Microgranular Variant of Acute Promyelocytic Leukemia in Children

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Purpose: **The microgranular variant (M3v) of acute promyelocytic leukemia (APL) rarely has been reported in a pediatric series of acute nonlymphoblastic leukemia (AnLL). We reviewed the clinical and biologic features of childhood M3v cases in our AnLL series.**

Patients and Methods: **From January 1970 to January 1991, 11 children with M3v were admitted and treated at our center. A diagnosis was made according to French-American-British (FAB) criteria. Morphologic examination, cytochemical analysis, and immunophenotyping were performed by a single pathologist. From January 1984, the diagnosis was confirmed by cytogenetic and, subsequently, by molecular analysis on frozen material.**

Results: **In our series, the overall incidence of children with APL was unusually high, 31.2% of the AnLL and M3v constituted one case in every four cases of APL. Even restriction of the analysis to the time when either cytogenetic and DNA studies confirmed the diagnosis, the incidence did not change. The immunophenotype of M3v cases was identical to that described for the hypergranular type, but an unexpected association of CD2 with M3v was shown. The onset was characterized by marked hyperleukocytosis (median WBC count, 87 × 10^9/L) unlike classic APL. Disseminated intravascular coagulation (DIC) was always present and severe. Hyperleukocytosis and DIC were responsible for the high incidence of deaths for hemorrhagic events in the first days after onset (eight of 11 patients).**

Conclusions: **In our experience, for unknown reasons, M3v may occur in childhood more than generally was considered. The clinical course and prognosis seem worse in M3v than in typical APL cases.**

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THE FRENCH-AMERICAN-BRITISH (FAB) classification recognizes a variant, FAB M3v, of acute promyelocytic leukemia (APL; FAB M3), which is characterized by bilobed cells, multilobular cells, or cells with reniform nucleus and cytoplasm with minimal or no granulations and generally is associated with a few typical M3 cells in the bone marrow.[1-5] However, M3v rarely has been diagnosed in pediatric series of acute nonlymphoblastic leukemias (AnLL) that were published by various centers.[6-14] We previously have reported the first few cases of M3v,[15,16] and stressed the gloomy prognostic significance of this form. In the present report we reviewed the clinical and biologic features of 11 cases of childhood M3v, the largest series studied so far.

PATIENTS AND METHODS

From January 1970 to January 1991, 141 pediatric patients were admitted and treated for newly diagnosed AnLL at our center. Diagnosis was made according to the FAB criteria using May-Grunwald Giemsa staining for morphologic examination on peripheral-blood and/or bone marrow smears. Morphologic diagnosis was performed retrospectively by three observers without preknowledge of patient identity in four of the M3v cases as previously described[16] and prospectively in the other seven. The number of children with APL was 44 (31.2% of AnLL). There were 11 M3v cases in children with APL (25% of APL).

Cytochemical stains for myeloperoxidase (MPO) or Sudan black and nonspecific esterase (alpha-naphtyl-acetate esterase, ANAE) were performed.[17] Phenotype analysis was performed by indirect immunofluorescence on bone marrow mononuclear cell suspensions. Briefly, 0.5 to 1 × 10^6 cells were incubated for 20 minutes at 4°C with the primary monoclonal antibody (MoAb), were washed twice with phosphate-buffered saline (PBS) plus 1% bovine serum albumin (BSA), and were stained with a fluorescein conjugated goat F(ab')2 antimouse immunoglobulin (Technogenetics, Trezzano s.N., Italy). After two washes in PBS-BSA, cells were examined under an epifluorescence microscope (Leitz, Wetzlar, Germany). The following MoAbs were used for phenotype analysis: J5/CD10, B4/CD19, BA-2/CD9, Leu9/CD7, T3/CD3, T11/CD2, OKM/CD11b, My7/CD13, My4/CD14, LeuM1/CD15, My9/CD33, and 7.2/anti-HLA-DR nonpolymorphic determinant. A reactivity of less than 20% was considered negative.

