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Menetelmä vatsasyövän osoittamiseksi

Förfarande för detektering av magcancer

(57) Tiivistelmä - Sammandrag - Abstract

Keksintö koskee menetelmä syövän osoittamiseksi tai seulomiseksi yksilöllä, jossa menetelmässä määritetään mainitun yksilön mahan limakalvon gastriittifenotyyppi, määritetään solufibronektiinipitoisuus mainitusta yksilöstä otetusta näytteestä, kuten seeruminäytteestä, valitaan cut-off-arvo solufibronektiinille ja verrataan solufibronektiinipitoisuutta valittuun cut-off-arvoon. Atrofisen gastriitin tila yhdessä solufibronektiinipitoisuuden, joka on yli valitun cut-off-arvon, kanssa on sitten osoitus mahasyövästä mainitulla yksilöllä.
Uppfinningen avser ett förfarande för att detektera eller sålla kancer hos en individ, vilket förfarande omfattar bestämning av gastritfenotypen av magslemhinnan hos nämnda individ, bestämning av den cellulära fibronektinkoncentrationen i ett prov, såsom ett serum prov av nämnda individ, val av ett cut-off-värde för cellulär fibronektin, och jämförelse av den cellulära fibronektinkoncentrationen med det valda cut-off-värdet. En kombination av ett tillstånd av atrof gastrit och en koncentration av cellulär fibronektin över det valda cut-off-värdet är då indikativ för magkancer hos nämnda individ.

METHOD FOR DETECTING GASTRIC CANCER

FIELD OF THE INVENTION

This invention relates to a method for diagnosing and/or screening gastric cancer in an individual. According to a further embodiment, the invention relates to a method which makes it possible to differentiate between a diagnosis for gastric and non-gastric cancer in an individual.

BACKGROUND OF THE INVENTION

Chronic gastritis is an extremely common disorder. It is estimated that nearly half of the world's population will get gastritis during their lifetime. Chronic gastritis is most often caused by *Helicobacter pylori* infection and can be considered an immunological reaction against this bacterium in a great majority of cases.

Chronic gastritis is a rather unique bacterial infection with characteristic chronicity and life-long duration. A spontaneous healing of gastritis, normalization of the gastric mucosa in both antrum and corpus, is a rare event. Usually the natural course of chronic gastritis is a sequence of alterations from inflammation to atrophy which significantly changes the structure and function of the gastric mucosa. The outcome of chronic gastritis represents several important disorders, all of which seem to show an association with a specific alteration and with some particular state in the course of gastritis.

Bacterial infection results in simple inflammation which consists of immunocompetent lymphocytes and plasma cells, and often granulocytes, in the gastric mucosa. Studies in children and young people suggest that this chronic inflammation is the prevailing initial phenotype of gastritis in early age. In the elderly, atrophy and intestinal metaplasia of the underlying mucosa are common phenomena and increase

in prevalence with age.

Studies suggest a slow progression of chronic gastritis into atrophy. Gastritis and subsequent atrophy are important causes of several functional and homeostatic impairments of the gastric mucosa. Atrophy results in failure of the secrection of acid, pepsinogens and gastrin from the corpus and antral mucosa along with the development of atrophy (loss of normal mucosal glands).

It is possible to electrophoretically distinguish 7 different pepsinogens from the gastric mucosa in humans. Of these the five fastest form the immunologically uniform group of pepsinogen I. The other two form the pepsinogen II group. The group I pepsinogens are synthezised only in the main cells and the mucous secreting cells of the corpus area of the stomach. In contrast thereto, group II pepsinogens are formed in the glands over the whole stomach area and to some degree also in the upper part of the duodenum in the Brunner's glands. In the serum of a healthy person the pepsinogen I concentration is approximately 6 times that of the pepsinogen II concentration. In atrophic gastritis of the corpus area of the stomach the serum pepsinogen I concentration decreases, whereas the serum pepsinogen II concentration remains at the previous level. Thus, the serum pepsinogen I concentration fairly well reflects the number of pepsinogen secreting cells in the corpus area of the stomach, and their condition. The more serious the atrophic gastritis of the corpus area of the stomach is, the lower is the serum pepsinogen I concentration. A low pepsinogen I concentration in the serum indicates severe atrophic corpus gastritis with a sensitivity of over 90 % and a specificity of almost 100 % (1).

Gastrin is secreted in the gastrointestinal tract in at least three different forms, the immunoreactive activity of all these forms being measured when serum gastrin is determined (total serum gastrin). Gastrin subtypes are the so-called minigastrin (G-14), little gastrin (G-17) and big gastrin (G-34). Physiologically most important are gastrin-17 and gastrin-34. The effect of gastrin-17 on the secretion of hydrochloric acid is 6 times that of gastrin-34. Gastrin is secreted from the so-called G-cells,

which appear both in antrum and in duodenum. The most important accelerators of gastrin secretion is the tonus of the vagus nerve and the protein degradation products. The secretion of gastrin is slowed down by a pH decrease of below 2.5. The gastrin secreted from the antrum is to over 90 % of the gastrin-17 type, whereas the duodenal gastrin is primarily of the gastrin-34 type (2). In a fasting situation, primarily gastrin-34 is found in the serum, whereas after a meal the serum gastrin is of the gastrin-17 type (3). The secretion of gastrin-17 can also be studied using the so-called protein stimulation test. In such a test, a blood sample after fasting is taken in the morning, whereafter the patient eats a protein rich standard meal and blood samples are taken at 15 minute intervals for two hours. The maximal increase is evident after appr. 20 minutes.

In atrophic antrum gastritis the mucous membrane of the antrum is atrophied and thus its gastrin-17 secretion decreases and its concentration in the serum is reduced. A reduced gastrin-17 concentration in the serum would thus be an indicator of antrum atrophy and of an increased risk of cancer in this area. In case the mucous membrane of the antrum is atrophied, there is a reduced response also in the protein stimulation test, which seems to be a more sensitive indicator of atrophy than the mere concentration determination. In the publication WO 96/15456, there is described a method for screening for the risk of cancer, by determining atrophy in the various parts of the stomach.

