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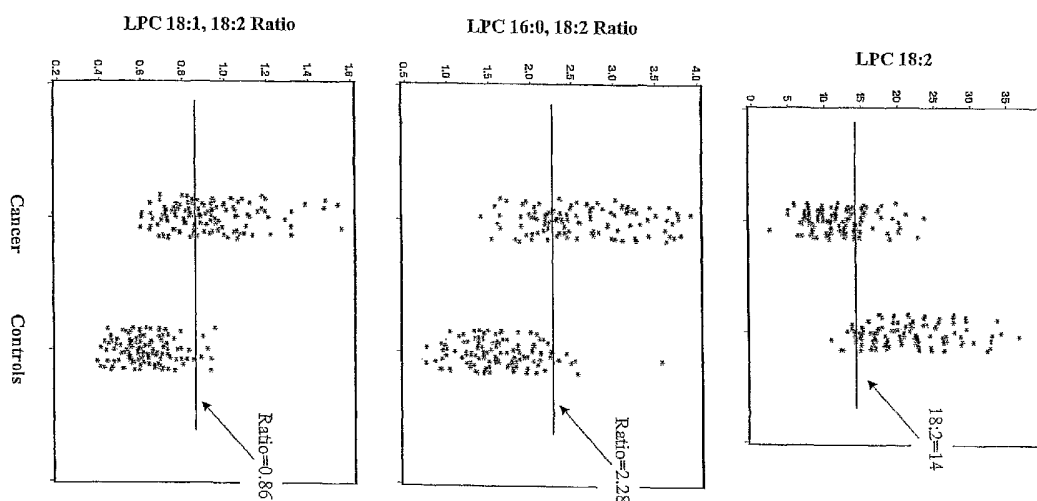
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(54) Title: METHODS OF DETECTING COLORECTAL CANCER



(57) Abstract: The present invention is directed to a method of detecting colorectal cancer in an individual comprising determining a level of lysophosphatylcholine (LPC) in the individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of colorectal cancer in the individual. The LPC can be, for example, LPC 18:2, LPC 18:1, LPC 16:0 or a combination thereof. In a particular embodiment, the sample the sample (e.g., blood, plasma, lymph and tissue) is obtained from the individual. The level of LPC in the individual can be detected using, for example, a liquid chromatography - mass spectrometry (LC-MS) detection method, an ultraviolet (UV) detection method, an enzymatic detection method and combinations thereof.

METHODS OF DETECTING COLORECTAL CANCER

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 5 60/618,787 filed October 14, 2004, and U.S. Provisional Application No. 60/690,604 filed June 14, 2005. The entire teachings of these applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Colorectal cancers (CRCs) are the third most common cancers in men and 10 women. Each year, more than 150,000 Americans are diagnosed with colorectal cancer (CRC) and approximately 57,000 die from this disease. It is the fourth most commonly diagnosed cancer and the second leading cause of cancer-related deaths in the United States (Society, A. C. Cancer Facts&Figures-2004. American Cancer Society Inc. (2004)). When CRCs are detected early, the 5-year relative survival rate 15 is 90%. However, only 37% of CRCs are detected at an early and localized stage. The 5-year survival rate for persons with distant metastases is only 8% (Society, A. C. Cancer Facts&Figures-2004. American Cancer Society Inc. (2004)).

Beginning at age 50, both men and women are recommended to follow one of the five testing schedules listed below:

- 20 • yearly fecal occult blood test (FOBT): a stool test that reveals small amounts of blood
- flexible sigmoidoscopy every 5 years

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- yearly fecal occult blood test plus flexible sigmoidoscopy every 5 years
- double-contrast barium enema every 5 years
- colonoscopy every 10 years

These tests are especially important for those with higher-than-average risk. Groups
5 identified with a high incidence of CRC include those with hereditary conditions, (e.g. familial polyposis or hereditary nonpolyposis colon cancer [HNPCC]), ulcerative colitis, personal or first-degree family history of CRC or adenomas, and personal history of ovarian or endometrial cancer).

Despite these screening methods, many patients present with late-stage disease
10 and have a poor prognosis (Shelton, B. K., *Semin Oncol Nurs* 18: 2-12. (2002)). While colonoscopy has been shown to be accurate with reported sensitivity and specificity values exceeding 95%, the cost, inconvenience, and discomfort associated with this test greatly reduce the degree of patient acceptance.

A number of biomarkers have been identified for the early detection of colon
15 cancer in colon tissues, feces, and serum (Srivastava, S., *et al.*, *Clin Cancer Res* 7:1118-26. (2001)). However, none of these markers provide a level of accuracy of detection comparable to colonoscopy. More recently, the detection of DNA mutations and/or amplification in stool has been evaluated as diagnostic markers (Calistri D, *et al.*, *Clin Gastroenterol Hepatol.*, 1(5):377-83 (2003); Dong SM, *et al.*, *J. Natl. Cancer Inst.*,
20 6;93(11):858-65 (2001)). In particular, the following website
<http://www.exactsciences.com/medical/pregen.html> posted the results of large-scale studies (total of 5,400 patients) using the PreGen-Plus (a non-invasive screening test designed to detect DNA alterations found in stool) compared with FOBT. Although the stool test demonstrated a sensitivity that was 4-fold greater than FOBT, the average
25 sensitivity of these tests from 9 studies was only ~57% (see the website and cited references). Thus, there continues to be an important need for a simple and highly reliable test for the early detection of colorectal cancers.

SUMMARY OF THE INVENTION

It has now been found that lysophosphatylcholines (LPC), choline-containing phospholipid species, can be used as biomarkers for colon cancer. For example, using 18:2-lysophosphatylcholine (LPC) and 18:1-LPC as markers, about 95% sensitivity and
5 about 97.5% specificity were obtained in the classification of colorectal cancer (CRC) among 38 patients with CRC and 40 healthy controls. The present invention is based, in part, on this discovery of effective markers, lysophosphatylcholines (LPC), which when present in an individual indicate the presence of CRC in the individual. As shown herein, methods of the present invention provide for a simple test (*e.g.*, a blood test) to
10 detect one or more markers in an individual.

Accordingly, the present invention is directed to a method of detecting CRC in an individual comprising determining a level of LPC in the individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of colorectal cancer in the individual.

15 In one embodiment, the present invention is directed to a method of detecting CRC in an individual comprising determining a level of LPC 18:2 in the individual wherein a level of LPC 18:2 in the individual that is lower than the level of LPC 18:2 in a control is indicative of CRC in the individual.

In another embodiment, the present invention is directed a method of detecting
20 CRC in an individual comprising determining a level of LPC 18:1 in the individual wherein a level of LPC 18:1 in the individual that is lower than the level of LPC 18:1 in a control is indicative of CRC in the individual.

In a particular embodiment, the present invention also relates to a method of detecting early stage CRC in an individual comprising determining a level of LPC in
25 the individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of early stage CRC in the individual.

Also encompassed by the present invention is a method of screening an asymptomatic individual for CRC. The method comprises determining a level of LPC

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in an individual. In the method, a level of LPC in the individual that is lower than the level of LPC in a control is indicative of CRC in the asymptomatic individual.

The present invention also provides a method of differentiating between a benign disease and a malignant CRC in an individual. The method comprises

5 determining a level of LPC in the individual. A level of LPC in the individual that is lower than the level of LPC in a control is indicative of malignant CRC in the individual, and a level of LPC in the individual that is essentially same as or higher than the control level is indicative of a benign CRC in the individual.

A method of monitoring a treatment regimen for CRC in an

10 individual is also included in the present invention. The method comprises monitoring a level of LPC in the individual. A level of LPC in the individual that is substantially the same as (substantially equal to) or higher than the level of LPC in a control sample is indicative of a successful treatment regimen, and a level of LPC in the individual that is lower than the level of LPC in the control is indicative of an

15 unsuccessful treatment regimen.

Also encompassed by the present invention is a kit for detecting the presence of LPC in a sample from an individual. The kit comprises a compound or an agent capable of detecting LPC 18:2, LPC 18:1, LPC 16:0 and a combination thereof in a sample. The kit can further comprise a means for determining the amount of LPC in

20 the sample; and/or a means for comparing the amount of LPC in the sample with a control.

The present invention provides a simple test (*e.g.*, blood test) to detect the LPC species (*e.g.*, LPC 18:2, LPC 18:1 and LPC 16:0), thereby detecting CRC, which is much easier to conduct than a colonoscopy. In addition, the methods of the

25 invention are highly accurate, reproducible, and sensitive for quantitative analyses of multiple forms of LPC species, and thus can reach the accuracy of detection comparable to a colonoscopy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical representation of the rule that results when LPC 18:2 and LPC 18:1 are combined into a single linear discriminator, which defines a case as cancer if: $\text{LPC 18:2} - 0.78 \times \text{LPC 18:1} \leq 6.2 \mu\text{M}$.

- 5 Figure 2 is a graphical representation of the rule that results when LPC 18:2 and LPC 18:1 are combined into a single linear discriminator, which defines a case as cancer if: $\text{LPC 18:2} - 0.8 \times \text{LPC 18:1} \leq 7.2 \mu\text{M}$.

- Figure 3 is a graphical representation showing empirical receiver operating characteristic (ROC) curves for three models: Model LPC 18:2; Model LPC 18:2 and
10 LPC 18:1; and Model LPC 18:2, LPC 18:1 and LPC 16:0 (see Table 7 below).

Figure 4 is a graphical representation showing LPC 18:2 and 18:1 data for Model LPC 18:1 and LPC 18:2 and superimposes the discriminator equation: $\text{LPC 18:2} - (0.79 \times \text{LPC 18:1}) = 5.57$.

- Figure 5 is a three-dimensional graphical representation showing LPC 18:2, LPC
15 18:1 and LPC 16:0 data for Model LPC 18:2, LPC 18:1 and LPC 16:0.

Figure 6 are graphical representations comparing cancer patients and controls with respect to LPC 18:2 levels alone, the ratios of LPC 16:0 to LPC 18:2, and the ratios of LPC 18:1 to LPC 18:2.

- Figure 7 is a graphical representation plotting LPC 18:2 levels against LPC 18:1
20 levels in cancer patients (C) and controls (H).

Figure 8 is a graphical representation showing empirical receiver operating characteristic (ROC) curves for LPC 18:2 alone, the ratios of LPC 16:0 to LPC 18:2, the ratios of 18:1 to 18:2, and a linear function of LPC 18:1 and LPC 18:2: $\text{LPC 18:2} - (0.79 \times \text{LPC 18:1}) < 5.57$.

- 25 Figure 9 is a graphical representation showing an empirical ROC curve for Model LPC 18:2 and LPC 18:1 and comparing it to an ROC curve based on Model LPC 18:2, LPC 18:1 and LPC 16:0 (see Table 10 below).

DETAILED DESCRIPTION OF THE INVENTION

In recent years, certain lysophospholipids have been recognized as important cell signaling molecules (Moolenaar, W. H., *Ann. NY Acad. Sci.* 905:1-10 (2000); Xu, Y., *et al.*, *Current drug targets-immune, endocrine & metabolic disorders* 3:23-32 (2003)). For example, lysophosphatidic acid (LPA) is an autocrine growth factor, which stimulates proliferation, adhesion, and migration of ovarian cancer cells (Xu Y, *et al.*, *Clinical Cancer Res.* 1:1223-1232 (1995); Xu Y, *et al.*, *Biochem. J.* 309: 933-940 (1995); Baudhuin LM, *et al.*, *Mol. Pharmacol.* 62:660-671 (2002); Sengupta S, *et al.*, *FASEB J.* 17:1570-1572 (2003)). LPA is elevated in plasma samples from patients with ovarian and other gynecological cancers, but not from patients with breast cancer and leukemias (Xu Y, *et al.*, *JAMA* 280:719-723 (1998); Sutphen R, *et al.*, *Cancer Epidemiol Biomarkers Prev.*, 13:1185-1191 (2004); Xu Y and Xiao, Y. US Patent No. 6,451,609 9/17/2002; Xu Y *et al.*, US Patent No. 5,994,141; Xu Y and Casey G. US Patent No. 5,824,555).

To identify potential blood plasma biomarker(s) for colon cancer, lipid factor levels in 38 patients with CRC and 40 healthy controls were analyzed using an electrospray ionization mass spectrometry (ESI-MS)-based method (Xiao, Y., *et al.*, *Ann. NY Acad. Sci.* 905: 242-59 (2000); Xiao, Y. J., *et al.*, *Anal Biochem* 290:302-13 (2001)). Plasma samples from pre-operational patients, which included four patients at an early stage (T1) of colon cancer, were isolated and analyzed for 20 individual choline-containing phospholipid species. Using 18:2-lysophosphatylcholine (LPC) and 18:1-LPC as markers, about 95% sensitivity and about 97.5% specificity were obtained in the classification of CRC. As described herein, a liquid chromatography - mass spectrometry (LC-MS) method has been developed to analyze LPC. The results described herein indicate that LPC 18:2, LPC 18:1 and LPC 16:0 levels in plasma represent useful and effective markers for CRC.

The present invention is based, in part, on the discovery that three LPC species: LPC 18:2, LPC 18:1 and LPC 16:0 are markers for CRC.

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In one embodiment, the combination of LPC 18:1 and LPC 18:2 markers can detect CRC with greater than about 90% sensitivity and specificity. In another embodiment, the combination of these two markers can detect CRC with greater than about 95% sensitivity and specificity. The LPC markers detected all 4 early-stage (T1) CRC among the 38 patient samples, thereby providing a clinically useful method for the early detection of CRC, which leads to an increase in survival rates of CRC patients.

The markers disclosed herein can be used to screen asymptomatic and symptomatic subjects and to differentiate between benign and malignant diseases. In addition, these markers can be used as prognostic markers and provide for therapeutic targets for CRC.