Morphologic examination, cytochemical analysis, and immunophenotyping were performed by a single pathologist at our center. Cytogenetic analysis was performed from January 1984. Chromosome preparations were obtained from 24- and 48-hour unstimulated bone marrow cell cultures that were stained with quinacrine mustard (banding technique); 40 metaphases were analyzed routinely and karyotype arranged according to the International System for Human Cytogenetic Nomenclature recommendations.[18]

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For DNA analysis, high molecular weight DNA was extracted from the mononuclear cells that were isolated by Ficoll-Hypaque centrifugation, digested to completion with *Bam* HI, *Hin*dIII, *Eco* RI, and *Bgl*1 restriction enzymes, size fractionated by electrophoresis through a 0.8% agarose gel, and transferred to nylon membranes. As previously reported,[19] DNA probes that were representative of the chromosome 17 (RAR-α locus) and 15 (PML) breakpoint regions were used and labeled to a high specific activity with ^{32}P.

Coagulopathy at the time of diagnosis was defined as a fibrinogen level less than 100 mg/dL, a prothrombin activity less than 70%, a partial thromboplastin time more than 45 seconds, and fibrin degradation products (FDP) more than 8 μg/mL. All patients received multiple transfusions of blood components (plasma, platelet concentrates, packed RBCs) according to the clinical course of the coagulopathy as well as laboratory tests, and, except for the patients who were treated in the 1970s, heparin treatment (100 to 400 U/kg daily intravenously [IV]) was administered until fibrinogen normalized, the platelet count stabilized, and cell lysis was completed. Five children (no. 7 to 11) also received tranexamic acid (500 mg IV three times per day) from the time that accelerated fibrinolysis was observed until it resolved. Febrile episodes were treated with broad-spectrum antibiotics and combined with amphotericin B if no response was obtained. As soon as possible, a central venous catheter was inserted to continue the administration of antiblastic drugs and blood components and to give total parenteral nutrition (no. 7 to 10).

The difference of therapeutic approaches was caused by the long period during which M3v was diagnosed; treatment details are given in the descriptions of the individual cases. The differences in the present features among patients with M3, M3v, and those with other AnLL subtypes were analyzed by χ^2 or t test for unpaired data.[20]

RESULTS

The typical morphologic features of M3v[2] were present in all 11 cases (Fig 1). In the bone marrow, a slight minority of the atypical cells showed the features of hypergranular leukemic promyelocytic cells, and cells with fagots of Auer rods were observed. Cytochemical stains for MPO or Sudan black were performed in all patients: intense positivity was observed for MPO in 98% to 100% of cells in all 10 (no. 2 to 11) tested and for Sudan black in the remaining (no. 1). A slight diffuse positivity for ANAE was observed in two of six cases that were tested in 80% and 20% of cells, respectively.

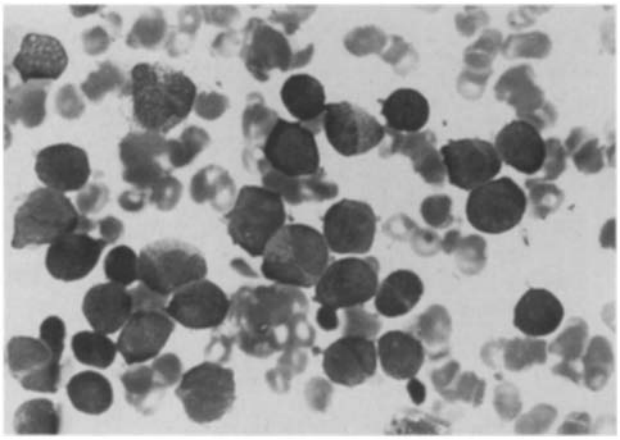

Fig 1. Patient no. 11. The M3 variant typical promyelocytes characterized by indented or bilobed nuclei. A cell with Auer rods is evident (MGG × 1,000).