Thus for the purpose of this invention, one distinguishes, both in the corpus and in the antrum mucosa, between the phenotypes: normal mucosa, gastritis (superficial or non-atrophic gastritis) and atrophic gastritis. Atrophic gastritis is, in turn, classified, in order of severity, in mild, moderate and severe atrophic gastritis.

Fibronectins (Fn) are large adhesive glycoproteins on cell surfaces and in extracellular matrix (4). They play a role in many biological functions; e.g., in adhesions and migration of the cells, and also in invasion, metastasis, and malignant transformations (4-8). The Fns are divided into two major forms: plasma (pFn) and

cellular fibronectins (cFn). The cFns differ from the pFns in having extra domain sequences (A and B) in the protein moiety (8). This difference enables the immunological differentiation of cFns from pFns by an EIA assay (9). Immunohistochemically, the cFn is mainly found in extracellular matrices but is also expressed in various malignant tumours (10,11).

Serum and plasma levels of cFn are shown to increase in cancer patients, particularly in the malignomas of the liver, pancreas and biliary tract, and in the malignancies of the digestive tract in general (12-22).

Further, as is shown below, early neoplastic lesions (gastric adenomas and dysplasias) show elevated cFn levels in high frequency, it is conceivable that a test for cFn could be helpful and applicable also in disclosure of early and curable cancers non-invasively. Such a test could thus be appointed to specific risk populations in which the cancer rate, and the rate of the precancerous lesions as well, is high; e.g., in subjects with advanced atrophic gastritis that is a serious precancerous condition for gastric cancer. Such risk population can be easily screened from the general population with simple blood tests (23,24). The post hoc application of the cFn test in such populations could be a tool to find and identify most of the patients who have an existing tumour or a premalignant lesion.

Furthermore, it would be advantageous to be able to provide a detection method according to which gastric cancer could be distinguished from non-gastric cancer. This would make it possible to quickly and purposefully direct a patient to further examinations and tests.

These and further objects are achieved with the method according to the invention for detecting or screening cancer or a precancerous condition in an individual, which method in its broadest sense comprises determining the gastritis phenotype of the gastric mucosa of said individual, determining the cellular fibronectin concentration in a sample from said individual, such as a serum sample, and

comparing the determined fibronectin concentration to a selected cut-off value, the determined phenotype in combination with the cellular fibronectin concentration in the sample as compared to the cut-off value, providing the evaluation basis for the detection or screening, i.e. for determining the presence and location of cancer or a precancerous condition, namely the presence of gastric or non-gastric cancer in said individual.

Thus the invention provides a method as defined above for detecting or screening gastric cancer or a gastric precancerous condition in an individual, which method comprises identifying an individual with atrophic gastritis, whereby a concentration of cellular fibronectin at or above the selected cut-off value being indicative of gastric cancer or precancerous condition in said individual.

The invention is specifically directed to a method for detecting or screening gastric cancer or a gastric precancerous condition in an individual, which method comprises the steps of

- determining quantitatively the pepsinogen I and gastrin-17 analyte concentrations in a sample from the said individual,
- selecting a cut-off value for respective analytes,
- comparing the pepsinogen I and gastrin-17 concentrations so determined with their respective selected cut-off value, the method comprising the additional step of
- determining quantitatively the cellular fibronectin analyte concentration in a sample from said individual,
- selecting a cut-off value for said analyte, and comparing the cellular fibronectin concentration so determined to the selected cut-off value, whereby a value for pepsinogen I and/or gastrin-17 below its respective cut-off value, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of gastric cancer or precancerous condition.

The invention is also directed in a broad sense to a method for detecting or

screening non-gastric cancer or a non-gastric precancerous condition, which comprises identifying an individual with normal mucosa or non-atrophic gastritis, whereby a concentration of cellular fibronectin at or above the selected cut-off value is indicative of the said individual having non-gastric cancer or precancerous condition.

The invention is specifically directed to a method for detecting or screening non-gastric cancer or a non-gastric precancerous condition in an individual, which method comprises the steps of

- determining quantitatively the pepsinogen I and gastrin-17 analyte concentrations in a sample from the said individual,
- selecting a cut-off value for respective analytes,
- comparing the pepsinogen I and gastrin-17 concentrations so determined with their respective cut-off value, the method comprising the additional step of
- determining quantitatively the cellular fibronectin analyte concentration in a sample from said individual,
- selecting a cut-off value for said analyte, and comparing the cellular fibronectin concentration so determined to the selected cut-off value, whereby a value for pepsinogen I and gastrin-17 at or above their respective cut-off values, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of non-gastric cancer or precancerous condition.

The method according to the invention thus allows for differentiating between a diagnosis of gastric and non-gastric cancer or precancerous condition in an individual, the method comprising determining the gastritis phenotype of the gastric mucosa of said individual, determining the cellular fibronectin concentration in a sample, such as a serum sample from the said individual, selecting a cut-off value for cellular fibronectin, comparing the cellular fibronectin concentration so determined to the selected cut-off value, a concentration of cellular fibronectin at or above the selected cut-off value in combination with atrophic gastritis being indicative of

gastric cancer or precancerous condition, and a concentration of cellular fibronectin at or above the cut-off value in combination with a normal mucosa or non-atrophic gastritis being indicative of non-gastric cancer or precancerous condition.

The invention thus concerns a method for differentiating between a diagnosis of gastric and non-gastric cancer or precancerous condition in an individual, the method comprising the steps of

- determining quantitatively the pepsinogen I and gastrin-17 analyte concentrations in a sample from the said individual,
- selecting a cut-off value for respective analytes,
- comparing the pepsinogen I and gastrin-17 concentrations so determined with their respective cut-off value, the method comprising the additional step of
- determining quantitatively the cellular fibronectin analyte concentration in a sample from said individual,
- selecting a cut-off value for said analyte, and comparing the cellular fibronectin concentration so determined to the selected cut-off value, whereby a value for pepsinogen I and/or gastrin-17 below its respective cut-off value, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of gastric cancer, and a value for pepsinogen I and gastrin-17 at or above their respective cut-off values, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of non-gastric cancer.