In the methods of the invention, the LPC can be, for example, LPC 18:2, LPC 18:1 or a combination thereof. Alternatively, the LPC can be LPC 18:2, LPC 18:1, LPC 16:0 or a combination thereof.

Typically, a sample (*e.g.*, whole blood, plasma, serum, lymph and tissue) is obtained from an individual and the level of LPC in the individual is detected using, for example, a liquid chromatography - mass spectrometry (LC-MS) detection method, an ultraviolet (UV) detection method, an enzymatic detection method and/or combinations thereof.

A LC-MS method to detect LPC has also been developed which can be used, for example, to detect LPC in samples such as blood. One or more of the following advantages are associated with the methods described herein:

- It is highly accurate and reproducible.
- No thin-layer chromatography is required.
- Only 20 μ L of plasma samples are needed for the test.
- Both plasma and serum samples can be used.
- UV detectors can likely be used to quantify the two LPC species. A HPLC system is approximately 10-15% of the cost of a MS system. Thus the cost of the test can be further reduced. In addition, the operation procedure is simpler for HPLC.

The methods described herein are highly accurate, reproducible, and sensitive for quantitative analyses of multiple forms of lysolipids. In a particular embodiment, the methods described herein provide for methods of detecting LPC. Accordingly, the present invention is directed to diagnostic, prognostic and therapeutic methods for CRC
5 (e.g., adenocarcinoma, carcinoid tumors, gastrointestinal stromal tumors, lymphomas, neuroendocrine carcinoma).

The level of LPC in an (one or more) individual or a sample from an individual can be determined qualitatively and/or quantitatively. In one embodiment, the level of LPC in the tested individual or sample can be compared to the level of LPC in a control.
10 For example, a level of LPC in the individual or sample that is lower than the level of LPC in a control (e.g., control individual or control sample) is indicative of CRC in the individual. Any suitable control sample can be used, wherein the level of LPC in the control sample is indicative of the level of LPC in an individual (one or more) that does not have CRC (e.g., the level of LPC in one or more healthy individuals). For example,
15 a suitable control can be established by assaying a large sample of individuals which do not have CRC and using a statistical model to obtain a control value (standard value). See, for example, models described in Knapp, R.G. and Miller M.C. (1992) *Clinical Epidemiology and Biostatistics*, William and Wilkins, Harual Publishing Co. Malvern, PA, which is incorporated herein by reference.

20 In addition, the concentration (level) of the LPC in the individual or sample can be determined and used as an indication of CRC in the tested individual or sample. In one embodiment of the invention, the LPC detected is LPC 18:2. A level of LPC 18:2 that is from about 14 μ M to about 15 μ M indicates that the individual has CRC. In a particular embodiment, if the level of LPC 18:2 is lower than about 15 μ M in the
25 individual, then the individual has CRC. In another particular embodiment, if the level of LPC 18:2 is lower than about 14 μ M in the individual, then the individual has CRC.

In another embodiment, the LPC detected is LPC 18:1. A level of LPC 18:1 that is from about 9 μ M to about 11 μ M indicates that the individual has CRC. In a particular

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embodiment, if the level of LPC 18:1 is lower than about 11 μ M in the individual, then the individual has CRC.

In yet another embodiment, a combination of LPC markers (e.g., LPC 18:1, LPC 18:2 and LPC 16:0) are detected in the methods of the invention. In a particular
5 example, the LPC detected is a combination of LPC 18:2 and LPC 18:1. As shown herein, combining LPC 18:2 and LPC 18:1 can be combined into a single linear discriminator to yield the following rule, which defines a case as cancer if:

$$\text{LPC 18:2} - 0.78 \times \text{LPC 18:1} \text{ is less than or equal to about } 6.2\mu\text{M}.$$

Using this rule LPC 18:2 plus LPC 18:1 had a sensitivity and specificity of about 95%
10 and about 97.5%, respectively. A graphical representation of this rule is given in Figure 1.

In another particular example, LPC 18:2 and LPC 18:1 can be combined into a single linear discriminator to yield the following rule, which defines a case as cancer if:

$$\text{LPC 18:2} - 0.8 \times \text{LPC 18:1} \text{ is less than or equal to about } 7.2\mu\text{M}.$$

15

Alternatively, LPC 18:2 and LPC 18:1 can be combined into a single linear discriminator to yield the following rule, which defines a case as cancer if:

$$\text{LPC 18:2} - 0.79 \times \text{LPC 18:1} \text{ is less than about } 5.57 \mu\text{M}.$$

A ratio of LPC 18:1 to LPC 18:2 can also be used for the methods of the
20 invention. In particular, in an individual, if:

$$\text{Ratio of LPC 18:1 to LPC 18:2 is greater than about } 0.86,$$

then CRC is detected in the individual.

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In yet a further embodiment, the LPC detected is a combination of LPC 18:2, LPC 18:1 and LPC 16:0. As shown herein, combining LPC 18:2, LPC 18:1 and LPC 16:0 into a single linear discriminator yields the following rule, which defines a case as cancer if:

- 5 $(1.09 \times \text{LPC } 18:2) - (0.65 \times \text{LPC } 18:1) - (0.13 \times \text{LPC } 16:0)$ is less than about $4.67 \mu\text{M}$.

A ratio of LPC 16:0 to LPC 18:2 can also be used for the methods of the invention. In particular, in an individual, if:

Ratio of LPC 16:0 to LPC 18:2 is greater than about 2.28,

- 10 then CRC is detected in the individual.

The methods of the invention employing these rules have high sensitivity and specificity. For example, the rule LPC 18:2 plus LPC 18:1, where $\text{LPC } 18:2 - 0.8 \times \text{LPC } 18:1$ is less than or equal to about $7.2 \mu\text{M}$, had sensitivity and specificity of about 100% and about 90%, respectively (see Example 1). Also, the rule LPC 18:2 plus LPC
15 18:1 plus LPC 16:0, where $(1.09 \times \text{LPC } 18:2) - (0.65 \times \text{LPC } 18:1) - (0.13 \times \text{LPC } 16:0)$ is less than about $4.67 \mu\text{M}$, had a sensitivity about 90% and specificity of about 95% (see Examples 2 and 3). In addition, as shown in Figure 2, using this rule the two LPC markers detected all 4 early-stage (e.g., T1) CRC among the 38 patients samples, thereby providing a clinically useful method for the early detection of CRC, which leads to an
20 increase in survival rates of CRC patients. Accordingly, the present invention is also directed to a method of detecting early stage CRC in an individual comprising determining a level of LPC in the individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of early stage CRC in the individual.

- 25 In addition, the present invention provides for a method of detecting recurrence of CRC in an individual that has been treated for CRC comprising determining a level of

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LPC in the individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of recurrence of colorectal cancer in the individual.

As used herein, early stage CRC generally refers to Type 0 or Type I CRC. The type (stage) of CRC is an indication of how far advanced the cancer is. In Tis (Type 0, T0), the cancer is in its earliest stage. It has not grown beyond the mucosa (inner layer) of the colon or rectum. This stage is also known as carcinoma in situ or intramucosal carcinoma. In Type I (T1), the cancer has grown through the mucosa and extends into the submucosa. In Type II (T2), the cancer has grown through the submucosa, and extends into the muscularis propria. In Type III (T3), the cancer has grown completely through the muscularis propria into the subserosa but not to any neighboring organs or tissues. In Type IV (T4), the cancer has spread completely through the wall of the colon or rectum into nearby tissues and/or organs. Recurrence of CRC (recurrent CRC) occurs when cancer comes back after treatment, and the cancer may recur in the colon or rectum or in another part of the body.

The present invention provides a method of screening an asymptomatic individual for CRC comprising determining a level of LPC in the individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of CRC in the asymptomatic individual.

The present invention also provides a method of differentiating between a benign disease and a malignant CRC in an individual (*e.g.*, a symptomatic individual, an asymptomatic individual). The method comprises determining a level of LPC in the individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of malignant CRC in the individual. Conversely, a level of LPC in the individual that is substantially the same as (similar to) or higher than the control level is indicative of a benign colorectal disease in the individual.

Also encompassed by the present invention is a method of monitoring a treatment regimen for CRC in an individual comprising monitoring a level of LPC in the individual. A level of LPC in the individual that is substantially the same as or higher

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than the level of LPC in a control sample is indicative of a successful treatment regimen; and a level of LPC in the individual that is lower than the level of LPC in the control is indicative of an unsuccessful treatment regimen.

The methods of the present invention provide for use of LPC 18:2, LPC 18:1, 5 LPC 16:0 and a combination thereof as prognostic markers for CRC. In one embodiment, the present invention provides for methods of monitoring an individual at risk for developing CRC (*e.g.*, an individual with familial polyposis or hereditary nonpolyposis colon cancer [HNPCC]), ulcerative colitis, personal or first-degree family history of CRC or adenomas, and personal history of ovarian or endometrial cancer; an 10 individual that produces lower than normal levels of LPC). The LPC level of an individual can be monitored at regular intervals (*e.g.*, once every 6 months; once a year; once every two years) in order to determine whether the levels of LPC in the individual change (*e.g.*, decreases, increases) over time. An indication that the level of LPC is decreasing over time in the individual is an indication that the individual is at risk for 15 developing, or has developed CRC.

In the methods of the present invention, the level of LPC in the individual can be determined from a sample (a test sample) of the individual. As used herein a "sample" includes any suitable biological sample which can be used in the methods of the present invention to detect LPC. For example, a sample includes tissues, cells, biological fluids 20 and extracts thereof obtained (*e.g.*, isolated) from an individual as well as present in an individual. Biological fluids include blood (*e.g.*, whole blood, packed red blood cells), serum, plasma, lymph, urine and semen. The level of LPC can also be detected in the individual without the need to remove or obtain a sample from the individual.

That is, in the methods of the present invention, the LPC of an individual can be 25 detected (directly, indirectly) *in vitro* or *in vivo*. Examples of *in vitro* techniques for detection of lipids such as LPC (*e.g.*, in a sample) are known in the art (Liebisch, G. *et al. Clin. Chem.*, 48:2217-2224 (2002); Hsu, F.F., *et al., J. Mass Spectrom.* 38:752-763 (2003); Hsu, F.F., *et al., J. Am. Soc. Spectrom.* 9:516-526 (1998); Han, X., *et al., J. Lipid Res.*, 44:1071-1079 (2003); Kerwin, J.L., *et al., J. Lipid Res.*, 35:1102-1114

(1994); Forrester, J.S., *et al.*, *Mol. Pharmacol.*, 65:813-821 (2004); Fang, N., *et al.*, *J. Agric. Food Chem.*, 51:6676-6682 (2003); Xiao, Y.J., *et al.*, *Anal. Biochem.*, 290:302-313 (2001); Hong, J., *et al.*, *Rapid Comm. in Mass Spectrom.*, 15(13):1120-1126 (2001)).

Such methods include mass spectrometry methods (e.g., electrospray ionization mass spectrometry (ESI-MS), liquid chromatography - mass spectrometry (LC-MS), fast atom bombardment tandem mass spectrometry); chromatography methods (e.g., high performance liquid chromatography (HPLC), gas chromatography (GC)); ultraviolet (UV) methods; immunoassay methods (e.g., immunoprecipitations, immunofluorescence); enzymatic assays (e.g., colorimetric assays) and combinations thereof.

Examples of *in vivo* techniques for detection of lipids such as LPC (e.g., in an individual) include magnetic resonance imaging (MRI) and nuclear magnetic resonance techniques (Leon, F., *et al.*, *J. Agric. Food Chem.*, 52(5):1207-1211 (2004); Kuliszkiewicz-Janus, M., *et al.*, *Anticancer Res.*, 16(3B):1587-1594 (1996); Merchant, T.E., *et al.*, *Brain Res.*, 649(1-2):1-6 (1994); Driscoll, D., *et al.*, *Int. J. Biochem.*, 26(6):759-767 (1994); Seijo, L., *et al.*, *Lipids*, 29(5):359-364 (1994); Phillipson, D.W., *et al.*, *J. Lipid Mediat.*, 7(2):155-167 (1993); Merchant, T.E., *et al.*, *Br. J. Cancer*, 63(5):693-698 (1991)).

The sample obtained from an individual can be analyzed immediately or the sample can be processed prior to detection of LPC, and depends upon the type of sample and the method of detection used to determine the level of LPC in the sample. In one embodiment, lipids are extracted from the sample. A variety of methods for extracting lipids from a sample are known in the art (see, for example, Xiao, Y., *et al.*, *Ann. NY Acad. Sci.*, 905:242-259 (2000); Xiao, X.J., *et al.*, *Anal. Biochem.*, 290:302-313 (2001)). The extracted lipids can then be subjected to a mass spectrometry method, such as EIS-MS, for detection of LPC.

The discovery that LPC species: LPC 18:1, LPC 18:2, and LPC 16:0 are markers for CRC also provides therapeutic targets for CRC. Thus, the present invention also provides for methods of treating CRC comprising administering to an individual in need

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thereof an agent that alters the metabolic pathway(s) of LPC 18:2, LPC 18:1, LPC 16:0, or combinations thereof. For example, an enzyme (*e.g.*, lipase) that is involved in the metabolic pathway of LPC 18:2, LPC 18:1 and/or LPC 16:0 can be contacted with an agent, such as an antagonist or agonist (*e.g.*, an antibody) that results in an increase in
5 the amount of LPC 18:2, LPC 18:1 and/or LPC 16:0 produced in an individual.

The discovery also provides for the ability to screen for agents which can be used for therapy of CRC. For example, an *in vitro* or *in vivo* assay that recapitulates the metabolic pathway of LPC 18:2, LPC 18:1 and/or LPC 16:0 can be used to identify an agent that results in an increase in the amount of LPC 18:2, LPC 18:1 and/or LPC 16:0.
10 Such agents could potentially be used to treat CRC.