Seven of 11 cases were studied by immunophenotyping and presented a similar antigenic profile that was characterized by the negativity virtually in all cases of HLA-DR. Unexpectedly, CD2 was positive in five of six cases; the other T-cell–related MoAbs were negative. Immunophenotypic data are summarized in Table 1.

From January 1984 to January 1991, 18 APL cases of 52 AnLL cases were diagnosed. During that time, the diagnosis of APL was confirmed by cytogenetic and, subsequently, by molecular analysis of frozen material. Chromosome preparations of bone marrow cells at the time of diagnosis were adequate for the analysis in 17 of 18 cases (12 M3, six M3v). All 17 (11 M3, six M3v) patients (100%) manifested the specific translocation between chromosome 15 and 17, which was t(15;17) (q22, q11-22). DNA analysis was performed in 15 (nine M3 and six M3v) of 18 patients. We demonstrated rearrangements of either RAR-α or PML loci, in all cases, and the results obtained in the first nine patients were reported previously.[19] No frozen cells were available from the M3v cases diagnosed before 1984 to perform retrospectic cytogenetic and molecular analysis. Table 2 lists M3v patients' characteristics at onset and outcome.

The M3v group consisted of nine females and two males (male:female ratio, 1:4.5) with a median age of 8.3 years (range, 1.7 to 13.4 years). At onset, all presented with an elevated leukocyte count (median, WBC count 87×10^9/L; range, 55 to 266×10^9/L), anemia of varying severity (median hemoglobin, 8.3 g/dL; range, 6.4 to 11.1 g/dL) and severe thrombocytopenia (median platelet count, 16×10^9/L; range, 8 to 31×10^9/L). Significant organomegaly was observed only in one case (spleen 5 cm below costal margin; patient no. 9). Only four patients underwent lumbar puncture (no. 3, 4, 7, and 9), which indicated neurologic involvement in one patient (no. 9). This investigation was not possible in the other patients because of the severe bleeding manifestations, but none of them presented clinical signs of CNS localization. All patients showed coagulation abnormalities before treatment and hemorrhagic diathesis (from cutaneous and mucous to severe intracranial hemorrhages). In the five patients (no. 6 to 10) who survived 48 hours after diagnosis, there was a mean increase in the

Table 1. Main Immunophenotypic Data in Seven Patients With M3v

Patient No.	DR	CD9	CD19	CD2	CD7	CD11b	CD13	CD14	CD15	CD33
5	0*	NT+	0	NT	NT	0	NT	NT	NT	NT
6	28	65	0	99	1	0	30	NT	3	78
7	14	99	0	90	1	8	90	21	1	99
8	7	99	0	30	0	5	99	1	9	98
9	8	99	0	80	0	15	85	1	0	95
10	1	2	0	7	0	6	83	1	0	96
11	12	95	NT	52	NT	0	60	NT	0	NT

Abbreviation: NT, not tested.
*Percent of positive cells.

WBC count of 127 × 10^9/L despite the start of treatment a few hours earlier.

Eight (73%) patients (no. 1 to 6, 10, and 11) died rapidly, 1 to 10 days after diagnosis, because of severe hemorrhagic events (cerebral hemorrhage, cases 1 to 3, 5, 6, and 10; pulmonary hemorrhage, cases 4 and 11). Three of these children had not received any antiblastic treatment (patients no. 1, 2, and 5) because of the rapidly worsening clinical course; death was within 24 hours of diagnosis. One patient (no. 3) started treatment with the drug combination of thioguanine (6TG; 100 mg/m^2 daily for 7 days), cytarabine (ARA-C; 100 mg/m^2 twice per day for 7 days), and daunorubicin (DNR; 60 mg/m^2 on days 5, 6, and 7), and died 6 days after diagnosis, after the first administration of DNR. Two patients (no. 4 and 6) received one dose of DNR IV (45 mg/m^2); both died within 48 hours.

