Cut-off-independent Tumour Marker Evaluation Using ROC Approximation

NORMAN BITTERLICH[1] and JOACHIM SCHNEIDER[2]

[1]Medizin und Service GmbH, Zwickauer Str. 227, D-09116 Chemnitz;
[2]Institut und Poliklinik für Arbeits- und Sozialmedizin der Justus-Liebig-Universität, Aulweg 129/III, D-35385 Giessen, Germany

Abstract. *Background: The analysis of tumour markers is based on the evaluation of data in relation to defined cut-off values. Changes in the method of determination or reference study group have led to different results. Cut-off-independent diagnostic evaluation of laboratory parameters can avoid laboratory-based and method-derived systematic errors. The decision guarantee (DG) is an appropriate parameter that can be determined using a defined reference population and its respective receiver operating characteristic (ROC) curve. The influence of ROC differences on the determination of DG is examined. Patients and Methods: A group of 281 consecutive patients with newly diagnosed, histologically confirmed lung cancer and a control group of 231 patients were examined. Histological classification of the tumour cases defined in 59 small-cell carcinoma, 102 squamous cell carcinomas, 66 adenocarcinomas and 54 large-cell carcinomas or mixed bronchial carcinomas without classification. The control group without tumours consisted of 23 healthy subjects, 125 patients with silicosis or asbestosis, 27 with chronic obstructive pulmonary diseases (COPD) and 56 suffering from inflammatory lung diseases. Results: Cytokeratin-19 fragments (CYFRA 21-1) was the most sensitive marker with a sensitivity of 57.3% and a specificity of 94.9%. Sensitivity and specificity influence each other. Related to the ROC curve, the method described here ensured the diagnosis of lung cancer on the basis of the data collected in comparison with a reference population. Thus, it was possible to determine with statistical certainty whether the evaluation of the sample data would lead to a diagnosis of lung cancer. Conclusion: The DG provides the basis for a laboratory- and method-independent support for a diagnosis including fairer information about the reference population in the data analysis. With feedback between laboratory results and the specialist physician's comprehensive clinical findings, the sensitivity and specificity can be continually monitored.*

Correspondence to: Priv.-Doz. Dr. Joachim Schneider, Institut und Poliklinik für Arbeits- und Sozialmedizin der Justus-Liebig Universität, Aulweg 129/III, D-35385 Giessen, Germany. Tel: +49 6419941303, Fax: +49 6419941309, e-mail: Joachim.Schneider@arbmed.med.uni-giessen.de

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The analysis of tumour markers as diagnostic tools is usually based on the evaluation of data in relation to defined cut-off values (1). The definition of the cut-offs considerably influences the diagnostic merit of this evaluation. Changes in the method of determination or reference population can lead to different results, and therefore data from different laboratories cannot be compared.

Cut-off-independent diagnostic evaluation of tumour markers or other laboratory parameters can avoid laboratory-based and method-derived systematic errors. The decision guarantee (DG) is an appropriate parameter that can be determined using a defined reference population and its respective receiver operating characteristic (ROC) curve (2).

The specificity is generally set at 95%, depending on the diagnostic test in question. The respective cut-off is determined and a sample is classified as malignant if the laboratory value of the tumour marker exceeds the cut-off value. Thus, each sample can be categorised as malignant or benign. The relationship of a value to the cut-off permits not only a qualitative characterisation of malignant or benign, but also describes the certainty with which a diagnosis of malignancy can be made. The difference between a value and the cut-off reflects to an extent the decision certainty or guarantee that accompanies this method of analysis.

The configuration of ROC curves are determined by the patient population on which they are based and an adequately large group of patients and controls is required for the development and optimisation of the classification method. The ROC curve is an indispensable component of the so-called sensitivity-adapted method of classification. The usefulness of this method can only be guaranteed when the ROC curve is correctly applied. Since quantitative errors are common, especially in cases where the sample number is low, the influence of these differences on the decision guarantee must be determined. The performance of this method in determining the diagnosis of lung cancer with certainty was evaluated in an individuum.