The present invention is also directed to kits for detecting the presence of LPC in a sample from an individual. For example, the kit can include a labeled compound or agent (*e.g.*, an antibody, an enzyme) capable of detecting LPC 18:2, LPC 18:1, LPC 16:0 or combinations thereof in a sample; means for determining the amount in the sample;
15 means for comparing the amount in the sample with a control (standard); and/or a suitable control. The component(s) can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect LPC 18:2, LPC 18:1, LPC 16:0 and/or combinations thereof.

EXEMPLIFICATION

20 Example 1. LPC 18:2 and LPC 18:1 as Markers for Colon and Rectal Cancers

A1. Materials and Methods

Blood samples were obtained from 38 patients with colorectal cancer and 40 healthy controls enrolled into the study. All patients had pathologically verified CRC and attended the department of Colorectal Surgery at the Cleveland Clinic Foundation.
25 Other eligibility criteria include age over 18 years and signed informed consent. There were no specific exclusion criteria, other than patient refusal to participate. All blood samples from patients were collected pre-operatively.

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All blood samples were processed as follows. Whole blood samples collected in EDTA-containing tubes were spun at 1,750g for 15 minutes at room temperature. Plasma samples were aliquoted into siliconized eppendorf tubes and frozen at minus 80°C until use.

5 A2. Lipid extraction and analyses of phospholipids

Lipids were extracted as described previously with some minor modifications (Xiao, Y., *et al.*, Ann. NY Acad. Sci. 905: 242-59 (2000); Xiao, Y. J., *et al.*, Anal Biochem 290:302-13 (2001)). To 0.5 mL plasma, 2 mL of MeOH/chloroform (2:1) and 0.1 mL of 6 N HCl were added. Samples were vortexed for 1 min and incubated
10 on ice for 10 min. 1 mL of chloroform and 1 mL of H₂O were added to separate the phases. Samples were vortexed for 0.5 min prior to centrifugation (2,000 g for 10 min). The lower phase was transferred to a new glass tube. To the upper phase remaining in the original tube, 1 mL of chloroform was added to extract more lipids and the tube was centrifuged (2,000 g for 10 min). The lower phase was transferred
15 into the same tube (with the lower phase extract) and the solvent was evaporated under nitrogen at 40°C. The dried lipids were suspended in 50 µL of solvent (MeOH:chloroform, 2:1), vortexed, and applied to a TLC plate. Two standards (18:1-LPA and 18:1-LPC) were applied to help in identifying the “LPA band” and the “LPC band” on each TLC plate. The TLC plates were developed in the solvent
20 system (chloroform:MeOH:AmOH, 65:35:5.5) until the solvent front is 1.5 inch from the top of the plate. The lipids from the “LPA band” and the “LPC band” were eluted with 2 mL of MeOH:chloroform (2:1) twice. The lipid solutions were dried under nitrogen at 30°C and lipids resuspended in 100 µL of MeOH for Mass tests.

A3. Analysis of LPC using a LC-MS method

25 As described herein, a method to detect lysophosphatidylcholine (LPC) in blood has been developed. 20 µL plasma is diluted by PBS (1X) to 0.5 mL, 2 mL of MeOH/chloroform (2:1) was added. The sample was vortexed for 1 min and incubated on ice for 10 min. 1 mL of chloroform and 1 mL of H₂O were added to separate the phases. The sample was vortexed for 0.5 min. After centrifugation

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(2000 g for 10 min), the lower phase was transferred to a new glass tube. One mL chloroform was added to the upper phase left in the original tube to further extract the lipids. The lower phase was transferred into the same tube and the solvent was evaporated under nitrogen at room temperature.

5 Mass spectrometry analyses were performed using a Quattro Ultima triple quadrupole ESI-MS (Micromass, Inc., Beverly, MA) with the Masslynx data acquisition system. Samples (20 μ L) were delivered into the ESI source through a LC system (Waters 2690, Waters) with an auto sampler.

Reversed-phase HPLC was conducted using a LC system with a C-18 column
10 (2.0 X 150 mm, 5 μ m SOD; Phenomenex, Rancho Palos Verdes, CA) at flow rate of 200 μ L/min. A gradient elution procedure was used for the separation of lysophospholipids (lyso-PLs). The solvent systems used were: A: methanol-water (50:50, v:v, containing 0.1% formic acid) and B: methanol (containing 0.1% formic acid). The separation was performed initially by isocratic elution with 100 % A for 5
15 min, followed by a linearly gradient from 100 % A to 100% B over 2 min, and then 100 % B for 70 min, followed by 100 %A over 3 min. It took approximately 80 min. to completely separate and elute all of choline-containing phospholipids in the lipid extract. When only LPC was tested, isopropanol was used to elute other choline-containing lipids and the analysis for each sample could be finished within 45 min.
20 Briefly, after 5 minutes of elution with solvent A and 2 minutes of gradient elution with solvents A and B (from 100% A to 100% B), 100% B was introduced for 18 minutes, followed by 100 % isopropanol for 18 minutes. Finally the column is re-equilibrated with solvent A for 2 minutes.

The Mass spectra instrument settings used were the same as described
25 previously (Xu, Y., *et al.*, *Current drug targets-immune, endocrine & metabolic disorders* 3:23-32 (2003)). Parent scanning and MS/MS analyses were performed to detect and confirm the structures of all lyso-PLs in blood samples. All quantitative analyses were performed in the multiple reaction monitoring (MRM) mode. Monitoring ions were at m/z 465 (parent ion)–184 (product ion) for SPC, 483-184
30 for lyso-PAF, 496-184 for 16:0-LPC, 440-184 for 12:0-LPC (taking as internal standard), 520-184 for 18:2-LPC, 522-184 for 18:1-LPC, 524-184 for 18:0-LPC,

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544-184 for 20:4-LPC, 568-184 for 22:6 LPC, 703-184 for 16:0-SM, 729-184 for 18:1-SM, 731-184 for 18:0-SM, 758-184 for 34:2-PC, 760-184 for 34:1-PC, 782-184 for 36:4-PC, 784-184 for 36:3-PC, 786-184 for 36:2-PC, 810-184 for 38:4-PC, 812-184 for 38:3-PC, 814-184 for 38:2-PC. The dwell time in the MRM mode was 100 ms and other conditions were the same as described previously.

A4. Statistical analyses

Levels of lipids were analyzed primarily using non-parametric methods. Associations between species were analyzed using Spearman rank correlations. Logistic regression models were used to identify combinations of phospholipids that had good sensitivity and specificity for distinguishing CRC patients from healthy controls. All tests of statistical significance were 2-sided and no adjustments for multiple comparisons were made. All data analyses were performed using SAS (Statistical Analysis Software, version 6.12, SAS Institute, Inc.).

A5. Results

15 Negatively-charged lysophospholipids were not markers for CRC

Thirty-eight subjects with colorectal cancer and 40 unaffected controls were recruited into the study. Overall 49 subjects (63%) were male and 29 (37%) were female. Initially both negatively- (LPA, shingosine-1-phosphate, and lysophosphatidylinositol) and positively-charged (choline-containing) lysophospholipids were analyzed in a subset of blood samples from 10 cases and controls. As levels of the negatively-charged lysophospholipids did not show a significant difference between samples from cancer patients and healthy controls, the analyses focused on the positively-charged phospholipids.

Tables 1 and 2 summarize the data of individual species of choline-containing phospholipids. Table 1 summarizes the species for all cancer patients versus all control subjects, and Table 2 summarizes the phosphatidylcholine (PC) species separately for males and females. In Tables 1 and 2 sphingosylphosphorocholine = SPC; lyso-platelet activating factor = LPAF; lysophosphatylcholine = LPC; sphingomyelin = SM; phosphatidylcholine = PC.

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As can be seen from Table 1, levels of lyso-platelet activating factor (LPAF), most of the lysophosphatidylcholine (LPC) species, and several of the PC species were significantly different (generally lower) between patients with cancer and unaffected controls ($p < .01$). The levels of sphingomyelin were not different between
5 two groups.

Levels of all PC species were lower in female cancer patients compared to female controls whereas the reverse was generally true for males, i.e. levels in male cancer patients tended to be greater than in male controls (Table 2). The reason for this difference between males and females was unclear.

10 LPC 18:2 was a good discriminator between cancer and non-cancer patients. Using a cutoff of 14.5 LPC 18:2 had a sensitivity of 92% and a specificity of 92.5%. To determine if additional species could improve these statistics, logistic regression with stepwise variable selection was used. The only additional factor that was found to improve the sensitivity and specificity of LPC 18:2 was LPC 18:1. Combining
15 LPC 18:2 and LPC 18:1 into a single linear discriminator yielded the following rule, defining a case as cancer if:

$$\text{LPC 18:2} - 0.78 \times \text{LPC 18:1} \leq \text{about } 6.2$$

Using this rule LPC 18:2 plus LPC 18:1 had a sensitivity and specificity of 95% and 97.5%, respectively. A graphical representation of this rule is given in the Figure 1.

20 Alternatively, LPC 18:2 and LPC 18:1 could be combined into a single linear discriminator to yield the following rule, which defined a case as cancer if:

$$\text{LPC 18:2} - 0.8 \times \text{LPC 18:1} \leq \text{about } 7.2 \mu\text{M}.$$

Using this rule LPC 18:2 plus LPC 18:1 had a sensitivity and specificity of about 100% and about 90%, respectively. As shown in Figure 2, the two LPC markers
25 detected all 4 early-stage (T1) CRC among the 38 patients samples, thereby providing a clinically useful method for the early detection of CRC, which could lead to an increase in survival rates of CRC patients.

Table 1. Cancer Patients versus Controls

		Cancer			Controls			p-value 1
Species		Mean $\pm$ s.d.	Median	Range	Mean $\pm$ s.d.	Median	Range	
SPC		0.024 $\pm$ 0.010	0.024	0-0.056	0.024 $\pm$ 0.010	0.024	0.005- 0.058	0.95
LPAF		0.161 $\pm$ 0.060	0.149	0.085- 0.300	0.232 $\pm$ 0.102	0.202	0.085- 0.567	.001
5	LPC 16:0	22.23 $\pm$ 6.34	21.78	10.42- 34.87	29.53 $\pm$ 9.64	28.61	10.27- 63.94	<.001
	LPC 18:0	7.74 $\pm$ 2.25	7.68	3.73- 11.95	10.82 $\pm$ 4.06	10.44	3.57- 23.27	<.001
	LPC 18:1	8.54 $\pm$ 2.17	8.55	4.78- 13.45	12.51 $\pm$ 4.13	11.85	6.68- 22.75	<.001
	LPC 18:2	10.62 $\pm$ 2.82	10.88	5.06- 17.43	20.24 $\pm$ 5.23	18.38	13.62- 34.21	<.001
	LPC 20:0	0.16 $\pm$ 0.05	0.15	0.08- 0.29	0.25 $\pm$ 0.12	0.23	0.09- 0.77	<.001
10	LPC 20:4	15.89 $\pm$ 4.09	15.96	9.53- 30.76	13.55 $\pm$ 4.55	13.12	5.85- 23.55	.05
	LPC 22:6	10.24 $\pm$ 3.57	9.30	5.05- 19.22	10.12 $\pm$ 4.35	9.44	3.91- 22.92	.76
	Total LPC	75.41 $\pm$ 17.55	72.52	43.12- 112.42	97.01 $\pm$ 23.59	91.49	65.76- 169.04	<.001
15	Unsat LPC	30.13 $\pm$ 8.45	29.73	14.25- 46.31	40.60 $\pm$ 13.48	39.91	13.97- 87.70	<.001
	Sat. LPC	45.29 $\pm$ 10.35	43.57	28.73- 73.46	36.41 $\pm$ 12.97	54.20	35.39- 87.82	<.001
	SM 16:0	146.04 $\pm$ 35.55	142.17	90.42- 269.16	148.76 $\pm$ 29.55	147.01	104.67- 240.57	.45
	SM 18:0	85.07 $\pm$ 27.55	80.60	48.26- 190.19	78.37 $\pm$ 21.87	74.39	41.01- 121.66	.33
	SM 18:1	30.50 $\pm$	28.42	18.70-	27.94 $\pm$	27.60	15.63-	.24

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		9.33		61.61	7.22		49.55	
20	Unsat	231.11 $\pm$	224.57	145.53-	227.13 $\pm$	225.73	151.99-	.95
	SM	60.80		459.35	46.54		356.87	
	Total SM	261.6 $\pm$	254.45	166.69-	255.06 $\pm$	249.01	167.61-	.87
		69.21		520.96	53.06		398.84	
	PC	350.24	346.79	195.61-	423.11 $\pm$	399.69	295.41-	.001
	18:1/16:0	+85.50		652.27	95.79		632.57	
25	PC	124.16 $\pm$	120.96	67.80-	157.91	150.16	107.10-	<.001
	18:2/16:0	31.24		208.15	+34.34		236.00	
	PC	182.38 $\pm$	172.88	90.68-	246.66 $\pm$	248.42	153.76-	<.001
	18:0/18:2	48.08		334.44	52.89		384.58	
	PC	231.34 $\pm$	216.30	144.54-	233.62 $\pm$	227.38	126.57-	.53
30	18:1/18:2	66.48		485.88	55.44		391.42	
	PC	135.62 $\pm$	119.97	78.87-	124.50 $\pm$	117.99	69.30-	.52
	18:2/18:2	45.63		297.17	33.02		201.71	
	PC	98.21 $\pm$	98.30	57.80 -	100.41 $\pm$	96.46	63.80-	.64
	18:1/20:2	28.03		214.38	23.76		162.04	
35	PC	43.04 $\pm$	40.96	23.88-	47.37 $\pm$	46.06	26.93-	.04
	18:0/20:2	12.44		88.93	10.99		76.76	
	PC	157.45 $\pm$	151.24	94.03-	141.20 $\pm$	139.21	83.74-	.10
	18:0/20:4	44.10		328.43	34.56		245.52	
	Total PC	1322.43	1277.9	810.54-	1474.77 $\pm$	1417.93	1003.3	.02
		$\pm$ 329.12	8	2609.64	290.48		4-	
							2232.01	
40	PC	141.11 $\pm$	130.56	78.87-	114.34 $\pm$	109.22	69.30-	.04
	18:2/18:2	46.50		297.17	31.55		190.31	
	1							
	PC	102.32 $\pm$	100.98	57.80-	90.99 $\pm$	87.50	63.80-	.05
	18:1/20:2	31.73		214.38	24.38		162.04	
45	1							
	PC	44.8 $\pm$	43.64	23.88-	42.87 $\pm$	40.49	26.93-	.48
	18:0/20:2	13.75		88.93	12.00		76.76	
	1							
	PC	162.88 $\pm$	153.82	94.03-	128.10 $\pm$	128.72	83.74-	.002

