 Summary of Lethal Complications

Nine protocol patients died from hemorrhage and/or leukostasis within the first 14 days after onset of therapy (median, fourth day). All these deaths were directly or indirectly related to the primary disease (median WBC 240000/mm^3) (see Sect. 4.6). It should be noted, however, that in four of these patients, the bleeding event occurred during rapid blast reduction (lysis) after the administration of anthracyclines. None of the children died from hemorrhage during CCR (Table 50).

Infection-induced deaths were rare. One child died after induction therapy with fever of unknown origin in BM depression following progressive renal failure. Three children died in CCR, 2–7 months after

Table 50. Lethal complications by therapy phase

Cause	Induction	Consolidation		Maintenance
		Phase 1	Phase 2	
	(*n*)	(*n*)	(*n*)	(*n*)
Hemorrhage	9[a]	–	–	–
Infections	1	2[c]	1[c]	–
Cardiotoxicity	3[b]	–	–	–

[a] Mainly leukemia related; see text.
[b] One patient died in CR.
[c] Death in CR.

onset of therapy, from uncontrollable infections (viral pneumonia, pulmonary aspergillosis, mucormycosis) already acquired during consolidation therapy. No maintenance therapy was carried out.

Finally, three children died from acute cardiac failure resulting from suspected anthracycline-induced cardiomyopathy, 5–6 weeks after onset of induction therapy.

5 Discussion

The therapy of acute leukemias has been increasingly successful within the last 20 years. There has already been a decisive breakthrough in the treatment of ALL and, at present, about 70% of the children with ALL can be cured [150]. While the prospects are not as favorable in AML, some studies have achieved long-term remissions in about 40% of mostly pediatric patients [41, 173]. This success has been predominantly due to new treatment protocols which differ in choice and combination of the various cytotoxic agents.

A comparison of different studies requires uniform criteria at enrollment. This is almost never achieved on the international level, since, apart from varying exclusion criteria, epidemiological factors such as ethnological characteristics and concurrent procedures differ. By contrast, studies AML-BFM-78 and -83 can certainly be compared because they agree almost completely in the composition of patient groups and in the participating hospitals, although advances in supportive care have to be taken into account. Furthermore, in comparison with other pediatric studies, the results of the AML-BFM studies have increased significance due to the considerably larger number of patients.

The following chapter will describe clinical features and FAB allocation in the AML-BFM studies and draw a comparison with other pediatric studies.

5.1 Clinical and Morphologic Characteristics of Childhood AML

Compared with ALL, AML typically occurs at a higher age (the distribution of AML peaks after age 55, [44]). The incidence in children under 15 years in the Federal Republic of Germany (averaged 1982–1986) is 0.6/100000 [90]; the corresponding value for ALL is 3.4/100000. Since childhood AML represents such a small proportion of the acute leukemias, there are only few reports on specific findings in these patients.

5.1.1 Epidemiological Factors

The characteristic age peak observed between age 2–5 in ALL is not found in AML. The two AML studies showed almost identical age distributions (Fig. 4a) with a slight peak in children under two years and a slow rise in frequency in children older than 10 years. Choi and Simone [35] described a similar distribution in 171 children with AML. However, the age distribution differs among the individual FAB types. We found that 32 out of 56 children under two years (57%) had FAB type M5. In children older than two years, the proportion of patients with FAB M5 (17%) corresponded to the percentage in adults (11–17%) [51, 115, 159].

The sex distribution of 1.2:1 for boys:girls was similar to the values given by Choi and Simone [35], whereas girls predominated in other pediatric studies (Table 51). Thus, boys and girls were more evenly distributed than in ALL. In FAB types M2 and M4 there was a clear predominance of boys, girls predominated in FAB type M5, and boys and girls were evenly distributed among FAB M1 patients (Table 9).

5.1.2 Clinical Data

The proportion of patients with hyperleukocytosis in AML-BFM-78 and -83, (23% and 21%) was high. Different authors have reported values between 13% and 21% in pediatric patients [35, 63], and only 11% in adults [177]. In childhood ALL, the proportion is 11% [74]. Initial platelet counts below 50000/mm^3 were found in more than 50% of the children (Table 51).

Varying threshold values are quoted in the literature with reference to hepatosplenomegaly. It can definitely be stated, however, that liver and spleen are not palpable in about 35%–40% of all AML patients [20, 35, our results]. In children with ALL, organs are impalpable in only 14% [20].

The data on lymph-node enlargement vary considerably, although palpably enlarged lymph-nodes are found less frequently in childhood AML than in ALL (47% vs. 76%) [20]. Nevertheless, 64% of the children studied by Choi and Simone [35] had palpable lymph-nodes. Differentiation of leukemia-related lymphadenopathy from the common non-malignant enlargement of lymph-nodes in children might, however, be difficult. Of the children in AML-BFM-83, 18% had considerably enlarged lymph-nodes, a percentage similar to that reported by Choi and Simone [35].

Predominantly asymptomatic CNS involvement was initially diagnosed in 5%–17% of children with AML (Table 51) [8, 120].

Table 51. Initial patient data of the AML-BFM studies in comparison with other pediatric AML studies

	AML-BFM	Boston [174]	St. Jude [35, 47]	
	78+83	VAPA+80-035	AML-76	1962–1973
No. of patients	333	125	95	171
Age, median (years)	9	no data	8.8	8
Sex (m:f)	1.2	0.7	0.8	1.2
Initial CNS involvement (%)	7	17[a]	15	14
Extramedullary organ involvement (%)	25	no data	no data	11
Hepatomegaly ≥5 cm (%)	23	no data	22	15 (>5 cm)
Splenomegaly ≥5 cm (%)	22	no data	17	19 (>5 cm)
Lymphadenopathy severe (%)	18[b]	no data	no data	22
WBC ≥ 100000/mm^3 (%)	22	18	19	21
Platelets <50000/mm^3 (%)	56	no data	no data	60
Hb, median (g/dl)	8,2	no data	no data	7
Auer rod positive (%)	48	no data	53	23
FAB M1 (%)	22	38	11	52
M2 (%)	21		40	
M3 (%)	3	6	6	4
M4 (%)	26	34	25	34
M5 (%)	24	14	15	
M6 (%)	3	4	2	2
other (%)	1	4	-	8

[a] Only study 80-035
[b] Only AML-BFM-83

The morphologic FAB classification yielded a higher proportion of children with FAB M5 in our trials than in other studies on childhood and adult AML. This can only be attributed to the fact that this particular subtype is especially frequent in children under two years who, compared with other pediatric AML studies, were overrepresented in our study. Tobelem et al. [161] have also pointed out that monoblastic leukemia is especially frequent in early childhood. A comparison, if at all possible in view of the relatively incomplete information available and the much smaller number of patients in most pediatric AML studies, demonstrates no large differences from our data, apart from the lower proportion of FAB M5 patients.

5.2 Characterization of the FAB Types M1–M7 in Children by Pretherapeutic Parameters

Apart from the clinical features, the cytomorphology of the acute leukemias is not only of special relevance for the prognosis in differentiating AML from ALL, but also in classifying the AML subgroups. For this reason, precise differentiation and group allocation prior to therapy are necessary.

Nonetheless, this cannot be done solely on the basis of morphology [169, 175]. Tests using the cytochemical stains PAS, acid phosphatase, POX, and nonspecific esterase are additionally required in the differential diagnosis of ALL as opposed to AML, and for the classification of AML subtypes. In recent years, new test methods provided by immunology, cyto- and molecular genetics have been applied in clinical practice, so that the identification of lymphoblasts or nonlymphoblasts is possible in all but a very few instances [38, 121].

In most cases of AML, several cell lines are affected, since they originate from a common hematopoietic stem cell. Therefore, pathological alterations of myeloblasts and monoblasts, erythroid precursor cells, and megakaryoblasts may be found at the same time. The cytomorphologic classification scheme, based on the FAB system of 1976, established an international standard [12]. However, since unambiguous allocation to the FAB groups was not always possible, the FAB classification was revised in 1985. Among other changes, the megakaryoblastic leukemia was included as FAB M7, and special variants of M3, M4, and M5 were defined [13, 14].

According to our analysis, the AML subtypes as defined by the FAB group were associated with specific clinical features. There were clear differences between the FAB types with monocytic differentiation (M4 and M5) and the nonmonocytic myelogenous types (FAB M1–M3). The

monocytic subtypes had in common a relatively high incidence of extramedullary organ involvement, hepatomegaly, and hyperleukocytosis (M4 more than M5), whereas initial CNS involvement was mostly seen in FAB M4 (Table 6 and Table 52).

One specific feature of the purely monocytic leukemias was the age distribution, with 40% of the children under two years old (Figs. 3 and 4).

According to the FAB criteria, the cytochemical stains POX and ANAE are relevant for the differentiation of monocytic from nonmonocytic FAB types. Our analysis, however, showed extensive overlap. The FAB types M1–M4 were always POX positive, as opposed to only some of the cases with FAB M5 (47%), in which this reaction also tended to be less strongly expressed. ANAE, which is specific for the monocytic FAB types, was rarely negative in these instances (3%), as opposed to the purely myelogenous leukemias where a negative reaction was frequent (41%) (Table 11 and Table 53).

Auer Rods. Auer rods are morphologic markers specific for the leukemic transformation of the granulocytic cell line. According to our results, they were predominantly found in FAB types M1–M3 with purely granulocytic differentiation (71%), and were very rarely seen in purely monocytic leukemia M5 (5%; Tables 11, 54). As expected, the propor-

Table 52. Allocation of characteristic pretherapeutic parameters to patients grouped according to FAB types (data from studies AML-BFM-78 and -83)

FAB	M1–M3	M4	M5	p (χ^2 test)	
Boys	88	49	37		
Girls	67	36	43		
Age <2 years	7	13	32	M1–4 vs. M5	$<.001$
$2-<10$ years	66	32	20		
≥ 10 years	82	40	28		
Extramed. organ involvement					
positive	22	25	30	M1–3 vs. M4,5	$<.001$
negative	128	54	42		
Liver ≤ 5 cm	144	66	54	M1–3 vs. M4,5	$<.001$
>5 cm	11	18	24		
CNS positive	7	14	2	M1–3,5 vs. M4	$<.001$
negative	140	66	75		
WBC $<20\times10^3/mm^3$	73	30	41		
$20-<100\times10^3/mm^3$	54	31	18		
$\geq 100\times10^3/mm^3$	28	24	20	M1–3 vs. M4,5	(.08)

Table 53. Cytochemical data of patients grouped by FAB types (AML-BFM-83)

	M1–M3	M4	M5	p (χ^2 test)
POX positive	73	43	24	n.a.
negative	–	–	21	
POX ≥ 80%	40	19	1	n.a.
< 80%	29	18	22	
ANAE positive	43	43	42	M1–M3 vs. M5
negative	30	–	3	$p < .0001$

tion of Auer rod positive patients with FAB M4 (44%), was intermediate to these values. All 10 children with FAB M3 showed the characteristic bundles of Auer bodies which have been studied extensively by Tulliez and Breton-Gorius [164]. Six out of eight patients with M6, and neither of two patients with M7 showed Auer rods. Thus, we can conclude that, in children as in adults, Auer rods are almost exclusively seen in granulocytically differentiated subtypes of AML.

Eosinophilia. In 1983, Arthur and Bloomfield [5] found structural alterations of chromosome 16 in AML and described them as partial deletion, del(16)(q22), and pericentric inversion, inv(16)(p13q22). Patients with such alterations showed initial eosinophilia of the BM and a morphologic picture of acute myelomonocytic leukemia (FAB M4). Le Beau et al. [103] also stressed the atypical morphology of eosinophils in FAB M4.

In our results, 3% or more eosinophils in the BM (eosinophilia) were predominantly observed in FAB types M2 (44%) and M4 (41%); (Tables 10, 11, 54), and only rarely in FAB M5 (5%). The spectrum of maturation of eosinophils ranges from completely immature, large eosinophilic blasts to mature, normal eosinophils, or abnormal eosinophils with basophilic cytoplasmic granules. Only children with a minimum of 3% eosinophils in the BM also showed atypical forms. The morphologic subtypes of these patients were mainly FAB M4, and, in some cases, purely granulocytic leukemias (FAB M1, M2; Tables 10, 11). One patient with FAB M2 showed a different alteration of the granules, i.e., bubbly granules. Cytogenetic findings have been submitted for eight patients with FAB M4; inv(16) was found in only one instance (see Sect. 4.5.6).