Patients and Methods

The study group consisted of a total of 512 individuals, including 281 consecutive patients (244 male, 37 female; 64.5±9.2 years of age) with newly diagnosed, histologically confirmed lung cancer. Histological classification of the primary lung tumour cases indicated 59 cases of small-cell carcinoma and 222 non-small cell carcinomas. Of the latter, there were 102 squamous cell carcinomas, 66 adenocarcinomas of the lung and 54 large-cell carcinomas or mixed bronchial carcinomas without classification. Exclusion criteria were cancer therapy and relapse, pulmonary metastases of extrapulmonary tumours, mesothelioma, sarcoma and lymphoma.

The tumour patients were compared with a control group of 231 patients (212 male, 19 female). Within this group, 23 were healthy subjects, and the others were patients with non-malignant lung disease, 125 patients had silicosis or asbestosis [criteria for diagnosis: see (3)], 27 male patients had chronic obstructive pulmonary diseases (COPD) and 56 patients suffered from inflammatory lung diseases.

After venipuncture the blood samples were centrifuged (550 g, 5 min) within 120 minutes. Sera were kept frozen at –18˚C until analyses were carried out. Analyses of carcinoembryonic antigen (CEA), cytokeratin-19 fragments (CYFRA 21-1), and neuron-specific enolase (NSE) were performed on sera using reagents from Roche Diagnostics GmbH, Mannheim, Germany, and were measured with an ES 600 ELISA analyzer (Roche).

Cut-off-based tumour marker profile evaluation. A laboratory analysis normally leads to a diagnosis with the help of a defined cut-off value. An analysed value is considered negative if it lies below the cut-off. A value over the cut-off is interpreted as a positive sign of disease. The diagnostic value of this classification is determined primarily by the definition of the cut-off value. This point is usually chosen such that 95% of the healthy subjects are defined as negative (specificity). Thus, for the control group in this investigation, 219 out of the 231 data points (94.8%) would be expected to be negative. The proportion of subjects determined to be positive for disease (sensitivity) characterises the comparable efficiency of this diagnostic method.

Receiver operating characteristics (ROC) curves. ROC curves were constructed using both patient and control subject serum marker levels to establish a sensitivity-specificity relationship of the measured marker.

The sensitivity and specificity for the 95th percentile levels of the control group were confirmed by ROC curves. To obtain the ROC curves for each marker, a new classification procedure was carried out for each data point at a given specificity. This function is independent of cut-off information (4).

In this analysis, achievable sensitivity is presented as a function of specificity in the absence of cut-off information. Thus, a direct graphic comparison of the classification by various analytical methods can be made.

Results

Adaptation of sensitivity in the evaluation of tumour marker data. In a cut-off-based, single marker analysis, the tumour marker CYFRA 21-1 proved to be the most sensitive marker for the diagnosis of lung cancer. With a specificity of 94.8%, a sensitivity of 57.3% was achieved. Accordingly, 219 of the control subjects were classified as negative (non-malignant) and 161 of the patients with diagnosed cancer as positive (malignant).

The ROC curve of the marker was generated (Figure 1). The influence of the actual value of the cut-off on the two diagnostic parameters specificity and sensitivity is well-known. When the chosen cut-off point is high, more data points are classified as negative. This means that the specificity rises with an increasing cut-off value, and the sensitivity declines in parallel.

The diagnostic parameters of specificity and sensitivity are an expression of the confidence of decision-making in diagnosis. A measured value can be viewed as a cut-off point, and the corresponding sensitivity and specificity can thus be determined. The diagnosis of malignancy is especially guaranteed if the specificity is high (because in this case more negative samples are classified as negative) or if the sensitivity is low (because in this case fewer positive samples are classified as being positive).

Since specificity and sensitivity influence each other, it is usually sufficient to evaluate one of these parameters. If sensitivity is determined, this can be normalised to the specificity in the following way: under the assumption that a value above the defined cut-off is positive and a value below is negative, a decision guarantee of 50% is assigned to this sensitivity value. In extreme situations, the designation is thus defined, at 100% sensitivity and 0% specificity, a reliable diagnosis of malignancy cannot be made, because all results would be classified as positive, and at 100% specificity and 0% sensitivity, a diagnosis of malignancy can be made with certainty, because all negative samples would be classified as negative, *i.e.* every positive classification is correct.