According to the invention the term 'individual' refers to a mammal, such as a human or an animal.

The sample is typically a body fluid sample, especially a serum sample, but also a urine, salivary or lacrimal fluid sample can be used.

The different methods for the determination of the PGI and G-17 are as such well

known to the person skilled in the art, and there are also kits commercially available for carrying out the determinations. Such methods are usually immunological methods, using mono- or polyclonal antibodies to the analytes. The detection methods for use include, for example, measuring absorbance, fluorescence or luminescence. It is also possible to carry out the measurements simultaneously, for example on the same microplate, in different wells thereon, which combined assay system provides for an especially convenient method of diagnosis.

The concept of cut-off values in assays involving the determination of analyte concentrations is well known to the person skilled in the art, and it generally means a value or a set of values chosen as a limit between the reference values (normal values) and the abnormal values for the test in question. Such cut-off values are method-specific and depend on the specificity and sensitivity chosen for the test method used for the determination of the analyte concentrations, see for example William J Marshall, Clinical Chemistry, Third Edition, 1995, Mosby.

In a stomach with a healthy mucosa, the serum pepsinogen I and the gastrin-17 concentrations are within their reference values, the reference values being, depending on the specificity and sensitivity agreed upon for the method in question, 25 - 120 μg/l for PGI. Also the gastrin-17 will be within its normal or reference values, which are in the range of 5 - 25 pmol/l when measured as the stimulated value, that is the value for gastrin-17 obtained after protein stimulation.

In a situation where the corpus is normal or the gastritis in the corpus is of superficial phenotype and that of the antrum is of moderate to severe atrophic phenotype, there is an increased risk for gastric cancer. In this situation, the pepsinogen I concentration is still at or above the upper limit of its reference value, but the gastrin-17 value will be low, such as at its lower reference value, or below its cut-off value for severe atrophy. The cut-off value for gastrin-17, which can be the unstimulated or protein stimulated gastrin-17, depends on the specificity and sensitivity of the method used, and can be determined by the person skilled in the

art. The cut-off value for gastrin-17 is typically 5 pmol/l (as the stimulated value, i.e. as the post-prandial gastrin-17). When testing for atrophy in the antrum area, the gastrin-17 measurement can be complemented with a protein stimulation test, by measuring first the gastrin-17 concentration in the serum at the base line situation (unstimulated) and then after protein stimulation, for example after a protein rich standard meal. A lack of response in this test indicates atrophy of the antrum area.

At increasing severity of atrophic corpus gastritis, with no or only superficial antrum gastritis, the serum pepsinogen I concentration falls below the cut-off value indicating an increased risk i.a. for cancer and pernicious anaemia. The cut-off value is, depending on the specificity and sensitivity of the chosen method, 20 - 30 μg/l, typically 25 μg/l. The gastrin-17 concentration is still above its reference value as indicated above.

At increasing severity of both antral and corpus atrophic gastritis, the serum pepsinogen I concentration is below its cut-off value indicating an increased risk of cancer, and the serum gastrin-17 concentration is at its lower reference limit, or below or at its cut-off value indicating an increased risk of cancer. These gastritis phenotypes, moderate and severe atrophic gastritis, are associated with a very high risk of gastric cancer.

According to the invention it has been shown that the mean values of cellular fibronectin are significantly higher in gastric cancer patients than in controls. Thus by applying a test for measuring the cFn levels to a group of individuals who are at risk for developing gastric cancer, that is individuals with atrophic corpus or antrum gastritis, or pangastritis, i.e. individuals with either a low PGI or gastrin-17, or both, it would be possible to screen those patients who are likely to have cancerous conditions, in particular gastric cancer. The test could also be used for detecting premalignant lesions of gastric cancer, that is in patients with gastric adenoma or definite dysplasia, which conditions are associated with elevated cFn levels as well, which typically, however, are lower than those encountered with

cancerous conditions. The test could also be used in patients with carcinoma other than gastric ones. Thus the test could be used to screen those patients the cancers of which can still be successfully treated. Also the test could used for follow-up after treatment.

cFn or its extra domain can be detected from body fluids, in particular serum, for example by using immunological methods with specific antibodies. Monoclonal antibodies for use in this invention can be prepared using conventional methods. Any suitable immunological method can be used in the invention. Such methods are for example enzyme immunoassays (EIA), enzyme linked immunosorbent assays (ELISA), radio immunoassays (RIA), fluorescence immunoassays (FIA), luminescence immunoassays (LIA), latex or other agglutination methods, particle containing immunoassays (PCIA), immunoblottings, immunoprecipitation methods or methods utilizing different kinds of biosensors. In the tests described below, a cut-off for serum cFn of 2.5 mg/l has been found useful.

According to an embodiment of the invention, the method includes a step of diagnosing said individual also for *Helicobacter pylori* infection, e.g. by determining *Helicobacter pylori* antibodies in a sample, such as a serum sample, form the individual. For the determination of *Helicobacter pylori* antibodies, a number of commercial kits are available (e.g. Orion Pyloriset EIA-G, Pyloriset EIA-A, EIA 2G by Roche, Pyloristat by Whittaker Bioproducts). Antigens can be prepared from *Helicobacter pylori* bacteria in various ways and they are also commercially available. The cut-off value for *Helicobacter pylori* antibodies can easily be determined by a person skilled in the art. According to one embodiment, one can use a value of 30 EIU as a cut-off for indicating the presence or absence of a *Helicobacter pylori* infection. Another alternative is to evaluate the presence or absence of a Helicobacter pylori infection by determining the presence of the antigen itself. Such measurement can for example be carried out on a stool sample from the individual to be tested, and assays, such as enzyme immunoassays are commercially available for this purpose (cf. Lancet 1999, 354, 30-33). It is also

possible to determine the presence of the antigen from the breath of the individual, by measuring the carbon dioxide content, and systems for this assay are also commercially available. A further alternative is to determine the presence or absence of the antigen in a biopsy sample taken from the individual.