Immunophenotypic Features. Initial attempts at immunophenotypic characterization of the AML FAB types met with little success [128,

Table 54. Distribution of Auer rods and eosinophilia in patients grouped by FAB types (AML-BFM-78 and -83)

FAB	M1–M3	M2	M4	M5	p (χ^2 test)	
Auer rods						
positive	94	49	34	3	M1–M3 vs. M4	.0001
negative	38	16	43	61	M1–M3 vs. M4	.0001
					M2 vs. M4	.0002
					M2 vs. M5	.0001
					M4 vs. M5	.0001
Eo in BM						
≥3%	39	28	29	3	M1–M3 vs. M5	.0002
<3%	90	36	41	55	M2 vs. M5	.0001
					M4 vs. M5	.0001

166], with two exceptions: binding of antibody Vie-64 to glycophorin A, which showed a well-defined reaction with the erythropoietic cell line [98], and the use of monoclonal antibodies against platelet glycoprotein which identified the megakaryocytic cell line [13]. Tests using a panel of monoclonal antibodies have only recently been successful in finding agreement with the FAB morphology and cytochemistry in more than 80% of cases [121].

Centralized immunologic testing in the AML-BFM studies was not started until 1983 and was not done for all patients. For this reason, the numbers of patients in the individual subgroups were small and do not allow any reliable interpretation with respect to FAB classification. Nevertheless, in two patients the diagnosis of FAB M7 could be established by immunologic typing.

The enzyme TdT is generally positive in ALL (except in B-ALL). Since about 5% of AML patients also show increased TdT values [88], this enzyme does not allow definitive differentiation of ALL from AML.

In AML-BFM-83, 11% of the children had TdT values above 10% (data were submitted for 68% of the study patients, Table 11). We included this parameter in our analysis in view of the fact that Bradstock et al. [24] discussed a poor prognosis of TdT positive AML as early as 1981, and Benedetto et al. [10] suggested in 1986 that TdT activity in AML indicates biphenotypy and may therefore be associated with a poor prognosis. We found that there was no significant prevalence of increased TdT values in any of the FAB types. The reaction was clearly positive in 19% of the children with purely myelocytic leukemias (FAB M1 and M2).

Cytogenetic Features. Cytogenetic studies are an additional diagnostic criterion for acute leukemias. Certain structural aberrations are associated with specific FAB types in AML, while other numerical and structural aberrations are found in several FAB types. Table 55 presents an overview of the relationships determined so far.

We found t(8;21), as described by Tricot et al. [163], in FAB M2 (five patients) but also in three children with FAB M1 (Table 12). This finding corresponds to the results published by Brodeur et al. [27], who also demonstrated t(8;21) in FAB types M1 and M4. Translocation 15;17 was only seen in FAB type M3 (3 patients), t(9;11) in four children with FAB M5. Other aberrations of the long arm of chromosome 11 (11q23), aside from t(9;11), were found in five children with FAB M4 and M5, and in one patient with M2. Chromosome 11 aberrations are frequently found in FAB M5, but have also been described in cases of M2 with a monocytic component [69].

Inv(16) was detected in only one patient with M4 and eosinophilia. Of the other seven patients with cytogenetic data available (Table 56), four karyotypes were normal, and three showed other aberrations. The results do not support the finding that most patients with FAB M4 and abnormal eosinophils show concurrent inv(16) or del(16) [15, 103].

Aberrations indicating a secondary leukemia, such as monosomy 7 and del(5) or -5, were found in only one child in our study.

Flow Cytometry. Since cytogenetic tests are difficult in their method and frequently fail for technical reasons, flow cytometry represents an im-

Table 55. Correlation of chromosomal abnormalities with morphologic subtypes and prognosis[a]

Chromosomal abnormality	FAB morphology	Relative prognosis
t(8;21)	M2	good
t(15;17)	M3	good
t(9;11) or 11q-	M4, M5	poor
inv or del(16)	M4	good
t(6;9)	M2, M4	-
+8	M1, M2, M4, M5, M6	-
-5/5q-, -7/7q-	Treatment induced or evolving from preleukemic syndrome	poor
t(9;22)-Ph[1]	M1, M2, M4, M5, M6	poor
t(4;11)	Hybrid; M5	poor
Aneuploid or pleomorphic	M1, M2, M4, M5, M6	poor

[a] In accordance with Champlin and Gale [33]

Table 56. Data on cytogenetics in M4 patients with eosinophilia compared to other patients with eosinophilia (AML-BFM-83)

Karyotype	M4 with Eo		Other FAB types with Eo	
No data submitted or test failure	9	(6)[a]	11	(2)
normal	4	(3)	3	(1)
inv(16)	1	(1)	–	–
t(8;21)	–	–	3	(1)
+22	1	(1)	–	–
11q23	2	(1)	–	–
other	–	–	3	(1)
Total	17	(12)	20	(5)

[a] Data in parentheses refer to atypical Eo.

portant supplementary method. Hiddemann et al. [76] demonstrated, in a fairly large number of children with acute leukemias, that cytogenetics and flow cytometry will offer useful supplementary information in the detection of karyotypic aberrations. Since the data in AML-BFM-83 are incomplete, however, it can only be stated that patients with aneuploidy often have an abnormal karyotype (eight out of nine patients, see Sect. 4.1.2).

5.3 Evaluation of the Relationship Between Pretherapeutic Parameters

The relationship between some of the pretherapeutic parameters and specific FAB subtypes was confirmed by association analysis. The purely granulocytic FAB types M1–M3 were associated with the following parameters: Auer rods, more than 80% POX positive blasts in the BM, eosinophilia, and the rare occurrence of extramedullary organ involvement and hepatomegaly, while the monocytically differentiated leukemias FAB M4 and M5 showed only a small proportion of cases with Auer rods or more than 80% POX positive blasts in the BM, and a higher frequency of extramedullary organ involvement and hepatomegaly.

The presence of myeloblasts and monoblasts in FAB M4 presents features specific for the granulocytic group (more than 80% POX positive blasts, Auer rods, eosinophilia) as well as features characteristic of the monocytic leukemias. Due to this intermediate position, the distinc-

tion between the two groups, M1–M3 and M4, M5 which was sometimes made in these studies was less pronounced.

Parameters characterizing the leukemic cell burden, such as hyperleukocytosis, hepatomegaly and probably initial CNS involvement as well, were also related. The association between an increased blast count on day 15 and hyperleukocytosis supports the prognostic value of an initially high WBC; the same is true for eosinophilia and a low blast count on day 15.

5.4 Prognostic Relevance of Pretherapeutic Parameters and Response Kinetics: Definition of Prognostic Groups

5.4.1 Clinical Parameters

In contrast to the numerous analyses which define clinical risk factors predicting remission rate and duration in adults, there are only few corresponding studies in children, due to the rarity of AML in this age group. An overview of the most important risk factors in children and adults with AML found thus far is shown in Table 57. The relative significance of inidividual variables for EFS and EFI in the two AML-BFM studies is presented in Tables 58 and 59. Variables with no influence have not been listed.

Among the general patient-inherent variables in adults, *age* is particularly important. Remission rates are especially low in the higher age groups [53, 129, 134, 137, 162]; remission duration, however, is not always much shorter than in younger patients [32, 94, 179].

In children, age below two years and especially infancy are regarded as risk factors in ALL and AML [154, 167]. The VAPA study [173], which presented good overall results, also stressed the poor prognosis for young children. According to our analyses, young age is not a risk factor. Treatment results in AML-BFM-78 showed no differences between age groups. An extension of EFI was seen in AML-BFM-83, compared to the preceding study, particularly in patients older than 10 years (Fig. 23b).

We have already pointed out that monocytic leukemia (FAB M5) predominates in children under two years old [40]. If children of various age groups who present this FAB type are compared, the results for young children are not particularly unfavorable. Patients under two have a higher frequency of complications in the beginning ([40] and Sect. 4.5.2), but remission failure is rare.

Another problem in infants with ALL and AML is the frequent initial involvement of the CNS [28, 167]. A higher incidence of CNS relapses has also been reported [28, 161, 173]. In our studies, children under two, as compared to other age groups, did not show a higher incidence of blasts in the CSF at presentation (two and zero patients in AML-BFM-78 and -83, respectively). Isolated or combined CNS relapses occurred in three and two of the under two-year-olds in AML-BFM-78 and -83. Isolated extramedullary relapses at other sites were observed in two patients of AML-BFM-78 and in one patient of AML-BFM-83.

Overall, young age was not found to be a risk factor in children with AML under BFM therapy. Instead, poor treatment results should be attributed to the high incidence of prognostically unfavorable ALL and AML types in this age group. ALL in infancy is often characterized by large tumor mass and CNS involvement; frequently, there are morphologic subtypes with immature common ALL antigen (cALLa) and/or TdT negative leukemic blasts [28]. In AML, FAB type M5 is frequent, which, regardless of age, constitutes a risk factor in most studies [161, 174].

Conclusions on the prognostic relevance of *sex* vary in the literature. Beguin et al. [9] report a low CR rate due to EDs during BM depression in women, whereas Weil et al. [171] describe clearly better results in females of all age groups. In our studies, the overall prognosis is the same for boys and girls. Only girls with FAB M1 have a lower CR rate than boys, due to a higher incidence of ED (Table 19, Fig. 20).

As in ALL [72], the *initial WBC or absolute blast count* in the peripheral blood has high prognostic relevance in adults and children with AML [95, 151, 174, 176]. This observation, however, needs to be investigated more closely with respect to the risk of ED, treatment failure, risk of relapse, and, in view of the heterogeneity of AML, morphologic status.

Different authors have suggested an increased risk of ED and treatment failure in adults and children with hyperleukocytosis [50, 85, 168]. This finding corresponds to the general oncological experience that larger tumors – or a large population of blast cells – show a poorer response to chemotherapy. It is evident in the subgrouping of prognostic variables shown in Table 57, that many of them are related to the proliferative activity of the blasts. The faster the leukemic clone is expanding, and the larger the cell pool at the time of diagnosis, the less favorable the course. In addition, Goldie et al. [64] presented mathematical models to show that the proportion of mono- and multiresistant cells is increased in a large tumor mass.

Our findings indicate that all patients with hyperleukocytosis carry a specific risk, varying by FAB type, of not achieving CR owing to early

Table 57. Prognostic factors in pediatric and adult AML patients

Variable	Favorable prognosis	Remission		Reference
		rate	duration	
Age (adults)	young	+	+	Rai et al. [134]; Whittaker et al. [176]; Passe et al. [126]
(children)	>2 years	Ø	+	Weinstein et al. [174][a]
Sex	male	+	Ø	Beguin et al. [9]
	female	+	Ø	Weil et al. [171]; Baehner et al. [7][a]
General condition	good	+	Ø	Vogler et al. [170]
Proliferation dependent variables				
initial blasts/WBC	low	+	+	Whittaker et al. [176]; Keating et al. [95]; Schwartz et al. [151]; Weinstein et al. [174][a]
Liver/spleen	normal	+	Ø	Henderson et al. [71]; Choi and Simone [35][a]; Dahl et al. [46][a]
extramedullary organ involvement	no	+	+	Plüss and Hitzig [131][a]
CNS involvement	no	+	+	Plüss and Hitzig [131][a]; Dahl et al. [46][a]
Thrombocytes	high	+	Ø	Whittaker et al. [176]; Vogler et al. [170]
Hemoglobin	high	+	+	Vogler et al. [170]
Labeling index	high	Ø	+	Dahl et al. [46][a]

Table 57. Continued

Variable	Favorable prognosis	Remission		Reference
		rate	duration	
Blast characteristics				
FAB type	M1, M2	+	+	Keating et al. [95]; Chessels et al. [34][a]
	M3	Ø	+	Brandmann et al. [25]; Keating et al. [95]; Arlin et al. [2]
	M4+Eo	+	+	Holmes et al. [80]
Auer rods	present	+	+	Mertelsmann et al. [116]; Passe et al. [126]
in vitro cell culture	cluster	+	+	Moore et al. [119]; Findley et al. [56]
in vitro drug resistance	low	+	+	Preisler [133]
Cytogenetics	normal, t(8;21)	+	Ø	Fourth international Workshop on Chromosomes in Leukemia [58]; Sandberg [145]
Biochemical variables				
LDH	<400 IU/dl	Ø	+	Keating et al. [94, 95]
Fibrinogen	<250 mg/dl	Ø	+	Keating et al. [94]
Parameter of disease course				
Blast reduction	rapide	Ø	+	Reizenstein et al. [138]; Keating et al. [95]
Time to remission	short	Ø	+	Keating et al. [94]
Hepatitis	yes	Ø	+	Foon et al. [57]

[a] Analyses of pediatric AML patients

lethal hemorrhage and/or leukostasis, or treatment failure (Table 22). Hyperleukocytosis has a particularly negative effect on the risk of ED due to hemorrhage and/or leukostasis in FAB type M5. In FAB M4 and, less obviously, in FAB M2, hyperleukocytosis is also associated with an increased number of NRs. A comparison of patient groups with a WBC of less than or over 20000/mm^3 shows a difference in CR rate only for AML-BFM-83 (Table 21).