Thus, a determination of the sensitivity-adapted guarantee of the diagnosis should be made. Based on a reference study group, for a measured value m, the specificity SP_m and sensitivity SE_m, which a cut-off with this value as threshold would produce is invastigated. Knowing the sensitivity SE* at 95% specificity, the decision guarantee (DG) can be calculated as follows:

$$DG(m) = \begin{cases} 0.5 \cdot \left(1 - \frac{SE_m - SE^*}{1 - SE^*}\right) & if \quad SE_m \geq SE^* \\ 0.5 \cdot \left(1 + \frac{SE^* - SE_m}{SE^*}\right) & if \quad SE_m < SE^* \end{cases}$$

The normalisation using SE* (or 1 – SE*) assures that the value of the decision guarantee lies between 0 and 1, and predictably for SE_m = 100%, takes on a value of 0, and for SE_m = 0%, a value of 1. As an example, a value of CYFRA 21-1 of 1.4 ng/ml was measured (manufacturer's cut-off value: 3.3 ng/ml CYFRA 21-1; manufacturer's cut-off values are determined at a specificity of 95% *versus* healthy persons). Applying the above formula, the decision guarantee was calculated to DG=0.114 and as a result, the interpretation of this marker value was "benign". In agreement with practical experience, the guarantee of making a decision for malignancy

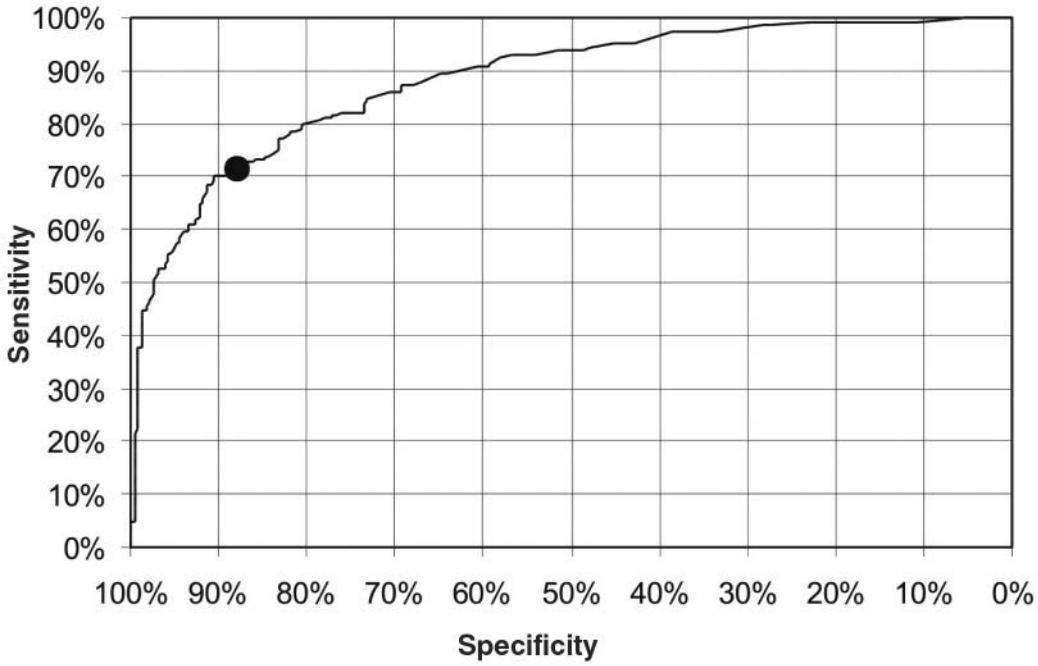

Figure 1. *ROC curve of the most sensitive single marker CYFRA 21-1 (sensitivity and specificity at the marked point corresponding to 1.5 ng/ml CYFRA 21-1).*

based on a CYFRA 21-1 value of 1.4 ng/ml is very low. Similarly for a CYFRA 21-1 value of 4.5 ng/ml the above formula reached a DG of DG=0.626. With a certainty of over 60% (62.6%), a diagnosis of malignancy could be made based on this CYFRA 21-1 value and in agreement with practical experience the diagnosis would be lung cancer.

