Abstract. The aim of this study was to improve diagnostic efficiency in the detection of gastro-intestinal cancers by using fuzzy logic modeling in combination with a tumor marker panel (CEA, CA72-4, CA19-9) including Tumor M2-PK. In this prospective study histologically confirmed colorectal (n=247), esophageal (n=86) and gastric cancer (n=122) patients were investigated and compared to control (n=53) persons without any malignant diseases. Tumor M2-PK was measured in plasma with an ELISA (ScheBoBiotech, Germany); all other markers were measured in sera (Roche, Germany). At 95% specificity, tumor detection was possible by the best single marker in colorectal cancer patients in 48% (Tumor M2-PK), in gastric cancers in 61% (CA72-4) and in esophageal cancers in 56% (Tumor M2-PK). A fuzzy logic rule-based system employing a tumor marker panel increased sensitivity significantly in colorectal cancers (p<0.001) to 63% (Tumor M2-PK and CEA), in gastric cancers (p<0.001) to 81% (Tumor M2-PK and CA72-4) and in esophageal cancers (p<0.02) to 74% (Tumor M2-PK and CA72-4). Adding a third marker further improved the sensitivity only marginally. Fuzzy logic analysis has proven to be more powerful than measurement of single markers alone or combinations using multiple logistic regression analysis of the markers. Therefore, with the fuzzy logic method and a tumor marker panel (including Tumor M2-PK), a new diagnostic tool for the detection of gastro-intestinal cancers is available.

Patients and Methods

Patients. In this prospective study, 455 consecutive patients (311 male, 144 female) with histologically confirmed gastro-intestinal cancers were examined. The group comprised 247 (148 male, 99 female) colorectal cancers (63±10.8 years), 122 (92 male, 30 female) gastric cancers (61±11.7 years) and 86 (71 male, 15 female) esophageal cancers (59±9.4 years).

The tumor patients were compared with a control group of 53 (32 male, 21 female) persons without any malignant diseases.
Methods. Blood was obtained from patients with histologically confirmed cancer. Blood samples were centrifuged (1000xg, 5 min) within 120 min. Sera were kept frozen at –85°C until analysis. For Tumor M2-PK quantifications, samples were collected as EDTA-blood, followed by centrifugation (1000xg, 10 min) and removal of the upper half of the supernatant plasma. The Tumor M2-PK was quantified in 10 µl EDTA-Plasma with a sandwich enzyme immunoassay (ScheBo® • Tech GmbH, Giessen, Germany). Tumor M2-PK was adsorbed in the wells of microtiter plates and coated with monoclonal antibodies specific for Tumor M2-PK. The antibodies do not cross-react with other isoforms of the pyruvate kinase. The test was performed according to the manufacturer’s instructions.

CEA, CA19-9 and CA72-4 analyses were performed in sera using reagents from Roche-Diagnostics Mannheim, Germany, and measured with an Elecsys® 1010 (Roche).

Mathematical analysis. The panel of markers which are associated with gastro-intestinal cancers were evaluated by fuzzy logic modeling.

Table I. Cut-off values of analyzed parameters. Comparison with manufacturer’s data. Manufacturer’s cut-off values are understood at a specificity of 95% versus healthy persons.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Tumor M2-PK (U/ml)</th>
<th>CEA (ng/ml)</th>
<th>CA19-9 (U/ml)</th>
<th>CA72-4 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-off  (manufacturer’s data)</td>
<td>15.0</td>
<td>5.0</td>
<td>30.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Cut-off (95% specificity for control subjects)</td>
<td>19.8</td>
<td>8.3</td>
<td>23.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

This basic method was first described by Zadeh in 1965 (11). Applications of fuzzy logic to problems in various fields have been realized employing rule- or pattern-based approaches, as described by Zimmermann (12) and Bocklisch and Bitterlich (13). Fuzzy logic...
draws nearer to realistic answers by replacing the inflexible "yes/no" by a topical adjustment in form of a "more or less" and by introducing linguistic nuances into the process of decision. This can be done by collecting statistical data and by comparing the probability density functions for the variables. The mathematical items were described in detail by Cruz and Beliakov (14). The method of choice for our fuzzy classifier is the rule-based procedure. The substitution of a graded ("fuzzy") function instead of a sharp threshold value of a given "yes-no" decision is an important characteristic: thus the coordination of a tumor marker level to the criterion e.g. "malignant", would be described in terms of "more...less" and not as a sharp cut-off value. Distinct, sharp values are assessed by so-called membership functions as measures for describing qualitative properties. For example, a marker concentration of CA72-4 exceeding the cut-off value of 3.2 ng/ml (manufacturers' data) is said to be "elevated", whereas a CA72-4 value below 2.0 ng/ml will be declared as "low", respectively (see Figure 1). Similarly, any other value can be assessed to corresponding qualitative expressions ("fuzzification"). Each test result can be correlated to the expression of cancer for a group of persons suffering from a specific disease. Our model uses membership functions of triangular shape describing the relation of each single marker to the term "malignant". By using triangular functions in our example, a test result of 2.8 ng/ml for CA72-4 is rated "normal" with an affiliation (membership) of 0.7 and, simultaneously, as "elevated" with an affiliation of 0.3 (see Figure 1).

