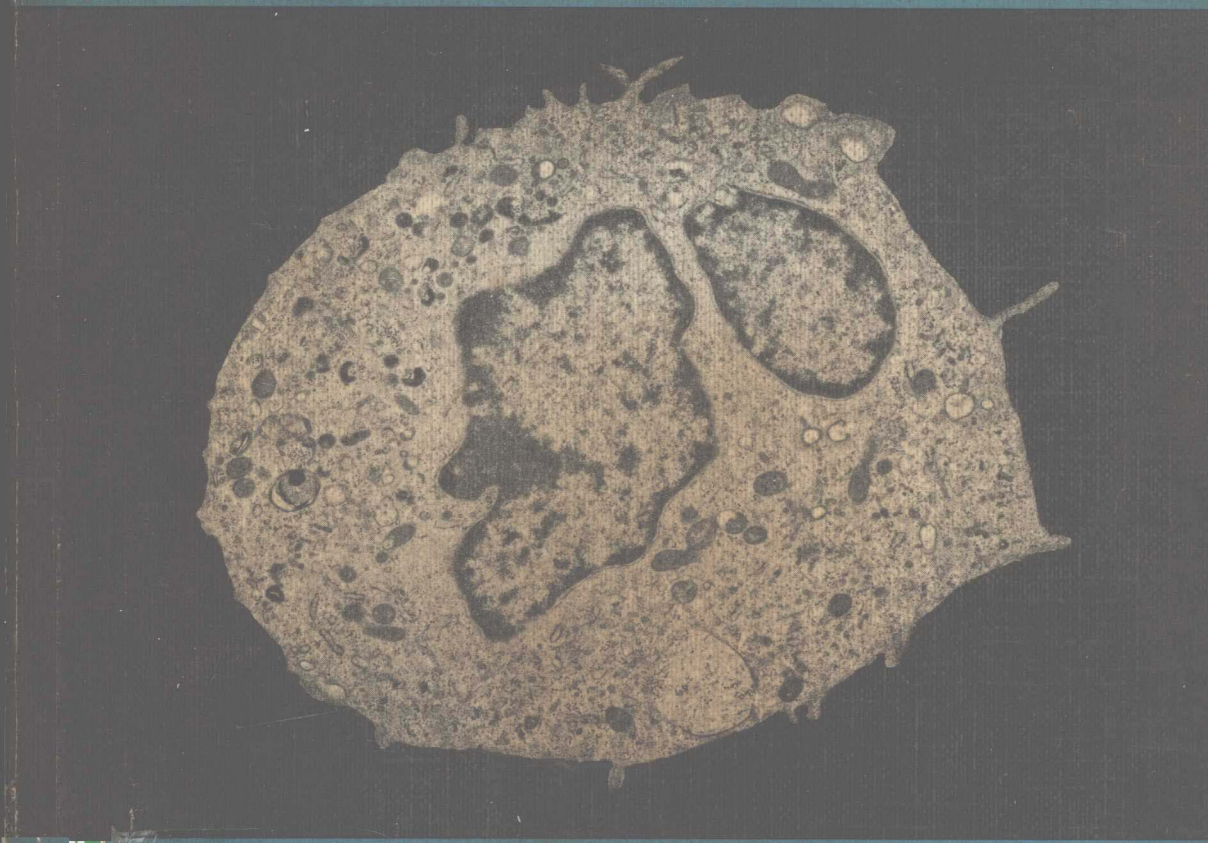
Unclassifiable Leukemias

Editors: Marcel Bessis and George Brecher



Springer-Verlag Berlin Heidelberg New York

Unclassifiable Leukemias

Editors: Marcel Bessis and George Brecher

With 81 Figures and 1 Color-Plate

Springer-Verlag Berlin Heidelberg New York 1975

Proceedings of a Symposium, held on October 11–13, 1974 at the Institute of Cell Pathology, Hôpital de Bicêtre, Paris, France

This monograph comprises the first issue of the new international journal **Blood Cells** (Editor: Marcel Bessis, Associate Editor: Robert I. Weed)

ISBN 3-540-07242-X Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-07242-X Springer-Verlag New York Heidelberg Berlin

Printed in Germany.

Type-setting, printing and bookbinding: Universitätsdruckerei H. Stürtz AG, Würzburg.

