colon carcinoma (13). These findings suggest that fructose malabsorption could be a risk factor in the development of these diseases.

Because bacterial metabolism alters folic acid status and intestinal bacterial colonization is altered by nutritional factors and carbohydrate malabsorption syndromes, dietary measurements should not rely solely on folic acid supplementation but should also consider carbohydrate malabsorption syndromes, especially fructose malabsorption. It is suggested that fructose malabsorption be considered in the elderly with folic acid deficiency. Further studies will be necessary to compare not only folic acid concentrations but also homocysteine, vitamin B12, and hematological indices in patients with and without fructose malabsorption.

References

Comparison of the Diagnostic Accuracy of Three Commercially Available Enzyme Immunoasays for Anti-p53 Antibodies, Jacques Rohayem,1* Karsten Conrad,1 Thomas Zimmermann,2 and Karl-Heinz Frank1 1Institute for Immunology and 2Department of Surgery, Medical Faculty “Carl Gustav Carus”, Technical University Dresden, Dresden 01101, Germany; *author for correspondence: fax 49-351-8832778, e-mail rohayem@rcs.urz.tu-dresden.de

The p53 tumor suppressor gene encodes a 53-kDa nuclear phosphoprotein that is thought to protect cells against the accumulation of genetic alterations (1). The p53 phosphoprotein is involved in cycle arrest, apoptosis, inhibition of tumor growth, and preservation of genetic stability (2). Abnormalities of the p53 gene are reported to be the most common genetic alterations in human cancer (3, 4). Mutated p53 gene encodes for mutant p53 proteins that may serve as targets of the host immune system as tumor-specific antigens (5). However, accumulation of the p53 protein in the cell is considered the main cause of anti-p53 antibody production (6). The presence of anti-p53 antibodies has been demonstrated in sera of patients with various cancers (7–9), including lung (10), breast (11, 12), liver (13), colorectal (14), and prostate cancer, and blood cell malignancies (15).

Anti-p53 antibodies initially were detected by immunoblot and immunoprecipitation with extracts of transformed cells as a source of antigen. In recent years, various ELISAs have been described that use mutant (8) or wild-type (16) p53 as antigen, solid-phase or sandwich methods, and prokaryotically or eukaryotically expressed p53 proteins.

Our purpose was to evaluate the potential of the tests to provide correct diagnostic classification (17). We studied three commercial ELISAs for anti-p53 antibodies by use of ROC curve analysis. We selected 72 patients presenting with suspicion of malignancy (unexplained body weight loss and chronic fatigue associated with chronic rectal hemorrhage and/or intermittent obstipation, hemoptysis, and/or pathognomonic aspect of tumoral development on chest radiographs). Ethics approval by the Ethics Commission of the University of Dresden (Germany) according to the Helsinki Declaration of 1975 as revised in 1996 as well as informed consent from the study subjects was obtained. Blood samples from patients with suspicion of lung cancer and/or other malignancies were collected during the control examination for occupational lung diseases at the Medical Opinion Community Niederdorf (Germany). Blood samples from patients with suspicion of colorectal malignancy were collected in the Department of Surgery at the University Hospital of Dresden (Germany). Forty-nine percent of the patients recruited presented symptoms of colorectal malignancy (men, n = 15; women, n = 20), 33.3% presented symptoms of lung malignancy, and 18.1% showed suspicion of melanoma (n = 1), blood cell malignancies (n = 2), or oral (n = 3), esophageal (n = 2), urogenital (n = 3), or hepatic (n = 2) cancer. The mean age (± SE) was 68 years (± 3 years) for the men and 71 years (± 4 years) for the women. Before therapy was started, blood samples were collected by venipuncture. After centrifugation, serum was stored at −28 °C. Later in the course of the disease, diagnosis of cancer was confirmed by histopathological examination.

Young, healthy blood donors, thus with a low probability to harbor “silent” cancer, were selected as a control population (n = 72). The mean age (± SE) was 19 years (± 1 year). Blood samples from healthy donors and patients with suspicion of cancer underwent the same procedure.

The anti-p53 antibody value was assessed for each serum sample with three different ELISAs: test A, a solid-phase ELISA using eukaryotically expressed wild-type p53 (PharmaCell; distributed by Coulter-Immuno-
tech); test B, a solid-phase ELISA using prokaryotically expressed wild-type p53 (Steinbeis Transfer Center; distributed by Dianova); and test C, a two-site sandwich ELISA using native p53 extracts from tumor cells (Orga-Med). We followed the recommendations of the manufacturer for each kit. In addition, Western blotting was performed to assess the usefulness of clinical laboratory detection of anti-p53 antibodies. The Western blot used prokaryotically expressed wild-type p53 protein. For descriptive statistics and ROC curve analysis, we used the Analyze-it™ package developed by Analyze-it Software.

The anti-p53 antibody values for healthy blood donors and cancer patients ranged from −4.6 to 16.03 (index), 0.004 to 2.915 (specific signal for anti-p53 antibody), and −0.27 to 1.763 (specific signal for anti-p53 antibody) for tests A, B, and C, respectively. The index used in test A refers to the quotient between the sample-specific signal for anti-p53 antibodies and the specific signal of the positive control. For tests A and C, the sample-specific signal for anti-p53 antibody was determined by subtracting the absorbance measured at 450 nm from two wells, with and without the p53 protein. For test B, the sample-specific signal for anti-p53 antibody was given by the absorbance measured at 450 nm.

Within-run and total imprecision were determined according to NCCLS instructions (18). The coefficients of variation (CVs) for tests A, B, and C were 15%, 6%, and 8%, respectively, for total imprecision. The CVs for tests A and B were 13% and 8%, respectively, for within-run imprecision.

