

The new DR-70 immunoassay detects cancer of the gastrointestinal tract: a validation study

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SUMMARY

Background: Malignant cells characteristically possess high levels of plasminogen activator, which induce local fibrinolysis. The DR-70 immunoassay is a newly developed test, which quantifies fibrin degradation products in serum by a proprietary antibody.

Aim: To evaluate the DR-70 immunoassay as a detection assay for the presence of gastrointestinal cancers.

Methods: We prospectively collected blood sera of 85 patients with histologically proven tumour and 100 healthy blood donors. Ten microlitres of the sera was used for the DR-70 immunoassay. Nineteen patients had a hepatocellular and 10 cholangiocellular carci-

noma, 13 cancer of the pancreas, 30 colorectal cancer, 10 stomach cancer and three cancer of the oesophagus. **Results:** Receiver–operator curve analysis revealed $<0.7 \mu\text{g/mL}$ as the best cut-off value to distinguish between patients with cancer and healthy controls. Using this cut-off value, the DR-70 immunoassay showed a good clinical performance with a sensitivity of 91% and a specificity of 93%. Patients with advanced tumour spread showed significantly higher DR-70 values than those with early-stage tumours ($P < 0.0003$).

Conclusion: The DR-70 immunoassay reliably differs between cancer patients and healthy controls. Therefore, it promises to become a useful test for the detection of cancer in clinical practice.

INTRODUCTION

Haemostasis and angiogenesis are tightly regulated physiological processes, which are deregulated in cancer growth.¹ Tumours have been shown to produce procoagulants and fibrinolytic factors. Activation of the extrinsic coagulation system and the fibrinolytic cascade within a tumour is thought to be related with growth, invasion and metastasis. The local thrombin generation and fibrin deposition and dissolution might be important in tumour growth and dissemination.^{2–4} Malignant cells characteristically possess high levels of plasminogen activator, which induces local fibrinoly-

sis.⁵ Plasminogen activator seems to be a prognostic factor in neoplasms.⁶

Fibrin degradation products have been reported to possess angiogenic, chemoattractant and anti-inflammatory activities.⁴ It is an established fact that fibrin degradation products are elevated in plasma of patients with malignancies.^{7–10} Therefore, the assumption seems to be reasonable that the assessment of fibrin degradation products might be clinically useful as a screening test for cancer.

The aim of this study was to evaluate the potential of the newly developed DR-70 immunoassay, which is based on the immunochemical detection of fibrin degradation products as a detection assay for gastrointestinal cancers. In addition, the quantitative value of DR-70 should be tested as a parameter of tumour load, progression and dissemination.

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METHODS

Patients and controls

After obtaining informed written consent, we prospectively collected blood sera of 85 cancer patients (27 females, 58 males; median age: 68 years, range: 37–89 years) with histologically proven malignant tumour and 100 healthy blood donors (32 females, 68 males; median age: 37 years, range: 18–66 years).

On admission, all patients underwent biochemical evaluation of the serum (blood cell count, aspartate aminotransferase, alanine aminotransferase, γ -glutamyltranspeptidase, alkaline phosphatase, total bilirubin, albumin and prothrombin activity). Patients with colorectal cancer were tested for carcinoembryogenic antigen (CEA). CEA levels >5 ng/mL were considered pathological. In serum of patients with hepatocellular carcinoma, alpha foetoprotein (AFP) was measured (normal level <10 μ g/L). Routine biochemical tests were carried out using commercially available tests. Staging examinations were performed including sonography and computer tomography of the abdomen and chest X-ray. Additionally, patients with oesophageal tumour underwent computer tomography of the chest and endosonography. The tumour extension and spread was classified using the organ-specific TNM classification. All sera were stored at -20°C until they were analysed using the DR-70 immunoassay.⁵

Nineteen patients had hepatocellular and 10 cholangiocellular carcinoma, 13 cancer of the pancreas, 30 colorectal cancer, 10 stomach cancer and three cancer of the oesophagus. In all 85 patients, the malignancy was assessed histologically after biopsy or after operative resection.