In order to illustrate the invention, the following tests for measuring serum levels of cellular fibronectin in the diagnosis of cancer, were performed.

Cancer series: The cancer series composed of two patient groups. The cancer series (I) consists of patients with gastric neoplasias, either precancerous lesions, such as adenomas or definite dysplasias of low or high grade (8 patients), or of those with an overt invasive gastric adenocarcinoma (39 patients). The cancer series (II) consists of 47 patients with overt invasive carcinomas other than the gastric ones. The details and demographics of the series (I) and (II) are presented in appended Table 1. The cancer diagnosis bases in all cases on histology (biopsy and/or operation specimens) and on clinical data.

Control series: The control series consists of 81 asymptomatic subjects without evidence of gastrointestinal diseases. Of these 40 were middle-aged or old (50-69 years) men representing a population based sample of asymptomatic males in Kotka-Vantaa cities in Southern Finland. The rest (41 persons) were asymptomatic volunteers representing all age groups. The detailed demographics of the control series are presented in Table 1.

Serum samples: The serum samples of the cancer series were collected in Helsinki University Central Hospital (HUCH), Jorvi Hospital, Espoo, Finland, in 1980-1986, and those of the control series in 1994-2000. The samples were taken in the morning after an overnight fast and were immediately frozen in -70°C until used.

Assay of cellular fibronectin (cFn):. The concentration of cFn in serum was measured by using sandwich enzyme immunoassay (EIA). 96-well microplates

(Maxisorb, Biohit Plc, Helsinki, Finland) were coated overnight at 4 °C with 4 μg/ml of monoclonal antibody (Mab) DH1 (Biohit PLc), specific for cFn. The plates were washed three times with 20 mM Phosphate buffered saline (PBS)-0,05% Tween 20, pH 7,0. The unspecific binding was blocked by incubation with 1% BSA in 10 mM PBS, pH 7.4 for 1h at 37 °C . The plates were washed as above. The serum samples were diluted 1:5 into 10 mM PBS, pH 7.4 with 1% BSA, 1mM EDTA, 0,02% Tween 20, and 100 μl of samples were added to the wells. After incubation for one hour at 37 °C the unbound material was removed by washing three times with washing buffer. Peroxidase conjugated Mab BE2 (Biohit Plc), recognising total Fn, was added to the wells and incubated 1h at 37 °C. After washing the bound enzyme was visualised by adding a substrate, tetramethyl-benzidine (TMB) to the wells. The formed colour was measured with microplate reader at 450 nm.

Statistics: In statistics, ordinary techniques were used. In assessing the accuracy of the cFn test, the case-control setting and the construction of the Receiver Operating Characteristic (ROC) curves were plotted by calculating the true positive fraction (sensitivity (%), and false positive fraction (100- specificity (%)) of the cFn at various cut off points (25). In estimation of the predictive values of the positive test result (PVP), the sensitivity and specificity figures were used by assumption that the expected prevalence rate of cancer in the study population is 5%.

The mean and median levels of serum cellular fibronectin (cFn) of cancer patients and non-cancer controls are presented in the appended Table 2 and in a box-and-whiskers format in Fig 1. The median is presented with the vertical line inside the box. Inside the boxes are 50% of the cases and inside the whiskers are 100% of the cases with normal distribution. Outliers (1.5x box length) and extreme cases (3x box length) are indicated.

The mean values of cFn were significantly ($P < 0.001$) higher in gastric cancer patients than in controls. This was also the case in patients with premalignant

lesions of gastric cancer; i.e., in patients with gastric adenoma or definite dysplasias (dysplasia of low or high grade), and in patients with carcinomas other than the gastric ones (Table 2).

The ROC-curves of serum cFn in the diagnosis of gastric carcinomas and non-gastric carcinomas are presented in Figs 2 and 3. The cFn values of 2.5 mg/l did show the most efficient cut-off level. In gastric carcinomas, by adopting this cut-off value, the serum cFn did show the sensitivity and specificity of 74% (CI95%: 61-81%) and 93% (87-98%), and in non-gastric carcinomas 70% (57-83%) and 93 % (87-98%), respectively.

The predictive values of high serum levels of cFn (2.5 mg/l or above, or 5.0 mg/l or above) for gastric carcinoma in relation to the prevalence of gastric cancer in the study population are presented in Fig 4. It appears that, by using the above cut-off levels, the predictive values of high cFn are 36% and 65% , respectively, if it is assumed that the prevalence of gastric cancer is 5% in the study population (indicated by the vertical line).

In the detection of carcinomas of various type and origin, the cFn showed high sensitivity values. There was some but insignificant variation between the cancer types. The cFn did show highest sensitivity in the diagnosis of the carcinomas of pancreas and biliary tract (Table 2). The serum levels of cFn were significantly ($P=0.002$) higher in gastric adenomas and dysplasias than in healthy controls. These cFn levels in gastric adenoma-dysplasia patients were slightly but insignificantly lower than those in patients with overt gastric carcinoma.
The application of cFn as a serum marker for cancer has been suggested and emphasised in some previous studies (20, 21). The present results suggest that the sensitivity of the pathological cFn levels (2.5 mg/l or above) is higher than 70% in gastric cancer, and that this high diagnostic accuracy is also the case in non-gastric cancers, such as, for example, colonic and pancreatic carcinomas. Moreover, at the best discrimination level (serum cFn 2.5 mg/l or above), the specificity of the cFn

was also high, over 90%.

Most of the available common cancer markers, such as CEA, Ca 19-9, etc., show sensitivity and specificity figures that are less than 70% or 90%, respectively (21). However, in addition to the sensitivity and specificity, the practical value of the test is dependent of the actual prevalence of cancer diseases in the population in which the test is intended to be used. Even in selected populations the cancer is quite infrequent finding which presupposes that the accuracy of the invented test must be high if the test is to be of effective diagnostic help.