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18:0/20:4 1	49.62		328.43	35.10		245.52	
Total PC	1359.83	1317.7	810.54-	1323.79 ±	1315.14	1003.3	.73
- Males	± 356.27	7	2609.64	250.34		4- 2232.01	

5 ¹ Wilcoxon rank sum test

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Table 2. PC Separately in Females and Males

		Cancer			Controls			
	Species	Mean $\pm$ s.d.	Median	Range	Mean $\pm$ s.d.	Median	Range	p- value ¹
	Females							
5	PC	328.49 $\pm$	296.56	268.76-	485.99 $\pm$	474.87	320.96-	.001
	18:1/16:0	65.45		450.24	92.97		632.57	
	PC	121.91 $\pm$	116.92	88.43-	178.15 $\pm$	173.88	116.15-	.001
10	18:2/16:0	28.81		172.23	33.83		236.00	
	PC	173.77 $\pm$	166.10	123.09-	273.64 $\pm$	268.60	185.90-	<.001
	18:0/18:2	39.87		239.29	44.58		384.58	
15	PC	213.49 $\pm$	192.82	163.43-	262.62 $\pm$	261.88	162.44-	.01
	18:1/18:2	54.67		352.56	50.41		391.42	
	PC	122.13 $\pm$	106.11	90.20-	136.91 $\pm$	141.62	75.90-	.08
20	18:2/18:2	42.42		237.65	31.23		201.71	
	PC	88.14 $\pm$	91.24	71.14-	111.92 $\pm$	109.37	83.85-	.001
	18:1/20:2	11.47		102.61	17.47		145.06	
25	PC	38.56 $\pm$	37.36	26.43-	52.86 $\pm$	53.62	39.85-	<.001
	18:0/20:2	7.05		52.88	6.42		61.81	
	PC	144.15 $\pm$	146.52	111.87-	157.21 $\pm$	156.17	108.74-	.16
30	18:0/20:4	22.77		189.38	26.92		213.51	
	Total PC -- Females	1230.63 $\pm$ 240.26	1126.8 8	980.94- 1692.7 5	1659.31 $\pm$ 224.94	1659.2 3	1305.4 5- 2074.99	.002
	Males							
25	PC	359.10 $\pm$	353.73	195.61-	371.67 $\pm$	363.97	295.41-	.35
	18:1/16:0	92.05		652.27	62.32		604.56	
	PC	125.08 $\pm$	122.25	67.80-	141.34 $\pm$	136.51	107.10-	.06
30	18:2/16:0	32.66		208.15	25.00		230.36	
	PC	185.88 $\pm$	175.34	90.68-	224.59 $\pm$	215.44	153.76-	.006
	18:0/18:2	51.33		334.44	49.53		376.40	
30	PC	238.60 $\pm$	235.01	144.54-	209.89 $\pm$	215.64	126.57-	.12
	18:1/18:2 ²	70.36		485.88	48.37		350.04	

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5	PC	141.11 $\pm$	130.56	78.87-	114.34 $\pm$	109.22	69.30-	.04
	18:2/18:2 ²	46.50		297.17	31.55		190.31	
	PC	102.32 $\pm$	100.98	57.80-	90.99 $\pm$	87.50	63.80-	.05
	18:1/20:2 ²	31.73		214.38	24.38		162.04	
	PC	44.8 $\pm$	43.64	23.88-	42.87 $\pm$	40.49	26.93-	.48
10	18:0/20:2 ²	13.75		88.93	12.00		76.76	
	PC	162.88 $\pm$	153.82	94.03-	128.10 $\pm$	128.72	83.74-	.002
	18:0/20:4 ²	49.62		328.43	35.10		245.52	
	Total PC –	1359.83	1317.7	810.54-	1323.79	1315.1	1003.3	.73
	Males ²	$\pm$ 356.27	7	2609.6	$\pm$ 250.34	4	4-	
				4			2232.01	

¹ Wilcoxon rank sum test² Median value in cancer patients < Median value in controlsExample 2. LPC, SPC, and L-PAF as Markers for Colon and Rectal Cancers

128 cancer patients and 125 control subjects were analyzed. Patients with
 15 adenomas were excluded. Cancer patients and control subjects differed with respect
 to gender, race, and age. That is, 69% (87/127) of cancer patients were male
 compared to 46% (58/125) of controls, $p < .001$ (Fisher's exact test); 89% of cancer
 patients were Caucasian compared to 71% of controls, $p < .001$ (chi-square test); and
 the median age of the cancer patients was 63 versus 44 for controls, $p < .001$
 20 (Wilcoxon rank sum test).

Table 3 summarizes disease characteristics of the cancer patients. Overall,
 62% of patients had rectal tumors; the majority of tumors were T₃ (53%); and most
 were N₀ (66%).

Table 3. Disease Characteristics

<u>Factor</u>	<u>N (%)</u>
Primary Site	
Colon	40 (31%)
Rectum	79 (62%)
Other ¹	9 (7%)
T-Stage	
T1, T2	50 (39%)
T3, T4	71 (55%)
Unknown	7 (5%)
N-Stage	
N ₀	85 (66%)
N ₁ , N ₂ , N ₃	36 (28%)
Unknown	7 (5%)
Grade	
Well, Moderate	90 (70%)
Poor	19 (15%)
Unknown	19 (15%)

¹Other – Rectosigmoid (n=6), anus (n=1), colon and rectum (n=1), rectum and cecum (n=1)

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Table 4a summarizes the prevalent LPC species (i.e. the species with the highest levels) in cancer patients and controls. Table 4b summarizes the prevalent species for specific disease characteristics. As can be seen from these tables, LPC

5 16:0 was the most prevalent species for both cancer patients and controls, and there were no differences with respect to which species was prevalent for any of the cancer characteristics examined.

Table 4a. Prevalent LPC Species - Cancer Patients vs Controls

	16:0	18:0	18:2	20:4
Cancer	123 (96%)	-0-	-0-	5 (4%)
Controls	111 (89%)	2 (2%)	9 (7%)	3 (2%)

10 Table 4b. Prevalent LPC Species - Cancer Patient Only

Factor	16:0	20:4
Primary Site		
Colon	37 (93%)	3 (8%)
Rectum	77 (97%)	2 (3%)
Other	9 (100%)	-0-
T-Stage		
T1, T2	50 (100%)	-0-
T3, T4	67 (94%)	4 (6%)
Unknown	6 (86%)	1 (14%)
N-Stage		
N0	82 (96%)	3 (4%)
N1, N2, N3	35 (97%)	1 (3%)
Unknown	6 (86%)	1 (14%)
Grade		
Well, Moderate	88 (98%)	2 (2%)
Poor	17 (89%)	2 (11%)
Unknown	18 (95%)	1 (5%)

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Table 5 summarizes the associations between age and different markers. As can be seen from this table, significant correlations were noted for most of the markers in both cancer patients and control subjects, however the “direction” of the associations were generally opposite. For example, LPC 18:1 tended to decrease with age in cancer patients, but increase in controls. It was not clear, however if this was an inherent difference between cancer patients and controls or if it was due at least in part to the age difference between the two groups.

Table 5. Spearman Rank⁴ Correlations Between Age and Markers

Marker	Cancer	Controls
LPC 16:0	-0.24 ²	0.22 ¹
LPC 18:0	-0.32 ³	0.58 ³
LPC 20:0	-0.15	0.34 ³
Saturated LPC	-0.28 ²	0.41 ³
LPC 18:1	-0.24 ²	0.27 ²
LPC 18:2	-0.27 ²	0.03
LPC 20:4	0.04	-0.33 ³
LPC 22:6	0.20 ¹	-0.20 ¹
Unsaturated LPC	-0.12	-0.08
Total LPC	-0.26 ²	0.25 ²
SPC	0.19 ¹	0.03
L-PAF	-0.08	-0.54 ³

¹ p<.05² p<.01³ p<.001⁴ Positive (negative) correlations imply the marker increases (decreases) with age

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With the exception of LPC 16:0, LPC 22:6, and the ratio of saturated to unsaturated LPC, there were no significant differences in marker levels between males and females. Females tended to have higher levels of LPC 22:6 than males ($p=.01$), whereas males tended to have higher levels of LPC 16:0 ($p=.04$). Males also tended
5 to have a higher ratio of saturated LPC to unsaturated LPC ($p=.02$). The Wilcoxon rank sum test was used for these comparisons.

Table 6 summarizes the marker data. As can be seen from this table, there are statistically significant differences between cancer patients and controls with respect to most of the markers examined. Interestingly, in terms of the absolute levels of the
10 LPC species, cancer patients and controls differed with respect to the unsaturated species but not the saturated ones.

Considering each marker individually, LPC 18:2 was the best single discriminator of cancer status. Using a cutoff of about 14.0 (i.e. categorized as cancer if $\text{LPC 18:2} < \text{about } 14.0$), the sensitivity and specificity of LPC 18:2 were 73% and
15 95%, respectively. Using logistic regression models to determine if additional markers could improve on the sensitivity of LPC 18:2 alone indicated that LPC 18:1 and likely LPC 16:0 were also important. Adding LPC 18:1 increased the sensitivity to 84% while adding both LPC 18:1 and LPC 16:0 increased the sensitivity to 90%. In both cases, the specificity remained 95%. The results of these analyses are summarized in
20 Table 7. Note that adjusting for gender and age, LPC 18:1 and 18:2, and LPC 16:0 remained important predictors of disease status.

Table 6. Markers

Marker	Cancer			Controls			p-Value ¹
	Mean $\pm$ s.d.	Median	Range	Mean $\pm$ s.d.	Median	Range	
LPC 16:0	33.071 $\pm$ 12.21	31.027	10.421-73.135	32.861 $\pm$ 8.999	31.486	10.266-66.130	0.7009
LPC 18:0	17.028 $\pm$ 9.124	15.313	3.734-47.988	17.441 $\pm$ 7.440	16.466	3.565-40.898	0.2981
LPC 20:0	0.262 $\pm$ 0.282	0.185	0-2.553	0.239 $\pm$ 0.137	0.213	0.046-0.766	0.6694
Sat. LPC	50.362 $\pm$ 20.79	45.786	14.253-116.928	50.541 $\pm$ 15.068	50.002	13.972-106.038	0.4763
LPC 18:1	10.764 $\pm$ 3.691	9.796	3.880-20.214	12.999 $\pm$ 3.907	12.487	6.504-26.728	<0.001
LPC 18:2	11.912 $\pm$ 3.929	11.612	2.491-23.679	21.084 $\pm$ 6.882	20.242	10.832-40.051	<0.001
LPC 20:4	11.008 $\pm$ 5.409	9.565	2.251-30.755	12.944 $\pm$ 4.545	12.635	4.455-25.519	0.0008
LPC 22:6	6.756 $\pm$ 3.617	5.949	0.762-19.223	8.819 $\pm$ 3.813	8.038	2.252-22.924	<0.001
Unsat. LPC	40.439 $\pm$ 11.27	40.189	10.213-81.891	55.846 $\pm$ 12.527	53.746	32.967-96.884	<0.001
Ratio of Sat to Unsat LPC	1.302 $\pm$ 0.530	1.255	0.479-2.672	0.924 $\pm$ 0.262	0.891	0.270-1.796	<0.001
Total LPC	90.801 $\pm$ 26.38	91.249	34.868-171.517	106.387 $\pm$ 23.719	101.978	65.758-195.589	<0.001
% LPC 16:0	0.360 $\pm$ 0.058	0.365	0.238-0.530	0.308 $\pm$ 0.043	0.310	0.156-0.421	<0.001
% LPC 18:0	0.179 $\pm$ 0.058	0.183	0.079-0.316	0.160 $\pm$ 0.050	0.159	0.054-0.298	0.0075
% LPC 20:0	0.003 $\pm$ 0.005	0.002	0-0.043	0.002 $\pm$ 0.001	0.002	0.001-0.007	0.0669
LPC 16:0 as % of Sat.	0.670 $\pm$ 0.058	0.674	0.544-0.786	0.660 $\pm$ 0.070	0.668	0.433-0.793	0.2942
LPC 18:0 as % of Sat.	0.324 $\pm$ 0.059	0.320	0.210-0.451	0.335 $\pm$ 0.069	0.328	0.201-0.560	0.2443
LPC 20:0 as % of Sat.	0.006 $\pm$ 0.008	0.004	0-0.062	0.005 $\pm$ 0.003	0.005	0.001-0.017	0.9486
% LPC 18:1	0.119 $\pm$ 0.023	0.115	0.081-0.198	0.122 $\pm$ 0.021	0.118	0.075-0.211	0.1218
% LPC 18:2	0.132 $\pm$ 0.024	0.130	0.071-0.197	0.199 $\pm$ 0.035	0.197	0.116-0.306	<0.001
% LPC 20:4	0.128 $\pm$ 0.066	0.107	0.029-0.291	0.125 $\pm$ 0.046	0.119	0.044-0.301	0.5877