DR-70 immunoassay

The fibrin degradation products were quantitatively measured using DR-70 kits (AMDL, Inc., Tustion, CA, USA) according to the manufacturer's instructions. The DR-70 assay is an enzyme-linked immunosorbent assay (ELISA)-based serological test utilizing removable strips in a 96-microwell format. Briefly, the wells are coated with polyclonal antibodies from the rabbit against DR-70 products of fibrin degradation. One hundred microlitres of patient sera (diluted 1:200) is incubated in the wells for 30 min. After washing, a second anti-DR-70 antibody conjugated to horse-radish peroxidase is added, which binds to the captured tumour marker.

After further wash steps, the colour reaction is started by adding 3,3',5,5'-tetramethylbenzidine to the wells. After stopping the reaction with 0.1 N hydrogenchloride, the intensity of the colour formed is read in a microplate reader at 450 nm. The concentrations of DR-70 in the sera are obtained from a standard curve, which results from the extinctions of calibrators provided with the kit. The concentrations of these DR-70 standards are 0, 0.625, 2.5, 5.0 and 10 μ g/mL.

Statistical analysis

Data are shown as median and range. Statistical analysis between groups was carried out using the Wilcoxon–Mann–Whitney *U*-test. Sensitivities and specificities of the immunoassay were calculated at various threshold concentrations. Thus, the best cut-off value for the DR-70 immunoassay was obtained performing receiver operating characteristics (ROC) curve analysis; 95% confidence intervals are given. $P < 0.05$ was considered to be statistically significant.

RESULTS

The results of the measured amounts of fibrinogen degradation products in patients and controls are given in Figure 1. The DR-70 values in cancer patients (2 μ g/mL; range: 0.4–18.6) significantly differed from the values in controls (0.37 μ g/mL; range: 0.3–1.11 μ g/mL; $P < 10^{-10}$). The results in the organ-related subgroups of cancer patients are also presented in Figure 1 and in Table 1.

Receiver operating characteristic curve analysis revealed ≤ 0.7 μ g/mL (Figure 2) as the best cut-off value to distinguish between patients with cancer and healthy controls. The area under the ROC curve was 0.965177.

Using ≤ 0.7 μ g/mL as the cut-off value, the DR-70 immunoassay showed a good clinical performance with a sensitivity of 90.6% (82.3–95.9%) and a specificity of 93.0% (86.1–97.1%). The positive predictive value was 91.7% (83.6–96.6%), the negative predictive value was 92.08% (85.0–96.5%) and the efficacy was 91.9% (87.0–95.4%).

According to the organ-specific TNM classification, 37 cancer patients were categorized as with limited disease ($T \leq 3$, $n < 2$, M0). Forty-eight patients presented with advanced cancer stages, i.e. T4 stadium