The decision guarantee and the cut-off-based evaluation are qualitatively equal, because the decision threshold 0.5 corresponds by definition to the cut-off value at 95% specificity. Every cut-off-based system of classification can be transformed to the basis of decision guarantee if the decision guarantee is used instead of the measured values and the general threshold of 0.5 is applied instead of a defined cut-off value (2).

Statistical differences in ROC curves in the application to a subpopulation. The determination of a decision guarantee using the above formula was substantially based on the ROC curve. To examine the influence of this curve on the parameter, the entire 512-member population (referred to below as ENTIRE) was divided into two subpopulations (P) of 256 subjects denoted P1 and P2, using a mathematical random generator. This random division applied to both the lung cancer patient group and the subjects without tumours. A Chi-square test demonstrated that differences in the two subpopulations were not statistically significant (p=0.657), Table I.

The ROC curves of the subpopulations were also similar to the curve for the ENTIRE group. Marked differences would not be expected here, since the two populations P1 and P2 originated from the same population and should be viewed as representative samples. Upon closer examination, however, there were some recognisable variations. The diagnostic performance at 95% specificity was 59.7% in P1 and 49.5% in P2 (while in the ENTIRE group it was 57.3%). Thus, around the relevant specificity, the curves for P1 and P2 had about a 10% difference in sensitivity.

Table I. *Composition of the subpopulations.*

Designation	Number of participants	Benign		Malignant (Lung cancer patients)	
		Number	Proportion	Number	Proportion
P1	256	118	46.1%	138	53.9%
P2	256	113	44.1%	143	55.9%
ENTIRE	512	231	45.1%	281	54.9%

From a statistical point of view, each concrete ROC curve was actually an estimate of the real situation (5). Each point on a ROC curve relates to specific values of the parameter's specificity and sensitivity for the data set on which the curve is based. Thus, along the curve there is a confidence interval within which the real curve is expected to lie, with a particular probability of error. It could therefore be assumed that, with a probability of error of 5%, there was no statistically significant difference between the P1 and P2 curves.

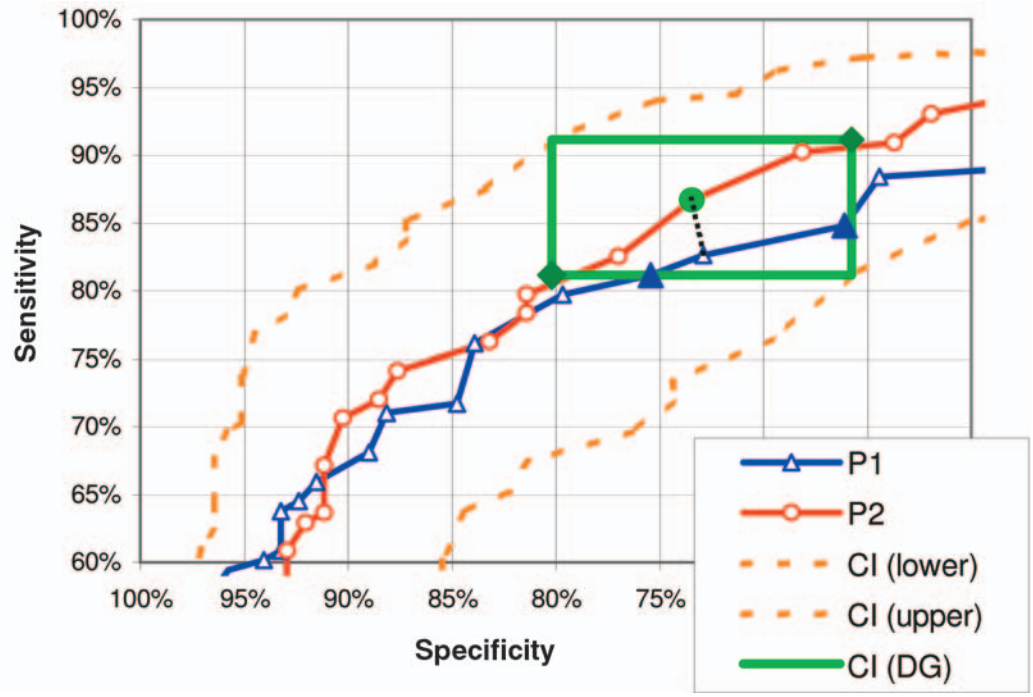

Figure 2. *Confidence interval around 73.5% specificity at 86.7% sensitivity in subpopulation P2 corresponding to a CYFRA 21-1 value of 1.5 ng/ml (marked point) in comparison to the curve for subpopulation P1. (CI=confidence interval, DG=decision guarantee).*

Statistically significant differences are only apparent at a specificity of over 98%. These effects result from individual cases with extreme values that lose their influence on the curve with increasing size of the population.