In the evaluation of the practical value of cFn, it was assumed that the test will be used in populations in which the expected prevalence rate of cancer is 5%. Concerning the gastric cancer rate alone, this is a realistic assumption: some previous studies have suggested that the prevalence rates of gastric neoplasia are 4-6% in risk populations such as patients with advanced atrophic gastritis (24,25). Based on the present sensitivity and specificity figures, the value of high serum cFn (2.5 mg/l or above) to predict gastric cancer (PVP) is 36% in a population in which the expected cancer prevalence is 5%; i.e., the cancer will be found and the test is correct in 36% of the cases with high cFn in the serum. This PVP will rise up to 65% if the cut-off value of cFn is increased to 5.0 mg/l. Since the cFn test also detects other common cancers (colon, pancreas, liver, biliary, etc.) with practically same accuracy than gastric cancers, the value of the cFn test as a clinical tumour marker is higher than the estimates based on gastric cancer alone may indicate.

The present series included eight patients with verified precancerous lesions. These are adenomas and/or dysplastic lesions which inevitably progress with time into the cancer. These tumours do not show evident invasion even though the dysplasia of high grade are considered carcinomas in situ by some pathologists (26,27). Surprisingly, also 5 of 8 patients with these tumours showed elevated cFn levels in the serum. In addition, the mean serum levels of cFn were significantly higher in the adenoma-dysplasia patients than those in healthy control subjects but were

somewhat lower than those seen in patients with invasive carcinomas. This finding suggests that the cancers raise the serum levels of cFn in early stage of their development. Some of the adenomas and dysplastic lesions may have had an early invasive component even though the biopsy material and tissue sampling of the tumours did not reveal that.

Table 1. Demographics of the series and controls.

SERIES	NUMBER OF CASES	M/F	AGE years Mean (SD)	AGE RANGE years
GASTRIC NEOPLASIAS				
ADENOMA	8	6/2	75 (9)	56-88
CARCINOMA	39	20/19	64 (14)	34-84
INTESTINAL	20	13/7	70 (12)	34-84
DIFFUSE	13	3/10	58 (15)	36-76
UNCLASSIFIED	6	472	61 (16)	40-76
TOTAL	47	26/21	66 (14)	34-88
NON-GASTRIC CANCERS				
COLO-RECTAL	14	9/15	62 (18)	23-88
LUNG-BRONCHIAL	3	370	77 (4)	74-82
PANCREAS-BILIARY	13	10/3	58 (14)	30-77
OVARIAL-GYNECOL	6	0/6	68 (11)	52-82
PROSTATIC	4	4/0	73 82)	69-74
OTHERS	7	¾	70 (16)	46-84
TOTAL	47	29/18	65 (15)	23-88
CONTROLS	81	62/19	57 (12)	25-80

Table 2. Serum concentrations of cellular fibronectin (cFn) in series and controls. Number and percentage of cases with cFn levels 2.5 mg/l or above (cut off value) is also indicated.

SERIES	NUMBER OF CASES	SERUM LEVEL OF CELLULAR FIBRONECTIN Mean (SD) mg/l	Median mg/l	2.5 or above No. and % of cases	
GASTRIC NEOPLASIAS					
ADENOMA	8	3.7 (3.6)	2.8	5	63%
CARCINOMA	39	4.7 (3.6)	3.5	29	74%
INTESTINAL	20	5.5 (3.6)	4.0	15	75%
DIFFUSE	13	3.5 (2.9)	2.7	8	62%
UNCLASSIFIED	6	4.2 (1.1)	3.5	34	72%
TOTAL	47				
NON-GASTRIC CANCERS					
COLO-RECTAL	14	3.9 (2.5)	3.6	9	64%
LUNG-BRONCHIAL	3	2.2 (1.1)	2.6	2	67%
PANCREAS-BILIARY	13	5.6 (4.3)	4.3	10	77%
OVARIAL-GYNECOL	6	3.3 (3.0)	2.5	4	67%
PROSTATIC	4	2.9 (1.4)	3.2	3	75%
OTHERS	7	4.8 (3.4)	4.3	5	71%
TOTAL	47	4.2 (3.2)	3.1	33	70%
CONTROLS	81	1.7 (1.7)	1.4	6	7%

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Claims

1. Method for detecting or screening cancer or a precancerous condition in an individual, which method comprises determining the gastritis phenotype of the gastric mucosa of said individual, determining the cellular fibronectin concentration in a sample from said individual, such as a serum sample, and comparing the determined fibronectin concentration to a selected cut-off value for cellular fibronectin, the determined phenotype in combination with the cellular fibronectin concentration providing the basis for the detection or screening.

2. The method according to claim 1 for detecting or screening gastric cancer or a gastric precancerous condition, comprising identifying an individual with atrophic gastritis, whereby a concentration of cellular fibronectin at or above the selected cut-off value is indicative of gastric cancer or a precancerous condition in said individual.

3. The method according to claim 2, comprising the steps of

- determining quantitatively the pepsinogen I and gastrin-17 analyte concentrations in a sample from the said individual,
- selecting a cut-off value for respective analytes,
- comparing the pepsinogen I and gastrin-17 concentrations so determined with their respective selected cut-off value, the method comprising the additional step of
- determining quantitatively the cellular fibronectin analyte concentration in a sample from said individual,
- selecting a cut-off value for said analyte, and comparing the cellular fibronectin concentration so determined to the selected cut-off value, whereby a value for pepsinogen I and/or gastrin-17 below its respective cut-off value, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of gastric cancer or a precancerous condition.