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% LPC 22:6	0.079±0.045	0.066	0.009-0.222	0.084±0.033	0.080	0.025-0.210	0.0267
LPC 18:1 as % of Unsat.	0.274±0.081	0.271	0.149-0.472	0.233±0.049	0.224	0.132-0.356	0.0002
LPC 18:2 as % of Unsat.	0.298±0.066	0.298	0.161-0.451	0.378±0.056	0.377	0.251-0.494	<.0001
LPC 20:4 as % of Unsat.	0.266±0.088	0.264	0.101-0.431	0.232±0.064	0.222	0.096-0.421	0.0043
LPC 22:6 as % of Unsat.	0.163±0.064	0.153	0.034-0.341	0.157±0.052	0.152	0.058-0.357	0.6080
SPC	0.012±0.010	0.007	0-0.056	0.031±0.122	0.013	0.001-1.344	0.0013
L-PAF	0.098±0.051	0.084	0.021-0.300	0.126±0.095	0.090	0.035-0.567	0.1684

¹ Wilcoxon rank sum test

Table 7. Logistic Regression Models

Model	-2 Log Likelihood	Discrimination Equation	Sensitivity ¹
LPC 18:2	180.6	Cancer if LPC 18:2<14.0	73%
LPC 18:2 LPC 18:1	119.9	Cancer if LPC 18:2 – (0.79 x LPC 18:1)<5.57	84%
LPC 18:2 LPC 18:1 LPC 16:0	108.0	Cancer if (1.09 x LPC 18:2) – (0.65 x LPC 18:1) – (0.13 x LPC 16:0)<4.67	90%

¹ Specificity held at 95%

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Figure 3 plots empirical receiver operating characteristic (ROC) curves for the three models described in Table 7. ROC curves plot sensitivity (y-axis) against 1-specificity (x-axis) using different cutoffs (e.g. the sensitivity and specificity described above using the 14.0 cutoff for LPC 18:2 alone would be one point on the ROC curve), and are often used to assess the performance of a “test”. In general, the steeper and closer to 1.0 (on the y-axis) a ROC curve is the better the test’s performance is. As can be seen from this figure, there was a significant improvement in performance using the LPC 18:2 plus LPC 18:1 model compared to LPC 18:2 alone. Adding LPC 16:0 to the LPC 18:2 plus LPC 18:1 model led to modest additional improvement.

LPC 16:0 was said to “possibly” be important because during internal validation in which 1000 bootstrap samples were generated and stepwise logistic regression models fit, LPC 16:0 was an important factor in 72% of the models (which is generally considered good), whereas LPC 18:1 and LPC 18:2 were each important factors in 99% of the models (even better). Also, the effect of LPC 16:0 in these models was somewhat mixed. That is, in 68.1% of the models LPC 16:0 had a positive impact (i.e. the likelihood of cancer increased as LPC 16:0 increased), but in 2.4% of the models it had a negative effect. In addition, as mentioned above, the addition of LPC 16:0 marginally improves the performance of LPC 18:2 and LPC 18:1 in terms of the ROC curve. Consequently, LPC 18:2 and 18:1 appeared to be the most important factors for discriminating between colorectal cancer patients and control subjects. It was determined that LPC 16:0 should likely also be considered.

Figure 4 plots the LPC 18:2 and 18:1 data for the two groups and superimposes the discriminator equation given in Table 7. Figure 5 plots the LPC 18:2, 18:1, and 16:0 data.

Using the LPC 18:2 plus 18:1 model 21 cancer patients were misclassified. Thirteen patients had rectal primaries, seven had colon primaries and one had a rectosigmoid tumor. Seven patients were stage T_3N_+ , five were T_3N_0 , one was T_2N_+ , seven were T_2N_0 , and one patient was T_1N_0 . Adding LPC 16:0 resulted in 16 misclassifications; all but one patient had also been misclassified using just LPC 18:2 and LPC 18:1. Ten patients had rectal tumors and six patients had colon primaries; four patients were stage T_3N_+ , five were T_3N_0 , and seven were T_2N_0 . All of the control subjects misclassified as cancers were 55 years of age or older.

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In addition to examining the ability of LPC, SPC, and L-PAF to discriminate between cancer and non-cancer patients (i.e. as a screening or diagnostic tool), the value of these markers as staging tools was also examined. No differences in marker levels were seen with respect to T or N stage, or grade. There were however
5 significant differences with respect to the primary site. Patients with colon primaries versus rectal primaries differed with respect to the levels of LPC 18:0, 18:1, 20:0, 20:4, 22:6, the ratio of saturated to unsaturated LPC, and SPC (all $p < .05$ based on the Wilcoxon rank sum test).

Example 3. Supplemental Analysis: LPC, SPC, and L-PAF as Markers for Colon and

10 Rectal Cancers

Below describes the supplemental analyses of the data discussed above that incorporates the ratios of the different LPC species – e.g. 16:0 to 18:2.

128 cancer patients and 125 control subjects were further analyzed. Patients with adenomas were excluded.

15 Table 8 summarizes the associations between age and the different markers and includes the ratio of LPC 16:0 to 18:2 and 18:1 to 18:2. As can be seen from this table significant correlations were noted for most of the markers in both cancer patients and control subjects, however the “direction” of the associations were generally opposite. For example, LPC 18:1 tended to decrease with age in cancer
20 patients, but increase in controls. It was not clear, however if this was an inherent difference between cancer patients and controls or if it was due at least in part to the age difference between the two groups. As before, there were some random correlations between the markers and gender. That is, considering only the control patients, LPC 16:0 and 18:1 were the only makers that differed by gender ($p = .05$ by
25 the Wilcoxon rank sum test in both cases).

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Table 8. Spearman Rank Correlations⁴ Between Age and Specific Markers

Marker	Cancer	Controls
LPC 16:0	-0.24 ²	0.22 ¹
LPC 18:0	-0.32 ³	0.58 ³
LPC 20:0	-0.15	0.34 ³
Saturated LPC	-0.28 ²	0.41 ³
LPC 18:1	-0.24 ²	0.27 ²
LPC 18:2	-0.27 ²	0.03
LPC 20:4	0.04	-0.33 ³
LPC 22:6	0.20 ¹	-0.20 ¹
Unsaturated LPC	-0.12	-0.08
Total LPC	-0.26 ²	0.25 ²
LPC 16:0, 18:2 Ratio	0.03	0.17
LPC 18:1, 18:2 Ratio	-0.10	-0.41 ³
SPC	0.19 ¹	0.03
L-PAF	-0.08	-0.54 ³

¹ p<.05² p<.01³ p<.001⁴ Positive (negative) correlations imply the marker increases (decreases) with age

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In contrast to gender, there appeared to be a number of differences in marker levels between the different racial groups (Caucasian, African American, and Asian controls). These included LPC 18:0, 18:1, 20:4, 22:6, the ratio of saturated to unsaturated LPC, and L-PAF (all $p < .05$ by the Kruskal-Wallis test). For example, the ratio of saturated to unsaturated LPC was 0.97 in Caucasian control subjects, 0.84 in Asian controls, and 0.64 in African American controls, suggesting that levels of saturated and unsaturated LPC were (very) approximately equal in Caucasians and Asians, whereas in African Americans there was a tendency for levels of unsaturated LPC to be approximately twice those of saturated LPC.

10 Table 9 summarizes the ratios of the various markers. As can be seen from this table, there were statistically significant differences between cancer patients and controls with respect to most of the ratios.

Considering each marker individually, LPC 18:2, the ratio of 16:0 to 18:2 and the ratio of 18:1 to 18:2 were the best single discriminators of cancer status. Using a cutoff of about 14.0 (categorized as cancer if LPC 18:2 < about 14.0) the sensitivity and specificity of LPC 18:2 were 73% and 95%, respectively. Using cutoffs of about 2.28 and about 0.86 for the two ratios (in this case categorize as cancer if a ratio was greater than the cutoff), the sensitivity and specificity of the 16:0 to 18:2 ratio was the same as that of 18:2 alone (i.e. 73% and 95%, respectively); the sensitivity of the 18:1 to 18:2 ratio was 58% (specificity was still 95%). Logistic regression models to determine if additional markers could improve on these markers yielded the same results as before, that is, the addition of LPC 18:1 and likely LPC 16:0 increased the sensitivity to of LPC 18:2 alone to 84% and 90%, respectively at 95% specificity (Table 10).

25 Figure 6 compares cancer patients and control subjects with respect to LPC 18:2 levels alone, the ratio of LPC 16:0 to 18:2, and the ratio of LPC 18:1 to 18:2. Although these markers were the best single discriminators, there was considerable overlap in their distributions between the two groups.

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Table 9. Markers (Ratios)

Marker	Cancer			Controls			p-Value ¹
	Mean $\pm$ s.d.	Median	Range	Mean $\pm$ s.d.	Median	Range	
LPC 16:0-18:0 Ratio	2.177 $\pm$ 0.594	2.122	1.206-3.739	2.105 $\pm$ 0.666	2.023	0.773-3.928	.25
LPC 16:0-18:1 Ratio	3.142 $\pm$ 0.786	3.154	1.658-5.719	2.618 $\pm$ 0.622	2.604	1.240-5.600	<.0001
LPC 16:0-18:2 Ratio	2.873 $\pm$ 0.909	2.709	1.409-6.822	1.614 $\pm$ 0.440	1.575	0.745-3.571	<.0001
LPC 16:0-20:4 Ratio	3.947 $\pm$ 2.701	3.543	0.824-15.982	2.872 $\pm$ 1.313	2.626	0.518-8.047	.01
LPC 16:0-22:6 Ratio	6.969 $\pm$ 6.555	5.534	1.128-56.783	4.414 $\pm$ 2.465	3.888	0.892-16.176	<.0001
LPC 18:0-18:1 Ratio	1.543 $\pm$ 0.528	1.530	0.635-2.974	1.351 $\pm$ 0.464	1.338	0.522-2.913	.004
LPC 18:0-18:2 Ratio	1.434 $\pm$ 0.620	1.383	0.505-3.655	0.850 $\pm$ 0.378	0.791	0.262-2.183	<.0001
LPC 18:0-20:4 Ratio	2.112 $\pm$ 1.615	1.562	0.276-8.089	1.559 $\pm$ 0.966	1.295	0.180-6.004	.09
LPC 18:0-22:6 Ratio	3.632 $\pm$ 3.115	2.730	0.355-21.122	2.376 $\pm$ 1.562	2.026	0.310-9.173	.01
LPC 18:1-18:2 Ratio	0.922 $\pm$ 0.201	0.881	0.599-1.558	0.623 $\pm$ 0.123	0.606	0.390-0.954	<.0001
LPC 18:1-20:4 Ratio	1.280 $\pm$ 0.837	0.970	0.380-4.033	1.141 $\pm$ 0.572	0.964	0.325-3.378	.83
LPC 18:1-22:6 Ratio	2.243 $\pm$ 1.918	1.688	0.437-13.297	1.737 $\pm$ 0.951	1.479	0.496-5.835	.16
LPC 18:2-20:4 Ratio	1.350 $\pm$ 0.767	1.062	0.422-3.977	1.819 $\pm$ 0.778	1.711	0.670-4.634	<.0001
LPC 18:2-22:6 Ratio	2.363 $\pm$ 1.700	1.926	0.493-11.008	2.787 $\pm$ 1.323	2.570	0.777-7.538	.0002
LPC 20:4-22:6 Ratio	1.759 $\pm$ 0.572	1.668	0.641-3.846	1.621 $\pm$ 0.633	1.599	0.529-4.109	.03

¹ Wilcoxon rank sum test

Table 10. Logistic Regression Models

Model	⁻² Log Likelihood	Discrimination Equation	Sensitivity ¹
LPC 18:2	180.6	Cancer if LPC 18:2<14.0	73%
16:0:18:2 Ratio	174.5	Cancer if Ratio of LPC 16:0 to 18:2>2.28	73%
18:1 to 18:2 Ratio	181.6	Cancer if Ratio of LPC 18:1 to 18:2>0.86	58%
LPC 18:2 LPC 18:1	119.9	Cancer if LPC 18:2 – (0.79 x LPC 18:1)<5.57	84%
LPC 18:2 LPC 18:1 LPC 16:0	108.0	Cancer if (1.09 x LPC 18:2) – (0.65 x LPC 18:1) – (0.13 x LPC 16:0)<4.67	90%

¹ Specificity held at 95%

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Figure 7 plots LPC 18:2 against 18:1 and demonstrates that the linear combination of these two markers (i.e. categorize as cancer if $\text{LPC 18:2} - 0.79 \times \text{LPC 18:1} < \text{about } 5.57$) does a better job at discrimination than either LPC 18:2 alone or the two ratios discussed. This can also be seen in Figure 8.

5 Figure 8 plots empirical receiver operating characteristic (ROC) curves for LPC 18:2 alone, the ratio of 16:0 to 18:2, the ratio of 18:1 to 18:2, and the linear function of LPC 18:1 and 18:2. As can be seen from this figure, the overall performance of LPC 18:2 and the two ratios were similar, however, a significant improvement was achieved by using the model based on $\text{LPC 18:2} - (0.79 \times \text{LPC 18:1})$.

10 Figure 9 plots the empirical ROC curve for the LPC 18:2 and 18:1 model again and compares it to the ROC curve based on the model in Table 10 that includes LPC 16:0 in addition to LPC 18:2 and 18:1. As before, a modest improvement was achieved by adding LPC 16:0.

As before, there were no differences in marker levels with respect to T-stage,
15 N-stage, or tumor grade; and there were differences between rectal and colon primaries with respect to a number of markers (LPC 18:0, 18:1, 20:0, 20:4, 22:6, the ratio of saturated to unsaturated LPC, most of the ratios summarized in Table 9, and SPC; all $p < .05$ based on the Wilcoxon rank sum test).

