Upsala J Med Sci 98: 293–298, 1993

6.1.1.3 Analytical Bias by Contamination from Hemolysis in Determination of Serum Lactate Dehydrogenase Isoenzyme 1 in Patients with Testis Germ Cell Tumors

Finn Edler von Eyben, Per Hyltoft Petersen, Ole Blaabjerg, Ebbe Lindegaard Madsen, Bent Nørgaard-Pedersen and Jørgen Arends

Departments of Clinical Chemistry, and Radiotherapy and Oncology, Odense University Hospital, Odense, and Department of Clinical Biochemistry, Statens Seruminstitut, Copenhagen, Denmark

ABSTRACT

We investigated the impact of correction for contamination from hemolysis on serum lactate dehydrogenase isoenzyme 1 (S-LD-1) determinations. A study of hemolysates from 7 control patients showed a mean correction factor for the contamination of 0.1 U/L S-LD-1 for each 1 mg/L serum hemoglobin (S-Hb). S-LD-1 in a series of blood samples from 44 patients (EJC 1992;28:410-5) would decrease median 24 U/L (range 8 - 70 U/L) if the measurements were corrected with this factor. So we advice to correct S-LD-1 determinations for the contamination with a common correction based on the S-Hb concentrations in the samples.

CLINICAL SITUATION

Patients with metastatic testicular germ cell tumors have a good chance of cure by platin-based combination chemotherapy (1). The outcome can be predicted at start of treatment from clinical characteristics such as tumor load and serum tumor marker levels (serum lactate dehydrogenase (S-LD, EC 1.1.1.27) and S-LD isoenzyme 1 (S-LD-1)) (1, 2). The patients either obtain longterm diseasefree survival (survivors) or die of tumor (nonsurvivors). The patients who are considered to have a high risk of a poor outcome may receive a more intense and toxic chemotherapeutic regimen than those considered to have a high chance of a good outcome (longterm diseasefree survival) from standard dose platin-based combination chemotherapy (1).

CHARACTERISTIC OF THE METHOD

S-LD-1 was analyzed by an immunochemical method with the Scandinavian determination of S-LD activity; serum hemoglobin concentration (S-Hb) with a spectrophotometric method (1).

Both the biological variation in the examined patients and the contamination caused by in vitro hemolysis in the analyzed blood sample may influence S-LD-1 measurements. Previously, we have estimated the contamination in blood samples from S-Hb and described a considerable variation for S-Hb (3). We have also described the impact on hemolysis from transportation of the unseparated samples (the preinstrumental phase) before the measurement (3). In the present study, we evaluate our correction for the measured contamination of the measured S-LD-1 value as an optimalization of the instrumental phase of the S-LD-1 determination.

MODEL FOR EVALUATION OF QUALITY SPECIFICATIONS

A series of 44 patients with metastatic testicular germ cell tumors where we measured S-LD-1 at start of chemotherapy illustrates aspects from correcting S-LD-1 determination for the contamination by hemolysis (1). The measured S-LD-1 was median 169 U/L (range 58 - 2795 U/L) and the median S-Hb 239 mg/L (range 79 - 695 mg/L). 33 became disease-free and 11 died of tumor.

We determined the between-subject variation in the individual correction factor in a study of blood from 7 cured patients (3). Fresh-drawn EDTA blood was centrifugated, the plasma was removed, and the erythrocyte concentrates were washed with normal saline (0.9% NaCl solution). After dilution 1:50 with distilled water concurrent values for LD-1 and Hb concentrations were determined in the hemolysates (3). The mean of the correction factors was 0.107 (0.107 U/L S-LD-1 for each 1 mg/L S-Hb), s = 0.011, and the 0.95 statistical coverage 0.085 - 0.129.

We used 0.1 as a common correction factor. This correction, however, does not consider the between-subject variation in the factor.