The example of the subpopulation P1 was used to demonstrate the calculation of the sensitivity-adapted decision guarantee. A CYFRA 21-1 value of 1.5 ng/ml was given and when used in subpopulation P1 as a cut-off, a sensitivity of 82.6% was obtained at a specificity of only 72.9%, by determining the number of correct positive and negative data points for a comparatively low threshold. Assuming the usual specificity of 95%, CYFRA 21-1 had a sensitivity of 59.7% in this population. According to the above formula, a decision guarantee was calculated for the value of 1.5 ng/ml for CYFRA: $DG_{P1}(1.5)=0.216$. This decision guarantee of about 22% means qualitatively that the sample came from a benign rather than a malignant tumour. A positive diagnosis (lung cancer) on the sole basis of the low CYFRA value of 1.5 ng/ml was possible, but less likely.

Decision guarantee as related to the reference population. If the value of 1.5 ng/ml was evaluated using the ROC curve of subpopulation P2, the diagnostic parameters were only slightly different from those for P1. By counting the correct positive and negative data sets in subpopulation P2, a sensitivity of 86.7% at a specificity of 73.5% was obtained. Applying the sensitivity of 49.5% at a specificity of 95%, the $DG_{P2}(1.5)$ value $=0.132$.

The marked difference in decision guarantee between 21.6% and 13.2% was due to the differences in the ROC curves of each subpopulation P1 and P2. The lower decision guarantee would appear plausible because the single marker had a lower diagnostic power at 95% specificity in subpopulation P2 compared to P1.

From the above formula, it can be seen that when the curves coincide exactly, then both estimates of DG will be the same. Therefore, the question is whether this difference in the curves can be eliminated or at least reduced in the calculation of the decision guarantee in subpopulation P2 based on the data of subpopulation P1. In this manner, the calculation could be made largely independent of the particular choice of (reference) population.

Regarding the spread of the confidence interval (6) around the point corresponding to the CYFRA 21-1 value of 1.5 ng/ml in P2, the values of the diagnostic parameters varied from 80.2% specificity at 81.1% sensitivity (Figure 2, diamond on the left side) to 65.8% specificity at 91.1% sensitivity (diamond on the right).

The statistical variability of approximately ±5.5% sensitivity and ±8.0% specificity demonstrated the need to quantify the decision guarantee in the practical application of diagnostic evaluations.

In the case of subpopulation P1, the confidence interval offered a solution, under the assumption made at the outset that populations P1 and P2 have similar ROC curves, the pair of data points on the ROC curve of P2 were related only to points on the ROC curve of P1 that were inside the P2 confidence interval for the CYFRA 21-1 value of 1.5 ng/ml that was being evaluated. In Figure 2 these points are included by the diagnostic parameters between 81.2% sensitivity at 75.4% specificity (triangle on the left) and 84.8% sensitivity at 66.1% specificity (triangle on right). In relation to subpopulation P1, this range in sensitivity was limited to the area from 81.2% (Figure 2, lower triangle) to SE_2=84.8% (Figure 2, upper triangle), accounting for approximately only ±1.8%. For these limits, the decision guarantee was calculated the upper limit was DG_{up}=0.233 and the lower limit was DG_{low}=0.188. Thus, without having to make calculations with actual data from subpopulation P2, the decision guarantee could be calculated in relation to subpopulation P1 using the confidence interval. Depending on the defined probability of error, the desired decision guarantee would be within the calculated limits. In addition, the narrow width of the respective area permited not only a definitive rejection of a malignant diagnosis in relation to subpopulation P2, but also in relation to P1.