Discussants

M. Baccarini
Servizio di Ematologia
Ospedale S. Orsola
40138 Bologna – Italy

D. Belpomme
Institut de Cancérologie et
d'Immunogénétique
Hôpital Paul Brousse
94800 Villejuif – France

J.M. Bennett
University of Rochester Cancer Center
1351 Mt Hope Avenue
Rochester, N.Y. 14620 – U.S.A.

Jean Bernard
Institut de Recherches sur les
Maladies du Sang
Hôpital Saint Louis
2, place du Docteur Fournier
75010 Paris – France

M. Bessis
Institut de Pathologie Cellulaire
Hôpital de Bicêtre
94270 Kremlin-Bicêtre – France

J.L. Binet
C.H.U. Pitié-Salpêtrière
Département d'Hématologie
91, boulevard de l'Hôpital
75013 Paris – France

G. Brecher
School of Medicine
Department of Clinical Pathology
and Laboratory Medicine
University of California
San Francisco, Calif. 94143 – U.S.A.

D. Catovsky
M.R.C. Leukaemia Unit
Royal Postgraduate Medical School
Hammersmith Hospital
London W12 – Great Britain

G. Flandrin
Institut de Recherches sur les
Maladies du Sang
2, place du Docteur Fournier
75010 Paris – France

D. Galton
M.R.C. Leukaemia Unit
Royal Postgraduate Medical School
Hammersmith Hospital
London W12 – Great Britain

F. Gavosto
Instituto di Semeiotica Medica
Universita di Torino
Strada San Vito, 34
10133 Torino – Italy

G. Haemmerli
Department of Cancer Research
Aussenstation Universität Zürich
Birchstrasse 95
8050 Zürich – Switzerland

M.W. Hess
Universität Bern
Pathologisches Institut
Freiburgstrasse 30
3010 Bern – Switzerland

L.G. Lajtha
Christie Hospital and Holt Radium Ins.
Paterson Laboratory
Manchester M20 9BX – Great Britain

M.A. Lichtman
University of Rochester
Medical Center
260, Crittenden Avenue
Rochester N.Y. 14642 – U.S.A.

P. Lortholary
Faculté de Médecine de Bobigny
74, rue Marcel Cachin
93000 Bobigny – France

J. Maldonado
Hematologic Electron Microscopy
Laboratory and Medical School
Mayo Clinic
Rochester, Minn. 55901 – U.S.A.

G. Mathe
Institut de Cancérologie et
d'Immunogénétique
Hôpital Paul Brousse
94800 Villejuif – France

H. MEL
Institut de Pathologie Cellulaire
Hôpital de Bicêtre
94270 Kremlin-Bicêtre – France
and:
Division of Medical Physics
Donner Laboratory
University of California
Berkeley, Calif. 94720 – U.S.A.

N. MOHANDAS
Institut de Pathologie Cellulaire
Hôpital de Bicêtre
94270 Kremlin-Bicêtre – France

M.A.S. MOORE
Sloan Kettering Institute
for Cancer Research
Donald S. Walker Laboratory
145 Boston Post Road
Rye, N.Y. 10580 – U.S.A.

N. MULLER-BERAT
Statens Seruminstitut
Amager Boulevard 80
2300 Copenhagen – Denmark

B. PEDERSEN
Cancer Research Institute
Radiumstationen
Norrebrogade 44 D.K.
8000 Aarhus – Denmark

R. PIERRE
Department of Laboratory Medicine
Mayo Clinic
Rochester, Minn. 55901 – U.S.A.

W. ROSSE
Institut de Pathologie Cellulaire
Hôpital de Bicêtre
94270 Kremlin-Bicêtre – France
and:
Duke University Medical Center
Durham, North Carolina 27710 – U.S.A.

F. SCHMALZL
Medizinische Universitätsklinik
6020 Innsbruck – Austria

M. SELIGMANN
Institut de Recherches sur les
Maladies du Sang
Hôpital Saint Louis
2, place du Docteur Fournier
75010 Paris – France

A. SHIBATA
Department of Internal Medicine
Akita University School of Medicine
6–10, Senshukubotamachi
Akita 010 – Japan

P.A. STRYCKMANS
Service de Médecine Interne
Institut Jules Bordet
1, rue Héger Bordet
Université de Bruxelles
Bruxelles 1000 – Belgium

C. SULTAN
Laboratoire Central d'Hématologie
Hôpital Henri Mondor
94 Créteil – France

J. TANZER
C.H.U. de Poitiers
Consultation d'Hématologie
86 Poitiers – France

B. THORELL
Karolinska Institutets
Patologiska Institution
Karolinska Sjukhuset
10401 Stockholm 60 – Sweden

J. TRUJILLO
Department of Clinical Chemistry
and Laboratory Medicine
The University of Texas
System Cancer Center
Texas Medical Center
Houston, Texas, 77025 – U.S.A.