We defined the optimal cut-off limit for S-LD-1 for the patients as the level where as many as possible of the nonsurvivors are included due to a high S-LD-1 in the group predicted to risk a poor outcome and as many as possible of the survivors in the group predicted to achieve a good outcome due to a low S-LD-1.

EVALUATION OF QUALITY SPECIFICATION

Median S-LD-1 in our series was decreased 24 U/L (range 8 - 70 U/L) as we corrected S-LD-1 for the contamination by hemolysis. After correction all nonsurvivors had S-LD-1

above 150 U/L. Figure 1 shows how analytical bias on this cut-off limit would influence the proportion with low values in the groups of survivors and non-survivors.

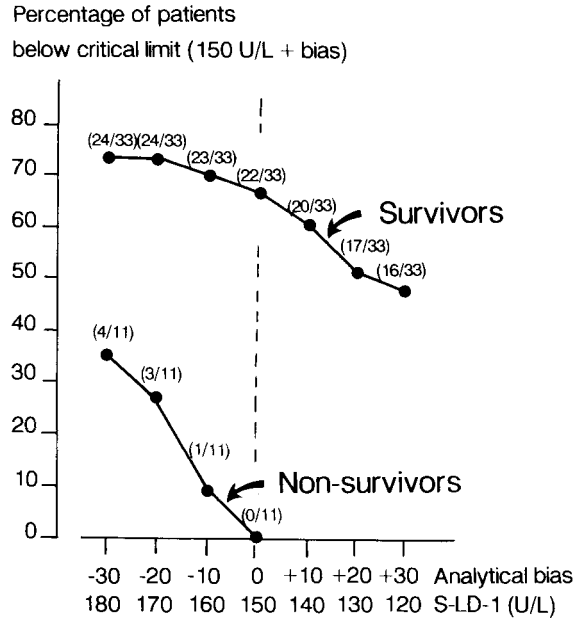

Figure 1 shows the impact from bias on proportion of survivors and nonsurvivors with S-LD-1 below a cut-off limit of 150 U/L if S-LD-1 is corrected for hemolysis by the factor 0.1.

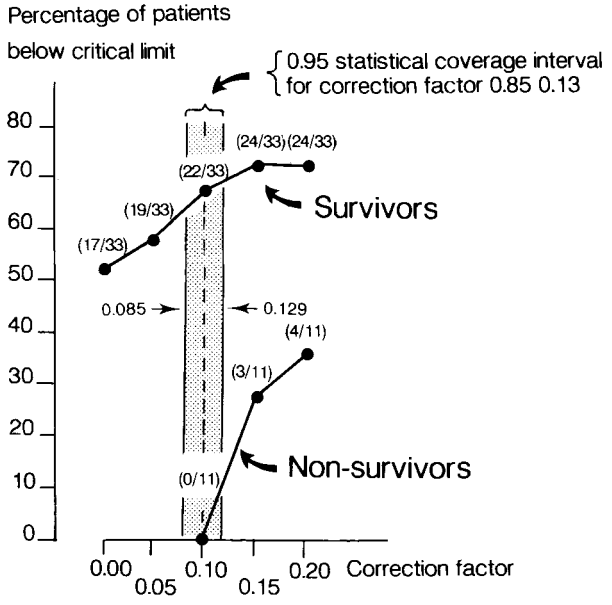

Figure 2 shows the impact from variation of correction factor within 0.95 statistical coverage interval for the correction factor (0.085-0.129) in the controls on proportion with S-LD-1 below the cut-off limit of 150 U/L for the survivors and the nonsurvivors.

Figure 2 shows how the proportion with low S-LD-1 values in the groups of survivors and non-survivors would vary with correction factors within the 0.95 statistical coverage interval for the correction factor in the controls. 11 died from tumor of 23 patients (48%) with

S-LD-1 above 150 U/L if the correction factor was 0.085. Similarly, 10 died from tumor of 21 patients (48%) with S-LD-1 above 150 U/L with 0.129 as correction factor. So the prediction of outcome from a pretreatment S-LD-1 varied only little within the 0.95 statistical coverage interval for the correction factor.