In patients no. 7 to 9, the coagulopathy was controlled and all three achieved complete remission (CR). They were allocated to protocol LAM 87 of the Italian Association of Pediatric Hematology and Oncology (Associazione Italiana di Ematologia ed Oncologia Pediatrica).[21,22] The induction phase of LAM 87 included a first cycle of DNR (45 mg/m^2 IV on days 1, 2, and 3) and ARA-C (200 mg/m^2/d in continuous IV infusion [CI] on days 1 to 7), which was followed by a second cycle with DNR (45 mg/m^2 IV on days 1 and 2) and ARA-C (200 mg/m^2 CI on days 1 to 5) 3 weeks later. During each cycle, intrathecal administration of ARA-C was performed at doses according to age (> 3 years, 70 mg). However, in the three patients, the antiblastic therapy was not given as prescribed by the protocol, but the doses and times of administration were modulated empirically according to the clinical conditions and laboratory tests of the patient with the aim of avoiding an excessively acute blast lysis and a consequent dramatic progression of disseminated intravascular coagulation (DIC) (patient no. 7, ARA-C 200 mg/m^2/d CI on days 1 to 3 and 6 to 9, and DNR 30 mg/m^2 IV on days 2, 8, and 10; patient no. 8, ARA-C 200 mg/m^2 CI on days 2 to 6, 8, and 9, and DNR 30 mg/m^2 IV on days 1, 8, and 9;

Table 2. Patient Characteristics at Onset and Outcome

Patient No.	Sex	Age (years)	Year of Diagnosis	Hemoglobin (g/dL)	WBC Count (10^9/L)	Platelets (10^9/L)	Cytogenetic Analysis	PTT (seconds)	Prothrombin Activity (%)	Fibrinogen (mg/dL)	FDP (mg/dL)	Overt Hemorrhage	Initial Therapy	Outcome
1	F	8.1	1972	8.3	266	16	NT	NA	NA	69	16	Yes	No	Hemorrhagic death (CNS) first day
2	F	7	1973	7.9	55	15	NT	NA	NA	50	25	Yes	No	Hemorrhagic death (CNS) first day
3	F	11.6	1974	6.4	84	16	NT	NA	45	153	50	Yes	DNR, 6TG, ARA-C	Hemorrhagic death (CNS) sixth day
4	F	12	1980	8.7	87	10	NT	70	43	157	25	Yes	DNR	Hemorrhagic death (lung) third day
5	F	12.6	1983	10.2	172	20	NT	NA	40	136	NA	Yes	No	Hemorrhagic death (CNS) first day
6	F	8.3	1988	7.6	81	22	t(15;17)	37	22	80	200	Yes	DNR	Hemorrhagic death (CNS) fifth day
7	M	6.2	1988	11.1	87	28	t(15;17)	27	31	97	640	Yes	DNR, ARA-C	Died in second CR after second BMT
8	F	13.4	1989	10.5	90	8	t(15;17)	25	35	146	16	Yes	DNR, ARA-C	Alive, CR, 17 months after AuBMT
9	F	8.7	1989	10.3	84.3	10	t(15;17)	26	40	100	32	Yes	DNR, ARA-C	Alive, CR, 14 months after AuBMT
10	M	2.4	1990	7.5	106.6	31	t(15;17)	26	50	130	64	Yes	DNR, ARA-C	Hemorrhagic death (CNS) tenth day
11	F	1.7	1991	6.8	114	27	t(15;17)	59	24	177	NA	Yes	DNR	Hemorrhagic death (lung) first day

*Abbreviations: NT, not tested; NA, not available; PTT, partial thromboplastin time; AuBMT, autologous bone marrow transplantation; F, female; M, male.