4. The method according to claim 1 for detecting or screening non-gastric cancer or a non-gastric precancerous condition, comprising identifying an individual with normal mucosa or non-atrophic gastritis, whereby a concentration of cellular fibronectin at or above the selected cut-off value is indicative of the said individual having non-gastric cancer or a precancerous condition

5. The method according to claim 4, comprising the steps of

- determining quantitatively the pepsinogen I and gastrin-17 analyte concentrations in a sample from the said individual,
- selecting a cut-off value for respective analytes,
- comparing the pepsinogen I and gastrin-17 concentrations so determined with their respective cut-off value, the method comprising the additional step of
- determining quantitatively the cellular fibronectin analyte concentration in a sample from said individual,
- selecting a cut-off value for said analyte, and comparing the cellular fibronectin concentration so determined to the selected cut-off value, whereby a value for pepsinogen I and gastrin-17 at or above their respective cut-off values, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of non-gastric cancer or precancerous condition.

6. The method according to claims 2 and 4 for differentiating between a diagnosis of gastric and non-gastric cancer or a precancerous condition in an individual, comprising determining the gastritis phenotype of the gastric mucosa of said individual, whereby a concentration of cellular fibronectin at or above the selected cut-off value in combination with atrophic gastritis is indicative of gastric cancer or precancerous condition, and a concentration of cellular fibronectin at or above the cut-off value in combination with a normal mucosa or non-atrophic gastritis is indicative of non-gastric cancer or precancerous condition.

7. The method according to claim 6, comprising the steps of

- determining quantitatively the pepsinogen I and gastrin-17 analyte concentrations in a sample from the said individual,
- selecting a cut-off value for respective analytes,
- comparing the pepsinogen I and gastrin-17 concentrations so determined with their respective cut-off value, the method comprising the additional step of
- determining quantitatively the cellular fibronectin analyte concentration in a sample from said individual,
- selecting a cut-off value for said analyte, and comparing the cellular fibronectin concentration so determined to the selected cut-off value, whereby a value for pepsinogen I and/or gastrin-17 below its respective cut-off value, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of gastric cancer or precancerous condition, and a value for pepsinogen I and gastrin-17 at or above their respective cut-off values, in combination with a cellular fibronectin concentration at or above its cut-off value, is an indication of non-gastric cancer or precancerous condition.

8. The method according to any one of the preceding claims, wherein the sample is a body fluid sample, especially a serum sample.

9. The method according to any one of the preceding claims, wherein the analytes are determined immunologically.

10. The method according to the claim 9, wherein the determination is carried out on a microplate.

11. The method according to any one of the preceding claims, wherein a detection method for determining the analytes is used which is based on measuring absorbance, fluorescence or luminescence.

12. The method according to any one of the claims 9 to 11, wherein for the determination of the pepsinogen I concentration, a polyclonal or monoclonal antibody to pepsinogen I is used.

13. The method according to any one of the claims 9 to 12, wherein for the determination of the gastrin-17 concentration, a polyclonal or monoclonal antibody to gastrin-17 is used.

14. The method according to any one of the preceding claims, wherein the cellular fibronectin is measured using an enzyme immunoassay method.

15. The method according to any one of the preceding claims which comprises the further step of determining a *Helicobacter pylori* infection in said individual, such as determining a *Helicobacter pylori* marker, such as antibodies, in a sample from said individual.

Fig 1

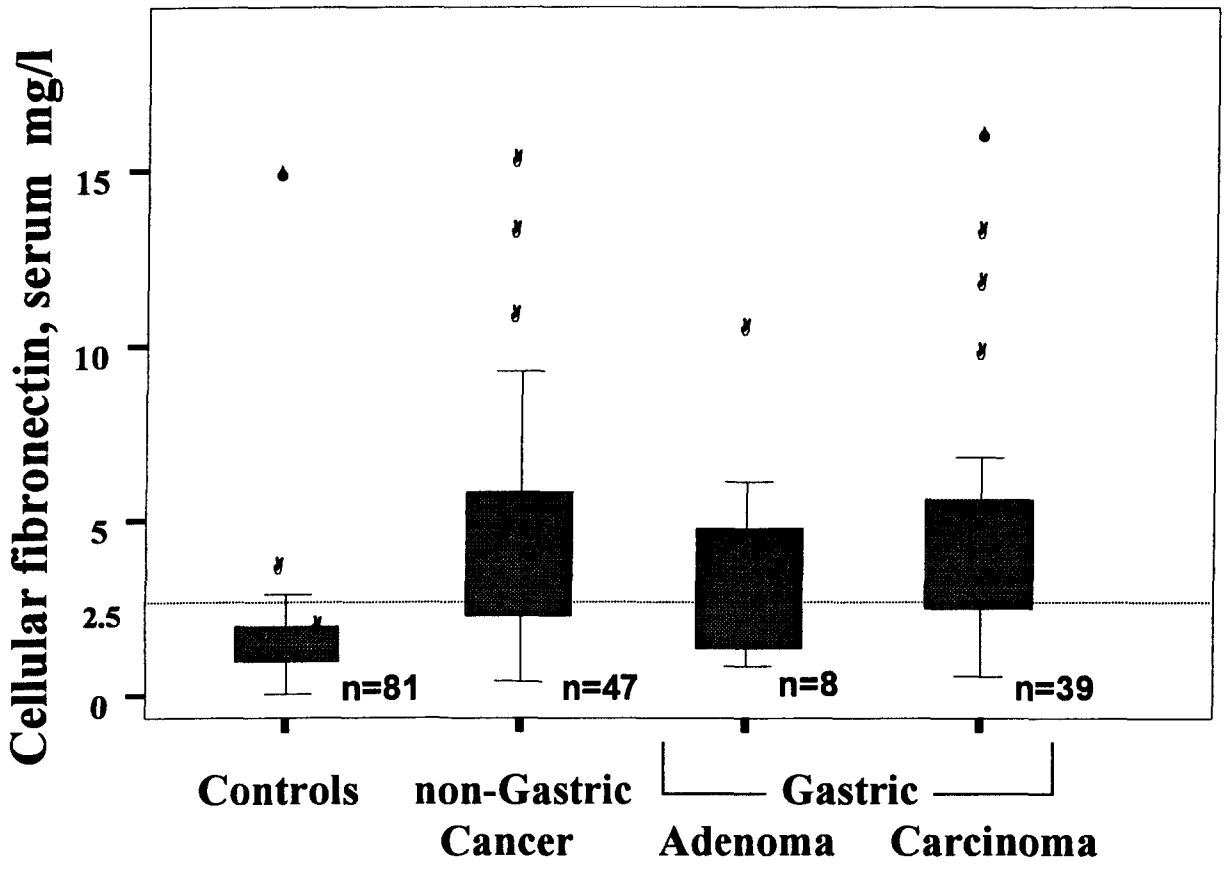

Cellular fibronectin, serum mg/l
15
10
5
2.5
0
n=81
n=47
n=8
n=39
Controls
non-Gastric Cancer
Gastric
Adenoma
Carcinoma