R.I. WEED
University of Rochester
School of Medicine and Dentistry
260, Crittenden Boulevard
Rochester N.Y. 14642 – U.S.A.

J. WHITE
University of Minnesota
Department of Pediatrics
Medical School
Box 284 – Mayo Memorial Building
Minneapolis, Minn. 55455 – U.S.A.

Table of Contents

Section I: Present Practice of Classification, its Utility and Limitations

Jean Bernard: Introduction 3

G. Flandrin, Jean Bernard: Cytological Classification of Acute Leukemias. A Survey of 1400 Cases 7

D.A.G. Galton, J.V. Dacie: Classification of the Acute Leukemias 17

G. Mathé, D. Belpomme, D. Dantchev, P. Pouillart, J.R. Schlumberger, M. Lafleur: Leukemic Lymphosarcomas: Respective Prognosis of the Three Types: Prolymphocytic, Lymphoblastic (or Lymphoblastoid) and Immunoblastic 25

G. Mathé, D. Belpomme, D. Dantchev, P. Pouillart, L. Navares, G. Hauss, J.R. Schlumberger, M. Lafleur: Search for Correlations between Cytological Types and Therapeutic Sensitivity of Acute Leukemias 37

Answers to Submitted Questions 53

Open Discussion of Section I 57

Section II: New Techniques Used in Cytological Diagnosis

L.G. Lajtha: Fluorescence Probe and Biochemical Characterization of Leukemic Cells 63
Discussion 67

B. Thorell: Automated Cell Population Analysis 71
Discussion 79

J.-C. Brouet, J.-L. Preud'Homme, M. Seligmann: The Use of B and T Membrane Markers in the Classification of Human Leukemias, with Special Reference to Acute Lymphoblastic Leukemia 81
Discussion 91

J.L. Binet, G. Dighiero, P. d'Athis, M. Feinermann, F. de Montaut, J. Seroude: Volumetric Determinations of Blood Cells in Acute Lymphoblastic Leukemia 95
Discussion 99

J.M. Bennett, C.E. Reed: Acute Leukemia Cytochemical Profile: Diagnostic and Clinical Implications 101
Discussion 109

D. Catovsky, B. Frisch, S. Van Noorden: B, T and "Null" Cell Leukemias. Electron Cytochemistry and Surface Morphology 115
Discussion 125

J.E. Maldonado: Giant Platelet Granules in Refractory Anemia (Preleukemia) and Myelomonocytic Leukemia: a Cell Marker? 129
Discussion 137

P.A. Frengley, W.A. Peck, M.A. Lichtman: Inhibition of Time-dependent Enhancement of Amino Acid Transport by Leukemic Leukocytes: a Possible Index of the Sensitivity of Cells to Drugs 139
Discussion 147

M.A.S. Moore: Marrow Culture – a New Approach to Classification of Leukemias 149
Discussion 159

J. Bull: Cytogenetic Studies of Marrow and Peripheral Blood Granulocyte Colonies in Treated Chronic Myelogenous Leukemia (presented by G. Brecher) Discussion 161

R.V. Pierre: Cytogenetic Studies in Preleukemia: Studies before and after Transition to Acute Leukemia in 17 Subjects 163
Discussion 171

J.M. Trujillo, M.J. Ahearn, A. Cork: Correlated Cytogenetic and Ultrastructural Studies in Acute Leukemia (Abstract) 173
Discussion 177

Section III: Pathophysiology of the Leukemic Cell

M. Bessis: Pathology of the Leukemic Cell or Reasons Why Some Leukemias are Unclassifiable 183
Discussion 189

D.F. Bainton: Abnormal Neutrophils in Acute Myelogenous Leukemia: Identification of Subpopulations Based on Analysis of Azurophil and Specific Granules (Read by G. Brecher) 191