The entire teachings of all of the references disclosed herein are incorporated
20 herein by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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CLAIMS

What is claimed is:

1. A method of detecting colorectal cancer in an individual comprising determining a level of lysophosphatylcholine (LPC) in the individual, wherein
5 a level of LPC in the individual that is lower than the level of LPC in a control is indicative of colorectal cancer in the individual.
2. The method of Claim 1 wherein the LPC is selected from the group consisting of: LPC 18:2, LPC 18:1 and a combination thereof.
3. The method of Claim 2, further comprising determining the level of LPC 16:0.
- 10 4. The method of Claim 1 wherein a sample is obtained from the individual and the sample is selected from the group consisting of: whole blood, plasma, serum, lymph and tissue.
5. The method of Claim 4 wherein the level of LPC in the individual is detected using a detection method selected from the group consisting of: a liquid
15 chromatography - mass spectrometry (LC-MS) detection method, an ultraviolet (UV) detection method, an enzymatic detection method and combinations thereof.
6. The method of Claim 5 wherein the LC-MS is an electrospray ionization mass spectrometry (ESI-MS).
- 20 7. The method of Claim 1 wherein the LPC detected is LPC 18:2.
8. The method of Claim 7 wherein a level of about 15 μ M or less of LPC 18:2 in the individual is indicative of colorectal cancer.

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9. The method of Claim 1 wherein the LPC detected is LPC 18:1.
10. The method of Claim 9 wherein a level of about 11 μ M or less of LPC 18:1 in the individual is indicative of colorectal cancer.
11. The method of Claim 1 wherein the LPC detected is a combination of levels of
5 LPC 18:2 and LPC 18:1.
12. The method of Claim 10 wherein if

LPC 18:2 - 0.78(LPC 18:1) is less than or equal to about 6.2 μ M,

then colorectal cancer is detected in the individual.
13. The method of Claim 10 wherein if

10 LPC 18:2 - 0.8(LPC 18:1) is less than or equal to about 7.2 μ M,

then colorectal cancer is detected in the individual.
14. A method of detecting colorectal cancer in an individual comprising
determining a level of lysophosphatylcholine (LPC) 18:2 in the individual
wherein a level of LPC 18:2 in the individual that is lower than the level of
15 LPC 18:2 in a control is indicative of colorectal cancer in the individual.
15. The method of Claim 14 wherein a sample is obtained from the individual and
the sample is selected from the group consisting of: whole blood, plasma,
serum, lymph and tissue.
16. The method of Claim 15 wherein the level of LPC 18:2 in the individual is

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detected using a detection method selected from the group consisting of: a liquid chromatography - mass spectrometry (LC-MS) detection method, an ultraviolet (UV) detection method, an enzymatic detection method and combinations thereof.

- 5 17. The method of Claim 16 wherein the LC-MS is an electrospray ionization mass spectrometry (ESI-MS).
18. The method of Claim 14 wherein a level of about 15 μ M or less of LPC 18:2 in the individual is indicative of colorectal cancer.
19. The method of Claim 14 further comprising detecting the level of LPC 18:1.
- 10 20. The method of Claim 19 wherein a level of about 11 μ M or less of LPC 18:1 in the individual is indicative of colorectal cancer.
21. A method of detecting colorectal cancer in an individual comprising determining a level of lysophosphatylcholine (LPC) 18:1 in the individual wherein a level of LPC 18:1 in the individual that is lower than the level of
15 LPC 18:1 in a control is indicative of colorectal cancer in the individual.
22. The method of Claim 21 wherein a sample is obtained from the individual and the sample is selected from the group consisting of: whole blood, plasma, serum, lymph and tissue.
23. The method of Claim 22 wherein the level of LPC 18:1 in the individual is
20 detected using a detection method selected from the group consisting of: a liquid chromatography - mass spectrometry (LC-MS) detection method, an ultraviolet (UV) detection method, an enzymatic detection method and combinations thereof.

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24. The method of Claim 23 wherein the LC-MS is an electrospray ionization mass spectrometry (ESI-MS).
25. The method of Claim 21 wherein a level of about 11 μ M or less LPC 18:1 in the individual is indicative of colorectal cancer.
- 5 26. The method of Claim 21 further comprising detecting the level of LPC 18:2.
27. The method of Claim 26 wherein a level of about 15 μ M or less of LPC 18:2 in the individual is indicative of colorectal cancer.
28. A method of detecting early stage colorectal cancer in an individual comprising determining a level of lysophosphatylcholine (LPC) in the
10 individual, wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of early stage colorectal cancer in the individual.
29. The method of Claim 28 wherein the LPC is selected from the group consisting of: LPC 18:2, LPC 18:1 and a combination thereof.
- 15 30. The method of Claim 29, further comprising determining the level of LPC 16:0.
31. The method of Claim 28 wherein a sample is obtained from the individual and the sample is selected from the group consisting of: whole blood, plasma, serum, lymph and tissue.
- 20 32. The method of Claim 31 wherein the level of LPC in the individual is detected using a detection method selected from the group consisting of: a liquid chromatography - mass spectrometry (LC-MS) detection method, an ultraviolet (UV) detection method, an enzymatic detection method and

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combinations thereof.

33. The method of Claim 32 wherein the LC-MS is an electrospray ionization mass spectrometry (ESI-MS).
34. The method of Claim 29 wherein the LPC detected is LPC 18:2.
- 5 35. The method of Claim 34 wherein a level of about 15 μ M or less of LPC 18:2 in the individual is indicative of colorectal cancer.
36. The method of Claim 29 wherein the LPC detected is LPC 18:1.
37. The method of Claim 36 wherein a level of about 11 μ M or less of LPC 18:1 in the individual is indicative of colorectal cancer.
- 10 38. The method of Claim 29 wherein the LPC detected is a combination of levels of LPC 18:2 and LPC 18:1.
39. The method of Claim 37 wherein if
- LPC 18:2 - 0.8(LPC 18:1) is less than or equal to about 7.2 μ M,
- then colorectal cancer is detected in the individual.
- 15 40. A method of screening an asymptomatic individual for colorectal cancer, comprising determining a level of LPC in an individual wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of colorectal cancer in the asymptomatic individual.
41. The method of Claim 40 wherein the LPC is selected from the group
- 20 consisting of: LPC 18:2, LPC and 18:1 and a combination thereof.

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42. The method of Claim 41, further comprising determining the level of LPC 16:0.
43. A method of differentiating between a benign disease and a malignant colorectal cancer in an individual comprising determining a level of LPC in the individual wherein a level of LPC in the individual that is lower than the level of LPC in a control is indicative of malignant CRC in the individual, and wherein a level of LPC in the individual that is substantially the same as or higher than the control level is indicative of a benign colorectal disease in the individual.
44. The method of Claim 43 wherein the LPC is selected from the group consisting of: LPC 18:2, LPC 18:1 and a combination thereof.
45. The method of Claim 44, further comprising determining the level of LPC 16:0.
46. A method of monitoring a treatment regimen for colorectal cancer in an individual comprising monitoring a level of LPC in the individual wherein a level of LPC in the individual that is essentially same as or higher than the level of LPC in a control sample is indicative of a successful treatment regimen, and wherein a level of LPC in the individual that is lower than the level of LPC in the control is indicative of an unsuccessful treatment regimen.
47. The method of Claim 46 wherein the LPC is selected from the group consisting of: LPC 18:2, LPC 18:1 and a combination thereof.
48. The method of Claim 47, further comprising determining the level of LPC 16:0.
49. A kit for detecting the presence of LPC in a sample from an individual comprising a compound or an agent capable of detecting an LPC selected from

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the group consisting of: LPC 18:2, LPC 18:1, LPC 16:0 and a combination thereof, in the sample.

50. The kit of Claim 49 further comprising a means for determining the amount of LPC in the sample; and/or a means for comparing the amount of LPC in the
5 sample with a control.
51. The kit of Claim 49 further comprising a control.
52. The kit of Claim 49 further comprising instructions for using the kit to detect LPC.

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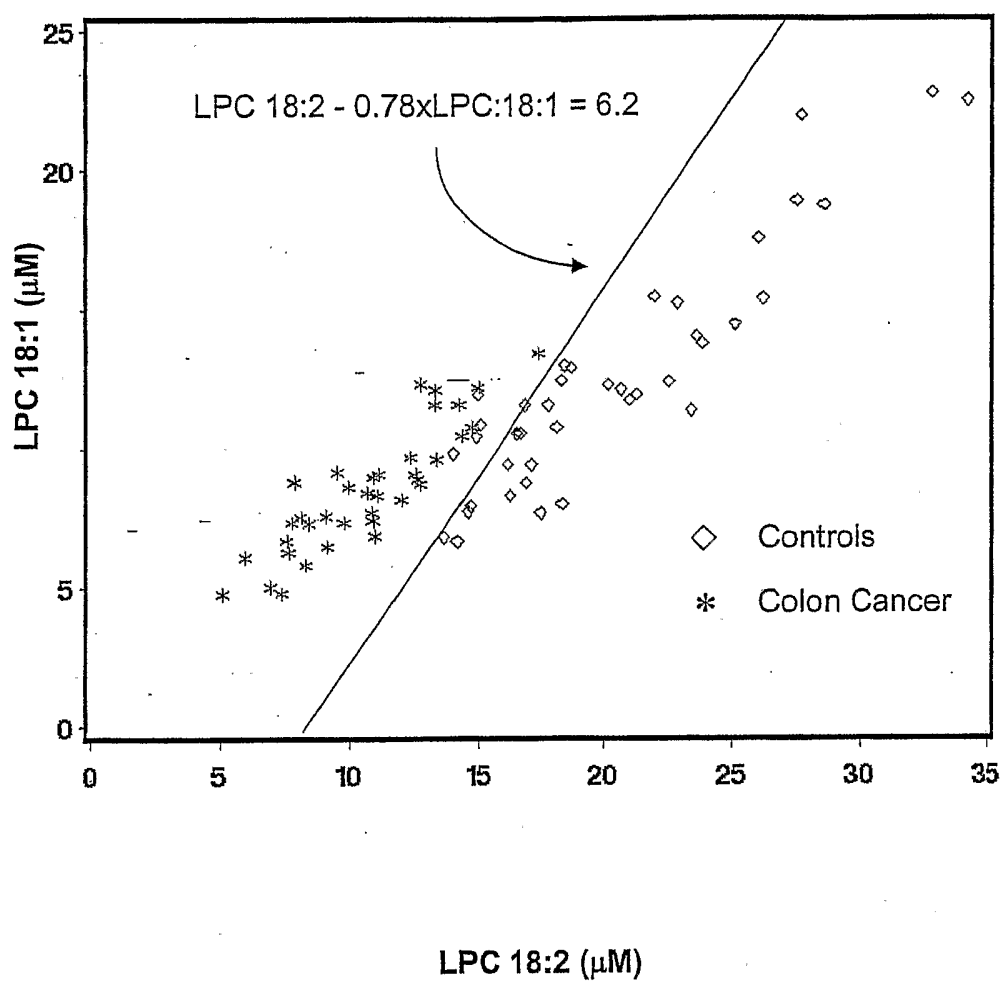


