To the editor:

**Estimation of cell membrane alteration after drug treatment by LDH release**

Renz et al. analyzed rapid release of cytochrome \(c\) after treatment of the Jurkat cells with agonistic anti-CD-95 monoclonal antibody, staurosporin, etoposide, and doxorubicine. Authors estimated cell death by release of large amounts of intracellular cytochrome \(c\) simultaneously with lactate dehydrogenase (LDH) in supernates of treated cells. LDH release in supernates of treated cells was not detected, although cytochrome \(c\) release and apoptosis by flow cytometry were detected.

The method for measuring the quantity of cytochrome \(c\) release was based on sensitive immunoprecipitation and subsequent immunoblotting. Contrary to this, LDH release in supernates from treated tumor cells was analyzed by routine LDH method, commonly used in clinical laboratory, based on consideration that LDH is a good clinical marker for estimation of tumor burden.

Determination of LDH activity by routine method for cell culture experiments is not suitable, due to its too-low sensitivity. Microassay for estimation of cell death process in vitro, by LDH release, was recommended. The assay is based on substrate mixture, using the small volume of culture cells or their supernates. The results are expressed as absorbance, not in international units, for better interpretation.

Determination of LDH release from cultured cells by microassay shows values that were significantly different, depending on cell type (tumor or normal), cell number, cell activation status, and separation process. Using corrections, LDH release assay also can be used as a sensitive indicator for natural killer cell activity estimation. Safety of evaluation of the vaccine and virus toxicity effects were performed by LDH micro assay as well.

Contrary to data reported by Renz et al, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) induced LDH release significantly from tumor, K-562, and Raji cells in a dose- and time-dependent manner rapidly after 2 hours, measured by a microassay. The percentage of LDH release from TNF-\(\alpha\)-treated tumor cells, expressed in terms of total intracellular enzyme activity, correlates with a decrease of intracellular enzyme activity, representing metabolic alteration; with a decrease of cell growth by \([3H]\)thymidine incorporation into DNA; and with a decrease of antigen expression, determined by flow cytometry.

The phenomenon of the cell membrane permeability for LDH was based on the high intracellular LDH values as well as an alteration in transport channels or pore forming during cell activation or the apoptosis process. The cytochrome \(c\) request transports through 2 intracellular compartments, including mitochondria membrane to cytoplasm and from cytoplasm to extracellular space, for detection. Cytochrome \(c\), a marker of mitochondrial alteration during apoptosis if it is released whenever the cell membrane is disintegrated, LDH as intracellular enzymes should be released as well.

The mechanisms for LDH and cytochrome \(c\) release during the apoptosis process were different and very complex. Although these 2 processes indicated diverse transports, probably including additional secretion or disintegration of the LDH molecule from cytoplasm to cell surface membrane, which were not definitively examined, exact measurements using sensitive and highly recommended assays are suggested.

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**References**

Response:

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

We are glad that our paper\(^1\) 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.\(^1\) 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\(^1\) described the diagnostic criteria for acute erythroleukemia. Of particular interest to us is the description of acute erythroid/myeloid leukemia. According to the WHO,\(^2\) 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.\(^3,4\) 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.\(^2\) In addition, the one case with more than 80% erythroid precursors revealed erythroid maturation and did not meet the criteria for pure erythroid leukemia.\(^2\) 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 erythroleukemia. 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 erythroleukemia 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\(^5,6\) 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 erythroleukemia. 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 erythroleukemia if the erythroid precursor percentage were determined to be 51% with the same myeloblast percentage. In