Response:

Comments on the estimation of cell membrane alteration after drug treatment by LDH release

We are glad that our paper attracted significant attention from the journal's readership. While we are thankful for the wise comments of Dr Jurisic about technical issues, we cannot agree with all the theses included. It is true that for our in vitro experiments the lactate dehydrogenase (LDH) microassay would have been the better choice; however, the method was not popular and we were not familiar with it at the time the experiments were performed. We also disagree with some of Dr Jurisic's points, particularly regarding 2 issues.

1. Dr Jurisic states that the immunoprecipitation-based cytochrome c assay is more sensitive than the LDH enzymatic assay. The detection of cytochrome c by immunoprecipitation is an undeniably sensitive method, but the LDH assay as an enzymatic method is also sensitive per se. Based on unit definition, we calculated that each single molecule of the released LDH (skeletal muscle-derived isozyme) performs about 42,000 enzymatic reactions within 1 second at 25°C. The reaction velocity is determined by the decrease in absorbance at 340 nm, resulting from the oxidation of nicotinamide adenine dinucleotide, so the signal is strongly amplified. In comparison, a single molecule of cytochrome c can be detected in our immunoprecipitation assay by only a single antibody (no significant amplification of the signal).

2. One has to underline some important differences between cytochrome c and LDH. Both molecules are localized in different cellular compartments: cytochrome c in the mitochondrial intermembrane compartment and LDH in the cytoplasm. The translocation of cytochrome c to the cytoplasm is a prerequisite for the initiation of the apoptotic process. LDH is already available there, and it is separated from the extracellular space by a single lipid (cellular) membrane. Also, the mechanisms of release of both molecules may differ significantly. With a molecular weight of approximately 140 kDa, LDH is about 10 times larger than cytochrome c (molecular weight approximately 14 kDa, inclusive of the coenzyme). Even if it is considered that single subunits of LDH are released separately and reaggregate extracellularly, still, based on the significant size difference, both molecules are likely released by different, yet-to-be-elucidated mechanisms.

Given the distinctions highlighted above, as well as the differences in the release kinetics (Renz et al.) Figure 2C, in vitro data: Table 1 and Figure 4, in vivo data), extracellularly detected cytochrome c and LDH likely indicate different ongoing cellular processes. Nevertheless, both methods are valuable indicators of cell damage in the clinic and under experimental conditions.

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Reference


To the editor:

Diagnostic criteria for acute erythroleukemia

The recent review article by Vardiman et al on the World Health Organization (WHO) classification of myeloid neoplasms described the diagnostic criteria for acute erythroleukemia. Of particular interest to us is the description of acute erythroid/myeloid leukemia. According to the WHO, acute erythroid/myeloid leukemia is defined as having at least 50% erythroid precursors in the entire marrow nucleated cell population and myeloblasts that account for at least 20% of the nonerythroid cell (NEC) population.

We recently have seen in our hospital/consultation service 5 patients (Table 1) with bone marrow aspirates revealing 4%-11.6% myeloblasts and erythroid precursors comprising 58.2%-83.6% of the nucleated cells within the marrow, based on a 500-cell differential count. The percentage of myeloblasts among the nonerythroid cells ranged from 22.6% to 28.4%. None contained more than 30% pronormoblasts, a finding that previously has been shown to be a negative prognostic indicator. None contained sufficient dysplasia to be classified as acute leukemia with multilineage dysplasia, a diagnosis that requires dysplasia in at least 50% of the cells of at least 2 lineages. In addition, the one case with more than 80% erythroid precursors revealed erythroid maturation and did not meet the criteria for pure erythroid leukemia. All 5 cases were diagnosed as acute erythroleukemia.

The transition from the French-American-British (FAB) classification of myeloid neoplasms to the WHO classification included a reduction from 30% to 20% in the required blast percentage within the marrow for a diagnosis of acute leukemia, based upon cohort data indicating similar therapeutic responses and outcomes using these 2 thresholds. To our knowledge, there are no analogous data specifically supporting the changes made to diagnostic criteria for acute erythroblastemia. The difference between 30% and 20% myeloblasts as a percentage of NECs in an erythroid-predominant myeloid neoplasm may not represent a true biologic difference but will certainly be used by clinicians making treatment decisions.