D. Catovsky, A.V. Hoffbrand, N.B. Ikoku, A. Petrie, D.A.G. Galton: Significance of Cell Differentiation in Acute Myeloid Leukemia 201

Discussion 213

F. Gavosto, P. Masera: Different Cell Proliferation Models in Myeloblastic and Lymphoblastic Leukemia. Contribution of Cell Kinetics to the Classification of Acute Leukemias 217
Discussion 223

B. Pedersen: Clonal Evolution and Progression in Chronic Myeloid Leukemia 227
Discussion 235

P. Stryckmans, L. Debusscher, T. Peltzer, M. Socquet: Variations of the Proliferative Activity of Leukemic Myeloblasts Related to the Stage of the Disease 239
Discussion 249

Section IV: Summary and General Discussion

Jean Bernard: Summing Up 255
G. Brecher: Summing Up 257
H.C. Mel: On Classification of Leukemia 259
General Discussion 263
Subject Index 269

SECTION I

PRESENT PRACTICE OF CLASSIFICATION, ITS UTILITY AND LIMITATIONS

Introduction

Jean BERNARD*

I should like to begin with an assumption and a paradox.

The assumption is that leukemia is a disease of a stem cell characterized by pathologic alterations of that cell and its progeny. All present research and discussions are centered around the leukemic cell. So is this symposium, which would not take place except for our primary interest in the leukemic cell. This does not preclude, of course, consideration of other definitions and other approaches to the problem.

By definition, then, the leukemic cells are abnormal cells and their metabolism and functions are presumed to be abnormal. Yet, the classification of the different types of leukemias is based upon the characteristics of normal cells. We talk of "lymphoblasts" and "myeloblasts" as predominant cell types in leukemia.

This leads to a double paradox. In the first place it is clearly illogical to classify abnormal cells by their resemblance to normal cells, since their very abnormality consists in not being normal. Yet, as a second paradox, the classification has had the happy consequence of aiding us in the treatment and prognosis of leukemia for the past 25 years.

A more detailed analysis shows that the consequence of this paradox are complex: while there exists a useful correlation between cellular types, treatment and prognosis, numerous problems and difficulties persist. The most serious of them concerns the "unclassified leukemias" which are the reason for this reunion.

Perhaps it is the inadequacy of classification by present day methodology which has limited progress in the treatment of acute leukemia in recent years. It is this thought which has inspired and, I believe, justifies this meeting. It has appeared to the organizers that the existence of unclassified leukemias should act as an incentive for a more profound analysis. In our attempt to resolve the present problem, we should be able to delineate its boundaries so that we may ask the right questions and define a better therapeutic approach based upon a more precise knowledge of the leukemic cell.

Three levels of discussion emerge as a result of to-days knowledge or ignorance:

1. The first level is that of the empirical cytologic methods currently used and based on the Giemsa stain and cytochemical methods in common use.

2. The second level is that of more precise markers which define certain characteristics of the leukemic cell, such as chromosome studies, immuno-chemical, cultural and kinetic methods.

* Institut de Recherches sur les leucémies et les maladies du sang, Hôpital Saint-Louis, 75010-Paris (France).

3. The third level which is more ambitious is the discovery of specific characteristics that really define the nature of the leukemic cell.

I would now like to introduce more directly the first level of discussion, the empirical level, which could be called "cuisine" without any pejorative intention. We know that "cuisine" is a very noble art.

The principal objective of this session, I believe, is a critical study of the successes (true or apparent) as well as a study of the difficulties and failures encountered. (The knowledge of the reasons for these failures is potentially very instructive).

This study demands an introduction and three parts, three stages.

The introduction is a statement, or the confirmation of an understanding which is both technical and semantic.

The technical analysis of empirical cytology is beyond the scope of this session. The semantic discussions which in the past have resulted in so many problems for the hematologist are also of no concern in this meeting.
(I recall, some 40 years ago, a discussion between Ferrata and Naegeli about the myeloblast which ended by Naegeli saying: "at my age, you will not change my opinions"). These discussions about nomenclature appeared important because certain names implied certain cytogenetic derivations. Even now, some recognize acute lymphoblastic leukemias and acute myeloblastic leukemias; others prefer to distinguish acute lymphoblastic leukemias and acute non-lymphoblastic leukemias, or acute leukemias with granulocytic differentiation (blasts) and acute leukemias without granulocytic differenciation (blasts).

