measuring human serum liver-type ARG in diagnosis of hepatic disorders (9). However, a lack of knowledge about the turnover of ARG has hampered a wider application of this enzyme for clinical diagnosis. As shown in Fig. 1, our results indicate that liver-type ARG is increased substantially in the acute phase of liver disorders.

We suggest that after induction of liver damage with carbon tetrachloride and ischemia-reperfusion injury, liver-type ARG, in comparison with the other enzymes tested in this study, leaks most profusely into the blood from the damaged liver. In an immunohistochemical study (data not shown), massive disappearance of liver-type ARG from hepatocytes immediately after liver damage was demonstrated. This further supports our hypothesis that liver-type ARG is an enzyme that reflects damage to hepatocytes regardless of the cause. The release of liver-type ARG into the bloodstream appears to differ from the release of AST and ALT (Fig. 1), supporting the leak-prone character of ARG.

ALT and AST are markers of liver cytolysis (4, 14, 15). During a short period after the injection of the chemical agent, liver-type ARG increased more rapidly than ALT (Fig. 1), indicating that the clinical value of the enzyme could be equal or superior to that of ALT, which supports an earlier study indicating that after partial hepatectomy, liver-type ARG increased more dramatically than ALT and AST (5).

OCT leaks out of hepatocytes into the bloodstream through the mitochondrial and plasma membranes, whereas ALT, which is a cytosolic enzyme, and liver-type ARG leak only through the plasma membrane. The difference in the pattern of increase of ARG compared with OCT and the transaminases may be attributable to their differential localization in hepatocytes, as well as the fact that liver-type ARG has a smaller molecular mass than other hepatic-marker enzymes (13, 16).

Our results indicate that among the hepatic enzymes tested in the present study, the release of liver-type ARG may be unique, particularly in the acute phase of hepatic-cellular injury, and that the measurement of ARG has its own clinical merit for detecting hepatic lesions.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (to M.I.). We thank Kiyoyuki Takahashi of Kyoto University for technical instruction and helpful advice for immunohistochemical staining.

References

6. Yu Y, Terada K, Nagasaki A, Takiguchi M, Mori M. Preparation of recombi-

Cerebrospinal Fluid Cytokeratins for Diagnosis of Patients with Central Nervous System Metastases from Breast Cancer, György Sélétormos* and Fleming Bach† (*1 Department of Clinical Biochemistry, Hillerød Hospital, Helsevej 2, DK-3400 Hillerød, Denmark; †2 Department of Oncology, Ålborg Hospital, DK-9000 Ålborg, Denmark; *author for correspondence: fax 45-48-24-00-67; e-mail geso@fa.dk)

In cases of breast cancer, the diagnoses of parenchymal brain metastases and leptomeningeal carcinomatosis are often delayed (1). New inexpensive biochemical diagnostic initiatives have suggested measurement of cytokeratin tumor markers in the cerebrospinal fluid (CSF) (2, 3). Each type of epithelial cell can be characterized by its cytokeratin polypeptide content because the expression pattern varies with the type of epithelium (4–6). Malignant epithelial tumors and their metastases maintain the cytokeratin expression typical for the tissue of origin (4, 7, 8). Cytokeratins 8, 18, and 19 are abundantly expressed in adenocarcinoma of the breast, only weakly expressed in epithelial cells of the choroid plexus, and not expressed in nerve, glial, ependymal, and meningeal cells (9). Because neither healthy nor malignant epithelial cells are known to secrete cytokeratins, how they appear in the CSF remains unexplained. Some authors have suggested that cytokeratins are released from the large fraction of dead and dying cells in growing tumors (5), whereas others have suggested that cytokeratins are markers of cell proliferation released during mitosis (10). In either
The diagnostic accuracy of medical tests and for leptomeningeal carcinomatosis. For classification of patients, we implemented the terminology described by Altman (12). The categorical variables true-positive (TP) marker test results, false-negative marker test results, false-positive (FP) marker test results, and true-negative marker test results were compared with Fisher’s test. Variables were statistically comparable between the single markers of TPA and TPS but were not statistically comparable between a single marker and the combination of TPA and TPS. A P value <0.05 was considered statistically significant. The confidence intervals for frequencies were calculated according to Wulf and Schlichting (13). The terminology of Zweig and Robertson (14) was used for calculating the ROC curves.

All 70 enrolled patients were eligible for the study, and no subjects were excluded. ROC curves of the correlated TP and FP rates for a series of cutoff points for each marker are provided in Fig. 1. We considered 65 and 95 units/L as tentative optimal cutoff values for TPS and TPA, respectively. When we used these values, the TP rates were 85% (TPS) and 74% (TPA), and the FP rate was 0% for both markers (Table 1).