patient no. 9, ARA-C 200 mg/m^2 CI on days 2 to 8, and DNR 30 mg/m^2 IV on day 2, 45 mg/m^2 IV on days 3 and 4 and 12.5 mg/m^2 IV on day 5). Remission was achieved in patients no. 7 and 8 after 43 and 25 days, respectively. Patient no. 9 was resistant to this induction regimen and obtained CR on day 101 after two cycles of 6TG (100 mg/m^2/d orally), ARA-C (200 mg/m^2/d CI), DNR (20 mg/m^2/d CI), etoposide (100 mg/m^2/d CI), and dexamethasone (2 mg/m^2 three times per day orally) for 4 consecutive days, with a 10-day interval. Subsequently, all three patients underwent autologous bone marrow transplantation (AuBMT). Patients no. 8 and 9 are still alive and disease-free 17 and 14 months after AuBMT, respectively. Patient no. 7 was in first remission when he underwent AuBMT 6 months after disease onset; he had a marrow relapse 8 months later, and attained another CR after two cycles of 6TG, ARA-C, DNR, etoposide, and dexamethasone as previously described for patient 9; 8 months after the relapse, he received a mismatched BMT from his mother and died 2 months later of encephalitis.

Patient no. 10 was also allocated to protocol LAM 87 and died on day 10, only 3 days after the first chemotherapy cycle with ARA-C was completed according to the protocol and DNR at reduced dose (15 mg/m^2 on 3 nonconsecutive days: days 4, 7, and 8). A central venous catheter was inserted when blastic lysis was complete and coagulation tests showed that DIC was controlled. Twenty-four hours later, the cardiac and respiratory conditions of the patient suddenly worsened and pulmonary thrombosis was diagnosed. Urokinase treatment was started, but cerebral hemorrhage intervened and the child died.

In patient no. 11, the treatment was modulated to allow her to survive, but a few hours after the administration of the first and only antiblastic drug received (DNR at reduced dose 15 mg/m^2), she died of pulmonary hemorrhage that was present before the treatment.

Clinical features at diagnosis of M3v were compared with those of M3 and other AnLL subtypes who were treated at our center in the same period. Table 3 summarizes the results. Statistical analysis showed a significant difference with respect to early death when M3v was compared with either M3 ($P < .001$) or AnLL subtypes ($P < .001$). Among the other features considered, the WBC count had a significantly higher result in M3v compared with M3 ($P < .001$). The number of children with coagulation abnormalities differed significantly between M3v and other AnLL subtypes ($P < .001$).

Table 3. Presentation Features of Children With M3, M3v, and Other AnLL Subtypes

	M3	M3v	Other Subtypes
No. of patients	33	11	97
Median age (year)	7.3	8.3	7.5
Males/females	19/14	2/9	60/37
Median hemoglobin (g/dL)	8.0	8.3	7.9
Median WBC count (10^9/L)	4.2	87	33.3
Median platelets (10^9/L)	27	16	32
No. of patients			
CNS involvement*	0	1	6
Splenomegaly†	0	1	10
Hepatomegaly†	0	0	8
Coagulation abnormalities	29	11	20
Early death‡	2	8	3

*Data on CNS involvement were available in 23, 4, and 86 cases of M3, M3v, and other AnLL subtypes, respectively.

†Organomegaly, more than 5 cm below costal margin.

‡Less than 10 days from onset.

DISCUSSION

In our series, the overall incidence of children with APL was unusually high, 31.2% of the AnLL. It is noteworthy that it did not change (32.7%) even when the analysis was restricted to the time (from January 1984 to January 1991) when either cytogenetic or DNA analysis confirmed the diagnosis of APL. The high percentage of APL observed at our center clearly was superior to that reported in the pediatric literature by other investigators both for FAB M3 and for FAB M3v and similar to that reported in studies of clusters limited to particular areas or a black population.[10,23,24] In pediatric age, the usual incidence of M3 is in approximately 4% to 7% of the AnLL.[8,9,14] There are only sporadic reports on M3v. In particular, it was underlined that the microgranular variant constituted 25% of the APL we observed, which was a similar proportion to that reported in adults.[25] A detailed epidemiologic study is necessary to identify the reasons of the unique distribution of AnLL subtypes we observed at our center.