The influence of the initial WBC on the risk of relapse must be evaluated in consideration of the chosen therapy. The relevance of the initial WBC is more apparent in AML-BFM-83 since an improvement of prognosis had been obtained for patients with low WBC (Tables 58, 59). Multivariate analysis indicated that the initial WBC was of decisive importance for the overall prognosis (EFS, Table 60). In AML-BFM-78, this parameter was the only independent risk factor. Analysis by FAB types in AML-BFM-83 showed an independent effect only in FAB M2.

Only a few studies emphasize the prognostic value of *hepato- and splenomegaly* [35, 71]. The AML-BFM studies did not determine any remarkable significance for these parameters (Tables A and B in the appendix). Only in AML-BFM-78 were remission rates reduced to 66%, as opposed to 83%, with hepatomegaly exceeding 5 cm below the costal margin. The result of the association analysis (Sect. 4.2) also showed a direct relationship between increased WBC and hepatomegaly.

The relevance of *infiltrations in extramedullary organs* involving sites other than the liver and spleen has rarely been discussed in the literature [131]. The comparison of AML-BFM-78 and -83 suggests that a lack of documentation might be responsible, since the increased inci-

Table 58. Relative significance of pretherapeutic parameters for prognosis in AML-BFM-78 (univariate – log rank test)

Variable		EFS	EFI
		p value	*p* value
WBC ($\times 10^3$/mm^3)	$<100 : \geq 100$	.01	(.17)
Extramed. organ involvement	neg:pos	.03	n.s.
CNS involvement	neg:pos	n.s.	(.07)
Hepatomegaly	≤ 5 cm : >5 cm	(.06)	n.s.
For the individual FAB types:			
FAB M2 WBC ($\times 10^3$/mm^3)	$<20 : \geq 20$	(.11)	(.07)
M4 WBC ($\times 10^3$/mm^3)	$<100 : \geq 100$	.03	n.d.
M5 organ involvement	neg:pos	.03	n.d.

Table 59. Relative significance of initial and response kinetic parameters for prognosis in AML-BFM-83 (univariate - log rank test)

Variable		EFS	EFI
		p value	*p* value
WBC ($\times 10^3/mm^3$)	$<20 : \geq 20$	.002	(.09)
	$<100 : \geq 100$	<.001	n.s.
POX	$<80\% : \geq 80\%$	<.001	<.0001
Auer rods	neg : pos	.001	.002
Eosinophils	$<3\% : \geq 3\%$	.01	.02
Age	<10 years : ≥ 10 years	n.s.	(.08)
Blasts day 15	$<10\% : \geq 10\%$	.001	(.09)
Remission	<10 weeks : ≥ 10 weeks	.04	
For the individual FAB types:			
FAB M1 Auer rods	neg : pos	<.0001	<.001
M2 WBC ($\times 10^3/mm^3$)	$<20 : \geq 20$	<.001	.001
	$<100 : \geq 100$	.02	n.d.
M4 WBC ($\times 10^3/mm^3$)	$<20 : \geq 20$	.02	n.s.
	$<100 : \geq 100$	(.13)	n.d.
M4 Eosinophils	$<3\% : \geq 3\%$	.007	.004
M5 WBC ($\times 10^3/mm^3$)	$<20 : \geq 20$	(.17)	n.s.
	$<100 : \geq 100$	.02	n.d.

dence of extramedullary organ infiltrates in AML-BFM-83 may be attributed solely to the fact that renal, skeletal and orbital infiltration were recorded in it, while in AML-BFM-78 they were not. According to our analysis, extramedullary organ involvement in FAB M5 increased the risk of ED due to hemorrhage and/or leukostasis (before or after onset of therapy, see Sect. 4.6). This parameter was less important for the achievement of CR (except in AML-BFM-78, for FAB types M4 and M5). Overall, the risk of relapse was independent of any initial extramedullary organ involvement, but sites of relapse are often found in extramedullary organs, either isolated or combined with BM infiltration. In AML-BFM-78, out of three isolated and five combined relapses (CNS relapses not included), four had had initial extramedullary organ involvement. In AML-BFM-83, out of one isolated and three combined relapses, three had had initial extramedullary organ involvement (no data available for one patient). Almost all extramedullary relapses occurred in the monocytic FAB types M4 and M5; only three out of 12 patients had a different morphologic type (Table 28).

A similar situation was found concerning *initial CNS involvement.* CNS involvement was not important for the achievement of CR, whereas the risk of relapse increased in initially CNS positive patients.

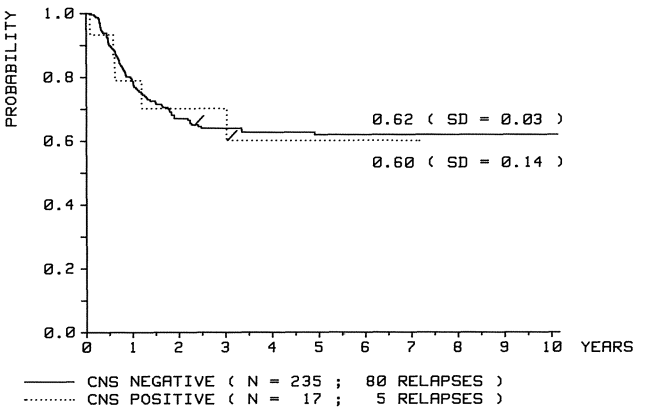


Fig. 50. Probability of EFI duration in initially CNS negative and CNS positive patients in AML-BFM-78 and AML-BFM-83. Combined analysis, CNS relapses censored

Again, evaluation of the relapse sites showed that initially CNS positive children were prone to CNS relapse. Exclusion of relapses with CNS involvement resulted in the disappearance of the difference in prognosis between initially CNS positive and CNS negative patients (Fig. 50). Again, extramedullary relapses in this particular site were most frequent in the monocytic FAB types (Table 22). Our results correspond to those of Dahl et al. [46], who found CNS relapses to be more frequent in children with CNS involvement (initially or within the first weeks before entering remission) than in others.

Thrombocytopenia and anemia were not risk factors in either of the two studies (Tables A and B in the appendix). Vogler et al. [170] have investigated these parameters by multivariate analysis and described them as independent variables. Their study was performed in adult patients and used triple randomization. A high labeling index, which represents the proportion of blasts in the DNA synthesis phase and thus is an indicator for proliferative activity, represents a risk factor according to the analysis by Dahl et al. [46]. Others have found no influence [1]. The labeling index was not investigated in our studies.

5.4.2 Morphologic Parameters

Among the various aspects of blast morphology, we investigated the correlation of FAB classification with other prognostic factors. In addi-

tion, the relevance of the variables, presence of Auer rods, eosinophilia, positive POX reaction, and TdT level was also analyzed. Any evaluation of the results of chromosomal analyses could only be descriptive.

In some respects the results obtained in the individual FAB types differed between the two AML-BFM studies. Both studies showed a high rate of ED in FAB type M5. The CR rates in the FAB types represented by larger numbers of patients (M1, M2, M4, and M5) differed only slightly between the two studies (Table 18). There were differences, however, in relapse risk: in AML-BFM-78, children with FAB M2 and M4 showed a high incidence of relapses, whereas in AML-BFM-83 it were M5 patients who had a high proportion of relapses (Figs. 13b, 17b). In AML-BFM-83, children with granulocytically differentiated FAB types, including the mixed type FAB M4, had a better prognosis than those with FAB M5 and all of the patients in the preceding study. This finding enables us to draw direct conclusions on the influence of therapy (see Sect. 5.8). In both studies, the FAB type M1 had the most favorable overall prognosis (Figs. 12a, 13a, 14a).

These conclusions on the FAB types correspond to reports in the literature indicating a better overall prognosis for FAB types with granulocytic, as opposed to monocytic, differentiation. The prognostic advantage is mainly represented by higher CR rates [34, 159]. Differentiated analyses determining the prognosis for the various FAB types under uniform therapy are usually not possible owing to small numbers of patients. However, according to our results, the individual FAB types cannot only be distinguished by characteristic parameters including clinical findings, but also by clinical course and prognosis. Prognostically relevant factors determined for the total group varied in their effects on the individual FAB types and their influence could be evaluated by multivariate analyses.

FAB M1. In both studies, patients with myelogenous leukemia without maturation were found to have a comparatively favorable prognosis, with CR rates exceeding 80%, and a probability of 57% (AML-BFM-78) and 74% (AML-BFM-83) of having a six-year EFI. Nevertheless, there was a subgroup of children with an unusually poor prognosis which we have previously described [143]; these patients had FAB M1 and an absence of Auer rods (Fig. 36a, b). Results of AML-BFM-83 showed a significant difference in EFS and EFI between Auer rod positive and negative patients. By multivariate analysis WBC had lost influence on prognosis; absence or presence of Auer rods remained the only independent factor.

Auer rods are found almost exclusively in leukemias with granulocytic differentiation. Since the prognosis of monocytic leukemias is less

favorable than that of granulocytic leukemias, overall analysis does not produce conclusive results on the prognostic relevance of Auer rods. We detected an improvement of the overall prognosis for Auer rod positive patients compared to Auer rod negative patients in AML-BFM-83, but not in AML-BFM-78 (Fig. 35, Table 29). Evaluation of the individual FAB types showed that significant differences between Auer rod positive and negative patients existed only for FAB type M1, and not for the other subtypes.

Several studies on adult patients reported higher CR rates in patients with Auer rods [95, 116, 126], although an extended duration of remission in Auer rod positive patients was found only in the study of Mertelsmann et al. [116]. These authors were also the only ones to discuss the relevance of Auer rods in the individual FAB types and in contrast to ourselves they found a significantly better prognosis in FAB type M2. However, this contrasting result may be due to differences in the composition of the patient group and the therapy applied. Moreover, the prognosis in adult AML patients was clearly less favorable than in our patients (less than 20% of the patients survived 5 years). In addition, the study by Mertelsmann et al. [116] employed a slightly modified version of the FAB classification. Their definition of FAB type M1 accepted a threshold value of 3% promyelocytes, whereas the original FAB definition applied in our study did not accept any maturation beyond the promyelocytic stage. This also explains the higher incidence of FAB M1 patients (20%) in study AML-BFM-83 than in the study by Mertelsmann et al. ([116], only 9% FAB M1 but 28% FAB M2). This shift means that there may not be any basic differences between our own results and those we have just cited.

The reasons for the prognostic significance of the Auer rods are still subject to speculation. Auer rods are morphologic markers specific for a granulocytic origin of leukemic transformation. They develop via pathologic fusion of lysosomal azure granules during granulocytic differentiation. Since the azure granules are formed during the promyelocytic stage, the relationship between the presence of Auer rods in granulocytically differentiated blasts with maturation is clear. Auer rod positive leukemia thus represents the classical AML with maturation, whereas the Auer rod negative AML shows either monocytic or only some evidence of granulocytic differentiation.