Figure 1

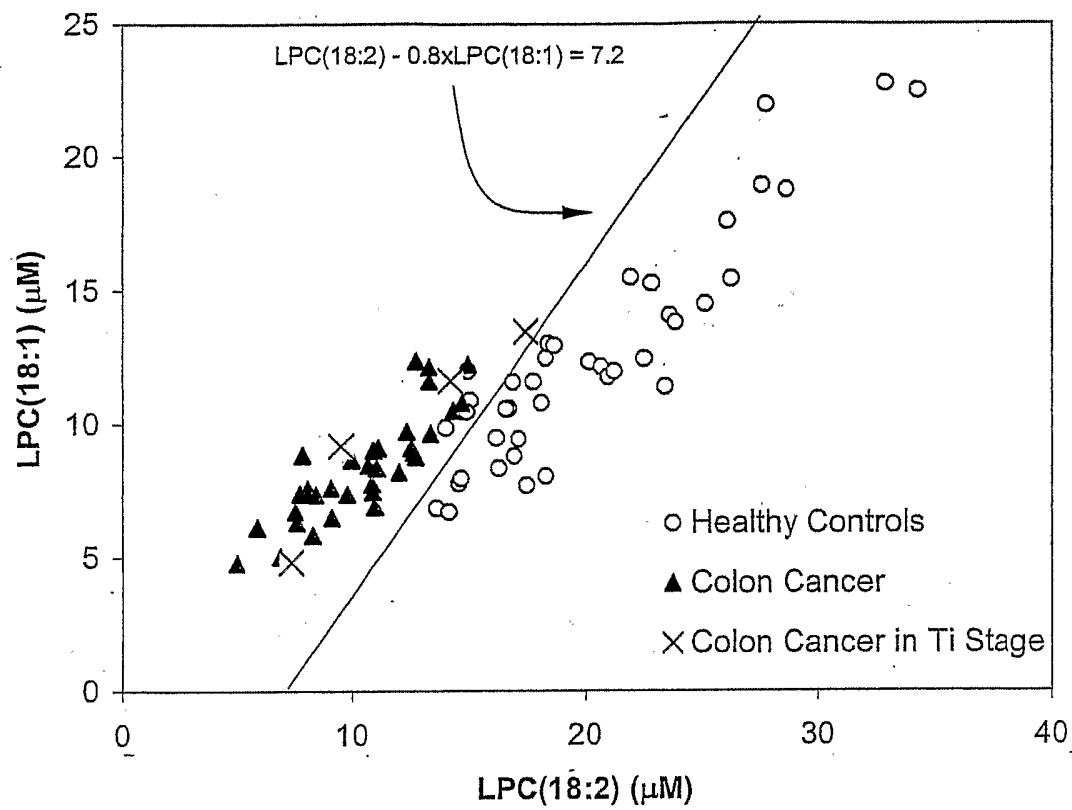


Figure 2

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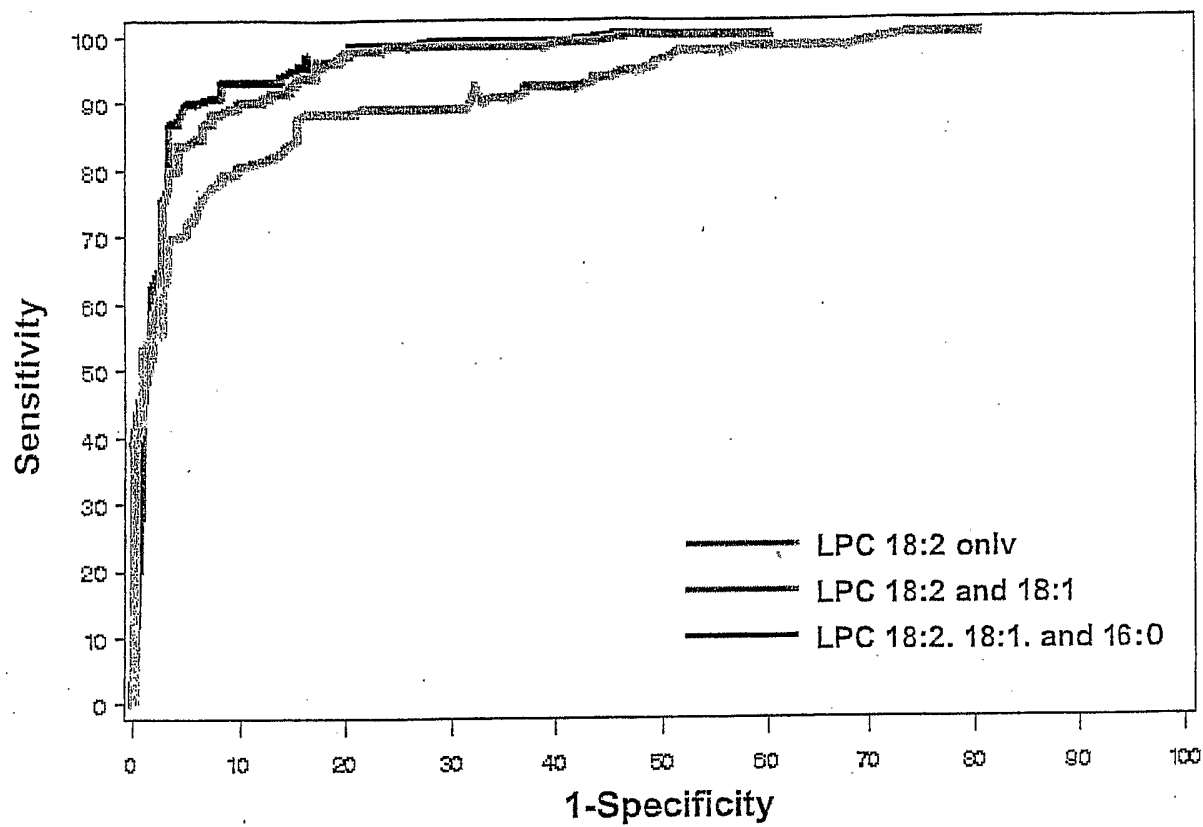


Figure 3

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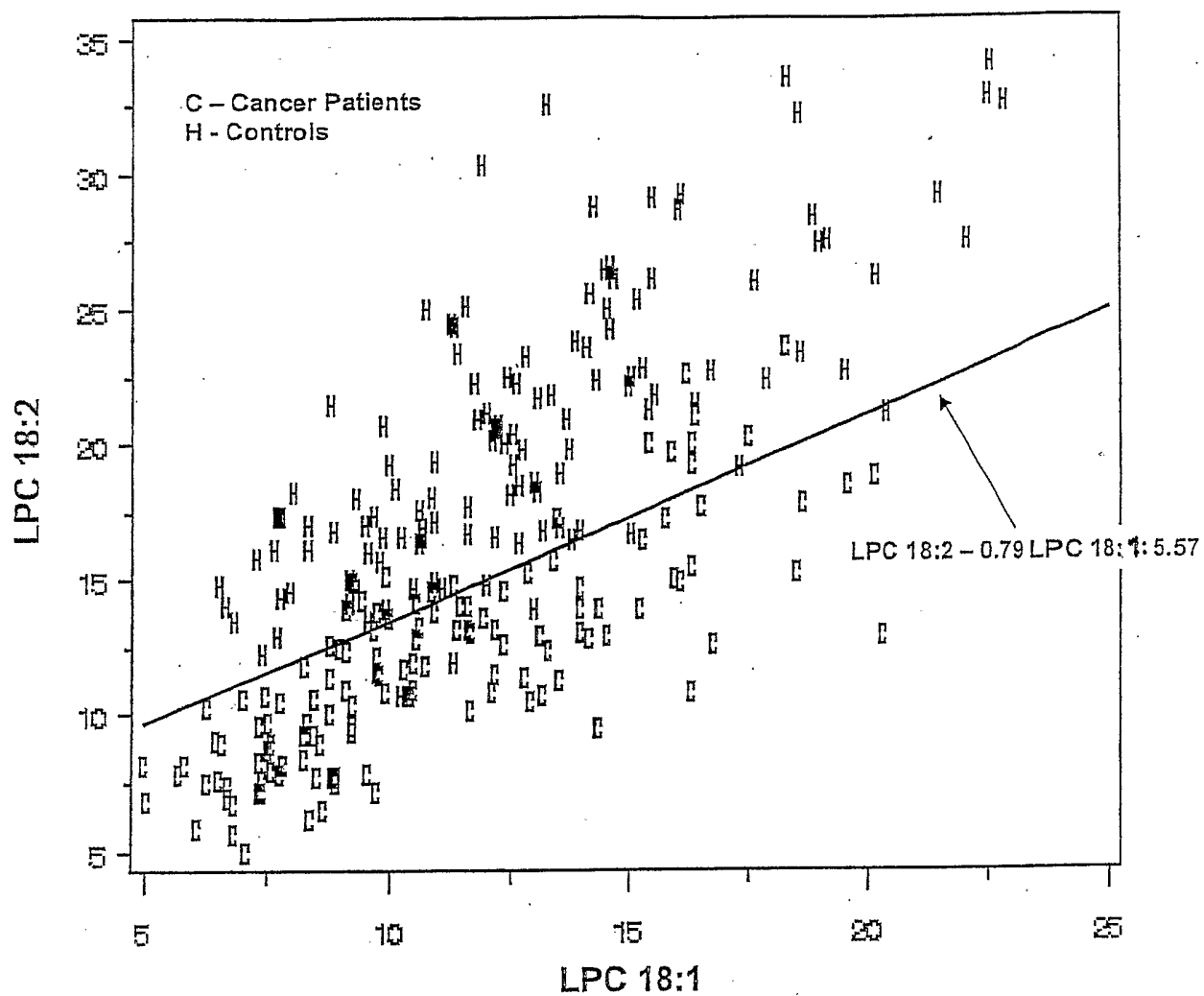


Figure 4

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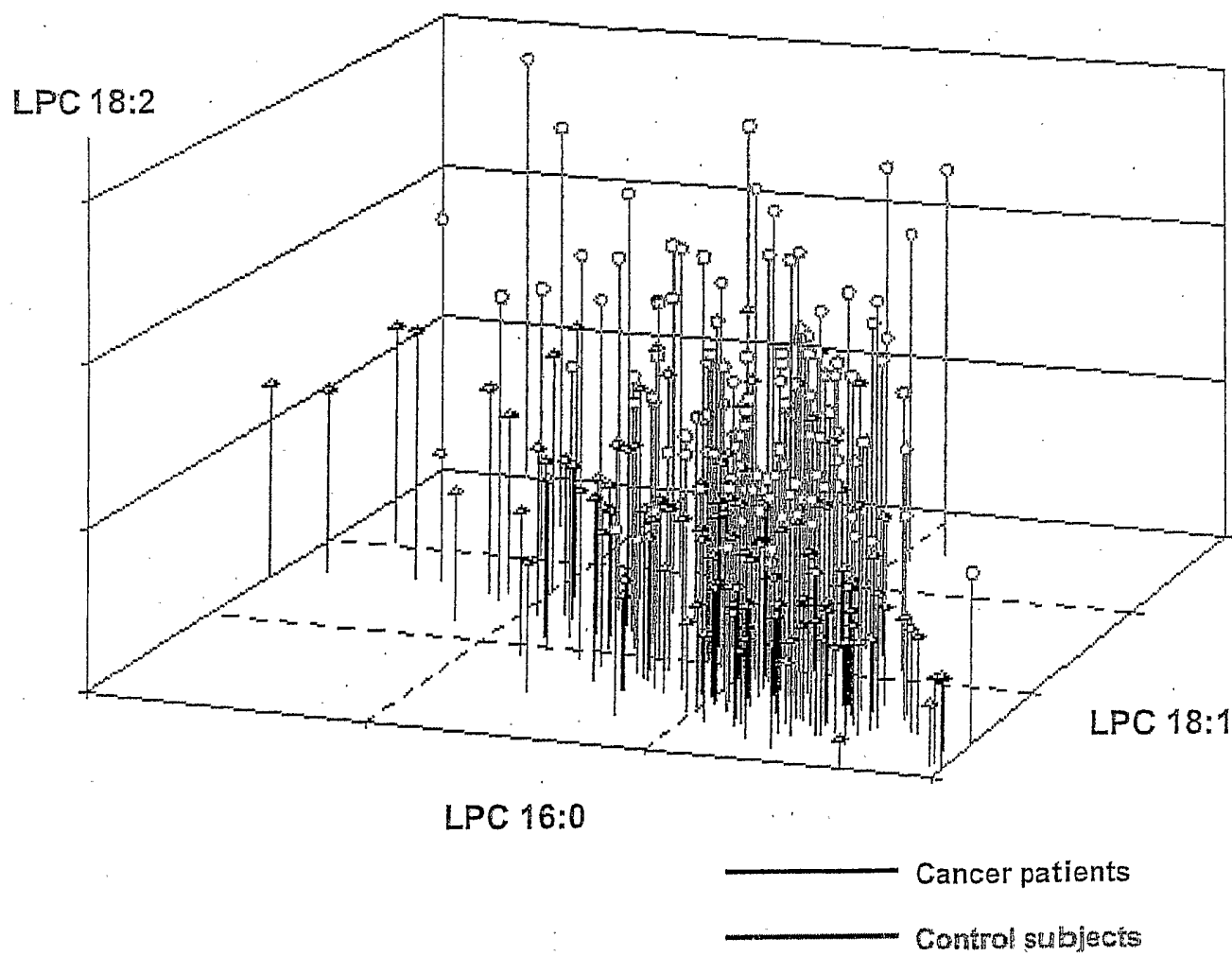


Figure 5

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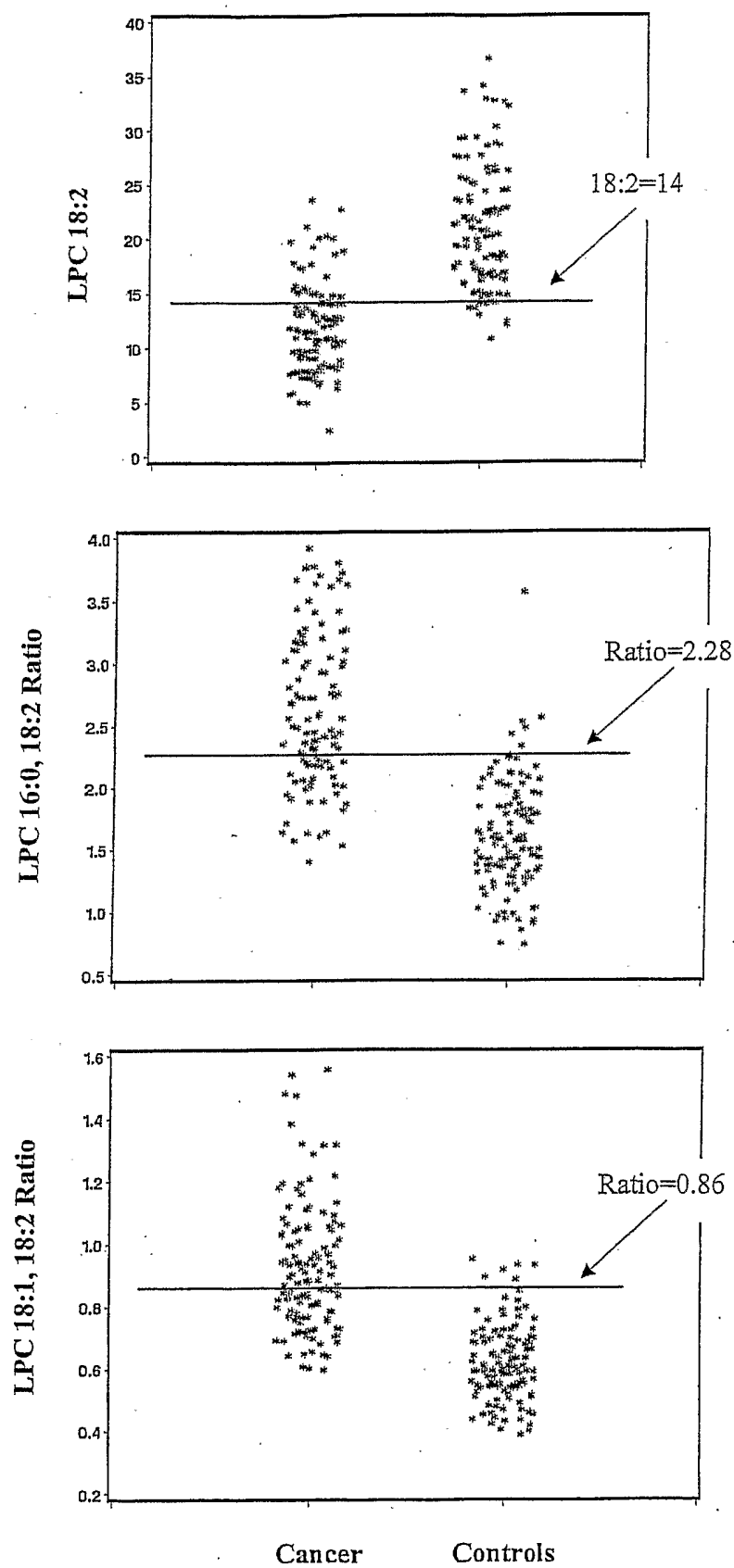