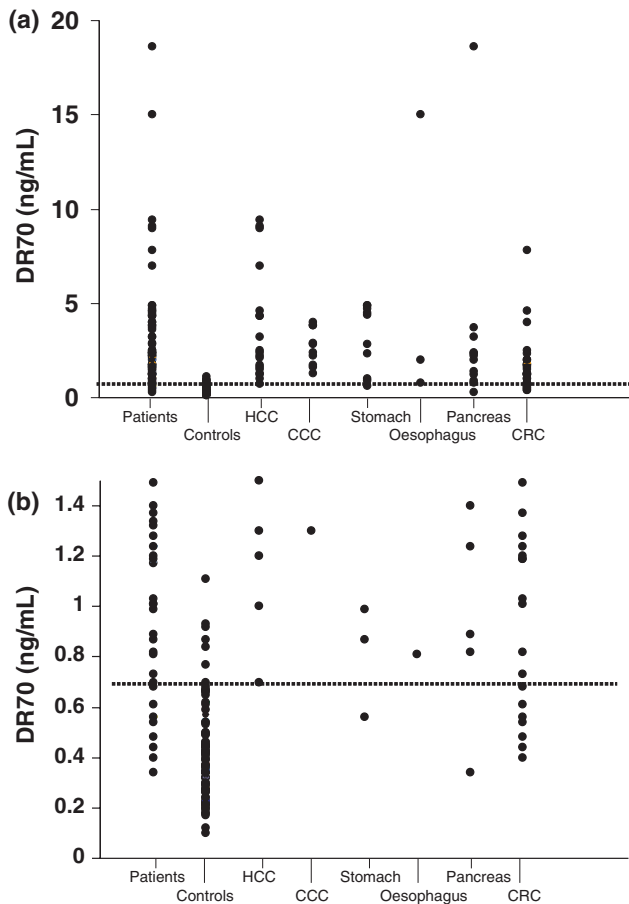


Figure 1. (a) Amount of serum DR-70 in controls and cancer patients. The organ-related subgroups are presented; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma; CRC, colorectal carcinoma. The dotted line presents the cut-off value $<0.7 \mu\text{g/mL}$. (b) Enlargement of (a) in the range around the cut-off value.

of the primary tumour and/or metastases of the lymph nodes ($n \geq 2$) and/or other organs (Mx).

Considering only patients with limited tumour disease, the test qualities of the DR-70 immunoassay were:

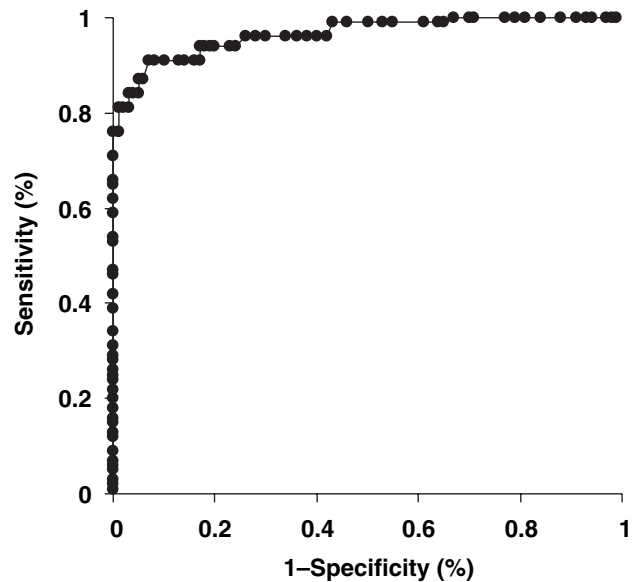


Figure 2. Receiver operating characteristics analysis evaluating different cut-off values for the DR-70 immunoassay.

sensitivity 89.2% (74.6–97.0%), specificity 93.0% (86.1–97.1%), predictive positive value 82.5% (67.2–92.7%), predictive negative value 95.9% (89.8–98.9%) and specificity 92.0% (86.1–95.9%).

Patients with advanced tumour spread showed significantly higher DR-70 values (median: $2.4 \mu\text{g/mL}$, range: $0.3\text{--}18.6 \mu\text{g/mL}$) than those with early stage-tumours (median: $1.3 \mu\text{g/mL}$, range: $0.5\text{--}4.4 \mu\text{g/mL}$, $P < 0.0003$; Figure 3).

Thirteen of 30 patients with colorectal cancer showed elevated CEA values in serum. The sensitivity of the CEA assay to detect colorectal carcinoma was 43.3% (25.5–62.6%).

In 18 of 19 patients with hepatocellular carcinoma, the AFP was elevated. Therefore, the sensitivity of AFP to detect hepatocellular carcinoma was 94.7% (74.0–99.8%).

Table 1. Performance of the DR-70 immunoassay in the groups with different types of cancer

Type of carcinoma	<i>n</i>	Median	Range	Sensitivity	95% Confidence interval
Hepatocellular	19	2.4	0.7–9.4	94.7	74.0–99.8
Cholangiocellular	10	2.6	1.3–4.0	100	69.1–100
Colorectal	30	1.2	0.4–4.0	80.0	61.4–92.3
Pancreatic	13	2.3	0.3–18.0	92.3	63.9–99.8
Gastric	10	3.6	0.6–4.9	90.0	55.5–99.8
Oesophageal	3	2.0	0.8–15.0	100	29.2–100