The assumption that Auer rod negative leukemias in FAB M1 represent particularly immature forms is supported by the fact that other signs of immaturity, such as a low number of POX positive blasts in the BM, were more frequent in our patients without Auer rods than in those with Auer rods [143]. Recently, Lee et al. [105] described AML with minimal differentiation as a special form. This morphologically

unclassifiable leukemia, in which, however, POX could be detected by electron microscopy, and which showed expression of myelogenous surface markers, has a poor prognosis. We may consequently assume that FAB type M1, negative for the presence of Auer rods, constitutes the same subentity.

FAB M2. According to our results, there was a significant association between overall prognosis and level of initial WBC in children with FAB M2 (Tables 21, 22). CR rates were lower in hyperleukocytosis (study AML-BFM-83) and the incidence of relapses increased with higher WBC (Fig. 28a, b).

In the multivariate analysis, initial WBC remained the only independent variable. Additionally, the negative effect of late CR was seen mostly in FAB M2 CR patients. Patients with FAB M2 (10 out of 22) represented the largest proportion among those achieving CR after a period of 10 weeks or more.

According to the results of the Fourth International Workshop on Chromosomes in Leukemia [58], t(8;21) is specific for the FAB type M2 and is associated with a favorable prognosis. Cytogenetic data were submitted for nine patients with FAB M2 in AML-BFM-83. All five children with t(8;21) remained in CCR. It is remarkable that four of these patients had a WBC below 20000/mm^3; the fifth child showed a WBC of 21000/mm^3. This potential association between low WBC and t(8;21) should be investigated in a larger number of patients.

FAB M3. Plasmatic coagulation defects with intravascular coagulation and excessive fibrinolysis, often causing severe or lethal hemorrhage during the initial phase, are conspicuous findings in the promyelocytic leukemias [2, 36, 92, 135]. Nevertheless, patients with this morphologic type survived longer once they achieved remission than patients with other FAB types [2, 92]. The results of 11 children with FAB M3 in the two AML-BFM studies confirmed the published data: four patients died early, but there was only one relapse (Table 18).

FAB M4. CR rates in adults with FAB M4 are comparatively low [159]. There was a higher number of NRs (11 out of 77, 14%) in FAB M4 than in any other FAB type (EDs not included). It should be taken into account, however, that FAB M4 patients had the highest median WBC, and that high WBC was a risk factor predicting for treatment failure in both studies.

Our results showed that eosinophilia was predominantly found in FAB types M2 and M4. The FAB type M4 with eosinophilia was characterized by the simultaneous presence of eosinophils in various steps

of maturation, together with atypical eosinophils. In both studies, the FAB M4 patient group with eosinophilia had a better prognosis than the group without. A significant difference in EFS and EFI was seen in AML-BFM-83, not in AML-BFM-78. After multivariate analysis, eosinophilia remained the only independent prognostic factor in FAB M4 (Tables 38, 60).

Remarkably, in AML-BFM-83, out of 12 patients with FAB M4 and atypical eosinophils 10 have remained in CCR (the other died from early hemorrhage and infection). In AML-BFM-78, four out of eight patients with this morphologic finding have remained in CCR (one ED, three relapses). In rare instances (three patients in AML-BFM-78 and five in AML-BFM-83), abnormal eosinophils were also seen in the FAB types M1 and M2. These children also had a favorable prognosis; only one patient, with unusual bubbly granules in the eosinophils, relapsed. The cytogenetic findings in the patients with M4 and abnormal eosinophils have already been discussed (Table 56).

Our results differ to a certain degree from the data published in the literature. Keating [96] discussed the prognostic significance of eosinophilia ($\geq 4\%$) in the BM in 1982; Holmes et al. [80] reported on 26 adult patients with M4 and abnormal eosinophils who all had simultaneous inv(16) (p13q22). Overall, the prognosis in this patient group was relatively favorable, but there was a remarkably high rate of CNS relapses [35%].

The rare finding of inv(16) in our patients may be due to methodological reasons [69]. Nonetheless, we can conclude that the morphologic finding of eosinophilia is a sufficiently good indicator of prognosis even without any cytogenetic finding. The comparatively low threshold value defining eosinophilia in the BM ($\geq 3\%$ instead of 4%) was chosen by us because atypical eosinophils were first detected with eosinophilia of 3%, although most patients with atypical eosinophils had a higher level of eosinophilia. Among the patients with FAB M1 and M2 with atypical eosinophils, three had t(8;21), which is also indicative of a good prognosis.

If the initial estimation of eosinophilia in the BM poses diagnostic difficulties, the suggestion by Holmes et al. [80] might be helpful, i.e. that eosinophilia in the BM in patients with initial hyperleukocytosis and close to 100% of blasts can only be detected after chemotherapy has been started and the number of blasts has been reduced.

Overall, our results show that children with FAB type M4 with eosinophilia represent a specific subgroup distinct from other M4 patients. With respect to pathogenesis, it can be assumed that these eosinophils are leukemic blasts. This assumption is supported by the enzymatic misdifferentiation of the eosinophilic granules described by Löffler in 1976

[111], and by the observation that Auer rod positive blasts in cell culture differentiated toward the eosinophilic line of maturation [102]. Cytogenetic analyses in some AML patients have recently proved the differentiation of leukemic blasts into different cell lines [97]. We found that many patients with eosinophilia also present with maturation of neutrophils and, in some cases, of the basophilic line. Le Beau et al. [104] observed that alteration of chromosome 16 led to division of the metallothionine gene cluster, thus probably influencing the proliferation and differentiation of cells of the granulocytic and monocytic line.

FAB M5. This FAB type is not only morphologically different from the other subtypes with mostly granulocytic differentiation, but also differs in clinical course. The high risk of ED and leukostasis is a problem, especially when associated with an initial high WBC and/or extramedullary organ involvement (see Sect. 5.6). Little can be said about the risk of nonresponse because the number of patients with FAB M5 and hyperleukocytosis surviving the first six weeks is very small.

In both studies, the risk of relapse was independent of the initial WBC. The difference in EFI between patients with and without initial extramedullary organ involvement was not significant ($p = 0.15$) when the patients of both studies were combined in the analysis (Fig. 34c). Due to the small number of patients, a combined analysis was necessary and also methodologically acceptable, since no improvement of prognosis was seen for FAB type M5 in AML-BFM-83 compared to the first study.

Patients with FAB types M4 and M5 had a higher incidence of initial extramedullary organ manifestations than children with other FAB types. Since isolated and combined extramedullary relapses occurred predominantly in patients who had such manifestations in the beginning as well, the comparatively frequent occurrence of extramedullary relapses in monocytic leukemias (Table 28) was not unexpected.

Monocytic leukemia is regarded as prognostically unfavorable both in children and adults [159, 161, 174]. Combined analysis of the Boston studies [174] showed the factor monocytic leukemia to be the most prominent risk factor in the multivariate analysis of results obtained in 125 children. The high rate of CNS relapses was particularly significant. We cannot confirm this result; however, treatment in our study did include CNS prophylaxis, which was absent in the VAPA study and consisted only of intrathecal application of ARA-C in study 80-035.

FAB M6. Acute erythroleukemia is characterized by abnormal proliferation of cells in preliminary stages of erythro- as well as myelopoiesis. Initially, a precise definition was not possible due to discrepancies be-

tween the acute and the chronic form and between the pure and the mixed erythro-myeloblastic form [48, 67]; the 1976 definition of acute erythroleukemia proposed by the FAB group [12] was also unsatisfactory. A clear differentiation of FAB M6 from refractory anemia with an excess of blasts (RAEB) was not possible until 1985 [14].

In both AML-BFM studies, patients with FAB M6 were extremely rare (10 out of 333). Therefore, little could be concluded except that children with FAB M6 did not have a poor prognosis when treated as in study AML-BFM-83.

FAB M7. Formerly, this rare form of leukemia was seldom diagnosed; BM aspiration often failed due to myelofibrosis. In these instances, when only peripheral blood could be examined, POX negative blasts used to be classified as undifferentiated leukemia.

Diagnosis is easier today because platelet POX can be detected by electron microscopy, and monoclonal or polyclonal, platelet-specific antibodies are available. In addition, BM biopsy is helpful. The FAB group published the following (excerpted) description of the characteristic morphologic features in 1985 [13]:

> The polymorphic blasts, mostly without granules, contain one to three prominent nucleoli. The cells resemble L1 or L2 cells, but vary considerably in size, in 20% to 30% reaching a cell diameter two to three times larger than normal. Often, cytoplasmatic blebs are seen. One should be aware of the possibility of megakaryocytic fragments in the periphery.

In AML-BFM-83, three patients were diagnosed with this type on the basis of morphology; in two of these children the diagnosis was supported by immunologic findings. Two of the children have achieved CR and one of them has remained in CCR for 28 months.

Two other patients with FAB M7 were allocated to the group of ineligible patients (Table 4). Initially there were doubts regarding diagnosis due to insufficient material; later on, the diagnosis was confirmed by BM biopsy and the children were treated according to a different therapy protocol. One child suffered an early relapse, the other achieved only partial remission, both died after BMT.

A literature review about this FAB type yields only case reports which mainly point to the poor prognosis and association with Down's syndrome in children [118, 147, 153].

5.4.3 Cytogenetics

The first cytogenetic studies in AML showed chromosomal aberrations in about 50% of cases [65], but it is currently assumed that better diag-

nostic procedures will detect clonal aberrations in most if not all AML patients [181]. In addition, specific aberrations have prognostic relevance and some are associated with particular FAB types [33, 152] (Table 55). Some frequent break points were found to be associated with the localization of oncogenes or fragile sites. These findings suggest that such chromosomal aberrations involve genetic regions which are relevant for the malignant process [6].

Cytogenetic findings were submitted for 59 patients of AML-BFM-83 (Table 12). The results concerning allocation by FAB types essentially confirm the data published in the literature. Only patients with FAB M4 and eosinophilia showed inv(16) less often than expected.

5.4.4 Response Kinetics

Response to chemotherapy can be measured by the decrease of blasts in the BM after induction or by the time until achievement of CR. All parameters reflecting fast cytoreduction, as opposed to those indicating slow response, are associated with an extended EFI. This was seen in adult AML patients [29, 96, 138] and could be confirmed by study AML-BFM-83.

5.4.5 Prognostic Groups in Childhood AML

Different degrees of association, as well as interactions of potential prognostic factors, require multivariate analysis in order to determine the independent effects of individual factors. One disadvantage of multivariate evaluation is that only patients with complete sets of data can be included, i.e., the selected group of patients may be distorted in composition.

Table 60 summarizes the relative significance of pretherapeutic parameters and of the response-kinetic parameter time until CR for the prognosis, since the importance of these interactions becomes apparent in multivariate analysis. The parameter initial WBC was an important factor for the total group of pediatric AML patients in both studies. However, in those patients who have achieved CR, the same parameter was no longer of great relevance. In study AML-BFM-83 the parameters eosinophilia and presence of Auer rods were favorable predictors; this was also true for age above 10 years. Late CR was associated with a particularly unfavorable course.

Combined analysis of FAB types M1–M4 showed that initial WBC and eosinophilia had independent prognostic significance. The in-

Table 60. Relative significance of pretherapeutic parameters and time until achievement of CR with respect to prognosis: summarized results of the Cox analyses (p values)

Variable / Patient group	WBC $\times 10^3/mm^3$ $<20 : \geq 20$	Eosinophils $<3\% : \geq 3\%$	Auer rods neg.:pos.	Age <10 years: ≥ 10 years	Time to achieve CR <10 weeks: ≥ 10 weeks
Study AML-BFM-78					
WBC $\times 10^3/mm^3$ ($<100 : \geq 100$)	.06	n.s.	n.s.	n.s.	–
Study AML-BFM-83					
Total group	.04	.005	.05	n.s.	–
CR group	n.s.	.002	.08	.004	.02
FAB M1–M4					
Total group	.008	.06	n.s.	n.s.	–
CR group	.01	.04	.08	.02	.03
FAB M1					
Total group	n.s.	n.p.	$<.001$	n.s.	–
FAB M2					
Total and CR group	$<.001$	n.s.	n.s.	n.s.	.04
FAB M4					
Total group	n.s.	.007	n.s.	n.s.	n.s.
FAB M5					
Total group	n.s.	n.s.	n.s.	n.s.	n.s.

fluence of initial WBC in these instances exceeded the effect observed in the total group of AML patients (M1–M7) and the negative effect of late CR was apparent.