Perhaps we can now forget the ancient, though still smoldering, controversy by reminding ourselves that classification does not in itself carry any cytogenetic significance and that we could, if we wished, speak of blasts type I, blasts type II, blasts type III, etc.

After this introduction, I should like to consider the utility of classification for treatment, list the problems encountered and propose future research.

1. The assessment of the utility of the classification must record the failures as well as the successes. The physicians who utilise throughout the world the cytological classification presume the existence of a correlation between cellular type, prognosis and treatment. We may ask: do these dogmatic correlations reflect reality? The answer must come from statistical studies done by those who treat many leukemias.

The analysis fo these correlations is not simple, because for a defined cell type, other factors play a part, such as age, the existence or non-existence of a preleukemic state, the variable degree of cytopenia, the volume of the tumoral mass. These factors are partly dependent and partly independent of cellular types.

In the cases where these correlations are partly verified, we must ask their meaning and how to explain that such an empirical approach can yield useful results.

The reasons explaining the positive value of a treatment are also very complex. We must consider other important factors in addition to cytolysis such as the ability of the bone marrow to recuperate after treatment.

This assessment must not limit to the two principal types of leukemia, lym-

phoblastic and myeloblastic, but also to subtypes or subclasses more recently described such as promyelocytic leukemia, monoblastic leukemia and perhaps subtypes of lymphoblastic leukemia. Certain correlations undoubtedly represent a success of the empirical method which has recognized the peculiar behavior of certain acute leukemias due to the metabolism of their blast cells: the severity of the promyelocytic leukemia may be ascribed to the thromboplastic activity of their blast cells; possibly the severity of the monoblastic leukemias is related to the nephro-toxicity of the lysosyme-rich blast cells; and the very frequent extramedullary localisation of leukemic lymphoblasts in the central nervous system, testis and ovaries may similarly find and explanation in the metabolism of the blast cells.

The failure of empirical cytology demands similar close attention. Treatment sometimes obscures a cytologic diagnostic. It has been frequently claimed that even short treatment may interfere with cytologic diagnostic and certainly every attempt should be made to fully assess the morphology before treatment is started. However the reasons why treatment may alter (?) morphologic diagnostic are not clear. In fact, in some cases partial maturation may occur during treatment and aid in classification. Why does this not happen always? May be this inquiry will allow us to better understand the activity of the drug used?

The review of our failures which is usually neglected, can be rewarding. How frequently does the cytologic diagnosis remain uncertain and what happens to these patients treated without a precise cytological identification? With what frequency do we have to modify our initial "clear-cut" diagnosis? What are the reasons for these failures? Are their frequencies identical from one center to another? Can we improve this situation? If so, by what means?

Our research must consider our limitations. Are we perhaps in a situation where progress is impossible? Can we imagine a more refined "cuisine", improvement of our empirical methods?

The progress of our discussion must arise from the different aspects revealed by such new methods. This meeting is designed to take the long view, not to meet short-term objectives. We do not expect to modify our methodology of treatment as such, but we hope, in the light of the discussion of this meeting, to open for the future new aspects of treatment that will have more succes.

Cytological Classification of Acute Leukemias
A Survey of 1400 Cases

G. FLANDRIN and Jean BERNARD*

ABSTRACT. **The separation of acute leukemia (AL) in several distinct cytological subtypes is of great practical value in establishing the prognosis of the patients and the choice of therapy. This morphological classification, though it is inadequate, is presently the best one to predict the efficiency of the therapeutic schedules. In this regard, the separation of acute leukemia into acute lymphoblastic leukemia (ALL) and acute non lymphoblastic leukemia has been for many years shown to be useful. The diagnosis of acute "myeloblastic" leukemia (AML), acute "promyelocytic" leukemia (APL) and acute "monocytic" or "monoblastic" leukemia (A Mono L) within the group of acute non lymphoblastic leukemias is now also of practical value.**

KEY WORDS: **Acute leukemias – Cytochemistry – Prognosis**

MATERIAL AND METHODS

From January 1968 to June 1974, 1409 untreated patients with AL were submitted to cytological subclassification in our laboratory.

