Reproducible measurements of AML blast p-glycoprotein function in 2 center analyses

The review article “Targeting the Multidrug Resistance-1 Transporter in AML” by Mahadevan and List 1 is thorough and thought-provoking, and we support its principal conclusions that p-glycoprotein (ppg) remains one of the most powerful prognostic factors in adult acute myeloid leukemia (AML) and that future trials of ppg modulators are called for. Since the preselection of patients with ppg-positive AML would target those who are most likely to benefit and would also improve the predictive power of modulator trials, it follows that ppg phenotyping should be carried out at diagnosis. However, Mahadevan and List were unable to indicate a suitable method for ppg measurement in the laboratory. They referred right back to the consensus recommendations of 1996 2 and to the French report of 1997 3 both of which highlighted discrepancies in methodology and analysis. Meanwhile, Broxterman and colleagues 4 published a report of a flow cytometric assay of functional ppg, which was reproducible in 2 centers in The Netherlands. In the United Kingdom, where ppg is being measured on AML trial patients in more than one laboratory, we built on the lessons of the Dutch and the French groups and decided to use a single standard operating procedure at participating laboratories. The Dutch protocol was adopted with the minor modification of adding CD45 staining to identify blasts. 5,6 Most samples were sent to one of 2 centers, Cardiff or Nottingham. Thirteen quality control samples have been shared between the 2 centers since our collaboration started in 1999. Discordant results were noted in 1 of 13 samples; 8 had negative or low values (less than 1.7), 2 had intermediate values (1.7-3.39), and 2 had high values (3.4). Furthermore, there was a remarkable similarity in the distributions of data. Figure 1 illustrates the distributions obtained on 120 samples, the first 60 analyzed at each center. At one stage in the trial a third center also participated in the functional ppg analysis. A Kolmogorov-Smirnoff test showed no significant difference in the distribution of PSC-833 modulation ratios at each of the 3 centers.

In contrast to the functional assays, antibody assays using MRK-16, also performed according to a standard operating procedure, showed significant differences in distribution between the 3 centers. While Mahadevan and List rightly pointed out that functional assays do not yield greater prognostic significance than antibody measurement in clinical trials, we have found that an important advantage of the functional assay lies in its greater sensitivity and reproducibility. We acknowledge that discrepancies do occur between phenotypic and functional ppg results, which are not fully understood. 7,8 However, in the United Kingdom LRF AML 14 trial, an additional advantage of using a ppg functional assay was that the agent being used in the trial, PSC-833, could also be used in the assay.

Using the Dutch protocol over several years has confirmed to us that this methodology is robust and could now be passed to the safe and reliable hands of hospital immunophenotyping laboratories. From receiving a marrow sample, the assay takes about 4 hours and has been undertaken with as few as 3 $\times$ 10$^6$ cells. Using this protocol, future trial organizers can have the choice of whether to give ppg modulators to an unsorted cohort or to ppg-positive patients only.

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References

To the editor:

Is immune thrombocytopenic purpura less common among black Americans?

While beginning a registry for immune thrombocytopenic purpura (ITP) in Oklahoma, we noted that the number of black patients seemed smaller than expected. At the same time we read a report suggesting that ITP is rare among black Africans and people of African ancestry in other parts of the world.1 However, in standard textbooks and in major review articles on ITP, race is not mentioned. Therefore, we systematically searched the Medline database for all publications on ITP from the United States to identify articles describing 10 or more patients that identified patients by race; we found only 6 articles.2-7 These articles report data from different regions of the United States and across many years.

In each of the 6 articles,2-7 the proportion of blacks among patients with ITP was lower than the proportion of blacks in the population. In 5 of the published studies, the 95% confidence interval (CI) for the observed percent of blacks among the ITP patients did not overlap with the percentage of blacks in the population.2,4-6,7 One of these articles commented on their observation that only 4 (6%) of 67 patients with ITP were black, compared with 25% of their hospital patients.2

A potential limitation of this study is our selection of census data. Although our calculations were based on United States census data for the cities from which the patients were reported and from a time near to patient accrual (Table 1), the percentage of blacks in the census data that we used may not accurately reflect the geographic origin of the reported patients if referral areas were large.