Fig 2

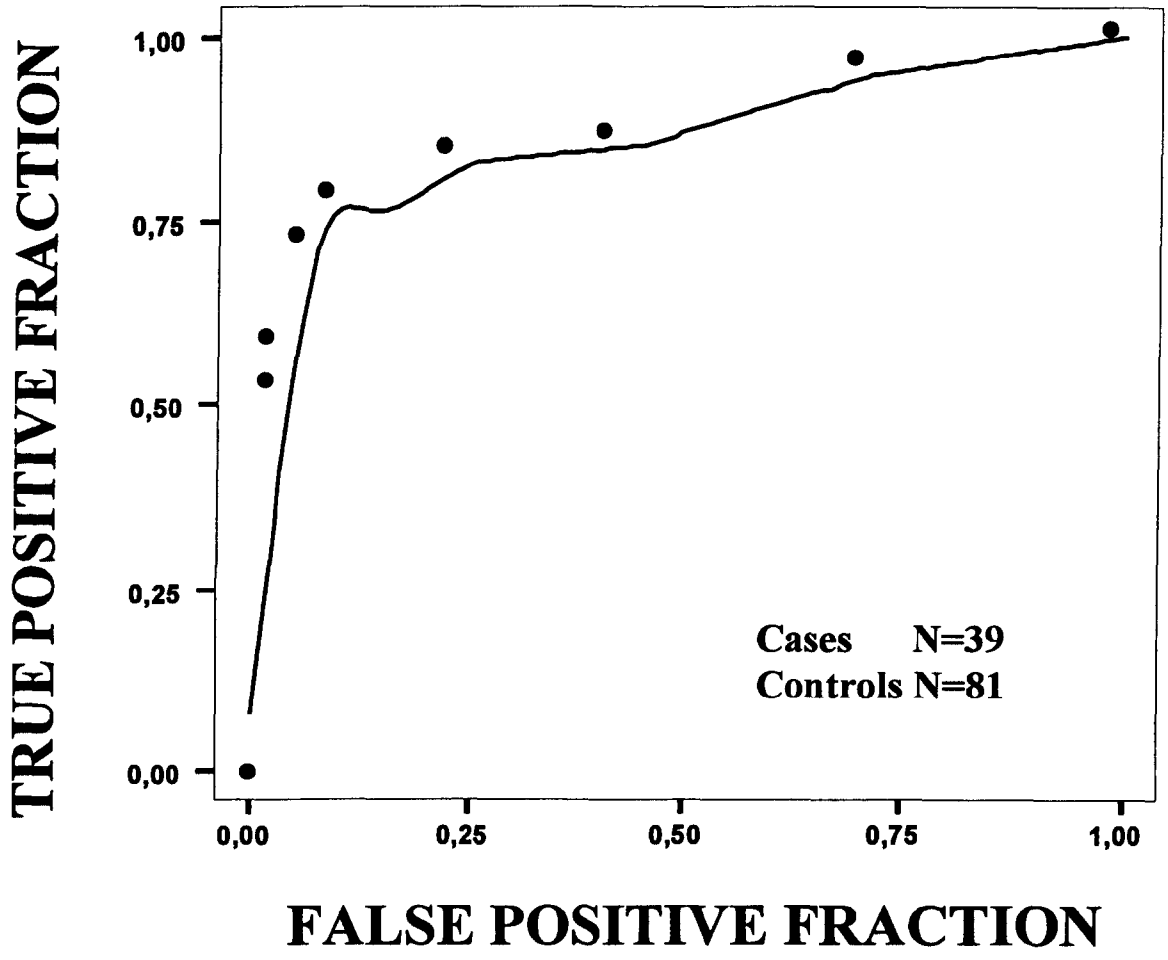
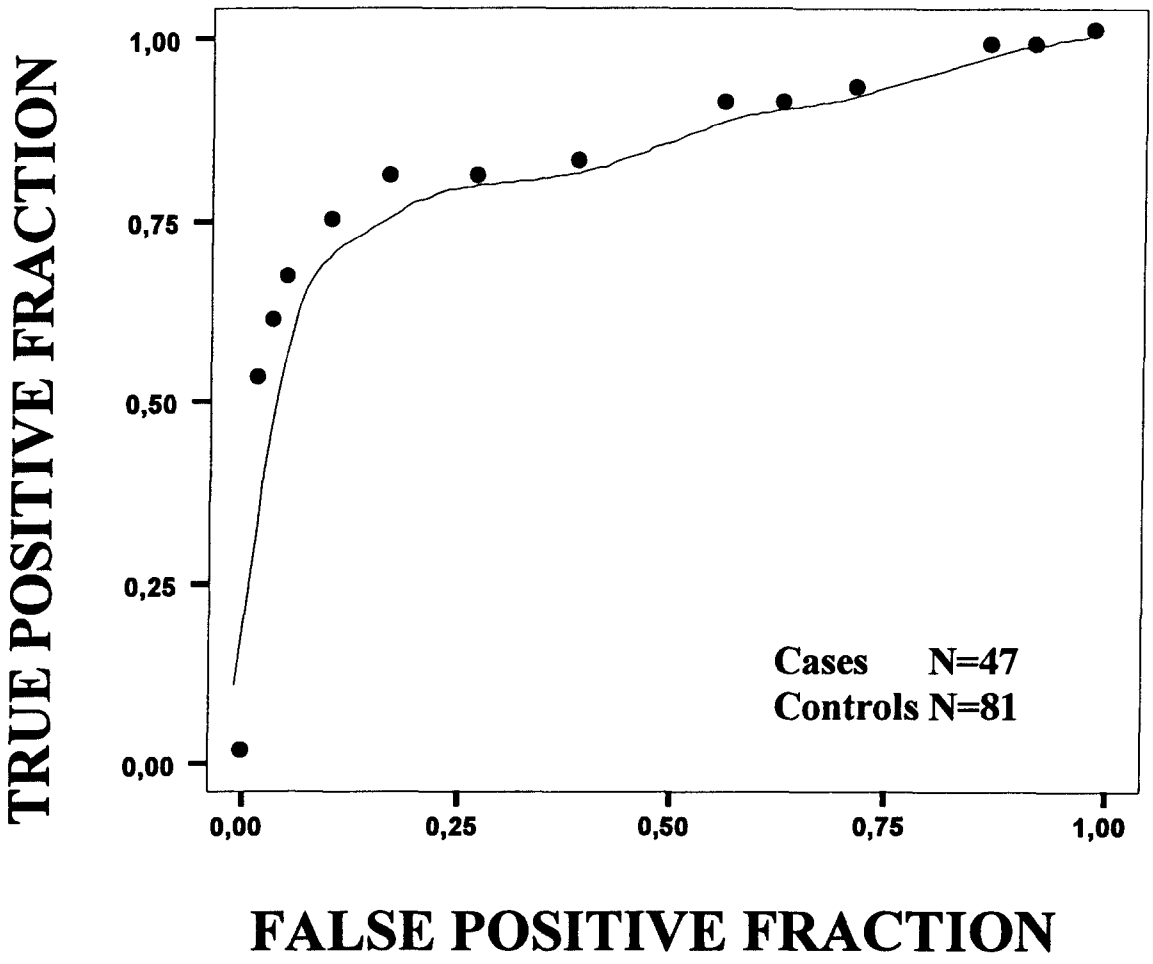