The cell variety in acute leukemia may be difficult to identify when the examination of blood and marrow is restricted to May-Grunwald-Giemsa (MGG) staining methods. Various cytochemical techniques have been shown to provide a more valuable approach in demonstrating characteristic features of abnormal cell lines [7]. The cytochemical reactions used are the following: Acid Periodic Schiff (PAS) reaction [14]; Peroxidase [14]; Naphtol AS-D Acetate Esterase [6] and the same reaction with Sodium Fluoride (Na F) [6] in diagnosing the specific Na F sensitive monocytic esterase. Sudan Black B [14] and Naphtol AS-D-Chloroacetate esterase reactions [18] were also used. Special cytochemical results in AL have been previously published [7].

RESULTS

From these 1409 AL patients the different cytological types are shown in Table 1.

Acute Lymphoblastic Leukemias (ALL) were characterized by the exclusive presence of blast cells which more or less are related morphologically to the "lymphoblasts" of the lymphoid tissue. They are defined by the absence of any evidence of cytoplasmic differentiation. They usually lack cytoplasmic granulations and are completely negative for peroxidase and Sudan Black B reactive material. Beyond the undifferentiated feature of blast cells, ALL is characterized

* Institut de Recherches sur les Maladies du Sang, 2, Place du Docteur Fournier, 75475 Paris, France.

Table 1. Different types of leukemias observed during 6 years and half. (Classification made by the same investigators)

	1968	1969	1970	1971	1972	1973	1974 first 6 months	Total	%
Acute lymphoblastic leukemia	93	91	87	53	91	104	67	586	41.5
Acute myeloblastic leukemia	98	65	61	92	60	81	28	485	34.4
Acute myeloblastic leukemia Partial infiltration (<40% blasts)	11	19	25	24	40	39	11	169	11.9
Erythroleukemia	6	5	6	10	4	2	2	35	2.4
Promyelocytic acute leukemia	10	7	9	6	4	6	8	50	3.5
Pure monocytic acute leukemia (monoblastic)	1[a]	0[a]	6	5	7	9	8	36	3.5[b]
Undifferentiated and rare cells types of acute leukemia	10	7	4	9	4	11	3	48	3.4
Total	229	194	198	199	210	252	127	1409	

[a] Before the systematic use of specific esterase reaction, "pure" monocytic leukemias were included in the acute myeloblastic group.
[b] % obtained on subjects from 1970 to 1974.

by the almost complete disappearance of maturing cells of the normal myeloid cell lines; the blast cells representing almost always nearly 100% of the cells in the bone marrow.

Among the non lymphoblastic AL we commonly distinguish acute myeloblastic (AML), acute "promyelocytic" (APL), acute "monocytic" or "monoblastic" leukemia (A Mono L). These cytological classes are characterized by the presence, in various proportions, of blast cells, which have enough cytological and cytochemical differentiation demonstrate to a relationship to their normal counterpart (myeloblast, monocyte etc.).

Acute myeloblastic leukemia (AML) is characterized by an excess of myeloblasts. These cells are characterized by azurophilic granulations; they sometimes contain Auer Rods and are positive peroxidase and Sudan Black B reactive material. And frequently they are positive in the Naphtol AS-D-Chloroacetate reaction.

Acute "promyelocytic" leukemias (APL) are characterized by special hypergranular "promyelocyte-like" cells, with a variable percentage of cells that contain characteristic bundles of large Auer rods [2].

Acute monocytic or monoblastic leukemias (A Mono L) are characterized by the nearly exclusive presence of monocytic precursors in both peripheral blood

Table 2. Cytochemical reactions used for acute leukemia classification

	ALL	AML	APmlL	AMoL
Peroxidase (or sudan black-B)	−	+ +	+ + + +	− to +
Naphtol AS-D chloro acetate	−	0 to + +	+ + +	−
N-AS-D acetate	±	± to + +	+ +	+ + + +
N-AS-D + Na F	no inhib.	no inhib.	no inhib.	inhib.
PAS	− to + +	− to +	− to ±	− to +
Acid-phosphatase	+ only granular	± diffuse	+ +	+ + + diffuse
β-glucuronidase	+ only granular	± diffuse		+ + diffuse

and bone marrow. These cells possess a strongly positive reaction for Naphtol AS-D esterase, and the reaction is strongly inhibited by Na-F [6, 4]. The schematic results of cytochemical reactions among AL are summarized in Table 2.