Evaluation of the individual FAB types separately showed the presence of Auer rods to be an independent positive prognostic parameter only in patients with FAB M1, while eosinophilia was relevant only in children with FAB M4. An explanation for the favorable prognosis of children older than 10 years in study AML-BFM-83 was not found. Even when patients with FAB M5, who have a poor prognosis and in most cases are younger than two years, are eliminated from the evaluation, an age effect remained.

In general, risk factors predicting for failure to achieve CR are also unfavorable prognostic variables predicting for relapse, since treatment failure is the main underlying cause in both cases. Generally, this is also true for the risk of ED due to hemorrhage and/or leukostasis, with one exception: patients with FAB type M3 have a high risk of early lethal bleeding complications even with low WBC, while the risk of relapse is low [2, 92, our results].

One other specific feature applies to the site of relapse. Patients with initial CNS involvement (Table 24) and other extramedullary organ involvement also have a higher risk of suffering an extramedullary relapse. Multivariate regression analysis showed no independent prognostic significance of initial CNS involvement. However, this is only true for the overall risk of relapse, and is not specific for the risk of suffering a relapse at one particular site.

Our results allowed us to define two prognostic groups in children with AML treated according to the therapy protocol AML-BFM-83

Table 61. Prognostic groups of AML in childhood according to AML-BFM-83

EFS/EFI[a]	Risk I >80%	Risk II <45%
FAB M1	Auer positive	Auer negative
FAB M2	WBC $<20\times10^3/mm^3$	WBC $\geq20\times10^3/mm^3$
FAB M3	all[b]	–
FAB M4	Eo ≥3%	Eo <3%
FAB M5	–	all
FAB M6	all[b]	–
FAB M7	–	(all)[b]
Additional factor for ‚Risk II‘: time to achieve CR		≥10 weeks

[a] Estimation for 6 years.
[b] Results based on a small number of patients.

(Table 61). In the low risk group (risk I) the probability of a six-year EFS and EFI exceeded 80% (Fig. 51). In 14% (8 of 59) of the children with pretherapeutic risk I criteria, the additional parameter late CR determined subsequent allocation to the high risk group (II). Risk group I included 37% of all the AML patients entering CR. The corresponding proportion in children with FAB M1–M4 is 45%.

Risk group II included close to two-thirds of the CR patients. The probability of a six-year EFS was 0.31 (SD 0.05), and 0.42 for a six-year EFI (SD 0.06). A clear difference between the two prognostic groups was apparent (Fig. 51). Definition of a patient group associated with the highest risk of relapse is impossible because neither multivariate nor individual life table analyses allow any relevant discrimination. Any in-

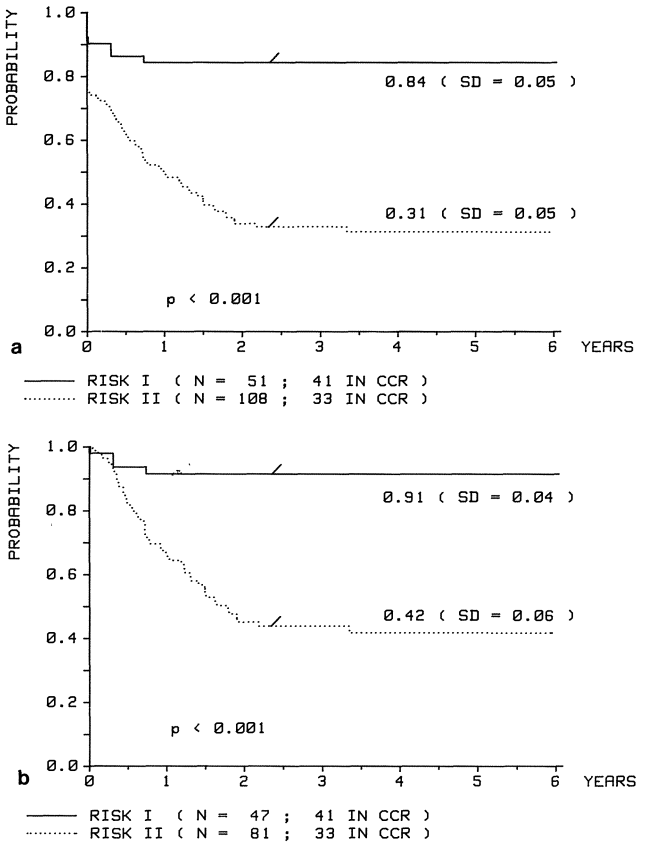


Fig. 51. **a** Probability of EFS duration in the individual risk groups, calculation for patients of AML-BFM-83. **b** Same for EFI duration

terpretation of the particularly high risk of relapse observed in children with FAB M4 without eosinophilia (Fig. 38a) should take into account the large standard deviation.

The definition of the two prognostic groups was based, in some subgroups, on small patient numbers but agrees with the report of the MIC cooperative group [152] about karyotypic-morphologic associations and prognosis (Table 59). t(8;21) is indicative of a good prognosis and is predominantly seen in FAB M2, but also in FAB M1 with Auer rods. Out of our five patients with t(8;21), four had a low WBC. All patients with FAB M3 had t(15;17), and for FAB M4 with eosinophilia inv(16) or del(16) is specific. Conversely, translocations and deletions with involvement of 11q23 characterize FAB M5 and indicate an unfavorable prognosis. Therefore, cytogenetic studies may offer a superior way of characterizing the low or high risk group. However, the method is difficult and test results are not readily available at the time of diagnosis.

Specification of prognostic groups is important for any future therapeutic approach, as they ensure that radical therapy, e.g. BMT, can be avoided in cases with a favorable prognosis and treatment can be tailored to the patient's requirements. Patients with FAB M5, for example, might receive different substances, and new elements of therapy might be introduced for high risk patients (see Sect. 5.8).

5.5 Influence of Therapy on the Prognostic Relevance of Pretherapeutic Parameters: Evaluating the Introduction of ADE Induction Therapy in AML-BFM-83 Study

One of the most important prognostic parameters in acute leukemias is the mode of therapy. This was especially apparent in the ALL-BFM studies. After introduction of risk-adapted induction and reinduction therapy, unfavorable pretherapeutic parameters lost significance [150].

The essential difference in study AML-BFM-83, as compared to study AML-BFM-78, consisted in the introduction of the intensive ADE induction therapy prior to the eight week induction/consolidation therapy. According to the results of the risk factor analysis, this new element of therapy did not lead to an increased CR rate but to an extended EFI (Fig. 10b). This improvement, however, did not apply to all pediatric AML patients, but only to those with FAB types with granulocytic differentiation (Fig. 52). Consequently, specific characteristics of granulocytic differentiation, such as presence of Auer rods or eosinophilia, were associated with an improved prognosis in AML-BFM-83,

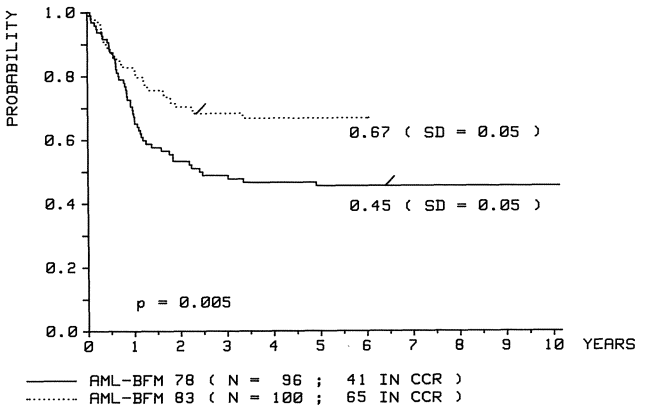


Fig. 52. Probability of EFI duration in patients with FAB M1–M4, AML-BFM-78 vs. AML-BFM-83

Table 62. Improvement of prognosis in AML-BFM-83 as compared to AML-BFM-78

	EFI (%)		*p* value
	AML-BFM-78	AML-BFM-83	
FAB M1–M4	45	67	.005
M1	57	74	(<.20)
M2	42	67	(.08)
M2	36	63	.04
Age ≥ 10 years	47	69	.03
WBC $<20\times10^3/mm^3$	51	68	(.11)
WBC $<20\times10^3/mm^3$ and FAB M1–M4	53	80	<.01
and FAB M1	58	81	(<.20)
and FAB M2	56	100	<.01
and FAB M4	33	74	(.07)
Auer rod positive	48	75	<.01
and FAB M1	60	88	(.06)
Eosinophils ≥3% in BM	45	81	<.01
and FAB M4	50	93	.03

as opposed to the preceding study (Table 62). A favorable effect on EFI was also observed in FAB M1–M4 patients with low WBC. No improvement was seen in AML-BFM-83 over study AML-BFM-78 as re-

gards the leukemias with purely monocytic differentiation (FAB M5) (Fig. 17b).

These results lead to the conclusion that a cell clone with granulocytic differentiation is effectively treated by use of ADE induction. Furthermore, in terms of methodology consecutive studies, as opposed to historical controls using retrospective evaluations or published data, are entirely justified. For example, one advantage of our consecutive studies was that the number of patients available for evaluation of the various prognostic groups within a given period was twice the number of patients available in a randomized study [79, 149].

5.6 Early Death Due to Hemorrhage and/or Leukostasis and Other Complications: Consequences for Initial Therapy

The definition of risk groups allows one to adjust the intensity of treatment to the individual risk. A therapy protocol can also be optimized by applying treatment modifications or special supportive measures in specific situations of danger for the patient. This is particularly true for children who carry the risk of hemorrhage and/or leukostasis during the initial phases of therapy.

In contrast to AML in adults, where EDs are mainly due to infections [52, 155], the most important problem during the initial phases of therapy in children is hemorrhage and/or leukostasis. Through our analysis, we identified a well-defined high risk group for early fatal hemorrhage and/or leukostasis (groups A and B). A second, smaller group of children showed different, but nonetheless specific, initial features and heterogeneous problems during induction (groups C and D).

Children with FAB M5 and concomitant hyperleukocytosis and/or organ infiltration (groups A and B) showed a particularly high risk of ED due to hemorrhage and/or leukostasis. Other studies have also discussed the prognostic significance of hyperleukocytosis as a risk factor predicting for hemorrhage and/or leukostasis [50, 85, 168]. The association between FAB M5 with extreme leukocytosis and extramedullary organ involvement has been described by Lester et al. [107] and Cuttner et al. [45], who also mentioned the hazard of leukostasis. Tobelem et al. [161] and McKenna et al. [114] have reported coagulation disorders with monocytic leukemias in all age groups.

The proportion of pediatric AML patients with FAB M5 is higher than in adults, and 40% of the children with this subtype are younger than two years. In this age group, the combination of FAB M5 with extramedullary organ involvement was especially prevalent [40]. Chil-

dren under the age of two, therefore, may not be at risk per se. The relationship between the same initial factors was not as obvious in children older than 10 years. It is of note, however, that there were twice as many girls as boys in this age group (12:6).

Lichtman and Rowe [109] described severe disorders of microcirculation with excessive leukocytosis, followed by bleeding, as the potential pathomechanism of hemorrhage and leukostasis. Rapid blast reduction with massive cytolysis associated with cytostatic therapy, particularly after the application of anthracyclines, induces tumor lysis and leads to imbalances of metabolism and hemostasis. A high blast count is accompanied by a high leukocrit. Correction of anemia, by whole blood or erythrocyte transfusion, leads to an increased cytocrit (=leukocrit+hematocrit) and may result in potentially fatal hyperviscosity [70, 109]. A similar set of events could explain the ED of patients in group B.

According to coagulation studies, low plasminogen activity predicts for early hemorrhage. The full picture of disseminated intravascular coagulation was seen in only one patient. The other patients with early hemorrhage had low fibrinogen levels in some instances, and raised levels of split products [160]. Autopsy findings include massive cellular infiltration of the interior organs, including the CNS. Leukostasis was described in four patients and perivascular infiltration in two others.

Our analysis points to a variety of possible diagnostic and therapeutic measures for patients with hyperleukocytosis (Table 63) which have already been described in detail [42, 43]. One of the most effective measures is initial exchange transfusion, which is superior to leukapheresis in children since volume shifts are smaller.