TRUE POSITIVE FRACTION
1,00
0,75
0,50
0,25
0,00
0,00
0,25
0,50
0,75
1,00
Cases N=39
Controls N=81
FALSE POSITIVE FRACTION

Fig 3


TRUE POSITIVE FRACTION
1,00
0,75
0,50
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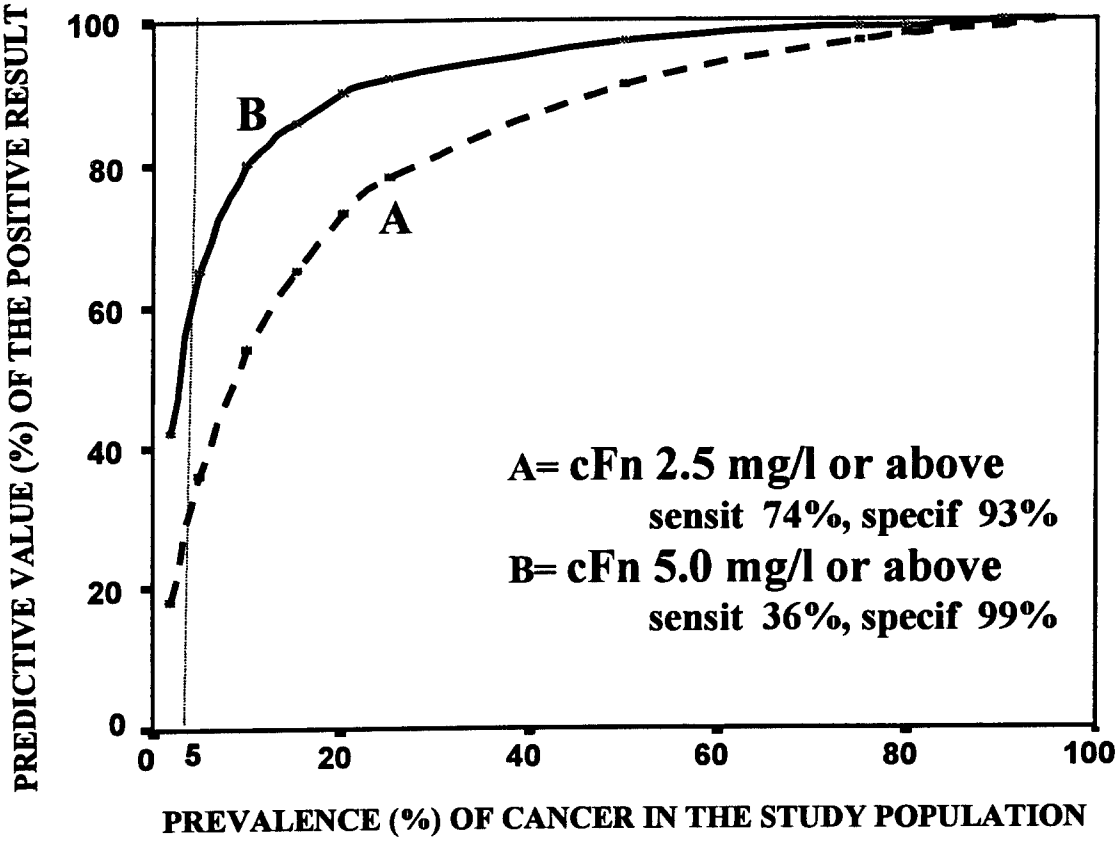
0,25
0,50
0,75
1,00
Cases N=47
Controls N=81
FALSE POSITIVE FRACTION

Fig 4


PREDICTIVE VALUE (%) OF THE POSITIVE RESULT
100
80
60
40
20
0
0
5
20
40
60
80
100
B
A
A= cFn 2.5 mg/l or above
sensit 74%, specif 93%
B= cFn 5.0 mg/l or above
sensit 36%, specif 99%
PREVALENCE (%) OF CANCER IN THE STUDY POPULATION