
Table 1. Plasma HCy over time from collection to separation of plasma from blood cells after collection in EDTA or fluoride tubes (n = 22).

<table>
<thead>
<tr>
<th>Time between collection and separation</th>
<th>EDTA</th>
<th>Fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD), μmol/L</td>
<td>Change (SD) from time 0, %</td>
</tr>
<tr>
<td>0 h</td>
<td>12.5 (6.3)</td>
<td>8.5 (4.1)</td>
</tr>
<tr>
<td>1 h</td>
<td>13.6 (7.0)</td>
<td>21.1 (5.4)</td>
</tr>
<tr>
<td>2 h</td>
<td>15.0 (7.1)</td>
<td>39.9 (9.9)</td>
</tr>
<tr>
<td>5 h</td>
<td>17.2 (7.4)</td>
<td>39.9 (9.9)</td>
</tr>
</tbody>
</table>

Our aim was to evaluate the protective effect of fluoride against spurious increases of in vitro HCy and to establish reference intervals for the AxSYM immunoassay (Abbott) for plasma HCy from blood collected into fluoride tubes.

The HCy assay was performed on the AxSYM analyzer according to the manufacturer’s instructions. Folic acid and vitamin B12 measurements were performed on the Elecsys analyzer (Roche Diagnostics) according to the manufacturer’s instructions.

For the study of a possible fluoride protective effect, blood was drawn from 22 individuals and collected in EDTA and fluoride-oxalate tubes (Terumo). Fluoride-oxalate tubes contained 6.75 mg of lyophilized sodium fluoride (54 mmol/L of blood). Plasma was separated immediately (centrifugation started within 2 min) and after 1, 2, and 5 h at room temperature. The HCy assay was performed on each aliquot. Two hundred and thirty women (mean age, 41 years; range, 24–59 years) and 126 men (mean age, 42 years; range, 23–63 years) who are workers in Erasme Hospital (Brussels) were included in the reference interval and vitamin correlation studies. The study was approved by the local ethics committee. For the establishment of plasma HCy reference values, fasting venous blood was drawn in fluoride-oxalate tubes transported on crushed ice to the laboratory, where plasma was immediately separated from blood cells and analyzed within 2 h. For vitamin B12 and folic acid measurements, serum was analyzed within 4 h after collection. Statistical analyses were performed with Analyze-It for Microsoft Excel.
Hyperhomocysteinemia is a well-documented cardiovascular risk factor (1–3). Because folic acid and vitamin B₁₂ supplementation can return plasma HCy concentrations to values within the reference intervals (13, 14), accurate measurement of this analyte is desired. Preanalytical factors are important. It has been shown that HCy concentrations increase by ~10% per hour in unseparated whole blood, whereas at 0 °C, no significant increase in HCy occurs during the first hour (4, 11). Nevertheless, in a recent study, only 16% of samples with HCy requested arrived at the laboratory within 1 h, and 28% of samples arrived at room temperature (15), showing a potential useful role for a preservative agent such as fluoride. Although several authors have described the protective effect of this additive (8, 9), in a recent study (16), plasma HCy increased by 9% after 1 h and 24% after 4 h in whole blood samples collected in EDTA tubes and stored at room temperature. In fluoride tubes, similar increases of 8–24% were observed. Hughes et al. (10) even suggested that the protective effect was only apparent. Our results did not confirm these data. In similar conditions, for fluoride tubes, we observed increases of 2.5% after 1 h, 6.5% after 2 h, and 14.8% after 5 h, whereas in EDTA tubes, we observed increases of 8.5% after 1 h, 21.1% after 2 h, and 40% after 5 h. This suggests that fluoride can be an essential additive, especially when plasma separation cannot be achieved quickly or when samples have not been kept at 0 °C. Like others, we found lower HCy concentrations in fluoride tubes than in EDTA tubes. Although some authors believe that these lower concentrations are caused by dilution of plasma as a result of dehydration of red cells in the presence of fluoride (4, 16), our data showing a much lower rate of increase in fluoride tubes compared with EDTA tubes suggest that this is truly a protective effect of fluoride. Age, sex, vitamin B₁₂, and especially folic acid status are known to be determinants of plasma HCy concentrations (1, 3, 16). Our results were in accordance with the literature. There was a statistically significant negative correlation between serum folate and plasma HCy and between serum vitamin B₁₂ and plasma HCy, but the correlation between age and HCy did not reach statistical significance. We found significantly higher HCy concentrations in men than in women (medians, 9.8 vs 8.3 μmol/L) and reference values (4.7–16.3 μmol/L) similar to, although slightly higher than, those commonly recommended (5–15 μmol/L) (1, 16).

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References

Hepatectomies were performed in 92 patients (47 women, 45 men). The mean (±SD) age was 57 ± 12 years, body weight was 70 ± 11 kg, the ratio of actual to ideal body weight was 1.13 ± 0.16 (1983 Metropolitan Tables), body surface area was 1.78 ± 0.15 m², and body mass index (weight/height²) was 25.0 ± 4.0 kg/m². Thirty-six patients had primary liver malignancy (23 with hepatocarcinoma, 10 with cholangiocarcinoma, 3 with other neoplasms), 35 had secondary hepatic malignancies (23 from colorectal cancer, 12 from other sources), and 21 had benign lesions. Eighteen patients had liver cirrhosis. Fifty-four patients were in ASA class I (7), in class II, 30 in class III, and 1 in class IV. No patient was on cholesterol-lowering medication. Hepatectomies consisted of 43 minor (<3 liver segments) and 49 major resections (3–6 segments). The mean number of resected segments was 3 ± 1. There were 17 associated bowel operations (resections for primary malignancy or Roux-en-Y biliary reconstructions). The duration of the operations was 390 ± 149 min, and the duration of normothermic liver ischemia (used in 61 patients) was 49 ± 28 min.

Seventy-one patients recovered without complications, whereas 15 had nonlethal complications: 9 had intra-abdominal or pulmonary sepsis, 5 had transient liver insufficiency, and 1 had a biliary fistula without sepsis. Diagnosis of sepsis was based on previously defined criteria (5). Six patients died. The study was carried out prospectively except for the inclusion of three nonsurvivors observed outside the prospective period; this improved the significance of results in nonsurvivors without bias because the pattern of death was similar in all cases (systemic sepsis with liver and/or respiratory insufficiency, progressing to multiple organ dysfunction syndrome). This patient population provided a continuous distribution of observations from minor to extreme surgical procedures (and degrees of postoperative illness) suited to assess correlates of hypcholesterolemia over a wide range of pathophysiologic abnormalities.

The database included 478 venous blood measurements. These were performed according to the clinical routine, without the need for consent, preoperatively and on postoperative days 1, 3, and 7 in all patients and thereafter only in those with complications until recovery or death. The following variables were considered: plasma cholesterol concentration, albumin, total protein, fibrinogen, creatinine, urate, alkaline phosphatase, γ-glutamyltranspeptidase, total and indirect bilirubin, prothrombin activity, hematocrit, hemoglobin, blood cell counts, number of resected liver segments, duration of the operation and of eventual liver ischemia, occurrence of cirrhosis, neoplastic disease, previous chemotherapy, associated bowel operations, sepsis, cholestasis, and substrate doses in patients on parenteral nutrition. Statistical analysis was based on least-squares regressions and “best fit” procedures selecting the simplest possible regressions controlling the largest possible variability of cholesterol, based on Mallows’ Cp criteria (8).

Plasma cholesterol decreased on postoperative days 1.