DESIGN OF CONTROL SYSTEM

All S-LD-1 measurements are corrected for the contamination by hemolysis as part of our internal control system regarding S-LD-1 determination for this group of patients. We routinely determine S-Hb and correct the S-LD-1 measurement according to the mean correction factor. Thereby, the imprecision from neglecting the inter-individual variation of the correction factor would not exceed the maximum allowable imprecision of the S-LD-1 assay unless the blood sample has a S-Hb above ≈ 650 mg/L (3).

Both the inter-laboratory variation for the S-LD-1 and S-Hb determinations and for the corrected S-LD-1 should be evaluated as part of the external control system (the instrumental phase). It should also limit the extent of hemolysis in the blood samples before determination of S-LD-1, especially if sent by mail (the preinstrumental phase).

DISCUSSION

S-LD-1 separates patients with metastatic testicular germ cell tumors at start of chemotherapy in two groups with different outcome. A pretreatment S-LD-1 may be used to prospectively to classify these patients in a good- and a poor-risk group. The corrections for contamination by hemolysis in our series were considerable (8-70 U/L S-LD-1) in relation both to the range of corrected reference values (52 - 114 U/L (3)) and the present cut-off limit (150 U/L). However, the patients should not be misclassified due to the contamination. Therefore we advice a routine correction in all samples with S-Hb <650 mg/L. Due to the grave consequences from a misuse of S-LD-1 in the tumor patients, the measured value should be optimally corrected for the contamination by hemolysis. Our control system allows us routinely to report the S-LD-1 results corrected for hemolysis to the clinician without any significant delay from the correction.

Our individual correction factors correspond with two previous studies of the contamination for the S-LD-1 determination (4,5).

Further retrospective and prospective studies of patients with testicular germ cell tumors might validate the model. It may also be studied in other clinical settings where it is recommended to determine S-LD-1, for instance concerning the diagnosis of myocardial infarction. The level of corrected cut-off values for clinical decisions should be specified for the clinical problems where determination of S-LD-1 is recommended.

CONCLUSION

S-LD-1 stratifies patients with metastatic testicular germ cell tumors in good- and poor-risk. All S-LD-1 measurements should be corrected for the contamination by hemolysis with a common correction factor of 0.1, if S-Hb is < 650 mg/L. The S-Hb should be determined and the contamination by hemolysis corrected without a significant delay in reporting the S-LD-1 value to the clinician.

It is possible to optimize the cut-off limit for S-LD-1 in this clinical situation. It is also possible to evaluate how varying correction factor and bias influence the stratification.

REFERENCES

1. von Eyben, F.E., Blaabjerg, O., Lindegaard Madsen, E., et al. Serum lactate dehydrogenase isoenzyme 1 and tumor volume are indicators of response to treatment and predictors of prognosis in metastatic testicular germ cell tumors. Eur J Cancer 1992;28:410-5.
2. von Eyben, F.E., Krag Jacobsen, G., Pedersen, H., et al. Multivariate analysis of risk factors in patients with metastatic testicular germ cell tumors treated with vinblastine and bleomycin. Invasion and Metastasis 1982;2:125-135.
3. von Eyben, F.E., Hyltoft Petersen, P., Lindegaard Madsen, E., et al. Contamination from hemolysis in measurements of lactate dehydrogenase isoenzyme 1 in serum. Submitted, 1992.
4. Hansen, J., Helbech, A. & Petersen, B. Retningslinjer for hæmolyse. Dept. Clin. Chem., Odense University Hospital, 1991-11-19.
5. von Eyben, F.E., Blaabjerg, O., Hyltoft Petersen, P., et al. Serum lactate dehydrogenase isoenzyme 1 as a marker of testicular germ cell tumor. J Urol 1988;140:986-990.

Correspondence:
Finn Edler von Eyben
Department of Internal Medicine
Fylkessjukehuset i Lærdal
N-5890 Lærdal
Norway