The value of a certain marker and the coherent malignancy is described by "if-then" rules as follows:

IF the value for CA72-4 is "low" or "normal", THEN the event/case is described as "benign".
IF the value for CA72-4 is "elevated", THEN the event/case is "uncertain".
IF the value for CA72-4 is "highly elevated", THEN the event/case is described as "malignant".

More than one rule can be applied at the same time for only one measured value. The more intense the membership of this value to the premise, the stronger the consideration of the corresponding rule ("inference"). For this purpose the MIN-MAX-algorithm is used.

Different applied rules may lead to different results. These intermediate results have to be summarized in the form of a starting figure ("defuzzification"). To achieve the final result, the contributory intermediate results are considered with respect to their individual importance/weight by applying the method of the focal point. The defuzzification employed the centre of gravity (COG) method to yield an output variable quantifying the distinctness of malignancy. The membership functions and the rules were defined with reference to the development data. The result is a multidimensional calculation in the form of specially adapted computer software. The complex information of the tumor marker panel is processed by means of this fuzzy logic modeling to generate an indicator for malignancy. The output variable ranges from 0% to 100% membership for occurrence of cancer. If the value of the "supermarker" was above 0.5 (>50% membership ) it was said to be "malign". Logistic regression analysis was computed with Statistica® available from StatSoft-Europe, Hamburg, Germany.
Figure 3. ROC curves comparing the performance of CEA, CA19-9, CA72-4 and Tumor M2-PK measurements in 53 control subjects and in 122 patients with gastric cancers. (AUC = Area under curve)

Figure 4. ROC curves comparing the performance of CEA, CA19-9, CA72-4 and Tumor M2-PK measurements in 53 control subjects and in 86 patients with esophageal cancers. (AUC = Area under curve)
Figure 5. ROC curves of the best tumor marker (Tumor M2-PK), the multiple logistic regression (Tumor M2-PK/CEA) and the fuzzy classifier in 53 control subjects and in 247 patients with colorectal cancers. (AUC = Area under curve)

Figure 6. ROC curves of the best tumor marker (CA72-4), the multiple logistic regression (CA72-4/Tumor M2-PK) and the fuzzy classifier in 53 control subjects and in 122 patients with gastric cancers. (AUC = Area under curve)
The Chi-square test was used to assess the statistical significance of differences between observed ratios. Since serum levels of the markers did not follow Gaussian distribution, the significance of differences between the groups was calculated by means of a nonparametric test (Mann-Whitney’s U-test). Values of $p<0.05$ were considered significant.

**Results**

**Analysis by ROC curves.** For this analysis, the patients suffering from colorectal, gastric or esophageal cancers were compared with the 53 control subjects. Table I reports the cut-off values at a specificity of 95% for the total of 53 controls. The sensitivity and specificity for the 95% levels of the control group were confirmed by ROC (receiver-operating characteristics) curves. To obtain the ROC curves, a new classification procedure has to be carried out for each data point at a given specificity. To demonstrate the accuracy, the AUCs (area under the curve) were calculated.

The ROC analyses are given comparing the performance of the single tumor marker measurements in 53 control subjects and in 247 patients suffering from colorectal cancers (Figure 2), in 122 patients with gastric cancers (Figure 3) and in 86 patients with esophageal cancers, respectively (Figure 4).

At a 95% specificity, Tumor M2-PK was the marker with a significantly higher sensitivity for the detection of colorectal cancers (48%) in comparison to CEA (sensitivity: 34%, $p<0.001$) or CA19-9 (sensitivity: 30%, $p<0.001$) (Figure 2). The area under the curves (AUC), an indicator for marker quality, was largest for Tumor M2-PK (AUC=0.820). The corresponding data were AUC=0.694 for CA19-9 and AUC=0.646 for CEA.