Patients with low peripheral blast count (group C) showed different pathophysiological mechanisms leading to hemorrhage. In acute promyelocytic leukemia, the high risk of bleeding is associated with disseminated intravascular coagulation and/or fibrinolysis due to release of coagulation-promoting substances from granules [49, 146].

Table 63. Provisions in case of hyperleukocytosis and danger of hemorrhage

1. Intensive care
2. Cautious hydration with exact balancing
3. No immediate blood transfusion (leave Hb <8 g/dl)
4. Alkalization of urine; allopurinol
5. Exchange transfusion
6. Fresh plasma in coagulation abnormalities
7. Infusion of platelets in thrombopenia
8. Hydroxyurea (2×20 mg/kg/day) for 2 days or Ara-C (40 mg/m^2/day)

Nonresponse and late response to treatment is accompanied by severe, long-lasting thrombo- and leukopenia. These patients, including those in group D, die in aplasia after several weeks of therapy; however, there were also two patients in group C without any blast cell reduction who died earlier, on day 12.

Hemorrhage is also associated with severe infections such as sepsis and pneumonia, (e.g., two children in group C) and is probably related to disseminated intravascular coagulation. Almost all deaths in group D were found to be directly related to the sequelae of BM depression, such as severe infections or thrombopenic bleeding.

5.7 Relationship Between Implementation of Therapy, Complications, and Course

Various problems are encountered in the analysis of implementation of therapy. First of all, the required prospective documentation of therapy data for each individual patient requires extraordinary effort – and without complete or nearly complete information the results of the analysis are useless. Secondly, the evaluation itself poses methodological problems which have not yet been resolved. For example, no solution has been found to the following questions [136]:

- Should evaluation of the total dose given to a patient relate to the cumulative dose given over one or all therapy phases, or to the actually administered proportion of the maximum dose as prescribed by the protocol?
- How should different substances, applied in combination, enter into the evaluation? Both simple addition of the various drugs resulting in a cumulative dose and the use of weighted averages of each individual substance are possible.
- How should the relationship between dose applied and duration of application be taken into consideration?

When agreement has been reached on these problems, methods for the correlation of implementation of therapy with therapy failure or survival will have to be found.

Studies on the therapy of breast cancer with adjuvant CMF chemotherapy (CP, methotrexate, 5-fluorouracil) using retrospective analyses established a relationship between either the total applied dose [22, 23] or the relative dose intensity (dose/body surface area/time) [83, 84] and the prognosis. In the first study by Bonadonna and Valagussa [22], it was possible to form three groups clearly differing in dose level ($\geq 85\%$,

65%–84%, <65% prescribed dose). Duration of therapy, however, did not enter the evaluation, which automatically eliminated patients with early treatment failure from the group receiving a high total dose [136]. Hryniuk and Bush [83] and Hryniuk and Levine [84] have analyzed the prescribed rather than the actual dose intensity of several CMF studies. The methodological problems were resolved by assuming equal cytostatic activity for the three substances and ascribing the main effect of the protocols to the varying total doses per time unit. Application of a similar procedure in the analysis of the MVPP therapy (mechlorethamine, vinblastine, procarbazine, prednisone) in Hodgkin's disease [66] showed that relapses occurred much sooner in the group with a dose intensity under 75%, which included more than half the patients.

Contrary to the above mentioned analyses, prospective data on actually applied doses and time intervals are available in our studies. However, protocol AML-BFM-83 does not represent a three-drug therapy repeated at four-week intervals; it provides for a combination of three phases with seven different substances and varying duration of therapy. The method of measuring dose intensity used by Hryniuk and Bush [83] and Green et al. [66] could not easily be applied, since dose intensity during the short induction was markedly higher than during the later phases of therapy. Moreover, the prescribed duration was generally followed in induction, whereas there were large deviations during consolidation. Consequently, the experience of other authors offered little on which to base our analysis. This report has presented mainly descriptive evaluations of the individual therapy phases and substances as well as resulting complications.

Our results allow three basic conclusions:

1. The protocol of AML-BFM-83 can be applied without problems in a majority of patients.
2. Deviations from the prescribed therapy in dosage and time mainly concern prospective NRs.
3. Especially in NRs, the implementation of therapy was complicated by severe infections.

DC in induction was protocol-appropriate in almost 80% of the patients; considerable deviations from the prescribed therapy occurred during consolidation in all patients who later turned out to be NRs, while 66% of the responders received more than 80% of the prescribed doses of all drugs in both phases of consolidation. As to maintenance therapy, which provided for breaks depending on the extent of leukopenia or thrombopenia, treatment of one-third of the patients had to be interrupted for more than two weeks, mostly due to thrombopenia.

When the duration of each individual therapy phase was taken into consideration, a decrease in severe complications from induction through consolidation to maintenance therapy was observed (Table 46). At a rate of seven out of 173 (4%), fatal complications due to therapy-induced toxicity, such as hemorrhage or infections during BM depression, or to cardiotoxicity, were less frequent in study AML-BFM-83 than in study AML-BFM-78 (11/149, 7%), and occurred following particularly severe infections (mucormycosis and aspergillosis). Deaths due to the cardiotoxic effect of cytostatic agents were first seen in study AML-BFM-83; they may be attributed to the introduction of the eight-day induction treatment which included a high DNR dose.

The methodological problems in calculating the influence of the implementation of therapy on the prognosis have not been completely solved [136]. Any estimation of the relevance of therapy compliance is therefore subject to reservations. Our life table analyses did not indicate any effect of the DC of individual substances on the relapse rate. The only remarkable result was the reduced DC in prospective NRs. But the patient groups with reduced DC were very small and consequently offered comparatively little information. The average DC of the various drugs in induction and consolidation was 96% (Tables 42 and 44). By comparison, the average dose intensity in the study on Hodgkin's disease analyzed by Green et al. [66], which was comparable to the DC in the induction of our study, was only 71%.

Comparison of the treatment results in AML-BFM-83 with those in AML-BFM-78 shows a clear difference in six-year EFI with $p = .03$ (AML-BFM-78, EFI = .47, SD .05; AML-BFM-83, EFI = .61, SD .04). The main difference in therapy between the two studies was the addition of the intensive ADE induction phase in AML-BFM-83. The change in therapeutic intensity was thus accompanied by a marked improvement in prognosis, whereas the comparatively small differences in DC within AML-BFM-83 ($< 80\%$ vs. $\geq 80\%$ prescribed dose) could not be associated with such an effect.

The results on the prognostic relevance of pretherapeutic parameters have already been discussed (Sect. 5.4). As to the estimation of the relative significance of DC and these parameters for prognosis, multivariate analyses are fairly questionable, since events that occur during treatment cannot be evaluated in the same manner as pretherapeutic parameters. Henze et al. [73] have discussed this complex relationship for childhood ALL. Out of eight cytostatic agents used during induction therapy, only the cumulative DNR dose had an influence on prognosis after the diagnostic risk factors had been compensated for. Using similar methods, this could not be demonstrated for any individual substance in our study.

Individual case histories show that a pretherapeutic finding which is of relevance for prognosis, such as hyperleukocytosis, affects the implementation of therapy, especially during the initial phase, and that infections often follow remission failure or late remission. This leads to the conclusion that it will be extremely difficult to provide additional intensification of initial therapy in this patient group of prospective NRs.

Our analysis confirms that the protocol of AML-BFM-83 could be applied with only minor deviations and a still acceptable rate of complications in the majority of patients. Due to the experience gained by the participating members of the study, the rate of lethal complications did not increase in comparison with AML-BFM-78, despite intensification of therapy. The results lead to conclusions which will serve to optimize the therapy schedules of further studies. These include less aggressive application of the anthracyclines during induction and shortening of the second phase of the consolidation (see also Sect. 5.8).

5.8 Perspectives for Future Prognosis-Adapted Therapy Strategies

The main objective of the analysis was to estimate the individual risk of children with AML at the time of diagnosis or as soon thereafter as possible. The results of the risk factor analysis allow one to estimate the overall risk of failure (ED, treatment failure, relapse, death in CCR) as early as the beginning of therapy on the basis of the variables WBC, eosinophilia, and Auer rods (Table 34).

As to the group of CR patients, the validity of risk estimation can be increased after the patients have entered CR, by including the parameter time until CR. Finally, inclusion of the morphologic FAB types as a parameter for group allocation allows one to form two risk groups for a stratified therapy. The allocation to these prognostic groups can be validated further by including cytogenetic parameters. Special analyses defining the risk of particular forms of treatment failure (ED, nonresponse, relapse, specific site of relapse) suggest specific therapeutic measures. The resulting consequences for the individual groups are discussed below. Some of them have already been included in the concept of AML-BFM-87.

5.8.1 Early Death Due to Hemorrhage and/or Leukostasis

The group of patients at an increased risk of ED due to hemorrhage and/or leukostasis has been identified on the basis of the results of our analysis. There are children with hyperleukocytosis and, in particular, patients with monocytic leukemias and concurrent hyperleukocytosis and/or extramedullary organ involvement (Table 39). In some children, severe bleeding occurred together with rapid blast cell reduction after anthracyclines. Further attempts should thus be made to prevent lethal complications by slow cytoreduction in connection with other prophylactic measures (Table 63). Exchange transfusion is regarded to be particularly valuable in this respect since, judging from individual case histories, it was successful in preventing early lethal bleeding in high risk patients (see Sect. 4.6).

Children with acute promyelocytic leukemia (FAB M3) who show no initial increase in WBC but are at special risk for disseminated intravascular coagulation, represent a special case. The prophylactic measures were similar to those taken in other high-risk patients, but the value of exchange transfusion has remained unclear.

5.8.2 Nonresponders

The group of NRs or partial responders is small in both AML-BFM studies (9% and 13%). In general, the risk of nonresponse is high in the same patients (risk group II) who are at high risk of relapse.

In addition, the evaluation of blast cell reduction in the BM on day 15 gave the best indication of the risk of nonresponse. The number of NRs is significantly increased with blast cell counts above 10% (Sect. 4.5.7). Unfortunately, the possibilities of therapy intensification for these patients are limited. The ADE therapy did not lead to any increase in remission rates in AML-BFM-83 as compared to the preceding study.

The analysis of the implementation of therapy shows severe complications and considerable deviations from the prescribed therapy during the second therapy phase (consolidation), especially in prospective NRs (partial responders included; see Sect. 4.7). Consequently, it will hardly be possible to achieve further intensification during the initial weeks of therapy in this group. This conclusion is in accordance with the findings of the Boston group: the initial use of high-dose ARA-C, especially when combined with a second cytotoxic drug, leads to much higher toxicity than administration at a later time when the patient is in CR

(Weinstein, personal communication). For these reasons, remission in patients with a high probability of nonresponse can only be attempted by continuation of therapy starting the second treatment phase early.

5.8.3 Relapses

The risk of relapse in children who are treated according to the therapy schedule of AML-BFM-83 can be estimated quite confidently on the basis of the defined prognostic groups (Table 61). This was previously not possible in children suffering from AML, and the risk of relapse in childhood AML was generally considered to be so high that all patients received maximum, i.e., uniform, therapy. Only the improvement of therapy results for a well-defined patient group has made it possible to differentiate between risk groups, thereby allowing stratification of therapy.

Stratification has the purpose of keeping toxicity at the same level for risk group I while reducing the relapse rate for risk group II. At present, there are two alternative ways to approach this goal: (1) introduction of additional phases of chemotherapy initially or soon after entering remission or, (2) BMT in first remission (allogeneic or autologous).

5.8.4 Additional Chemotherapy

Therapy was intensified in AML-BFM-83 by addition of the ADE induction. A further increase of therapy intensity during this phase is hardly justified because patients with a favorable overall prognosis would be endangered. Therefore, intensification soon after entering CR should be considered instead.

A new therapeutic approach to intensification during remission is high-dose ARA-C (HD-ARA-C) therapy which has been investigated since 1976 [144]. Phase II trials have demonstrated that HD-ARA-C alone or in combination with a second cytotoxic agent induced remission in adult patients with refractory AML (44%–60%) [3, 75, 78]. Remarkable results have been reported by Hiddemann et al. [77] and Freund et al. [61], who achieved remissions in about 50% of negatively selected adult AML patients (NRs or early relapsed) by applying HD-ARA-C and mitoxantrone or VP-16.