Our awareness of potential racial disparities was stimulated by our recent demonstration, from the Oklahoma Thrombotic Thrombocytopenic Purpura Hemolytic Uremic Syndrome (TTP-HUS) Registry, of an increased proportion of blacks among patients with TTP and severe ADAMTS13 deficiency. The age- and sex-standardized incidence rate ratio of blacks to nonblacks was 9.3 (95% CI, 4.3-19.9).8 The apparent opposite racial disparity among patients with ITP and TTP is intriguing, but different racial disparities also occur among other autoimmune disorders. For example, the prevalence of systemic lupus erythematosus (SLE) is greater among blacks,9 while the prevalence of anticardiolipin antibodies preceding SLE is greater among whites.10

If a racial disparity among patients with ITP is confirmed, it may indicate a genetic influence on the etiology of ITP. Alternative explanations may be that the diagnosis of ITP is made less often among blacks because of disparities in health care11 or because the presence of petechiae and ecchymoses may not be appreciated.

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Table 1. Frequency of blacks among patients with immune thrombocytopenic purpura

<table>
<thead>
<tr>
<th>Study (site)</th>
<th>Publication y</th>
<th>Y of patient accrual</th>
<th>Patients</th>
<th>% black*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block et al2 (Chicago)†</td>
<td>1966</td>
<td>1948-1964</td>
<td>67</td>
<td>4 (6.0, 1.7-14.6)</td>
</tr>
<tr>
<td>Wilde et al2 (Pittsburgh)‡</td>
<td>1967</td>
<td>1949-1966</td>
<td>43</td>
<td>2 (4.7, 0.6-15.8)</td>
</tr>
<tr>
<td>Akwari et al4 (Durham)§</td>
<td>1987</td>
<td>1975-1985</td>
<td>100</td>
<td>20 (20.0, 12.7-29.2)</td>
</tr>
<tr>
<td>Guthrie et al5 (Augusta)</td>
<td>1988</td>
<td>1954-1983</td>
<td>40</td>
<td>12 (30.0, 16.6-46.5)</td>
</tr>
<tr>
<td>George et al6 (United States)¶</td>
<td>2003</td>
<td>1997-2000</td>
<td>66</td>
<td>5 (7.6, 2.5-16.8)</td>
</tr>
<tr>
<td>Aledort et al6 (United States)**</td>
<td>2004</td>
<td>2000-2001</td>
<td>158</td>
<td>9 (5.7, 2.6-10.5)</td>
</tr>
<tr>
<td>Unpublished review (Oklahoma)††</td>
<td>2004</td>
<td>2004</td>
<td>87</td>
<td>4 (4.6, 1.3-11.4)</td>
</tr>
</tbody>
</table>

Data summary for all published articles from the United States describing 10 or more patients that identified patients by race.

* Census data are percentages for one race reported as black non-Hispanic.
† Census data for Chicago for 1960 were obtained from the Reference Department of the Illinois State Library, Springfield, IL.
‡ Census data for Pittsburgh for 1960 were obtained from the Bureau of State Library-Law/Government Publication, State Library of Pennsylvania, Harrisburg, PA.
§ Census data for Durham for 1980 were obtained from the Missouri State Census Data Center (http://oseda.missouri.edu/mscdc/census/us/trend/places/S37NC/ P370750), August 17, 2004.
¶ Census data for Richmond County, which includes Augusta, for 1980 were obtained from the East Central Georgia Regional Library, Augusta, GA.
† This study reported 70 adult patients, ages ≥ 18 years, from 20 United States sites that enrolled 1 to 10 patients. The 4 patients from our site are omitted to avoid analysis of duplicate patients; they are included in our Oklahoma data. Census data for adults ages ≥ 18 years were obtained from the United States Census Bureau; Census 2000, PL 94-171 Summary File, using American Fact Finder; http://factfinder.census.gov (July 27, 2004). Data for the cities where the studies were performed were averaged in proportion to the number of patients enrolled from each city. Data on race were not included in the published article6 but were obtained from the original case report forms.
** This study reported data on race for 185 adult patients, ages ≥ 18 years, from 11 sites that enrolled 4 to 46 patients. The 4 patients from our site are omitted to avoid analysis of duplicate patients; they are included in our Oklahoma data. The 23 patients from Canada also are omitted since this analysis is limited to the United States census data for adults ages ≥ 18 years that were obtained from the United States Census Bureau; Census 2000, PL 94-171 Summary File, using American Fact Finder; http://factfinder.census.gov (July 27, 2004). Data for the cities where the studies were performed were averaged in proportion to the number of patients enrolled from each city. Data from the individual sites were not included in the published article6 but were obtained from Amgen, Thousand Oaks, CA.
†† Unpublished data from our record review to begin an Oklahoma ITP Registry. Census data were obtained from the United States Census Bureau; Census 2000 Summary File 1; using American Fact Finder; http://factfinder.census.gov (August 16, 2004). Data for individual Oklahoma counties were averaged in proportion to the number of patients living in each county.
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