In gastric cancers the sensitivity of Tumor M2-PK (sensitivity: 57%) was comparable with CA72-4 (sensitivity: 61%) (Figure 3). The sensitivity of both markers were significantly higher than CA19-9 (sensitivity: 46%; $p<0.01$) or CEA (sensitivity: 24%; $p<0.001$). The area under the curves for gastric cancers were calculated for CA72-4 (AUC=0.852), followed by Tumor M2-PK (AUC=0.848), CA19-9 (AUC=0.714) and CEA (AUC=0.566).

In esophageal cancers, being nearly equivalent, Tumor M2-PK (56%) and CA72-4 (54%) were the most sensitive markers (Figure 4). The sensitivity of CA19-9 in the detection of esophageal cancers was calculated as 28% and was significantly lower than the sensitivity of Tumor M2-PK ($p<0.001$) or CA72-4 ($p<0.001$). CEA provided no relevant information in the detection of esophageal cancers (sensitivity: 15%). The area under the curves for esophageal
cancers were calculated for Tumor M2-PK (AUC=0.816), CA72-4 (AUC=0.764), CA19-9 (AUC=0.669) and CEA (AUC=0.656).

For testing the marker combinations including fuzzy-classification, the parameters with the best malignant-benign discrimination were chosen. For gastric as well as for esophageal cancers, the highest sensitivities were found for Tumor M2-PK and CA72-4, respectively, while for colorectal carcinomas for Tumor M2-PK and CEA. Besides this, all other tumor marker combinations were suitable for discriminating malignant and benign cases applying fuzzy classification. However, other combinations were not superior and, therefore, the data were not shown.

To generate one point of the ROC curves for multiple logistic regression analysis, the possibility for colorectal, gastric or esophageal cancer diagnosis varied between 0 and 1 and the data counted below the "cut-off" were declared "benign" and above the "cut-off" "malignant". This resulted in a curve, which is different from a simple combination based on the manufacturer’s cut-off for healthy persons (Figures 5-7).

Regarding colorectal cancer patients, the sensitivity using the multiple logistic regression analysis with the marker combination Tumor M2-PK/CEA increased significantly (p<0.001). The area under the curve (AUC) was largest for the fuzzy classifier (AUC=0.856), followed by the multiple logistic regression analysis (AUC= 0.834). In comparison to the best single marker for gastric cancers (CA72-4: 61%), the sensitivity (at 95% specificity) employing multiple logistic regression analysis increased significantly (p<0.05) to 75% for the two-marker combination Tumor M2-PK/CA72-4, and with fuzzy techniques to 81% (p<0.01) for the same marker combination (Figure 6). The differences between the multiple logistic regression analysis and fuzzy classification were not yet significant (p=0.280) but, by calculating the area under the curves, the superiority of fuzzy classification (AUC=0.947 vs. AUC=0.910) was still impressive.

In esophageal cancer patients, the marker with the highest sensitivity was Tumor M2-PK (sensitivity: 56%). The multiple logistic regression analysis increased sensitivity not significantly (p=0.43) to 63% (Figure 7). However, a significant further improvement (p<0.02) of sensitivity was observed using the fuzzy classifier based on the measurement of the same parameters. At this high specificity, the fuzzy classification revealed a sensitivity of 74%, which exceeds that of the best performing single marker Tumor M2-PK by about 18% (p<0.02) and the multiple logistic regression analysis by 11% (not significant). By calculating the areas under the curves, the superiority of fuzzy classification using tumor marker panels was obvious (AUC=0.900).
Adding a third marker to the presented two-marker panels did not improve the sensitivity. The ROC curves created with three different markers were comparable with the two-marker model, therefore the data are not shown.

Generally, the fuzzy classifier was able to detect a higher rate of gastro-intestinal cancer patients.

**Analysis by tumor extent.** Because increasing tumor marker concentrations were observed with progressive diseases, the following analysis were done for tumors with distant metastases and without distant metastasis separately. Of the total collective there were 131 colorectal cancers, 76 gastric cancers and 65 esophageal cancers without distant metastasis and the remaining patients with generalized metastasized cancers: 116 colorectal, 46 gastric and 21 esophageal cancers. In this analysis, the sensitivities of the best single marker, the multiple logistic regression analysis and the fuzzy classification were calculated for patients with and without distant metastasis in colorectal, gastric or esophageal cancers. The specificity of 95% referred to the control subjects.

Tumor M2-PK alone detected about 47% of localized colorectal cancers. In progressive cancers, the sensitivity rose marginally to 50% (Figure 8). The multiple logistic regression analysis showed no significantly higher sensitivities (54% resp. 62%). With the fuzzy classifier the calculated sensitivity was 55% in patients without distant metastasis. In comparison to the best single marker (Tumor M2-PK: 50%), the sensitivity was significantly higher ($p<0.0005$) in patients with progressive colorectal cancers (73%).