TPS was determined in CSF samples from 60 patients, TPA was determined in CSF samples from 59 patients, and both TPS and TPA were determined in CSF samples from 49 patients. With regard to the number of patients without metastases (FP plus true-negative marker test results), the numbers of individuals shown for specificity vary among the three columns in Table 1, not because of different cutoff values but because of the procedure used to calculate data for specificity. When investigating the accuracy of the markers to identify and exclude any CNS metastases, we classified increased marker concentrations associated with parenchymal brain metastases, as well as with leptomeningeal carcinomatosis as TP test results. When investigating the accuracy of the markers to identify and exclude parenchymal brain metastases, we clas-
sified increased marker concentrations associated with leptomeningeal carcinomatosis as FP results. When investigating the accuracy of the markers to identify and exclude leptomeningeal carcinomatosis, we classified increased marker concentrations associated with parenchymal brain metastases as FP results. Neither TPS nor TPA was able to discriminate breast cancer metastases involving the meninges from those confined to the brain parenchyma (Table 1). With regard to any CNS metastases, TPS and TPA supplied similar diagnostic information (P > 0.1, Fisher’s test), and each marker identified ~80% of patients with CNS metastases.

We found that there was no diagnostic gain when we combined TPS and TPA because none of the patients had increased concentrations of TPS when they had TPA concentrations within reference values, or vice versa; therefore, only one of these markers should be measured. Given the high positive predictive value, the confidence intervals, and the low costs of a cytokeratin test, our data support the view that cytokeratin measurements may be of use as part of a diagnostic protocol for patients suspected of CNS metastases. Thus, breast cancer patients with increased cytokeratin concentrations associated with healthy CNS scan results and the absence of tumor cells in the CSF may have to be evaluated in terms of initiating or continuing further systemic treatment to avoid unnecessary toxicity associated with an ineffective chemotherapy. On the basis of the presented results, we find the data sufficiently encouraging to further investigate the use of cytokeratins as part of a diagnostic strategy in patients suspected of CNS metastases from breast cancer.

Table 1. Diagnostic accuracy \(^a\) of cytokeratin measurements in CSF obtained from breast cancer patients suspected of CNS metastases.

<table>
<thead>
<tr>
<th>Sensitivity, %</th>
<th>Parenchymal brain metastases</th>
<th>Leptomeningeal carcinomatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any CNS metastases</td>
<td>85 (68–95)</td>
<td>88 (64–99)</td>
</tr>
<tr>
<td>TPS</td>
<td>74 (52–90)</td>
<td>69 (39–91)</td>
</tr>
<tr>
<td>TPS + TPA</td>
<td>82 (60–95)</td>
<td>85 (55–98)</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>100(^b) (87–100)</td>
<td>70 (54–83)</td>
</tr>
<tr>
<td>TPS</td>
<td>100(^b) (90–100)</td>
<td>83 (62–89)</td>
</tr>
<tr>
<td>TPS + TPA</td>
<td>100(^b) (87–100)</td>
<td>81 (64–92)</td>
</tr>
<tr>
<td>Positive predictive value, %</td>
<td>100(^b) (88–100)</td>
<td>54 (34–72)</td>
</tr>
<tr>
<td>TPS</td>
<td>100(^b) (80–100)</td>
<td>53 (28–77)</td>
</tr>
<tr>
<td>TPS + TPA</td>
<td>100(^b) (81–100)</td>
<td>61 (36–83)</td>
</tr>
<tr>
<td>Negative predictive value, %</td>
<td>84 (67–95)</td>
<td>94 (79–99)</td>
</tr>
<tr>
<td>TPS</td>
<td>86 (71–95)</td>
<td>90 (77–97)</td>
</tr>
<tr>
<td>TPS + TPA</td>
<td>87 (70–96)</td>
<td>94 (79–99)</td>
</tr>
</tbody>
</table>

\(^a\) Value (95% confidence interval).
\(^b\) Value (95% confidence interval).

### References


### Adaptation of an Enzyme Immunoassay to Assess Urinary Cotinine in Nonsmokers Exposed to Tobacco Smoke, Denis Roche,\(^1,2\) Françoise Callais,\(^1,2\) Patrice Reun-gaot,\(^1\) and Isabelle Momas\(^*\)

Nicotine and its metabolites (1), expired carbon monoxide, and thiocyanates (2) are the most widely used smoking biomarkers. Among these biomarkers, urinary cotinine has been one of the most representative and specific for tobacco smoke exposure (3–5) with regard to active or passive smoking. The methods most frequently used for cotinine quantification are gas chromatography (6) and HPLC (7), coupled or not with mass spectroscopy (8, 9). These methods, however, are difficult to use in large-scale epidemiological studies because they require specialized laboratories. In 1973, Langone et al. (10) proposed the assessment of cotinine by RIA, but RIAs also require specialized laboratories. This last technique was then extended to ELISA (11) and fluorescence polarization immunoassay (12).

Recently, an enzyme immunoassay (EIA) that is easier to perform (13) was developed to measure cotinine concentrations between 100 and 200 μg/L, a range that...