Some other cytological classes of AL are traditionally used:

Erythroleukemia

Erythroleukemia (or Di Guglielmo syndrome) is used for some kinds of AML with a very high level of abnormal erythroblasts in bone marrow ($>40\%$).

Acute Myelomonocytic Leukemias

Acute myelomonocytic leukemias are usually characterized as AML with apparent monocytic proliferation mixed with an excess of myeloblasts. In absence of precise criteria for this last group, we did not separate them from the main group of AML (see Table 1).

Since 1968, we have distinguished a special class from the general group of AML when the percentage of myeloblasts was relatively low ($<40\%$). We considered these cases as "partial myeloblastosis" or refractory anemia with low excess of myeloblasts [5]. The clinical experience in these cases showed that chemotherapy was less effective than in the usual cases of AML.

A few other patients remain as undifferentiated or unclassifiable cases or as very exceptional subtypes (megakaryocytic leukemia, basophilic leukemia etc.). Table 1 shows that the frequency of the major sub-groups of AL was relatively constant for $6^1/_2$ years when patients were diagnosed by the same observers, using the same techniques and the same criteria for classification. Such a reproductibility appears to be a good argument for the objectivity of this type of morphological classification. The regular frequency of rare diseases such as APL or A Mono L are of great value in this regard (Table 1).

The comparison of the frequency of AL subtypes to the results of other groups of hematologists is difficult to evaluate; the relative frequency of each cytological subgroup is primarly dependent on the proportion of children and adults among the patients studied. The results given in Table 1 correspond to a population of patients with 44% children (below 18 years) and 56% adults (above 18) (from statistics on 921 patients) [7]. Forty percent of all the patients had ALL and 60% non lymphoblastic AL; among the cases of ALL 83% were children and among non lymphoblastic AL 81% were adults. If we group the patients by both age and cellular subclass, 33% were children with ALL 11% chidren with non lymphoblastic leukemia, 7% adults with ALL, and 49% adults with non lymphoblastic leukemia.

More precise age analyses among the cellular subclass (ALL, AML, APL, A Mono L) are demonstrated in Fig. 1; 80% of the patients with ALL were below 18 years. The frequency of both AML and APL was about one same at all ages and the A Mono L had a bimodal distribution which we will emphasize further.

The aim of this cytological classification is to attempt to find therapeutic and prognostic correlations. Only the 4 main classes (AML, APL, A Mono L, ALL) are presently of practical use and they were utilised for analysis of prognosis results and of chemotherapy. Fig. 2 shows the major differences which exist among these main cylological classes in the ability to obtain the first complete remission (CR). Fig. 2 indicates the particular behaviour of patients with A

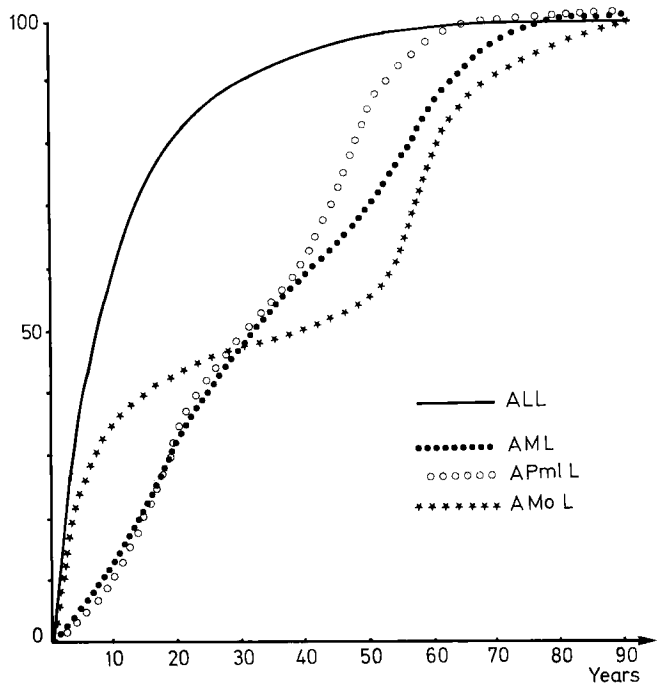


Fig. 1. Age repartition (in %) in the four main groups of acute leukemia (ALL Acute lymphoblastic leukemia; AML Acute myeloblastic leukemia; A Pml Acute promyelocytic; AMoL Acute monocytic leukemia)