The results of our pilot study using HD-ARA-C in combination with mitoxantrone (HAM) in refractory childhood AML [142] are listed in

Table 64. Results of pilot study (HAM) in 21 children with refractory AML (30. Sept. 87)

Patient group	n	ED	NR	PR	CR	in CCR
Refractory AML	7	1	–	3	3	2
Early relapse	9	1	1	3	4[a]	2[b]
Late relapse	4	1	–	–	3	1
Secondary AML	1	–	–	–	1	–
Total	21	3	1	6	11	5

[a] Three of these four patients received BMT in 2nd CR.
[b] In 2nd CCR after BMT.

Table 64. Of 21 children 11 achieved CR again. At the time of the analysis, five of these had remained in remission for 2–18 months.

The 24 children who received HAM in remission, according to the preliminary version of therapy study AML-BFM-87, had a rate of lethal complications of 12.5%, which was comparable to the pilot study, while no life-threatening complications were observed with the combination HD-ARA-C/VP-16 used in eight children whose disease was therapy-resistant and in another 14 during remission. Of the eight patients with therapy-resistant AML seven entered remission.

According to these results, stratification of chemotherapy might be performed by administering therapy according to the schedule of AML-BFM-83 in patients of risk group I, and giving additional intensification with HD-ARA-C combined with VP-16 to those in risk group II. Increased use of VP-16 is also supported by the finding that this substance is especially effective in patients with FAB M5 [122, 123]. Since no improvement in prognosis was obtained by introduction of ADE therapy (study AML-BFM-83) in children with FAB M5, the use of different cytotoxic agents seems to be indicated. Apart from VP-16 and VM-26, combination with an alkylating substance is suggested, and supported by the findings of Hurd et al. [86], and Kalwinsky et al. [91]. Thus, an additional method of stratifying therapy might be a shift in the relative emphasis on drugs for myeloblastic or monoblastic leukemias.

5.8.5 Allogeneic BMT in First CR

The value of allogeneic BMT in first CR in children and adolescents suffering from AML can be estimated on the basis of comprehensive American studies from Seattle and New York [124]. The probability of

a five-year leukemia-free survival is about 65% [31]. As to the reasons for failure, 10%–20% are attributable to transplantation-induced immunologic complications such as acute and chronic graft-versus-host disease and associated infections. The morbidity rate is also high, due to the sequelae of the conditioning radiotherapy and chronic graft-versus-host disease [158].

For these reasons, BMT in first CR has been used sparingly in the Federal Republic of Germany until now. Moreover, the results of chemotherapy producing a probability of 60% for the six-year EFI correspond to those of BMT. Comparison of nine children in studies AML-BFM-78 and -83 (see Sect. 4.3), who underwent allogeneic BMT in first CR, with the remaining patients on chemotherapy shows no difference (Fig. 53). The life table analysis included all study patients whose period of survival in CCR was at least as long as the corresponding median duration in the group of BMT patients at the time of BMT (9.5 months after diagnosis).

In risk group I as defined by the present evaluation, the prospective result of more than 80% six-year EFI achieved with chemotherapy alone is so favorable that allogeneic BMT in first CR is not indicated. The prognosis in risk group II was clearly less favorable (Fig. 51b; six-year EFI 42%), so that BMT in first CR might be superior to continued chemotherapy in CCR. As to stratification, the use of allogeneic BMT in first CR can be recommended in patients of risk group II with an

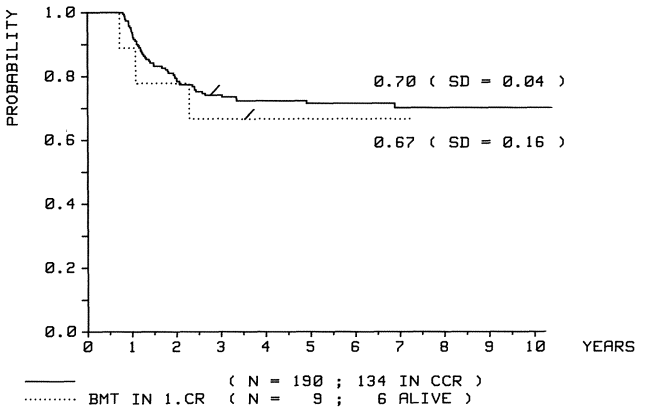


Fig. 53. Probability of survival after BMT compared with chemotherapy in patients who stayed in CCR for a minimum of 9.5 months after diagnosis. Combined analysis of AML-BFM-78 and AML-BFM-83

HLA identical donor, and the same procedure should be considered in patients of risk group I after relapse. The results of BMT also depend on prognostic factors, suggesting that high-risk patients might also have to expect comparatively less favourable results after BMT [62].

5.8.6 Autologous BMT

Autologous BMT is still experimental. Yaeger et al. [180] achieved a survival rate of 43% when they performed this treatment in second and third CR in 25 predominantly adult patients, with a mean follow-up period of 400 days. The results of BMT in children in first remission are within the same range [110].

According to these results, autologous BMT in first CR should be reserved to exceptional instances. Children suffering from AML who do not have an HLA identical sibling donor and who have an especially high risk of relapse should be considered; according to our analysis, there is no other eligible subgroup to be clearly separated out from risk group II. Children with FAB M4 without eosinophilia have an especially high risk of relapse (which, however, is not significantly higher than in other patients in risk group II), and the same is probably true for patients with the rare FAB type M7, where almost exclusively negative results were seen [118]. For this reason, autologous BMT in first CR might be considered in this small group of patients.

In general, harvesting of BM in first CR from all AML patients constitutes a procedure which might allow one to perform autologous BMT after a relapse.

The analysis of relapsed patients in studies AML-BFM-78 and -83 showed that, except in a few instances after chemo- and radiotherapy, only the patients with allogeneic BMT had a chance of survival (Fig. 11) (autologous BMT after relapse has not been performed until recently).

5.8.7 Measures for Reduction of Toxicity

In order to reduce toxicity during consolidation limitation of this therapy phase to six weeks seems indicated, since breaks in therapy due to infections and bleeding under BM depression cannot be avoided, especially during the last two weeks (see Sect. 4.7). This reduction is also justified in view of the possibility of intensification in remission.

In three children of study AML-BFM-83, the introduction of ADE therapy led to acute therapy-resistant cardiac failure, most probably due to anthracycline-induced cardiomyopathy, 5–6 weeks after therapy had started. The DNR dose of 180 mg/m^2 given within three days, was comparatively high; moreover, it was given as bolus injections of 60 mg/m^2. According to Legha et al. [106], the cardiotoxicity of the anthracyclines is clearly lower with continuous infusion than with bolus injection. Pharmacokinetic studies have shown that maximum plasma concentration is lower with continuous infusion but the total anthracycline plasma level remains the same [141]. These findings suggest that cardiotoxicity should be reduced by protracted infusion of anthracyclines.

5.8.8 Modification of the CNS 'Prophylaxis'

The AML-BFM-78 and -83 studies showed isolated or combined CNS relapses in 8% and 5%, respectively, of the CR patients with prophylactic CNS irradiation and intrathecal MTX or ARA-C application (Table 12), while other pediatric studies showed CNS relapse rates of 3%–18% without CNS 'prophylaxis' or with intrathecal MTX or ARA-C administration alone [34, 46, 113, 174]. The relatively high proportion of CNS relapses in the VAPA study, which differs from other studies in an extended remission duration, demonstrates the need for CNS 'prophylaxis' with improved overall results [148].

According to our analyses, initially CNS positive patients have an increased risk of relapse in the CNS. These patients should therefore be given cranial irradiation as a routine measure. In the remaining children, the value of prophylactic cranial irradiation might be investigated in a controlled study. This would be especially justified if all patients received a new form of CNS 'prophylaxis' by application of HD-ARA-C. High CSF levels would be maintained over a long period of time, because ARA-C elimination in the CSF is slower than in the periphery [99]. When radiation treatment is abandoned, severe sequelae such as brain tumors, disseminated leukoencephalopathy and late neurophysiological and intellectual side effects may be prevented [68].

5.8.9 Concluding Remarks

The two consecutive studies AML-BFM-78 and -83 which differ mainly in only one element of therapy, demonstrate that it is possible to compare consecutive studies, each producing substantial results.

For pediatricians participating in such studies, cooperation has positive effects on the care of their patients. One fact among others reflecting the increase in experience is the reduction of therapy-induced deaths over the years (Table 15). For the patients, participation in such a cooperative study means optimum state-of-the-art treatment and availability of a study center which can be consulted in case of complications or special questions.

For further therapy studies, parallel basic research in areas such as cytogenetics and immunology or pilot studies in relapsed patients will be important and may lead to an even more precise definition of risk groups in AML.

6 Summary

The first cooperative studies on the therapy of childhood AML, combining 33 pediatric hospitals, have been run in the Federal Republic of Germany since 1978. The present analysis was based on the data of 333 patients under the age of 17 from studies AML-BFM-78 and AML-BFM-83 and sought to develop criteria for estimating each individual's risk of early death, nonresponse, and relapse, at the time of diagnosis or soon after, in order to gain a basis for a risk-adapted treatment strategy.

The results of the two consecutive studies showed a marked improvement of the prognosis in childhood AML during the last 10 years. In both studies, 80% of the children achieved complete remission. In AML-BFM-78, the probability of a six-year continuous complete remission (six-year event-free interval) was 47%, SD 5%, compared with 61%, SD 4%, in AML-BFM-83 ($p < .05$).

The pretherapeutic patient data allowed the definition of specific features for the individual FAB groups. One of these was the clinical finding of extramedullary organ involvement (including liver enlargement) in the FAB types M4 and M5 with predominantly monocytic differentiation (M4/M5 vs. M1–M3, $p < .01$). In FAB M5, the high proportion (40%) of children under the age of two was conspicuous, as was the high frequency of hyperleukocytosis and initial CNS involvement in FAB M4 (M4 vs. non-M4, $p < .01$). In the FAB types M1–M3, with purely granulocytic differentiation, morphologic features such as more than 80% POX positive blasts in the bone marrow (M1–M3 vs. M4/M5, $p < .001$) and the presence of Auer rods (M1–M3 vs. M4/M5, $p < .001$) were specific findings. Eosinophilia in the bone marrow was mainly seen in the FAB types M2 and M4 (M2/M4 vs. M1/M3/M5, $p < .01$).

The combination of various pretherapeutic parameters and the inclusion of response dynamics allow for the first time definition of two patient groups with clear differences in prognosis but which received identical treatment based on the protocol of AML-BFM-83 (Table 65).

Table 65. Prognostic groups of AML in childhood according to AML-BFM-83

EFS/EFI[a]	Risk I >80%	Risk II <45%
FAB M1	Auer positive	Auer negative
FAB M2	WBC $<20\times10^3/mm^3$	WBC $\geq20\times10^3/mm^3$
FAB M3	all[b]	-
FAB M4	Eo ≥3%	Eo <3%
FAB M5	-	all
FAB M6	all[b]	-
FAB M7	-	(all)[b]
Additional factor for ‚Risk II': time to achieve CR		≥10 weeks

[a] Estimation for 6 years.
[b] Results based on a small number of patients.

The probabilities of a six-year event-free survival and interval were 84% (SD 5%) and 91% (SD 4%), respectively, in the low risk group (group I). This group included 37% of the AML patients entering complete remission. Specific features were predominance of granulocytic differentiation in the leukemic blast population: the presence of Auer rods in FAB M1, low white blood count in FAB M2, eosinophilia in the bone marrow in FAB M4, and all patients with the FAB type M3, indicating a favorable prognosis. The high risk group of patients (group II) had probabilities of 31% (SD 5%) with respect to six-year event-free survival and 42% (SD 6%) for event-free interval. In these patients, the blasts showed either monocytic or only minimal differentiation, as in the FAB type M1 without Auer rods. The leukemic cell load was larger than in low risk children (median WBC $30000/mm^3$ vs. $13000/mm^3$).