Figure 6

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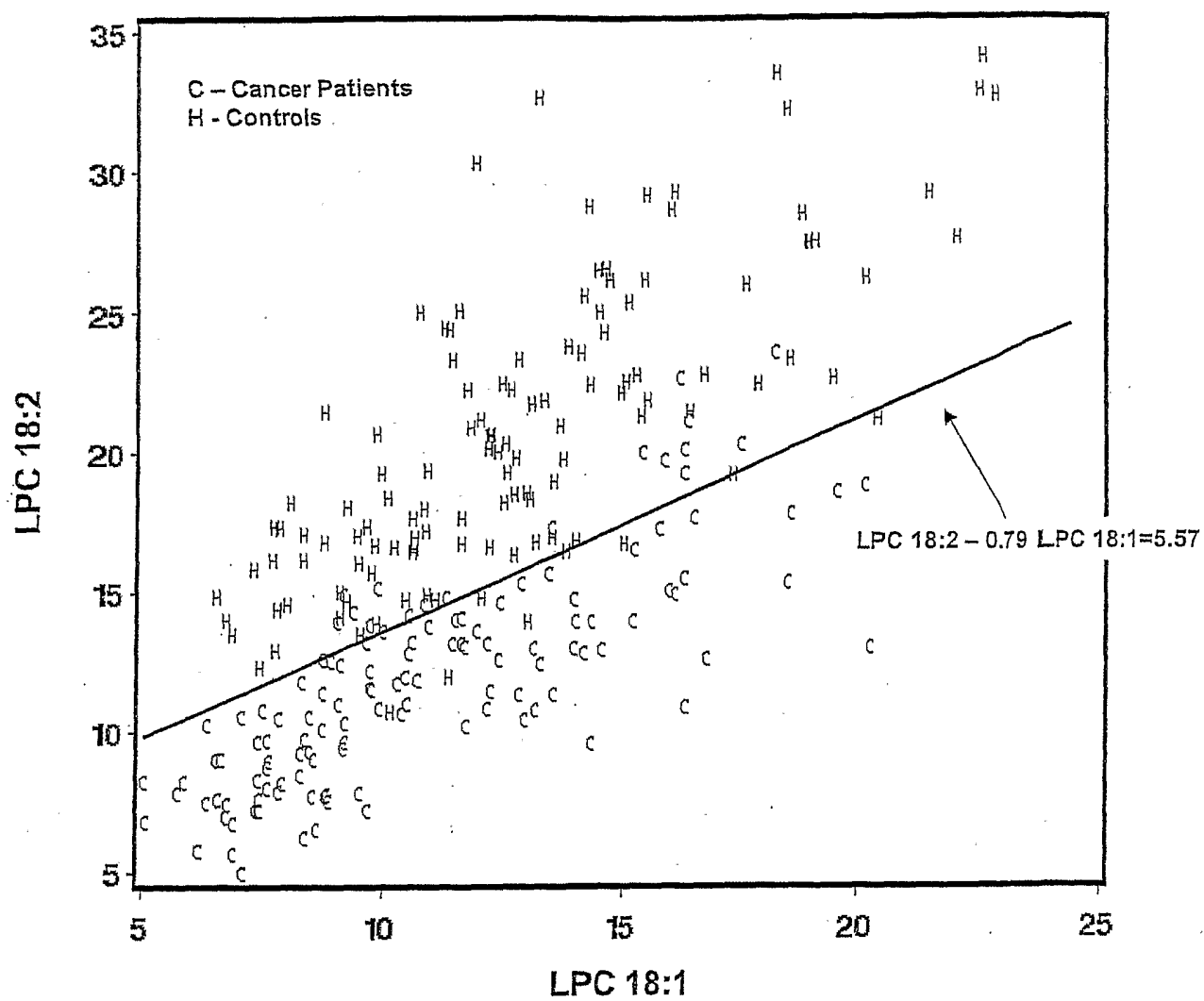
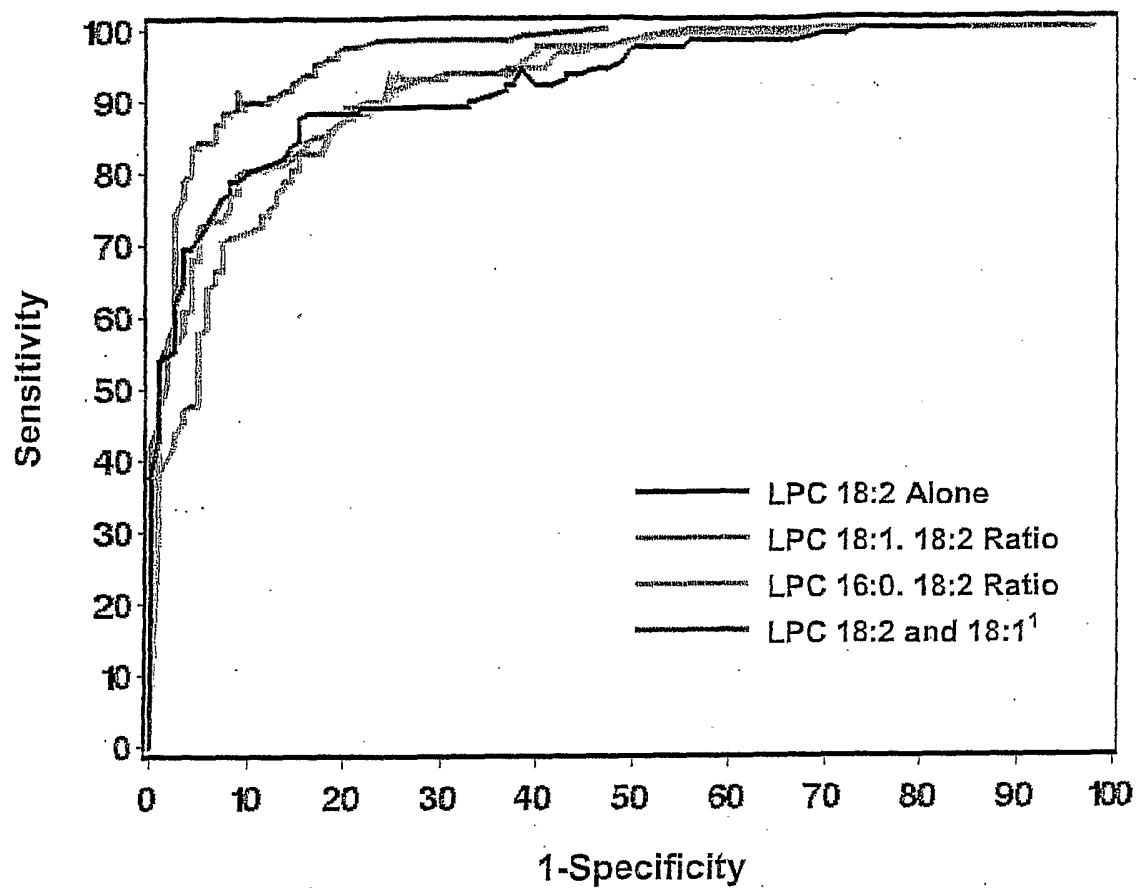


Figure 7

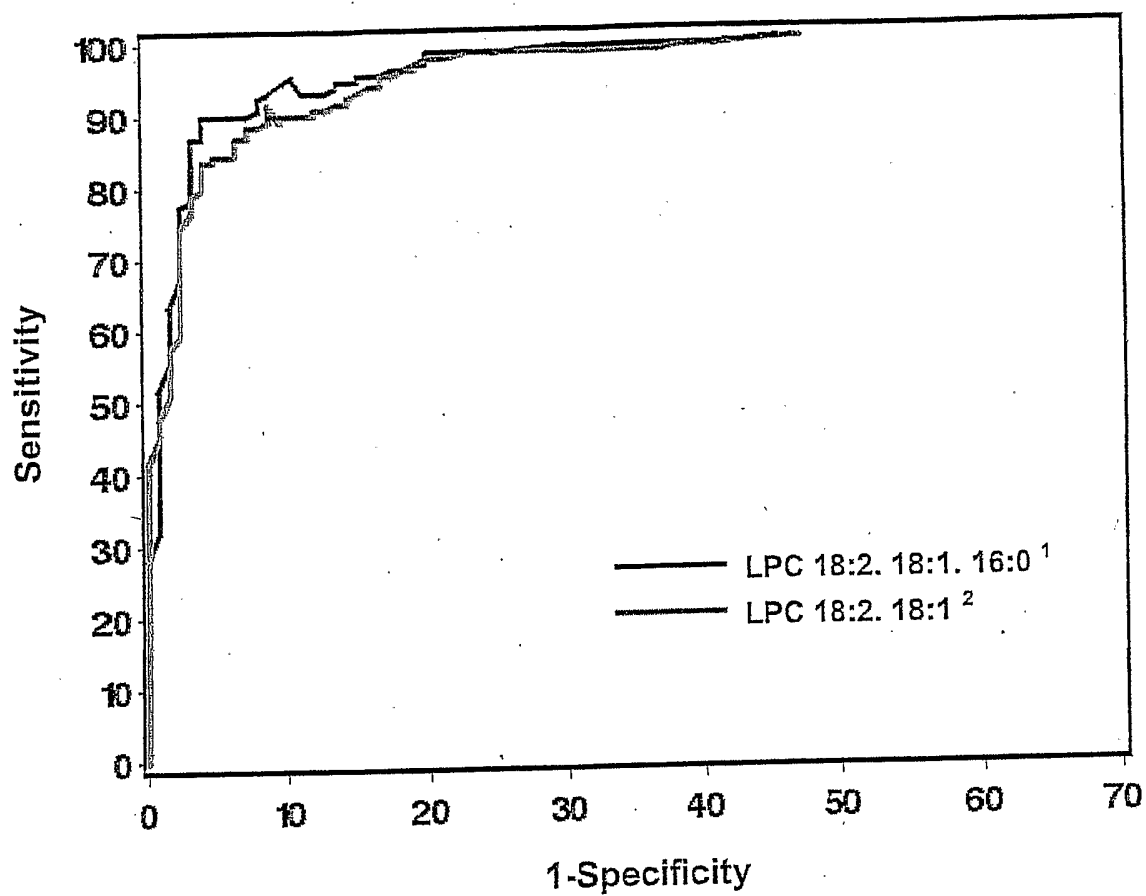
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¹ $\text{LPC 18:2} - 0.79 \times \text{LPC 18:1} < 5.57$

Figure 8

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$$^1 (1.09 \times \text{LPC } 18:2) - (0.65 \times \text{LPC } 18:1) - (0.13 \times \text{LPC } 16:0) < 4.67$$

$$^2 \text{LPC } 18:2 - 0.79 \times \text{LPC } 18:1 < 5.57$$

Figure 9

INTERNATIONAL SEARCH REPORT

Int. application No
PCT/US2005/037049

A. CLASSIFICATION OF SUBJECT MATTER
G01N33/574 G01N33/92

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARIANO-A ET AL: "Ganglioside and phospholipid patterns in human gastrointestinal tumors" BULLETIN OF MOLECULAR BIOLOGY AND MEDICINE, vol. 19, no. 2-4, 1994, pages 115-126, XP008059882 ISSN: 0391-481X	1,4
Y	the whole document ----- -/--	1-48

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

15 February 2006

Date of mailing of the international search report

01/03/2006

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INTERNATIONAL SEARCH REPORT

Inte application No
PCT/US2005/037049

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	"Choline glycerophospholipids" 'Online! 12 August 2004 (2004-08-12), pages 1-4, XP002367454 INTERNET Retrieved from the Internet: URL: http://web.archive.org/web/20040812062002/http://www.cyberlipid.org/phlip/pgly03.htm > 'retrieved on 2006-02-13! page 1 page 3, paragraph 5	1-48
Y	EHEHALT R ET AL: "Phosphatidylcholine and lysophosphatidylcholine in intestinal mucus of ulcerative colitis patients. A quantitative approach by nanoElectrospray-tandem mass spectrometry." SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY. AUG 2004, vol. 39, no. 8, August 2004 (2004-08), pages 737-742, XP008059885 ISSN: 0036-5521 the whole document	5,6,16, 17,23, 24,32,33
X	US 2002/107197 A1 (XU YAN ET AL) 8 August 2002 (2002-08-08) abstract; example 4	49-52
A	MERCHANT T E ET AL: "PHOSPHOLIPID PROFILES OF HUMAN COLON CANCER USING 31P MAGNETIC RESONANCE SPECTROSCOPY" INTERNATIONAL JOURNAL OF COLORECTAL DISEASE, SPRINGER VERLAG, BERLIN, DE, vol. 6, no. 2, 1991, pages 121-126, XP001000922 ISSN: 0179-1958 the whole document	1-48
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A	WO 2004/071267 A (ROCHE DIAGNOSTICS GMBH; F.HOFFMANN-LA ROCHE AG; BERNDT, PETER; HAGMANN) 26 August 2004 (2004-08-26) abstract; claim 5	1-48

INTERNATIONAL SEARCH REPORT

Information on patent family members

In  al application No
PCT/US2005/037049

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 2002107197	A1	08-08-2002	NONE	
WO 2004071267	A	26-08-2004	NONE	