In a further mathematical evaluation of the decision guarantee, for example in multiparametric diagnostic methods, it was necessary to define a concrete value instead of a range for the decision guarantee. For an estimate of the DG, it would appear intuitively appropriate to choose a point on the ROC curve of the reference population that was closest to the data point pair in the diagram to be evaluated. Such a neighbouring point could always be determined, independent of the statistical quality. In the above example, this point in subpopulation P1 was at a sensitivity of 82.6% with a specificity of 72.9%. The dotted line in Figure 2 between the two ROC curves marks these neighbouring points. Since, as a basis of comparison, a pair of parameters was chosen that also corresponded to the CYFRA 21-1 value of 1.5 ng/ml in P1, the estimated decision guarantees in both subpopulations P1 and P2 were in agreement.

Discussion and Conclusion

Lung cancer is one of the most prominent causes of cancer death in Western industrialised countries, particularly among the male population. Thus, a comprehensive primary diagnostic program is of great importance for successful therapy.

Patients with lung cancer particularly in the early stages often do not exhibit specific symptoms. Dyspnoea, cough and thoracic pain are considered to be nonspecific early signs, while hemoptysis may indicate an already advanced stage of lung cancer. Relapsing infectious diseases of the respiratory system in combination with a smoking history or occupational exposure to lung carcinogens might be a hint for further examinations. Diagnostics for lung cancer include medical history and physical examination, clinical laboratory tests, chest radiography, computed tomography or magnetic resonance imaging of the chest, abdomen and the brain, bronchoscopy, sputum cytology, biopsy, bone scan, preoperative pulmonary function studies, and eventually positron emission tomography, bone marrow biopsy and thoracentesis (7-13).

Ideally diagnostic procedures should be conducted rapidly, costs for staff and equipment should be kept low and moreover there should not be any complications for the patient. These are the reasons why examinations for tumour markers could be a promising tool, because these necessitate only a blood test (14). The sensitivities reported in the literature for CYFRA 21-1 are comparable with our results (15-22).

The development of data evaluation methods for use in diagnostics is generally based on reference populations. The algorithms are optimised for the actual data of these populations, and the diagnostic performance of the method is tested by applying it to data for validation. The routine use of this method implies that the conditions for development and application are similar. The use of the sensitivity-adapted decision guarantee ensures that systematic differences in laboratory results will be eliminated. Factors resulting from the specific makeup of the patient collective should receive special attention. Changes in the ROC curves can be observed when the proportion of patients having tumours of various stages shifts or when certain types of tumours predominate. The ROC curves are in good agreement for similar populations. The method described here offers a practical way to evaluate the decision guarantee of a diagnosis using ROC curves. Additionally this it is possible to determine with statistical certainty whether the evaluation of the sample data would lead to a diagnosis of lung cancer.

Use of the sensitivity-adapted decision guarantee opens new avenues for quality assurance. Fairer information about the reference population is included in the data analysis. Thus, the requirement is met for determining the statistical comparability of the user's situation with that of the reference population in order to ensure the correct qualitative application. With a systematic feedback between laboratory results and the specialist physician's comprehensive clinical findings, the sensitivity and specificity of the laboratory parameter in question can be continually monitored. Marked changes in these diagnostic parameters should always be investigated. It is especially important to verify that such an effect does not arise through a change in the method of measurement. Thus, this method of data analysis could supplement the normal measures of quality assurance already in place in the laboratory.

The diagnostic parameters can be determined for a given laboratory value with reference to the appropriate population. The confidence interval can be calculated based on sensitivity and specificity. If none of the points of the ROC curve of the reference population fall within this interval, it must be assumed that the basic characteristics of the populations are statistically so different that a generalisation of the decision guarantee should be viewed with scepticism. Under such conditions, the mathematical algorithms can be formally applied, but a meaningful interpretation of quantitative results cannot be guaranteed.

If the ROC curve of the reference population lies inside the confidence interval, the method described here ensures a quantitative result for the decision guarantee. Even more importantly, a diagnosis of malignancy can be made not only on the basis of the data collected but in comparison with a reference population. Thus, it is possible to determine with statistical certainty whether the evaluation of the sample data would lead to the diagnosis of lung cancer. This provides the basis for a laboratory- and method-independent support for a diagnosis.

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