For the total group of patients treated according to the protocol of AML-BFM-83, the results of the multiple stepwise regression analysis indicated that the parameters initial leukocyte count, eosinophilia, and Auer rods were independent prognostic factors. With individual analysis of the FAB types, however, only Auer rods in FAB M1, leukocytes and time of complete remission in FAB M2, and eosinophilia in FAB M4 remained independent variables. Remarkably, there was no parameter of prognostic value in the FAB type M5. Thus, in each individual case of variation of one of these variables, it will be possible to determine the individual's risk of failure as compared to other children who show the same prognostic criteria in all other respects.

Comparison of studies AML-BFM-78 and AML-BFM-83 showed an improvement of prognosis after introduction of ADE induction treat-

ment, in FAB types with predominantly granulocytic differentiation (six-year EFI, FAB M1–M4, AML-BFM-78: 45%, SD 5%; AML-BFM-83: 67%, SD 5%, $p < .01$), but not in FAB M5 (six-year EFI, FAB M5, AML-BFM-78: 63%, SD 11%; AML-BFM-83: 40%, SD 9%, n.s.).

Evaluation of implementation of therapy and related complications will permit estimation of protocol compliance and provide the basis for treatment modifications in subsequent studies.

Patients at risk of suffering early death from hemorrhage and/or leukostasis are closely defined by our analysis. These are children with hyperleukocytosis (with a lethality rate of 31%) and, especially, patients with monocytic leukemia and concurrent hyperleukocytosis (lethality 70%) or extramedullary organ involvement (lethality 40%).

Our findings support a stratified treatment approach for later studies, whereby low-risk patients are not exposed to unnecessary danger, but children at high risk can receive intensified therapy with additional chemotherapy or bone marrow transplantation during first complete remission. Another possibility resulting from a differentiated therapy would provide for variation in emphasis or application of different or new substances in leukemias with monocytic and myelocytic differentiation.

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8 Appendix

The following tables present an overview of therapy results based on initial clinical and hematological features, and additionally parameters of response kinetic such as 'blasts day 15', and 'time of CR'. Further cytochemical, immunological and flowcytometric data have been added in study BFM-83.

Table A. AML-BFM-78: Results based on initial clinical and hematological features, and response rate after 10 weeks (March 1989)

	Study patients	ED before therapy	Protocol patients	ED	NR (+PR)	CR		Death in CCR	BMT in 1st CR	lfu	In CCR	EFS (SD)		EFI (SD)	
	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(%)	(*n*)	(*n*)	(*n*)	(*n*)	(%)	*p*	(%)	*p*
Total	151	2	149	17	13	119	80	6	2	4	52	37 (4)		46 (5)	
Sex male	81	1	80	8	6	66	83	5	2	2	26	35 (5)		42 (6)	
female	70	1	69	9	7	53	77	1	–	2	26	40 (6)		52 (7)	
Age <2 years	23	–	23	5	–	18	78	–	–	1	9	41 (11)		53 (12)	
2–<10 years	55	1	54	3	5	46	85	3	1	1	19	37 (7)		43 (7)	
>10 years	73	1	72	9	8	55	76	3	1	2	24	36 (6)		47 (7)	
WBC $<20\times10^3/mm^3$	71	–	71	8	6	57	81	2	1	2	28	41 (6)		51 (7)	
≥20–$100\times10^3/mm^3$	46	–	46	3	1	42	88	3	1	2	18	43 (8)	.02	48 (8)	
$>100\times10^3/mm^3$	34	2	32	6	6	20	63	1	–	–	6	19 (7)		30 (10)	
Hemoglobin ≤8 g/dl	67	–	67	9	8	50	75	2	1	1	21	33 (6)		45 (7)	
>8 g/dl	84	2	82	8	5	69	84	4	1	3	31	40 (6)		47 (6)	
Platelet count															
$\leq50\times10^3/mm^3$	93	–	93	13	7	73	78	5	–	2	33	37 (5)		47 (6)	
$>50\times10^3/mm^3$	58	2	56	4	6	46	82	1	2	2	19	37 (7)		45 (8)	
Liver ≤5 cm	120	1	119	9	11	99	83	5	2	3	44	39 (5)	.07	47 (5)	
>5 cm	30	1	29	8	2	19	66	1	–	1	7	25 (8)		39 (11)	
no data submitted	1	–	1	–	–	1		–			1				
Spleen ≤5 cm	125	1	124	14	11	99	80	5	1	3	45	38 (4)		48 (5)	
>5 cm	25	1	24	3	2	19	79	1	–	1	7	32 (10)		41 (12)	
no data submitted	1		1			1			1		–				

Table A. Continued

	Study patients	ED before therapy	Protocol patients	ED	NR (+PR)	CR		Death in CCR	BMT in 1st CR	lfu	In CCR	EFS (SD)		EFI (SD)	
	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(%)	(*n*)	(*n*)	(*n*)	(*n*)	(%)	*p*	(%)	*p*
CNS negative	134	1	133	15	12	106	80	5	2	4	48	39 (4)		49 (5)	.07
positive	13	–	13	2	–	11	85	1	–	–	3	23 (12)		27 (13)	
no data submitted	4	1	3		1	2					1				
Organ negative	122	1	121	8	10	103	85	6	2	4	46	41 (5)	.03	48 (5)	
positive	27	–	27	9	3	15	56	–	–	–	6	22 (8)		40 (13)	
no data submitted	2	1	1	–	–	1		–	–	–	–				
Auer negative	61	1	60	9	7	44	73	4	–	2	17	30 (6)		41 (8)	
positive	68	–	68	4	4	60	88	1	2	1	27	42 (6)		48 (7)	
no data submitted	22	1	21	4	2	15	71	1	–	1	8	39 (11)		53 (13)	
Eo in BM															
<3%	76	1	75	6	7	62	83	4	2	3	27	38 (6)		46 (6)	
≥3%	36	–	36	4	3	31	82	–	–	1	13	39 (8)		45 (9)	
no data submitted	39	1	38	7	3	26	72	2	–	–	12	33 (8)		50 (10)	
Remission															
<10 weeks						103		6	2	4	45			48 (5)	
≥10 weeks						16		–	–	–	7			44 (12)	

PR, partial responders

Table B. AML-BFM-83: Results by pretreatment clinical and hematological features, and parameters of response kinetic (March 1989)

	Study patients	ED before therapy	Protocol patients	ED	NR (+PR)	CR		Death in CCR	BMT in 1st CR	lfu	In CCR	EFS (SD)			EFI (SD)		
	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(%)	(*n*)	(*n*)	(*n*)	(*n*)	(%)		*p*	(%)		*p*
Total	182	9	173	12	22	139	80	4	7	–	82	49	(4)		61	(4)	
Sex male	99	3	96	4	11	81	84	1	1	–	50	53	(6)		63	(6)	
female	83	6	77	8	11	58	75	3	6	–	32	45	(6)		59	(7)	
Age < 2 years	33	2	31	2	4	25	81	0	0	–	14	45	(9)		56	(10)	
2- < 10 years	69	1	68	5	7	56	82	3	2	–	30	46	(6)		56	(7)	.09
> 10 years	80	6	74	5	11	58	78	1	5	–	38	54	(6)		69	(6)	
WBC $< 20 \times 10^3/mm^3$	84	1	83	2	6	75	88	2	4	–	49	61	(6)	.04	68	(6)	
$\geq 20\text{-}100 \times 10^3/mm^3$	60	0	60	2	9	49	86	2	3	–	25	44	(7)	.05	53	(7)	
$> 100 \times 10^3/mm^3$	38	8	30	8	7	15	50	0	0	–	8	27	(8)		53	(13)	
Hemoglobin ≤ 8 g/dl	85	5	80	6	10	64	80	1	1	–	38	49	(6)		62	(6)	
> 8 g/dl	93	4	89	6	11	72	81	3	6	–	41	48	(6)		61	(6)	
no data submitted	4	–	4	–	1	3		–	–	–	3						
Platelet count																	
$\leq 50 \times 10^3/mm^3$	92	1	91	9	9	73	80	2	3	–	46	53	(5)		65	(6)	
$> 50 \times 10^3/mm^3$	89	8	81	3	13	65	80	2	4	–	35	44	(6)		56	(6)	
no data submitted	1	–	1	–	–	1		–	–	–	1						
Liver ≤ 5 cm	154	6	148	9	19	120	81	4	6	–	70	49	(4)		60	(5)	
> 5 cm	26	2	24	3	3	18	75	0	1	–	12	53	(10)		71	(11)	
no data submitted	2	1	1	–	–	1		–	–	–	–						

Table B. Continued

	Study patients (*n*)	ED before therapy (*n*)	Protocol patients (*n*)	ED (*n*)	NR (+PR) (*n*)	CR (*n*)	(%)	Death in CCR (*n*)	BMT in 1st CR (*n*)	lfu (*n*)	In CCR (*n*)	EFS (SD) (%)	*p*	EFI (SD) (%)	*p*
Spleen ≤5 cm	155	5	150	10	21	119	79	4	6	–	69	48 (4)		60 (5)	
>5 cm	23	3	20	2	1	17	85	0	1	–	11	59 (11)		69 (12)	
no data submitted	4	1	3	0	0	3		0	0	–	2				
CNS negative	159	6	153	9	16	128	84	3	7	–	74	50 (4)		60 (4)	
positive	10	1	9	0	3	6	67	1	0	–	3	33 (16)		50 (20)	
doubtful	7	1	6	2	2	2	33	–	–	–	2				
no data submitted	6	1	5	1	1	3	60	–	–	–	3				
Organ negative	112	2	110	6	13	91	83	1	5	–	56	52 (5)		63 (5)	
positive	52	5	47	4	7	36	77	2	2	–	21	48 (7)		62 (8)	
no data submitted	18	2	16	2	2	12	75	1	–	–	5				
Blasts day 15															
<10%			113	1	7	105	93	2	5	–	63	58 (5)	.001	63 (5)	.09
≥10%			30	1	8	21	72	2	2	–	9	27 (10)		38 (14)	
no data submitted			30	10	7	13	43	–	–	–	10				
Remission															
before consolidation						97		3	4	–	60	–		64 (5)	
after (in)						37		1	2	–	20	–		53 (9)	
no data submitted						5		–	1	–	2				
Remission															
<10 weeks						117		4	6	–	73	–		65 (4)	.04
≥10 weeks						22		–	1	–	9	–		38 (12)	

Table C. AML-BFM-83: Results by pretreatment morphological, cytochemical, immunological and flowcytometric data (March 1989)

	Study patients (*n*)	ED before therapy (*n*)	Protocol patients (*n*)	ED (*n*)	NR (+PR) (*n*)	CR (*n*)	(%)	Death in CCR (*n*)	BMT in 1st CR (*n*)	lfu (*n*)	In CCR (*n*)	EFS (SD) (%)	*p*	EFI (SD) (%)	*p*
Total	182	9	173	12	22	139	80	4	7	–	82	49 (4)		61 (4)	
Auer rod negative	85	6	79	4	14	61	77	2	4	–	27	36 (5)	.001	46 (6)	.002
positive	69	1	68	4	5	59	87	1	3	–	42	65 (6)		75 (6)	
no data submitted	28	2	26	4	3	19	73	1	–	–	13				
Eo in BM <3%	120	5	115	7	17	91	78	3	5	–	48	43 (5)	.01	54 (5)	.02
≥3%	37	1	36	2	2	32	88	–	2	–	24	72 (8)		81 (7)	
no data submitted	25	3	22	3	3	16	73	1	–	–	10	48 (11)		67 (12)	
TdT <10%	109	7	102	7	13	82	80	2	4	–	49	50 (5)		62 (5)	
≥10%	14	–	14	–	3	11	79	–	1	–	6	33 (16)		42 (20)	
no data submitted	59	2	57	5	6	46	81	2	2	–	27				
POX in BM <80%	74	6	68	5	9	54	79	1	5	–	21	33 (6)	.001	41 (7)	.00002
≥80%	60	1	59	4	5	50	85	1	–	–	43	73 (6)		86 (5)	
no data submitted	48	2	46	3	8	35	59	2	2	–	18				
DNA index =1	49	–	49	5	7	37	76	2	3	–	18	38 (7)		50 (8)	
</>1	20	2	18	–	5	13	72	–	1	–	7	43 (12)		59 (14)	
no data submitted	113	7	106	7	10	89	84	2	3	–	57				

9 Subject Index