in a temperature-controlled environment. In this format, multiple replicate measurements can be made, increasing the precision of the assay and allowing the use of quality-control materials in each assay. Although the CD assay may never become a routine clinical assay for the assessment of lipid peroxidation, it can now be applied to specimens from large clinical trials and epidemiologic surveys.

We thank Mark Witham of Bio-Tek Instruments, Inc. (Winoooski, VT) and Ken Brown of Bio-Resources, Inc. (St. Louis, MO) for providing access to preproduction versions of the PowerWave 200 instrument.

References


Evaluation of the Measurement of Lysate Homocysteine in Patients with Symptomatic Arterial Disease and in Healthy Volunteers, Richard Brandl,1 Reiner Probst,2 Burkhard Müller,3 Sibylle Powarzynski,3 Peter Carl Maurer,3 and Dieter Neumeier2 (Klinikum rechts der Technischen Universität München, 1 Institute of Vascular Surgery and 2 Institute of Clinical Chemistry and Pathobiology, Ismaninger Strasse 22, 81675 Munich, Germany; *author for correspondence: fax 49-89-41404875)

Because of the increasing interest in routine clinical measurement of plasma total homocyst(e)ine (tHcy), it is necessary to simplify the critical preanalytical phase, especially the centrifugation step required immediately after blood collection to separate homocysteine-producing and -releasing blood cells from plasma (1–4). To overcome this procedural problem, which leads to falsely increased tHcy results when sample transport is prolonged, we recently developed a blood collection system that stabilizes tHcy in lysed whole blood (lysHcy) for at least 2 days at room temperature without requiring a centrifugation step (5). Because of the dilution with
plasma with intracellular liquid, the lysHcy concentrations measured in the lysate system are lower than the homocysteine concentrations measured in the tHcy system; therefore, the aim of the present study was to evaluate the measurement of lysHcy in patients with symptomatic arterial disease and healthy volunteers, using the determination of tHcy as a reference method. tHcy and lysHcy determinations were compared with multiple linear regression analysis, taking into account age, and the concentrations of tHcy as a reference method. tHcy and lysHcy concentrations were slightly higher (246 ± 151 vs 257 ± 87 pmol/L) in the volunteers (P < 0.05). Creatinine was significantly higher (85 ± 49 vs 69 ± 13 μmol/L) in patients (P < 0.005), who were significantly older than volunteers, with a mean age of 66 ± 12 years compared with 41 ± 15 years.

The tHcy cutoff was 8.5 μmol/L and was calculated from the regression equation \[ c_{\text{tHcy}} = 0.59 + 0.53 c_{\text{tHcy}} \] obtained from linear regression analysis of all tHcy concentrations. The area under the ROC curve (AUC) for tHcy (AUC, 0.77; 95\% confidence interval, 0.72–0.81) was not significantly different (P < 0.05) from tHcy for patients and volunteers, who were significantly older than volunteers, with a mean age of 66 ± 12 years compared with 41 ± 15 years.

The lysHcy cutoff was 8.5 μmol/L and was calculated from the regression equation \[ c_{\text{lysHcy}} = 0.59 + 0.53 c_{\text{lysHcy}} \] obtained from linear regression analysis of all lysHcy concentrations (n = 315) and the generally accepted tHcy cutoff concentration of 15.0 μmol/L (9).

The tHcy cutoff (15 μmol/L) was exceeded by 42.3\% of the patients and 15.4\% of the volunteers; the lysHcy cutoff (8.5 μmol/L) was exceeded by 46.8\% of the patients and 13.2\% of the volunteers. The areas under the ROC curves (AUC) for tHcy (AUC, 0.77; 95\% confidence interval, 0.72–0.81) and lysHcy (AUC, 0.76; 95\% confidence interval, 0.71–0.81).

Multiple linear regression analysis (Table 1) showed that both tHcy and lysHcy correlated positively with creatinine (P ≤ 0.005) and age (P < 0.005) and correlated differences between volunteers and patients were significant for lysHcy (P < 0.005) and tHcy (P < 0.005). The bottoms of the boxes represent the 25th, and the tops of the boxes represent the 75th percentile. The lines within the boxes represent the 50th percentile (median value).

Fig. 1. Box-and-whiskers plot comparing lysHcy and tHcy concentrations in healthy volunteers and patients.

Differences between volunteers and patients were significant for lysHcy (P < 0.005) and tHcy (P < 0.005). The bottoms of the boxes represent the 25th, and the tops of the boxes represent the 75th percentile. The lines within the boxes represent the 50th percentile (median value).
negatively with folate \( (P < 0.005) \). Differences in the regression coefficients for lysHcy and tHcy reflected the different concentration ranges of these systems. Neither lysHcy nor tHcy depended significantly on vitamin B\(_{12}\) concentrations. Goodness of fit statistics for tHcy and lysHcy were 0.249 and 0.250 for \( r^2 \).