When the 20% blast percentage cut-off is incorporated into the criteria for erythroblastemia described above, the diagnosis of acute leukemia can be rendered with a relatively low myeloblast percentage. In our cases, the most extreme example was 4% myeloblasts within the bone marrow. In addition, the prevalence of erythroid hyperplasia within cases of myelodysplasia may make this scenario more commonplace than is currently recognized, since erythroid-predominant cases will require at most 10% total myeloblasts to fulfill criteria for acute erythroblastemia. A patient with 49% erythroid precursors and 10% myeloblasts would be diagnosed as having refractory anemia with excess blasts, type 2 (RAEB-2); the same patient easily could be diagnosed with acute erythroblastemia if the erythroid precursor percentage were determined to be 51% with the same myeloblast percentage. In
some cases, other causes of erythroid hyperplasia with slightly elevated blast counts also could be diagnosed incorrectly as acute erythroleukemia.

In the absence of data supporting the changes included in the WHO classification, we should critically assess the appropriateness of assigning acute leukemia diagnoses to patients with fewer than 10% (and in one of our cases, fewer than 5%) myeloblasts. Cases of acute erythroleukemia with a low percentage of myeloblasts deserve further study to help determine if there are other relevant clinical, morphologic, and cytogenetic discriminators.

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Table 1. Cell differential, cytogenetic results, and outcomes for patients with acute erythroleukemia

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age, sex</th>
<th>Blasts, % of total</th>
<th>Erythroid precursors, %</th>
<th>Blasts, % of NEC</th>
<th>Cytogenetics</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61, M</td>
<td>4.0</td>
<td>83.6</td>
<td>24.0</td>
<td>Abnormal*</td>
<td>Alive 3 months after diagnosis, no therapy</td>
</tr>
<tr>
<td>2</td>
<td>44, M</td>
<td>7.0</td>
<td>75.4</td>
<td>28.4</td>
<td>Normal male</td>
<td>Relapsed</td>
</tr>
<tr>
<td>3</td>
<td>60, M</td>
<td>7.4</td>
<td>67.2</td>
<td>22.6</td>
<td>Abnormal†</td>
<td>Died of disease 2 months after diagnosis, standard chemotherapy</td>
</tr>
<tr>
<td>4</td>
<td>23, M</td>
<td>11.6</td>
<td>58.2</td>
<td>27.0</td>
<td>Normal male</td>
<td>Clinical remission, S/P ALLO-BMT‡</td>
</tr>
<tr>
<td>5</td>
<td>81, F</td>
<td>7.0</td>
<td>75.0</td>
<td>28.0</td>
<td>Normal female</td>
<td>Lost to follow-up</td>
</tr>
</tbody>
</table>

M indicates male; and F, female.

*Abnormal karyotype included 45-46, XY, −5, −7, add (8)(p21)[2], −15[1], add(17)(p11.2), −21[1], +mar [13].
†Abnormal karyotype included 41-45, X, −Y, del(5)(q33), −16, add(21)(q22), dic(17;20)(p13;q11.2)−20, add(20)(q11.2).
‡Status after autologous bone marrow transplantation.

References


To the editor:

CYP2C9 exon 4 mutations and warfarin dose phenotype in Asians

It is well established that functionally defective variant alleles of CYP2C9 have a major impact on anticoagulation-related outcomes during warfarin therapy. A recent paper by Leung et al2 reported the discovery of 4 new single nucleotide polymorphisms (SNPs) in the coding region of the CYP2C9 gene: L208V, Q192P, H184P, and I181L, with allele frequencies of 0.09-0.75 that may be associated with a low warfarin maintenance dose in whites to elicit the same degree of anticoagulation. 3 Asian subjects from 3 racial groups. The reverse primer exhibits a 100% match to exon 4/intron 4 sequences within CYP2C9.

In assessing variations in the experimental protocols that might underlie the interlaboratory differences, we re-examined the primers chosen for amplification of exon 4 by Leung et al. The first 10 bases of the forward primer and 8 bases of the reverse primer (5’ to 3’) appeared to have been included to incorporate a unique restriction endonuclease site into the resulting amplicon. Analysis of the remaining primer sequence revealed that the forward primer exhibits a 100% match to exon 4 sequences within CYP2C8, CYP2C9, CYP2C18, and CYP2C19. The reverse primer exhibits a 100% match to exon4/intron4 sequences within CYP2C9 and CYP2C19, although the 3’ end of the primer shows considerable identity to sequences with CYP2C18 and CYP2C19 and might well have resulted in some amplification at those loci as well. In any event, it is clear that the primers used by Leung et al are not adequate for SNP discovery, as they will result in a mixed template from multiple loci. These observations call into question the presence of the L208V, Q192P, H184P, and I181L variants of