CORRESPONDENCE

DIAGNOSIS AND INCIDENCE OF ACUTE PROMYELOCYTIC LEUKEMIA (FAB M3 AND M3 VARIANT) IN CHILDHOOD

To the Editor:

In the last 20 years an unusually high incidence of acute promyelocytic leukemia (APL) has been observed at the Monza pediatric oncology center of the University of Milan in northern Italy (Clinica Pediatrica dell'Universita di Milano, Ospedale San Gerardo, Monza, Italy) (MZ). During this period at MZ, 30% of pediatric acute myeloblastic leukemia (AML) cases have been classified as APL, and the microgranular variant of APL (FAB M3v) has constituted 25% of APL cases. A similar high incidence of APL has been reported previously only in small studies limited to patient clusters in restricted non-western geographic areas and/or to South American populations and a comparable incidence of M3v is unreported. Unlike classic APL, M3v is always characterized by hyperleukocytosis and very severe coagulopathy; death from central nervous system (CNS) or lung hemorrhage is frequent within the first 10 days after diagnosis, and correct diagnosis of M3v is essential for proper treatment. Because the role of trans-retinoic acid (TRA) in M3v has been inadequately studied, and correct diagnosis of M3v is essential for proper treatment. Because the role of trans-retinoic acid (TRA) in M3v has been inadequately explored, obviously it should be evaluated in future treatment trials requiring accurate diagnosis of M3v. Although cytogenetic and molecular studies allow precise diagnosis of all APL cases and immunophenotypic and molecular studies may help in recognition of M3v cases, these studies are not performed routinely in all cases in most centers. Entry in current M3 treatment protocols is still usually determined by morphology, which remains an essential diagnostic tool in this disease. The general consensus among morphologists is that, in experienced hands, microscopic diagnosis of classic APL is reproducible and easy; by contrast, the morphologic features of M3v can raise difficulty for separation from classic APL on the one hand, and from FAB M2, M4, and M5 on the other. The relative rarity of M3v may contribute to its misdiagnosis.

To confirm the high incidence of APL at MZ and to evaluate these related diagnostic issues, a working group was formed by representatives of MZ, the Cooperative Acute Myeloid Leukemia Berlin-Frankfurt-Munster Therapy Study Group (BFM), and St Jude Children’s Research Hospital (SJCRH). A comparison of the historical incidence of APL in these three institutions is presented in Table 1.

In the MZ APL series, cytogenetic analysis was not performed in some cases, especially older cases in the series. To evaluate the reliability of morphologic diagnosis of APL and recognition of M3v in these three series, and whether differences in morphologic criteria exist among the three institutions, a blind evaluation of slide material from the MZ childhood APL series was undertaken. Twenty-two bone marrow slides of classic APL and M3v from MZ, stained with May-Grunwald-Giems, and 15 additional AML cases chosen for resemblance to M3 and M3v were reviewed by experienced morphologic observers from each group (MZ, BFM, and SJCRH) without preknowledge of the cases. The original MZ diagnosis was accepted as the reference diagnosis because of the availability of cytogenetics (it documented in 11 M3 and 4 M3v cases), molecular biologic analysis, clinical information, cytochemistry review, treatment outcome, and serial morphology review. Concordance for diagnosis of classic APL was very good (Table 2): MZ classic M3 versus BFM 17/17, and MZ versus BFM 100% (17/17), and MZ versus BFM 91%. SJCRH classified a single MZ M2 as classic M3. Of the 5 cases classified as M3v by MZ, BFM classified 2 as classic M3, 2 as M3v, and 1 as unclassified, whereas SJCRH classified 3 as classic M3 and 2 as M3v. Both SJCRH and BFM classified the same MZ M1 as M3v.

This high concordance for M3 cases with blind morphologic review suggests that at least the vast majority of M3 cases lacking cytogenetic analysis in the MZ series are correctly diagnosed; the high incidence of APL in the MZ series is accurate, and the low incidence of M3 in the BFM and SJCRH series (Table 1) cannot be attributed to the failure of morphologic diagnosis. Further confirmation was shown when the large majority of AML cases at SJCRH during this period received successful cytogenetic analysis, with appropriately high concordance of cytogenetics and morphology in APL. Many SJCRH cases have been confirmed with molecular analysis of chimeric PML/RARA mRNA, and in cases with complete analysis at MZ there is a similarly high concordance of cytogenetics, morphology, and molecular study. The cause of this high incidence of APL at MZ is unknown, but it has been sustained over a 22-year period of observation.

As for M3v, although there were few cases in the slide review, concordance was low. Unexpectedly, most discordance was not due to failure in recognition of APL, but rather to the separation of classic APL and M3v. A possible explanation is that morphologic features of M3v are more pronounced in peripheral blood than in bone marrow, where morphology may resemble classic hypergranular APL. Material for our blind review consisted of bone marrow smears; to diagnose M3v it may be advisable to also review peripheral smears.

In conclusion, we confirm by cross-review the high incidence of

Table 1. APL Incidence in MZ, BFM, and SJCRH Series

<table>
<thead>
<tr>
<th></th>
<th>MZ</th>
<th>BFM</th>
<th>SJCRH</th>
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<tbody>
<tr>
<td>AML (no. of cases)</td>
<td>151</td>
<td>543</td>
<td>390</td>
</tr>
<tr>
<td>APL (no. of cases) (%)</td>
<td>46 (30.4)</td>
<td>21 (3.8)</td>
<td>25 (6.4)</td>
</tr>
<tr>
<td>FAB M3v/M3</td>
<td>12/34</td>
<td>0/21</td>
<td>1/24</td>
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Table 2. Results of Blind Morphologic Review

<table>
<thead>
<tr>
<th>Original Diagnosis</th>
<th>MZ</th>
<th>M3v</th>
<th>Other</th>
<th>BFM</th>
<th>SJCRH</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3 (17)</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>M3v (5)</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2*</td>
</tr>
<tr>
<td>Other AML (15)</td>
<td>14*</td>
<td>11</td>
<td>14</td>
<td>1</td>
<td>1*</td>
</tr>
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* No FAB diagnosis, 1 case.
† BFM and SJCRH both called the same case M3v.
‡ No FAB diagnosis, 2 cases.

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APL in pediatric patients at MZ, and show a need to standardize diagnosis of M3v and its separation from classic hypergranular APL. Although morphologic and clinical features remain the major criteria to distinguish M3v from classic hypergranular M3, additional laboratory features have been suggested as possibly helping in this distinction (CD2 positivity in the MZ pediatric M3v series and a predominance of BCR3 breaks in PML in M3v cases). These parameters require confirmation by additional study. In this regard, we plan to continue our collaboration by prospectively studying peripheral blood and bone marrow smears of cytogenetically defined cases from our three groups, comparing morphologic analysis with results of immunophenotyping and molecular biologic studies.

REFERENCES
Diagnosis and incidence of acute promyelocytic leukemia (FAB M3 and M3 variant) in childhood [letter]

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