In gastric cancer patients, the tumors were detectable with CA72-4 in 57%. An advantage of multiple logistic regression analysis compared to the best single marker was found. However, the results were not significant. The fuzzy logic approach was still superior. A substantial gain in sensitivity was found in patients with gastric cancers. With a sensitivity of 75%, the fuzzy classifier was significantly ($p<0.03$) higher in comparison to the best single marker in patients without distant metastasis. The highest sensitivity of 91% was seen in gastric cancer patients with distant metastasis by using the fuzzy classifier and the markers CA72-4 and Tumor M2-PK (n.s.).

The difference of the fuzzy classification vs. best single marker was 19% in patients without and 9% in patients with distant metastasis of esophageal cancers. Neither fuzzy classification nor multiple logistic regression analysis increased sensitivity in esophageal cancer patients significantly. Nevertheless the advantage of the fuzzy classification was also seen in this cancer type.

Generally the fuzzy classifier was able to detect a higher rate of the malignant diseases independent of tumor type and tumor extent.

**Discussion**

The concept we employed was based on the measurement of a panel of markers related to gastro-intestinal cancers and its evaluation by fuzzy logic mathematical modeling. The markers under investigation were associated with colorectal, gastric or esophageal cancers (CEA, CA19-9, CA72-4). In addition, Tumor M2-PK, a novel tumor metabolic marker, was analyzed.

The sensitivities reported in the literature for single tumor markers are comparable with our results (15-20). Tumor M2-PK was the most sensitive index of colorectal- and esophageal cancers, where CA72-4 showed highest sensitivities in gastric cancers (8). Cancers without distant metastasis were generally detectable at lower sensitivities (8, 16). In advanced gastric cancers, sensitivities of CA72-4, CA19-9 and CEA were 37.5%, 17.9% and 35.7%, respectively (16). Increasing sensitivities in advanced gastro-intestinal cancers could also be demonstrated by our data (Figure 8).

Several combinations of markers were used to improve the diagnostic procedures in detection of gastro-intestinal cancers. The sensitivity of two combinations increased to 56.5% in gastric cancer patients (17). In the report of Schulze (8), a combination of Tumor M2-PK and CA19-9, CA72-4 or CEA further increased sensitivity. Also, in lung cancer patients, the multiple marker panel proved to be more sensitive and specific than any single marker, but it was of limited value in discriminating malignant from benign diseases (21). Rising sensitivities were associated with reduced specificities. Therefore, marker combinations were not estimated as useful tools for cancer screening (22). Even the powerful and widely used logistic regression and recursive partitioning methods for discrimination sometimes did not prove to be superior (22, 23). This could also be confirmed in gastro-intestinal tumors by our results.

The new fuzzy classifier provided more information concerning the occurrence of gastro-intestinal cancers. In our study, we tested a panel of established tumor markers and Tumor M2-PK.

The combination of the markers Tumor M2-PK and CEA in colorectal cancers and the combination Tumor M2-PK and CA72-4 in gastric or esophageal cancers led to an increase in sensitivity. Adding a third marker to the presented two-marker panels did not improve the sensitivity. With respect to costs, a three-marker panel can not be recommended so far.

On the basis of our data, we evaluated the multiple logistic regression analysis and additionally a fuzzy logic classification for the best two markers. For a given specificity (95%), the corresponding cut-off values were varied until the combination with the best sensitivity was determined. Mathematical methods *i.e.* multiple logistic
regression analysis gave better results than the use of single markers, but the fuzzy approach was still superior.

For the discrimination of malignant vs. non-malignant diseases, the fuzzy classifier increased sensitivity compared to the best single marker over 15% in colorectal (Figure 5), over 20% in gastric (Figure 6) and over 18% in esophageal cancers (Figure 7). Also, the multiple logistic regression analysis including the same marker combinations was surpassed by about 5% in colorectal, about 6% in gastric and about 11% in esophageal cancers. As can be derived from Figures 4-7, the area under the ROC curves was always largest for the fuzzy classifier. It is notable that the fuzzy classifier significantly improved the sensitivity of the tumor marker panel in all gastrointestinal tumors. The advantage of the fuzzy classification was not only seen in progressive cancer patients, but also in patients without distant metastasis (Figure 8). These characteristics may be of certain interest in monitoring localized and possibly curable gastro-intestinal cancers.

Fuzzy classification is a mathematical procedure for a non-invasive analytical method. With this new method including the marker Tumor M2-PK, a promising tool is available to improve diagnostic efficiency in gastrointestinal tumors.

References


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