We consider that a diagnosis of FAB M3v may be based on morphologic findings, which are extremely characteristic, and on intense positivity for MPO. Moreover, the demonstration of t(15;17) with RAR and PML rearrangements represents an important diagnostic step.[19,26-31] Ultrastructural investigations, which fundamentally have been important to confirm the promyelocytic nature of the atypical elements present in this leukemia,[4] strictly are not necessary for diagnostic confirmation except in some doubtful cases when cytogenetic and molecular data cannot be obtained.

Our data show that the immunophenotype of M3v is

identical to that described for the hypergranular variant, HLA-DR, CD11b, and CD15 negativity, which is associated with CD9 positivity being the most typical feature.[32,33] Otherwise in the series of patients with hypergranular M3 reviewed by Drexler,[34] CD11b and CD15 positivity was found in a proportion of patients (53% of cases for CD11b and 69% for CD15). Positivity for the T-associated MoAb CD2 in five of six M3v cases was an unexpected finding. In contrast, CD2 was expressed in only one of nine pediatric cases with hypergranular M3 in our series (data not shown). CD2 positivity already has been reported in acute myeloid leukemia,[35] but to our knowledge this is the first report that suggests a strong association of CD2 with M3v.

The hematologic characteristic of M3v at onset, in addition to DIC, was hyperleukocytosis, whereas the hypergranular form is usually accompanied by low leukocyte counts. Induction failure because of early hemorrhagic death is elevated in many APL series and accounts for the small number of patients with FAB M3v who achieve a CR.[25] Hyperleukocytosis at the onset of disease was confirmed recently as a predictor in APL of early hemorrhagic death.[36,37] In our series, one of the most striking features was the different incidence of early death; only 6% (two of 33) of the patients with classic APL versus 73% (eight of 11) of those with M3v died in the first 10 days of chemotherapy from hemorrhagic events. The clinical course of M3 patients (data not shown) in our experience was quite different, although they received the same treatment of coagulopathy, and the same supportive care and antiblastic therapy through the years: high induction rate (79%), low induction failure, relatively long remission (median, 447 days), and high relapse rate with only five of 33 patients alive and disease-free from 1 to 14 years after the first CR was achieved. The start of treatment in M3v patients should not be delayed because the disease rapidly worsens, and has a tendency to increase markedly the leukocyte count in the first 24 to 48 hours. Possibly treatment modulation, as reported in the present study, would promote patient survival, but new therapeutic approaches should be explored. Recently, all-*trans*-retinoic acid (ATRA) has been used in the treatment of APL[38,39] and has shown promising results. Hyperleukocytosis has been the most important ATRA-related effect. The role of increased hyperleukocytosis in the fatal evolution of a case of M3v that was treated with ATRA could not be excluded.[38] ATRA should be used cautiously in patients with hyperleukocytosis, ie, those with M3v. Maximal support therapy (blood components, broad-spectrum antibiotics, total parenteral nutrition), which certainly has improved in last decade, also promotes survival. Although heparin has been used as a standard method in the management of DIC in APL,[25,40] the benefit of its use has not been proven, and other investigators are reluctant to administer it to thrombocytopenic patients.[41] Also tranexamic acid[42] is of uncertain benefit. It is noteworthy that no thrombotic manifestations were observed in patients who were treated with antifibrinolytic agents in a recent retrospective study; however, the same study failed to demonstrate any beneficial effects of heparin or antifibrinolytics in the reduction of the incidence of early death.[37]

In conclusion, our experience shows that M3v may occur in childhood more than generally was considered. The clinical and hematologic characteristics, particularly the morphologic and cytochemical, and, more recently, molecular studies[19,42,43] enable a rapid diagnosis to be made. Often, the precipitous clinical course and the prognosis seem worse than in typical forms of APL, and, as in an adult setting may be attributed to the marked hyperleukocytosis and severe DIC. New therapeutic approaches may open new perspectives in this rare and dramatic form of leukemia.

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