Analytical correlation was performed by plotting the analytical results for 72 patients with various cancers by tests (B vs A, C vs A, and B vs C). We used only the results from patients with cancer so that the low anti-p53 antibody value was not overrepresented in the correlation. The Pearson correlation coefficient was 0.81 (P < 0.0001), 0.82 (P < 0.0001), and 0.94 (P < 0.0001), respectively. The regression analyses according to Deming’s unbiased method (19) were B_{450 nm} = 0.041 + 0.15A_{index}, C_{450 nm} = 0.081A_{index}−0.0023, and B_{450 nm} = 0.081 + 1.52C_{450 nm}.

The ROC curves are shown Fig. 1. Test B achieved high true-positive rates with low false-positive rates, whereas the ROC plots of tests A and C showed low discriminative ability between true- and false-positive rates, reflected by the low areas under the curves (AUCs). The AUC for each test as well as the 95% confidence interval (95% CI) are shown in Table 1. Comparison of ROC curves within a single population showed the highest AUC for test B, with significant statistical difference and confidence intervals (Table 1). As mentioned above, the sample-specific signal was determined for tests A and C by subtracting the absorbance measured at 450 nm from two wells, with and without the p53 protein. To assess the usefulness of this procedure, we performed for both tests an ROC analysis considering the signal obtained from the wells coated with the p53 protein only. The resulting AUCs for tests A and C were low (0.505 and 0.547, respectively) and showed 95% CIs that overlapped the previous ones (95% CIs, 0.410–0.601 for test A and 0.451–0.644 for test C).

Among the three tests used, the solid-phase ELISA using prokaryotically expressed wild-type p53 (Dianova) showed the highest diagnostic accuracy (AUC = 0.902), with a significant difference from other tests (Table 1). Furthermore, the overlapping 95% CIs of the AUCs showed that the diagnostic accuracy of tests A (PharmaCell) and C (Orga-Med) was similar.

The results obtained by Western blotting showed a strong discrepancy to the ELISA values and were considered unreliable for two reasons. First, the wild-type p53 used in the Western blot exhibited linear epitopes, therefore lacking the conformation of the wild-type p53 used in the ELISAs. Thus, the comparison of the results of an experimental Western blot with commercially available ELISAs seems rather uncertain. The autoantibodies occurring during autoimmune diseases often are directed against epitopes of the native three-dimensional conformation of the protein. Second, the Western blot used...
prokaryotically expressed wild-type p53, thus introducing a bias.

A possible explanation of the low classification performance is the analytical specificity, i.e., the ability of an assay to produce a measurable response only for the analyte of interest (20). One weakness of tests A and C is that negative values are obtained at various anti-p53 antibody concentrations. Thus, a higher signal in the well without p53 protein and/or a lower signal in the well with p53 protein gives a final negative result. Considering this, the lack of specific binding to the p53 protein seems to be responsible for the low diagnostic accuracy of tests A and C. Indeed, the specificity of the entire assay depends on the specific binding of the analyte, and thus the anti-p53 antibody, in the environment in which the analytical reaction takes place (21).

One limitation of our results was the control group that was selected. Ideally, the control population should consist of age-matched individuals presenting clinical suspicion of malignancy but whose histopathology remained negative for cancer. Because anti-p53 antibodies were reported to be detectable in the preneoplastic stages of lung cancer (15), young healthy blood donors were selected as a control population because such individuals have a low probability to harbor silent cancer. Accordingly, among the three ELISAs assessed, the anti-p53 ELISA (Dianova) showed the highest discrimination ability between young healthy individuals and patients (ages, 66 to 75 years) with various cancer types. However, these findings do not imply that the anti-p53 ELISA (Dianova) will also be useful in differentiating cancer patients from patients with other diseases that have similar symptomatology.

References


Stability of Blood Homocysteine and Other Thiols: EDTA or Acidic Citrate? Jean-Frédéric Salazar,1 Bernard Herbeth,2 Gérard Siest,1,2 and Pierre Leroy1* (1 Centre du Médicament, Faculté des Sciences Pharmaceutiques et Biologiques, B.P. 403-54001 Nancy Cedex, France; 2 Centre de Médecine Préventive, UPRES, B.P. 7-54001 Vandoeuvre-lès-Nancy Cedex, France; * author for correspondence: fax 33-(0)3-83-32-13-22, e-mail pierre.leroy@pharma.u-nancy.fr)

Homocysteine (Hcy), a thiol-containing amino acid resulting from demethylation of methionine (Met), is relevant to the risk of vascular diseases (1). In plasma, total homocysteine (tHcy) includes the free reduced and oxidized forms as well as the Hcy bound by disulfide bonds in proteins. tHcy is frequently increased in patients with coronary, cerebrovascular, or peripheral arterial diseases; the association is independent of most other risk factors for atherosclerosis (2). The simultaneous measurement of other thiols is of interest because most of them are metabolically related and disturbances of their concentrations can correspond to disorders of metabolism. Hcy may either be catabolized to cysteine (Cys) or remethylated to Met (3). In addition, Cys and γ-glutamylcysteine are precursors to glutathione (GSH), and cysteinylglycine (CysGly) is a breakdown product of GSH; this latter plays a major role in defense against oxidative and free radical-mediated cell injury, and its measurement permits the evaluation of oxidative status of cells and tissues (4).

It should be expected that less rigorous blood sampling and treatment conditions are needed than those involved for thiol redox status evaluation (5). For plasma, storage conditions have little influence on tHcy values: tHcy in plasma is stable at −20 °C for at least 3 months and after nine freeze/thaw cycles (6). In contrast, in whole blood, an increase of tHcy is observed after collection because of ongoing metabolism and time-dependent release from erythrocytes (7). The artificial increase in plasma tHcy...