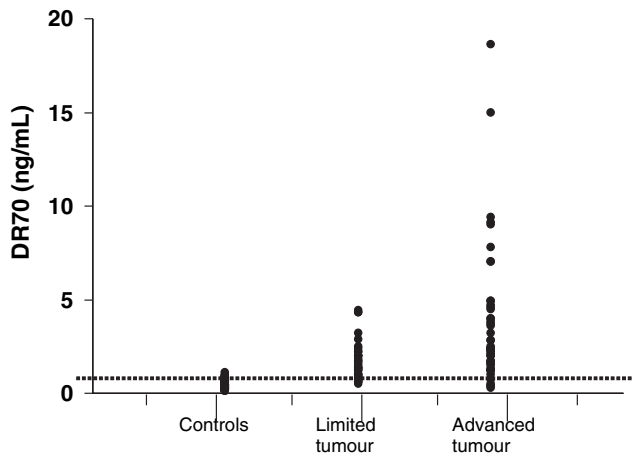


Figure 3. DR-70 levels in cancer patients with limited tumour extension or advanced tumour spread.

DISCUSSION

In our findings, DR-70 levels were significantly higher in all types of gastrointestinal cancer tested than in healthy controls. Therefore, DR-70 cannot be considered as an organ-specific tumour marker. But as the immunoassay detects multiple cancers with a high degree of sensitivity and specificity, it could be clinically used as a global serological cancer detection tool, not limited to specific tumour types.

The quantitative comparison of DR-70 levels in patients with early stages of cancer and in those with advanced tumour progression shows that DR-70 levels increase with the stage and dissemination of the malignant disease. The level of fibrin degradation products was positively correlated with tumour load and number of metastatic sites. The association between markers of fibrin degradation and tumour stage suggests that the increase of DR-70 in the individual is a clinically important marker for progression and points towards a relation between haemostasis and tumour progression.

The results of our study confirm the findings of Wu *et al.*,⁷ who tested patients with different types of cancer (lung, breast, stomach and rectum). Wu *et al.* found the best sensitivities in patients with stomach cancer (92.6%), while the DR-70 test kit performed with lower sensitivity in patients with rectum cancer (66.7%).

In few cases, which were not included in this study, we exemplarily investigated whether other pathological conditions, which also induce fibrinolysis, might result in increased DR-70 levels and false-positive tests. Four patients with a deep vein thrombosis and/or acute lung emboli did not show increased DR-70 levels. Moreover,

six patients with inflammatory processes or systemic immunogenic diseases (Crohn's disease three, diverticulitis one, appendicitis one, lupus erythematoses one) were negative in the DR-70 immunoassay. Although the knowledge about the performance of the DR-70 immunoassay in disorders with activated coagulation and fibrinolysis is still limited, we do not have indications that these conditions might lead to false-positive results. But further studies in patients with dysbalanced coagulation system are required.

The good clinical performance of DR-70 immunoassay and the sharp discrimination between cancer patients and healthy controls in our study might partly be based on the fact that the majority of our patients (56%) presented with advanced tumour disease. For a screening assay, however, reliability in cases with early stages of cancer is important. The purpose of a screening test is to detect subjects, in whom therapeutic consequences will still have an effect on survival, i.e. patients with early-stage tumours.

Considering only patients with early-stage tumours, satisfying test results were obtained using the DR-70 immunoassay in our study (sensitivity 89.2%, specificity 93.0%). But before the DR-70 immunoassay can be recommended as a global screening test for the presence of malignancy, further studies with a higher number of patients in early stages of cancer disease are required.

When we compared the DR-70 immunoassay with conventional tumour markers, DR-70 turned out to be superior to CEA in the detection of patients with colorectal cancers and equivalent to AFP in patients with hepatocellular carcinoma. A recent study by Blackwell *et al.*¹¹ demonstrated that circulating D-dimer levels were better predictors of overall survival and disease progression than carcinoembryonic antigen levels in patients with metastatic colorectal carcinoma.

The laboratory performance of the DR-70 immunoassay takes about 2 h and requires skilled technical personnel and equipment found in most hospital and commercial laboratories.

In conclusion, the DR-70 immunoassay reliably differs between patients with cancer of the gastrointestinal tract or the hepatobiliary system and healthy controls. Therefore, it promises to become a useful cancer detection tool in clinical practice. In addition, there is an association between the quantitative DR-70 value and the stage of tumour extension. This points at an applicability of the DR-70 immunoassay as a prognostic factor in the clinical course of malignant diseases.

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