Several well-designed cross-sectional and case-control studies have clearly shown evidence that tHcy is a major independent risk factor for PAOD, cardiovascular morbidity, and death \( (10–12) \). The determination of tHcy has been shown to be sensitive to preanalytical handling \( (1–4) \), and in daily routine, the determination of tHcy commonly lacks standardized preanalytical processing conditions. An accurate determination of tHcy is desirable for several reasons. Several studies of carotid and coronary atherosclerosis, myocardial infarction, and venous thrombosis indicate that there is a linear relationship between tHcy concentrations and risk, rather than a threshold value, and that tHcy is pathologically active even at concentrations below the currently discussed cutoff of 15 \( \mu \text{mol/L} \) \( (13, 14) \). Verhoeof et al. \( (15) \) and Nygard et al. \( (12) \) found that increases of tHcy concentrations of up to 3 or 5 \( \mu \text{mol/L} \) produced odds ratios or mortality ratios between 1.35 and 1.9. The mean increase of tHcy in EDTA blood is approximately 10% per hour if blood cells are not separated after blood collection \( (7, 16) \). This corresponds diagnostically to an estimated 1.3-fold increase in risk for a patient if blood is left standing for 3 h, given an initial tHcy concentration of 10 \( \mu \text{mol/L} \). To avoid this overestimation of risk, the aim of the present study was to establish the robust lysHcy method \( (5) \) for a safe and clinically reliable determination of tHcy for routine clinical use.

We did not use the ROC to derive a cutoff for two reasons: the sensitivity and specificity for both tHcy and lysHcy are quite low because of a significant overlap (Fig. 1) of the respective distributions, and the two groups are not comparable in age. The association of tHcy and creatinine, described in 1992 by Chauveau et al. \( (17) \), could be confirmed for both tHcy and lysHcy determinations (Table 1). In 13.8% of patients, creatinine concentrations exceeded the respective cutoff concentrations for males and females and were accompanied by increased tHcy \( (20.3 \pm 7.8 \mu \text{mol/L}) \) and lysHcy \( (11.7 \pm 4.4 \mu \text{mol/L}) \). Of this group, 74% had tHcy concentrations >15 \( \mu \text{mol/L} \) and 70% had lysHcy concentrations >8.5 \( \mu \text{mol/L} \).

Creatinine concentrations were not increased in any of our volunteers. Although the folate concentrations were similar in the patients and volunteers, regression analysis showed that there was a close relationship between folate and tHcy or lysHcy (Table 1). The current lack of commercialized blood collection systems for lysHcy determination will be overcome in the near future: Bio-Rad Germany currently is establishing a similar system that uses stabilized lysed capillary blood instead of lysed venous blood.

In summary, our data show that the prevalence of increased lysHcy is increased in patients with systemic atherosclerosis. Because of the 2-day stability of lysHcy in our blood collection system and the good comparability of tHcy and lysHcy determinations, the latter is the more reliable indicator for atherosclerotic risk assessment in the clinical routine, especially if rapid sample transport from the patient to the laboratory is not guaranteed.

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### References

6. European Carotid Surgery Trialists' Collaborative Group. MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70%–99%) or with mild (0%–29%) carotid stenosis. Lancet 1991;337:1235–43.

### Table 1. Comparison of multiple linear regression analyses of all measured values \( (n = 315) \), with lysHcy and tHcy as dependent variables, and vitamin B\(_{12}\), folate, creatinine, and age as independent variables.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>lysHcy Coefficient</th>
<th>SE</th>
<th>tHcy Coefficient</th>
<th>SE</th>
<th>lysHcy ( P )</th>
<th>tHcy ( P )</th>
</tr>
</thead>
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<tr>
<td>Vitamin B(_{12})</td>
<td>−0.00113</td>
<td></td>
<td>−0.00336</td>
<td></td>
<td>0.00112</td>
<td>0.00194</td>
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<tr>
<td>Folate</td>
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<td></td>
<td>−0.60026</td>
<td></td>
<td>0.07071</td>
<td>0.12290</td>
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<tr>
<td>Creatinine</td>
<td>1.28686</td>
<td>2.16932</td>
<td>0.44191</td>
<td>0.76763</td>
<td>0.0039</td>
<td>0.0051</td>
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<tr>
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<td>0.12451</td>
<td>0.01223</td>
<td>0.02121</td>
<td>0.0000</td>
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